Toxic effect of cyanobacterial blooms on the grazing activity of *Daphnia magna* Straus

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Abstract

The investigations aimed to determine the impact of cyanobacteria *Microcystis aeruginosa* (Kütz.) Kütz. and *Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet et Flah., both toxic algae, on the grazing intensity of *Daphnia magna* Straus. In order to determine the parameter permitting the quantitative determination of the grazing intensity of herbivorous organisms, methods based on the following techniques were applied: microscopy, to determine the degree of gut fullness; spectrophotometry, to determine the levels of chlorophyll *a* and its degradation products in the food composition; high-performance liquid chromatography (HPLC) to determine the content of exogenous and endogenous carotenoids. Each of these methods confirmed that the tested algae species inhibited grazing intensity in *D. magna* Straus. The most obvious effects were obtained when *M. aeruginosa* (Kütz.) Kütz. was used as food. With these cyanobacteria, the gut fullness indicator did not exceed 58%, and the chlorophyll *a* content in the digestive system of the tested *D. magna* was three times lower than that in the control organisms. It seems that the defensive reaction of organisms was a reversible process. However, the possibility of a long-term, sublethal influence of cyanobacteria on the physiology and internal processes of this species cannot be ruled out.

1. Introduction

As the natural ecological balance in coastal seas has been disturbed, especially in bays and shelf areas, the frequency of large-scale blooms of algae, including toxic cyanobacteria, has increased in the Baltic Sea (Huebel & Huebel 1995, Leppänen et al. 1995, Paerl 1999). The effects of

cyanobacterial toxins, which belong to the group of hepato- and neurotoxins, have been investigated mainly with regard to mammals (Shirai et al. 1986, Carmichael et al. 1990). However, it has been demonstrated that some of these substances also have a lethal, sublethal or allelopathic impact on many aquatic invertebrates (DeMott et al. 1991, Haney et al. 1995, Engstroem et al. 2000). This applies in particular to herbivorous zooplankton organisms, which are directly endangered by contact with toxins. Numerous physiological and metabolic processes in particular organisms are usually affected (Vasconcelos 1990, Reinikainen et al. 1995): motor activities are limited, reduced grazing intensity, reproduction and the development of juvenile stages are inhibited.

There is a need for a better understanding of the interactions between algal toxins and herbivorous zooplankton, and to investigate the defensive mechanisms which animals have developed. For example, the weakened toxic effect of cyanobacteria on herbivorous invertebrates is explained by the low nutritional values of these algae, as well as by their availability in minute amounts (because of their shape, colony size and coenobia) which causes the natural elimination of cyanobacteria in the diets of zooplankton (Holm et al. 1983).

This paper presents the results of investigations, the aim of which was to determine the impact of selected cyanobacteria species belonging to the group of toxic algae (i.e. *Microcystis aeruginosa* (Kütz.) Kütz. and *Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet et Flah.) on the grazing intensity of typically herbivorous cladocerans *Daphnia magna* Straus (Cladocera).

2. Materials and methods

2.1. Material and conditions for cultivation of experimental organisms

Daphnids *D. magna* were caught in July 2000 in the coastal area of the Vistula Lagoon. After the organisms had been selected, they were placed in 5-litre aquaria containing water from the sampling station and were cultivated at a temperature of 19–20°C and a pH of 7.1–7.5. The cladocerans were fed on two kinds of algae – *Scenedesmus* and *Chlorella* – and baking yeast (Lakota 1963). After the required amount of experimental material had been obtained, some 500 individuals were moved to each of nine aquaria (triplicate control and three replicates with *A. flos-aquae* and three replicates with *M. aeruginosa*) containing filtered water from the sampling areas. The organisms were starved, and they remained in the aquarium until they began to form an *ephippium*; this indicated a significant decrease in the

quality of their living conditions which, in this case, was the stress induced by lack of food, since the other cultivation parameters remained constant.

