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Plankton hitch-hikers on naturalists' instruments as silent intruders of aquatic ecosystems: current risks and possible prevention

Łukasz Wejnerowski[1], Tümer Orhun Aykut[2], Aleksandra Pełechata[1], Michał Rybak[3], Tamara Dulić[4], Jussi Meriluoto[4], Marcin Krzysztof Dziuba[5]

1 *Department of Hydrobiology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland* **2** *Department of Biology, Institute of Science, Istanbul University, Istanbul, Turkey* **3** *Department of Water Protection, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland* **4** *Biochemistry, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland* **5** *Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, USA*

Corresponding author: Łukasz Wejnerowski (wejner@amu.edu.pl)

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Abstract

Organism dispersal is nowadays highly driven by human vectors. This also refers to the aquatic organisms that can often silently spread in and invade new waters, especially when human vectors of dispersal act without brakes. Thus, it is mandatory to continuously identify human-mediated mechanisms of organism dispersal and implement proper biosecurity treatments. In this study, we demonstrate how the plankton net – one of the basic instruments in the equipment of every plankton sampling person is a good vector for plankton dispersal and invasions. We also demonstrate whether keeping the net in an ethanol solution after sampling is a proper biosecurity treatment, and what kind of treatments are implemented by people worldwide. The first simulation shows that bloom-forming cyanobacteria can easily infiltrate into the new environment thanks to the nets, and can prosper there. However, ethanol-based biosecurity treatment efficiently prevented their spread and proliferation in the new environment. The second simulation, based on wild plankton from an eutrophic lake, indicates that a plethora of phyto- and zooplankton taxa can infiltrate into the new waterbody through the net and sustain themselves there if the net is only flushed in the waterbody and left to dry after sampling (an approach that is commonly

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used by naturalists). Here, we also show that native plankton residents strongly shape the fate of hitchhikers, but some of them like cyanobacteria can successfully compete with residents. Survey data alert us to the fact that the vast majority of biologists use either ineffective or questionable biosecurity treatments and only less than a tenth of samplers implement treatments based on disinfectant liquids. Our results emphasize that the lack of proper biosecurity methods implemented by the biologists facilitates the spread and invasions of plankton including also invasive species of a great nuisance to native ecosystems. Considering that naturalists usually use different instruments that might also be good vectors of plankton dispersal, it is necessary to develop proper uniform biosecurity treatments. No longer facilitating the plankton spread through hydrobiological instruments is the milestone that we, plankton samplers worldwide, should achieve together in the nearest future if we want to continue our desire to explore, understand, protect and save nature.

Keywords
Abiotic resistance, accidental species introduction, aquatic biomonitoring, biosecurity treatment, invasive species

Introduction

Dispersal is one of the main forces that shape the diversity of plankton communities. Numerous plankton species possess various morphological structures (hooks, threads, spines, suckers) and adaptations (buoyancy regulation, resting cells and eggs, gelatinous sheaths) that make them easy to be transported via different vectors for short and long distances. Thanks to dispersal, plankton organisms can invade new habitats, which may lead to establishing a stable population in a new waterbody. Thereby, plankton dispersal has an important influence on the biodiversity of aquatic ecosystems.

Plankton organisms can disperse outside of their existing distributions using different natural vectors: wind, extreme events, channels or rivers, other organisms such as birds, semi-aquatic and land animals (Havel and Shurin 2004; Bergström et al. 2008; Solarz et al. 2020). The dynamic change of the structure and composition of ecosystems has always occurred as an outcome of natural processes. However, nowadays organism dispersal is highly driven by increased human activity and human-made devices, instruments and actions (Havel and Shurin 2004). For example, Carlton and Geller (1993) were some of the first researchers to show that aquatic organisms can be easily transported across the oceanic barriers in ships' ballast water. Simulations performed by Hyun et al. (2016) revealed that some of the plankton travellers could survive the journey in ballast water and establish stable populations in the new environment. In some circumstances, e.g. other climatic conditions in relation to native conditions, silent invaders can express specific phenotypic adaptations (Miglietta and Lessios 2009). Nonindigenous plankton species can also get into new waters during the stocking of fish in the water bodies. Such a mode of introduction has been hypothesized for exotic cladoceran *Daphnia lumholtzi*, which appeared in some North American lakes at the beginning of the 1990s (Sorensen and Sterner 1992; Havel and

Hebert 1993) and currently is widely distributed in many different aquatic habitats in North America (Benson et al. 2022).

