Toxic effect of harmful algae on zooplankton in the Curonian Lagoon (the Baltic Sea)

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**Abstract**

The Curonian Lagoon is the largest freshwater coastal lagoon of the Baltic Sea. The phytoplankton and zooplankton samples were taken every month from April till October at 6 stations in 2007-2010. To distinguish between live and dead organisms zooplankton’s samples were vitally stained using aniline blue. Dead organisms have been found out in populations of all dominating zooplankton species. During the period of mass development harmful Cyanobacteria and after it in July-October 2008-2010 the percentage of dead organisms from abundance and biomass of zooplankton increased in 5-13 times (up to 8.3-19.4%) with compared the percentage of dead organisms in July-October 2007 (1.5-1.8%), when biomass of Cyanobacteria was very low. Sensitivity of zooplankton’s taxonomical groups to “bloom” of Cyanobacteria and influence of toxins (according increasing proportion dead organisms in period mass developing Cyanobacteria) increased in line Cyclopoida→Calanoida→Rotifera→Cladocera. Minimal percentage of dead organisms was observed at the station in which organic pollution, eutrophication and biomass of harmful Cyanobacteria (especially *Microcystis* spp.) were at lower level. One of the main possible reasons of increasing proportion of dead organisms in zooplankton is the influence of toxins *Microcystis* spp. We obtained a significant correlation between proportion of dead organisms in zooplankton and biomass of toxic *Microcystis* spp. (*r*=0.62-0.81). Green algae can presumably compensate negative effect of Cyanobacteria, the correlation between proportion Chlorophyta in total biomass phytoplankton and proportion of dead organisms in zooplankton was negative (*r*=0.51-0.60). Thus, the proportion of dead organisms in zooplankton may be the sensitive indicator to shown the influence of harmful algae.

**Keywords:** Curonian Lagoon, zooplankton, mortality, *Microcystis* spp., toxic effect, harmful algae

**Introduction**

The study of non-consumption mortality of zooplankton is currently very topical. Cause of its increase can be both physical (temperature, wind speed, turbidity, flow rate) and chemical (oxygen, toxic substances) factors, and also biological (diseases, parasites and epibionts) and trophic (quantity and quality of food) factors (Dubovskaya, 2009). Also one of the factors increasing the non-consumption mortality may be the influence of cyanobacterial toxins, which at the present time are not yet fully investigated.

The Curonian Lagoon is a large shallow freshwater lagoon (area 1584 km²; water capacity 6.2 km³; average depth 3.8 m) of the Baltic Sea which is strongly affected by anthropogenic pressure. The phenomenon of mass development of harmful algae (mostly Cyanobacteria) is regularly observed in the lagoon; the biomass of these Cyanobacteria in
some years is considerably higher than the level that determines secondary pollution of the water body. Over recent years, mass development of harmful algae has started happening more often. For instance, from the early 1980s, phytoplankton biomass in summer was always at the level 10–30 g/m³. Intensive bloom of Cyanobacteria (phytoplankton biomass over 100 g/m³) was observed since the last 1980s to current period. The phytoplankton biomass exceeded 100 g/m³ was observed annually during the summer period at some stations in 2002-2010 (except 2007).

In periods mass development of harmful algae (from July to September), potentially toxic species become abundant in the phytoplankton and account for 22–89% of the total phytoplankton biomass. A number of investigators (Belykh et al., 2009; Karmaikl et al., 1993; Paldaviiene et al., 2009) cyanobacterial toxins in the waters of the Curonian Lagoon, thus confirming the presence of toxic Cyanobacteria in this water body. The hepatotoxins, which produced by toxic strains of Cyanobacteria from genus Microcystis, were found in the Curonian Lagoon in 2006. The concentration of microcystins in water was in 26 times higher than the norm established by the WHO (Belykh et al., 2009; Karmaikl et al., 1993; Paldavičiene et al., 2009). Mass development of harmful algae is one of the most unfavorable factors for hydrobionts, animals and humans and have a strong negative effects on whole ecosystem of the Curonian Lagoon. Large accumulations of cyanobacterial toxins have a depressing effect on zooplankton, leading to its mortality.

