Sinice jako czynniki wpływające na zespoły orzęsków w płytkich zbiornikach wodnych

Cyanobacteria as drivers of *Ciliata* assemblages in shallow inland waters

Joanna Kosiba

Kraków, 2021

AUTOR: **MGR INŻ. JOANNA KOSIBA**

Instytut Ochrony Przyrody Polskiej Akademii Nauk

Al. Adama Mickiewicza 33, 31-120 Kraków

PROMOTOR: **DR HAB. ELŻBIETA WILK-WOŹNIAK, PROF. IOP PAN**

Instytut Ochrony Przyrody Polskiej Akademii Nauk

Al. Adama Mickiewicza 33, 31-120 Kraków

SPIS TREŚCI

SPIS PUBLIKACJI

1. Kosiba J., Wilk-Woźniak E., Krztoń W., Strzesak M., Pociecha A., Walusiak E., Pudaś K., Szarek-Gwiazda E. 2017. *What underpins the trophic networks of the plankton in shallow oxbow lakes?* Microbial Ecology 73 (1): 17-28, DOI: 10.1007/s00248-016-0833-6; IF=4,552; lista MEiN=100 pkt,

2. Kosiba J., Krztoń W., Wilk-Woźniak E. 2018. *Effect of Microcystins on Proto- and Metazooplankton is more evident in artificial than in natural waterbodies.* Microbial Ecology 75: 293-302, DOI: 10.1007/s00248-017-1058-z; IF=4,552; lista MEiN=100 pkt,

3. Kosiba J., Wilk-Woźniak E., Krztoń W. 2019. *The effect of potentially toxic cyanobacteria on ciliates (Ciliophora).* Hydrobiologia 1 (827): 325-335, https://doi.org/10.1007/s10750-018-3783-9; IF=2,694; lista MEiN=100 pkt,

4. Kosiba J., Krztoń W. 2021. *Insight into the role of cyanobacterial bloom in the trophic link between ciliates and predatory copepods.* Artykuł zaakceptowany do druku w Hydrobiologia, DOI: 10.1007/s10750-021-04780-x; IF=2,694; lista MEiN=100 pkt.

FINANSOWANIE BADAŃ

Badania wchodzące w skład rozprawy doktorskiej sfinansowano z dotacji dla doktorantów Studium Doktoranckiego Nauk Przyrodniczych PAN przy Instytucie Botaniki im. W. Szafera Polskiej Akademii Nauk w Krakowie, z dotacji dla młodych pracowników naukowych Instytutu Ochrony Przyrody Polskiej Akademii Nauk w Krakowie oraz ze środków statutowych Instytutu Ochrony Przyrody Polskiej Akademii Nauk w Krakowie.

Niniejsza rozprawa doktorska została przygotowana w trakcie trwania studiów doktoranckich w Studium Doktoranckim Nauk Przyrodniczych PAN w Krakowie.

PODZIĘKOWANIA

Przede wszystkim pragnę podziękować mojej opiekunce naukowej czyli dr hab. Elżbiecie Wilk-Woźniak, prof. IOP PAN za całokształt opieki nad moją rozprawą doktorską od początku do końca oraz za wyrozumiałość, życzliwość, bezdyskusyjne wsparcie, nieustającą motywację i nieocenioną pomoc na poszczególnych etapach tej drogi. Dziękuję serdecznie za wszelakie wskazówki, bezcenne sugestie i możliwość konsultacji.

Na wstępie chciałam również podziękować dr Krystynie Kalinowskiej (Instytut Rybactwa Śródlądowego w Giżycku), dr Evelinie Griniene (Klaipeda University) i dr hab. Januszowi Fydzie, prof. UJ (Uniwersytet Jagielloński w Krakowie) za konsultacje wprowadzające w świat orzęsków.

Dziękuję kolegom i koleżankom z Zakładu Biologii Wód Instytutu Ochrony Przyrody PAN w Krakowie, w szczególności kolegom z zespołu: mgr Wojciechowi Krztoniowi i dr inż. Edwardowi Walusiakowi za nieocenioną pracę w terenie przy poborze prób. Dr hab. Antoniemu Amirowiczowi, prof. IOP PAN za zawsze racjonalne porady merytoryczne. Dr hab. Piotrowi Skórce, prof. IOP PAN za cenne rady statystyczne i konsultacje „trudnych" przypadków. Kolegom i koleżankom z pokoju za sympatyczną atmosferę w pracy.

Chciałam również podziękować dr Łukaszowi Wejnerowskiemu (Uniwersytet Adama Mickiewicza w Poznaniu) za pomoc przy hodowli sinicy *Planktotrix agardii* do eksperymentu laboratoryjnego i dr Mateuszowi Sobczykowi (Uniwersytet Jagielloński w Krakowie) za wskazówki dotyczące hodowli orzęsków. Dziękuję również za wykonanie analiz toksyn sinicowych dr hab. Tomaszowi Jurczakowi, prof. UŁ (Uniwersytet Łódzki) i mgr Krzysztofowi Pudasiowi (MPWiK S.A. w Krakowie).

Na końcu pragnę podziękować wszystkim tym, w szczególności mojemu Mężowi, Rodzinie oraz Znajomym, którzy w różnym stopniu wspierali i motywowali na poszczególnych etapach tworzenia tej rozprawy.

STRESZCZENIE

Zakwity wody spowodowane masowym rozwojem sinic są zagrożeniem dla integralności biocenoz i funkcjonowania zbiorników wodnych. Wiele gatunków sinic ma zdolność wydzielania toksycznych metabolitów, które mogą oddziaływać na mikroorganizmy wodne modyfikując ich skład gatunkowy, liczebność czy biomasę oraz kumulować się w łańcuchu troficznym. Zależności pomiędzy orzęskami, będącymi ważnym składnikiem planktonu, a sinicami były dotychczas tematem rzadko poruszanym w porównaniu do zależności metazooplankton – sinice. Dopiero stosunkowo niedawno nastąpiło poszerzenie modelu PEG (Plankton Ecology Group; Sommer i in., 2012), w którym uwzględniono orzęski jako ważnych konsumentów bakterii i fitoplanktonu.

Celem mojej rozprawy doktorskiej była ocena czy masowy rozwój sinic jest czynnikiem wpływającym na zespoły orzęsków w płytkich eutroficznych zbiornikach wodnych. W swojej pracy sprawdziłam czy orzęski, jako ważny składnik zooplanktonu, tworzą alternatywną ścieżkę przekazywania węgla i energii w sieciach troficznych płytkich starorzeczy **(artykuł nr 1: Microbial Ecology 2017)**. Przeanalizowałam również wpływ zakwitów sinicowych i wykazałam, że mogą one modyfikować skład i wielkość zespołów orzęsków w eutroficznych zbiornikach wodnych, szczególnie tych najbardziej zagrożonych antropopresją **(artykuł nr 2: Microbial Ecology 2018)**. Za pomocą eksperymentów laboratoryjnych sprawdziłam czy toksyny sinicowe mogą być czynnikiem regulującymi rozwój zarówno pojedynczych gatunków, jak i całych zespołów **(artykuł nr 3: Hydrobiologia 2019)**. Udowodniłam także, że zakwity sinicowe wzmacniają zależność orzęski–drapieżne widłonogi **(artykuł nr 4: zaakceptowany do druku w Hydrobiologia)**.

Poznanie zachowań orzęsków w obecności sinic pozwoliło na poszerzenie wiedzy o transferze węgla w sieci troficznej w okresach występowania zakwitów sinicowych oraz umożliwiło budowanie nowych hipotez pozwalających dokładniej poznać zjawisko zakwitów sinicowych. Wyniki moich badań są ważne w aspekcie zmian jakie zachodzą w ekosystemach wodnych wskutek zmian klimatycznych. Ocieplenie klimatu jest czynnikiem wzmagającym rozszerzanie obecności zakwitów sinicowych.

SUMMARY

Cyanobacterial blooms caused by the massive growth of cyanobacteria in waterbodies are the serious threat to the integrity of biocenoses and the functioning of freshwaters. Many species of cyanobacteria have the ability to release cyanotoxins that can negatively affect aquatic organisms by modifying their species compositions, abundance or biomass and accumulate in the trophic chain. Relationships between ciliates, an important component of zooplankton, and cyanobacteria have so far been rarely discussed in comparison with metazooplankton–cyanobacteria relationships. Only recently has there been an extension of the PEG model (Plankton Ecology Group; Sommer et al., 2012) to include ciliates as important consumers of bacteria and phytoplankton.

The aim of my doctoral thesis was to evaluate whether mass cyanobacterial growth is a factor affecting ciliate assemblages in shallow eutrophic waterbodies.

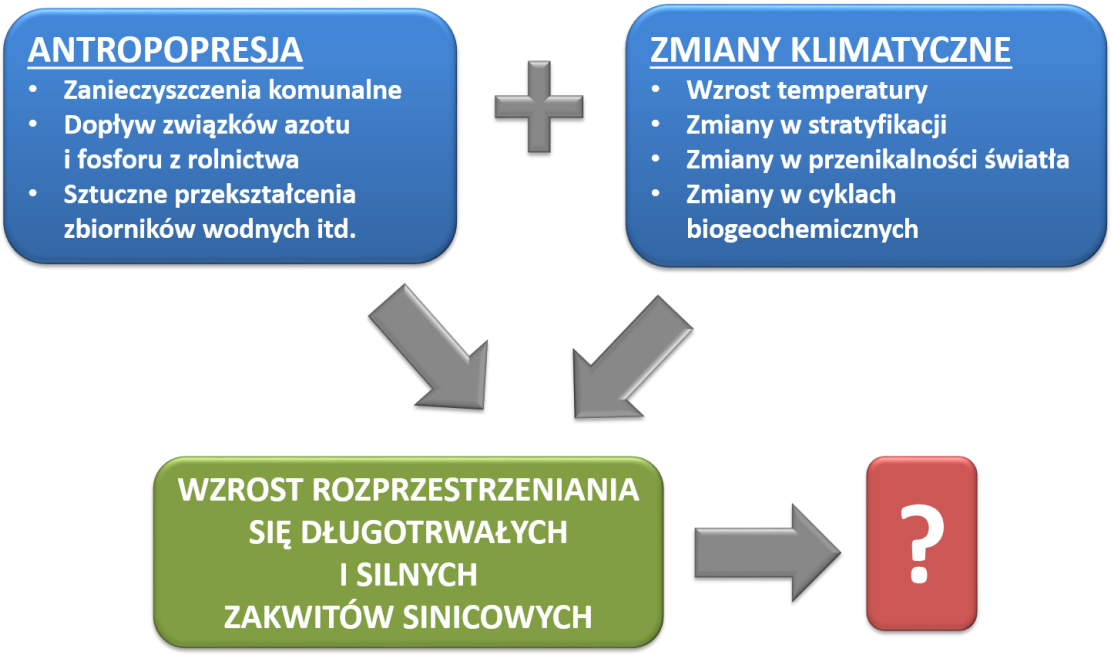
In my work, I checked whether ciliates, as an important component of zooplankton, creates an alternative pathway for carbon and energy transfer in the trophic networks of shallow oxbow lakes **(article 1: Microbial Ecology 2017)**. I have analyzed the effect of cyanobacterial blooms, and demonstrated that they can modify the composition and size of ciliate assemblages in eutrophic waterbodies, especially those most threatened by anthropopressure **(article 2: Microbial Ecology 2018).** Using laboratory experiments, I tested whether cyanobacterial toxins can be a factor regulating the growth of both individual species and entire assemblages of ciliates **(article 3: Hydrobiologia 2019).** I also demonstrated that cyanobacterial blooms enhance the ciliates–predator copepod relationship **(article 4: accepted for publication in Hydrobiologia)**.

Understanding the behavior of ciliates in the presence of cyanobacteria allowed us to extend our knowledge on carbon transfer in the trophic network during cyanobacterial blooms and allowed me to develop new hypotheses to understand more precisely the phenomenon of cyanobacterial blooms. The results of my study are important in terms of changes that occur in aquatic ecosystems due to climate change. Global warming is a factor that increases the proliferation of cyanobacterial blooms.

WSTĘP

Niewielkie eutroficzne zbiorniki wodne są ważnymi elementami krajobrazu, zwiększając jego różnorodność. To miejsca cechujące się dużymi walorami przyrodniczymi, z wysoką różnorodnością biologiczną, ale też są potencjalnie bardzo narażone na niekorzystne działania człowieka i zagrożone szybkim zanikiem. Ich wartość przyrodnicza została zauważona w skali całego kontynentu europejskiego, klasyfikując takie zbiorniki w kategorii siedlisk o szczególnym znaczeniu dla Wspólnoty Europejskiej – jako siedlisko 3150 *Natural eutrophic lakes with Magnopotamion or Hydrocharition-type vegetation*, w Polsce jako *Starorzecza i naturalne eutroficzne zbiorniki wodne ze zbiorowiskami z Nympheion, Potamion* (Wilk-Woźniak i in., 2012; Interpretation manual of Europen Union habitats, 2013).

Płytkie i niewielkie zbiorniki wodne są szczególnie narażone na antropopresję, której efektem może być przyspieszenie eutrofizacji, prowadzącej do zaniku jakości i wartości przyrodniczej siedliska. Jednym ze wskaźników wzrostu eutrofizacji jest występowanie zakwitów sinicowych (Paerl i in., 2011; O'Neil i in., 2012; Huisman i in., 2018). W zbiornikach wód eutroficznych jest to zjawisko naturalne, jednak wzrost ich intensywności i przedłużające się trwanie wskazuje na pogarszające się cechy siedliska. Antropopresja i zmiany klimatu są dwoma głównymi czynnikami odpowiedzialnymi za zwiększanie się intensywności zakwitów sinicowych wraz z wydłużonym czasem ich trwania (Paerl i Huisman, 2008, 2009; Ryc. 1).



Ryc. 1. Główne czynniki odpowiedzialne za wzmacnianie częstotliwości i długości trwania zakwitów sinicowych

Takie intensywne i długotrwałe zakwity powodują zaburzenia w funkcjonowaniu ekosystemów wodnych prowadząc do spadku ich bioróżnorodności, zarówno w udziale poszczególnych grup organizmów np. zooplanktonu (Kosiba et al., 2018), a także grup funkcjonalnych (Krztoń i in., 2019; Krztoń i Kosiba, 2020). Ponadto bieżące badania dowodzą, że akumulacja i dekompozycja biomasy zakwitów sinicowych wpływa na strukturę mikrobiologiczną w ekosystemach wodnych, indukując tym samym metylotroficzne ścieżki obiegu materii i produkcji metanu (CH_4) w jeziorach eutroficznych. Rozkład sinic powoduje wytwarzanie trimetyloaminy ($N(CH_3)_3$), zwiększa zasięg środowiska beztlenowego i względną liczebność metanobakterii (archeowce). Zmiany te sprzyjają produkcji i emisji metanu z osadów jeziornych (Zhou i in., 2021).

Biorąc pod uwagę, że ekosystemy wodne znajdujące się w strefie klimatu borealnego i kontynentalnego są szczególnie wrażliwe na zmiany klimatyczne (Donis i in., 2021), to poznanie zależności zachodzących w eutroficznych płytkich zbiornikach wodnych jest konieczne, aby proponowane sposoby zarządzania takimi ekosystemami były jak najbardziej efektywne, przyczyniając się tym samym do łagodzenia skutków zmian klimatycznych, w tym do obniżenia produkcji i emisji metanu.

Zakwity sinicowe nie stanowią etapu końcowego procesu wzmożonej eutrofizacji lecz stanowią jego punkt środkowy (Wilk-Woźniak, 2020). Sinice to fotosyntetyzujące bakterie, obecne na Ziemi od przynajmniej 3 mld lat (Kaufman, 2014). Przez ekologów planktonu zaliczane są do fitoplanktonu, będąc producentami pierwotnymi. Bezpośrednio z fitoplanktonem, w tym z sinicami, związane są grupy innych mikroorganizmów: wirusy, bakterie, grzyby wodne, protista niefotosyntetyzujące, metazooplankton. Spośród tych grup szczególnie ważną grupą zaliczaną do protista są orzęski (*Ciliophora*).

Orzęski są kluczowym elementem wodnych sieci troficznych. Orzęski planktonowe tworzą protozooplankton, który jest częścią zooplanktonu. Protozooplankton pełni funkcję konsumentów: sinic, bakterii, glonów, grzybów wodnych, innych protista, a także drobnego metazooplanktonu (Boechat i Adrian, 2005; Weisse i Sonntag, 2016). Z drugiej strony stanowi pożywienie dla metazooplanktonu, głównie widłonogów i wioślarek (Jack i Gilbert, 1997; Weisse, 2006). Ze względu na istotne znaczenie w transferze węgla w sieciach troficznych, grupa ta zasługuje na szczególną uwagę. Badania zależności sinice–zooplankton mają swoją długą historię (Burns, 1987; Haney, 1987; Fulton i Paerl, 1988), jednak uwaga badaczy najczęściej skupiała się na metazooplanktonie (Gliwicz, 1990; Sellner i in., 1994; Wejnerowski i in., 2015), a protozooplankton jako istotny element sieci troficznych przez długi czas był zaniedbywany. Dopiero kiedy na przełomie lat 80. i 90. XX wieku odkryto "pętlę mikrobiologiczną" (np. Jumars i in., 1989; Weisse i in., 1990; Porter, 1996, Sommer i in., 2012), protozooplankton uznano za ważny składnik sieci troficznej (Christoffersen i in., 1990; Kalinowska, 2004; Zingel i in., 2007).

Badania naukowe dowodzą, że kiedy fitoplankton (w tym sinice) nie może być eliminowany przez metazooplankton w klasycznym liniowym łańcuchu, wówczas większą rolę odgrywają bakterie i protista, w tym orzęski (Agasid et al., 2013). Ponadto orzęski mają wpływ na przebieg dekompozycji materii organicznej, obieg węgla i składników odżywczych, odgrywają bardzo ważną rolę troficzną w zbiorowiskach peryfitonu, a przede wszystkim stanowią również duży udział w strukturze zbiorowisk zooplanktonu (Gates, 1984; Kalinowska, 2004; Mieczan, 2005). Jednakże ze względu na trudności w identyfikacji orzęsków (Foissner i Berger, 1996, 1999) badania nad tą grupą są wciąż w fazie pionierskiej i prowadzone są jedynie przez nieliczne zespoły badawcze (Wiąckowski in., 2001, Pajdak-Stós i in., 2017, Sherr i Sherr, 2002; Agasild i in., 2013; Boas i in., 2020; Napiórkowska-Krzebietke i in., 2021). Uzyskane wyniki mają istotne znaczenie dla zrozumienia procesów zachodzących w ekosystemach wodnych i ich zmian.

CEL BADAŃ

Ze względu na ważność tematu podjęłam w swoich badaniach problem wpływu zakwitów sinicowych na orzęski. Celem badań było ustalenie: *Czy sinice są czynnikiem istotnie wpływającym na zespoły orzęsków w płytkich eutroficznych zbiornikach wodnych*?

W celu odpowiedzi na to pytanie założyłam przetestowanie następujących hipotez:

H1. Orzęski są istotnym komponentem zooplanktonu i tworzą alternatywną ścieżkę przekazywania węgla i energii w sieciach troficznych (**artykuł nr 1:** Kosiba J., Wilk-Woźniak E., Krztoń W., Strzesak M., Pociecha A., Walusiak E., Pudaś K., Szarek-Gwiazda E. 2017. ***What underpins the trophic networks of the plankton in shallow oxbow lakes?*** Microbial Ecology 73 (1): 17-28, DOI: 10.1007/s00248-016-0833-6; IF=4,552; lista MEiN=100 pkt).

H2. Zakwity sinicowe powodują zmiany jakościowe i ilościowe zespołów orzęsków (**artykuł nr 2:** Kosiba J., Krztoń W., Wilk-Woźniak E. 2018. ***Effect of Microcystins on Proto- and Metazooplankton is more evident in artificial than in natural waterbodies.*** Microbial Ecology 75: 293-302, DOI: 10.1007/s00248-017-1058-z; IF=4,552; lista MEiN=100 pkt), a toksyny sinicowe są czynnikami regulującymi rozwój zarówno pojedynczych gatunków, jak i całych zespołów (**artykuł nr 3:** Kosiba J., Wilk-Woźniak E., Krztoń W. 2019. ***The effect of potentially toxic cyanobacteria on ciliates (Ciliophora).*** Hydrobiologia 1 (827): 325-335, https://doi.org/10.1007/s10750-018-3783-9; IF=2,694; lista MEiN=100 pkt).

H3. Zakwity sinicowe wzmacniają zależność orzęski bakteriożerne–widłonogi (**artykuł nr 4:** Kosiba J., Krztoń W. 2021. ***Insight into the role of cyanobacterial bloom in the trophic link between ciliates and predatory copepods.*** (Artykuł zaakceptowany do druku w Hydrobiologia, IF=2,694; lista MEiN=100 pkt).

Prezentowana praca doktorska obejmuje cztery publikacje naukowe dotyczące badań terenowych i laboratoryjnych nad zespołami orzęsków obecnych w protozooplanktonie w kontekście zakwitów sinicowych.

TEREN BADAŃ

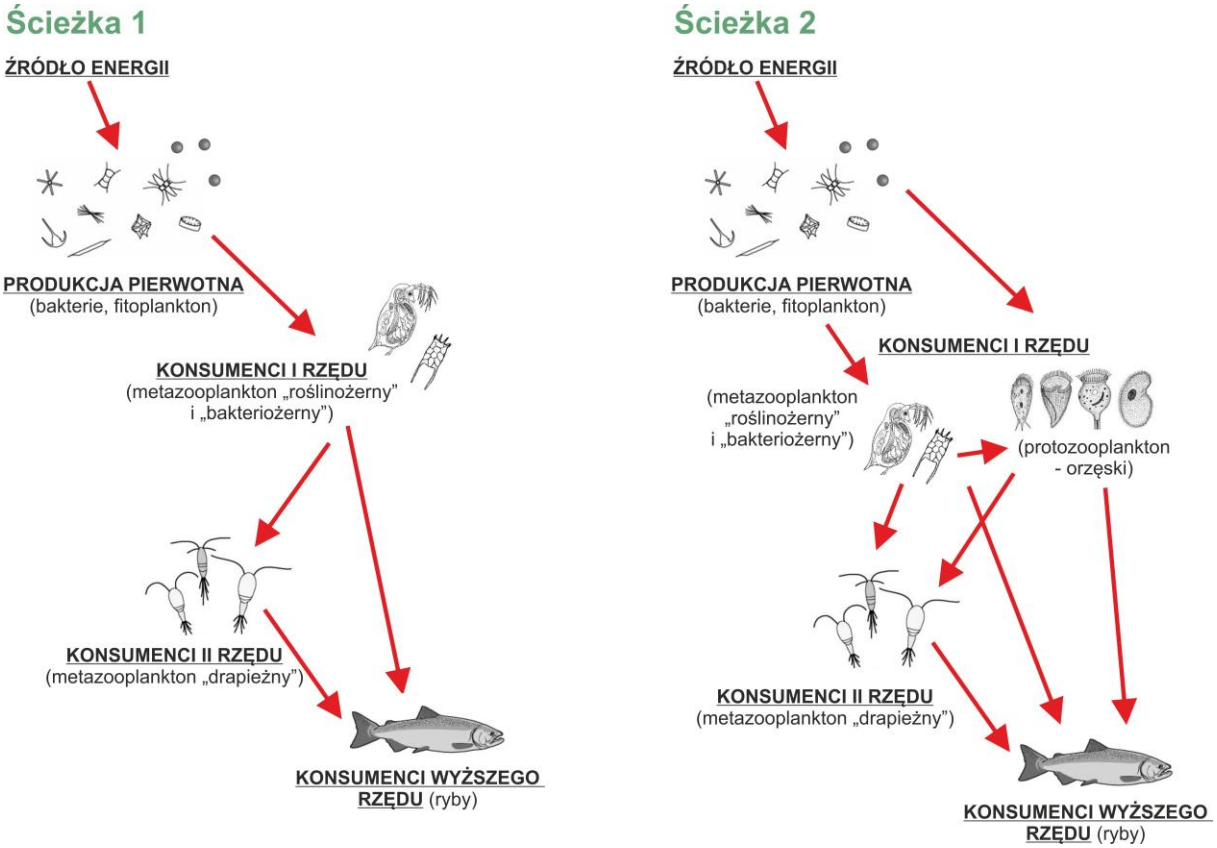
Badania terenowe prowadzone były w sezonie wegetacyjnym w latach 2014 i 2017 w okolicach Krakowa w siedmiu płytkich eutroficznych zbiornikach wodnych. Do badań terenowych wybrano pięć niewielkich i płytkich starorzeczy Wisły: dwa zlokalizowane w Podgórkach Tynieckich: Tyniec 1 (50°01’47’’N, 19°49’40’’E) i Tyniec 2 (50°01'28"N 19°48'48"E), dwa w Jeziorzanach: Jeziorzany 1 (49°59’46”N 19°46’52”E) i Jeziorzany 2 (49°59’44”N 19°47’11”E) oraz jedno w Piekarach (50°00’50”N 19°47’36”E). Dwa kolejne zbiorniki to dwa sztucznie wybudowane płytkie stawy: Podkamycze 1 (50°05’11’’N, 19°50’02’’E) i Podkamycze 2 (50°04’60”N, 19°50’05”E).

Starorzecza funkcjonują w cyklu naturalnych sezonowych zmian, co oznacza obecność zakwitów sinicowych w okresie lata i/lub jesieni, natomiast w sztucznie wybudowanych stawach stwierdza się długotrwałe i silne zakwity sinicowe obecne od wiosny do późnej jesieni. Zgodnie z klasyfikacją troficzną Carlsona (Carlson i Simpson, 1996) wszystkie badane zbiorniki klasyfikowane są jako zbiorniki eutroficzne.

WYNIKI

W artykule nr 1 (Microbial Ecology 2017, hipoteza 1) oceniłam zależności jakie wystąpiły w sieci troficznej zbiorowisk planktonowych w płytkich zbiornikach wodnych w celu zrozumienia w jaki sposób węgiel jest przekazywany pomiędzy różnymi grupami tworzącymi plankton.

W artykule tym zwróciłam szczególną uwagę na ważność zespołów orzęsków w sieciach troficznych zbiorników wodnych. Wyniki badań potwierdziły, że transfer węgla następuje dwiema równoległymi ścieżkami: 1) fitoplankton – metazooplankton lub 2) fitoplankton – orzęski – metazooplankton (Ryc. 2). Na podstawie prostych korelacji został stworzony model sieci troficznej w małych płytkich starorzeczach. Pokazane relacje między organizmami planktonowymi wskazują zarówno bezpośrednie, jak i pośrednie związki, takie jak konkurencja o zasoby pokarmu czy drapieżnictwo. Zgodnie z koncepcją pętli mikrobiologicznej rozpuszczony węgiel organiczny uwalniany przez fitoplankton jest wykorzystywany przez bakterie, które następnie są wyżerowywane przez pierwotniaki, będące z kolei pokarmem dla drapieżnego metazooplanktonu. Wyniki badań potwierdziły istotny udział orzęsków w całkowitej biomasie zooplanktonu. Orzęski stanowiły od 6,7% do 44,5% całkowitej biomasy zooplanktonu w badanych zbiornikach wodnych i były to w głównej mierze gatunki glonożerne i bakterio-glonożerne.

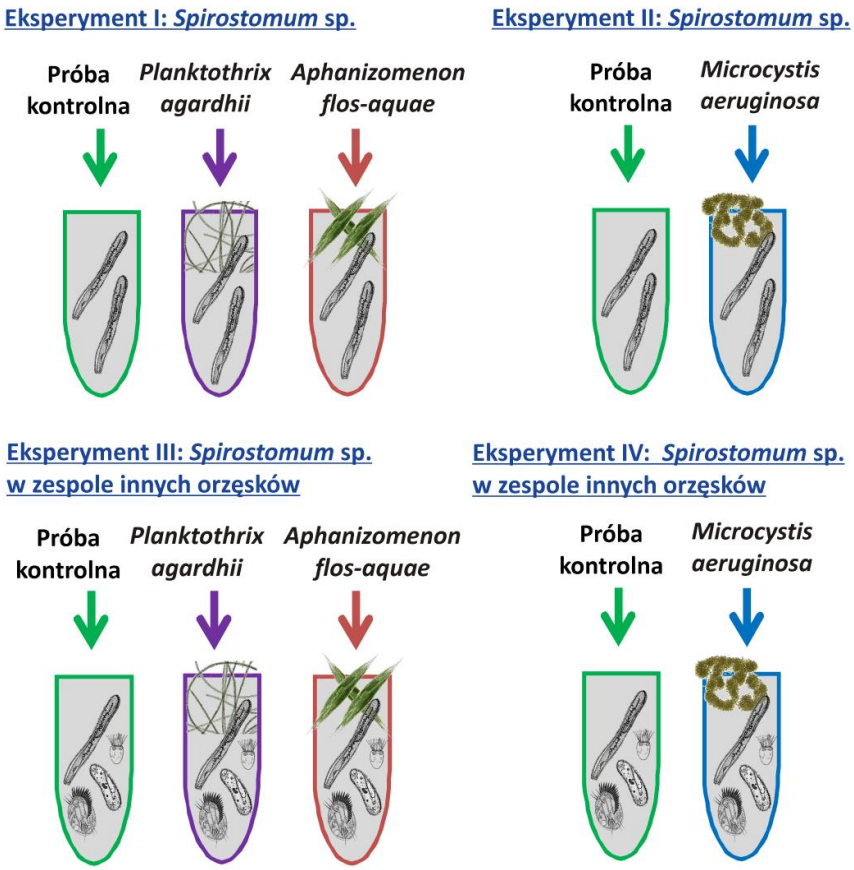


Ryc.2. Alternatywne ścieżki transferu węgla w planktonie

W celu zbadania wpływu zakwitów sinicowych na orzęski przeprowadziłam badania terenowe i laboratoryjne **(hipoteza 2)**. Wyniki badań terenowych pokazały, że reakcje zespołów orzęsków nie były jednakowe w badanych zbiornikach. **W pracy nr 2 (Microbial Ecology 2018, hipoteza 2)** wykazałam, że im dłużej trwa zakwit sinicowy, tym większe zmiany następują w zbiorowiskach zooplanktonu (zarówno proto- jak i metazooplanktonu). W zbiornikach wodnych z silnymi i długotrwałymi zakwitami oraz wysoką koncentracją i długotrwałą obecnością mikrocystyn (toksyny sinic) w wodzie (stawy Podkamycze 1 i Podkamycze 2), parametry populacyjne meta- i protozooplanktonu (zagęszczenie, biomasa, bogactwo gatunkowe) ulegały redukcji, a struktura gatunkowa zbiorowisk wykazała tendencję zmniejszenia różnorodności. Zbiorniki te są środowiskami sztucznymi poddanymi silniejszej antropopresji w porównaniu do badanych starorzeczy. Wyniki badań wykazały dodatkowo, że zbiorniki bardziej narażone na antropopresję były też bardziej narażone na silne i długotrwałe zakwity sinicowe w porównaniu do zbiorników funkcjonujących w naturalnym cyklu rocznym, poddanych mniejszej antropopresji (płytkie starorzecza: Piekary i Tyniec).

Wyniki badań terenowych sugerowały, że toksyny sinicowe mogą wpływać na jakościowe i ilościowe parametry zespołów orzęsków. W celu potwierdzenia tej obserwacji przeprowadziłam eksperyment laboratoryjny – **praca nr 3 (Hydrobiologia 2019, hipoteza 2)**. Wykonałam badania dotyczące reakcji pojedynczego gatunku orzęska *Spirostomum* sp. oraz tego samego gatunku w uproszczonym zespole, składającym się z 4-gatunków: *Spirostomum* sp., *Euplotes patella*, *Strobilidium* sp., *Paramecium aurelia*-complex) w zależności od gatunku sinicy oraz obecności/nieobecności toksyn w biomasie sinic. *Spirostomum* sp. został wybrany do badań, ponieważ w literaturze przedmiotowej gatunek *Spirostomum ambiguum* wykazywany jest jako wrażliwy na toksyny (Tarczyńska i in., 2001) i używany w testach toksyczności (np. test Spirotox: Nałęcz-Jawecki, 2004).

Przeprowadziłam cztery eksperymenty laboratoryjne (Ryc. 3) z udziałem trzech gatunków sinic, mających potencjalną zdolność produkcji toksyn i różniących się morfologią: *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek, *Microcystis aeruginosa* (Kützing) Kützing, *Aphanizomenon flos-aquae* Ralphs ex Bornet (Flahault). Eksperymenty podzieliłam na dwie części i sprawdziłam reakcję na obecność poszczególnych gatunków sinic: *P. agardhii* i *A. flos-aqaue* (sinice filamentowe) oraz *M. aeruginosa* (sinica kokkalna, kolonijna). Określiłam także obecność lub brak toksyn w biomasie sinic. W biomasie *P. agardhii* oraz *M. aeruginosa* wykazano obecność toksyn (mikrocystyny), a w biomasie *A. flos-aquae* nie stwierdzono ich obecności, jak również nie stwierdzono obecności anatoksyny-a.