Because humans will continue to interact with aquatic environments, it is impossible to completely stop the human-aided dispersal of aquatic organisms. What we can do is minimise the risk of occurrence of human-mediated organism dispersal events by the implementation of biosecurity methods in human activity. For example, ballast water is subjected to different disinfection techniques before discharge (Hess-Erga et al. 2019), which consequently lowers the risk of spreading the ship's nonindigenous hitchhikers in the new environment. However, we should be aware that many other kinds of activity in the aquatic environment that neglect a proper biosecurity method(s) might facilitate the spread of aquatic organisms. The problem increases if the species with a high invasive potential are present in the environment and on human-mediated vectors of an organism dispersal act without any biosecurity treatments. This may facilitate fast expansion of invasive organisms in a given region, which consequently hinders water bodies management, threatens the native species (Bøhn et al. 2008), can support a spread of new diseases (Martín-Torrijos et al. 2021), and generally leads to a deterioration in the ecosystem quality (Pinero-Rodríguez et al. 2020). Thus, continuous identification of other human-mediated mechanisms of plankton organism dispersal as well as development of proper biosecurity treatments are necessary.

In this study, we checked the possibility of the spread of lake plankton between different water bodies through a plankton net during sampling campaigns and whether disinfection of the plankton net with ethanol after sampling might be an efficient method to prevent the transfer of plankton net hitch-hikers into the next waterbody to be sampled. There were two experiments performed. In the first one, we tested whether plankton sampling with a net could facilitate the spread of some filamentous cyanobacteria and their potential invasion of new water bodies; we also tested if the use of biosecurity would prevent their spreading. Our attention was focused on cyanobacteria – organisms that often constitute a dominant component of plankton in different types of aquatic habitats and form dense blooms, generating an avalanche of problems to the functioning and usage of aquatic ecosystems (Waajen et al. 2014; Krztoń et al. 2019; Moustaka-Gouni and Sommer 2020). Many cyanobacteria are known producers of potent toxins with noxious effects on humans as well as on many aquatic and terrestrial organisms, which further highlights the need to control their spread. In the second experiment, we assessed the spreading success of plankton net hitch-hikers from an eutrophic lake in the filtrate from another lake containing or lacking a resident green alga *Tetradesmus obliquus*. In addition to the experimental part of the work, we performed a short survey among plankton samplers worldwide. In this way, we got an insight into what kind of biosecurity treatments they use during plankton sampling via nets, and what has been changed in the way of naturalists' actions during their work since the recalls of some scientists (Talling 1951; Padisák 2003; Padisák et al. 2016) about the problem of plankton dispersal through hydrobiological instruments.

Methods and materials

Dispersal of cyanobacteria via a plankton net, the fate of hitch-hikers, and possible prevention

We checked whether plankton sampling by a net with and without biosecurity could facilitate the spread of cyanobacteria and their invasion of new water bodies. Four laboratory strains of common bloom-forming filamentous cyanobacteria species such as *Aphanizomenon gracile*, *Aphanizomenon flos-aquae*, *Planktothrix agardhii*, and *Raphidiopsis raciborskii* (Table 1) were used for this test.

Table 1. Basic information on the origin (lake/reservoir name, GPS coordinates) of examined strains of cyanobacteria and their toxins (symbols +, –, n.e. indicate that a given toxin was detected, not detected or not examined, respectively).

Toxin	*A. flos-aquae*		*A. gracile*		*P. agardhii*		*R. raciborskii*	
	AMU-DH-6		AMU-DH-1		SAG 6.89		SAG 1.97	
	Sulejów Reservoir		Lake Buszewskie		Lake Lough Neagh		Lake Balaton	
	51°26'0"N, 19°55'25"E		52°32'42"N, 16°22'47"E		54°11'0"N, 10°26'45"E		46°48'51"N, 17°45'52"E	
Anabaenopeptins	–	(†)	+	(‡)	+	(†)	+	(‡)
Anatoxin-a	–	(†)	–	(‡)	–	(†)	–	(‡)
β-Methylamino-L-alanine	–	(†)	–	(‡)	–	(†)	–	(‡)
Cylindrospermopsin	–	(†)	–	(¥)	–	(¶)	–	(¶)
Desmethyl microcystin-LR	–	(†)	–	(¥)	+	(¶)	–	(¶)
Desmethyl microcystin-RR	–	(†)	–	(¥)	+	(¶)	–	(¶)
Microcystin-LR	–	(†)	–	(¥)	+	(§, ¶)	–	(¶)
Microcystin-RR	–	(†)	–	(¥)	+	(§, ¶)	–	(¶)
Microcystin-YR	–	(†)	–	(¥)	+	(§, ¶)	–	(¶)
Microcystin-LF	–	(†)	–	(¥)	–	(¶)	–	(¶)
Microcystin-LW	–	(†)	–	(¥)	–	(¶)	–	(¶)
Microcystin-LY	–	(†)	–	(¥)	–	(¶)	–	(¶)
Nodularin	–	(†)	–	(†)	–	(†)	*n.e.*	
Saxitoxins	–	(†)	–	(‡)	–	(†)	–	(‡)

