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Effect of cyanobacteria on fish and crustacean growth

Miina Karjalainen, Marko Reinikainen, Lisa Spoof, and Jussi Meriluoto

Abstract

The aim of this study was to evaluate the potential harmful effects of cyanobacteria-exposed food web on two planktivorous animals: a fish larva (pike, *Esox lucius*) and a mysid shrimp (*Neomysis integer*). The planktivores were fed with natural zooplankton community, pre-exposed to filtrate and toxin of the toxic cyanobacterium *Nodularia spumigena*. In addition, radiolabelled nodularin (^{3}H-dihydronodularin) was used in separate experiments where vectorial transfer of nodularin was measured.

During 11-day exposures we observed that dissolved nodularin, toxin produced by *Nodularia spumigena*, was transferred to fish and mysids through zooplankton. Our main results showed that the growth of pike larvae was slower when larvae were fed with zooplankton exposed to cyanobacterial filtrates, whereas no effect on mysid growth was observed. The reduced growth of pike larvae was also observed by measuring C:N -ratios, which were lower in the nodularin treatment than in the control. Based on our results we suggest that dissolved cyanobacterial toxins released during bloom decay have a negative impact on larval fish growth through zooplankton, without a direct contact with cyanobacteria.

Keywords: *Nodularia spumigena*, nodularin, fish larvae, mysids, growth

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Introduction

Toxic cyanobacterial blooms are a frequently occurring phenomenon in the Baltic Sea during late summer. The negative effects of toxic cyanobacteria on Baltic Sea zooplankton have been studied both in the laboratory (e.g. Koski et al. 1999) as well as in the field (e.g. Sellner et al. 1994, 1996). It has been shown experimentally that zooplankton, after feeding on natural plankton assemblage containing toxic *Nodularia spumigena*, can contain nodularin (Kozlowsky-Suzuki et al. 2003). However, very little is known about the effect of food-web mediated transfer of toxins to planktivores.

We hypothesise that cyanobacterial toxins or other bioactive compounds produced by cyanobacteria can be transferred via zooplankton to planktivores, and that this exposure via food can have negative, sublethal effects on them.

Materials and methods

We used hatchery-reared pike larvae (*Esox lucius*) and field-collected mysid shrimps (*Neomysis integer*) in our experiments. Zooplankton community used in the feeding experiments was collected with 90 µm plankton net with horizontal tows from 0,5 m depth at Tvärminne Zoological Station, Gulf of Finland. In zooplankton exposures a *Nodularia spumigena* extract (strain AV1, prof. Kaarina Sivonen) and purified nodularin (Biomol Research Laboratories, Inc., USA) were used, in concentrations about 20 µg l^{-1}. Zooplankton for food was exposed to either crude extract of *Nodularia spumigena*, or purified nodularin for 16 h prior to the experiments, whereafter they were rinsed with filtered seawater before they were offered as food for the experimental animals.

Pike larvae were fed with pre-exposed zooplankton for 11 days and their growth was measured twice during the experiment by weighing them carefully in a vial filled with filtered seawater, and the growth of mysids was monitored by measuring the length of their moulting cycle. Their pellet production was measured, and after the experiments also the C:N-ratios of pike larvae and mysids were analysed.

Radiolabelled nodularin (100µg l^{-1}) was used in additional set of experiments, where the transfer of toxin was observed during 48 h. The zooplankton community was exposed to ^{3}H-dihydronodularin for 16 h before offering them as food for the animals, and mysids and pike larvae were collected to toxin analyses after 12 and 48 hours.

Results

Zooplankton diet pre-exposed to *N. spumigena* exract decreased the growth of pike larvae (ANOVA F=5.439, p=0.011, post hoc Tukey p= 0.013, fig.1). The amount of pellets produced by pike larvae decreased in both treatments (ANOVA F=4.269, p=0.026), in *N. spumigena* treatment post hoc Tukey p=0.049, in nodularin treatment p=0.038, (fig. 2).