The grazing intensity of the daphnids was tested with respect to two species of cyanobacteria, *M. aeruginosa* and *A. flos-aquae*, which belong to a group of algae which have toxic blooms (Leppänen et al. 1995). They were collected in August, when they occur on a large scale in the Gulf of Gdańsk (*M. aeruginosa*) and the Vistula Lagoon (*A. flos-aquae*). In order to preserve similar food fractions, the algae were filtered through a 20 μm mesh plankton net and subjected to ultrasound in order to destroy larger

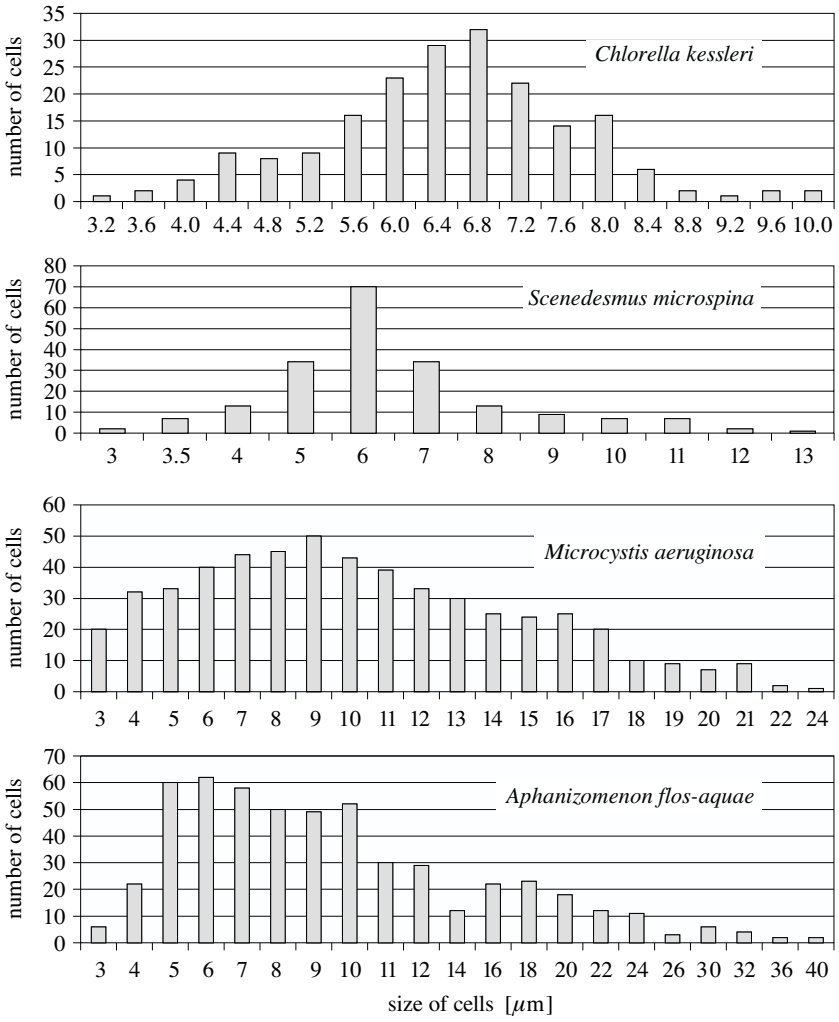

Fig. 1. Size distribution of algal cells (and coenobia) used as food during the cultivation of cladocerans *Daphnia magna* Straus

coenobia. The following mixture of algae was used as food in the control aquarium: *Chlorella* and *Scenedesmus* (*Chlorella kessleri* Fott et Novakova and *Scenedesmus microspina* Martens et Pankov, monocultures). The cell size distributions, including the coenobia in both cyanobacteria and green alga *S. microspina*, are presented in Fig. 1.

In the experimental and control aquaria, the initial algae biomass was 2.0–3.1 μm^3 cm^{-3} (this parameter describes the volume of cells in 1 cm^3 of water in the aquarium).

2.2. Methods

Microscopy, spectrophotometry and high performance liquid chromatography (HPLC) were applied in order to determine the grazing intensity parameter.

2.2.1. Microscope-based technique

An inverted microscope with polarised light was used (Axiovert 35) to determine the degree of gut fullness in daphnia. A method was devised to record the digestive tract photographically and to determine the degree of gut fullness (expressed in %) from the photographs. The application of this method is exemplified in Fig. 2.

During each experiment, 20 individuals were observed every 15 minutes over a period of 45 minutes after feeding. It was found that under optimal experimental conditions, the intestines of adult individuals were completely filled with food within 30 minutes.

2.2.2. Spectrophotometric method

About 100 individuals were collected from each aquarium 15 and 45 minutes after feeding to determine the content of chlorophyll *a* and pheopigment. This was done using the standard extraction method described by Edler (1979) and the quantitative analysis described by Jeffrey & Humphrey (1975) (the chlorophyll *a* concentration was recalculated per gram of dry weight, unlike the cited formula in which it is given per water volume). In order to avoid error by determining chlorophyll *a* in exogenous phytoplankton cells in the sample, the material was placed, before extraction, in a funnel with a 200 μm mesh net and rinsed with filtered water in order to remove the algae. The material remaining after this extraction was used to determine the dry weight.