Ryc. 3. Schemat przeprowadzonych eksperymentów laboratoryjnych. Eksperyment I: sinice filamentowe + *Spirostomum* sp. Eksperyment II: sinica kokkalna + *Spirostomum* sp. Eksperyment III: sinice filamentowe + zespół orzęsków. Eksperyment IV: sinica kokkalna + zespół orzęsków.

W eksperymencie przeprowadzonym na pojedynczym gatunku stwierdziłam, że *Spirostomum* sp. zwiększył liczebność w próbach z sinicami toksycznymi, niezależnie od ich morfologii i wielkości koncentracji toksyn w biomasie sinic. Natomiast gdy *Spirostomum* sp. był składową uproszczonego zespołu, jego liczebność malała we wszystkich trzech przypadkach – niezależnie od gatunku sinicy. Wyniki wskazują zatem, że toksyny nie były czynnikiem ograniczającym rozwój orzęska. Czynnikiem tym natomiast mogła być konkurencja o pokarm z gatunkami orzęsków cechującym się mniejszymi rozmiarami, które mogą bardziej efektywnie wyjadać bakterie rozwijające się na biomasie sinic.

Wyniki eksperymentu wskazują, że sinice mogą być czynnikiem modyfikującym dynamikę i skład gatunkowy orzęsków pośrednio poprzez modyfikację środowiska, uruchamiając zmiany w zależnościach pomiędzy gatunkami orzęsków w istniejących zbiorowiskach (np. konkurencja o pokarm) lub połączenia poziome (lateralne; Weisse i Sonntag, 2016) dotyczące np. konkurencji wewnątrzgatunkowej lub komunikacji pomiędzy osobnikami (np. wydzielanie substancji niosących informacje).

Jako konsumenci bakterii i fitoplanktonu orzęski łączą producentów pierwotnych z wyższymi poziomami troficznymi. Duże drapieżne widłonogi mogą efektywnie konsumować orzęski (Kalinowska i in., 2015). Drapieżnictwo widłonogów na orzęskach jest dobrze udokumentowane w badaniach ekosystemów morskich (np. Calbet i Saiz, 2005), ale słabo w ekosystemach wód słodkich. Dlatego ważnym elementem moich badań było poznanie zależności ofiara (orzęski) – drapieżnik (widłonogi) **(hipoteza 3, artykuł nr 4 zaakceptowany do druku).** Celem badań było ustalenie czy zakwity sinicowe modyfikują relacje między orzęskami i widłonogami. Ponieważ eutrofizacja ma tendencję do wzmacniania sprzężeń między proto- i metazooplanktonem (Ger i in., 2016), spodziewałam się znaleźć istotną zależność między całkowitą biomasą orzęsków obecnych w planktonie, a całkowitą biomasą drapieżnego metazooplanktonu (widłonogów) w czasie zakwitów sinicowych. Uzyskane wyniki analiz wskazały, że zależności orzęski – drapieżne widłonogi w eutroficznych ekosystemach wodnych są istotną drogą transferu węgla, funkcjonującą zarówno w okresach bez zakwitu sinic, jak i podczas zakwitu. Zależność biomasy drapieżnych widłonogów od biomasy glonożernych i bakterio-glonożernych orzęsków, przy jednoczesnym braku zależności od biomasy bakteriożernych orzęsków, wskazuje, że węgiel wiązany przez bakterie może tylko częściowo być przekazywany przez połączenie orzęski–widłonogi. Wyniki badań wskazują, że ważnym czynnikiem decydującym o kierunku transferu węgla mogą być strategie życiowe orzęsków. Większe i swobodnie pływające gatunki orzęsków są chętniej zjadane przez widłonogi, w przeciwieństwie do gatunków mniejszych i osiadłych. Tłumaczę to tym, że widłonogi drapieżne mają zdolność detekcji ruchu wody wytworzonego przez pływające orzęski (Kiørboe i Visser, 1999). W warunkach zakwitu, kiedy przejrzystość wody jest niewielka, cecha ta pozwala na zlokalizowanie poruszającej się ofiary, a gatunki osiadłe stają się trudniejsze do złowienia.

WNIOSKI

Badania, które przeprowadziłam pozwoliły odpowiedzieć na pytanie: *Czy sinice mogą być czynnikiem istotnie wpływającym na zespoły orzęsków w płytkich eutroficznych zbiornikach wodnych?*

Ponieważ orzęski stanowią jeden z głównych elementów sieci troficznej wód, a struktura ich zbiorowisk zależna jest od stopnia trofii i zanieczyszczenia zbiornika wodnego, poznanie zależności sinice–orzęski jest ważne zarówno w samym aspekcie poznawczym, jak i dla interpretacji możliwych kierunków zmian w ekosystemach wodnych spowodowanych zmianami klimatycznymi (wydłużające się i bardziej intensywne zakwity sinic).

W swojej pracy zastosowałam podejście do wzajemnych zależności pomiędzy organizmami jako sieci, a nie prostego łańcucha pokarmowego oraz holistyczne rozumienie funkcjonowania organizmów jako asocjacji (sinice + mikroorganizmy z nimi związane jako cały nierozerwalny kompleks), co jest podejściem nowatorskim w badaniach laboratoryjnych.

Wyniki moich badań potwierdziły, że orzęski będące konsumentami bakterii, glonów, drapieżnikami innych orzęsków, a nawet niektórych metazoa, są kluczowym elementem sieci troficznej. Rozszerzenie badań dotyczących relacji sinice–zooplankton o protozooplankton poszerza wiedzę o procesach i zależnościach w ekosystemach wód słodkich w czasie zakwitów sinicowych. Zrozumienie ko-ewolucyjnej dynamiki sinic i zwierząt planktonowych może okazać się krytycznym elementem poznania czynników regulujących zakwity (Ger i in., 2016) oraz czynników regulujących obecność i dynamikę zwierząt planktonowych. Wyniki moich badań potwierdziły możliwość przepływu materii organicznej w sieci troficznej w obrębie planktonu na dwa sposoby: 1) przepływ materii od bakterii i fitoplanktonu bezpośrednio do metazooplanktonu i 2) przepływ materii od bakterii i fitoplanktonu przez protozooplankton do metazooplanktonu (**Praca nr 1:** Kosiba i in. 2017; **Praca nr 4:** Kosiba i Krztoń 2021 [zaakceptowany do druku]).

Badania terenowe pokazały iż długotrwały zakwit sinic wiązał się z redukcją zagęszczenia, biomasy oraz bogactwa gatunkowego i zmniejszaniem różnorodności (wzrostem ujednolicenia, homogeniczności) proto- i metazooplanktonu (**Praca nr 2:** Kosiba i in. 2018). Wyniki tych badań wskazały, że ekosystemy sztuczne były bardziej podatne na długotrwałe zakwity sinicowe oraz że zbiorowiska zwierząt planktonowych wykazały większe zubożenie gatunkowe (homogenizacja zbiorowisk) proto- i metazooplanktonu. To ważne odkrycie wskazujące, że zbiorowiska planktonu zwierzęcego w naturalnych ekosystemach wodnych są mniej podatne na zaburzenia jakim są zakwity sinicowe.

Z kolei badania eksperymentalne (**Praca nr 3:** Kosiba i in. 2019), mające na celu wyjaśnienie czy toksyny sinicowe wpływają na dynamikę liczebności orzęsków, wykazały, że obecność

toksyn w biomasie sinic nie oddziaływała negatywnie na liczebność pojedynczego gatunku orzęska. Z kolei liczebność orzęsków była związana z gatunkiem testowanej sinicy i z obecnością lub brakiem innych gatunków orzęsków. Przeprowadzając eksperyment zastosowałam **podejście holistyczne** zakładając, że sinice wraz z asocjacjami (inne mikroorganizmy żyjące w cyjanosferze) stanowią nierozerwalną całość oddziałującą na inne organizmy (Codd, 2018). Szczepy sinic wyizolowane ze środowisk naturalnych zawierają naturalnie występujące kompleksy mikroorganizmów i dlatego przeprowadzenie eksperymentów z takimi szczepami daje wyniki odzwierciedlające procesy zachodzące w warunkach naturalnych i wiarygodne odpowiedzi na pytania o wzajemne zależności. Stwierdzoną modyfikację składu i dynamiki zbiorowisk orzęsków można interpretować jako bezpośrednie oddziaływanie nie tylko samych sinic lecz organizmów pozostających w asocjacjach lub jako pośredni efekt (konkurencja międzygatunkowa, powiązania lateralne). **Połączenie badań terenowych i laboratoryjnych było dodatkowym atutem weryfikacji hipotezy 2**.

Nowym zagadnieniem poruszonym w moich badaniach była ocena zależności protozooplankton–widłonogi drapieżne w ekosystemach słodkowodnych (**Praca nr 4:** Kosiba i Krztoń, 2021, zaakceptowany do druku). Wyniki badań wykazały, że zakwit sinicowy wzmacniał relację ofiara–drapieżnik.

PODSUMOWANIE

Przeprowadzone badania przyczyniły się do poszerzenia wiedzy dotyczącej wpływu zakwitów sinicowych na orzęski planktonowe oraz do opisania nowych zależności w obrębie zooplanktonu modyfikowanych przez obecność zakwitów sinicowych. Wykazałam, że sinice wpływają na zmianę struktury i dynamiki liczebności oraz biomasy ważnego elementu zooplanktonu, jakim jest protozooplankton, do tej pory często pomijany w badaniach dotyczących funkcjonowania sieci troficznych w ekosystemach wodnych oraz w badaniach dotyczących ekologii planktonu.

LITERATURA

Agasild H., Zingel P., Karus K., Kangro K., Salujoe J., Noges T. 2013. Does metazooplankton regulate the ciliate community in a shallow eutrophic lake? Freshwater Biology 58(1): 183-191.

Boechat I.G., Adrian R. 2005. Biochemical composition of algivorous freshwater ciliates: you are not what you eat. Microbiol Ecology 53(3): 393-400.

Burns C.W. 1987. Insights into zooplankton-cyanobacteria interactions derived from enclosure studies. New Zealand Journal of Marine and Freshwater Research 21: 477-482.

Calbet A., Saiz E. 2005. The ciliate-copepod link in marine ecosystems. Aquatic Microbial Ecology 38(2): 157-167.

Carlson R.E., Simpson J. 1996. A Coordinator's Guide to Volunteer Lake Monitoring Methods. North American Lake Management Society. 96 pp.

Christoffersen K., Riemann B., Hansen L.R., Klysner A., Sørensen H.B. 1990. Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microbial Ecology 20: 253–272.

Codd G.A. 2018. The antibiotic resistome: Broadening the recognition of cyanobacterial blooms hazards. The Phycologist 95: 11.

Donis D., Mantzouki E., McGinnis D.F., Vachon D., Gallego I., Grossart H.P., ..., Rodríguez V. 2021. Stratification strength and light climate explain variation in chlorophyll a at the continental scale in a European multilake survey in a heatwave summer. Limnology and Oceanography.doi.org/10.1002/lno.11963.

Foissner W., Berger H. 1996. A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators In rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biology 35(2): 375-482.

Foissner W., Berger H., Schaumburg J. 1999. Identification and Ecology of Limnetic Plankton Ciliates, Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, München.

Fulton R.S., Paerl H.W. 1988. Effects of the blue-green alga Microcystis aeruginosa on zooplankton competitive relations. Oecologia 76(3): 383-389.

Gates M.A. 1984. Quantitative importance of ciliates in the planktonic biomass of lake ecosystems. Hydrobiologia 108(3): 233-238.

Ger K.A., Urrutia-Cordero P., Frost P.C., Hansson L.A., Sarnelle O., Wilson A.E., Lürling M. 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic world. Harmful Algae 54: 128-144.

Gliwicz Z.M. 1990. Why do cladocerans fail to control algal blooms? Hydrobiologia 200–201: 83–97. doi:10.1007/BF02530331.

Haney J.F. 1987. Field studies on zooplankton-cyanobacteria interactions. New Zealand Journal of Marine and Freshwater Research 21: 467–475.

Huisman J., Codd G.A., Paerl H.W., Ibelings B.W., Verspagen J.M., Visser P.M. 2018. Cyanobacterial blooms. Nature Reviews Microbiology 16(8): 471-483.

Jack J.D., Gilbert J.J. 1997. Effects of Metazoan Predators on Ciliates in Freshwater Plankton Communities 1. Journal of Eukaryotic Microbiology 44(3): 194-199.

Jumars P.A., Penry D.L, Baross J.A., Perry M.J., Frost B.W. 1989. Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in ani-mals. Deep Sea Research Part A. Oceanographic Research Papers 36(4): 483-495.

Kalinowska K. 2004. Bacteria, nanoflagellates and ciliates as components of the microbial loop in three lakes of different trophic status. Polish Journal of Ecology 52(1): 19-34.

Kalinowska K., Ejsmont-Karabin J., Rzepecki M., Kostrzewska-Szlakowska I., Feniova I.Y., Palash A., Dzialowski A.R. 2015. Impacts of large-bodied crustaceans on the microbial loop. Hydrobiologia 744(1): 115–125.

Kaufman A. 2014. Cyanobacteria at work. Nature Geosci 7: 253–254. https://doi.org/10.1038/ngeo2128.

Kiørboe T., Visser A.W. 1999. Predator and prey perception in copepods due to hydromechanical signals. Marine Ecology Progress Series 179: 81-95.

Kosiba J., Wilk-Woźniak E., Krztoń W. 2018. Effect of Microcystins on Proto- and Metazooplankton is more evident in artificial than in natural waterbodies. Microbial Ecology 75: 293-302. doi: 10.1007/s00248-017-1058-z.

Krztoń W., Kosiba J., Pociecha A., Wilk-Woźniak E. 2019. The effect of cyanobacterial blooms on bio- and functional diversity of zooplankton communities. Biodiversity and Conservation 28(7): 1815-1835. doi.org/10.1007/s10531-019-01758-z.

Krztoń W., Kosiba J. 2020. Variations in zooplankton functional groups density in freshwater ecosystems exposed to cyanobacterial blooms. Science of the Total Environment 730: https://doi.org/10.1016/j.scitotenv.2020.139044.

Mieczan T. 2005. Periphytic ciliates in littoral zone of three lakes of different trophic status. Polish Journal of Ecology 53(4): 489-502.

Nałęcz-Jawecki G. 2004. Spirotox—*Spirostomum ambiguum* acute toxicity test—10 years of experience. Environmental Toxicology: An International Journal 19(4): 359-364.

Napiórkowska-Krzebietke A., Kalinowska K., Bogacka-Kapusta E., Stawecki K., Traczuk P. 2021. Persistent blooms of filamentous cyanobacteria in a cormorant-affected aquatic ecosystem: Ecological indicators and consequences. Ecological Indicators 124: 107421.

Nature ENV. 2013. EUROPEAN COMMISSION DG ENVIRONMENT. https://ec.europa.eu/environment/nature/legislation/habitatsdirective/docs/Int_Manual_EU28.pdf

O'Neil J.M., Davis T.W., Burford M.A., Gobler C.J. 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14: 313–334. doi:10.1016/J.HAL.2011.10.027

Paerl H.W., Huisman J. 2008. Blooms like it hot. Science 320: 57-58.

Paerl H.W. Huisman J. 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports 1(1): 27–37. https://doi.org/ 10.1111/j.1758-2229.2008.00004.x.

Paerl H.W., Hall N.S., Calandrino E.S. 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. The Science of the Total Environment 409(10): 1739–1745. doi:10.1016/J.SCITOTENV.2011.02.001.

Pajdak-Stós A., Sobczyk M., Fiałkowska E., Kocerba-Soroka W., Fyda J. 2017. The effect of three different predatory ciliate species on activated sludge microfauna. European journal of protistology 58: 87-93.

Porter K. G. 1996. Integrating the Microbial Loop and the Classic Food Chain Into a Realistic Planktonic Food Web. In: Polis G. A., Winemiller K.O. (eds) Food Webs. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-7007-3_5.

Sellner K.G., Olson M.M., Kononen K. 1994. Copepod grazing in a summer cyanobacteria bloom in the Gulf of Finland. Hydrobiologia 292(1): 249-254.

Sherr E.B., Sherr B.F. 2002. Significance of predation by protist in aquatic microbial food web. Antonie van Leeuwenhoek 81: 293- 308.

Sommer U., Adrian R., De Senerpont Domis L., Elser J.J., Gaedke U., Ibelings B., Jeppesen E., Lürling M., Molinero J.C, Mooij W.M,E. Van Donk E. 2012. Beyond the plankton ecology group (PEG) model: mechanisms driving plankton succession. Annual Review of Ecology Evolution and Systematics 43: 429-448. doi:10.1146/ ANNUREV-ECOLSYS-110411-160251.

Tarczyńska M., Nałęcz-Jawecki G., Romanowska-Duda Z., Sawicki J., Beattie K., Codd G. & Zalewski M. 2001. Tests for the toxicity assessment of cyanobacterial bloom samples. Environmental Toxicology 16(5): 383–390.

Weisse T., Müller H., Pinto-Coelho R.M., Schweizer A., Springmann D., Baldringer G. 1990. Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnology and Oceanography 35: 781-794.

Weisse T. 2006. Freshwater ciliates as ecophysiological model organisms - lessons from Daphnia, major achievements, and future perspectives. Archiv fur Hydrobiologie 167: 371–402.

Weisse T., Sonntag, B. 2016. Ciliates in planktonic food webs: communication and adaptive response. In Biocommunication of ciliates: 351-372. Springer, Cham.

Wejnerowski L., Cerbin S., Dziuba M.K. 2015. Thicker filaments of Aphanizomenon gracile are more harmful to Daphnia than thinner Cylindrospermopsis raciborskii. Zoological Studies 54(1): 2. doi:10.1186/S40555-014-0084-5.

Wiąckowski K., Ventelä A.M., Moilanen M., Saarikari V., Vuorio K., Sarvala J. 2001. What factors control planktonic ciliates during summer in a highly eutrophic lake? Hydrobiologia 443: 43-57.

Wilk-Woźniak E., Gąbka M., Pęczuła W., Burchardt L., Cerbin S., Glińska-Lewczuk K., Gołdyn R., Grabowska M., Karpowicz M., Klimaszyk P., Kołodziejczyk A., Kokociński M., Kraska M., Kuczyńska-Kippen N., Ligęza S., Messyasz B., Nagengast B., Ozimek T., Paczuska B., Pełechaty M., Pietryka M., Piotrowicz R., Pociecha A., Pukacz A., Richter D., Walusiak E., Żbikowski J. 2012. 3150-Starorzecza i naturalne eutroficzne zbiorniki wodne ze zbiorowiskami z Nympheion, Potamion. Monitoring siedlisk przyrodniczych. Przewodnik metodyczny, Mróz W. (red.) GIOŚ, Warszawa 2: 130-149.

Wilk-Woźniak E. 2020. An introduction to the ‘micronet’ of cyanobacterial harmful algal blooms (CyanoHABs): cyanobacteria, zooplankton and microorganisms: a review. Marine and Freshwater Research 71(5): 636-643.

Zingel P., Agasild H., Noges T., Kisand V. 2007. Ciliates are the dominant grazers on pico-and nanoplankton in a shallow, naturally highly eutrophic lake. Microbial Ecology 53(1): 134-142.

Zhou C., Peng Y., Yu M., Deng Y., Chen L., Zhang L., ..., Wang G. 2021. Severe cyanobacteria accumulation potentially induces methylotrophic methane producing pathway in eutrophic lakes. Environmental Pollution: 118443.

ARTYKUŁ 1:

Kosiba J., Wilk-Woźniak E., Krztoń W., Strzesak M., Pociecha A., Walusiak E., Pudaś K., Szarek-Gwiazda E. 2017. ***What underpins the trophic networks of the plankton in shallow oxbow lakes?*** Microbial Ecology 73 (1): 17-28, DOI: 10.1007/s00248-016-0833-6; IF=4,552; lista MEiN=100 pkt.

Microb Ecol (2017) 73:17–28
DOI 10.1007/s00248-016-0833-6

MICROBIOLOGY OF AQUATIC SYSTEMS

What Underpins the Trophic Networks of the Plankton in Shallow Oxbow Lakes?

J. Kosiba[1] · E. Wilk-Woźniak[1] · W. Krztoń[1] · M. Strzesak[1] · A. Pociecha[1] · E. Walusiak[1] · K. Pudaś[2] · E. Szarek-Gwiazda[1]

Received: 10 March 2016 / Accepted: 8 August 2016 / Published online: 20 August 2016
© The Author(s) 2016. This article is published with open access at Springerlink.com

Abstract The aim of this study was to determine the relationships in the microbial trophic network underpinning them about communities of plankton ciliates in shallow oxbow lakes of the Vistula River in southern Poland (Jeziorzany 1, Jeziorzany 2, Piekary, Tyniec). The plankton components (phytoplankton, ciliates, zooplankton) were grouped by dietary preference. The studied oxbows differed in physicochemical parameters and in phytoplankton. Cyanobacteria dominated in the total biomass of phytoplankton in the Tyniec oxbow, big green algae (>30 μm) in Piekary and Jeziorzany 1, and euglenoids in Jeziorzany 2 oxbow. The dominance pattern of ciliates and zooplankton were similar in all oxbows. Algivorous ciliates were the main dominant ciliates, and among zooplankton the dominant ones were herbivores that feed on small algae (<30 μm). The oxbows differed significantly in total phytoplankton biomass, cyanobacteria biomass, euglenoid biomass, small green algae (<30 μm) biomass, total biomass of zooplankton, biomass of zooplankton feeding on bacteria + algae, and biomass of zooplankton feeding on big algae (>30 μm). There was no significant differences in ciliate biomass between oxbows. In redundancy analyses, the variability at the trophic groups of plankton was described by explanatory variables in 42.3 %, and positive relationships were found: e.g., between omnivorous zooplankton biomass, the biomass of ciliates feeding on bacteria + algae, and NH_4 level; between euglenoid biomass and dinoflagellate biomass; and between cyanobacteria biomass and bacterivorous ciliate biomass. Spearman correlation analysis revealed several relationships between different groups of plankton. In general, phytoplankton group shows more connection among themselves and with different zooplankton groups, e.g., phytoplankton biomass with herbivorous zooplankton biomass (−0.33); and cyanobacteria biomass with dinoflagellate biomass (0.65). Ciliates showed more connections among their trophic groups (e.g., algivorous ciliate biomass with omnivorous ciliate biomass, 0.78) and with zooplankton trophic groups (e.g., biomass of algivorous + bacterivorous ciliates with biomass of predator zooplankton, −0.36). Simple correlations analysis revealed the trophic food web network connectivity among plankton organisms, indicating the flow of organic matter from phytoplankton to zooplankton and from ciliates to zooplankton. Our study sheds light on the trophic relations among plankton ciliates, which are neglected in research but often form a large percentage of zooplankton biomass. In the studied oxbows, ciliate forms 6.7 % of total zooplankton biomass in Jeziorzany 1 and up to 44.5 % of it in the Piekary oxbow.

Keywords Trophic networks · Ciliates · Zooplankton · Phytoplankton · Oxbow lakes

✉ E. Wilk-Woźniak
wilk@iop.krakow.pl

[1] Institute of Nature Conservation, Department of Freshwater Biology, Polish Academy of Sciences, al. A. Mickiewicza 33, 31-120 Krakow, Poland

[2] Central Laboratory, Municipal Water and Sewage Company, Lindego 9, 30-148 Krakow, Poland

Introduction

Microorganisms are basic components functioning in all water ecosystems playing role in maintenance of nutrient cycles. Our understanding of aquatic microbial ecology, particularly the interactions in those trophic networks, is still far from sufficient. To study them, network analyses employ quantitative food web models which describe the energy flow of an

ecosystem and provides information about how the nature of the ecosystem has changed over time.

This type of research is especially needed for oxbow lakes, one of the most endangered landscape elements, which are disappearing due to river regulation, dam building and alteration of rivers and floodplains [1]. Oxbows are important habitats and refuges for microorganisms [2, 3]; they increase biodiversity and play an important role in maintaining gene pools [4].

Studies of the relationship among water organisms have a long history (e.g., [5–9]) and often focus on single relationship (in laboratory experiments; e.g., [10]) or simple trophic relationship (Fig. 1). For the management and maintenance of healthy water ecosystem, the interaction between the smallest components of trophic network in freshwaters must be known. Oxbow lakes tend to be naturally eutrophic. According to some authors, production in such ecosystems depends on "new nutrients," and the classical pelagic food chain plays a more important role [11] than recycling of nutrients via microbial loops; the latter is more important in oligotrophic ecosystems [12], though some studies have confirmed the importance of microbial loops in eutrophic ecosystems as well [9, 13].

Thirty years ago, the PEG model [14] explained the role of abiotic and biotic factors as significant drivers of phytoplankton and zooplankton development in lakes, but today still we do not have a full grasp of the processes occurring in oxbow ecosystems. Because they are hydrologically variable, as lotic, lentic and semilotic types [15], the interactions among the components of their food webs are dependent on hydrological pulses [16]. A model of microbiological food web connections during different hydrological phases was recently proposed [17], but hydrological factors are not the only one regulating plankton relationships. Interbiotic relations between different components of plankton are also important.

The aim of this study was to determine the relationships in the trophic network of plankton components in shallow oxbow lakes, in order to improve our understanding of how carbon and energy is transferred among the microbial organisms inhabiting them.

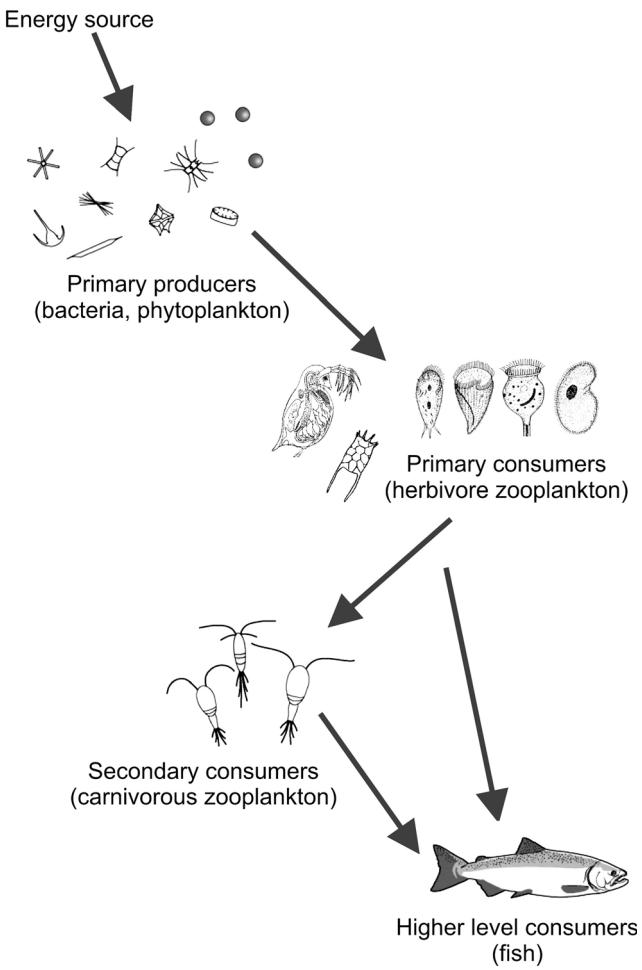


Fig. 1 Scheme of trophic relationships in water ecosystems

Materials and Methods

Samples were collected from four oxbow lakes of Poland's largest river, the Vistula: Jeziorzany 1 (J1), Jeziorzany 2 (J2), Piekary (P) and Tyniec (T). These lakes are located in southern Poland in or near the city of Krakow, and are small, covering ca. 1.5–5.7 ha (Table 1).

Samples were collected from the deepest part of each reservoir from May to October 2014, each month prior to cyanobacterial bloom formation and every week during bloom growth. We collected 108 samples for biological analyses (36 phytoplankton samples, 36 ciliate samples, 36 zooplankton samples). For physicochemical analyses, we collected 72 samples: 36 samples at 1 m depth and 36 samples near the lake bottom but finally used only the samples from 1 m depth for those tests. Water temperature, oxygen saturation, pH, conductivity and chlorophyll a concentration were measured in situ with a YSI 6600 V2 multiparameter sonde. Samples for analysis of anions (HCO_3^-, SO_4^{2-}, Cl^-, NO_3^-, PO_4^{3-}) and cations (Ca^{2+}, Mg^{2+}, Na^+, K^+, NH_4^+) were immediately transported to the laboratory. Ion concentrations were measured with a Dionex Ion Chromatograph (DIONEX, IC25 Ion Chromatograph; ICS-1000, Sunnyvale, CA, USA) in the laboratory of the Institute of Nature Conservation, Polish Academy of Sciences.

Samples for biological parameters were taken from 1 m depth using a 5 L Ruttner sampler and were concentrated from 10 L with a plankton net (mesh size 10 μm for phytoplankton and ciliates, and 50 μm for the rest of zooplankton).

Table 1 Geographical coordinates and chosen parameters of the studied oxbows

Parameter		Oxbows			
		J1	J2	P	T
Geographical coordinates		49°59′46.0″N 19°46′52.5″E	49°59′43.7″N 19°47′10.6″E	50°00′50.1″N 19°47′35.7″E	50°01′47.0″N 19°49′39.8″E
Area [ha]		2.21	2.19	1.56	5.75
Max. depth [m]		2.40	5.50	4.00	3.00
Temperature [°C]	Range (mean)	12.7–23.3 (18.5)	14.7–25.0 (20.7)	8.7–24.3 (17.3)	9.3–24.7 (17.9)
	CV	23	23	27	26
pH	Range	7.1–7.6	7.2–8.1	6.4–8.3	6.8–8.3
	CV	3	4	7	6
Oxygen saturation [%]	Range (mean)	27.4–94.6 (60.9)	75.7–115.2 (95.2)	53.1–100.8 (53.1)	41.0–169.6 (88.3)
	CV	40	14	24	43
Conductivity [μS cm^{-1}]	Range (mean)	748–773 (802.0)	682–697 (690.8)	481–958 (653.0)	1268–1360 (1297.5)
	CV	1	1	19	2
HCO_3^- [mg/L]	Range (mean)	229.8–306.9 (281.0)	202.9–280.2 (257.6)	196.4–265.1 (242.2)	224.9–317.0 (283.8)
	CV	11	12	8	11
SO_4^{2-} [mg/L]	Range (Mean)	43.3–65.9 (52.9)	44.6–64.2 (51.8)	21.2–78.1 (36.7)	75.9–100.1 (84.7)
	CV	14	14	40	8
NO_3^- [mg/L]	Range (mean)	0.23–0.95 (0.58)	nd-1.15 (0.47)	0.18–1.03 (0.39)	nd-1.06 (0.53)
	CV	53	110	62	46
NH_4^+ [mg/L]	Range (mean)	0.005–0.320 (0.140)	0.009–0.219 (0.071)	0.025–0.557 (0.183)	0.029–0.780 (0.220)
	CV	106	111	88	101
PO_4^{3-} [mg/L]	Range (mean)	nd-0.030 (0.008)	nd-0.068 (0.026)	nd-0.190 (0.060)	nd-0.490 (0.150)
	CV	169	122	92	108
Mg^{2+} [mg/L]	Range (mean)	4.60–8.11 (7.04)	4.30–7.94 (6.91)	6.50–16.75 (13.06)	11.90–21.83 (18.94)
	CV	19	21	20	13
Chl *a* [μg/L]	Range (mean)	3.1–39.7 (21.2)	6.2–24.2 (13.2)	3.7–94.4 (32.3)	11.0–140.0 (37.3)
	CV	72	53	89	89

n.d. undetectable level, *CV* coefficient of variation

Since all the oxbows were relatively shallow and polymictic, no epilimnion, metalimnion, or hypolimnion were present. We took biological samples from 1 m depth because preliminary studies in previous years (unpubl. data) had shown that the diversity and biomass of plankton organisms, and especially phytoplankton, were highest at that depth, a finding supported by studies of ciliates and zooplankton: ciliates that are mixotrophic or consume algae prefer the upper levels of water [18, 19]; during the summer, the hypolimnetic refuge is not available to migratory zooplankton due to anoxic conditions [20].

Samples for quantitative analyses were immediately fixed with Lugol's solution for algae and ciliates, and with 4 % formaldehyde for the rest of the zooplankton. Samples for phytoplankton, ciliates, and zooplankton (rotifers, cladocerans, copepods) were taken separately. Additional fresh samples, not fixed but concentrated as described above, were taken for species composition analysis of live material. Phytoplankton species were identified and counted in a modified chamber (0.4 mm high, 22 mm diameter). Phytoplankton biomass was calculated from the cell numbers and specific volumes [21].

