THE EFFECT OF CHLORIDE CONTENT IN WATER ON THE TOXICITY OF SODIUM NITRITE FOR SPINY-CHEEK CRAYFISH (*ORCONECTES LIMOSUS* RAF.)

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ABSTRACT

Toxicity of sodium nitrite for the spiny-cheek crayfish (*Orconectes limosus*) was assessed in relation to the concentration of chlorides diluted in water. Results of the tests confirmed a favourable effect of chloride content in water on the tolerance of spiny-cheek crayfish to nitrites. The $96hLC_{50}$ value of 4.8 mg l^{-1}, 17.7 mg l^{-1}, 34.7 mg l^{-1}, 50.5 mg l^{-1}, 74.4 mg l^{-1}, and 96.6 mg l^{-1} $N\text{-}NO_2^-$ found out in individual tests corresponded, respectively to a chloride content of 11, 40, 100, 200, 300 and 400 mg l^{-1} Cl^- in diluting water. Linear relationship of the $96hLC_{50}$ values on chloride concentration (y = 0.23 ×+ 7) was proven statistically (R = 0.995).

Key-words: nitrite, crayfish, chlorides, water quality, toxicity test.

EFFET DE LA CONCENTRATION EN CHLORIDES DE L'EAU SUR LA TOXICITE DU NITRITE DE SODIUM POUR L'ECREVISSE AMÉRICAINE (*ORCONECTES LIMOSUS* RAF.)

RÉSUMÉ

La toxicité du nitrite de sodium vis-à-vis de l'écrevisse américaine *(Orconectes limosus*) a été évaluée en relation avec la concentration des chlorides dilués dans l'eau. Les résultats des expériences ont confirmé l'effet favorable de la concentration des chlorides dans l'eau sur la tolérance des écrevisses américaines envers les nitrites. Les valeurs du $96hLC_{50}$ de 4,8 mg l^{-1}, 17,7 mg l^{-1}, 34,7 mg l^{-1}, 50,5 mg l^{-1}, 74,4 mg l^{-1}, et 96,6 mg l^{-1} de $N\text{-}NO_2^-$ trouvées dans les expériences correspondent respectivement à une teneur de l'eau en chlorides de 11, 40, 100, 200, 300 et 400 mg l^{-1} Cl^-. Une relation linéaire entre les valeurs du $96hLC_{50}$ et les concentrations en chlorides de l'eau (y = 0.23 × + 7) a été prouvée statistiquement (R = 0,995).

Mots-clés : nitrates, écrevisses, chlorides, qualité de l'eau, test de toxicité.

INTRODUCTION

Nitrite ($N\text{-}NO_2^-$) is an intermediate product in the oxidation of ammonia. Nitrite is usually found together with nitrate and ammonia in water. It usually occurs in small concentration considering its chemical and biochemical instability. In aquatic ecosystem,

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nitrite concentrations are elevated by pollution with nitrogenous wastes and imbalances in bacterial nitrification and denitrification processes. Nitrite is transformed by nitrification to nitrate under aerobic condition. The average concentration of N-NO_2^- in ground water ranges from 0.004-0.179 mg l^{-1}. Higher concentrations (more than 1 mg l^{-1}) occur in wastewater (PITTER, 1999) and could increase also in intensive aquaculture and recirculation systems usually in starting filters. The nitrification process is used to decrease ammonia concentration that is the main product of fish metabolism (SVOBODOVÁ *et al.*, 2003). In fish, nitrite is absorbed at the gills and oxidizes the iron in the haemoglobin molecule to methemoglobin. The result of nitrite poisoning is hypoxia caused by a reduction in the blood's oxygen carrying ability. It is likely that the same type of reaction that occurs with the iron of haemoglobin also occurs with the copper in crustacean hemocyanin (COLT and ARMSTRONG, 1981)