2.2.3. HPLC analysis – identification of pigments

Chromatographic analysis was carried out before feeding and after the experiment, i.e. after 45 minutes. The conditions of HPLC distribution are

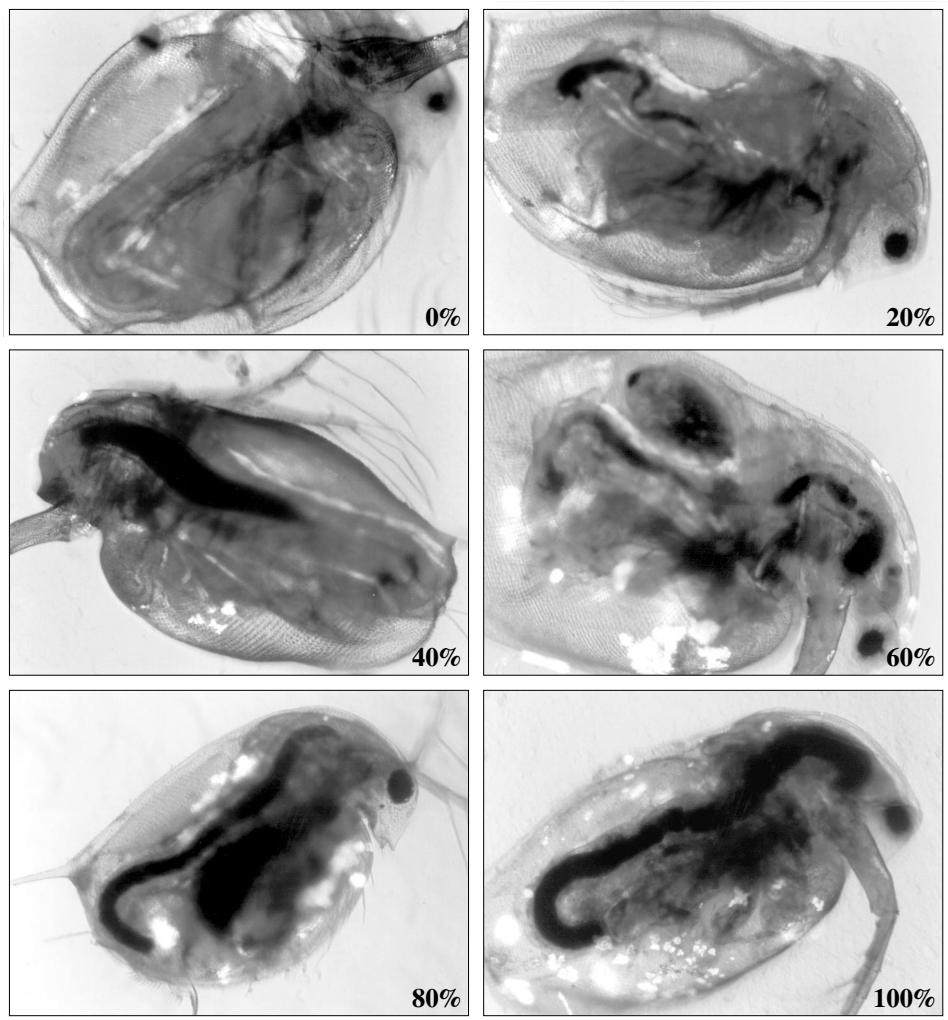

Fig. 2. Degree of gut fullness (in %) in *Daphnia magna* Straus established by polarised light microscopy

described by Łotocka (1998, 1999). Where plant pigments were numerous, additional HPLC distribution was carried out according to the procedure described by Mantoura & Llewellyn (1983).

The experiment was repeated three times in the same way (all techniques) at intervals of 2 days (the same types of water and organisms were used).

All data are reported as mean $\pm$SD. The differences between means were analysed using Student's t-test.