The purpose of this study is to analyze the seasonal and interannual dynamics, as well as the spatial distribution of relative mortality parameters in the zooplankton of the Curonian Lagoon in comparison with the development of harmful Cyanobacteria.

Materials and methods

Plankton samples were taken in 2007-2010 every month from April till October in the central part of the lagoon at six standard monitoring stations of the Atlantic Research Institute of Fishery and Oceanography (Fig. 1) using a 6-l Van Dorn water sampler from the depths of 0.5, 1.5, and 3.0 m. Zooplankton was filtered using a plankton net with a mesh size of 64 μm. For each zooplankton sample, 6 to 18 l of water were filtered through the plankton net. Immediately after sampling, the zooplankton was stained with aniline blue to distinguish dead individuals from living ones (Dubovskaya, 2008; Bickel et al., 2008; Dubovskaya et al., 2003; Gladyshev et al., 2003; Seepersad, Crippen, 1978). The staining was performed aboard the vessel, so as to exclude the additional mortality of zooplankters during the transport of samples. After staining, the zooplankton samples were washed with water and preserved in 4% formaldehyde with sucrose (Haney, Hall, 1973). Laboratory processing was performed by the standard method (Kiselev, 1969; Methodological guidelines..., 1984) living (unstained and partially stained) and dead (entirely stained) zooplankters were calculated separately (Dubovskaya, 2008). Biomass zooplankton was calculated from size structure and abundance of species (Methodological guidelines..., 1984).

The phytoplankton samples were analyzed by the standard method in a Nageotte chamber. The dominant species were defined as being 10% of the total phytoplankton biomass and more (Method of …, 1975.).

Over 200 phytoplankton and 700 zooplankton samples were collected and processed in the course of the study. Proportions (%) of the abundance/biomass of dead individuals in
the total abundance/biomass of living and dead zooplankters were used as parameters of zooplankton mortality (Dubovskaya, 1987; Dubovskaya et al., 1999).

Fig. 1. The schematic map of the Curonian Lagoon (Baltic Sea). Numbers indicate sampling stations.
Results

Phytoplankton

Over the investigation period, a total of 320 species and subspecies of phytoplankton have been found in the phytoplankton of the Curonian Lagoon, the most abundant was Chlorophyta, Bacillariophyta and Cyanobacteria.

In Curonian Lagoon in spring the diatoms dominated in biomass (67–86%). The species Actinocyclus normanii, Asterionella formosa, Cyclotella meneghiniana, Stephanodiscus hantzschii, Stephanodiscus astrea v. astraea, Aulacosira islandica formed the spring dominant complexes. In earlier summer the diatoms, cryptophytes, green algae, filamentous and colonial Cyanobacteria appeared. The phytoplankton community in summer and autumn was mostly made up dominating species of diatoms Actinocyclus normanii, Stephanodiscus astrea v.astraea (50–62%) and potential toxic filamentous Cyanobacteria (30–43%) Aphanizomenon flos – aquae, Planktothrix agardhii, Anabaena sp., Microcystis wesenbergii, Microcystis viridis (Fig.2).
Fig. 2. Seasonal changes of complexes dominant species of phytoplankton in 2007-2010: a-spring, b-summer, c-autumn. Total phytoplankton biomass (right y axis); share of dominant species (left y axis).

The biomass of *Microcystis* spp., which capable producing of hepatotoxins, in summer at some stations was 18 g/m³ (Fig. 3).

Fig. 3. Changes of total phytoplankton biomass (right y axis) and biomass of toxic *Microcystis* (left y axis) in July-September 2007-2010.
This species makes a significant contribution to the biomass in average over the study period, the maximum concentration of this species was observed in north-western part of the Curonian Lagoon (Fig 5 a).