Freshwater fish and crustaceans are hyperosmotic to their environment. They actively take in ions via the gills to balance that lost in their urine and the passive outflow by gills. Nitrite has an affinity for the active chloride uptake mechanism by chloride cells in the gills (MAETZ, 1971). Chloride cells excrete ammonia or H^+ ions for Na^+ ions and bicarbonate (HCO_3^-) for Cl^- ions (LOVE, 1980). NO_2^- has affinity to Cl^-/HCO_3^- exchanging. Part of Cl^- demand is replaced by NO_2^- when it is in water. Fish with higher speed of chloride uptake by gills (rainbow trout, perch, pike) are more sensitive to nitrites then fish with lower speed of chloride uptake (eel, common carp, tench) (WILLIAMS and EDDY, 1986). The competition between chloride and nitrite ions transport across the gill membrane explains because the higher concentration of Cl^- protects fish against toxic impact of nitrite (JENSEN, 2003). The positive effect of chloride for fish tolerance to nitrites has been demonstrated in other studies in fish (SVOBODOVÁ *et al*., 1987; HILMY, 1987; ATWOOD *et al*., 2001; HUERTAS *et al*., 2002; TAVARES and BOYD, 2003; FULLER *et al*., 2003) and for crayfish (JEBERG and JENSEN, 1994; BEITINGER and HUEY, 1981). CRAWFORD and ALLEN (1977) showed that toxicity of nitrite depended on water salinity. In seawater, 50-100 times lower mortality was observed than in fresh water in same concentration of nitrite. The relationship between nitrite toxicity and chloride concentration is linear (RUSSO and THURSTON, 1977). The effect of nitrite on chloride regulation in *Pacifastacus leniusculus* was studied by HARRIS and COLEY (1991).

Other anions also have a positive impact on decreasing nitrite toxicity, mainly bromide (EDDY *et al*., 1983). Carbonate and nitrate also have a significant impact but they are not as effective as chloride and bromide (LEWIS and MORRIS, 1986).

The $96hLC_{50}$ value for nitrite in freshwater fish ranges from 0.66 to 200 mg l^{-1}, while values for crustaceans range from 8.5 to 15.4 mg l^{-1} (BOYD, 1990 *in* ROUSE, KASTNER and REDDY, 1995). Only WICKINS (1976) mentioned a high tolerance to nitrite in prawns ($48hLC_{50}$=170 mg l^{-1} N-NO_2^-). ROUSE, KASTNER and REDDY (1995) found the $96hLC_{50}$ values for nitrite in hatching redclaw crayfish, *Cherax quadricarinatus* of 1.03 mg l^{-1} ($24hLC_{50}$=1.4, $48hLC_{50}$=1.1, $72hLC_{50}$=1.1 mg l^{-1}). HYMEL (1985) in ROUSE, KASTNER and REDDY (1995) reported that the $96hLC_{50}$ value for nitrite toxicity to juvenile *Procambarus clarkii* was 5.94 mg l^{-1}, LIU *et al*. (1995) reported a 4.7 mg l^{-1} nitrite toxicity to juvenile redclaw and POLICAR *et al*. (2003) reported a $48hLC_{50}$ value for one year old noble crayfish of 6.7-33.3 mg l^{-1} NO_2^-. These higher values could be related to age and bigger size of these species (ROUSE, KASTNER and REDDY, 1995). MALONE and BURDEN (1988) in ROUSE, KASTNER and REDDY (1995) recommended maintaining nitrite levels below 0.5 mg l^{-1} in recirculation systems used for holding adult *P. clarkii*.