Ciliates were determined taxonomically from living material in a 1-mL chamber with a glass cover, according to Foissner and Berger [22, 23]. The total biomass of ciliates (mg/L) was calculated according to Jerome et al. [24], Menden-Deuer and Lessard [25], Wiąckowski et al. [26] and Putt and Stoecker [27].

Zooplankton samples were analyzed in a 0.5-mL chamber. Average of five counts were calculated. The species were identified with keys [28–31]. Dry weight was calculated using a regression equation for body length and weight for each species [32–36]. Because phytoplankton and ciliates were calculated as fresh biomass, zooplankton dry mass was recalculated according to the index proposed by Bottrell et al. [34].

The above analyses employed a Nikon H550L light microscope at 40–1000 × .

To describe the network structure, microorganisms were divided by trophic group: primary producers (phytoplankton), protozoan consumers (ciliates), and metazoan consumers (zooplankton - rotifers, cladocerans, copepods). Producers were subdivided into size and trophic classes: cyanobacteria (only large colonies or trichomes were present in the collected samples), big diatoms (>30 μm), small diatoms (<30 μm), big green algae (>30 μm), small green algae (<30 μm), and mixotrophic algae. Mixotrophic algae were grouped as follows: cryptophytes (sparse phagotrophic species), golden brown algae (equal use of phagotrophy and phototrophy; e.g., *Dinobryon* [37]), dinophytes, and euglenoids. Ciliates were grouped as follows: species that feed on algae, bacterivorous species, algivorous and bacterivorous species, and omnivorous species [22, 23]. Zooplankton group was divided into species that feed on the seston and bacteria, species that feed on algae >30 μm, species that feed on algae <30 μm, predators, and omnivorous species [38].

The basic statistics used for data analysis were range (minimum–maximum), average, standard deviation (SD) and coefficient of variation (CV). The Kruskal–Wallis test was used to determine the significance of differences in biomass between the different plankton components of oxbows. Spearman correlations were used to build a model to explain the relationships between plankton components, and redundancy analysis (RDA) was used to build a model to explain the relationships between plankton components and physicochemical parameters. Statistica 10.0 and CANOCO 5 for Windows were used for these statistical analyses. The data were log-transformed. The manual forward selection procedure was run using the Monte Carlo permutation test. Variables having a conditional effect that was significant at $p < 0.05$ were included.

Results

Physicochemical Factors

All the oxbows are in the same geographical zone and are exposed to the same climate, but showed differences in physicochemical parameters (Table 1).

The shallowest oxbow was J1 (2.4 m) and the deepest was J2 (5.5 m). Table 1 represents the parameters bearing any relation to plankton components as assessed by RDA. Variation (CV) of water temperature in J1 and J2 was similar, and was higher in P oxbow and T oxbow. Water pH showed a similar tendency. Variation of oxygen saturation was highest in J1 and T. Mean conductivity was highest in the water of T, and variation of conductivity was highest for P. Mean NH_4^+ and PO_4^{3-} concentrations were highest in T, and NO_3^- was highest in J1. Other parameters also differed oxbows from each other.

Phytoplankton

The phytoplankton consisted of cyanobacteria, golden brown algae, cryptomonads, dinoflagellates, euglenoids, diatoms, and green algae. Golden brown algae and cryptomonads were found only occasionally in single samples. The mean total biomass of phytoplankton was highest in T and lowest in J2. Variation of total phytoplankton biomass was highest in P (Table 2).

The pattern of dominance in the total biomass of phytoplankton was somewhat similar for J1 and J2, however differed between the oxbows (Fig. 2):

J1: big green algae > euglenoids > dinoflagellates > small green algae.
J2: euglenoids = big green algae > cyanobacteria.
P: big green algae > euglenoids > cyanobacteria > dinoflagellates.
T: cyanobacteria > euglenoids > dinoflagellates.

Ciliates

The plankton ciliates consisted of the following groups: (1) algivorous ciliates (*Oligotrichida*: *Codonella cratera*, *Tintinidium* sp.; *Prostomatida*: *Coleps spetai*); (2) bacterivorous ciliates (*Peritrichia*: *Epistylis* sp., *Vorticella* sp.; *Hypotrichia*: *Aspidisca* sp.); (3) mixed type of feeding – ciliates that feed on algae and bacteria (*Oligotrichida*: *Strobilidium* sp.; *Peritrichia*: *Vorticella campanula*); and (4) omnivorous species (*Hymenostomata*: *Cinetochilum margaritaceum*, *Paramecium bursaria*; *Hypotrichia*: *Euplotes patella*; *Prostomatida*: *Coleps hirtus*: *Heterotrichida*: *Stentor* sp.). Mean total biomass of plankton ciliates and variation of total biomass were highest for P and lowest for J1 (Table 2).

The pattern of dominance in the total biomass of ciliates was similar for all oxbows (Fig. 3):

J1: algivorous ciliates > omnivorous ciliates > bacterivorous ciliates > algivorous and bacterivorous ciliates
J2: algivorous ciliates > algivorous and bacterivorous ciliates > omnivorous ciliates > bacterivorous ciliates
P: algivorous ciliates > omnivorous ciliates > bacterivorous ciliates
T: algivorous ciliates > omnivorous ciliates > bacterivorous ciliates

For all oxbows taken together, algivorous ciliates were dominant, followed by omnivorous ciliates. Bacterivorous and bacterio-algivorous ciliates had lower shares of total ciliate biomass.

Table 2 Biomass (mg/L) of phytoplankton, plankton ciliates and zooplankton in oxbows—basic statistics

	J1			J2			P			T		
Statistic	Phyto	Ciliates	Zoo	Phyto	Ciliates	Zoo	Phyto	Ciliates	Zoo	Phyto	Ciliates	Zoo
Min-max	4.8–28.5	0.07–1.1	1.9–9.3	1.8–12.4	0.1–2.6	0.5–6.3	1.0–30.6	0.1–26.7	4.1–19.1	3.9–163.3	0.07–12.0	4.0–12.4
Average	14.8	0.5	6.7	6.5	1.0	4.0	11.9	8.0	9.9	65	3.7	8.1
SD	9.9	0.4	2.6	4.4	0.9	1.9	10.8	9.5	4.5	44. 5	3.9	2.8
CV (%)	67	75	39	68	90	48	90	119	46	69	108	34

SD standard deviation, *CV* coefficient of variation

Zooplankton

Zooplankton consisted of the following trophic groups: (1) seston-feeding and bacterivorous animals (rotifers: *Brachionus angularis*, *B. diversicornis*, *B. urceolaris*, *Filinia longiseta*, *Keratella cochlaris*, *K. tecta*, *Polyarthra major*, *P. remata*, *P. vulgaris*, *Pompholyx sulcata*; copepods: nauplii), 2) herbivorous animals that feed on small algae (<30 μm) (rotifers: *Brachionus calyciflorus*, *Kellicotia longispina*, *Keratella quadrata*, *Trichocerca similis*; cladocerans: *Bosmina longirostris*, *Chydorus sphaericus*, *Diaphanosoma brachyurum*, *Eubosmina coregoni*, *E. gibera*, *E. longispina*, *Moina micrura*; copepods: *Acanthocyclops venustus*, *Cyclops vicinus*, *Eurytemora affinis*, copepodites); (3) herbivorous animals that feed on algae larger than 30 μm (cladocerans: *Daphnia ambigua*, *D. cucullata*, *D. longispina*, copepods: *Eudiaptomus gracilis*); (4) predators (cladocerans: *Leptodora kindtii*; copepods: *Cyclops abyssorum*, *C. strennus*, *Thermocyclops crassus*); and (5) omnivorous species (rotifers: *Asplanchna priodonta*, *Gastropus minor*, *Trichocerca capucina*; copepods: *Mesocyclops leuckartii*, *Metacyclops gracilis*).

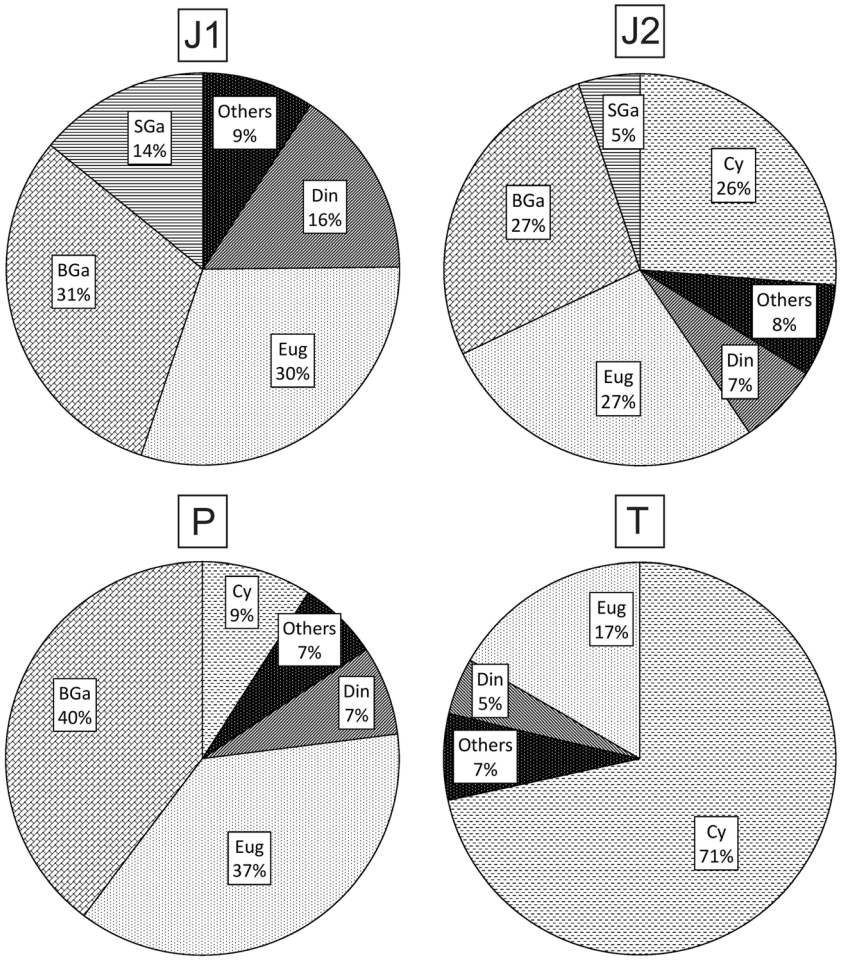


Fig. 2 Percentage shares of different phytoplankton groups in total phytoplankton biomass in the four studied oxbow lakes. Abbreviations: *J1: Din* dinoflagellates, *Eug* euglenoids, *BGa* big green algae, *SGa* small green algae, *Others* cyanobacteria, golden brown algae, diatoms. *J2: Din* dinoflagellates, *Eug* euglenoids, *BGa* big green algae, *SGa* small green algae, *Cy* cyanobacteria, *Others* golden brown algae, diatoms. *P: Din* dinoflagellates, *Eug* euglenoids, *BGa* big green algae, *Cy* cyanobacteria, *Others* small green algae, golden brown algae, diatoms, cryptomonads. *T: Cy* cyanobacteria, *Din* dinoflagellates, *Eug* euglenoids, *Others* green algae, diatoms, cryptomonads

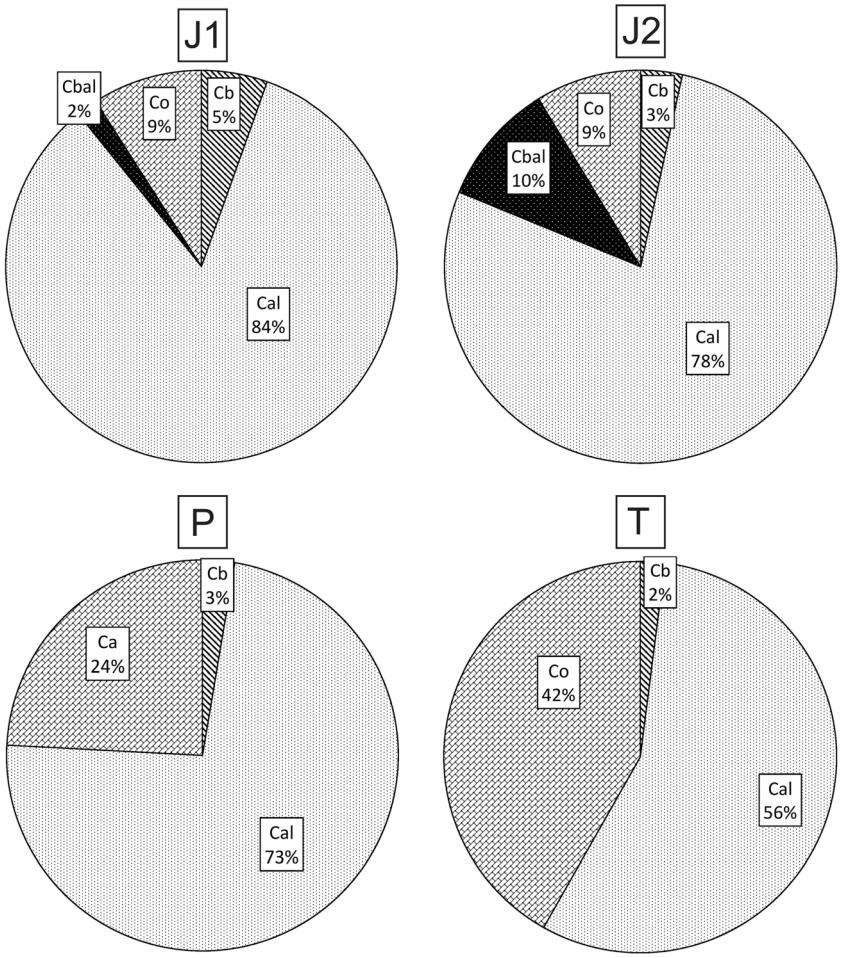


Fig. 3 Percentage shares of different ciliate groups in total ciliate biomass in the four studied oxbow lakes. Abbreviations: *J1: Cal* algivorous ciliates, *Cbal* algivorous and bacterivorous ciliates, *Co* omnivorous ciliates, *Cb* bacterivorous ciliates, *J2: Cal* algivorous ciliates, *Cbal* algivorous and bacterivorous ciliates, *Co* omnivorous ciliates, *Cb* bacterivorous ciliates, *P: Cal* algivorous ciliates, *Co* omnivorous ciliates, *Cb* bacterivorous ciliates. *T: Cal* algivorous ciliates, *Co* omnivorous ciliates, *Cb* bacterivorous ciliates

Variation of total zooplankton biomass was highest for J2 and P, and lowest for T (Table 2).

Herbivores that feed on algae smaller than 30 μm were dominant in all oxbows. Three oxbows (J1, J2, P) showed a similar pattern of dominant species; T differed from the others (Fig. 4):

J1: herbivores that feed on small algae (<30 μm) > seston-feeding and bacterivorous animals > predators > omnivores.

J2: herbivorous animals that feed on small algae > seston-feeding and bacterivorous animals > herbivorous animals that feed on big algae > predators > omnivores.

P: herbivorous animals that feed on small algae > seston-feeding and bacterivorous animals > omnivores > predators > herbivorous animals that feed on big algae.

T: herbivorous animals that feed on small algae > predators > herbivorous animals that feed on big algae > omnivores > seston-feeding and bacterivorous animals.

Total Plankton

There were significant differences in total phytoplankton biomass between J2 and T and between P and T (Table 3), in cyanobacterial biomass between J1 and T, in euglenoid biomass between J2 and T, in the biomass of small green algae between J1 and P and between P and T, in total zooplankton biomass between J2 and T, in the biomass of zooplankton that feeds on the seston and bacteria between J1 and T and between P and T, and in the biomass of zooplankton that feeds on big algae between J1 and T. Neither total ciliate biomass nor the biomass of any ciliate group differed between oxbows.

Statistical Analysis

Spearman correlation revealed several relationships between different groups of plankton (Table 4). RDA analysis showed relationship between different groups and abiotic parameters. The explanatory variables described

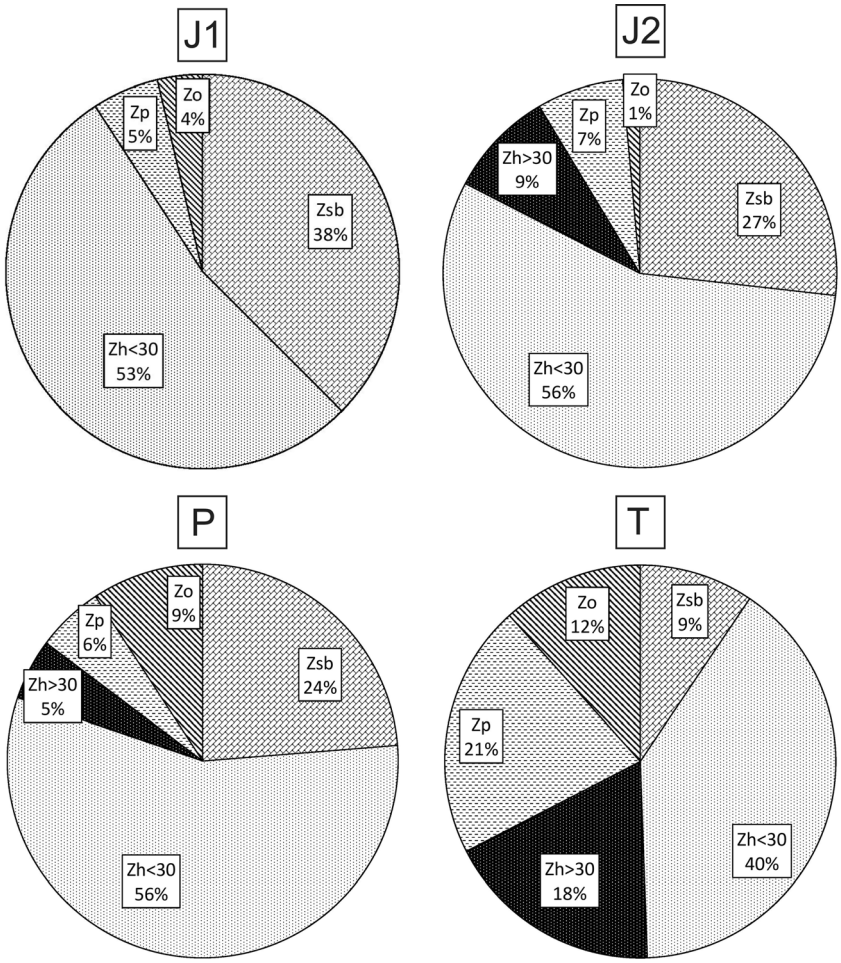


Fig. 4 Percentage shares of different zooplankton groups in total zooplankton biomass in the four studied oxbow lakes. Abbreviations: *J1: Zsb* seston and bacterivorous animals, *Zh < 30* herbivorous animals that feed on small algae, *Zp* predators, *Zo* omnivorous zooplankton. *J2: Zsb* seston and bacterivorous animals, *Zh < 30* herbivorous animals that feed on small algae, *Zh > 30* herbivorous animals that feed on big algae, *Zp* predators, *Zo* omnivorous zooplankton. *P: Zsb* seston and bacterivorous animals, *Zh < 30* herbivorous animals that feed on small algae, *Zh > 30* herbivorous animals that feed on big algae, *Zp* predators, *Zo* omnivorous zooplankton. *T: Zsb* seston and bacterivorous animals, *Zh < 30* herbivorous animals that feed on small algae, *Zh > 30* herbivorous animals that feed on big algae, *Zp* predators, *Zo* omnivorous zooplankto

42.3 % variability at plankton trophic groups in oxbow lakes (Fig. 5). We noted the following groups of positive relationship: (*a*) the biomass of big green algae, the biomass of herbivorous zooplankton that feeds on small algae (<30 μm), the biomass of omnivorous zooplankton, the biomass of ciliates that feed on bacteria and algae, and the concentration of NH_4^+; (*b*) the biomass of small green algae, the biomass of zooplankton that

Table 3 Statistically significant differences between various components of plankton and between oxbows (Kruskal–Wallis test; *z* statistic value; *p* level of significance)

Biomass	Oxbow lake	*z*	*p*
Total biomass of phytoplankton	Jeziorzany 2 - Tyniec	3.097	0.012
H (3, *N* = 36) = 13.56	Piekary - Tyniec	2.979	0.017
Biomass of cyanobacteria	Jeziorzany 1- Tyniec	3.336	0.005
H (3, *N* = 36) = 13.14			
Biomass of euglenoids	Jeziorzany2 - Tyniec	2.721	0.039
H (3, *N* = 36) = 8.77			
Biomass of small green algae	Jeziorzany 1 - Piekary	3.970	<0.000
H (3, *N* = 36) = 22.65			
Total biomass of zooplankton	Jeziorzany 2 - Piekary	3.315	0.006
H (3, *N* = 36) = 11.44			
Biomass of zooplankton feed on bacteria + algae	Jeziorzany1 - Tyniec	2.707	0.041
H (3, *N* = 36) = 11.95	Piekary - Tyniec	2.830	0.028
Biomass of zooplankton feed on big algae	Jeziorzany 1 - Tyniec	3.831	<0.000
H (3, *N* = 36) = 14.96			

Table 4 Statistically significant Spearman correlations between various trophic groups of plankton occurring in the studied oxbow lakes ($p < 0.05$)

Biomass	Biomass	Coefficient
Phytoplankton in total	Herbivorous animals feed on small algae (dimension < 30 μm)	−0.33
	Herbivorous animals feed on big algae (dimension > 30 μm)	0.36
	Predator zooplankton	0.49
Ciliates in total	Euglenoids	0.33
Zooplankton in total	Golden brown algae	−0.33
	Algae- and bacterivorous ciliates	−0.63
Algivorous ciliates	Omnivorous ciliates	0.78
Algae- and bacterivorous ciliates	Zooplankton in total	−0.63
	Predator zooplankton	−0.36
	Herbivorous animals feed on small algae	−0.47
Omnivorous ciliates	Algivorous ciliates	0.78
	Herbivorous animals feed on small algae	0.45
	Cryptomonads	0.35
	Euglenoids	0.41
Zooplankton feed on seston + bacteria	Herbivorous animals feed on big algae	−0.33
Herbivorous animals feed on algae smaller dimension than 30 μm	Phytoplankton in total	−0.34
	Algae- and bacterivorous ciliates	−0.47
	Omnivorous ciliates	0.45
	Cyanobacteria	−0.33
	Dinoflagellates	−0.37
	Small green algae (dimension < 30 μm)	−0.45
Herbivorous animals feed on algae bigger dimension than 30 μm	Phytoplankton in total	0.36
	Zooplankton feed on seston + bacteria	−0.34
	Predator zooplankton	0.42
	Cyanobacteria	0.43
	Euglenoids	0.40
Predator zooplankton	Phytoplankton in total	0.49
	Algae- and bacterivorous ciliates	−0.36
	Herbivorous animals feed on big algae	0.42
	Cyanobacteria	0.37
	Golden brown algae	−0.37
	Big green algae (dimension >30 μm)	0.51
Cyanobacteria	Golden brown algae	−0.35
	Dinoflagellates	0.65
Dinoflagellates	Euglenoids	0.47

feeds on big algae (>30 μm), conductivity, and oxygen concentration; (*c*) the biomass of euglenoids, the biomass of big diatoms, the biomass of dinoflagellates, and the biomass of golden brown algae; (*d*) the biomass of cyanobacteria and cryptomonads, the biomass of small diatoms, the biomass of bacterivorous ciliates, the biomass of algivorous ciliates, the biomass of omnivorous ciliates, the biomass of zooplankton that feeds on the seston and bacteria, and the concentrations of PO_4^{3-}, SO_4^{2-}, HCO_3^-, and Mg^{2+}; (*e*) the biomass of predator zooplankton was correlated with the NO_3^- concentration. Negative relationships were found between groups *a* and *c* and between groups *b* and *d*.

Discussion

In the cascade model, the structure of the food web is determined by the trophic position of the component species: species in a higher trophic position can consume only species that occupy a lower position. The theoretical cascade model has been adopted in empirical studies, and now the trophic positions of species are commonly used to estimate food web structure and trophic connectivity [39]. Based on the biomass of various components of the plankton and the biomass of trophic groups, we constructed a model of the trophic network in small, shallow oxbow lakes.

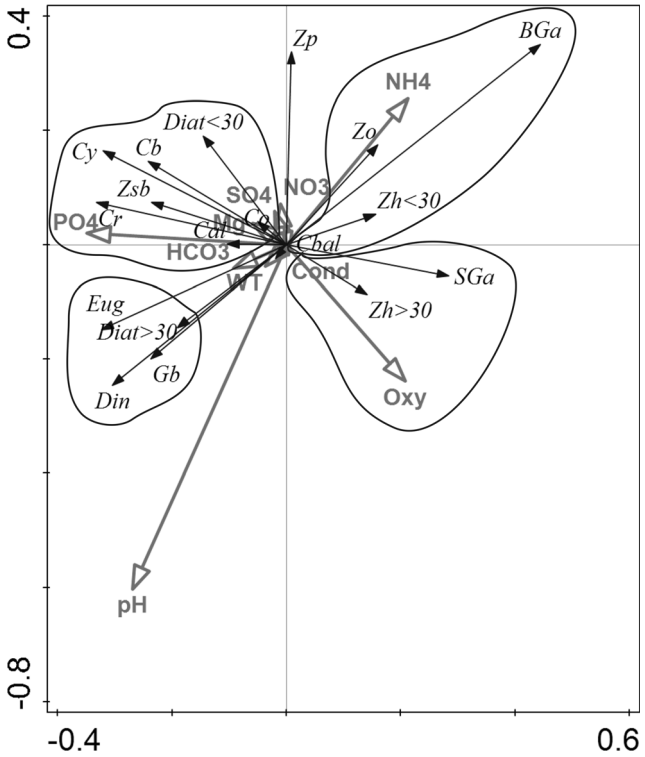


Fig. 5 Redundancy analysis (RDA) biplot of the relationships between trophic groups of plankton components and environmental factors (constrained partial analysis, partial RDA). Partial variation was 110.9344; the explanatory variables accounted for 42.3 %; adjusted explained variation was 1.1 %; eigenvalues: 0.0765 (axis 1); 0.0311 (axis 2), 0.0229 (axis 3), 0.0194 (axis 4); explained variation (cumulative): 17.54; 24.67; 29.92; 34.36; pseudocanonical correlations: 0.8543; 0.7360; 0.7225; 0.5692; explained fitted variation (cumulative): 41.48; 58.33; 70.74; 81.24; permutation test results: on first axis, pseudo-$F = 3.0$, $P = 0.186$; on all axes, pseudo-$F = 1.0$, $P = 0.440$. Abbreviations: *Din* dinoflagellates, *Eug* euglenoids, *BGa* big green algae, *SGa* small green algae, *Cy* cyanobacteria, *Gb* golden brown algae, *Diat > 30* big diatoms, *Diat < 30* small diatoms, *Cr* cryptomonads. *Cal* algivorous ciliates, *Cbal* algivorous and bacterivorous ciliates, *Co* omnivorous ciliates, *Cb* bacterivorous ciliates. *Zp* predator zooplankton, *Zo* omnivorous zooplankton, *Zh < 30* herbivorous zooplankton that feeds on small algae, *Zh > 30* herbivorous zooplankton that feeds on big algae, *Zsb* zooplankton that feeds on the seston and bacteria

Phytoplankton forms the first trophic level that directly responds to changes in abiotic parameters [40]. Differences in physicochemical parameters such as conductivity and the concentrations of phosphates, nitrate nitrogen, and ammonia nitrogen resulted in clear differences in phytoplankton composition between the studied oxbow lakes. The differences at higher levels (e.g., ciliates, zooplankton) were not as conspicuous. We found differences in ciliate biomass and variability, but the dominance of trophic groups of ciliates was similar in the different oxbows, as the dominance of zooplankton trophic groups, which differed only for the zooplankton in the Tyniec oxbow (highest trophic status). There were significant differences in the biomass of trophic groups between the oxbows for some phytoplankton and zooplankton groups, but not for ciliates. It appears that ciliates are generalists, in that they can consume multiple resources [41]. Pelagic ciliates are the main component of the microzooplankton, forming up to 34 % of the total zooplankton biomass in eutrophic lakes and up to 62 % of it in hypertrophic lakes [42, 43]. In our study, the share of plankton ciliates in total zooplankton biomass ranged from 6.7 % in Jeziorzany 1 to 44.5 % in the Piekary oxbow.

In redundancy analysis, physicochemical factors explained 42.3 % of the variability in the trophic groups of plankton. Simple correlations allowed us to delineate trophic network connectivity among the plankton organisms, implying direct and indirect relationships such as competition, predation, coexistence, disturbance, and resource heterogeneity (Fig. 6a–c and 7). Predation was shown by a negative correlation between total phytoplankton biomass and the biomass of herbivorous zooplankton that feeds on small algae (<30 μm). An indirect relationship was seen between total phytoplankton biomass and the biomass of predator zooplankton; the positive correlation suggests an undisclosed link (herbivorous animals) between phytoplankton and predators. The positive correlation between total phytoplankton biomass and the biomass of zooplankton that feeds on big algae (>30 μm) indicates that an increase in zooplankton that feeds on big algae promotes an increase in the total biomass of phytoplankton, and vice versa.

The positive correlation between the total biomass of ciliates and that of euglenoids (Figs. 6b and 7) is explained by their coexistence or by their food resource heterogeneity (heterotrophy and autotrophy). Moreover, both groups are mobile and can seek food by moving in the water.

The negative correlation between total zooplankton biomass and the biomass of ciliates that feed on algae + bacteria (Figs. 6c and 7) showed a direct relationship reflecting predation of plankton animals on ciliates. Field and laboratory experiments have shown that the impact of grazing on the ciliate stock by copepods is greatest when the phytoplankton concentration is low and when it is dominated by small phytoflagellates [44].

The negative correlation between zooplankton biomass and the biomass of golden brown algae is unclear and difficult to explain.

We found a number of more specific relationships between particular trophic groups of plankton (Fig. 7). Different phytoplankton groups were related to each other: cyanobacteria to dinoflagellates, and dinoflagellates to euglenoids. There were other relationships between phytoplankton groups and different trophic groups of zooplankton. Only euglenoids and cryptomonads were correlated with omnivorous ciliates. In general, phytoplankton groups showed more connections with different zooplankton groups and among themselves, but ciliate groups showed more connections among themselves and with zooplankton groups. These simple relationships support the notion that ciliates transfer organic matter to zooplankton. According to the microbial loop concept, the dissolved

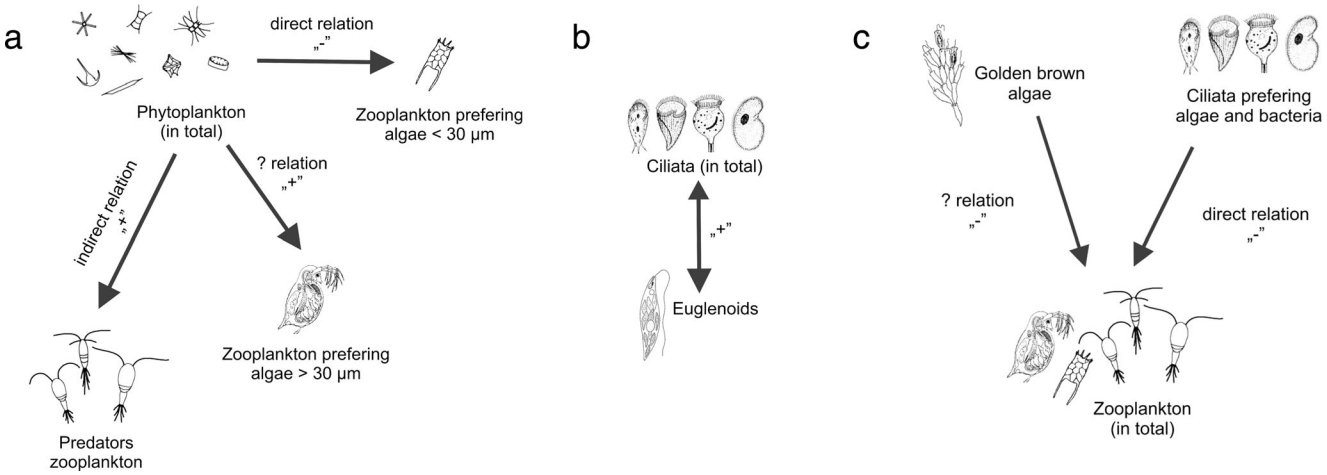


Fig. 6 Model of the relationships between the plankton in oxbow lakes (only significant ones shown): **a** total biomass of phytoplankton; **b** total biomass of ciliates; and **c** total biomass of zooplankton

organic carbon released by phytoplankton is used by bacteria, which are then preyed upon by protozoa that are subsequently consumed by zooplankton [45, 46].