Due to the fact that the Cyanobacteria are the main part of the total biomass of phytoplankton in summer, the spatial distribution of biomass repeated pattern of spatial distribution of the total biomass of phytoplankton. Maximum values were observed in the center of the southern part of the Lagoon in area of № 6 stations, the minimum in the area of station № 12. (Fig. 5 b, d). Due to the fact that the spatial distribution of the Cyanobacteria biomass depends on wind direction, significant concentrations of Cyanobacteria have been observed in south part of lagoon’s coast near the station № 3, at the river Deima flow. The lowest percentage of Cyanobacteria was observed in the area of station № 12 (Fig. 5 c).

Seasonal dynamics of phytoplankton biomass was characterized by a summer maximum in August and was due to an increase in the biomass of Cyanobacteria that resulted to "bloom" of water. Phytoplankton biomass varied from year to year, the lowest values were in 2007, the highest in 2008 and 2010 (Fig. 4).

![Seasonal changes the biomass of phytoplankton in Curonian Lagoon in 2007-2010.](image)

The average total biomass over the vegetation period was 27 g/m³ in 2007, 58 g/m³ in 2008, 40 g/m³ in 2009 and 46 g/m³ in 2010.

**Zooplankton**

Over the investigation period, a total of 90 species and subspecies of invertebrates have been found in the plankton of the Curonian Lagoon, including 45 of Rotifera, 27 of Cladocera, and 18 of Copepoda.

The complex of species dominant in abundance included *Keratella cochlearis* (Gosse), *K. quadrata* (O.F. Müller), *Chydorus sphaericus* (O.F. Müller), *Eubosmina coregoni* Baird, *Mesocyclops leuckarti* (Claus), and naupliial Copepoda. Rotifera were prevalent in zooplankton.
in regards to abundance. The highest total abundance of zooplankton was observed in May, when *Keratella quadrata* and *K. cochlearis* were dominant. The total abundance of zooplankters in different horizons differed visibly only in spring, when the highest abundance was observed in the middle layer. The average values of the total zooplankton abundance were close over the vegetation periods of 2007 and 2008 (433 ± 23 and 413 ± 43 thousand ind./m³, respectively) and over the vegetation periods of 2009 and 2010 (190 ± 10 and 195 ± 17 thousand ind./m³, respectively).

In biomass, the zooplankton was dominated by *Asplanchna herricki* Guerne, *Eubosmina coregoni*, *Chydorus sphaericus*, *Daphnia galeata* G.O. Sars, *Diaphanosoma mongolianum* Ueno, *Eudiaptomus graciloides* Lilljeborg, and *Mesocyclops leuckarti*. In all years of investigations, Cladocera accounted for most of the total biomass (56–83%). The highest total biomass of zooplankton in 2007, 2008 and 2010 was observed in June: in 2007 and 2010 this was due to an increase in *Daphnia galeata*; in 2008 it was because of increases in *Asplanchna herricki*, *Eubosmina coregoni*, and *Daphnia galeata*. In 2009 the highest total biomass of zooplankton was observed in May also due to an increase in *Daphnia galeata*. In 2007, the average total biomass over the vegetation period was 2.5 ± 0.3 g/m³; in 2008 – 5.4 ± 0.6 g/m³, in 2009 – 4.0 ± 0.5 g/m³ and in 2010 – 2.1 ± 0.3 g/m³.

Dead individuals were found in populations of 38 species. Species prevalent among them (on different dates and at different stations) included both those dominant in the plankton (*Asplanchna herricki*, *Keratella quadrata*, *Daphnia galeata*, *Chydorus sphaericus*, *Diaphanosoma mongolianum*, *Eubosmina coregoni*, *Mesocyclops leuckarti*, and *Eudiaptomus graciloides*) and those of relatively low abundance and biomass (*Brachionus angularis* Gosse, *Conochilus unicornis* Rouss, *Kellicottia longispina* (Kellicott), *Pollyarthra vulgaris* (Carlin), *Trichocerca capucina* (Wierz. et Zach.), and *Cyclops vicinus* Uljan).

The highest absolute abundance among dead individuals was recorded in *Keratella quadrata*, *Chydorus sphaericus*, *Mesocyclops leuckarti*, and nauplial Copepoda; the maximal biomass was found in *Asplanchna herricki*, *Daphnia galeata*, *Diaphanosoma mongolianum*, *Mesocyclops leuckarti*, and *Eudiaptomus graciloides*.