Low levels of nitrite could produce sub lethal effects where the fitness of the species might be reduced because of either ionic imbalance of extra cellular fluids or increased energy costs of maintaining additional transport sites (HARRIS and COLEY, 1991). No mortality was observed in redclaw crayfish in 10 mg l^{-1} NO_2^- ($24hLC_{50}$ = 42.9 mg l^{-1} NO_2^-, $48hLC_{50}$ = 37.1 mg l^{-1} NO_2^-, $96hLC_{50}$ = 25.9 mg l^{-1} NO_2^-) (MEADE and WATTS, 1995). ROUSE, KASTNER and REDDY (1995) presented that 24 hours exposure to nitrite concentrations of 0.4 mg l^{-1} NO_2^- and 0.6 mg l^{-1} NO_2^- decreased subsequent growth of redclaw crayfish by 17% and 67% and increased mortality by 5% and 48%, respectively.

The aim of this study was to examine acute toxicity of nitrite in spiny-cheek crayfish and to verify the positive effect of chloride on increasing crayfish tolerance to higher concentrations of nitrite in water.

MATERIAL AND METHODS

The methodology of acute toxicity test in fish (ČSN EN ISO 7346-1) was carried out for acute toxicity tests. One-year-old spiny-cheek crayfish of 38.7 ± 5.3 mm (27-55 mm) of total body length, 11.9 ± 1.8 mm (7-18 mm) of carapace length and 1.6 ± 0.7 g (0.45-4.75 g) of body weigh were collected from Kořensko dam (Vltava river).

Altogether, six toxicological tests with various chloride concentrations in water were performed. One of them was without chloride addition (content of chlorides in diluting water = 11 mg l^{-1} Cl^-). The others were with added chloride concentration in water (final concentrations = 40, 100, 200, 300, 400 mg l^{-1} Cl^-). Two day-old aerated drinking water (pH 7.2, ANC (acid neutralizing capacity) 0.5 mmol l^{-1}, COD_{Mn} (permanganate index) 2.2 mg l^{-1}, $\Sigma Ca^{2+} + Mg^{2+}$ 14.0-15.0 mmol l^{-1}, N-NH_4^+ 0.03 mg l^{-1}, N-NO_3^- 5.2 mg l^{-1}, N-NO_2^- ≤ 0.01 mg l^{-1}, Cl^- 11.0 mg l^{-1}, SO_4^{2-} 51.5 mg.l^{-1}, P-PO_4^{3-} ≤ 0.01 mg l^{-1}) was used as a diluting water.

Sodium nitrite ($NaNO_2$) was used as the toxicant for the nitrite bioassay. Test concentrations for nitrites were:

- 2.0, 4.1, 10.1, 20.3, 41.6, 60.9 mg l^{-1} N-NO_2^- at 11, 40 and 100 mg l^{-1} Cl^-;
- 20.3, 40.6, 60.9, 81.2,101.0, 121.7 mg l^{-1} N-NO_2^- at 200 and 300 mg l^{-1} Cl^-;
- 20.3, 40.6, 60.9, 81.2, 101.0, 121.7, 142.0 and 162.3 mg l^{-1} N-NO_2^- at 400 mg l^{-1} Cl^-

Chloride was dosed as a NaCl.

Totally, 1 100 crayfish were used for the experiment (25 crayfish were used for each chloride and nitrite concentration). The crayfish were stocked to 25-litre aquarium filled with 15 litres of water. Water was not exchange. Plastic spirals were provided as shelters to minimize cannibalism. Crayfish were stocked in the diluting water without the substance being tested.

Exposure time was 96 hours. Crayfish were not fed during the test. Crayfish mortality, oxygen content, pH value and temperature were observed 1 hour after stocking and then daily.

Water pH value was not adjusted and ranged from 7.05 to 7.84. Temperature during the test ranged from 17°C to 19°C, and the oxygen saturation was more than 80%.

Values of $96hLC_0$, $96hLC_{50}$, $96hLC_{100}$ nitrite toxicity were calculated by probits analysis using software EKOTOX v 5.1 (INGEO Liberec CZ). The $96hLC_{50}$ value was statistically evaluated by regression analyses using Microsoft Excel.