The simple positive correlation observed between the biomass of cyanobacteria and dinoflagellates is supported by laboratory experiments demonstrating allelopathic interactions between dinoflagellates and toxic cyanobacteria [47]. Simple positive relationships between dinoflagellates and euglenoids might be explained as coexistence. We speculate that because both of these organisms are mobile

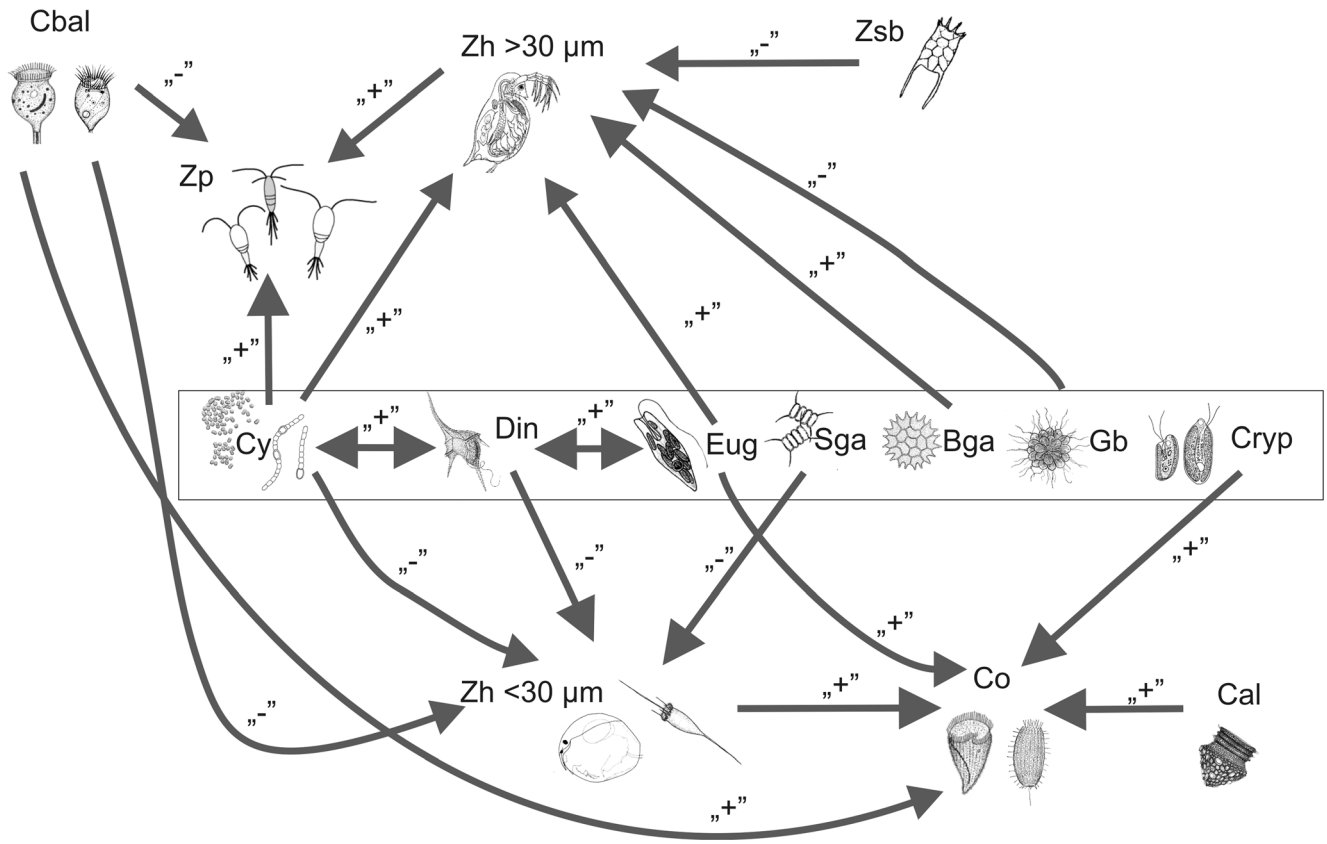


Fig. 7 Model of the trophic network among plankton components in small shallow oxbow lakes. Abbreviations: *Din* dinoflagellates, *Eug* euglenoids, *BGa* big green algae, *SGa* small green, *Cy* cyanobacteria, *Gb* golden brown algae, *Cr* cryptomonads. *Cal* algivorous ciliates, *Cbal* algivorous and bacterivorous ciliates, *Co* omnivorous ciliates. *Zp* predator zooplankton, *Zh < 30 µm* herbivorous zooplankton that feeds on small algae, *Zh > 30 µm* herbivorous zooplankton that feeds on big algae, *Zsb* zooplankton that feeds on the seston and bacteria; "-" negative relation, "+" positive relation

and mixotrophic, they can use alternative methods of feeding and do not compete.

The negative correlation between the biomass of herbivorous zooplankton that feeds on small algae and the biomass of small green algae (<30 μm) are explained by grazing, and the negative correlation between the biomass of herbivorous zooplankton and that of ciliates that feed on bacteria and algae can be explained by competition.

Many studies have suggested that the biomass of some herbivorous zooplankton species (mostly *Daphnia* species) decreases during cyanobacterial blooms [48]. Often a negative correlation between the biomass of herbivorous zooplankton and that of dinoflagellates and cyanobacteria is explained as a lack of a food source for zooplankton. This would seem to make the positive relationships we found between these organisms and cyanobacterial biomass difficult to explain. However, recent reports increasingly suggest that *Daphnia*–cyanobacteria relationships are more complicated than previously thought and that a decrease in the daphnid population during cyanobacterial blooms is not necessarily the result of toxins [49]. Moreover, short-term exposure to toxic cyanobacteria can improve the fitness of *Daphnia magna* for further exposure to toxic prey during development. This trait might be transferred to offspring via maternal effects, or such an adaptation might be clone-specific [50].

The negative correlation between the biomass of herbivorous zooplankton species that feed on big algae (>30 μm) and that of zooplankton species that feed on the seston and bacteria may suggest some unknown type of competition. Animals that feed on the seston and bacteria are an important link in the transfer of carbon from bacterial biomass to macrozooplankton [51, 52], and might compete with ciliates which also transfer organic matter from bacteria in a microbial loop. This possibility will be the focus of our future work.

Predation may also explain the negative relationship between the biomass of predator zooplankton and the biomass of ciliates that feed on bacteria and algae. Copepods, which traditionally have been considered to be herbivores, are in fact omnivores which also feed on heterotrophic protists and are inefficient at feeding on prey less than 5–10 μm in size [53]. Large-bodied copepods can effectively consume protists (heterotrophic nanoflagellates and ciliates), rotifers, and cladocerans [54].

Simple relationships allowed us to outline the trophic network among plankton components in the four small shallow oxbow lakes we studied. The network was underpinned by adding plankton ciliates, which are often neglected in such studies. In general, the relationships indicated the flow of organic matter from phytoplankton to zooplankton and from ciliates to zooplankton.

Acknowledgments We thank the anonymous reviewers for their helpful comments and suggestions and PhD Piotr Skórka for discussion on statistics. This work was supported by the Institute of Nature Conservation, Polish Academy of Sciences (Kraków, Poland) through grant funding for PhD students and young scientists and through the Institute's statutory funds. The authors are grateful to Michael Jacobs for editing of the manuscript.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Heintz MD, Hagemeier-Klose M, Wagner K (2012) Towards a risk governance culture in flood policy—findings from the implementation of the "Floods Directive" in Germany. Water 4(1):135–156
2. Obolewski K, Glińska-Lewczuk K (2011) Effect of oxbow reconnection based on the distribution and structure of benthic macroinvertebrates clean. Soil Air Water 39:853
3. Gadzinowska J (2013) Plankton communities in oxbow lakes of the River Vistula (Oświęcim Basin) with bottom sediments heterogeneously contaminated with heavy metals. Limnol Review 13(2): 93–104
4. Schindler S, Sebesvari Z, Damm C, Euller K, Mauerhofer V, Schneidergruber A et al (2014) Multifunctionality of floodplain landscapes: relating management options to ecosystem services. Landscape ecol 29(2):229–244
5. Pomeroy LR (1974) The ocean's food web, a changing paradigm. BioScience 24:499–504
6. Porter KG, Pace ML, Battey JF (1979) Ciliate protozoans as links in freshwater planktonic food chains. Nature 277:563–565
7. Arndt H (1993) Rotifers as predators on components of microbial web (bacteria, heterotrophic flagellates, ciliates). Hydrobiologia 255⁄256:231–246
8. Gilbert JJ, Jack JD (1993) Rotifers as predators on small ciliates. Hydrobiologia 255⁄256: 247–253
9. Chróst RJ, Adamczewski T, Kalinowska K, Skowrońska A (2009) Abundance and structure of microbial loop components (bacteria and protists) in lakes of different trophic status. J Microbiol Biotechnol 19(9):858–868
10. Archbold JHG, Berger J (1985) A qualitative assessment of some metazoan predators of *Halteria gradinella*, a common freshwater ciliate. Hydrobiologia 126:97–102
11. Porter KG, Pearl H, Hodson R, Pace ML, Priscu J, Riemann B, Scavia D, Stockner JG (1988) Microbial interactions in lake food webs. In: Carpenter SR (ed) Complex interactions in lake communities
12. Weisse T, Müller H, Pinto-Coelho RM, Schweizer A, Sprigmann D, Baldringer G (1990) Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnol Oceanogr 35:781–794
13. Christofersen K, Riemann B, Hansen LR, Klysner A, Sorensen HB (1990) Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microb Ecol 20:253–272
14. Sommer U, Gliwicz ZM, Lampert W, Duncan A (1986) The PEG-model of seasonal succession of planktonic events in fresh waters. Arch Hydrobiol 106(4):433–471

15. Glińska-Lewczuk K, Burandt P (2011) Effect of river straightening on the hydrochemical properties of floodplain lakes: observations from the Łyna and Drwęca Rivers, N Poland. Ecol Eng 37(5):786–795
16. Grabowska M, Glińska-Lewczuk K, Obolewski K, Burandt P, Kobus S, Dunalska J, Kujawa R, Goździejewska A, Skrzypczak A (2014) Effects of hydrological and physicochemical factors on phytoplankton communities in floodplain lakes. Pol J Environ Stud 23(3):713–725
17. Segovia BT, Pereira DG, Bini LM, de Meira BR, Nishida VS, Lansac-Tôha FA, Velho LFM (2015) The role of microorganisms in a planktonic food web of a floodplain lake. Microb Ecol 69(2): 225–233
18. Taylor WD, Heynen ML (1987) Seasonal and vertical distribution of ciliaphora in lake Ontario. Can J Fish Aquat Sci 44:2185–2191
19. Amblard C, Carrias JF, Bourdier G, Maurin N (1995) The microbial loop in a humic lake: seasonal and vertical variations in the structure of different communities. Hydrobiologia 300(301):71–84
20. Shapiro J, Lamarra VA, Lynch M (1975) Biomanipulation: an ecosystem approach to lake restoration. In: Brezonik PL, Fox JL (eds) Proceedings of a symposium on water quality management through biological control. Univ. of Florida, Gainesville, pp 85–96
21. Rott E (1981) Some results from phytoplankton counting intercalibrations. Schweiz Z Hydrol 43:34–62
22. Foissner W, Berger H (1996) A user-friendly guide to the ciliates *(Protozoa, Ciliophora)* commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biol 35(2):375–482
23. Foissner W, Berger H, Schaumburg J (1999) Identification and ecology of limnetic plankton ciliates. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, München
24. Jerome CA, Montagnes DJS, Taylor FJR (1993) The effect of the quantitative protargol stain and Lugols and Buinos fixatives on cell size: a more accurate estimate of ciliate species biomass. J Euk Microbiol 40:254–259
25. Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol Oceanogr 45:569–579
26. Wiąckowski K, Doniec A, Fyda J (1994) An empirical study of the effect of fixation on ciliate cell volume. Mar Microb Food Webs 8(1–2):59–69
27. Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol Oceanogr 34:1097–1103
28. Dussart B (1967) Les Copépodes des eaux contonentals d'Europe occidentale: Calanoides et harpacticopides. Boubée & Cie, Paris
29. Dussart B (1969) Les Copépodes des eaux continentals d'Europe occidentale: Cyclopoides et biologie. Boubée & Cie, Paris
30. Flößner D (2000) Die Haplopoda und Cladocera (Ohne Bosminidae) Mitteleuropas. Backhuys, Leiden
31. Ejsmont-Karabin J, Radwan S, Bielańska-Grajner I (2004) Rotifers. Monogononta - atlas of species. Polish Freshwater Fauna. University of Łódź, Łódź, pp 77–447 [in Polish]
32. Cummins KW, Costa RR, Rowe RE, Moshiri GA, Scanlon RM, Zajdel RK (1969) Ecological energetics of a natural population of the predaceous zooplankter *Leptodora kindtii*. Oikos 20:189–223
33. Dumont HJ, Van de Velde I, Dumont S (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton, and benthos of continental waters. Oecol (Berl) 19:75–97
34. Bottrell HH, Duncan A, Gliwicz ZM, Grygierek E, Herzig A, Hillbricht-Ilkowska A, Kurasawa H, Larsson P, Weglenska T (1976) A review of some problems in zooplankton production studies. Norw J Zool 24:419–456
35. Ruttner-Kolisko A (1977) Suggestions for biomass calculation of plankton rotifers. Arch Hydrobiol Beih Ergebn Limnol 8(7):1–76
36. Pearsson G, Ekbohm G (1980) Estimation of dry-weight in zooplankton populations—methods applied to crustacean populations from lakes in the Kuokkel Area, Northern Sweden. Arch Hydrobiol 89:225–246
37. Sanders RW, Porter KG, Caron DA (1990) Relationship between phototrophy and phagotrophy in the mixotrophic chrysophyte *Poterioochromonas malhamensis*. Microb Ecol 19(1):97–109
38. Wilk-Woźniak E, Pociecha A, Bucka H (2001) Phytoplankton-zooplankton interactions, size relations and adaptive responses. A short review. Ecohydrol Hydrobiol 1(4):511–517
39. Boyce DG, Frank KT, Leggett WC (2015) From mice to elephants: overturning the 'one size fits all'paradigm in marine plankton food chains. Ecol Lett 18(6):504–515
40. Dunson WA, Travis J (1991) The role of abiotic factors in community organization. Am Nat 138:1067–1091
41. MacArthur RH (1972) Geographical ecology: patterns in the distribution of species. Princeton Univ. Press
42. Pace M, Orcutt JD Jr (1981) The relative importance of protozoans, rotifers and crustaceans in a freshwater zooplankton community. Limnol Oceanogr 26:822–830
43. Beaver JR, Crisman TL (1990) Seasonality of planktonic ciliated protozoa in 20 subtropical Florida lakes of varying trophic state. Hydrobiologia 190:127–135
44. Jakobsen HH, Hansen P (1997) Prey size selection, grazing and growth response of the small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate *Balanion comatum*-a comparative study. Mar ecol-prog ser 158:75–86
45. Sherr EB, Sherr BF (1988) Role of microbes in pelagic food webs: a revised concept. Limnol Oceanogr 33:1225–1227
46. Kalinowska K (2004) Bacteria, nanoflagellates and ciliates as components of the microbial loop in three lakes of different trophic status. Pol J Ecol 52(1):19–34
47. Vardi A, Schatz D, Beeri K, Motro U, Sukenik A, Levine A, Kaplan A (2002) Dinoflagellate-cyanobacterium communication may determine the composition of phytoplankton assemblage in a mesotrophic lake. Curr Biol 12(20):1767–1772
48. Hietala J, Laurén-Määttä C, Walls M (1996) Sensitivity of *Daphnia* to toxic cyanobacteria: effects of genotype and temperature. Freshwater Biol 37:299–306
49. Wojtal-Frankiewicz A, Kruk A, Frankiewicz P, Oleksińska Z, Izydorczyk K (2015) Long-term patterns in the population dynamics of *Daphnia longispina*, *Leptodora kindtii* and cyanobacteria in a shallow reservoir: a self-organising Map (SOM) approach. PLoS One 10(12):e0144109
50. Lyu K, Guan H, Wu C, Wang X, Wilson AE, Yang Z (2015) Maternal consumption of non-toxic *Microcystis* by *Daphnia magna* induces tolerance to toxic *Microcystis* in offspring. Freshwater Biol, DOI:10.1111/fwb.12695
51. Stockner JG, Shortreed KS (1989) Algal picoplankton and contribution to food webs in oligotrophic British Columbia Lakes. Hydrobiologia 173:151–166
52. Wilk-Woźniak E, Pociecha A, Amirowicz A, Gąsiorowski M, Gadzinowska J (2014) Do planktonic rotifers rely on terrestrial organic matter as a food source in reservoir ecosystems? Int Rev Hydrobiol 99(1–2):157–160
53. Löder MG, Meunier C, Wiltshire KH, Boersma M, Aberle N (2011) The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. Mar Biol 158(7):1551–1580
54. Kalinowska K, Ejsmont-Karabin J, Rzepecki M, Kostrzewska-Szlakowska I, Feniova IY, Palash A, Dzialowski AR (2015) Impacts of large-bodied crustaceans on the microbial loop. Hydrobiologia 744(1):115–125

ARTYKUŁ 2:

Kosiba J., Krztoń W., Wilk-Woźniak E. 2018. ***Effect of Microcystins on Proto- and Metazooplankton is more evident in artificial than in natural waterbodies.*** Microbial Ecology 75: 293-302, DOI: 10.1007/s00248-017-1058-z; IF=4,552; lista MEiN=100 pkt.

Microb Ecol
DOI 10.1007/s00248-017-1058-z

MICROBIOLOGY OF AQUATIC SYSTEMS

Effect of Microcystins on Proto- and Metazooplankton Is More Evident in Artificial Than in Natural Waterbodies

J. Kosiba[1] · **W. Krztoń**[1] · **E. Wilk-Woźniak**[1]

Received: 13 May 2017 / Accepted: 22 August 2017
© The Author(s) 2017. This article is an open access publication

Abstract The increasing proliferation of cyanobacterial blooms prolongs the impact of cyanobacteria on aquatic fauna, potentially altering trophic relationships. We hypothesized that any effect of dissolved microcystins (toxins produced by cyanobacteria) on plankton assemblages would be more evident in artificial reservoirs and ponds than in natural ones. The concentrations of dissolved microcystins in the waters we studied ranged widely from 0.07 to 0.81 μg/L. We showed that the artificial ponds were subjected to more frequent and longer-lasting harmful algal blooms. The plankton occurring in them were exposed to significantly higher concentrations of dissolved microcystins than those in natural oxbow lakes. Using a general linear model (GLM) regression, our study identified a significant relationship between dissolved microcystins and both the density and biomass of particular zooplankton groups (ciliates, rotifers, cladocerans, copepods). The density, biomass, and richness of the animal plankton were significantly lower in the artificial ponds than in the natural oxbow lakes. The impact of microcystins and the length of time that they remained in the water caused structural homogenization of the plankton.

Keywords Plankton · Oxbow lakes · Cyanobacterial blooms

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00248-017-1058-z) contains supplementary material, which is available to authorized users.

✉ E. Wilk-Woźniak
wilk@iop.krakow.pl

[1] Department of Freshwater Biology, Institute of Nature Conservation, Polish Academy of Sciences, Al. Adama Mickiewicza 33, 31-120 Krakow, Poland

Introduction

Growing en masse in water, cyanobacteria create a phenomenon known as cyanobacterial blooms. Local and global warming and increasing anthropogenic eutrophication and pollution of water have led to the proliferation of harmful algal blooms (HABs) that show accelerated and prolonged activity [1]. Because "cyanoHABs" are toxic, cause hypoxia, decrease biodiversity, and disrupt food webs [2, 3], they present a serious threat to water ecosystems.

The most threatened ecosystems are those in small, shallow reservoirs, ponds, and oxbow lakes, which are biodiversity hotspots, serve as water migration corridors, diversify the landscape, and provide habitats for many rare and valuable species [4–6]. Because they are naturally eutrophic, these types of waterbodies naturally host cyanobacterial blooms, but the increasing proliferation of such blooms adds a new factor: it prolongs the impact of cyanobacteria on aquatic fauna, potentially altering trophic relationships, damaging these exceptionally important ecosystems, and compromising their ecosystem services.

Cyanobacteria change trophic interactions through several mechanisms. First, they are a poor food source due to their large size, low digestibility [7] and lack of long-chain polyunsaturated fatty acids (PUFAs) [8]. Second, they produce toxins. The most common of the several types of cyanotoxins are microcystins. Microcystins are produced by and retained in cyanobacterial cells during the growth and stationary phases of blooms [9]. When the blooms decay and their cells deteriorate, metabolites are released, raising the concentration of toxins in the water. The presence of microcystins is reported in 50 to 90% of samples taken during bloom events [10]. Toxins released in the water can remain there for up to 3 weeks

Published online: 02 September 2017

[11], causing harm even after the cyanobacteria are gone. More than 100 microcystin analogues are known [12]. The analogues differ in toxicity; microcystin-LR (MC-LR) has been found to be the most toxic one, followed by microcystin-YR (MC-YR) and microcystin-RR (MC-RR) [13]. It is well known that microcystins harm humans and other mammals by altering cell metabolism and triggering a cascade of events that leads to cell necrosis or apoptosis [14]. Such effects do not require direct contact with cyanobacteria cells and occur even if the toxins cannot readily diffuse across the plasma membrane. There is evidence that hydrophobic toxins (e.g., MC-YR) can affect membranes that have packing defects [15]. Some hydrophobic microcystins can, by pinocytosis, penetrate the cell along with other material associated with the plasma membrane [16].

Dissolved cyanobacterial toxins released during bloom decay have negative effects on feeding and on the growth of fish larvae [17]. Cyanotoxins may be transferred to higher trophic levels through primary consumers such as protozooplankton [18] and metazooplankton [19]. Relatively little is known about the response of plankton to toxins, especially to dissolved toxins. It is difficult to draw conclusions about the processes and relationships that operate during CyanoHAB events, and effects measured in the laboratory may not always mirror the natural processes that occur in the field [20].

Finally, cyanotoxins may harm humans following chronic exposure to low concentrations of microcystins via consumption of contaminated water and food (e.g., agricultural products, fish, prawns, mollusks), dermal exposure, and inhalation [14].

Some species feed on cyanobacteria and are exposed to the toxins present in cyanobacterial cells. Many more species are exposed to cyanotoxins dissolved in the water. It is ever more important to understand how the presence of dissolved microcystins affects the structure and trophic network of plankton communities. Some field and laboratory studies have shown that toxins dissolved in the water affect the protozooplankton and metazooplankton living there [21–23].

Protozooplankton and metazooplankton organisms are basic and critical parts of the food web in aquatic ecosystems, able to transfer carbon to higher levels [24]. We studied the effect of dissolved microcystins on the shape of protozooplankton and metazooplankton assemblages in small waterbodies. With increasing anthropopression, we will see further proliferation of CyanoHABs. We need to know exactly how plankton assemblages will be affected by those blooms. For this study, we postulated that the effect of dissolved microcystins on plankton assemblages would be more pronounced in artificial waterbodies than in natural ones.

Material and Methods

Study Area and Materials

This study used samples from four waterbodies in which cyanobacterial blooms occur: two natural oxbow lakes (Piekary, P; Tyniec, T) formed by the Vistula River and two artificial ponds (Podkamycze 1, P1; Podkamycze 2, P2) (Fig. 1). All the studied waterbodies are relatively small, covering 1.56–17.28 ha and ranging in maximum depth from 2.5 and 4.0 m. They all are classified as eutrophic [25] and are near each other, so their weather conditions are very similar.

Sampling Procedure

Samples were collected from the central point of each waterbody between May and October 2014. Sampling was done each month before cyanobacterial blooms formed and then each week during bloom events. In total, 64 sample sets were collected for biological analyses (cyanobacteria, ciliates, metazooplankton) and to determine the concentration of microcystins in the water. Because the studied oxbow lakes are shallow and polymictic, they were not stratified into epilimnion, metalimnion and hypolimnion.

Although, the Ruttner sampler is not a perfect device for quantifying zooplankton abundance [26, 27], but it is broadly use in ecological studies [28]; therefore, we decided to use it.

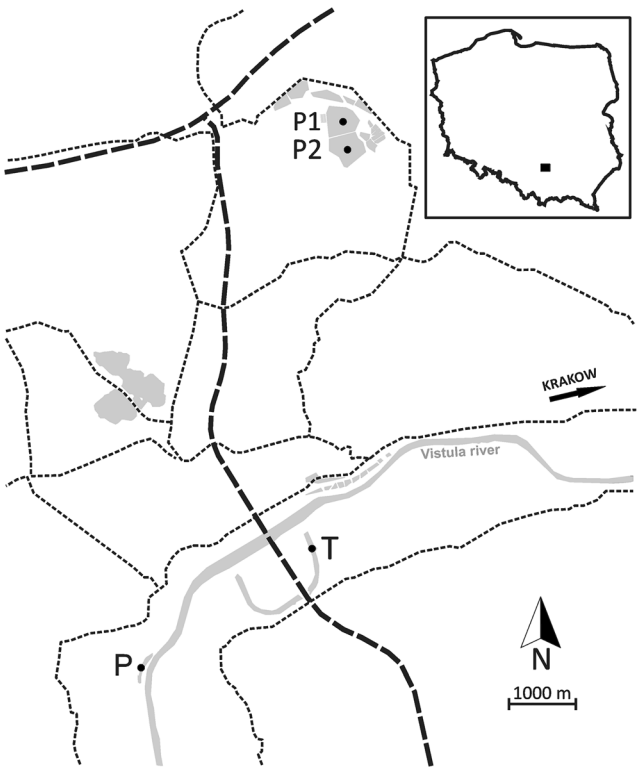


Fig. 1 Locations of the studied waterbodies

Samples were taken at 1 m depth using a 5-L Ruttner sampler and were concentrated from 10 L with plankton nets (mesh sizes 10 μm for cyanobacteria and ciliates; 50 μm for metazooplankton). Immediately after collection, the samples were fixed for quantitative analyses (with Lugol's solution for algae and ciliates; with 4% formaldehyde for metazooplankton). Additional fresh (not fixed) samples were concentrated as described above, and the live material was taken for species composition analysis. See [24] for the keys used for taxonomic identification of cyanobacteria. The living ciliates were identified in 1 mL chambers with glass covers, according to [28] and [29], and their density was averaged from three counts. Total biomass of ciliates was calculated according to [30–33].

Metazooplankton samples were analyzed in 0.5 mL chambers, and their density was calculated as means of five counts. The keys we used for identification of animal species are listed in [23]. Dry weight was calculated by a regression equation defining the body length and weight of each species (see [23] for references). Because the phytoplankton and ciliates were calculated as fresh biomass, zooplankton dry mass was recalculated according to the index proposed by [34]. All microscopy of phytoplankton, ciliates, and metazooplankton employed a Nikon H550L light microscope at × 40–× 1000.

Toxin Analysis

Microcystin concentrations (analogues: MC-LR, MC-RR, MC-YR) were determined by high-performance liquid chromatography (HPLC) using an Agilent 1100 apparatus with a diode matrix (DAD) in the Central Laboratory of the Municipal Water and Sewage Company in Krakow, Poland [35].

Statistical Analysis

The Mann-Whitney *U* test was used to ascertain the statistical significance of differences between the artificial ponds and natural oxbow lakes. The factors analyzed included the microcystin concentrations and the population parameters for the protozooplankton (*Ciliata*), metazooplankton, and particular groups of metazooplankton (*Cladocera*, *Copepoda*, *Rotifera*). Canonical correspondence analysis (CCA; constrained ordination) was applied to analyze the effect of type of waterbody on species composition; the same weight was given to each species in the analysis, regardless of the count of a given species in the samples.

We applied a set of generalized linear models (GLMs) to determine whether the density and biomass of the protozooplankton and metazooplankton depended on the dissolved microcystins, using Poisson error distributions for the density and biomass data from the different plankton groups. GLM residuals were graphically examined to test the model assumptions (residual distribution, independence, homoscedasticity). Finally, we used partial residual plots to visualize

Table 1 Basic information about the type of waterbody, cyanobacterial blooms, and microcystin concentrations

	Piekary	Tyniec	Podkamycze 1	Podkamycze 2
Geographical coordinates	50° 00′ 50.1″ N, 19° 47′ 35.7″ E	50° 01′ 47″ N, 19° 49′ 39.8″ E	50° 05′ 11″ N, 19° 50′ 01.6″ E	50° 04′ 59.6″ N, 19° 50′ 05.4″ E
Type of reservoir	Natural	Natural	Artificial	Artificial
Max depth (m)	4.0	3.0	3.0	2.5
Area (ha)	1.56	5.75	16.82	17.28
Trophic class	Eutrophic	Eutrophic	Eutrophic	Eutrophic
Period of bloom	From August to October	From August to October	From May to November	From May to November
Species created blooms	*Oscillatoria tenuis*, *Dolichospermum planctonicum*, *D. spiroides*, *Microcystis wesenbergii*	*Aphanocapsa* sp., *Microcystis aeruginosa*, *M. ichtyblabe*, *M. wesenbergii*, *Woronichiania naegeliana*, *Aphanizomenon* sp.	*Aphanizomenon flos-aque* with *M. aeruginosa*	*Aphanizomenon flos-aque* with *M. aeruginosa*
Presence of microcystins dissolved in water	All of October	Beginning of September and end of October	From end of June to August and from mid-September to end of October	From end of June to beginning of August and from mid-September to end of October
Concentration of toxins (MCtot)	Min.–max. = 0.00–0.21 μg/L; Avg. = 0.07 μg/L; SD = 0.09 μg/L	Min.–max. = 0.00–0.25 μg/L; Avg. = 0.03 μg/L; SD = 0.08 μg/L	Min.–max. = 0.00–0.67 μg/L; Avg. = 0.17 μg/L; SD = 0.21 μg/L	Min.–max. = 0.00–0.81 μg/L; Avg. = 0.19 μg/L; SD = 0.24 μg/L

Avg. average, *max.* maximum, *min.* minimum, *SD* standard deviation

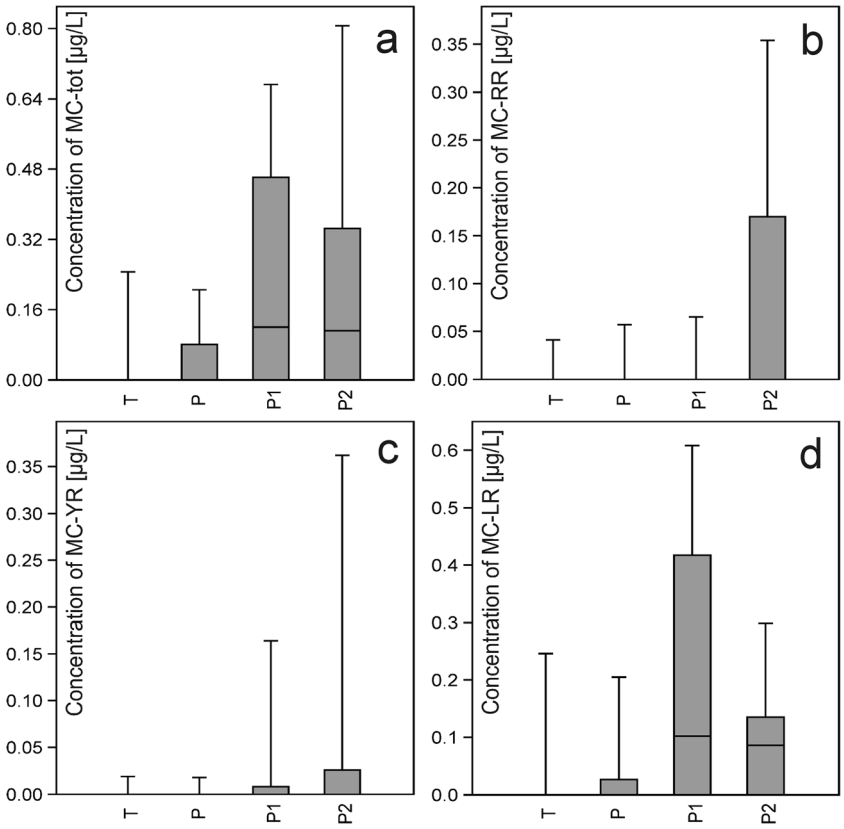


Fig. 2 **a**–**d** Dissolved microcystin concentrations (µg/L) in the waterbodies. **a** MCtot. **b** MC-RR. **c** MC-YR. **d** MC-LR. Dark horizontal lines represent medians; boxes enclose 25th and 75th percentiles

significant relationships between the density or biomass of the protozooplankton and metazooplankton and the dissolved microcystins. According to [36], both of the methods we used are good options for spatial modeling of species distributions.

