Biochemical Systematics and Ecology 41 (2012) 98–103

Contents lists available at SciVerse ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Effect of long-term nitrite exposure on the cladoceran *Daphnia obtusa*: Survival, moults, and reproduction

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ARTICLE INFO

Article history:
Received 21 August 2011
Accepted 9 December 2011
Available online 11 January 2012

Keywords:
Daphnia obtusa
Life history traits
Logistic model
Nitrite
Reproduction

ABSTRACT

Increasing concentrations of nitrite in surface waters may cause toxicity to aquatic organisms. The purpose of this study was to assess the effect of nitrite on life history traits of cladoceran zooplankton. The key species *Daphnia obtusa*, isolated from natural waters, was selected as the test organism. Results showed that survival time, number of moults, clutches per female, offspring per clutch, and total offspring per female decreased significantly with increasing nitrite concentration during the 21-day experiment, and their EC_{50}s were 6.03, 4.78, 5.66, 1.80, and 1.59 mg L^{-1} NO_2-N, respectively. The time to first eggs and the time to first clutch were significantly delayed with increasing nitrite concentration, whereas the size at first clutch was significantly decreased with increasing nitrite concentration. We conclude that nitrite is toxic to *D. obtusa* as increased nitrite resulted in mortality, decreased moults, reduced growth, delayed maturation, lower reproduction, and, eventually, population decline.

1. Introduction

Nitrite is a toxic intermediate of the bacterial oxidation of nitrogenous wastes (e.g. ammonia) in the aquatic environment. Generally, nitrite occurs at low levels because it is rapidly converted into nitrate. However, during an imbalance in the process of the nitrogen cycle or the degradation of massive organisms, nitrite levels may rise in freshwaters (Jensen, 2003). Elevated nitrite concentrations in the environment are a potential problem because nitrite has a well-documented toxicity to animals (e.g. Lewis and Morris, 1986; Jensen, 2003). Aquatic animals are at higher risk of nitrite intoxication than terrestrial animals, as nitrite in the surrounding water is actively exchanged across the gill epithelium and can accumulate to very high concentrations in the body fluids (Bath and Eddy, 1980; Jensen, 1990, 1996). Studies on fish and crustaceans have revealed that nitrite induces a large variety of physiological disturbances (Jensen, 2003).

There are a number of studies about the toxic effects of nitrite on different species of fish and crustaceans (e.g. Lewis and Morris, 1986; Alcaraz et al., 1999; Cheng and Chen, 2000; Jensen, 2003; Kroupova et al., 2005). Generally, crustaceans are less sensitive to nitrite than fish due to the differential effects of nitrite on their respiratory pigments (Armstrong et al., 1976). Compared to many publications on the toxicity of nitrite to the relatively larger crustaceans, such as shrimp and crayfish (e.g. Armstrong et al., 1976; Mevel and Chamroux, 1981; Jensen, 1990; Cheng and Chen, 1998; Tseng and Chen, 2004; Sowers et al., 2004), there are only a few published studies concerned with the toxicity of nitrite on small-sized crustaceans, i.e. cladocerans (Dave and Nilsson, 2005; Hannas et al., 2010; Xiang et al., 2010; Yang et al., 2011).

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doi:10.1016/j.bse.2011.12.006

Cladocerans are generally a dominant group in freshwater zooplankton where they may contribute up to 80% of the secondary production (Mangas-Ramirez et al., 2001) and are important trophic components of aquatic food webs constituting a major food source for fish and invertebrate predators. They are subjected to strong variations in the physical and chemical variables in natural waters, sensitive to a vast majority of toxic substances, and can rapidly respond to different environmental changes. As increasing concentrations of nitrite in surface waters are becoming a worldwide concern, the elevated concentration of nitrite in aquatic ecosystems could affect the population dynamics of cladocerans. Thus, in this study a key species, *Daphnia obtusa*, was selected and isolated from natural waters and used as a test organism to investigate effect of long-term nitrite exposure on the survival, moults, and reproduction of cladocerans. Models describing concentration-response relationships are becoming increasingly popular in ecotoxicological research (Chèvre et al., 2005), as they provide estimates of biological meaningful parameters that allow us to make quantitative predictions about the nature of toxins in the environment. In this study, thus, concentration-response functions of the relevant life history parameters were fitted where appropriate.