Symbols: † this study is the first report; ‡ Falfushynska et al. (2021); § Kosiba et al. (2019); ¶ Wejnerowski et al. (2018); ¥ Wejnerowski et al. (2020). Detailed methodology and data of toxicological analyses performed for the purpose of this study are described in Suppl. material 1.

Twenty-day-old cyanobacterial cultures were inoculated into 70 L of distilled water. After 5 min. of gently mixing the volume of the container, we collected three subsamples of water for chlorophyll-*a* and microscopic analyses of cyanobacteria (respectively: 80 ml × 3, 1.5 ml × 3). Subsequently, 10 handmade plankton nets (mesh size: 100 µm; Suppl. material 2) were separately and fully immersed in water with cyanobacteria for 5 seconds. A large mesh size has been deliberately chosen to make the experiments more rigorous and leading to stronger conclusions (smaller mesh size of the net potentially means a higher risk of plankton retention on the net). The volume of the container was mixed for 30 seconds before each net was dipped. Net sample buckets were open

during the procedure, which enabled an immediate outflow of water after pulling the net out of water. Five randomly selected nets were then left freely in empty plastic bags for 3 hours (treatment: Biosecurity(–)). This procedure simulated an approximate travel time from one sampling point to another and was estimated arbitrarily. The rest of the nets were entirely immersed for 3 hours in a 30% ethyl alcohol solution in distilled water (treatment: Biosecurity(+)) to see if it can effectively prevent the spread of cyanobacteria. After that, every net was separately immersed for 5 seconds in a beaker filled with 2.5 L of WC medium (Guillard and Lorenzen 1972). The beakers were then placed in a walk-in phytotron chamber (Conviron, Winnipeg, Canada) for seven days at a defined temperature (20 ± 0.5 °C), light intensity (40–50 μmol photons m^{-2} s^{-1}), and photoperiod (16:8 hours light:dark cycle). At the beginning and the end of the incubation, samples from each beaker were collected for chlorophyll-*a* (80 ml) and microscopic analyses (1.5 ml). Before sampling, the content of each beaker was gently mixed for 10 seconds.

Dispersal of the wild plankton via a net and the fate of hitch-hikers

Here, we checked whether plankton sampling with a net without biosecurity could facilitate the spread of lake phyto- and zooplankton and their invasions of new water bodies. For this purpose, a shallow postglacial open lake – Gopło (central Poland) was visited and sampled in October 2019. We chose this lake because of its high susceptibility to cyanobacterial blooms known from the past several decades (Burchardt and Podolski 1992). At the time of our sampling campaign, the lake was dominated by *Planktothrix agardhii*. Detailed characteristics of the Gopło Lake, including cyanotoxin presence, are outlined in Suppl. material 3.

At first, we collected three 300 ml subsamples of the lake water for a chlorophyll-*a* analysis and a 1 L sample for a microscopic analysis. Second, 15 plankton nets (the same nets as used in the first experiment) with open sample buckets were separately and fully immersed for 5 seconds in Gopło's waters at a depth of ~1.5 m. Subsequently, they were placed freely in plastic bags in a car Volkswagen Caddy III cargo (Volkswagen Group, Wolfsburg, Germany).

After 4.5 hours, the nets were returned to the laboratory. Five randomly selected nets were separately immersed for 5 seconds in beakers filled with 3 L of distilled water. After gently mixing, half of the volume was taken from each beaker and fixed with Lugol's solution for a qualitative/quantitative phyto- and zooplankton analysis. This procedure was to reveal Gopło's plankton net hitch-hikers, which infiltrated into a new waterbody. The remaining ten nets were separately immersed for 5 seconds in beakers filled with 3 L of filtrate (GF/C filters, Whatman, Maidstone, United Kingdom) water from another eutrophic lake – Kierskie (western Poland; GPS coordinates: 52°28'25.6"N, 16°46'59.7"E). Five of them were additionally inoculated with 30 ml of a green alga *Tetradesmus obliquus* (strain SAG 276-3a). This procedure was to see the fate of Gopło's potential plankton net hitch-hikers in a new waterbody when a resident is present. All beakers were maintained in a walk-in phytotron for ten days.