All of our analyzed data were log-transformed. The statistical analyses employed Statistica 12 (descriptive statistics, Mann-Whitney *U* test), Past 3.10 (box plots), and Canoco 5.04 (CCA, GLM).

Results

Cyanobacterial Blooms and Microcystins

Cyanobacterial blooms were observed in all four waterbodies. The blooms persisted for up to 3 months in the two oxbow lakes (P, T) and for up to 6 months in the two artificial ponds (P1, P2). Cyanobacterial toxins (microcystins) occurred in the water of all studied waterbodies but varied in concentration and duration (Table 1; Fig. 2).

The dissolved microcystin concentrations were highest in the artificial ponds (P1, P2) and varied the most in P2 (Fig. 2a); the concentrations were lower and more uniform in the natural oxbow lakes (P, T) (Fig. 2b–d). The microcystin forms differed in their patterns of occurrence: in the artificial ponds, the highest

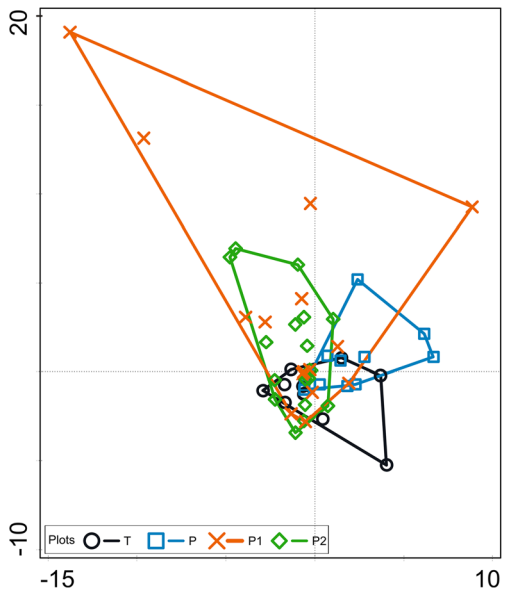


Fig. 3 CCA plot diagram. Composition of *Ciliata* assemblages, samples, and waterbodies. The samples are grouped as follows: blue envelope—Piekary oxbow lake (natural reservoir); black envelope—Tyniec oxbow lake (natural reservoir); brown and green envelopes—Podkamycze 1 and 2 (artificial ponds). Total variation = 3.24; explanatory variables account for 4.0%. Eigenvalues for axis 1 = 0.067 and for axis 2 = 0.042. Permutation test results: on first axis pseudo-F = 1.2, P = 0.81; on all axes pseudo-F = 0.8, P = 0.836. Explained fitted variation (cumulative) for axis 1 = 51.94 and axis 2 = 84.33.

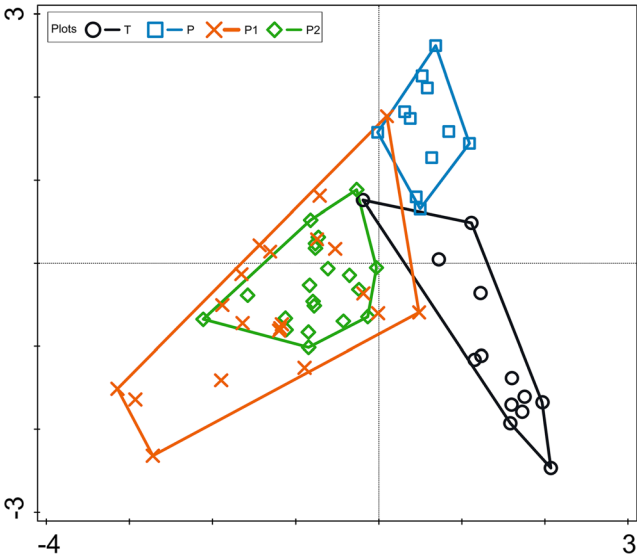


Fig. 4 CCA plot diagram. Composition of metazooplankton assemblages, samples, and waterbodies. Samples are grouped as follows: blue envelope—Piekary oxbow lake (natural reservoir); black envelope—Tyniec oxbow lake (natural reservoir); brown and green envelopes—Podkamycze 1 and 2 (artificial ponds). Total variation = 2.74; explanatory variables account for 13.9%. Eigenvalues for axis 1 = 0.207 and for axis 2 = 0.097. Permutation test results: on first axis pseudo-F = 4.9, P = 0.002; on all axes pseudo-F = 3.2, P = 0.002. Explained fitted variation (cumulative) for axis 1 = 54.29 and axis 2 = 79.81

concentration of MC-LR was found in P1 and the highest concentration MC-RR and MC-YR in P2 (Fig. 2b–d).

The differences in dissolved microcystin concentrations between the natural oxbow lakes and the artificial ponds were statistically significant (for MCtot Mann-Whitney U test, $z = -3.00$ and $p < 0.000$; for MC-LR Mann-Whitney U test, $z = -2.43$ and $p = 0.015$).

Zooplankton Structure

The zooplankton organisms were divided into protozooplankton (*Ciliata*) and metazooplankton (*Cladocera*, *Copepoda*, *Rotifera*). We recorded 15 *Ciliata* taxa and 54 metazooplankton taxa (see supplementary data). The average number of *Ciliata* taxa was lower than the average number of metazoan taxa, but Spearman rank correlations showed a positive relationship between the number of *Ciliata* taxa and the number of metazooplankton taxa ($r = 0.46$, $p < 0.05$).

CCA partially differentiated the protozooplankton of the natural waterbodies (P, T) from that of the artificial ponds (P1, P2) along the first axis based on the species composition of the samples, but those results were not statistically significant (Fig. 3).

CCA of the metazooplankton showed differences in species composition between the natural (P, T) and artificial (P1, P2) waterbodies along the first axis based on the species composition of the samples. Those differences were statistically significant (Fig. 4).

Zooplankton vs. Dissolved Microcystins

GLM showed statistically significant negative relationships between the biomass and the density of several zooplankton groups and the concentrations of MCtot and MC-LR (Tables 2 and 3), but not for MC-RR or MC-YR.

Table 2 GLM, biomass of protozooplankton, and particular groups of metazooplankton and microcystins (MCtot and MC-LR) dissolved in water

Response variable	Predictors	Fitted model deviance	Null deviance	Model AIC	Model test F	p	B intercept/MC tot or MC-LR	s.e. intercept/MC tot or MC-LR	T value intercept/MC tot or MC-LR
Total biomass of *Ciliata*	MCtot	320.77	348.89	409.54	28.1	< 0.000	1.20/− 3.17	0.09/0.71	12.33/− 4.41
Total biomass of *Ciliata*	MC-LR	305.27	330.70	389.57	25.4	< 0.000	1.26/− 4.27	0.09/1.03	13.03/− 4.14
Total biomass of metazooplankton	MCtot	227.74	260.30	429.18	32.6	< 0.000	2.29/− 1.59	0.05/0.30	41.48/− 5.21
Total biomass of metazooplankton	MC-LR	226.40	245.93	405.97	19.5	< 0.000	2.19/− 1.81	0.06/0.44	37.27/− 4.06
Biomass of *Copepoda*	MCtot	131.33	135.99	285.17	4.7	0.035	1.40/− 0.83	0.08/0.40	16.51/− 2.06
Biomass of *Copepoda*	MC-LR	125.63	132.64	265.16	7.0	0.010	1.44/− 1.52	0.08/0.61	16.88/− 2.46
Biomass of *Cladocera*	MCtot	315.85	361.06	434.71	45.2	< 0.000	1.51/− 3.66	0.08/0.67	17.99/− 5.44
Biomass of *Cladocera*	MC-LR	297.31	313.73	395.70	16.4	< 0.000	1.18/− 3.19	0.09/0.91	11.96/− 3.49

Only statistically significant relationships are show

Table 3 GLM, density of protozooplankton, and particular groups of metazooplankton and microcystins (MCtot and MC-LR) dissolved in water

Response variable	predictors	Fitted model deviance	Null deviance	Model AIC	Model test *F*	*p*	*B* intercept/ MC tot or MC-LR	s.e. intercept/ MC tot or MC-LR	*T* value intercept/MC tot or MC-LR
Total density of *Ciliata*	MCtot	17,002,628.19	19,021,611.61	1.7e+007	2.019e+006	< 0.000	12.03/− 4.41	0.0004/0.004	27,099.8/− 1103.35
Total density of *Ciliata*	MC-LR	16,044,830.96	17,942,766.49	1.605e+007	1.898e+006	< 0.000	12.09/− 6.15	0.0004/0.006	27,638.6/− 1050.42
Total density of Metazooplankton	MCtot	77,365.64	79,328.20	7.782e+004	1963	< 0.000	7.28/− 0.92	0.005/0.022	1616.07/− 42.12
Total density of Metazooplankton	MC-LR	75,349.29	76,393.39	7.577e+004	1044	< 0.000	7.29/− 0.93	0.005/0.030	1611.15/− 30.98
Density of *Copepoda*	MCtot	5339.32	5643.18	5704.77	303.9	< 0.000	5.34/− 0.96	0.012/0.058	451.08/− 16.54
Density of *Copepoda*	MC-LR	4988.59	5366.55	5321.14	378.0	< 0.000	5.38/− 1.57	0.012/0.087	450.06/− 18.08
Density of *Cladocera*	MCtot	3739.79	4301.09	4022.47	561.3	< 0.000	4.50/− 2.50	0.019/0.12	241.49/− 20.51
Density of *Cladocera*	MC-LR	3150.87	3381.86	3398.86	231.0	< 0.000	4.24/− 2.39	0.021/0.175	198.68/− 13.60
Density of *Rotifera*	MCtot	86,520.67	87,833.35	8.694e+004	1313	< 0.000	7.05/− 0.83	0.005/0.024	1397.82/− 34.62
Density of *Rotifera*	MC-LR	82,454.17	83,053.02	8.283e+004	598.9	< 0.000	7.08/− 0.764	0.005/0.0326	1408.89/− 23.64

Only statistically significant relationships are shown

Population Parameters of Proto- and Metazooplankton Assemblages

The richness, total density, and total biomass of *Ciliata* species in the natural oxbow lakes (P, T), having lower microcystin concentrations, were significantly higher than in the artificial ponds (P1, P2), having higher microcystin concentrations (Fig. 5a–c).

The richness and density of metazooplankton species were significantly higher in waterbodies that had shorter-duration cyanobacterial blooms and lower microcystin concentrations (Fig. 6a–c), but total metazooplankton biomass did not show such a correlation. The natural and artificial waterbodies differed significantly for biomass of *Rotifera* (Fig. 6d–f) and *Copepoda* (Fig. 6g–i), but surprisingly not for biomass of *Cladocera* (Fig. 6j–l).

Discussion

Microcystins are a group of toxins often present in water, as they are produced by species that commonly occur there (e.g., species of the genera *Planktothrix*, *Microcystis*, *Aphanizomenon*, *Nostoc*, *Anabaena*) [37]. In the studied waterbodies, we found three microcystin analogues: MC-YR, MC-RR, and MC-LR. The first two occurred at small concentrations, and for them, we found no significant differences between the waterbodies nor any relationships with plankton parameters. Only dissolved MC-LR was

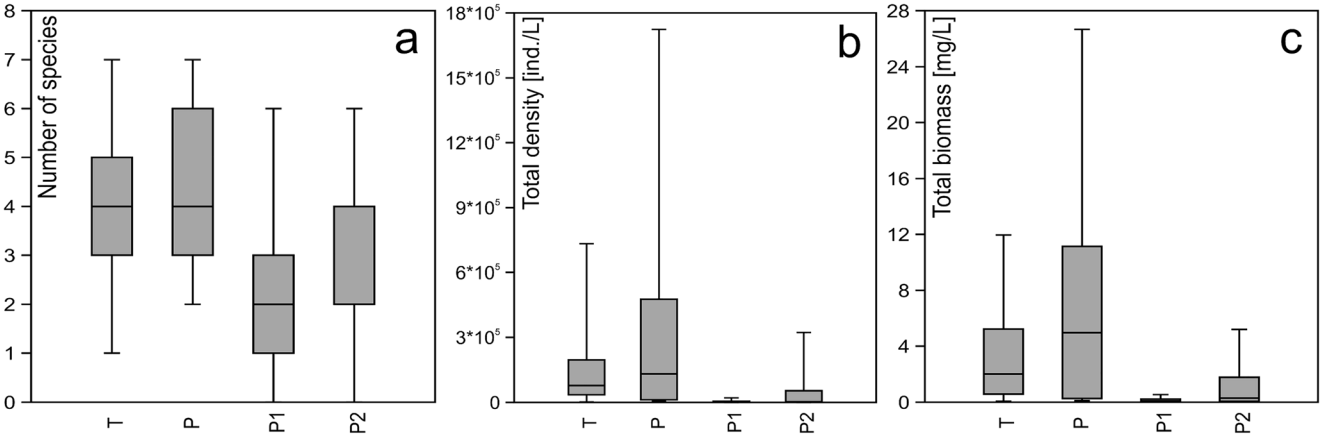


Fig. 5 Box plots for **a** number of species, **b** total density, and **c** total biomass of *Ciliata* in particular waterbodies. Mann-Whitney *U* test showed statistically significant differences between the natural (P, T) and artificial waterbodies (P1, P2) for all parameters (number of species $z = 4.215$, $p < 0.000$; density $z = 4.833$, $p < 0.000$; biomass $z = 4.472$, $p < 0.000$). Dark horizontal lines represent medians; boxes enclose 25th and 75th percentiles; whiskers represent 5th and 95th percentiles

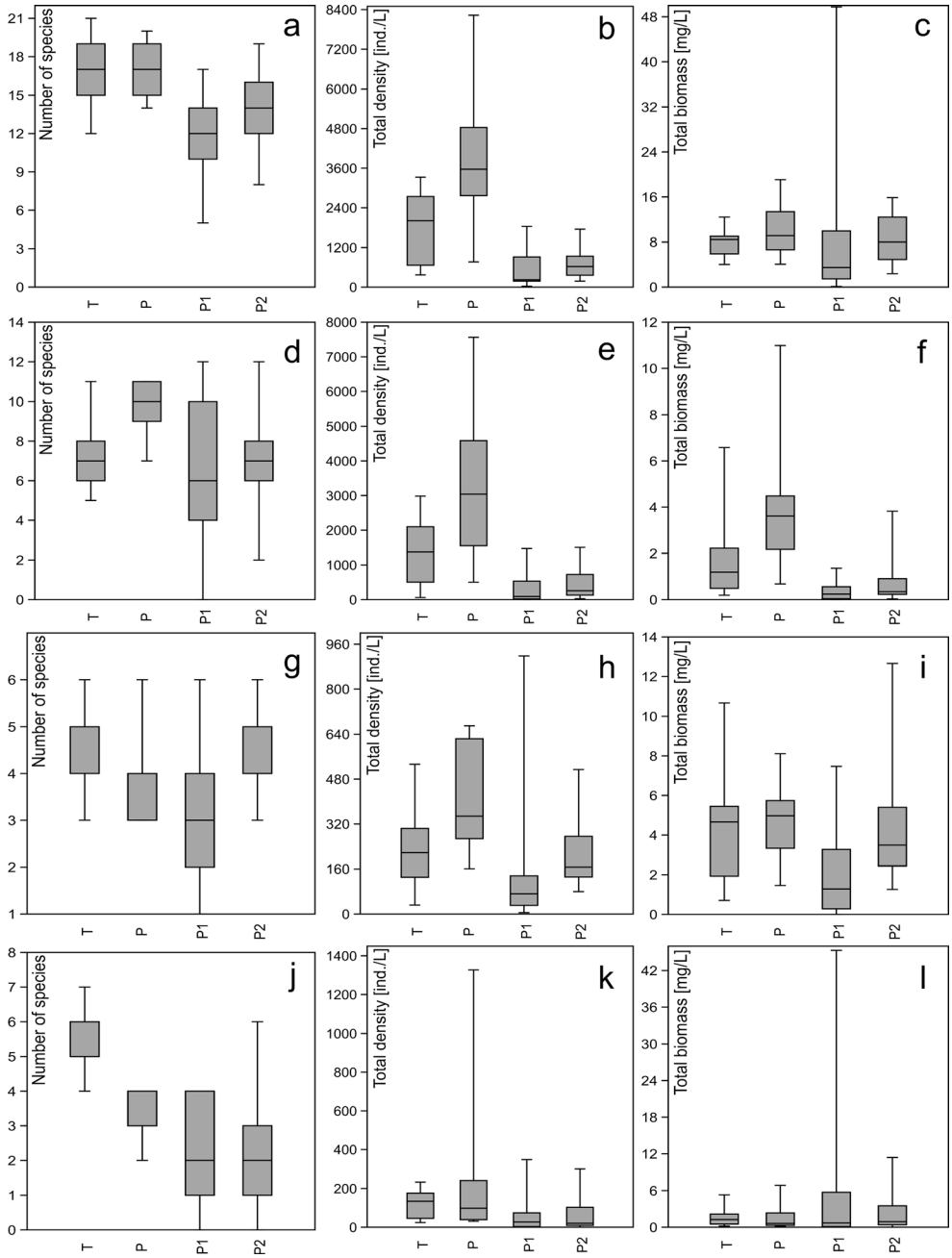


Fig. 6 Box plots. **a** Total number of metazooplankton species (Mann-Whitney *U* test $z = 5.001$, $p < 0.000$). **b** Total density of metazooplankton (Mann-Whitney *U* test $z = 5.235$, $p < 0.000$). **c** Total biomass of metazooplankton (not statistically significant). **d** Total number of *Rotifera* species (Mann-Whitney *U* $z = 2.039$, $p = 0.041$). **e** Total density of *Rotifera* (Mann-Whitney *U* test $z = 5.151$, $p < 0.000$). **f** Total biomass of *Rotifera* (Mann-Whitney *U* test $z = 4.937$, $p < 0.000$). **g** Total number of *Copepoda* species (not statistically significant). **h** Total density of *Copepoda* (Mann-Whitney *U* test $z = 3.314$, $p < 0.000$). **i** Total biomass of *Copepoda* (Mann-Whitney *U* test $z = 2.364$, $p = 0.018$). **j** Total number of *Cladocera* species (Mann-Whitney *U* test $z = 5.077$, $p < 0.000$). **k** Total density of *Cladocera* (Mann-Whitney *U* test $z = 3.842$, $p < 0.000$). **l** Total biomass of *Cladocera* (not statistically significant). Dark horizontal lines represent medians; boxes enclose 25th and 75th percentiles; whiskers represent 5th and 95th percentiles

associated with the parameters of the plankton, both protozooplankton (*Ciliata*) and metazooplankton. Differences in hydrophobicity can make microcystins differ in the way that they are taken up by animals. They may be ingested with food [38] or may bind to membranes and penetrate cells by pinocytosis [16]. The microcystins affected the plankton animals in different ways in the studied waterbodies. We showed that they were more harmful to these organisms in the artificial ponds than in the natural oxbow lakes. There were significant differences in dissolved MC-LR concentration between the natural and artificial waterbodies. MC-LR is known to be the most potent toxin [39]; we infer that the significantly higher and longer-persisting concentrations of that analogue in the artificial ponds shaped the structure of the ciliate and metazooplankton assemblages.

Species-specific adaptations in zooplankton have led to variation of the observed responses to cyanobacteria blooms [40] and cyanobacterial toxins. In the literature, information about the response of ciliates [21, 22, 41, 42], rotifers [43, 44], copepods [20, 45], and cladocerans [46, 47] to cyanotoxins is contradictory and unclear. Our GLM analyses showed significant negative correlations between the dissolved microcystins and both the density and the biomass of *Ciliata*. Other research indicates that cyanobacterial blooms generally affect communities of ciliates by lowering their diversity: only a

few ciliate species were found to develop during the culminating stage of cyanobacterial blooms [48].

The richness, total biomass, and density of *Ciliata* species in particular samples were significantly lower in the two artificial ponds (P1, P2), where microcystins occurred at significantly higher concentrations and remained in the water longer than in the oxbow lakes (P, T). The composition of *Ciliata* assemblages in particular samples was more uniform in the ponds and assumed a more typical structure in the oxbow lakes (CCA). That uniformity or homogeneity of *Ciliata* assemblages in the artificial ponds reflects their longer exposure to dissolved cyanotoxins. The more typical structure of the assemblages found in the oxbow lakes reflects the operation of an ecosystem in which toxins are present at lower concentrations and for a shorter period.

The response of the metazoan assemblages was similar to that of the ciliate assemblages. GLM regression showed negative relationships between dissolved microcystins and both the density and the biomass of the metazooplankton. We found significantly fewer species and lower total density of metazooplankton in the ponds (P1, P2) than in the oxbow lakes (P, T), but surprisingly we did not find significant differences in total biomass.

Since metazooplankton organisms form a heterogeneous group consisting of various subgroups, we also analyzed data from particular groups. We found a significant relationship between microcystins and the density of *Rotifera* and a decrease in the number of species, total density, and total biomass of rotifers in the ponds, which had higher dissolved microcystin concentrations.

Copepod biomass was also negatively correlated with dissolved microcystin concentration. However, copepods are able to discriminate between toxic and nontoxic cyanobacteria [44], but they can assimilate toxins directly from the water or via ciliates [49, 50], and they may adsorb toxins and then transfer them to higher trophic levels [51]. Analyses of copepod biomass and density showed statistically significant differences between the ponds (P1, P2) and the oxbow lakes (P, T), in line with laboratory studies [45] which showed that an elevated concentration of microcystins reduced the survival of *Eurytemora affinis*.

The relationship between toxins and *Cladocera* is even more complicated. It has been demonstrated that *Daphnia* species may adapt to the presence of toxins [47]. Small cladocerans such as *Bosmina* may not be sensitive to the effects of microcystins. *Bosmina* and *Daphnia* are species that ingest toxic cyanobacteria, leading to microcystin accumulation [52, 53] and transferring them to higher trophic levels [54]. In our study, *Cladocera* showed significant negative correlations with microcystins, mainly MC-LR. There were significant differences in the total density but not the biomass of *Cladocera* between the artificial and natural waterbodies: the oxbow lakes showed higher density of *Cladocera* species but their biomass was higher in the ponds. This suggests that the large cladocerans (*Daphnia*) in our waterbodies were adapted to higher concentrations of those toxins.

Conclusion

We demonstrated that in waterbodies with higher and longer-persisting microcystin concentrations, various parameters (density, biomass, richness) of the zooplankton population decreased, and the structure of the species assemblages tended toward uniformity. The studied artificial ponds were more exposed to harmful cyanobacterial blooms, and for a longer period, than the natural oxbow lakes. The general problem can be expressed in this way: increasing artificiality of the aquatic environment (transformation, destruction, creation of new waterbodies) + eutrophication + global warming = increased proliferation of toxic cyanobacterial blooms + homogenization of plankton species structure.

Acknowledgements We thank Dr. Edward Walusiak for assistance with sampling, Krzysztof Pudaś for analyses of microcystins, and the anonymous reviewers for helpful comments and suggestions. Michael Jacobs line-edited the manuscript for submission.

Funding Information This study was supported by the Institute of Nature Conservation, Polish Academy of Sciences, through its statutory fund and a grant for young scientists and Ph.D. candidates.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. Paerl HW, Gardner WS, Havens KE, Joyner AR, McCarthy MJ, Newell SE, Qin B, Scott JT (2016) Mitigating cyanobacterial harmful algal blooms in aquatic ecosystems impacted by climate change and anthropogenic nutrients. Harmful Algae 54:213–222
2. Carmichael WW (2001) Health effects of toxin producing cyanobacteria: the cyano HABs. Hum. Ecol. Risk. Assess. 7: 1393–1407
3. Moustaka-Gouni M, Vardaka E, Michaloudi E, Kosmas KA, Tryfon E, Mihalatou H, Gkelis S, Lanaras T (2006) Plankton food web structure in a eutrophic polymictic lake with a history of toxic cyanobacterial blooms. Limnol. Oceanogr. 51:715–727
4. Dembowska EA, Napiórkowski P (2015) A case study of the planktonic communities in two hydrologically different oxbow lakes, Vistula River, Central Poland. J. Limnol. 74:2008–2015
5. Wilk-Woźniak E, Ligęza S, Shubert E (2014) Effect of water quality on phytoplankton structure in oxbow lakes under anthropogenic and non-anthropogenic impacts. Clean Soil Air Water 42:421–427
6. Goździejewska A, Glińska-Lewczuk K, Obolewski K, Grzybowski M, Kujawa R, Lew S, Grabowska M (2016) Effects of lateral

connectivity on zooplankton community structure in floodplain lakes. Hydrobiologia 774:7–21
7. Wilson AE, Sarnelle O, Tillmanns AR (2006) Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: metaanalyses of laboratory experiments. Limnol. Oceanogr. 51:1915–1924
8. Martin-Creuzburg D, Wacker A, Von Elert E (2005) Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. Oecologia 144:362–372
9. Rapala J, Sivonen K, Lyra C, Niemelä SI (1997) Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. Appl. Environ. Microbiol. 63: 2206–2212
10. Sivonen K, Bartram J (1999) Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management. E. and F.N. Spon, New York
11. Miller MA, Kudela RM, Mekebri A, Crane D, Oates SC, Tinker MT, et al. (2010) Evidence for a novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea otters. PLoS One 5(9):e12576
12. Buratti FM, Manganelli M, Vichi S, Stefanelli M, Scardala S, Testai E, Funari E (2017) Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Arch. Toxicol. 91:1049–1130
13. Jakubowska N, Szeląg-Wasielewska E (2015) Toxic picoplanktonic cyanobacteria—review. Mar Drugs 13:1497–1518
14. Campos A, Vasconcelos V (2010) Molecular mechanisms of microcystin toxicity in animal cells. Int. J. Mol. Sci. 11:268–287
15. Lankoff A, Kolataj A (2001) Influence of microcystin-YR and nodularin on the activity of some proteolytic enzymes in mouse liver. Toxicon 39:419–423
16. Vesterkvist PS, Meriluoto JA (2003) Interaction between microcystins of different hydrophobicities and lipid monolayers. Toxicon 41:349–355
17. Karjalainen M, Reinikainen M, Spoof L, Meriluoto JAO, Sivonen K, Markku V (2005) Trophic transfer of cyanobacterial toxins from zooplankton to planktivores: consequences for pike larvae and mysid shrimps. Environ. Toxicol. 20:354–362
18. Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie Van Leeuwenhoek. Int J Gen Mol Microbiol 81:293–308
19. Irigoien X, Harris RP, Head RN, Cummings D, Harbour B, Myer-Harms B (2000) Feeding selectivity and egg production of *Calanus helgolandicus* in the English Channel. Limnol. Oceanogr. 45:44–54
20. Ger KA, Teh SJ, Baxa DV, Lesmeister S, Goldman CR (2010) The effects of dietary *Microcystis aeruginosa* and microcystin on the copepods of the upper San Francisco estuary. Freshw. Biol. 55: 1548–1559
21. Ransom RE, Nerad TA, Meier PG (1978) Acute toxicity of some bluegreen algae to the protozoan *Paramecium caudatum*. J. Phycol. 14:114–116
22. Zurawell RW, Chen H, Burke JM, Prepas EE (2005) Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. J Toxicol Environ Health B Crit Rev 8:1–37
23. Krztoń W, Pudaś K, Pociecha A, Strzesak M, Kosiba J, Walusiak E, Szarek-Gwiazda E, Wilk-Woźniak E (2017) Microcystins affect zooplankton biodiversity in oxbow lakes. Environ. Toxicol. Chem. 36:165–174
24. Kosiba J, Wilk-Woźniak E, Krztoń W, Strzesak M, Pociecha A, Walusiak E, Pudaś K, Szarek-Gwiazda E (2017) The trophic network in shallow oxbow lakes exposed to cyanobacterial blooms. Microb. Ecol. 73(1):17–28
25. Carlson RE, Simpson J (1996) A coordinator's guide to volunteer lake monitoring methods. North American Lake Management Society, Madison
26. Patalas K (1954) Zespoly skorupiakow pelagicznych 28 jezior pomorskich [Communities of pelagic crustacean of 28 Pomeranian Lakes]. Ekol Pol 2(1):61–88
27. Kvam OV, Kleiven OT (1995) Diel horizontal migration and swarm formation in Daphnia in response to Chaoborus. Hydrobiologia 307(1–3):177–184
28. Foissner W, Berger H (1996) A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshw. Biol. 35:375–482
29. Foissner W, Berger H, Schaumburg J (1999) Identification and ecology of limnetic plankton ciliates. Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft, München
30. Jerome CA, Montagnes DJS, Taylor FJR (1993) The effect of the quantitative protargol stain and Lugols and Buinos fixatives on cell size: a more accurate estimate of ciliate species biomass. J Eukryot Microbiol 40:254–259
31. Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnol. Oceanogr. 45:569–579
32. Wiąckowski K, Doniec A, Fyda J (1994) An empirical study of the effect of fixation on ciliate cell volume. Mar Microb Food Webs 8:59–69
33. Putt M, Stoecker DK (1989) An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnol. Oceanogr. 34:1097–1103
34. Bottrell HH, Duncan A, Gliwicz ZM, Grygierek E, Herzig A, Hillbricht-Ilkowska A, Kurasawa H, Larsson P, Węgleńska T (1976) A review of some problems in zooplankton production studies. Norw J Zool 24:419–456
35. Meriluoto J, Codd GA (2005) Cyanobacterial monitoring and cyanotoxin analysis. Acta Acad Abo 65:1–145
36. Guisan A, Weiss SB, Weiss AD (1999) GLM versus CCA spatial modeling of plant species distribution. Plant Ecol. 143:107–122
37. Van Apeldoorn ME, Van Egmond HP, Speijers GJ, Bakker GJ (2007) Toxins of cyanobacteria. Mol. Nutr. Food Res. 51:7–60
38. Wiegand C, Pflugmacher S (2005) Ecotoxicological effects of selected cyanobacterial metabolites: a short review. Toxicol. Appl. Pharmacol. 203:201–218
39. Gupta N, Pant SC, Vijayaraghavan R, Lakshmana Rao PV (2003) Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 188:285–296
40. Gustafsson S, Hansson L-A (2004) Development of tolerance against toxic cyanobacteria in *Daphnia*. Aquatic Ecol 38:37–44
41. Fabbro L, Baker M, Duivenvoorden L, Pegg G, Shiel R (2001) The effects of the ciliate *Paramecium* cf. *caudatum* Ehrenberg on toxin producing *Cylindrospermopsis* isolated from the Fitzroy River. Australia Environ Toxicol 16:489–497
42. Ward CJ, Codd GA (1999) Comparative toxicity of four microcystins of different hydrophobicities to the protozoan, *Tetrahymena pyriformis*. J. Appl. Microbiol. 86:874–882
43. Gilbert JJ (1996) Effect of food availability on the response of planktonic rotifers to a toxic strain of the cyanobacterium *Anabaena flos-aquae*. Limnol. Oceanogr. 41:1565–1572
44. Fulton RS, Paerl H (1987) Effects of colonial morphology on zooplankton utilization of algal resources during blue-green algal (*Microcystis aeruginosa*) blooms. Limnol. Oceanogr. 2:634–644
45. Reinikainen M, Lindvall F, Meriluoto JAO, Pepka S, Sivonen K, Spoof L, Wahlsten M (2002) Effects of dissolved cyanobacterial toxins on the survival and egg hatching of estuarine calanoid copepods. Mar. Biol. 140:577–583
46. Hansson LA, Gustafsson S, Rengefors K, Bomark L (2007) Cyanobacterial chemical warfare affects zooplankton community composition. Freshw. Biol. 52:1290–1301

47. Wojtal-Frankiewicz A, Bernasińska J, Frankiewicz P, Gwoździński K, Jurczak T (2014) Response of *Daphnia*'s antioxidant systems to spatial heterogeneity in cyanobacteria concentrations in a lowland reservoir. PLoS One 9:e112597
48. Tirjaková E, Krajčovičová K, Illyová M, Vdačny P (2016) Interaction of ciliate communities with cyanobacterial water bloom in a shallow, hypertrophic reservoir. Acta Protozool. 3:173–188
49. Karjalainen M, Reinikainen M, Lindvall F, Spoof L, Meriluoto JAO (2003) Uptake and accumulation of dissolved, radiolabeled nodularin in Baltic Sea zooplankton. Environ. Toxicol. 18:52–60
50. Agasild H, Zingel P, Karus K, Kangro K, Salujoe J, Noges T (2013) Does metazooplankton regulate the ciliate community in a shallow eutrophic lake? Freshw. Biol. 58(1):183–191
51. Sopanen S, Uronen P, Kuuppo P, Svensen C, Rühl A, Tamminen T, et al. (2009) Transfer of nodularin to the copepod Eurytemora affinis through the microbial food web. Aquat. Microb. Ecol. 55(2):115–130
52. Watanabe MM, Kaya K, Takamura N (1992) Fate of the toxic cyclic heptapeptides, the microcystins, from blooms of Microcystis(cyanobacteria) in a hypertrophic lake. J. Phycol. 28:761–767
53. Thostrup L, Christoffersen K (1999) Accumulation of microcystin in Daphnia magna feeding on toxic Microcystis. Arch. Hydrobiol. 145:447–467
54. Tönno I, Agasild H, Kõiv T, Freiberg R, Noges P, Noges T (2016) Algal diet of small bodied crustacean zooplankton in a cyanobacteria-dominated eutrophic lake. PLoS One 11:e0154526

ARTYKUŁ 3:

Kosiba J., Wilk-Woźniak E., Krztoń W. 2019. ***The effect of potentially toxic cyanobacteria on ciliates (Ciliophora).*** Hydrobiologia 1 (827): 325-335, https://doi.org/10.1007/s10750-018-3783-9; IF=2,694; lista MEiN=100 pkt.