3. Results

The results of the investigations focused on the degree of gut fullness of cladocerans fed on cyanobacteria (*M. aeruginosa* (Kütz.) Kütz. and *A. flos-aquae* (L.) Ralfs ex Bornet et Flah.) are presented in Fig. 3. The mean gut fullness index in the control aquaria was significantly different

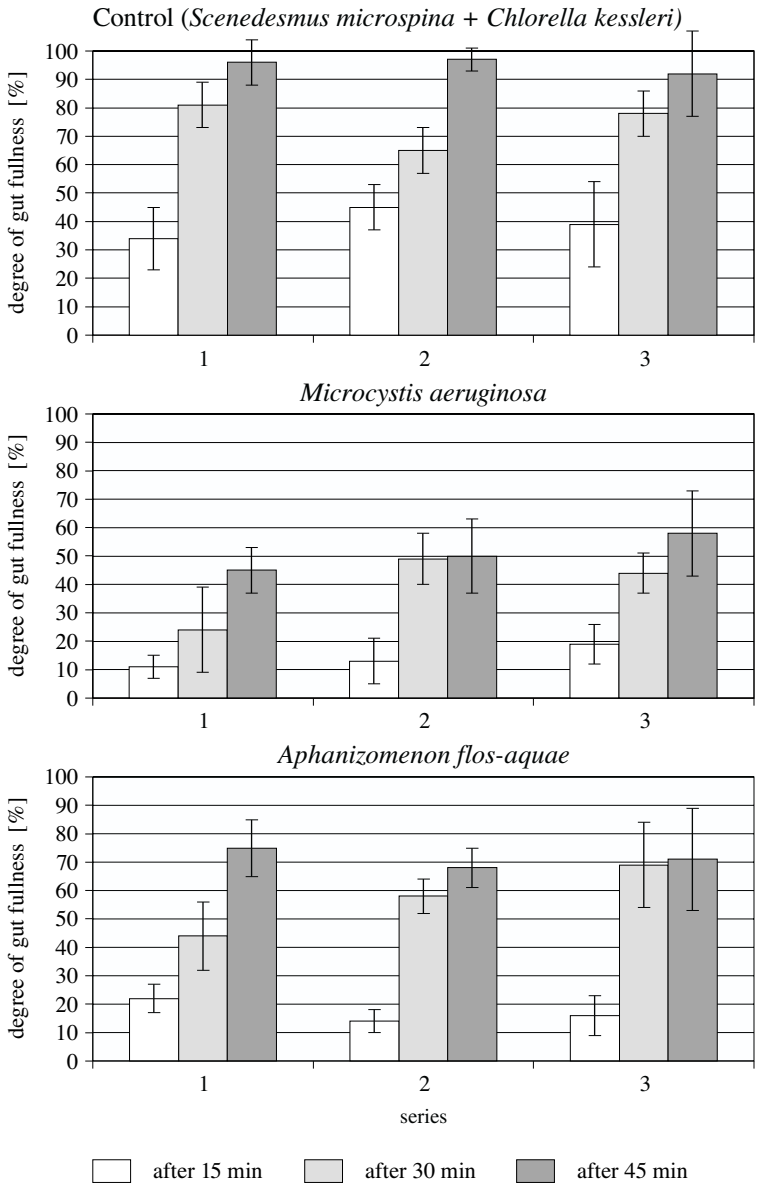

Fig. 3. Degree of gut fullness (in %) in *Daphnia magna* Straus (± SD, n = 20)

from that in the other aquaria. The lowest index of digestive tract fullness 45 minutes after feeding was noted when *M. aeruginasa* was used as food; its maximum value did not exceed 58% ($p < 0.001$), while in the control aquarium it was 97%. The *A. flos-aquae* values of this index were below 75% ($p < 0.001$).

The results of chlorophyll *a* analyses in the gut of *D. magna* were similar to the gut fullness indices (Table 1). The highest content of chlorophyll *a* and its degradation products in the digestive tracts of cladocerans was recorded in the control aquarium 45 minutes after feeding (mean 97.0 μg g^{-1} dry wt.); this value fell to a minimum when food consisted of cyanobacteria *M. aeruginosa* (mean 33.4 μg g^{-1} dry wt.) ($p < 0.001$).

Table 1. Content of chlorophyll *a* and its degradation products in the gut of *Daphnia magna* Straus

Duration of experiment	Content of chlorophyll *a* and pheopigment (in μg g^{-1} dry wt.)		
	Control	*Aphanizomenon flos-aquae*	*Microcystis aeruginosa*
after 20 minutes	58.7 ± 12.0* range 45.4–74.0	32.5 ± 5.9* range 24.7–40.5	29.3 ± 4.5* range 23.7–37.3
after 45 minutes	97.0 ± 7.6* range 87.1–109.2	51.1 ± 8.0* range 42.6–64.2	33.4 ± 3.9* range 27.5–39.8

* mean ± SD (n = 9).