On day 0 and after ten days, beakers were sampled for a chlorophyll-*a* analysis (100 ml) and an assessment of *P. agardhii* biomass (1.5 ml). At the endpoint, we have also collected 50 mL samples for nutrient analyses and 1 L samples for a qualitative/quantitative analysis of the whole phyto- and zooplankton community structure. This procedure was to see the successes and failures of Gopło's potential plankton net hitch-hikers after ten days in the new water bodies.

Sample analyses

Chlorophyll-a concentration

Samples were filtered through GF/C filters using a glass vacuum filtration kit and analysed spectrophotometrically following grinding of the filters using a mortar and subsequent 24 hours extraction in 90% acetone (Wetzel and Likens 2000).

Cyanobacteria and green algae biomasses

Samples (1.5 ml) collected from the experiments were analysed using an inverted microscope Leica DM IL LED (Leica Microsystems, Wetzlar, Germany) equipped with a digital camera Jenoptik ProgRes Speed Xtcore3 (Jenoptik Optical Systems, Jena, Germany). Trichomes of *A. gracile*, *P. agardhii*, and *R. raciborskii* from the first experiment and *P. agardhii* from the second experiment, were counted in a Sedgewick-Rafter chamber (Cole-Parmer, Vernon Hills, United States of America). Morphometry of cyanobacterial trichomes was assessed using ProgRes image capture software (Jenoptik Optical Systems, Jena, Germany). The thickness (n = 15) and length (n = 30) of randomly selected trichomes of each species were measured in each sample. Then, the average biovolume of each species was calculated using a formula for computing the volume geometric shape corresponding to the geometric shape of a given species (Vadrucci et al. 2007). Abundance and biovolume data were used to calculate the initial and final biomass of each species according to the following formula:

$$B(mm^3L^{-1}) = Abundance \times Biovolume$$

Phyto- and zooplankton community composition

Samples (1 L) collected from Experiment 2 were subjected to sedimentation for a month, subsequently concentrated up to 50 ml and additionally fixed with Lugol's solution. The qualitative (the number of taxa and species composition) and quantitative (abundance expressed as the number of individuals of a given taxon/species L^{-1}) analyses of the Gopło's phytoplankton and Gopło's hitch-hikers were performed using an Olympus BX 51 microscope with Nomarski differential contrast (Olympus, Tokyo, Japan). Individuals (single cells, coenobia, colonies, filaments with a length of 100 μm)

were counted in Fuchs-Rosenthal counting chamber (height: 0.2 mm, area: 0.0625 mm^2) at varied magnifications, i.e. microplankton in the whole chamber (512 fields) using 200× and nannoplankton in 256 fields using 400×. Phytoplankton identification was based on current references (e.g., Siemińska 1964; Starmach 1968; Ettl 1983; Ettl and Gärtner 1988; Hindák 1988; Starmach 1989; Popovský and Pfiester 1990; Komárek and Anagnostidis 1998, 2005; Wołowski and Hindák 2003; Komárek 2013; Lange-Bertalot et al. 2017).

The qualitative (the number of taxa and species composition) and quantitative (abundance expressed as the number of individuals of a given taxon/species L^{-1}) analyses of Gopło's zooplankton hitch-hikers were performed using a light microscope Axioskop 2 MOT+ (Carl Zeiss Light Microscopy, Oberkochen, Germany). Zooplankters of three groups (Cladocera, Copepoda, Rotifera) were determined and counted in a Sedgewick-Rafter chamber. If necessary, some animals were dissected after analysing the content of the whole chamber to be completely certain of the identification. Zooplankton identification followed Amoros (1984), Bielańska-Grajner et al. (2015), Błędzki and Rybak (2016).

Plankton sampling – survey

A short online survey was designed directly to gather information from plankton samplers worldwide about what they do with the plankton net after sampling and what biosecurity treatment they use. Questions in the survey were close-ended and addressed the following issues: frequency of plankton sampling with a net, the number of sampling points a day, the used methods of biosecurity after sampling, profession and degree status of the respondent (Suppl. material 4). In the case of some questions, participants had the opportunity to provide a short open-ended response to clarify or specify the response.