Hydrobiologia (2019) 827:325–335
https://doi.org/10.1007/s10750-018-3783-9

PRIMARY RESEARCH PAPER

The effect of potentially toxic cyanobacteria on ciliates (Ciliophora)

Joanna Kosiba · Elżbieta Wilk-Woźniak · Wojciech Krztoń

Received: 15 June 2018 / Revised: 27 July 2018 / Accepted: 24 September 2018 / Published online: 10 October 2018
© The Author(s) 2018

Abstract The most frequently observed cyanotoxins are microcystins. They trigger a cascade of events leading to cellular responses. The hypothesis of the study was that cyanobacteria affect ciliates as solitary species and as assemblages. The aim of our study was to determine whether ciliates respond to cyanobacteria because of the presence of cyanotoxins (microcystins—MC). We set up experiments with toxic (*Planktothrix agardhii* and *Microcystis aeruginosa*) and non-toxic (*Aphanizomenon flos-aquae*) cyanobacteria, solitary *Spirostomum* sp. (Ciliophora), and a simple ciliate assemblage. Predicted values showed statistically significant increase during the solitary *Spirostomum* sp. abundance in the presence of toxic *P. agardhii* (MC total concentration in cells 323.9 µg/l) and *M. aeruginosa* (MC total concentration in cells 31.9 µg/l) but a decrease in the presence of non-toxic *A. flos-aquae*. The abundance of *Spirostomum* sp., being a component of ciliate assemblage, decreased significantly in the presence of all the three species of cyanobacteria due to competition from small-sized ciliate species that graze bacteria more effectively compared to large-cell-sized *Spirostomum*. We conclude that toxic cyanobacteria may affect ciliates in various ways, not necessarily because of production of toxins. As a consequence of the presence cyanotoxins, a cascading effect of passing carbon in the food web might be induced.

Keywords Microcystins · *Spirostomum* sp. · Cyanotoxins · Ciliophora

Handling editor: Judit Padisák

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10750-018-3783-9) contains supplementary material, which is available to authorized users.

J. Kosiba (✉) · E. Wilk-Woźniak · W. Krztoń
Institute of Nature Conservation, Polish Academy of Sciences, al. Adama Mickiewicza 33, 31-120 Kraków, Poland
e-mail: kosiba@iop.krakow.pl

Introduction

Cyanobacteria are prokaryotic, autotrophic organisms which, developing in mass, create water blooms. One potentially hazardous consequence of cyanobacterial blooms is the production of toxins (e.g. Carmichael, 1994). Various types of cyanotoxins are produced by different species of cyanobacteria, e.g. species from genera: (a) *Anabaena*, *Aphanizomenon* and *Oscillatoria* produce anatoxin-a; (b) *Aphanizomenon*, *Planktothrix*, *Anabaena*, *Cylindrospermopsis* and *Lyngbya* produce saxitoxins; (c) *Microcystis*, *Planktothrix*, *Dolichospermum* and *Aphanizomenon* produce microcystins; (d) *Nodularia* produces nodularins; and (e) *Cylindrospermopsis* and *Aphanizomenon* produce

cylindrospermopsins (Codd et al., 1995, 2005; Sivonen & Jones, 1999; Buratti et al., 2017).

Among toxins, hepatotoxins, and especially microcystins (MCs), which are heptapeptides (Carmichael, 1992), are the most frequently observed and have been well studied (Zurawell et al., 2005; Mantzouki et al., 2018). They are endotoxins (Rapala et al., 1997) and are not released into water until cell lysis (Rohrlack & Hyenstrand, 2007).

Microcystins induce abnormal signalling in multiple pathways mediated by protein phosphatase 2 (PP2A), resulting in increased protein phosphorylation that triggers a cascade of events leading to a series of cellular responses such as: modification of the cytoskeleton and disruption of actin filaments, oxidative stress, induction of apoptosis, and reduced DNA repair or cell proliferation leading to tumour promotion (Codd et al., 1995).

Primary consumers of cyanobacteria (such as protists, rotifers, copepods and cladocerans) can be directly contaminated by consuming cyanobacterial cells (Ferrão-Filho & Kozlowsky-Suzuki, 2011). Next, predators on protists and animals can be indirectly contaminated and spread the MCs to other organisms via trophic transfer (Ibelings et al., 2005; Gołdyn et al., 2010; Kozlowsky-Suzuki et al., 2012). However, cyanotoxins may be passed into the food web not only because they are cumulated in the invertebrates that consume cyanobacteria cells but also because toxins might be bound to the outside part of cells of protists and animals. This way, cyanotoxins may also be transferred via planktonic animals to higher levels of the food chain.

Although a great deal of research has shown the negative effect of toxic cyanobacteria on metazooplankton (Lampert, 1987; Wilson et al., 2006), much less has been focused on protists (Maršálek & Bláha, 2004; Combes et al., 2013; Tirjaková et al., 2016).

Protists are an important component of the water trophic network, particularly in lakes where cyanobacterial filaments or colonies are too large to be eaten by zooplankton (Havens, 1998). However, more recently, their role has been much appreciated as a very important link in the microbial loop (Sommer et al., 2012). A prominent group of protists constitutes ciliates that are a key component in energy transfer from microbial elements to the higher trophic levels in the food chain (Sherr & Sherr, 1994).

An understanding of the functionality of different group of species and changes in the food web network of water ecosystems is important because a reduction or alteration of the planktonic population can cause a deficit or an unbalance in food availability for higher levels of the trophic web (Zaccaroni & Scaravelli, 2008). Based on the assumption that a decrease in the number of ciliates in water bodies containing toxic cyanobacteria is a result of cyanobacteria producing toxic compounds that induce harmful effects to these organisms (Martins et al., 2011), we hypothesised that cyanobacteria affect ciliates as solitary species and as assemblages. Our previous field studies showed that cyanobacterial blooms significantly lowered diversity, density and biomass of ciliate communities (Kosiba et al., 2018). The aim of our study was to determine whether ciliates respond to cyanobacteria because of the presence of cyanotoxins (microcystins).

Materials and methods

To determine whether potentially toxic cyanobacteria affect ciliates, we set up four experiments (Fig. 1). For the experiments, we used the biomass of species as follows: (1) three species of potentially toxic cyanobacteria (*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault, *Microcystis aeruginosa* Kütz. and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek); (2) solitary species of ciliates (*Spirostomum* sp.); and (3) a simple ciliate assemblage consisting of four species (*Spirostomum* sp., *Euplotes patella* (Müller), *Strobilidium* sp. and *Paramecium aurelia*-complex). Among the ciliates, we chose *Spirostomum* sp. because the species from this genus were found to be sensitive to cyanotoxins (Tarczyńska et al., 2001). Cyanobacteria biomasses used for experiments were obtained as (a) biomass of the strain from culture (*P. agardhii*—strain SAG 6.89), and (b) biomass of the species collected from their natural habitats. *A. flos-aquae* and *M. aeruginosa* were taken during the peak of blooms. *A. flos-aquae* was collected from a shallow, eutrophic pond (Podkamycze 1), located close to Kraków (southern Poland; 50°05′11″N, 19°50′01.6″E), and *M. aeruginosa* was collected from a shallow, eutrophic oxbow lake (Tyniec 1), located in Kraków (50°01′47″N, 19°49′39.8″E).

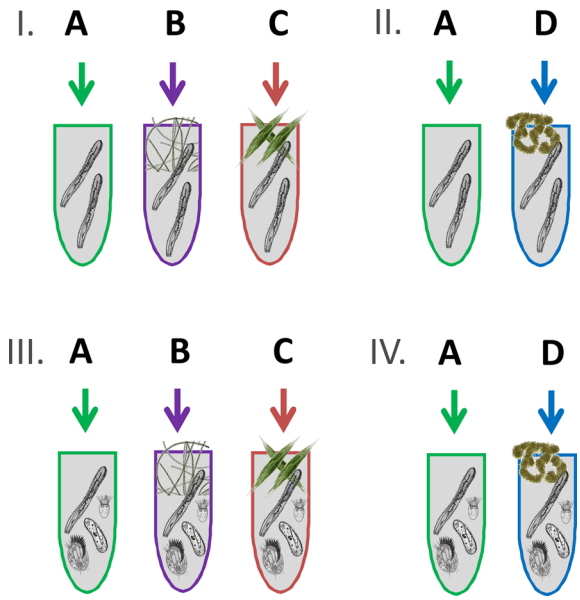


Fig. 1 Design of the experiments. I. Experiment 1. Solitary *Spirostomum* versus filamentous cyanobacteria—A: control sample: *Spirostomum* sp. + Żywiec brand mineral water; B: *Spirostomum* sp. + *P. agardhii*; C: *Spirostomum* sp. + *A. flos-aquae*; II. Experiment 2. Solitary *Spirostomum* versus chroococcal cyanobacteria: A: control sample: *Spirostomum* + Żywiec brand mineral water; D: *Spirostomum* sp. + *M. aeruginosa*; III. Experiment 3. *Spirostomum* in the ciliates assemblage versus filamentous cyanobacteria: A: control sample: *Spirostomum* in the ciliates assemblages + Żywiec brand mineral water; B: *Spirostomum* as a component of assemblage + *P. agardhii*; C: *Spirostomum* as a component of assemblage + *A. flos-aquae*; IV. Experiment 4. *Spirostomum* in the ciliates assemblage versus chroococcal cyanobacteria: A: control sample: *Spirostomum* in the ciliates assemblages + Żywiec brand mineral water; D: *Spirostomum* as a component of assemblage + *M. aeruginosa*

The control sample contained in Experiments 1 and 2: solitary *Spirostomum* cells + Żywiec brand mineral water, and in Experiments 3 and 4: ciliates assemblage + Żywiec brand mineral water.

Planktothrix agardhii (strain SAG 6.89) was obtained from the laboratory of the Department of Hydrobiology Adam Mickiewicz University in Poznań. This strain was bought from the SAG collection in Goettingen. *P. agardhii* was cultured using the method: 'batch culture' WC medium in the cell culture flasks Greiner BioOne, at the temperature of 21°C, and light intensity of 25 µmol m^{-2} s^{-1}. The biomass of cyanobacteria (*A. flos-aquae* and *M. aeruginosa*) was concentrated from 10 L of water from each water body using plankton net (mesh size 50 µm) during the blooms. The clonal strains of *Aphanizomenon flos-aquae* and *Microcystis aeruginosa* were isolated from the biomass of cyanobacteria. *A. flos-aquae* strains were maintained in modified AF-6 medium (Andersen, 2001) without the addition of nitrogen compounds, and *M. aeruginosa* strains were grown in a modified MWC medium with the addition of selenium (MWC + Se; Johansson et al., 2016) under a 12:12-h light–dark cycle and at a light intensity of approximately 30 µmol m^{-2} s^{-1} using cool, white fluorescent illumination (Philips TL-D 36W/840) at 20°C. The strains were deposited in the culture collection of algae and cyanobacteria at the Nature Research Centre in Vilnius (Lithuania) (Koreivienė et al., 2016). Those strains will be used for the further experiments.

In the present experiments, we wanted to imitate natural conditions; therefore, cyanobacteria were not purified from bacteria. Trichomes (*A. flos-aquae*) and colonies (*M. aeruginosa*) were picked up from samples and conditioned in the 1-l glass beakers in water from its natural habitat under the following conditions: daylight, regime 12:12, temperature 21°C, during one day. For this purpose, 5 l of water from the pond for *A. flos-aquae* and 5 l of water from the oxbow lake for *M. aeruginosa* were filtered using Whatman filters GF/C in order to remove all organisms. The remaining filtered water was kept in the refrigerator in sterilised glass flasks for the experiments. Before adding it to the experiments, it was kept in the laboratory atmosphere until warming up to 21°C. Trichomes and colonies of cyanobacteria were taxonomically identified, checked for conditions (healthy/not healthy/lysis of the cells) and measured under a Nikon Eclipse 80i light microscope at a magnification of × 40. The number of trichomes and colonies were counted in the 1-ml planktonic chamber with glass cover, using the same microscope equipped with a Nikon DS-Fi1 camera and the program NIS-Elements BR v. 3.22.12. The trichomes and colonies were healthy and in good condition. No lysis of cells were observed. They did not gather into clumps.

Spirostomum sp. was obtained from culture maintained in the Department of Hydrobiology of Jagiellonian University in Kraków, and was cultured in spring water (Żywiec Zdrój brand) and fed with buckwheat. Prior to the experiment, *Spirostomum* sp. cultures were maintained under the conditions: the

daylight, regime 12:12, and temperature 21°C in a Pol-Eko ILN 53/115/240 incubator. A simple assemblage of ciliates containing *Spirostomum* sp. was maintained in the laboratory of the Institute of Nature Conservation, cultured in spring water (Żywiec Zdrój brand) and fed with straw.

Because the blooms of *A. flos-aquae* and *M. aeruginosa* were created at different times, we set up two experiments as follows: Experiments 1 and 3 tested the effect only of the filamentous species, *P. agardhii* and *A. flos-aquae* (Fig. 1; I and III), and Experiments 2 and 4 tested the effect of the chroococcal species—*M. aeruginosa* (Fig. 1; II and IV).

The experiment was performed in Corning® Cell Wells containing 10 ml of medium. The medium was used as follow: (a) for control samples—spring water (Żywiec Zdrój brand); (b) for samples with *P. agardhii*—filtered water from culture containing *P. agardhii*; (c) for samples with *A. flos-aquae*—filtered water from the pond (natural habitat); and (d) for samples with *M. aeruginosa*—filtered water from the oxbow lake (natural habitat).

Every well was fed the similar number of ciliates (150 individuals of *Spirostomum* in the first and second experiments and 10–40 individuals of *Spirostomum* in the third and fourth experiments), and cyanobacteria trichomes or colonies (*P. agardhii* 50,000 trichomes ml^{-1}; *A. flos-aquae* 660,000 trichomes ml^{-1}; *M. aeruginosa* 40,000 colonies ml^{-1}), which were filled with drops of the incubated culture using pipets.

The assemblage of ciliates contained four species (*Spirostomum* sp., *Euplotes patella*, *Strobilidium* sp. and *Paramecium aurelia*-complex). The ciliates were fed at the beginning of the experiment (0 day): each well was inserted with 1 grain of buckwheat (experiments with solitary *Spirostomum*) or a blade of straw (1 cm; experiments with simple assemblages of ciliates).

Because microcystins are stable in the dark (Chorus & Bartram, 1999), in order to keep the cyanotoxins stable for as long as possible, we decided to perform the experiments in the dark. The experiments were kept at a constant temperature of 21°C. Each experiment was performed in triplicate. The experiments were conducted over 14 days. The plates with cells were constantly gently shaken. Individuals of ciliates were counted on the following days: 0, 3rd, 7th, 10th and 14th. Samples for ciliates counting were taken by micropipets (1 ml). The cells were fixed by adding Lugol's iodine solution and were not returned to the wells. The number of cells was counted under the Nikon Eclipse 80i light microscope at a magnification of × 20 in the 1-ml planktonic chamber with glass cover.

In order to obtain knowledge about the concentration of microcystins, an analysis of toxins in the cyanobacteria cells and dissolved in the water was performed. Because *A. flos-aquae* are able to produce anatoxin-a, we also checked *A. flos-aquae* for its presence. For the analysis of toxins (microcystins and anatoxin-a) in the cyanobacterial cells, HPLC analyses were conducted. Immediately after sampling, 1-l samples of water containing cyanobacteria (samplings were done at the same places and time as was done while gathering the cyanobacteria biomass), and 1-l samples of water from the *Planktothrix* culture were filtered through Whatman filters GF/C. For a detailed description of the method and equipment used for the analysis, see Kaczkowski et al. (2017).

For the microcystins dissolved in the water, we used 1-l samples of water from the *P. agardhii* culture and water collected from the pond and oxbow lake. 1-l water samples were filtered using Whatman GF/C filter papers to separate cyanobacterial cells from the water. Extracellular microcystins were concentrated in Baker C_{18} solid-phase extraction (SPE) cartridges (Deventer, Netherlands; sorbent mass: 500 mg) and eluted using 90% MeOH containing 0.1% trifluoroacetic acid (TFA) according to the methods of Meriluoto & Codd (2005).

The microcystins were analysed using an Agilent 1100 apparatus with a diode matrix (DAD) (Quinn & Keough, 2002). The concentrations of microcystins dissolved in the water were determined in the Central Laboratory of the Municipal Water and Sewage Company in Kraków, Poland. In both cases, the microcystins were identified using the microcystin standards MC-LR, MC-RR and MC-YR based on their characteristic absorption spectra and retention times.

Intra- and extracellular toxins were measured in biomass and in the same water used for the experiments.

Statistics We used a generalized linear model (GLM) to test the relationship and differences among the relationships between *Spirostomum* sp. and different species of potentially toxic cyanobacteria. The

generalized linear model (GLM) is an extension of the simple linear regression model for a continuous response variable given one or more continuous and/or categorical predictors. It includes multiple linear regression, as well as analysis of variance and analysis of covariance (Quinn & Keough, 2002). We calculated the GLM using the Poisson distribution; the dependent variables were a) cyanobacteria species and b) cyanobacteria species + day of experiment. Plots of the predicted values were created using the 'ggeffects' package (Lüdecke, 2017). Data were considered statistically significant at $P < 0.05$. All statistical analyses were performed by means of R v. 3.4.2 (R Core Team, 2017).

Results

The biomass of cyanobacteria used for the experiments showed the presence of microcystins for *P. agardhii* and *M. aeruginosa*, but not for *A. flos-aquae*. We did not find demethylated variants of microcystins in the *P. aghardii* biomass and also did not find anatoxin-a in the biomass of *A. flos-aquae* (Table 1). Microcystins were present as three analogues: microcystin-LR (MC-LR), microcystin-RR (MC-RR) and microcystin-YR (MC-YR). In the *Microcystis aeruginosa* cells, MC-LR and MC-RR were having a similar concentration, but in the *Planktothrix agardhii* cells, the highest concentration was found for MC-RR. The strain of *P. agardhii* was highly toxic.

Microcystins were also found as dissolved in the water from the *P. agardhii* culture and in the water from the oxbow lakes where *M. aeruginosa* bloomed, but not in the water from the pond where *A. flos-aquae* bloomed (Table 1).

The *Spirostomum* sp. population versus biomasses of different species of cyanobacteria

In the experiments, we examined the effects of toxic and non-toxic cyanobacteria biomasses on the population of *Spirostomum* as solitary species (Experiments 1—filamentous cyanobacteria and 2—chroococcal cyanobacteria; for the experiment description, see "Materials and methods" and Fig. 1).

The GLM showed an increase in the number of *Spirostomum* individuals in the control sample (no cyanobacteria, no toxins) and in the sample with highly toxic *P. agardhii*, but a decrease in the sample with non-toxic *A. flos-aquae* (Experiment 1, Table 2, Fig. 2A). In the second experiment, the GLM analysis showed stable populations of *Spirostomum* (Table 2) in the control sample (no cyanobacteria, no toxins), and an increase in the number of *Spirostomum* individuals in the sample with toxic *M. aeruginosa* (Table 2, Fig. 2B).

The number of *Spirostomum* individuals changed in the samples with the presence of particular cyanobacteria species (Table 2). All the differences were statistically significant except for the experiment with *A. flos-aquae* (Table 2). We also found strong statistical differences in the number of *Spirostomum* individuals in the presence of cyanobacteria species over time (Table 2). The number of *Spirostomum* individuals increased over time in the samples with *P. agardhii* and *M. aeruginosa*, but decreased in the sample with *A. flos-aquae* (Fig. 2A and 2B).

Spirostomum sp. as a component of ciliate assemblages versus cyanobacteria

In these experiments, we examined the effect of cyanobacteria biomass on *Spirostomum* as a component of a simple ciliate assemblage consisting of four species (Experiments 3—filamentous cyanobacteria and 4—chroococcal cyanobacteria).

Table 1 Concentrations of microcystins (μg/l) in the cells of cyanobacteria and dissolved in the water used for the experiments

Number of experiment	Sample	MC-RR (in cells)	MC-YR (in cells)	MC-LR (in cells)	MC-tot	
					In cells	Dissolved in water
1, 3	*P. agardhii*	282.6	11.9	29.4	323.9	29.9
1, 3	*A. flos-aquae*	0.0	0.0	0.0	0.0	0.0
2, 4	*M. aeruginosa*	14.3	2.8	14.8	31.9	0.4

Table 2 Results of the generalized linear model (GLM) for solitary *Spirostomum* sp.

Treatment	Estimate	SE	*Z*	*P*
Intercept (Control 1) Experiment 1	5.030	0.031	159.654	< **0.001**
Planktothrix 1	− 0.427	0.045	− 9.343	< **0.001**
Aphanizomenon 1	− 0.164	0.058	− 2.798	< **0.01**
Control 1: day	0.087	0.003	28.057	< **0.001**
Planktothrix 1: day	0.088	0.004	20.802	< **0.001**
Aphanizomenon 1: day	− 0.612	0.028	− 21.360	< **0.001**
Intercept (Control 2) Experiment 2	5.600	0.027	205.899	< **0.001**
Microcystis 2	− 0.161	0.034	− 4.676	< **0.001**
Control 2: day	− 0.018	0.003	− 5.525	< **0.001**
Microcystis 2: day	0.207	0.003	53.680	< **0.001**

Estimates of the GLM coefficients and their standard errors (SE) are presented. *Z*—GLM test statistic, *P*—statistical significance are emboldened

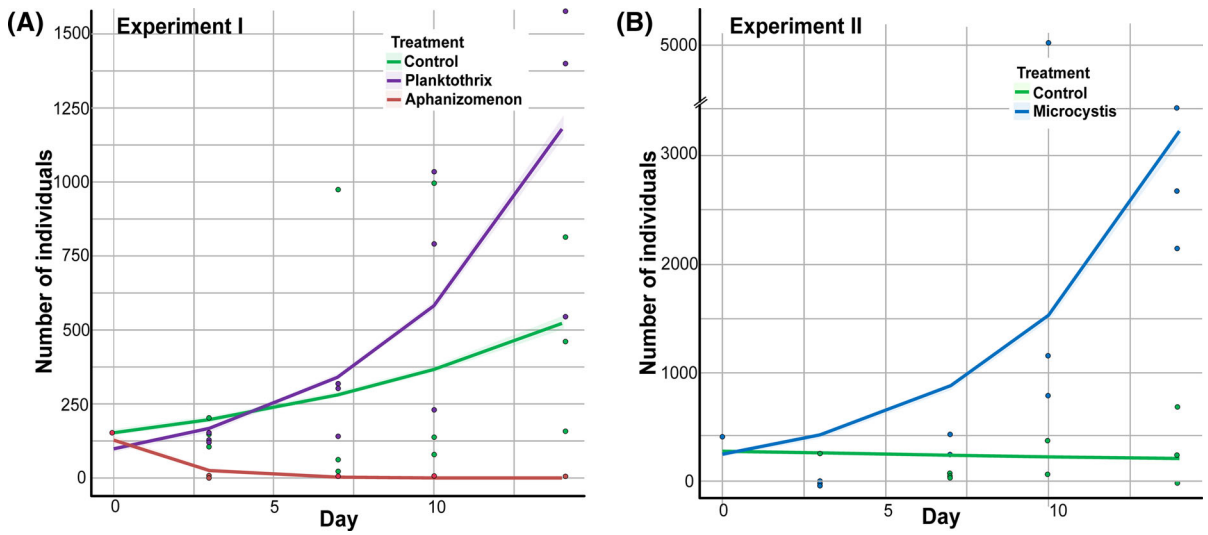


Fig. 2 The predicted number of individuals for *Spirostomum* sp. (solitary specimen): (**A**) Experiment 1: green line and dots—control sample, violet line and dots—sample with the addition of *P. agardhii*, red line and dots—sample with the addition of *A. flos-aquae*; (**B**) Experiment 2: green line and dots—control sample, blue line and dots—sample with the addition of *M. aeruginosa*

The GLM analysis showed a decrease of the *Spirostomum* population being a component of ciliate assemblages in the presence of cyanobacteria, for both toxic and non-toxic types (Fig. 3A, B).

The statistically significant differences were found for the number of *Spirostomum* individuals being a component of ciliate assemblage in the presence of *A. flos-aquae* (non-toxic) and *M. aeruginosa* (toxic), but not in the presence of *P. agardhii* (highly toxic, Table 3). A statistically significant decrease of the number of *Spirostomum* individuals in time was observed in the presence of all three species of cyanobacteria. The strongest decrease was found for *A. flos-aquae*, a weaker one for *P. agardhii*, and the weakest for *M. aeruginosa* (Table 3).

We observed weak but significant decrease of *Euplotes patella* (Table 4, Fig. 4, Supplementary material) over time in the presence of *P. agardhii* and weak and also significant increase of *E. patella* in the presence of *A. flos-aquae* and *M. aeruginosa*. For *Strobilidium* sp. (Table 5, Fig. 5, Supplementary material), we observed weak and significant decrease in the presence of *A. flos-aquae* and *P. agardhii*, but increase in the presence of *M. aeruginosa*. However, for *Paramecium aurelia*-complex (Table 6, Fig. 6, Supplementary material), we found statistically significant increase in the presence of *A. flos-aquae* and

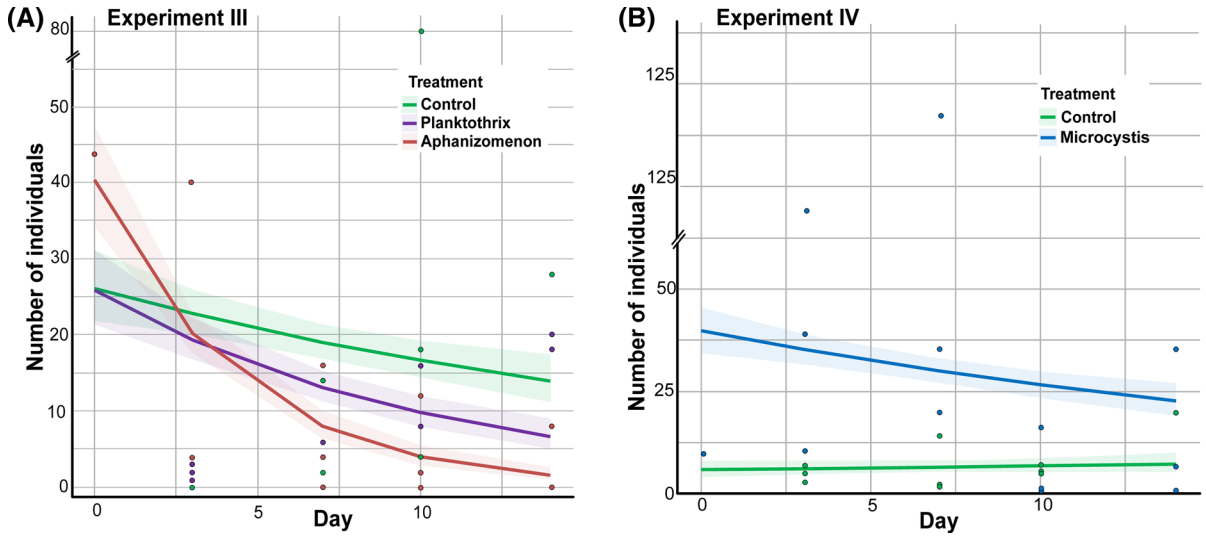


Fig. 3 The predicted number of individuals of *Spirostomum* sp. (as a component of ciliate assemblage): (A) Experiment 3: green line and dots—control sample, violet line and dots—sample with the addition of *P. agardhii*, red line and dots—sample with the addition of *A. flos-aquae*; (B) Experiment 4: green line and dots—control sample, blue line and dots—sample with the addition of *M. aeruginosa*

Table 3 Results of the generalized linear model (GLM) for *Spirostomum* sp.

Sample	Estimate	SE	*Z*	*P*
Intercept (Control 1) Experiment 3	3.259	0.090	36.187	< **0.001**
Planktothrix 1	− 0.006	0.130	− 0.047	0.962
Aphanizomenon 1	0.436	0.122	3.577	< **0.001**
Control 1: day	− 0.044	0.012	− 3.728	< **0.001**
Planktothrix 1: day	− 0.052	0.018	− 2.767	< **0.01**
Aphanizomenon 1: day	− 0.186	0.023	− 8.046	< **0.001**
Intercept (Control 2) Experiment 4	1.760	0.178	9.854	< **0.001**
Microcystis 2	1.923	0.192	9.977	< **0.001**
Control 2: day	0.015	0.020	0.745	0.456
Microcystis 2: day	0.0554	0.022	− 2.454	< **0.05**

Spirostomum sp. being a component of ciliate assemblage. Estimates of the GLM coefficients and their standard errors (SE) are presented. *Z*—GLM test statistic, *P*—statistical significance are emboldened

P. agardhii but no significant changes in the presence of *M. aeruginosa*.

Discussion

The toxic effect of cyanotoxins on vertebrates is well known, but they are also harmful for invertebrates, e.g. planktonic animals, and may be responsible for the appearance of clonal subpopulations of invertebrates such as *Daphnia* sp. (e.g. Schwarzenberger et al., 2013). Although planktonic animals are subjected to cyanotoxins because of the uptake of toxic cells, toxins dissolved in the water may also have a negative effect (Reinikainen et al., 2002), because some microcystin congeners may cross cell membranes by other mechanisms, including diffusion (Chorus & Bartram, 1999).

There are no clear responses of zooplankton to cyanotoxins, and especially of ciliates. Information in

the literature is contradictory: for example, the toxicities of *Fischerella epiphytica*, *Gleotrichia echinulata* and *Nostoc linckia* were demonstrated to *Paramecium caudatum* (Ransom et al., 1978). On the other hand, a different response of *Tetrahymena pyriformis* to microcystins (Ward & Codd, 1999) was shown, with no lethal effect of microcystins to *Nassula* sp. that grazed on toxic *Planktothrix agardhii* (Combes et al., 2013). *Nassula* sp. is also considered as a species grazing on *Aphanizomenon* and *Anabaena* and may reduce cyanobacterial blooms (Canter et al., 1990). Also, *Paramecium* cf. *caudatum* was found to be a successful grazer of toxin-producing *Cylindropsermopsis* (Fabbro et al. 2001). However, cyanobacteria–ciliates relationships might be modyfied because of differences in cyanobacteria cells, shape and size, which determine the capability of defending cyanobacteria themselves against protozoan grazers (Pajdak-Stós et al., 2001; Fiałkowska & Pajdak-Stós, 2014).

Our studies indicate that microcystins in cells and dissolved in water do not harm ciliates, even when concentrations of toxins are high. On the other hand, some studies reported that the high toxicity of extract containing anatoxin-a was harmful to *Tetrahymena thermophila* (Sierosławska et al., 2010). It illustrates that cyanotoxin–ciliate relationships should be studied as species-specific phenomena. Our studies also showed that the abundance of *Spirostomum* sp. increased in the presence of toxic cyanobacteria with a high to extremely high concentration of microcystins in the cells and also with high concentrations of microcystins dissolved in the water. On the other hand, the abundance of *Spirostomum* decreased in the presence of *A. flos-aquae*, strain which did not produce either microcystins or anatoxin-a. These results indicate that the factor that negatively affected *Spirostomum* was neither the presence nor the high concentration of toxins (microcystins, anatoxin-a). In such a case, it might be another direct factor such as the presence of different kinds of metabolites produced by *A. flos-aquae* (Řezanka & Dembitsky, 2006) that affected *Spirostomum* abundance, or some other indirect factor. In our opinion, a better explanation of this phenomenon is the indirect relation between cyanobacteria and ciliates. There are known ciliates–bacteria relationships but they are not well understood (Boscaro et al., 2018). Cyanobacterial bloom may significantly induce an increase in bacterial production (Kuosa & Kivi, 1989), which could be a food source for bacterivorous ciliates, e.g. *Spirostomum*. Bacteria growing up on different cyanobacteria species might be specific, and may inhibit the growth of specific ciliates species. Pearman et al. (2016) observed that the bacterial and protists community changed because of cyanobacterial bloom. At least, some species of ciliates appear to selectively feed upon other bacteria if offered a choice (Caron et al., 1991).