In mysid experiments neither their moulting cycle (ANOVA F=0.209, p=0.814) nor pellet production (ANOVA F= 2,381, p=0.208) was affected by cyanobacterial treatments (fig.3).

Isotope experiment revealed, that the label from the toxin was transferred to the fish and mysids via their food (fig. 4). The carbon to nitrogen ratio that was measured from the in pike larvae right after the experiments were over, had decreased in nodularin treatment (ANOVA F=3.760, p=0.023, post hoc Tukey p=0.013, fig. 5).

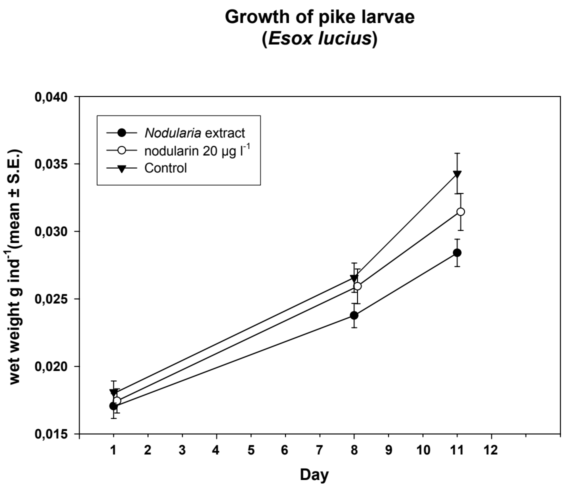

Figure 1. The growth of pike larvae during the 11-day experiment as wet weight (mean ± S.E.) with different zooplankton pre-treatments, *Nodularia spumigena* –extract, purified nodularin and control with filtered seawater.

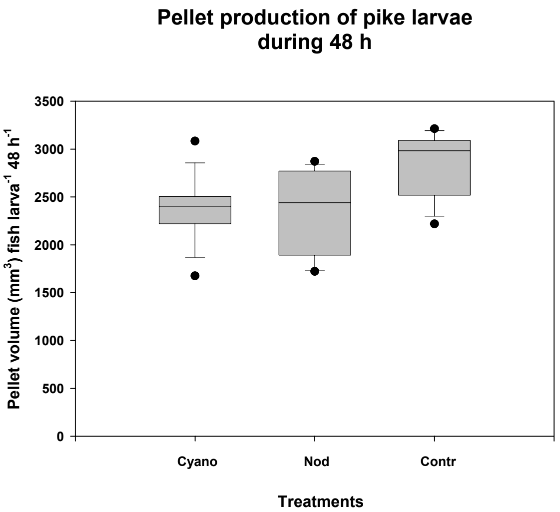

Figure 2. The pellet production of pike larvae during 48 hours period. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate 90th and 10th percentiles, respectively. Outlying points are presented as dots. Treatments: Cyano = zooplankton pre-exposed to *N. spumigena* extract, Nod = zooplankton pre-exposed to purified nodularin, Contr = control zooplankton with filtered seawater.

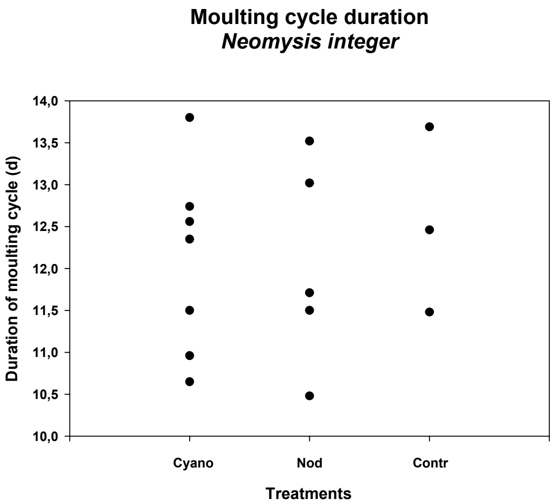

Figure 3. Moulting cycle duration of *Neomysis integer*. Treatments: Cyano = zooplankton pre-exposed to *N. spumigena* extract, Nod = zooplankton pre-exposed to purified nodularin, Contr = control zooplankton with filtered seawater.