In populations of the dominant species of zooplankton, the proportion of dead individuals reached 14 and 18% in abundance and biomass respectively (in *Mesocyclops leuckarti*) in 2007, 26% (in *Keratella cochlearis*) in abundance and 49% (in *Chydorus sphaericus*) in biomass in 2008; 23% (in *Daphnia galeata*) in abundance and 48% (in *Asplanchna herricki*) in biomass in 2009, 47% and 51 (both in nauplia Calanoida) in 2010.

Sensitivity of zooplankton’s taxonomical groups to “bloom” of Cyanobacteria and influence of toxins (according increasing proportion dead organisms in period mass developing Cyanobacteria) increased in line Cyclopoida→Calanoida→Rotifera→Cladocera.

The proportion of dead individuals in the total abundance and biomass of zooplankton varied in different stations and horizons and over the vegetation period. During the vegetation period in 2007-2010, the highest proportion of dead individuals in the total abundance and biomass of zooplankton was recorded in different horizons, but usually their proportion was highest at a depth of 1.5 m, though the differences were not statistically significant.

In 2007, the proportion of dead individuals at different sampling stations varied from 0.4 to 7.8% of the total abundance and from 0.2 to 9.0% of the total biomass of zooplankton.
In 2008, the range of this variation increased to 0.2–47.8 and 0.1–62.5%, respectively. In 2009, the proportion of dead individuals varied from 0.5 to 24.2% of the total abundance and from 0.2 to 28.5% of the total biomass of zooplankton. And in 2010 the range of this variation was maximal to 4.4–76.2 and 0.3–63.0%, respectively.

The highest proportions of dead zooplankters in the total abundance and biomass were recorded in western part of the lagoon (at station 7), where the processes of pollution and eutrophication are more pronounced than at the other stations, and the lowest proportions were recorded in the north-eastern part of the lagoon (at station 12), which is strongly influenced by the river runoff. In the northeastern part of the lagoon (at station 12) the minimum of biomass Microcystis spp., biomass Cyanobacteria, total biomass of phytoplankton and minimal proportion of Cyanobacteria in total biomass phytoplankton were also observed (Fig. 5).
Fig. 5. Maps the spatial distribution: a – biomass of *Microcystis* spp., g/m$^3$; b – biomass of Cyanobacteria, g/m$^3$; c – proportion Cyanobacteria in total biomass of phytoplankton, %; d – total biomass of phytoplankton, g/m$^3$; e – proportion of dead individuals in the total abundance of zooplankton, %; f – proportion of dead individuals in the total biomass of zooplankton, %.

During the vegetation period of 2007, alterations of increasing and decreasing proportions of dead zooplankters in the total abundance and biomass were observed. The highest proportions of dead organisms were recorded in April and August (Fig. 6). The average proportions of dead individuals in the total abundance and biomass of zooplankton (1.6 ± 0.3 and 1.8 ± 0.3%, respectively) in the period of increased phytoplankton abundance and immediately after this period (from July to October) were close to those recorded during the vegetation period (2.6 ± 1.2 and 2.3 ± 0.7%, respectively).

In 2008, the proportion of dead individuals in the total abundance of zooplankton continuously increased to September and then decreased (Fig. 6a). The proportion of dead zooplankters in the total biomass of zooplankton increased from April to October (Fig. 6 b). The average proportion of dead zooplankters in the total abundance and biomass of zooplankton (8.4 ± 3.5 and 8.2 ± 3.4%, respectively) in the period of mass development of harmful algae and immediately after this period (from July to October) were higher by a factor of 5 to 6 than in the same months of 2007. The average proportion of dead individuals over the vegetation period of 2008 was 6.1 ± 1.5% by abundance and 6.9 ± 2.6% by biomass, which was higher by a factor of 2 to 3 than in 2007.