Fig. 4 presents an example of an HPLC chromatogram of pigments isolated from cladocerans *D. magna* (from individuals collected directly before feeding). Astaxanthin was dominant (75% of the total carotenoid content) in the adult individuals of this species in the population analysed. The other pigments present were canthaxanthin (10.6%), β,β-carotene (9.1%) and astaxanthin mono- and diester (5.3%). The composition of pigments changed significantly when the majority of the organisms had full guts, for example, those from the control aquarium (Fig. 5).

Based on chromatographic analyses, the ratio of plant pigments to the total carotenoid content in cladocerans *D. magna* was established as a percentage for each of the three investigative methods. The analyses were carried out 45 minutes after feeding, and the results are presented in Table 2. Under optimal feeding conditions, plant pigments constituted almost 60% of the total carotenoid content isolated from *D. magna*. Under the other

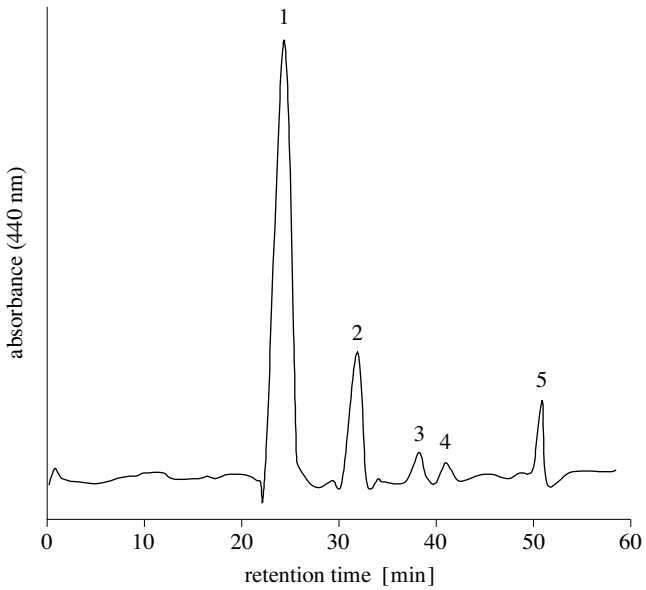

Fig. 4. HPLC chromatogram of carotenoids isolated from cladocerans *Daphnia magna* Straus after a period of starvation. Pigment fractions: 1 – astaxanthin; 2 – canthaxanthin; 3, 4 – astaxanthin esters; 5 – β,β-carotene

Table 2. The percentage of plant pigments in the total composition of carotenoids in *Daphnia magna* Straus based on food type

Type of food	[%]
Control (*Chlorella kessleri* + *Scenedesmus microspina*)	58.9 % ± 12.3* range 39.8–70.3
Microcystis aeruginosa	32.2 % ± 4.9* range 26.7–40.1
Aphanizomenon flos-aquae	43.2 % ± 5.2* range 35.7–49.2

* mean ± SD (n = 9).

experimental conditions, the contribution of pigments from the diet was considerably lower, which may indicate inhibition of grazing processes. In the case of *A. flos-aquae*, the mean percentage of plant pigments was 43.2% ($p < 0.001$) in *D. magna* (45 minutes after feeding) and 32.2% ($p < 0.001$) in *M. aeruginosa*. After 96 hours, mature females in the control aquarium with access to proper food produced summer eggs, while in the other aquaria females with an *ephippium* were prevalent.