RESULTS

Nitrite toxicity test in diluting water

The $96hLC_{50}$ value for N-NO_2^- was 4.8 mg l^{-1}. The first mortality was observed after 24-hour exposition. Overall, 12% crayfish died in 4.1 mg l^{-1} N-NO_2- and 100% crayfish died in 60.9 mg l^{-1} N-NO_2^-. Massive crayfish mortality was observed also in concentration of 10.1, 20.3 and 40.6 mg l^{-1} N-NO_2^- with total mortality after the next 24 hours. No mortality or behavioural changes were observed in the control group and in a $N\text{-}NO_2^-$ concentration of 2.0 mg l^- (Table I).

Table I

Acute nitrite toxicity (cumulative mortality) for *O. limosus* related to time of exposure in a chloride concentration of 11 mg l^{-1} Cl^- (diluting water).

Tableau I

Toxicité aiguë des nitrites (mortalité cumulative) pour *O. limosus* en relation avec l'exposition à une concentration en chlorides de 11 mg l-1 Cl- dilués dans l'eau.

Concentration of $N\text{-}NO_2^-$ (mg l^{-1})	Cumulative mortality (%)				
	Time of exposition				
	1 hour	24 hours	48 hours	72 hours	96 hours
0.0 (control group)	0	0	0	0	0
2.0	0	0	0	0	0
4.1	0	12	12	12	12
10.1	0	72	100	100	100
20.3	0	84	100	100	100
40.6	0	96	100	100	100
60.9	0	100	100	100	100

Toxicity test with higher level of chloride in diluting water

The results of acute toxicity tests at the variable Cl^- concentrations are summarised in Table II.

The Figure 1 show that higher concentrations of chlorides in water markedly increased crayfish tolerance to nitrite.

While a $N\text{-}NO_2^-$ concentration of 10.1 mg l^{-1} with 11 mg l^{-1} of chloride induced 100% mortality after 24 hours, the same nitrite concentration with 40 mg l^{-1} of chloride did not induce any mortality after 96 hours exposition.

No mortality was observed in 400 mg l^{-1} of Cl^- in 60.9 mg l^{-1} of $N\text{-}NO_2^-$ concentration after 96 hours exposition (Figure 1).

The $96hLC_{50}$ value of 4.8 mg l^{-1}, 17.7 mg l^{-1}, 34.7 mg l^{-1}, 50.5 mg l^{-1}, 74.4 mg l^{-1}, and 96.6 mg l^{-1} $N\text{-}NO_2^-$ found out in individual tests corresponded, respectively to a chloride content of 11, 40, 100, 200, 300 and 400 mg l^{-1} Cl^- in diluting water (Table III).

Linear dependence was found for $96hLC_{50}$ of sodium nitrite in various chloride concentrations (Figure 2).

The regression equation was calculated as:

$$y = 0.23 \times + 7,$$

with

x = chloride concentration in diluted water (mg l^{-1} Cl^-),
y = $96hLC_{50}$ value (mg l^{-1} $N\text{-}NO_2^-$).

The $96hLC_0$ and the $96hLC_{100}$ values increased similarly as the $96hLC_{50}$ value relate to chloride concentration.