In 2009, the highest proportion of dead individuals in the total abundance of zooplankton was in April, July and October, maximal proportion of dead individuals in biomass was in July (Fig. 6). The average proportion of dead zooplankters in the total abundance and biomass of zooplankton (8.1 ± 1.9 and 8.9 ± 1.9%, respectively) from July to October were close over 2008 and 2009 and were also higher by a factor of 5 to 6 than in the same months of 2007. The average proportion of dead individuals over the vegetation period
of 2009 was 6.9 ± 1.5% by abundance and 6.7 ± 2.6% by biomass, which was higher by a factor of 3 than in 2007.

Fig. 6. Seasonal dynamics of proportion of dead individuals (%) in the total abundance of zooplankton (a) and total biomass of zooplankton (b).
In 2010, the proportion of dead individuals in the total abundance and in the total biomass of zooplankton increased from April to July and then decreased (Fig. 6). The average proportion of dead zooplankters in the total abundance and biomass of zooplankton (19.9 ± 5.1 and 13.2 ± 4.5%, respectively) from July to October were higher by a factor of 7 to 13 than in the same months of 2007. The average proportion of dead individuals over the vegetation period of 2010 was 16.1 ± 2.6% by abundance and 12.8 ± 2.6% by biomass, which was higher by a factor of 5 to 6 than in 2007.

Thus, during the period of mass development harmful Cyanobacteria and after it in July-October 2008-2010 the percentage of dead organisms from abundance and biomass of zooplankton increased in 5-13 times (up to 8.1-19.9%) with compared the percentage of dead organisms in July-October 2007 (1.6-1.8%), when biomass of Cyanobacteria was very low. Minimal percentage of dead organisms was observed at the station in which organic pollution, eutrophication and biomass of harmful Cyanobacteria (especially Microcystis spp.) were at lower level.

The significant correlations were obtained between proportion of dead organisms in zooplankton and biomass of toxic Microcystis spp. (r=0.62-0.81), and between proportion Chlorophyta in total biomass phytoplankton and proportion of dead organisms in zooplankton (r=0.51-0.60).

Discussion

Dead individuals were recorded in all populations of all dominant zooplankton species; in some periods they reached a considerable proportion in their abundance and biomass, once again confirming the importance of taking into account the proportion of dead individuals in water bodies. Some invertebrate species were represented by only a few specimens, so that dead individuals of these species could not be found due to the small sample size.

The method of staining zooplankton with aniline blue used by different investigators in several water bodies and watercourses revealed different ranges of variation and average numbers of dead zooplankters in the total abundance of zooplankton. The author’s data fall within the average values of this variation, but the highest values recorded in the Curonian Lagoon in the years, when the higher Cyanobacteria blooms were observed are closer to those observed in unstable or disturbed ecosystems.

Different proportions of dead individuals among zooplankters of different systematic groups probably reflect their different sensitivities both to factors related to eutrophication and to cyanobacterial toxins. Cladocera (especially large species of the genus Daphnia) are known to be sensitive to different pollutants and toxins, which is the reason for their wide usage as test objects in aquatic toxicology (Shcherban’, 1982, 1992). The influence of cyanobacterial toxins can be one of the causes of increased zooplankton mortality (Dubovskaya, 2009). The toxic effect of Cyanobacteria on zooplankton, especially Cladocera (genus Daphnia), is known and has been confirmed by a number of experiments in the laboratory and in the natural environment (Fulton, Paerl, 1982; Lampert, 1981; Moustaka-Gouni et al., 2006; Nizan et al., 1986; Rohrlack et al., 2001).
In July-September potentially toxic phytoplankton species abundantly develop in the phytoplankton of the Curonian Lagoon. A number of investigators (Belykh et al., 2009; Karmakl et al., 1993; Paldavičiene et al., 2009) found cyanobacterial toxins in the waters of the Curonian Lagoon, thus confirming the presence of toxic Cyanobacteria in this water body.