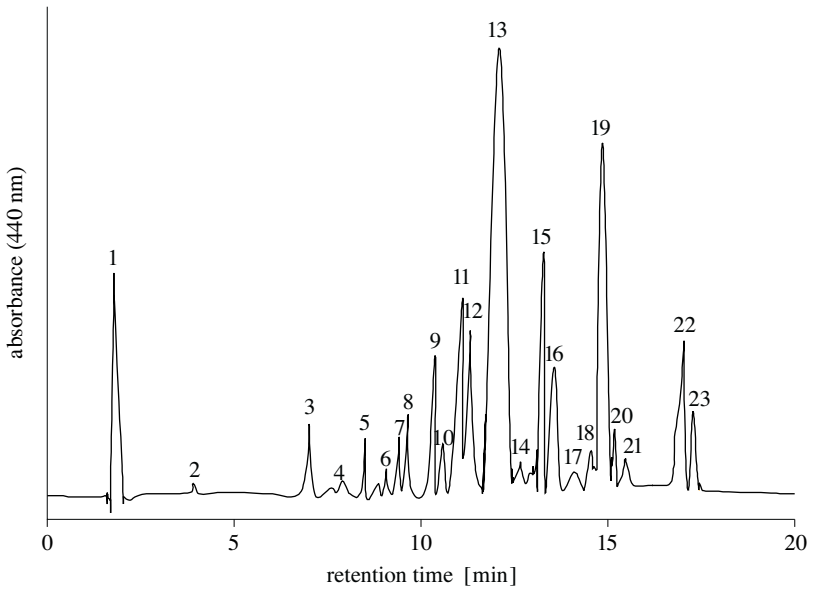

Fig. 5. HPLC chromatogram of pigments (chlorophylls and carotenoids) isolated from cladocerans *Daphnia magna* Straus in the control aquarium (HPLC condition after Mantoura & Llewellyn 1983). Pigment fractions: 1 – solvent front; 2 – unknown; 3 – chlorophyllide *a*; 4 – unknown carotenoid; 5 – neoxanthin; 6 – unknown neoxanthin-like pigment; 7 – unknown carotenoid; 8 – violaxanthin; 9 – antheraxanthin; 10 – unknown carotenoid; 11, 12 – astaxanthin; 13 – lutein and zeaxanthin; 14 – unknown astaxanthin-like pigment; 15 – canthaxanthin; 16 – unknown carotenoid; 17 – astaxanthin esters; 18 – chlorophyll *a* allomer; 19 – chlorophyll *a*; 20 – chlorophyll *a* epimer; 21 – echinenone; 22 – pheophytin *a*; 23 – β,β-carotene

4. Discussion and conclusions

The investigations on the grazing intensity of daphnia *D. magna* Straus were carried out using three independent methods. For this species, polarised light microscopy is simple, quick and reliable. The accuracy of the results can be improved using a densitometer; this was done with a number of samples.

Determining the levels of chlorophyll *a* and its degradation products in herbivorous organisms is a common way of investigating zooplankton grazing intensity (Christoffersen & Jespersen 1986, Dagg et al. 1989, Makino et al. 1996). Far more information regarding grazing processes, e.g. food selectivity and rate of digestion, can be obtained by determining chlorophylls

and carotenoids with HPLC, a technique which is coming into greater favour (Quiblier-Lloberas et al. 1996, Descy et al. 1999, McLeroy-Etheridge & McManus 1999).

Each of the methods used during the experiments confirmed the limiting impact of the cyanobacteria *M. aeruginosa* and *A. flos-aquae* on the grazing intensity of *D. magna*. According to the literature data, both algae species are capable of synthesising hepatotoxins such as microcystin (Plumley 1997, Willen & Mattsson 1997) and neurotoxins such as aphantoxin (Pereira et al. 2000). It is assumed that these types of relationships are formed by the algae as a defence mechanism against intensive grazing by herbivorous plankton (Uye & Takamatsu 1990, Carlsson et al. 1995). This is confirmed by investigations indicating that toxins appear in environments (or their concentrations increase) concurrently with the growth of herbivorous populations (Fulton & Paerl 1987, Haney et al. 1995). Although much is already known regarding the effects of elemental microcystin on mice and small crustaceans, the fall in egg production, the inhibition of embryonic development, the effects of aphantoxin on zooplankton and their mechanisms are still poorly understood (Shurtleff 1987, Hanazato 1996, Schaeffer et al. 1999). Furthermore, current studies indicate that aphantoxin is produced mainly by freshwater cyanobacteria (Bumke-Vogt et al. 1999, Ferreira et al. 2001). In the Baltic Sea, aquatic blooms formed by the potentially toxic species *A. flos-aquae* did not contain this toxin (Sivonen et al. 1989). So far, the toxicity of *A. flos-aquae* from the Vistula Lagoon has not been investigated.

The observed effect of inhibited grazing may be due to temporary damage to the mobility system which precludes grazing. This appears to be a transient effect, since organisms moved to an aquarium devoid of cyanobacteria fed and developed in a similar manner to those in the control aquarium. However, this type of sublethal toxic effect requires long-term observation, and the internal mechanisms of the effect have yet to be discovered.

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