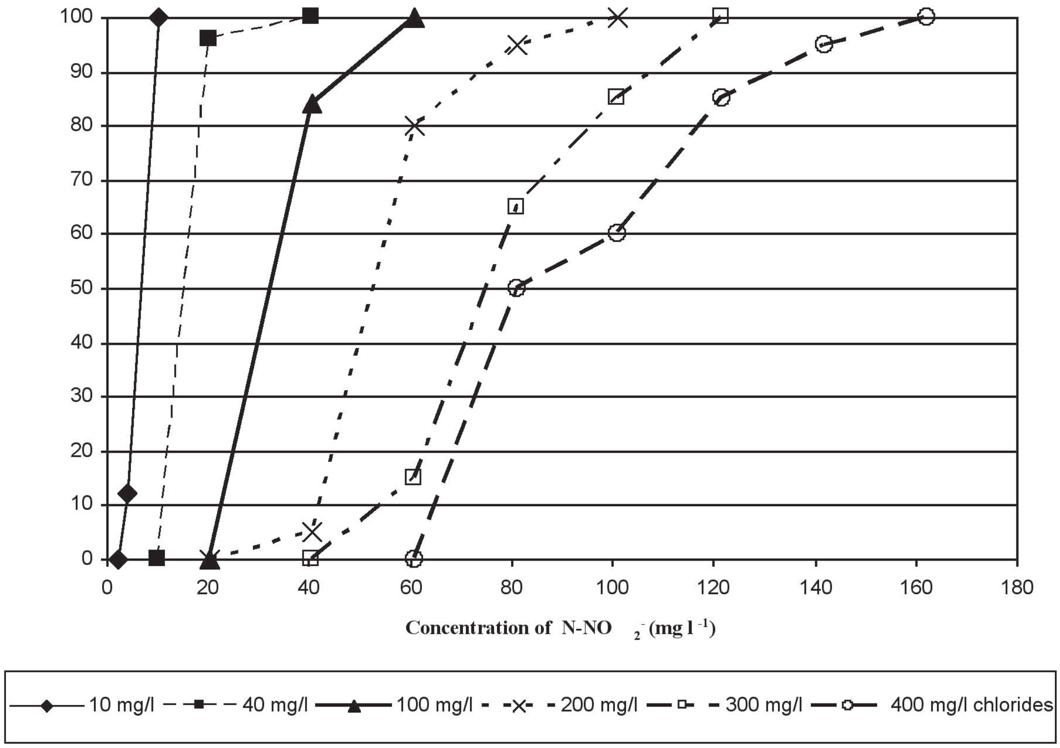

Figure 1
Impact of chlorides to toxicity of nitrites for *O. limosus*. (96-h exposure).

Figure 1
Effet de la toxicité des nitrites sur *O.limosus* (96 h d'exposition).

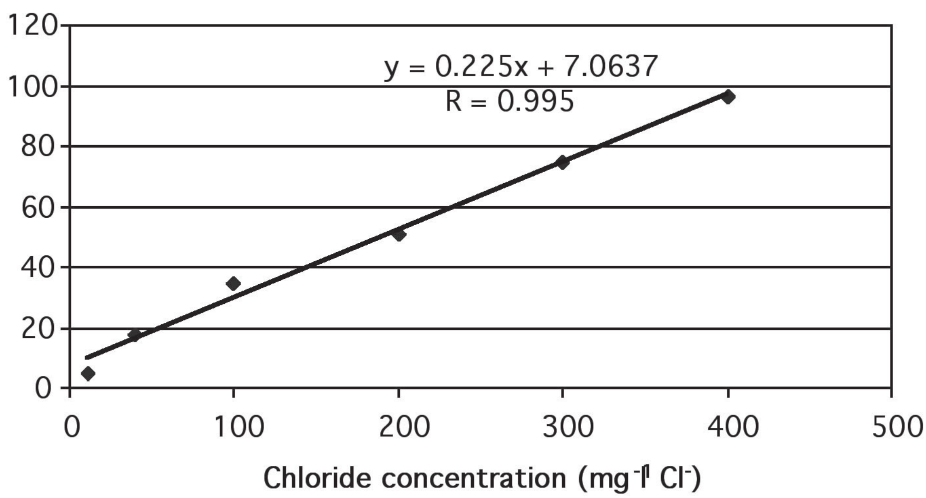

Figure 2
Relationship of chlorides to 96hLC$_{50}$ N-NO$_2^-$ value.

Figure 2
Effet des chlorides sur la valeur de 96hLC$_{50}$ N-NO$_2^-$.

Table II
Impact of chlorides to toxicity of nitrites for *O. limosus*. (96-h exposure).

Tableau II
Effet de la toxicité des nitrites sur *O. limosus* (96 h d'exposition).