In the period mass development of harmful algae, the proportion of dead cladocerans in their total abundance and biomass in the Curonian Lagoon significantly increased by a factor of 5-13. It was probably the deterioration of conditions due to this period (possibly including increased toxicity) that cladocerans reacted to by increased mortality, which resulted in an increased proportion of dead animals. Since copepods, especially cyclopoids, are more tolerant to pollution of different kinds (Andronikova, Raspopov, 2007; Vinberg, 1979), the proportion of dead Cyclopoida did not increase significantly in the year of higher water bloom; however, the proportion of dead nauplial copepods considerably increased, which is probably explained by their increased sensitivity to cyanobacterial toxins.

One of the main possible reasons of increasing proportion of dead organisms in zooplankton is the influence of toxins Microcystis spp. We obtained a significant correlation between proportion of dead organisms in zooplankton and biomass of toxic Microcystis spp. \( r=0.62-0.81 \). Green algae can presumably compensate negative effect of Cyanobacteria, the correlation between proportion Chlorophyta in total biomass phytoplankton and proportion of dead organisms in zooplankton was negative \( r=-0.51-0.60 \).

According to the literature only impact toxins of Microcystis spp. exactly proved on the zooplankton (Bernardi, Giussani, 1990; Nizan at al., 1986; Fulton, Paerl, 1987; Rohrlack et al., 2001), which is confirmed by our data. On the other hand, in experiments (Hanazato at al, 1988; Chen, Xie, 2004) was showed that green algae can reduce the negative effect of blue-green algae, which confirms the dependence obtained by us.

Higher proportions of dead zooplankters were recorded in April and from July to October. The high proportion of dead individuals at the beginning of the vegetation period is probably related to hydrological factors: at this time of year, active wind induced mixing of the lagoon’s waters is observed, while the water temperature is low and food for zooplankton is deficient. Another factor explaining the high proportion of dead individuals in the spring may be mass development of harmful algae in the previous year (Aleksandrov, 2009).

Mass development of harmful algae is related to the abundant production of organic matter, which enters the water after phytoplankton destruction and leads to the secondary pollution of the water body (Aleksandrov, Dmitrieva, 2006). The effect of this secondary pollution can probably still be observed in the next year. The increased proportions of dead individuals in summer-autumn period are apparently related to the abundant development of phytoplankton, with a high proportion of potentially toxic species.

The possible negative impact of toxins is also confirmed by the fact that in July-October 2008-2010 the percentage of dead organisms from abundance and biomass of zooplankton increased in 5-13 times, when bloom of Cyanobacteria was observed. The negative influence of the bloom of toxic Cyanobacteria, leading to an increased proportion of dead zooplankters, was recorded earlier in the open part of the Baltic Sea (Shchuka, 2002).
Conclusions

The Curonian Lagoon currently is hypertrophy water body. The phenomenon of mass development of harmful algae (mostly Cyanobacteria) is regularly observed in the lagoon; the biomass of these Cyanobacteria in recent years is considerably higher than the level that determines secondary pollution of the water body.

During the period of mass development harmful Cyanobacteria and after it in July-October 2008-2010 the percentage of dead organisms from abundance and biomass of zooplankton increased in 5-13 times (up to 8.3-19.4%) with compared the percentage of dead organisms in July-October 2007 (1.5-1.8%), when biomass of Cyanobacteria was very low. Sensitivity of zooplankton’s taxonomical groups to “bloom” of Cyanobacteria and influence of toxins (according increasing proportion dead organisms in period mass developing Cyanobacteria) increased in line Cyclopoida→Calanoida→Rotifera→Cladocera. Minimal percentage of dead organisms was observed at the station in which organic pollution, eutrophication and biomass of harmful Cyanobacteria (especially Microcystis spp.) were at lower level. One of the main possible reasons of increasing proportion of dead organisms in zooplankton is the influence of toxins Microcystis spp. We obtained a significant correlation between proportion of dead organisms in zooplankton and biomass of toxic Microcystis spp. Green algae can presumably compensate negative effect of Cyanobacteria, the correlation between proportion Chlorophyta in total biomass phytoplankton and proportion of dead organisms in zooplankton was negative. Thus, the proportion of dead organisms in zooplankton may be the sensitive indicator to shown the influence of harmful algae.

Literature


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