Another indirect relation might be an effect of the ability of cyanobacteria to produce biologically active compounds with cyanotoxins showing antibacterial, antiviral, antifungal, and anticancer activities (Bhateja et al., 2006). In such a case, the toxic species are responsible for destroying some adverse microorganisms (viruses, bacteria, pathogens, etc.) that promote the development of some species of ciliates. Antibacterial activity changes according to the cyanobacteria species. These bioactive substances may possibly lead to specific bacterial flora affecting the composition of bacterial communities (Skulberg, 2000) and, as a consequence, promoting the development or decrease of specific species of ciliates.

An increase of ciliates abundance in the presence of toxic cyanobacteria is possible due to some aquatic bacteria having the ability to metabolise high MC concentrations; MCs are a source of dissolved organic carbon for bacteria, which enables an increase of the bacteria's population (Sellner, 1997; Sopanen et al., 2009) and, as a consequence, ciliates. On the contrary, a lack of toxins will cause a decrease of bacteria and, furthermore, a decrease of ciliates.

Bacterial growth can be also limited when nitrogen limitation occurs (Casamatta & Wickstrom, 2000). The presence of *A. flos-aquae* indicates a deficiency of nitrogen in the water and may indicate a scarcity of bacteria, explaining the decrease of *Spirostomum* abundance.

The indirect relation between *Spirostomum* and cyanobacteria is confirmed by the experiment with simple assemblage of ciliates versus toxic/non-toxic cyanobacteria species. In this experiment, *Spirostomum* abundance decreased in the presence of toxic (*M. aeruginosa*, *P. agardhii*) and non-toxic (*A. flos-aquae*) cyanobacteria. This trend indicates an effect of competition between *Spirostomum* and the remaining smaller ciliates species (*Euplotes patella* and *Strobilidium* sp. in the presence of *M. aeruginosa*, and *Paramecium aurelia*-complex and *E. patella* in the presence of *A. flos-aquae*, but *Paramecium aurelia*-

complex in the presence of *P. agardhii*) that feed more effectively (compared with the large *Spirostomum*) on bacteria (Christoffersen et al., 1990). In a shallow lake in Denmark, the importance of small ciliates as transformers of carbon from bacteria growing after *Aphanizomenon* bloom was found (Christoffersen et al., 1990).

In general, we found that the reaction of solitary species of ciliates (*Spirostomum*) to the presence of toxic cyanobacteria is not the same as *Spirostomum* being a component of ciliates assemblage. *Spirostomum* as a single species did not show a negative response to toxic cyanobacteria and toxins dissolved in the water. On the other hand, a decrease of *Spirostomum* abundance being in the ciliate assemblage might be an effect of competition between different species of ciliates. However, our observation is contrary to the one that was shown by Tirjaková et al. (2016) in a small eutrophic pond. In those studies, *Spirostomum* dominated among ciliate communities during cyanobacterial bloom, along with *Euplotes* and *Paramecium* which were also used in our experiments. Therefore, Tirjaková et al. (2016) stated that *Spirostomum* can be considered as a good competitor in natural communities, also possibly because it is microaerophilic and can withstand under a wide range of oxic/anoxic conditions (literature cited in Tirjaková et al., 2016). In the above-mentioned studies, *Spirostomum* abundances were positively correlated with *Euplotes* abundances, which is also opposite to our observation. The differences might be explained by different food preferences and/or by a broader range of food sources in the macrocosm (e.g. the presence of different species of bacteria, etc.), and/or by different structures of ciliates communities (e.g. the presence more species in the pond), and/or by the presence of metazooplankton which may effect ciliates (in pond) or lack of it (in the experiment).

Finally, the results of our studies show that cyanobacterial blooms affected ciliates not necessarily as a direct result of toxins–ciliates relationships, but because cyanobacteria and toxins induce changes in the water food network.

Conclusions

In conclusion, toxic cyanobacteria may positively or negatively affect the *Spirostomum* population (and other ciliates species) not only because of direct toxic effect but because of indirect interaction as well.

The same species of ciliates may react in a different way to the presence of cyanobacterial blooms, not only because of cyanotoxins but also because of ciliate assemblages composition and the competition between species and the existence of species-specific interactions (e.g. ciliates–ciliates, ciliates–cyanobacteria, ciliates–bacteria).

Acknowledgements The present study was supported by the Institute of Nature Conservation, Polish Academy of Sciences as a statutory activity and as a grant for Young Scientists for J. Kosiba; and by the Institute of Botany, Polish Academy of Sciences as a grant for PhD students granted for J. Kosiba. The authors would like to thank senior researcher J. Koreivienė, PhD (Nature Research Centre, Vilnius, Lithuania) for the isolation of cyanobacterial strains and keeping them in the algal and cyanobacterial culture; the PhD students: M. Sobczyk (Jagiellonian University) and Ł. Wejnerowski (Adam Mickiewicz University in Poznan) for their sharing of ciliates and cultures of *P. agardhii*; Prof. P. Skórka and Prof. D. Chmura for consultation with statistical analyses; and E. Walusiak, PhD for help in the field samples collection. The analysis of cyanotoxins in biomass was performed by T. Jurczak, PhD (Department of Applied Ecology, University of Łódź), and in water by K. Pudaś (Municipal Water and Sewage Company, Kraków). We would also like to thank anonymous reviewers for sharing your doubts and valuable comments and suggestions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Andersen, R. A., 2001. Algal Culturing Techniques. Elsevier, Amsterdam.

Bhateja, P., T. Mathur, M. Pandya, T. Fatma & A. Rattan, 2006. Activity of blue green microalgae extracts against in vitro generated Staphylococcus aureus with reduced susceptibility to vancomycin. Fitoterapia 77: 233–235.

Boscaro, V., S. I. Fokin, G. Petroni, F. Verni, P. J. Keeling & C. Vannini, 2018. Symbiont replacement between bacteria of different classes reveals additional layers of complexity in

the evolution of symbiosis in the ciliate *Euplotes*. Protist 169(1): 43–52.

Buratti, F. M., M. Manganelli, S. Vichi, M. Stefanelli, S. Scardala, E. Testai & E. Funari, 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Archives of Toxicology 91(3): 1049–1130.

Canter, H. M., S. I. Heaney & J. W. G. Lund, 1990. The ecological significance of grazing on planktonic populations of cyanobacteria by the ciliate *Nassula*. New Phytologist 114(2): 247–263.

Carmichael, W. W., 1992. Cyanobacteria secondary metabolites—the cyanotoxins. Journal of Applied Bacteriology 72(6): 445–459.

Carmichael, W. W., 1994. The toxins of cyanobacteria. Scientific American 270: 78–86.

Caron, D. A., E. L. Lim, G. Miceli, J. B. Waterbury & F. W. Valois, 1991. Grazing and utilization of chroococcoid cyanobacteria and heterotrophic bacteria by a protozoa in laboratory cultures and a coastal plankton community. Marine Ecology Progress Series 76(3): 205–217.

Casamatta, D. A. & C. E. Wickstrom, 2000. Sensitivity of two disjunct bacterioplankton communities to exudates from the cyanobacterium *Microcystis aeruginosa* Kützing. Microbial Ecology 40(1): 64–73.

Chorus, L. & J. Bartram, 1999. Toxic Cyanobacteria in Water. E&FN Spon, London/New York.

Christoffersen, K., B. Riemann, L. R. Hansen, A. Klysner & H. B. Sørensen, 1990. Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microbial Ecology 20(1): 253–272.

Codd, G. A., C. Edwards, K. A. Beattie, L. A. Lawton, D. L. Campbell & S. G. Bell, 1995. Toxins from cyanobacteria (blue–green algae). In Wiessner, W., E. Schnepf & R. C. Starr (eds), Algae, Environment and Human Affairs. Biopress Ltd., Bristol: 1–17.

Codd, G. A., L. F. Morrison & J. S. Metcalf, 2005. Cyanobacterial toxins: risk management for health protection. Toxicology and Applied Pharmacology 203(3): 264–272.

Combes, A., M. Dellinger, S. Cadel-six, S. Amand & K. Comte, 2013. Ciliate *Nassula* sp. grazing on a microcystin-producing cyanobacterium (*Planktothrix agardhii*): impact on cell growth and in the microcystin fractions. Aquatic Toxicology 126: 435–441.

Fabbro, L., M. Baker, L. Duivenvoorden, G. Pegg & R. Shiel, 2001. The effects of the ciliate *Paramecium* cf. *caudatum* Ehrenberg on toxin producing *Cylindrospermopsis* isolated from the Fitzroy River, Australia. Environmental Toxicology 16(6): 489–497.

Ferrão-Filho, A. D. & B. Koslowsky-Suzuki, 2011. Cyanotoxins: bioaccumulation and effects on aquatic animals. Marine Drugs 9: 2729–2772.

Fiałkowska, E. & A. Pajdak-Stós, 2014. Chemical and mechanical signals in inducing *Phormidium* (Cyanobacteria) defence against their grazers. FEMS Microbiology Ecology 89(3): 659–669.

Gołdyn, R., E. Szeląg-Wasielewska, K. Kowalczewska-Madura, R. Dondajewska, A. Budzyńska, S. Podsiadłowski, P. Domek & W. Romanowicz-Brzozowska, 2010. Functioning of the Lake Rusałka ecosystem in Poznań (western Poland). Oceanological and Hydrobiological Studies 39(3): 65–80.

Havens, K. E., 1998. Size structure and energetics in a plankton food web. Oikos 81: 346–358.

Ibelings, B. W., K. Bruning, J. Jonge, K. Wolfstein, D. Pires, J. Postma & T. Burger, 2005. Distribution of microcystins in a lake foodweb: no evidence for biomagnification. Microbial Ecology 49(4): 487–500.

Johansson, K. S., K. Luhrig, J. Klaminder & K. Rengefors, 2016. Development of a quantitative PCR method to explore the historical occurrence of a nuisance microalga under expansion. Harmful Algae 56: 67–76.

Kaczkowski, Z., A. Wojtal-Frankiewicz, I. Gągała, J. Mankiewicz-Boczek, A. Jaskulska, P. Frankiewicz, K. Izydorczyk, T. Jurczak & M. Godlewska, 2017. Relationships among cyanobacteria, zooplankton and fish in sub-bloom conditions in the Sulejow Reservoir. Journal of Limnology 76(2): 380–396.

Koreivienė, J., J. Kasperovičienė, K. Savadova, J. Karosienė & I. Vitonytė, 2016. Collection of pure cultures of algae and cyanobacteria for research, teaching and biotechnological applications (Nature Research Centre, Lithuania). Botanica Lithuanica 22: 87–92.

Kosiba, J., W. Krztoń & E. Wilk-Woźniak, 2018. Effect of microcystins on proto-and metazooplankton is more evident in artificial than in natural waterbodies. Microbial Ecology 75: 293–302.

Koslowsky-Suzuki, B., A. E. Wilson & A. S. Ferrão-Filho, 2012. Biomagnification or biodilution of microcystins in aquatic foodwebs? Meta-analyses of laboratory and field studies. Harmful Algae 18: 47–55.

Kuosa, H. & K. Kivi, 1989. Bacteria and heterotrophic flagellates in the pelagic carbon cycle in the northern Baltic Sea. Marine Ecology Progress Series 53: 93–100.

Lampert, W., 1987. Laboratory studies on zooplankton–cyanobacteria interactions. New Zealand Journal Marine Freshwater Research 21(3): 483–490.

Lüdecke, D., 2017. ggeffects: Create Tidy Data Frames of Marginal Effects for 'ggplot' from Model Outputs. R package version 0.3.0. https://CRAN.R-project.org/package=ggeffects.

Mantzouki, E., M. Lürling, J. Fastner, L. de Senerpont Domis, E. Wilk-Woźniak, J. Koreiviene, et al., 2018. Temperature effects explain continental scale distribution of cyanobacterial toxins. Toxins 10(4): 156.

Maršálek, B. & L. Bláha, 2004. Comparasion of 17 biotests for detection of cyanobacterial toxicity. Environmental Toxicology 19: 310–317.

Martins, J., L. Peixe & V. M. Vasconcelos, 2011. Unraveling cyanobacteria ecology in wastewater treatment plants (WWTP). Microbial Ecology 62(2): 241–256.

Meriluoto, J. & G. A. Codd, 2005. Toxic: Cyanobacterial monitoring and cyanotoxin analysis. Acta Academiae Aboensis 65(1): 1–149.

Pajdak-Stós, A., E. Fiałkowska & J. Fyda, 2001. *Phormidium autumnale* (Cyanobacteria) defense against three ciliate grazer species. Aquatic Microbial Ecology 23(3): 237–244.

Pearman, J. K., L. Casas, T. Merle, C. Michell & X. Irigoien, 2016. Bacterial and protist community changes during a phytoplankton bloom. Limnology and Oceanography 61(1): 198–213.

Quinn, G. P. & M. J. Keough, 2002. Experimental Design and Data Analysis for Biologists. Cambridge University Press, Cambridge.

R Core Team, 2017. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ransom, R. E., T. A. Nerad & P. G. Meier, 1978. Acute toxicity of some bluegreen algae to the protozoan *Paramecium caudatum*. Journal of Phycology 14: 114–116.

Rapala, J., K. Sivonen, C. Lyra & S. I. Niemelä, 1997. Variation of microcystins, cyanobacterial hepatotoxins, in *Anabaena* spp. as a function of growth stimuli. Applied and Environmental Microbiology 63: 2206–2212.

Reinikainen, M., F. Lindvall, J. A. O. Meriluoto, S. Repka, K. Sivonen, L. Spoof & M. Wahlsten, 2002. Effects of dissolved cyanobacterial toxins on the survival and egg hatching of estuarine calanoid copepods. Marine Biology 140: 577–583.

Řezanka, T. & V. M. Dembitsky, 2006. Metabolites produced by cyanobacteria belonging to several species of the family Nostocaceae. Folia Microbiologica 51(3): 159–182.

Rohrlack, T. & P. Hyenstrand, 2007. Fate of intracellular microcystins in the cyanobacterium Microcystis aeruginosa (Chroococcales, Cyanophyceae). Phycologia 46(3): 277–283.

Schwarzenberger, A., S. D'Hondt, W. Vyverman & E. von Elert, 2013. Seasonal succession of cyanobacterial protease inhibitors and *Daphnia magna* genotypes in a eutrophic Swedish lake. Aquatic Sciences 75(3): 433–445.

Sellner, K. G., 1997. Physiology, ecology, and toxic properties of marine cyanobacterial blooms. Limnology and Oceanography 42: 1089–1104.

Sherr, E. B. & B. F. Sherr, 1994. Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microbial Ecology 28: 223–235.

Sierosławska, A., A. Rymuszka, R. Kalinowska, T. Skowroński, A. Bownik & B. Pawlik-Skowrońska, 2010. Toxicity of cyanobacterial bloom in the eutrophic dam reservoir (Southeast Poland). Environmental Toxicology and Chemistry 29(3): 556–560.

Sivonen, K. & G. Jones, 1999. Cyanobacterial toxins. In Chorus, I. & J. Bartram (eds), Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. E&FN Spon, London and New York: 43–112.

Skulberg, O. M., 2000. Microalgae as a source of bioactive molecules – experience from cyanophyte research. Journal of Applied Phycology 12: 341–348.

Sommer, U., R. Adrian, L. De Senerpont Domis, J. J. Elser, U. Gaedke, B. Ibelings, E. Jeppesen, M. Lürling, J. C. Molinero, W. M. Mooij & E. Van Donk, 2012. Beyond the Plankton Ecology Group (PEG) model: mechanisms driving plankton succession. Annual Review of Ecology, Evolution, and Systematics 43: 429–448.

Sopanen, S., P. Uronen, P. Kuuppo, C. Svensen, A. Rühl, T. Tamminen, E. Granéli & C. Legrand, 2009. Transfer of nodularin to the copepod *Eurytemora affinis* through the microbial food web. Aquatic Microbial Ecology 55(2): 115–130.

Tarczyńska, M., G. Nałęcz-Jawecki, Z. Romanowska-Duda, J. Sawicki, K. Beattie, G. Codd & M. Zalewski, 2001. Tests for the toxicity assessment of cyanobacterial bloom samples. Environmental Toxicology 16(5): 383–390.

Tirjaková, E., K. Krajčovičová, M. Illyová & P. Vd'ačný, 2016. Interaction of ciliate communities with cyanobacterial water bloom in a shallow, hypertrophic reservoir. Acta Protozoologica 3: 173–188.

Ward, C. J. & G. A. Codd, 1999. Comparative toxicity of four microcystins of different hydrophobicities to the protozoan, *Tetrahymena pyriformis*. Journal of Applied Microbiology 86: 874–882.

Wilson, A. E., O. Sarnelle & A. R. Tillmanns, 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: meta-analyses of laboratory experiments. Limnology and Oceanography 51(4): 1915–1924.

Zaccaroni, A. & D. Scaravelli, 2008. Toxicity of fresh water algal toxins to humans and animals. In Evangelista, V., L. Barsanti, A. M. Frassanito, V. Passarelli & P. Gualtieri (eds), Algal Toxins: Nature, Occurrence, Effect and Detection. Springer, Dordrecht: 45–89.

Zurawell, R. W., H. Chen, J. M. Burke & E. E. Prepas, 2005. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. Journal of Toxicology and Environmental Health. Part B 8(1): 1–37.

ARTYKUŁ 4:

Kosiba J., Krztoń W. 2021. ***Insight into the role of cyanobacterial bloom in the trophic link between ciliates and predatory copepods.*** Artykuł zaakceptowany do druku w Hydrobiologia, DOI: 10.1007/s10750-021-04780-x; IF=2,694; lista MEiN=100 pkt.

Joanna Kosiba*, Wojciech Krztoń

Insight into the role of cyanobacterial bloom in the trophic link between ciliates and predatory copepods

Institute of Nature Conservation, Polish Academy of Sciences, al. Adama Mickiewicza 33, 31-120 Kraków, Poland
*corresponding author: e-mail: kosiba@iop.krakow.pl; phone: +48 12 3703502

Conflict of Interest: The authors declare that they have no conflict of interest.

Abstract

An important group of protozooplankton, the ciliates, are a crucial component of aquatic food webs. They are the main grazers on bacteria and algae transferring carbon to higher levels of the food web (metazooplankton and fish fry). Changes in the quality and quantity of protozooplankton can modify the quality and quantity of metazooplankton, especially predatory copepods, causing changes in energy transfer and the matter cycle. Observable climate change is one of the most significant factors promoting the increase of cyanobacterial blooms. Therefore, the aim of this study was to find out how cyanobacterial blooms modify relationships between ciliates (prey) and copepods (predator), and to discover possible pathways of changes in freshwater food webs. We analysed the relationship between the biomass of predatory copepods and feeding guilds of ciliates (algivorous, bacterivorous, bacteri-algivorous). The relationship of predators biomass with algivorous and bacteri-algivorous ciliate biomasses, with a simultaneous lack of relationship with bacterivorous ciliate biomass, demonstrates that bacterial fixed carbon may be only partially contributing to the total energy passed through this link. Results demonstrated that the bloom enhanced the relationship between prey and predator. Larger and free-swimming ciliate species appear to play a greater role in energy transfer than smaller sedentary species.

Keywords:

ciliates, predatory copepods, feeding guilds, cyanobacterial blooms, shallow freshwaters

Introduction

Zooplankton species, both proto- and metazooplankton, are directly or indirectly dependent on primary producers (Pace & Lovett, 2013), which in all waterbodies are phytoplankton. One particular group included by ecologists as phytoplankton are cyanobacteria. Many species of cyanobacteria are capable of releasing cyanotoxins which can negatively affect other organisms or become concentrated via bioaccumulation, thus threatening not only aquatic but also terrestrial organisms (Papadimitriou et al., 2010; Martins et al., 2011; Paldavičienė et al., 2015). Apart from toxins, cyanobacteria are capable of secreting other harmful metabolites (e.g. Codd, 1995; Chorus, 2001; Puharinen, 2021). Additionally, it is believed that due to their low content of polyunsaturated fatty acids (PUFA) cyanobacteria are not a good food source for zooplankton (Elert et al., 2003; Wilson et al., 2006), although they do in fact produce amino acids, and also contain large amounts of proteins, carbohydrates, lipids, minerals, vitamins, and pigments (Pagels et al., 2021). Therefore, from the other hand, they might also be an attractive food for zooplankton (Wilk-Woźniak, 2020). However, it can ultimately be seen that one of the negative effects of cyanobacterial blooms is a decrease in biodiversity of water ecosystems, including in particular the zooplankton group (Kosiba et. al., 2018) and changes in their functional groups (Krztoń et al., 2019; Krztoń & Kosiba, 2020).

For many years, studies of primary producer-consumer relationships in aquatic ecosystems were conducted on an incomplete set which included two elements: phytoplankton (primary producers) and metazooplankton (primary consumers). It was only in the late 1980s and early 1990s that the "microbial loop" was discovered (e.g. Jumars et al., 1989; Weisse et al., 1990) – protozooplankton, including ciliates, were recognised as an important component of the food web (Christoffersen et al., 1990; Kalinowska, 2004; Zingel et al., 2007). However, due to difficulties in identifying ciliates, studies on this group are still in a pioneering phase and are being conducted by only a few research groups (Sherr & Sherr, 2002; Agasild et al., 2013; Boas et al., 2020; Napiórkowska-Krzebietke et al., 2021), although the results obtained are important for understanding the changes taking place in aquatic ecosystems, which are particularly sensitive ecosystems which react quickly to climate change (Firth & Fisher, 2012). One of the significant changes predicted as a result of global warming is the proliferation of toxic species of cyanobacteria, more frequent and longer-lasting blooms, with increased toxicity (Paerl & Huisman, 2009), especially in aquatic ecosystems of continental and boreal climate zones (Mantzouki et al., 2018). Therefore, studies of the mutual relationships among basic elements of the food webs of aquatic ecosystems will allow us to indicate what changes are generated by cyanobacterial blooms and how aquatic ecosystems may, in the future, react to climate changes.

Protozooplankton is a crucial component that transfers carbon to higher levels of food webs (Sherr & Sherr, 2002; Sommer et al., 2012; Agasild et al., 2013; Kosiba et al., 2017). An important group of protozooplankton, the ciliates (phylum Ciliophora; Warren et al., 2016), consists of species that are involved in the complex of interactions contributing substantially to carbon and nutrient turnover and the diet of primary consumers (Sherr & Sherr, 2007). Ciliates are one of the important part of the Harmful Algal Blooms "micronet" (Wilk-Woźniak, 2020), which might elongate the trophic chain in carbon and nutrient transfer. The ciliates are main grazers on bacteria, unicellular algae, filamentous cyanobacteria, and they may also be important in the transformation of ultrafine organic matter into a particle size range more available to metazooplankton (Porter et al., 1979). They are good food for predatory metazooplankton (Sanders & Wickham, 1993). However, the impact of predation on ciliates has been underestimated because most ciliates have soft bodies and this material is difficult to detect in the gut contents of their potential predators (Jack & Gilbert, 1997). Among metazooplankton, predatory copepods are an important group, since large-bodied predatory copepods can effectively consume ciliates (Laybourn-Parry et al., 1988; Kalinowska et al., 2015). Copepod predation on ciliates is well documented in studies of marine systems (e.g. Calbet & Saiz, 2005) but not many studies exist for freshwaters. For the most part, the simple trophic relationship between ciliates and predatory metazooplankton has been studied (Porter at al., 1979; Archbold & Berger, 1985). Eutrophic shallow lakes can be suitable for studying trophic interactions between predatory copepods and ciliates because of the dominance of a few species which replace each other during the seasonal cycle in waterbodies (Jeppesen et al., 1997, Jürgens et al., 1999). Copepods are also important because they are the main food source for fish fry, high amounts of these metazooplankton organisms are vital for fish in their period of infancy (Güher, 2002).

Changes in the quality and quantity of protozooplankton can modify the quality and quantity of metazooplankton, and especially copepods, causing changes in energy transfer and the matter cycle. Therefore, the aim of this study was to find out how cyanobacterial blooms modify the relationships between ciliates and copepods (predators). Here we explore the relationships between planktonic predators and their prey in order to determine the contribution of three different feeding guilds of ciliates in energy transfer in the aquatic food web, in non-bloom and bloom circumstances. We define feeding guilds consistent with Stroud et al. (2015): "A group of

species that exploit the same class of resources in a similar way. Guilds are a specialized kind of functional group centred on resource use and its associated processes".

We tested the following hypotheses: Hypothesis 1: The biomass of predatory copepods is significantly correlated with the biomass of ciliates during periods of bloom because during cyanobacterial blooms carbon transfer takes place from the bacteria via the ciliates to the predatory zooplankton. Hypothesis 2: During cyanobacterial blooms the biomass of bacterivorous ciliates and bacteri-algivorous ciliates is significantly higher than in non-bloom periods, but the biomass of algivorous ciliates is significantly lower than during non-bloom periods. Hypothesis 3: There will be an effect of the biomass of individual guilds of ciliates on the predatory copepods.

Here we expect that during bloom periods, the biomass of bacterivorous ciliates will have a significant effect on the biomass of predatory copepods. On the other hand, the biomass of algivorous ciliates will not have a significant effect on the biomass of predatory copepods during bloom periods, but will have a significant effect during non-bloom periods. We also expect, that the biomass of mixed-feeding bacteri-algivorous ciliates will have a significant effect on the biomass of predatory copepods in both periods.

Material and Methods

Study area and sampling procedure

The study was conducted in four, northern temperate waterbodies in Southern Poland (Kraków) (Table 1). Two of them are natural oxbow lakes (Tyniec 1, T1; Tyniec 2, T2) formed by the Vistula River, and the further two are artificial ponds (Podkamycze 1, P1; Podkamycze 2, P2). All the studied waterbodies are relatively small, covering 5.75-17.28 ha with a maximum depth from 1.9 - 3.0 m (Table 1). They are all classified as eutrophic according to Carlson & Simpson (1996) and are near each other, so the weather conditions are similar and do not affect the possible differences of functioning of the waterbodies. All of these waterbodies are prone to cyanobacterial blooms, defined as visible discolouration of water (Huisman et al., 2018), and cyanobacteria biomass exceeded 3 mg/L (Nebaeus, 1984). Samples were collected during the period May-October, in 2014 and 2017, every other week, covering periods before cyanobacterial blooms and during the blooms. Samples were collected from the central point of each waterbody, at a depth of 1 m. In total, 101 sample sets were collected for biological analyses: phytoplankton with cyanobacteria, protozooplankton (ciliates) and metazooplankton (rotifers, cladocerans, copepods) but for further analysis only the group of predatory copepods was used. Basic physical and chemical parameters (max. depth, water temperature, pH, conductivity, oxygen saturation and concentration of chlorophyll *a*) of the studied waterbodies were measured *in situ* with a YSI 6600 V2 Multiparameter Probe.

Samples for phytoplankton, ciliates, and metazooplankton were taken separately. The samples were taken using a 5-L Ruttner sampler from a volume of 10 L of water and were concentrated with a planktonic net (10 μm for cyanobacteria and ciliates and 50 μm for metazooplankton). The samples for quantitative analyses were fixed (Lugol's solution for phytoplankton and ciliates, and 4% formaldehyde for metazooplankton). Phytoplankton, excluding of cyanobacteria, were identified with the use of the keys listed in Wilk-Woźniak (2009), and counted in a modified chamber (0.15 ml). Cyanobacteria were identified using the keys: Komárek & Anagnostidis (1998), Komárek & Anagnostidis (2005), Komárek (2013). Phytoplankton biomass was calculated as a biovolume by comparing specimens with their geometrical shapes according to Rott (1981). The ciliates were identified in 1 mL chambers with glass covers according to Foissner & Berger (1996; 1999) and the total biomass of ciliates (mg/L) was calculated according to Putt & Stoecker (1989), Jerome et al. (1993), Wiąckowski et al. (1994a). The averages of three counts were calculated. Metazooplankton samples were analysed in 0.5 mL chambers. The averages of five repetitions were counted. Species were identified according to Ejsmont-Karabin et al. (2004) and Błędzki & Rybak (2016). Dry weight was calculated using a regression equation defining the body length and weight for each species (Cummins et al., 1969; Dumont et al., 1975; Ruttner-Kolisko, 1977; Pearsson & Ekbohm, 1980). Because phytoplankton and protozooplankton were calculated as fresh biomass, metazooplankton dry mass was recalculated according to the index proposed by Bottrell et al. (1976). All microscopy analysis of phytoplankton, ciliates, and metazooplankton employed a Nikon H550L light microscope at ×40–×1000.

Ciliates were divided into feeding guilds (Hopkins et al. 1993; definition of guilds in Stroud et al., 2015) based on trophic groups separated in Kosiba et al. (2017) as: algivorous ciliates, bacterivorous ciliates and mixed feeding ciliates (algae and bacteria; Kosiba et al., 2017; Krztoń & Kosiba, 2020; Table 2).

Statistical Analysis

GLM was used for testing the relationship between total biomass of predatory copepods and total biomass of ciliates. Further, we analysed differences in biomass of predatory copepods and biomass of guilds of ciliates between the periods with and without bloom. Counts were expressed as median values, with 25th and 75th percentiles in a box plot diagram. Next, we also used a generalized linear model (GLM) to test the model of the relationship between the biomass of predatory copepods and feeding guilds of ciliates: a) algivorous ciliates, b) bacterivorous ciliates, c) mixed feeding (algae and bacterivorous) ciliates. Analyses were done for periods with and without blooms and also tested the alone effect of cyanobacterial bloom on the biomass of predatory metazoans. Next, we tested the interaction how the bloom affects the relationship between predatory metazoans and their prey.All statistical analyses were performed by means of R-studio, R v. 4.0.2 (R Core Team, 2020).

Results

We found cyanobacterial blooms in all four waterbodies. The length of duration of these phenomena was different in each waterbody, however the shortest lasted 1 month and the longest 5 months. Altogether, we collected 101 samples: 31 samples during a cyanobacterial bloom, and 70 samples in the periods without cyanobacterial blooms. 18 species of ciliates and 10 species of predatory metazooplankton (copepods) were identified in the study material. Among predatory copepods the following species were present: *Acanthocyclops robustus* Sars, 1863, *Acanthocyclops trajani* Mirabdullayev & Defaye, 2004, *Acanthocyclops venustus* Norman & Scott T., 1906, *Cyclops abyssorum* Sars, 1863, *Cyclops strenuus* Fischer, 1851, *Cyclops vicinus* Uljanin, 1875, *Mesocyclops leuckarti* Claus, 1857, *Thermocyclops crassus* Fischer, 1853, *Thermocyclops dybowskii* Landé, 1890 and *Thermocyclops oithonoides* Sars, 1863. The species of ciliates divided into feeding guilds were presented in Table 2.

The total biomass of predatory copepods was positively linked to the total biomass of ciliates, both, during bloom periods ($p=0.0168$) and in non-bloom periods ($p=0.0961$). However, further analyses did not show statistically significant differences between the biomass of any groups of planktonic animals (bacterivorous ciliates, algivorous ciliates, mixed type feeding ciliates, predatory copepods) in the periods without and with blooms (Fig. 1).
Statistical analysis (GLM) showed a positive significant effect of the biomass of two feeding guilds: 1) algivorous ciliates ($p =0.029$) and 2) bacteri-algivorous ciliates ($p=0.023$) on the biomass of predatory copepods, during periods without cyanobacterial blooms (Table 3). For the cyanobacterial bloom effect alone, we did not find a significant relationship with predatory metazoans biomass. For algivorous and mixed-feeding ciliates (in interaction with present of cyanobacterial bloom) the bloom enhanced the relationship with predators (as evidenced by the increase in estimate for the groups tested). In contrast, no statistically significant effect of the biomass of bacterivorous ciliates on the biomass of predatory copepods was noted, either during periods of bloom or periods without bloom, either in interaction (Table 3).

Discussion

The role of ciliated protozoans in fluxing primary production during cyanobacterial blooms has been already indicated, however this topic is still understudied and it is not fully understood (Ger et al., 2016). Ciliates are an important component of the ‘microbial loop’, transferring matter and energy during cyanobacterial blooms from bacteria to metazooplankton predators (Johnke et al., 2017) and therefore contributing to the biogeochemical cycling of nutrients (Berman et al., 1987). Feeding the bacteria and algae (Engström-Öst et al., 2013; Gaedke et al., 2002) protozooplankton transfers the carbon to metazoans. Ciliates may be grazed upon by predatory metazoans (Gifford, 1991; Wickham, 1995), which may have essential importance during cyanobacterial bloom periods (Ger et al., 2016).