$N\text{-}NO_2^-$ concentration ($mg\ l^{-1}$)	Cl^- concentration ($mg\ l^{-1}$)					
	11.0	**40.0**	**100.0**	**200.0**	**300.0**	**400.0**
0.0 (control group)	0%	0%	0%	0%	0%	0%
2.0	0%	0%	0%	-	-	-
4.1	12%	0%	0%	-	-	-
10.1	100%	0%	0%	-	-	-
20.3	100%	96%	0%	0%	0%	0%
40.6	100%	100%	84%	5%	0%	0%
60.9	100%	100%	100%	80%	15%	0%
81.2	-	-	-	95%	65%	50%
101.0	-	-	-	100%	85%	60%
121.7	-	-	-	100%	100%	85%
142.0	-	-	-	-	-	95%
162.3	-	-	-	-	-	100%

Table III
Acute nitrite toxicity (LC_0, LC_{50} and LC_{100} values) for *O. limosus* **related to chloride concentrations.**

Tableau III
Toxicité des nitrites (LC_0, LC_{50} and LC_{100} values) pour *O. limosus* **en relation avec la concentration en chloride.**

Chloride concentration ($mg\ l^{-1}\ Cl^-$)	$96hLC_0$ ($mg\ l^{-1}\ N\text{-}NO_2^-$)	$96hLC_{50}$ ($mg\ l^{-1}\ N\text{-}NO_2^-$)	$96hLC_{100}$ ($mg\ l^{-1}\ N\text{-}NO_2^-$)
11	2.2	4.8	10.7
40	8.9	17.7	35.5
100	20.1	34.7	59.9
200	22.8	50.5	112.1
300	41.4	74.4	133.7
400	55.05	96.6	169.5

DISCUSSION

The $96hLC_{50}$ value for nitrite in one-year-old spiny-cheek crayfish ranged from 4.8 to 96.6 mg l^{-1} $N\text{-}NO_2^-$, according to chloride concentration in diluting water in our test.

Very similar results of $48hLC_{50}$ found Harris and Coley (1991) in *P. leniusculus* – 31 mg l^{-1} NO_2^-, but in their study the chloride concentration was 0.5 mmol.l^{-1} Cl^- (17.5 mg l^{-1}) compared to 11 mg l^{-1} Cl^- used in the present study.

Crayfish mortality displayed already after 24-hours exposition as HUEY *et al.* (1980) and EDDY *et al.* (1983) reported for fish. High impact of chloride ions to nitrite accumulation in noble crayfish body was presented by JEBERG and JENSEN (1994). We found remarkable differences in nitrite toxicity in low and high level of chloride content in water.

We found positive impact of chlorides also in higher chlorides concentration (400 mg l^{-1} Cl^-) than the concentration recommended in *Procambarus simulans* by BEITINGER and HUEY (1981) (300 mg l^{-1} Cl^-).

BOYD (1990) in ROUSE *et al*. (1995) reported in freshwater crustaceans $96hLC_{50}$ values in range from 8.5 to 15.4 mg l^{-1} $N\text{-}NO_2^-$.

Our results are in agreement with this author but comparison is very difficult without information about chloride or other anions concentration in diluting water.

Very interesting is to compare our result with result presented by RUSSO and THURSTON (1977) for rainbow trout. They found linear relationship between nitrite toxicity and chloride concentration in water. They reported that increasing chloride concentration in water by 1 mg l^{-1} Cl^- increase $96hLC_{50}$ value by 0.29 mg l^{-1} $N\text{-}NO_2^-$, that corresponded with our result of 0.225 mg l^{-1} for the spiny-cheek crayfish.

CONCLUSION

The $96hLC_{50}$ values for nitrite in one-year-old spiny-cheek crayfish ranged from 4.8 to 96.6 mg l^{-1} $N\text{-}NO_2^-$ and were related to the chloride concentration in diluting water in our test. Higher concentration of chlorides in water markedly increased crayfish tolerance to nitrite.

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