2. Materials and methods

D. obtusa was originally isolated from Lake Taihu, China and maintained in the laboratory for several months prior to this study. The animals were cultured in beakers at 25 °C under fluorescent light at an intensity of 40 μE m^{-2} s^{-1} with a light–dark period of 12:12 h and fed *Scenedesmus obliquus* which was cultured axenically in liquid BG-11 medium under the same conditions as culturing the animals.

Newborns (<24 h-old) taken from a single mother (F_0) in the stock culture were isolated and grown individually in 50-mL beakers under the conditions described above and fed daily *S. obliquus* (5.0×10^4 cells mL^{-1}). These newborns (F_1), which served grandmothers to the experimental animals, were transferred daily to new beakers. Newborns from the first and second clutches were removed and not used. Newborns (F_2) from the third clutch served as experimental mothers and were transferred individually to 50-mL beakers. Subsequently, newborns (F_3) from the third clutch of the experimental mothers were used as experimental animals. Experimental animals were placed randomly in each of the 50-mL beakers. Each beaker contained only one animal to avoid density effects, and each treatment was made up of four replicates. The concentrations of nitrite test solutions (NO_2–N) in the beakers were set as 0, 0.1, 0.5, 1.0, 2.0, 4.0, and 8.0 mg L^{-1}. To keep the nitrite concentrations constant, the test solutions in each beaker were replaced with fresh ones every two days. Nitrite test solutions were prepared by dissolving sodium nitrite ($NaNO_2$) in de-chlorinated tap water. The experimental conditions were identical to those used during culturing.

The experimental duration was 21 days, during which survival and moults were monitored daily. Dead individuals were confirmed under a microscope and then removed. Moult was examined by counting shed carapaces. Size was measured from above the eye to the base of the tail spine using an inverted microscope and an image analysis system. Offspring production was measured daily; once counted these were removed. Also recorded were: the time to first batch of eggs appearing in the brood pouch; the time to first clutch; the size at first batch of eggs; the size at first clutch; the number of clutches per female; the number of offspring in each clutch; and the total number of offspring per female.

All data are presented as means ± 1 SEs and were evaluated by one-way analysis of variance (ANOVA) followed by Duncan multiple range test ($\alpha = 0.05$). Chronic effect concentrations are not given as NOECs and LOECs but rather as EC50s because the latter are considered having higher precision (Dave and Nilsson, 2005). Thus, the three-parameter logistic model, $Y = a/(1+(X/X_0)^b)$, was chosen to fit the data of life history indices if appropriate, where Y is value of the index, X is the NO_2–N concentration, a is value of the index at zero NO_2–N concentration (theoretic maximum value at zero NO_2–N concentration), X_0 is concentration that reduces value of the index by 50% (i.e. EC_{50}), b is the form parameter determining shape of curve. All statistical analyses were carried out with SigmaPlot 11.0.

3. Results

There was no mortality in the control and the treatments of lower nitrite concentrations (NO_2–N: 0, 0.1, 0.5, 1, and 2 mg L^{-1}) during the 21-day experiment. Only in the two highest nitrite concentrations (4 and 8 mg L^{-1}) was mortality observed during the 21-day period; thus, mean survival time decreased with increasing nitrite concentration when nitrite concentration was higher than 2 mg L^{-1} (Fig. 1a). The mean survival time in the highest concentration (8 mg L^{-1}) was only 5 days and significantly lower than those in the control and the other treatments ($P < 0.001$). The three-parameter logistic model fitted the data well; according to the model, the EC_{50} for survival time was about 6 mg L^{-1} during the 21-day experiment.

The mean number of moults was 14 in the control and decreased with increasing concentrations; there were only 2.25 moults in the 8 mg L^{-1} NO_2–N concentration. A significant difference was detected between the lower concentrations and the higher concentration treatment (Fig. 1b, $P < 0.001$). Also, the three-parameter logistic model fitted the data well. According to the model, the theoretic maximum moult at zero concentration (control) was 13.82 times in the 21-day experiment; the EC_{50} for number of moults was 4.78 mg L^{-1}.

The time to first eggs in the brood pouch was significantly delayed with increasing nitrite concentration (Fig. 2a, $P < 0.001$). In the high concentration of 4 mg L^{-1} NO_2–N, the mean time to first eggs was 4.75 days and was significantly longer than those in low NO_2–N concentrations (less than 4 days). Nitrite also increased the time to first clutch from 4.95 days in the control to 6.7 days in 4 mg L^{-1} NO_2–N (Fig. 2b, $P < 0.001$). There was no significant difference in size at first batch of eggs