Predatory metazooplankton are able to reduce the abundance of ciliate communities and depend on the potential of reproduction and the mortality rate of ciliates which are imposed by the predators (Gilbert & Jack, 1993; Wiąckowski et al., 1994b). Wickham & Gilbert (1991; 1993) showed that metazooplankton suppress ciliates through predation and mutual competition and that ciliates are an important source of nutrients and may thus facilitate utilization of organic carbon by predatory metazooplankton (Stoecker & Capuzzo, 1990). Since eutrophication tends to strengthen coupling between protozooplankton and metazooplankton (Ger et al., 2016), we expected to find a significant relationship between the total biomass of ciliates and the total biomass of predatory metazooplankton (copepods) during cyanobacterial blooms. Meanwhile, the results of our studies demonstrated a significant relationship between both groups during both periods (bloom and non-bloom). This demonstrates that the ciliate-copepod link is important for aquatic ecosystems, independently of bloom occurrence conditions.

Therefore, we have taken a deeper look into the ciliate-copepod link, with emphasis on the feeding preferences of ciliates (feeding guilds). We expected a special importance of bacterivorous ciliates, considering a study by Christoffersen et al. (1990), who found that ciliates under a body size of 50 μm removed 19–39% of bacterial production. Therefore, we expected that differences in biomass of bacterivorous ciliates would be significantly higher in the bloom period in comparison with the non-bloom period. However, our results did not support our thesis. Moreover, the biomass of other ciliate guilds (algivorous and bacteri-algivorous type feeding) and predatory copepods did not demonstrate statistically significant differences between periods with and without bloom. Furthermore, we did not find any statistically significant effect of total biomass of ciliates on predatory copepod biomass, independently of bloom or non-bloom periods. However, we found such a positive effect for the biomass of algivorous and mixed type feeding ciliates on predatory copepod biomass. Therefore, we decided to include interactions in the GLM analyses to test how the bloom affects the relationship between predatory metazoans and their prey. During the bloom periods, this effect was stronger compared to non-bloom periods. It seems that the bloom enhanced the relationship between prey and predator. We believe that the copepods showed little interest in the small bacterivorous ciliates when they had access to a better food source in the form of larger species of ciliates from other guilds (mixed feeding and algivorous). It appears that the size of the ciliate, as a food unit, is of considerable significance. In studies to date, all species of zooplankton have showed the highest preferences for grazing for ciliates in the size range 20–55 μm (Adrian & Schneider-Olt, 1999). In our studies, algivorous and bacteri-algivorous ciliates consisted of bigger species (35-100 μm), whereas bacterivorous were of a smaller size (20-60 μm). Therefore, the predatory metazooplankton preferred ciliates not belonging to the bacterivorous guild as a food source, which explains the relationships observed by us. Of importance in grazing preferences may also be the capacity for movement, which up until now has been seen as a better life strategy for avoiding predators (Bray, 2001). The mechanism of prey capture (Kiørboe, 2011) of metazoan predators included in our study (cyclopoid copepods) is ambush feeding. Therefore, we explfain our results from the perspective of the possibility of prey detection. Hydrodynamic disturbances allow predators to detect motile prey and then rapidly jump. Studies conducted to date have suggested that ciliates may weaken the predatory behaviour of metazooplankton by actively fleeing (Gilbert & Jack, 1993; Gilbert, 1994; Burns & Gilbert, 1993; Broglio et al., 2001). However, the results of our study may demonstrate the reverse.

We compared the way of moving (do they swim or not – Table 2; Lisicki et al., 2019) and the type of food (bacterivorous, algivorous, mixed type of feeding) of chosen species of ciliates. We found that algivorous and mixed type feeding ciliates are free-swimming species, but the majority of bacterivorous ciliates are sedentary (*Epistylis* sp., *Opercularia* sp., *Vorticella convallaria* Linnaeus, 1758 and *Vorticella* sp.). However, some of bacterivorous ciliates may also graze while swimming on bacteria formed on organic matter. Species belonging to algi- and bacteri-algivorous (mixed type feeding) guilds moved through the water column relatively quickly, making them easy to recognize by the ambush feeding predatory copepods. The ability to recognize prey due to water movements generated by this prey is all the more significant when the aquatic environment is of low visibility, a characteristic feature during periods of cyanobacterial bloom. The actively grazing copepods are thus quite able to feed themselves in environments of low water visibility by sensing motion in the water.

Therefore, larger body size and the ability to move quickly paradoxically may not be the best strategy for predator avoidance. According to Kiørboe & Visser (1999), larger copepods usually select larger prey, because they make stronger disturbances in the water, so they are easier to perceive and to be caught by the copepods, which use mechanoreceptors to detect their prey. Wiąckowski et al. (1994b) have similar observations on larger ciliates, which were more often attacked by adult predatory copepods. Usually, predatory copepods behave in ways predicted by the optimal foraging theory and select prey that maximize their trophic benefit (Wiąckowski & Kocerba-Soroka, 2017). In a system such as the one we studied, the ability to move actively may be a worse survival strategy than sedentary foraging, as it generates motion in the water which informs predators of the presence of prey and requires greater energy inputs.

Our results suggest that the ciliate–predatory copepod link in eutrophic aquatic ecosystems is a significant pathway of energy flow, functioning both under non-bloom and bloom periods. The relationship of predatory copepod biomass with algivorous and bacteri-algivorous ciliates biomasses, with a simultaneous lack of relationship with bacterivorous ciliate biomass, suggests that bacterial fixed carbon may be only partially contributing the total energy passed through this link. An important factor controlling this process might be the life strategies and behaviour of ciliates. Larger and free swimming ciliate species appear to play a greater role in energy transfer than smaller and sedentary species. Further more detailed information on the mutual relationship between ciliates and predatory copepods may be provided by study involving stable isotopes.

Conclusion

Hypothesis 1 was not confirmed by this study as there was a significant relationship between ciliate and copepod biomasses both under bloom conditions and in periods without blooms. Hypothesis 2 was not confirmed by this study as individual guilds of ciliate biomass did not display statistically significant differences between the two periods (bloom / non-bloom). Hypothesis 3 and our expectations were partially confirmed by the study. The biomass of bacterivorous ciliates did not significantly affect the biomass of predatory copepods. The biomass of algivorous ciliates affected the biomass of predatory copepods in both periods (bloom/non-bloom), though this effect was stronger in the bloom period than in the period without bloom. The biomass of mixed feeding bacteri-algivorous ciliates had a significant effect on the biomass of predatory copepods in both periods, but also stronger during the bloom. This proves that the bloom enhanced the relationship between prey and predator. The results suggested that the larger, more mobile ciliates (primarily those from the algivorous and bacteri-algivorous guilds) are preyed upon by copepods, while smaller, more sedentary ciliates (primarily bacterivorous) most likely constitute a less desirable food source for predatory copepods.

Acknowledgements

We thank Dr. Edward Walusiak for assistance with sampling as well as Prof. Elżbieta Wilk-Woźniak for their valuable and constructive suggestions during the development of this work. We would also like to thank anonymous reviewers and Editor for sharing your doubts and valuable comments and suggestions.

Funding Information

This study was supported by the Institute of Nature Conservation, Polish Academy of Sciences, through its statutory fund and a grant for young scientists and Ph.D. candidates, for the PhD students: Joanna Kosiba and Wojciech Krztoń.

References

Adrian, R. & B. Schneider-Olt, 1999. Top-down effects of crustacean zooplankton on pelagic microorganisms in a mesotrophic lake. Journal of Plankton Research 21(11): 2175-2190.

Agasild, H., P. Zingel, K. Karus, K. Kangro, J. Salujoe & T. Noges. 2013. Does metazooplankton regulate the ciliate community in a shallow eutrophic lake? Freshwater Biology 58(1): 183-191.

Archbold J. H. G. & J. Berger, 1985. A qualitative assessment of some metazoan predators of Halteria gradinella, a common freshwater ciliate. Hydrobiologia 126: 97-102.

Berman T. M. Nawrocki, G. T. Taylor , & D. M. Karl, 1987. Nutrient flux between bacteria, bacterivorous nanoplanktonic protists and algae. Marine microbial food webs 2(2): 69-81.

Błędzki, L. A & J. I. Rybak, 2016. Freshwater crustacean zooplankton of Europe: Cladocera & Copepoda (Calanoida, Cyclopoida) key to species identifcation, with notes on ecology, distribution, methods and introduction to data analysis. Springer, New York.

Boas, L. D. A. V., M. V. X. Senra, K. Fernandes, A. M. da Anunciação Gomes, R. J. P. Dias, E. Pinto & A. L. Fonseca, 2020. In vitro toxicity of isolated strains and cyanobacterial bloom biomasses over Paramecium caudatum (ciliophora): Lessons from a non-metazoan model organism. Ecotoxicology and Environmental Safety 202: 110937;

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson& T. Weglenska, 1976. A review of some problems in zooplankton production studies. Norwegian Journal of Zoology. 24: 419-456.

Bray, D., 2001. Cell movements: from molecules to motility. Garland Science.

Broglio, E., M. Johansson & P. R. Jonsson, 2001. Trophic interaction between copepods and ciliates: effects of prey swimming behavior on predation risk. Marine Ecology Progress Series 220: 179-186.

Burns C.W. & J. J. Gilbert, 1993. Predation on ciliates by freshwater calanoid copepods: rates of predation and relative vulnerabilities of prey. Freshwater Biology 30: 377-393.

Calbet A, & E. Saiz, 2005. The ciliate-copepod link in marine ecosystems. Aquatic Microbial Ecology 38(2): 157-167.

Carlson, R. E. & J. Simpson, 1996. A coordinator's guide to volunteer lake monitoring methods. North American Lake Management Society, Madison.

Chorus, I., 2001. Cyanotoxins: Occurrence, Causes, Consequences. Berlin: Springer-Verlag.

Christoffersen, K., B. Riemann, L. R. Hansen, A. Klysner, & H. B. Sørensen, 1990. Qualitative importance of the microbial loop and plankton community structure in a eutrophic lake during a bloom of cyanobacteria. Microbial Ecology 20: 253–272.

Codd, G. A., 1995. Cyanobacterial toxins: occurrence, properties and biological significance. Water Science Technology 32: 149-156.

Cummins K. W., R. R. Costa, R. E. Rowe, G. A. Moshiri, R. M. Scanlon & R. K. Zajdel, 1969. Ecological energetics of a natural population of the predaceous zooplankter *Leptodora kindtii*. Oikos 20: 189-223. doi: 10.2307/3543189.

Dumont, H. J., I. Van de Velde & S. Dumont, 1975. The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton, and benthos of continental waters. Oecologia (Berlin): 19: 75-97. doi: 10.1007/BF00377592.

Ejsmont-Karabin, J., S. Radwan & I. Bielańska-Grajner, 2004. Rotifers. Monogononta-atlas of species. Polish freshwater fauna. University of Łódź, Łódź: 77-447.

Elert, E. V., D. Martin-Creuzburg & J. R. Le Coz, 2003. Absence of sterols constrains carbon transfer between cyanobacteria and a freshwater herbivore (*Daphnia galeata*). Proceedings of the Royal Society of London - B. Biological Sciences 270(1520), 1209–1214. doi:10.1098/ RSPB.2003.2357.

Engström-Öst, J., R. Autio, O. Setälä, S. Sopanen & S. Suikkanen, 2013. Plankton community dynamics during decay of a cyanobacteria bloom: a mesocosm experiment. Hydrobiologia 701: 25-35.

Firth, P. & S. G., Fisher, 2012. Global climate change and freshwater ecosystems. Springer Science & Business Media.

Foissner, W. & H. Berger, 1996. A user-friendly guide to the ciliates *(Protozoa, Ciliophora)* commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. Freshwater Biology 35(2): 375-482.

Foissner, W. & H. Berger, 1999. Schaumburg J. Identification and ecology of limnetic plankton ciliates. München: Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft.

Gaedke, U., S. Hochstädter & D. Straile, 2002. Interplay between energy limitation and nutritional deficiency: empirical data and food web models. Ecological Monographs 72(2): 251-270.

Ger, K. A., P. Urrutia-Cordero, P. C. Frost, , L. A. Hansson, O. Sarnelle, A. E. Wilson & M. Lürling, 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic world. Harmful algae 54: 128-144.

Gifford, D. J., 1991. The protozoan-metazoan trophic link in pelagic ecosystems. Journal of Protozoology 38: 81-86.
Gilbert, J. J. & J. D. Jack, 1993. Rotifers as predators on small ciliates. Hydrobiologia 255/256: 247-253.
Gilbert, J. J., 1994. Jumping behavior in the Oligotrich ciliate Strombilidium velox and Halteria grandinella, and its significance as a defence against rotifer predators. Microbial Ecology 27:189-200.
Güher, H., 2002. Cladocera and Copepoda (Crustacea) Fauna of Lake Terkos (Durusu). Turkish Journal of Zoology 26(3): 283-288.
Hopkins, T. L., T. M. Lancraft, J. J. Torres, & J. Donnelly, 1993. Community structure and trophic ecology of zooplankton in the scotia sea marginal ice zone in winter (1988). Deep Sea Research Part I: Oceanographic Research Papers 40(1): 81–105. doi:10.1016/0967-0637(93)90054-7.
Huisman, J., G. A. Codd, H. W. Paerl, B. W. Ibelings, J. M. Verspagen, P. M. Visser, 2018. Cyanobacterial blooms. Nature Reviews Microbiology 16(8): 471-483.
Jack, J. D. & J. J. Gilbert, 1997. Effects of Metazoan Predators on Ciliates in Freshwater Plankton Communities 1. Journal of Eukaryotic Microbiology 44(3): 194-199.
Jeppesen, E., J. P. Jensen, M. Søndergaard, T. Lauridsen, L. J. Pedersen & L. Jensen, 1997. Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. In Shallow Lakes' 95: 151-164. Springer, Dordrecht.
Jerome, C. A., D. J. S. Montagnes & F. J. R. Taylor, 1993. The effect of the quantitative protargol stain and Lugols and Buinos fixatives on cell size: a more accurate estimate of ciliate species biomass. Journal of Eukaryotic Microbiology 40: 254-259. doi: 10.1111/j.1550-7408.1993.tb04913.x.
Johnke, J., A. Chatzinotas, H. Harms & J. Boenigk, 2017. Killing the killer: predation between protists and predatory bacteria. FEMS Microbiology Letters 364: fnx089.
Jumars, P. A., D. L. Penry, J. A. Baross, M. J. Perry & B.W. Frost, 1989. Closing the microbial loop: dissolved carbon pathway to heterotrophic bacteria from incomplete ingestion, digestion and absorption in animals. Deep Sea Research Part A. Oceanographic Research Papers 36(4): 483-495.
Jürgens, K., O. Skibbe & E. Jeppesen, 1999. Impact of metazooplankton on the composition and population dynamics of planktonic ciliates in a shallow, hypertrophic lake. Aquatic Microbial Ecology 17(1): 61-75.
Kalinowska K., J. Ejsmont-Karabin, M. Rzepecki, I. Kostrzewska- Szlakowska, I. Y. Feniova, A. Palash & A. R. Dzialowski, 2015. Impacts of large-bodied crustaceans on the microbial loop. Hydrobiologia 744(1): 115-125.
Kalinowska, K., 2004. Bacteria, nanoflagellates and ciliates as components of the microbial loop in three lakes of different trophic status. Polish Journal of Ecology 1(52).
Kiørboe, T. & A. W. Visser, 1999. Predator and prey perception in copepods due to hydromechanical signals. Marine Ecology Progress Series 179: 81-95.
Kiørboe, T., 2011. How zooplankton feed: mechanisms, traits and trade-offs. Biological Reviews 86(2): 311-339.
Komárek, J., 2013. Cyanoprokaryota, 3. Teil / 3rd part: Heterocytous Genera. Süsswasserflora von Mitteleuropa 19/3, Springer-Verlag, Berlin, Germany.
Komárek, J. & K. Anagnostidis, 1998. Cyanoprokaryota, 1. Teil: Chroococcales. Süsswasserflora von Mitteleuropa 19/1, Gustav Fischer Verlag, Stuttgart, Germany.
Komárek, J. & K. Anagnostidis, 2005. Cyanoprokaryota, 2. Teil/ 2nd Part: Oscillatoriales. Süsswasserflora von Mitteleuropa 19/2, Elsevier, Spektrum Akademischer Verlag, Heidelberg, Germany
Kosiba, J., E. Wilk-Woźniak & W. Krztoń, 2018. Effect of Microcystins on Proto- and Metazooplankton is more evident in artificial than in natural waterbodies. Microbial Ecology 75: 293-302, doi: 10.1007/s00248-017-1058-z.
Kosiba, J., E. Wilk-Woźniak, W. Krztoń, M. Strzesak, A. Pociecha, E. Walusiak., K. Pudaś & E. Szarek-Gwiazda, 2017. What underpins the trophic networks of the plankton in shallow oxbow lakes?. Microbial Ecology 73(1): 17-28. DOI: 10.1007/s00248-016-0833-6.
Krztoń, W. & J. Kosiba, 2020. Variations in zooplankton functional groups density in freshwater ecosystems exposed to cyanobacterial blooms. Science of the Total Environment 730: https://doi.org/10.1016/j.scitotenv.2020.139044.
Krztoń, W., J. Kosiba, A. Pociecha & E. Wilk-Woźniak, 2019. The effect of cyanobacterial blooms on bio- and functional diversity of zooplankton communities. Biodiversity and Conservation 28(7): 1815-1835. doi.org/10.1007/s10531-019-01758-z.
Laybourn-Parry, J., B. A. Abdullahi & S. Tinson, 1988. Temperature dependent energy partitioning in the benthic copepods Acanthocyclops viridis and Macrocyclops albidus. Canadian Journal of Zoology 66: 2709-2714.
Lisicki, M., M. F. Velho Rodrigues, R. E. Goldstein & E. Lauga, 2019. Swimming eukaryotic microorganisms exhibit a universal speed distribution. eLife 8. e44907. https://doi.org/10.7554/eLife.44907.

Mantzouki, E., M. Lürling, J. Fastner, L. de Senerpont Domis, E. Wilk-Woźniak, J. Koreivienė,... & T. P. Warming, 2018. Temperature effects explain continental scale distribution of cyanobacterial toxins. Toxins 10(4): 156.

Martins, J., L. Peixe & V. M. Vasconcelos, 2011. Unraveling cyanobacteria ecology in wastewater treatment plants (WWTP). Microbial Ecology 62(2): 241-256.

Napiórkowska-Krzebietke, A., K. Kalinowska, E. Bogacka-Kapusta, K. Stawecki & P. Traczuk, 2021. Persistent blooms of filamentous cyanobacteria in a cormorant-affected aquatic ecosystem: Ecological indicators and consequences. Ecological Indicators 124: 107421.

Nebaeus, M., 1984. Algal water-blooms under ice-cover: with 1 figure and 2 tables in the text. Verhandlungen der Internationalen Vereinigung fur Theoretische und Angewandte Limnologie 22(2): 719-724.

Pace, M. L. & G. Lovett, 2013. Primary production: the foundation of ecosystems. In Weathers, K. C., D. L. Strayer & G. E. Likens (eds) Fundamentals of ecosystem science. Second edition. Elsevier Academic Press, London, UK, 27-51.

Paerl, H. W. & J. Huisman, 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environmental Microbiology Reports 1(1): 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x.

Pagels, F., V. Vasconcelos & A. C. Guedes, 2021. Carotenoids from Cyanobacteria: Biotechnological Potential and Optimization Strategies. Biomolecules 11(5): 735.

Paldavičienė, A., A. Zaiko, H. Mazur-Marzec & A. Razinkovas-Baziukas, 2015. Bioaccumulation of microcystins in invasive bivalves: a case study from the boreal lagoon ecosystem. Oceanologia 57(1): 93-101.

Papadimitriou, T., I. Kagalou, V. Bacopoulos & I. D. Leonardos, 2010. Accumulation of microcystins in water and fish tissues: an estimation of risks associated with microcystins in most of the Greek Lakes. Environmental toxicology *25*(4): 418-427.

Pearsson, G. & G. Ekbohm, 1980. Estimation of dry-weight in zooplankton populations—methods applied to crustacean populations from lakes in the Kuokkel Area, Northern Sweden. Archiv für Hydrobiologie. 89: 225-246.

Porter, K. G., M. L. Pace & J. F. Battey, 1979. Ciliate protozoans as links in freshwater planktonic food chains. Nature 277(5697): 563-565.

Puharinen, S. T, 2021. Good Status in the Changing Climate?—Climate Proofing Law on Water Management in the EU. Sustainability 13(2): 517.

Putt, M. & D. K. Stoecker, 1989. An experimentally determined carbon: volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnology and Oceanography 34: 1097-1103. doi: 10.4319/lo.1989.34.6.1097.

R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria URL. https://www.R-project.org/.

Rott, E., 1981. Some results from phytoplankton counting intercalibrations. Schweizerische Zeitschrift fur Hydrologie 43: 34-62.

Ruttner-Kolisko, A., 1977. Suggestions for biomass calculation of plankton rotifers. Archiv für Hydrobiologie. Beihefte, Ergebnisse der Limnologie 8(7): 1-76.

Sanders, R. W. & S. A. Wickham, 1993. Planktonic protozoa and metazoa: predation, food quality and population control. Marine Microbial Food Webs 7: 197-223.

Sherr, E. B. & B. F. Sherr, 2007. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. Marine Ecology Progress Series 352: 187-197.

Sherr, E. B. & B. F. Sherr, 2002. Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology 81: 293-308.

Stoecker, D. K. & J. M. Capuzzo, 1990. Predation on protozoa: its importance to zooplankton. Journal of Plankton Research 12: 891-908.

Stroud, J. T., M. R. Bush, M. C. Ladd, R. J. Nowicki, A. A. Shantz & J. Sweatman, 2015. Is a community still a community? Reviewing definitions of key terms in community ecology. Ecology and evolution 5(21): 4757-4765.

Warren, A., G. F. Esteban & B. J. Finlay, 2016. Chapter 2 - Protozoa. In Thorp J. H. & D. C. Rogers (eds), Thorp and Covich's Freshwater Invertebrates (Fourth Edition), Academic Press 5-37, ISBN 9780123850287, https://doi.org/10.1016/B978-0-12-385028-7.00002-0.

Weisse T., H. Müller, R. M. Pinto-Coelho, A. Schweizer, D. Springmann & G. Baldringer, 1990. Response of the microbial loop to the phytoplankton spring bloom in a large prealpine lake. Limnology and Oceanography 35: 781-794.

Wiąckowski, K. & W. Kocerba-Soroka, 2017. Selective predation by a harpacticoid copepod on ciliates in phytotelmata: a laboratory experiment. Hydrobiologia 790: 13-22. https://doi.org/10.1007/s10750-016-2941-1.

Wiąckowski, K., A. Doniec & J. Fyda, 1994a. An empirical study of the effect of fixation on ciliate cell volume. Marine Microbial Food Webs 8(1–2): 59-69.
Wiąckowski, K., M. T. Brett & C. R. Goldman, 1994b. Differential effects of zooplankton species on ciliate community structure. Limnology and Oceanography 39: 486-492.
Wickham, S. A. & J. J. Gilbert, 1991. Relative vulnerabilities of natural rotifer and ciliate communities to cladocerans: laboratory and field experiments. Freshwater Biology 26(1): 77-86.
Wickham, S. A. & J. J. Gilbert, 1993. The comparative importance of competition and predation by Daphnia on ciliated protists. Archiv für Hydrobiologie 126(3): 289-313.
Wickham, S. A. 1995. Trophic relations between cyclopoid copepods and ciliated protists: complex interactions link the microbial and classic food webs. Limnology and Oceanography 40(6): 1173-1181.
Wilk-Woźniak E., 2009. Zmiany populacyjne w zbiorowiskach glonów planktonowych oraz ich strategie życiowe w warunkach ekosystemów wodnych sztucznie zmienionych (Changes in phytoplankton communities and the life strategies of planktonic algae in artificially changed aquatic ecosystems), Studia Naturae 55: 1-132 (in Polish).
Wilk-Woźniak, E., 2020. An introduction to the 'micronet'of cyanobacterial harmful algal blooms (Cyano-HABs): cyanobacteria, zooplankton and microorganisms: a review. Marine and Freshwater Research 71(5): 636-643.
Wilson, A. E., O. Sarnelle & A. R. Tillmanns, 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: meta-analyses of laboratory experiments. Limnology and Oceanography 51(4): 1915-1924.
Zingel, P., H. Agasild, T. Nõges & V. Kisand, 2007. Ciliates are the dominant grazers on pico- and nanoplankton in a shallow, naturally highly eutrophic lake. Microbial Ecology 53: 134-142.

Tables

Table 1 Basic information about the studied waterbodies

	PODKAMYCZE 1	**PODKAMYCZE 2**	**TYNIEC 1**	**TYNIEC 2**
Geographical coordinates	50°05'11''N, 19°50'01.6''E	50°04′59.6″N, 19°50′05.4″E	50°01'47''N, 19°49'39.8''E	50°01'28.1"N 19°48'47.7"E
Type of waterbody	artificial	artificial	natural	natural
Trophic class	eutrophic	eutrophic	eutrophic	eutrophic
Max depth (m)	3.0 m	2.5 m	3.0 m	1.9 m
Area (ha)	16.82 ha	17.28 ha	5.75 ha	8,61 ha
Period with cyanobacterial bloom (cyanobacteria biomass >= 3 mg/L)	**2014:** July **2017**: from August to September	**2014:** from June to October **2017**: from July to October	**2014:** from August to October **2017**: from September to October	 **2017**: from July to October
Mean cyanobacteria biomass [mg/L] in all samples	1.20	29.27	3.19	8.53
Max cyanobacteria biomass [mg/L] in all samples	9.47	419.18	12.83	30.14
Species of cyanobacteria present in studied waterbodies	**2014:** *Aphanizomenon flosaquae* (Ralfs ex Bornet & Flahault, 1886) with *Microcystis aeruginosa* (Kützing) Kützing, 1846 and *Dolichospermum* sp.	**2014:** *Aphanizomenon flosaquae* with *Microcystis aeruginosa* **2017:** *Microcystis aeruginosa, Microcystis*	**2014:** *Aphanocapsa* sp., *Microcystis ichthyoblabe* (G.Kunze) Kützing, 1843, *Microcystis wesenbergii*, *Woronichinia*	**2014:** not available

	2017: *Aphanizomenon flosaquae*, *Dolichospermum flosaquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek, 2009, *Microcystis aeruginosa*, *Microcystis viridis* (A.Braun) Lemmermann, 1903, *Microcystis* sp., *Gomphosphaeria* sp.	*wesenbergii* (Komárek) Komárek ex Komárek 2006, *Woronichinia naegeliana* (Unger) Elenkin 1933, *Gomphosphaeria* sp., *Aphanizomenon flosaquae*, *Snowella* sp., *Gleocapsa* sp., *Merismopedia tenuissima* Lemmermann, 1898, *Aphanocapsa* sp., *Oscillatoria* sp.,	*naegeliana*, *Aphanizomenon flosaquae*, *Microcystis aeruginosa*, *Phormidium* sp. **2017:** *Microcystis aeruginosa*, *Microcystis wesenbergii*, *Microcystis* sp., *Snowella* sp., *Snowella lacustris* (Chodat) Komárek & Hindák, 1988, *Aphanizomenon* sp., *Cuspidothrix issatschenkoi* (Usacev 1938) Rajaniemi et al., 2005, *Oscillatoria* sp., *Planktothrix* sp., *Chroococcus* sp.	**2017:** *Microcystis aeruginosa*, *Microcystis wesenbergii*, *Woronichinia naegeliana*, *Aphanizomenon* sp., *Snowella* sp.
Cyanobacteria dominated in waterbodies and creating blooms (cyanobacteria biomass >= 3 mg/L)	**2014:** *Aphanizomenon flosaquae* **2017:** *Microcystis aeruginosa*	**2014:** *Aphanizomenon flosaquae* **2017:** *Microcystis aeruginosa*	**2014:** *Microcystis ichthyoblabe*, *Microcystis wesenbergii*, *Woronichinia naegeliana* **2017:** *Microcystis aeruginosa*	**2014:** not available **2017:** *Microcystis aeruginosa*, *Microcystis wesenbergii*

Table 2 Chosen feeding guilds of protozooplankton.

Feeding guilds	Species	Lifestyle
Algivorous ciliates	*Codonella cratera* Leidy, 1887	Free-swimming
	Coleps spetai Foissner, 1984	Free-swimming
	Tintinidium sp.	Free-swimming
Bacterivorous ciliates	*Aspidisca* sp.	Crawling
	Epistylis sp.	Sedentary
	Non-identified ciliate	Free-swimming
	Small *scuticociliata*	Free-swimming
	Tetrahymena sp.	Free-swimming
	Vorticella convallaria	Sedentary

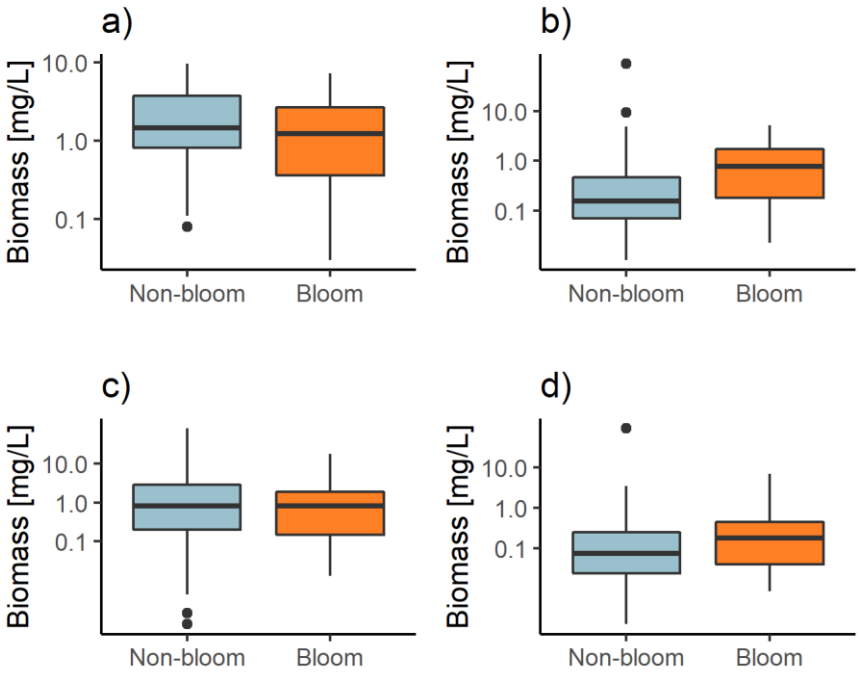
	Linnaeus, 1758 *Vorticella* sp. *Opercularia* sp.	Sedentary Sedentary
Mixed feeding (algae and bacteria) ciliates	*Coleps hirtus* (Müller, 1786) Nitzsch, 1827 *Frontonia* sp. *Holophrya* sp. *Paramecium* sp. *Stentor* sp. *Strobilidium* sp. *Strombidium* sp.	Free-swimming Free-swimming Free-swimming Free-swimming Free-swimming Free-swimming Free-swimming

Table 3 The effect of the biomass of individual feeding guilds of ciliates during periods of cyanobacterial bloom and periods without bloom on the biomass of predatory metazooplankton (copepods). In table are groups of ciliates tested with predatory metazooplankton; statistical significance are emboldened.

Predictor	Effects	Estimate	Std Error	t value	p (>|t|)
Algivorous ciliates	Intercept	1.867	0.262	7.115	<0.001
	Biomass of ciliates (without bloom)	**0.055**	0.025	2.221	**0.029**
	Cyano bloom	-0.578	0.555	-1.042	0.300
	Biomass: Cyano bloom	**0.490**	0.295	1.662	**0.099**
Bacterivorous ciliates	Intercept	2.030	0.277	7.324	<0.001
	Biomass of ciliates (without bloom)	-0.020	0.025	-0816	0.417
	Cyano bloom	-0.428	0.519	-0.824	0.412
	Biomass: Cyano bloom	0.165	0.114	1.453	0.150
Mixed (algae and bacteria) feeding ciliates	Intercept	1.880	0.259	7.254	<0.001
	Biomass of ciliates (without bloom)	**0.054**	0.023	2.311	**0.023**
	Cyano bloom	-0.372	0.489	-0.760	0.449
	Biomass: Cyano bloom	**0.460**	0.238	1.934	**0.056**

Figure captions

Fig. 1 Box plots for periods without and with cyanobacterial bloom for a) predatory copepods, b) algivorous ciliates, c) bacterivorous ciliates, d) mixed feeding (algae and bacterivorous) ciliates. The horizontal lines represent the median, the boxes represent 1st and 3rd percentiles, the vertical lines represent range, and the points represent outliers. The blue boxes represent the values of each group for the period without cyanobacterial bloom, the orange boxes represent the values for the period with cyanobacterial bloom.



502