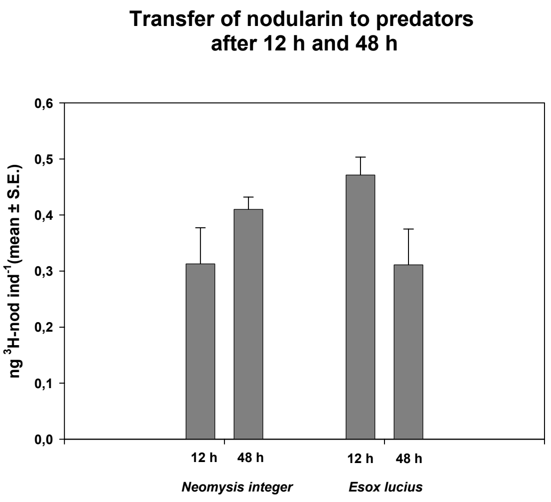

Figure 4. Concentration of radiolabelled nodularin (mean ± S.E.) in mysids and in pike larvae after feeding zooplankton pre-exposed to ^{3}H-dihydronodularin.

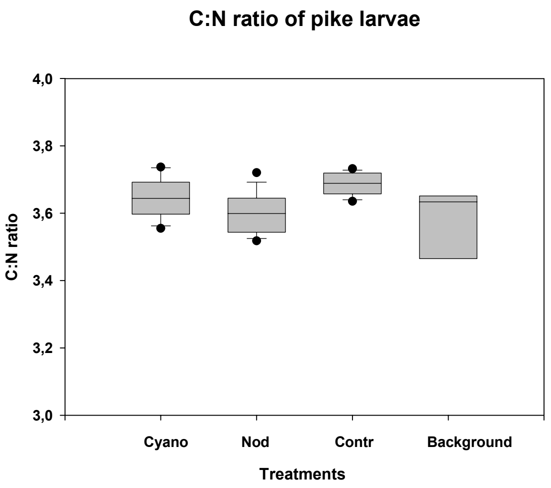

Figure 5. The C:N ratio of pike larvae in different treatments (Cyano = zooplankton pre-exposed to *N. spumigena* extract, Nod = zooplankton pre-exposed to purified nodularin, Contr = control zooplankton with filtered seawater) and at the beginning of the experiment (Background). Box plot symbols as in figure 2.

Discussion

Growth of pike larvae decreased during 11-day exposure when fed with *N. spumigena* pre-treated zooplankton. This indicates, that either pre-exposure to cyanobacterial extract decreases the quality of zooplankton as a food source possibly by adding toxins and bioactive compounds to their tissues, or fish larvae feed less on exposed zooplankton. One possibility is that fish larvae have higher metabolic costs when digesting food containing these cyanobacterial compounds, which in turn would reflect in their growth rate. The crude extract of *N. spumigena* had a stronger effect on the growth of pike larvae than purified nodularin, possibly since cyanobacteria produces other bioactive compounds other than hepatotoxins that may be toxic to aquatic organisms. The transfer of toxins via zooplankton to planktivores was verified with radiolabelled nodularin, which could be detected in the tissues of both pike larvae and mysids after 48h.

Pellet production of pike larvae decreased in both treatments. Previous studies with copepods have revealed, that ingestion of cyanobacteria decreases the pellet production (e.g. Lehtiniemi et al.

2002). Whether this is due to avoidance of non-palatable food items, or actually a sign of metabolic costs to the organism remains to be solved by further research. However, the decreased C:N –ratio found in fish tissues after the experiments with purified nodularin showed that their growth was impaired by pre-treated zooplankton.

The tolerance of mysids to harmful effects of cyanobacterial metabolites during this study was in accordance with earlier experiments. The tolerance of mysids to *N. spumigena* has been previously shown by Engström et al. 2001, indicating that invertebrates are more tolerant to cyanobacterial toxins than vertebrates.

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