The survey was targeted at samplers who had an activity in the field of water research visible in the form of a scientific publication(s) indexed in the Web of Science citation database (Clarivate Analytics PLC, Philadelphia, United States of America) between the years 2000–2019. We used the keyword "plankton" in the WoS database search box to find proper participants for our survey. The scientists whose e-mails showed up in the database for the above search range were invited to respond to the survey (10,306 potential respondents). The survey was conducted in English and hosted on the SurveyLab online platform (www.surveylab.com; 7 Points Sp. z o.o., Warsaw, Poland). Each participant could complete the survey only once between March 2 and March 15, 2020.

The survey was voluntary and anonymous. Due to the hosting platform, we avoided collecting sensitive data from respondents. Also, we were unable to identify the respondents and link them to their answers. The exception was when a respondent sent an e-mail directly to the handling author of the survey with additional comments, suggestions, and the desire for a broader discussion.

In the analysis of the survey data, we did not include respondents sampling the marine environment. We also excluded from the analysis those respondents that answered to Q3 ("How many water bodies (e.g. lakes, reservoirs, ponds) do/did you sample for plankton using plankton net per day?") that they sample/sampled always one water body per day while later (Q5: "Do/Did you use different nets for each sampled lake when you sample/sampled more than one on the same day?") they did not mark the answer "I do/did not sample more than one lake on the same day". For the same reason, we excluded from the analysis those respondents that marked another answer than "Always one per day" to Q3 while later to Q5, they marked "I do/did not sample more than one lake on the same day".

Statistical analyses

Statistical analyses and graphs were conducted using the R software version 4.1.2 (R Core Team 2021), RStudio version 2021.9.2.382 (R Studio Team 2022). A Welch's paired t-test was applied to test the difference between chlorophyll-*a* concentration, the biomass of particular cyanobacteria strains at the start- and endpoint of the cultivation in Biosecurity(–) treatment (Experiment 1). Here, we used *rstatix* package (Kassambara 2020). Test assumptions were checked visually (Q-Q plots) and tested with formal methods (normality checked by the Shapiro–Wilk's test for the differences). The chlorophyll-*a* data in Biosecurity(–) contained one extreme outlier. Square-root transformation eliminated the problem and enabled us to run a classically paired t-test. Two outliers were detected in *P. agardhii* biomass data of Biosecurity(–) treatment, and data transformation did not solve it. Thus, *P. agardhii* biomass between two points of the cultivation was compared using Yuen's trimmed means test for two dependent samples using the *yuend* function in the *WRS2* package (Mair and Wilcox 2020). Data obtained from the Biosecurity(+) treatment were self-evident, and there was no necessity to seek a statistical confirmation.

In the case of experiment 2, Yuen's trimmed means test for two dependent samples was applied to test the difference in chlorophyll-*a* concentration between the start- and endpoint of the incubation of Gopło's hitch-hikers without a resident (Resident(–)). This test was used because these data contained one outlier, and data transformation did not solve the problem. The difference in chlorophyll-*a* concentration between the start- and endpoint of the incubation of Gopło's hitch-hikers with a resident *T. obliquus* (Resident(+)) was tested using Welch's paired t-test. The difference between Resident(–) and Resident(+) treatments in the biomass yield of Gopło's hitch-hiker *P. agardhii* at the endpoint of the incubation was tested using Yuen's trimmed means test. It was due to the presence of one outlier in the Resident(–) treatment.

Data were visualized using *GGally* (Schloerke et al. 2021), *ggplot2* (Wickham 2016), and *gridExtra* (Auguie 2017) package.

Results

Dispersal of cyanobacteria via a plankton net, the fate of hitch-hikers, and possible prevention

Cyanobacteria successfully spread into the new environment and withstood there when no biosecurity was applied (Biosecurity(–)), as positive values of the chlorophyll-*a* concentration and biomass of each species of cyanobacteria were detected immediately after the inoculation (Fig. 1). The chlorophyll-*a* concentration in beakers of the Biosecurity(–) treatment additionally increased by the end of the incubation ($t = -13.4$, $DF = 4$, $p = .0001$) (Fig. 1). The biomass of *A. gracile* increased almost threefold within the seven days after the inoculation ($t = -4.07$, $DF = 4$, $p = .01$), while the biomass of *P. agardhii* and *R. raciborskii* was stable (respectively: $t = 1.31$, $df = 2$, $p = .32$; $t = 1.36$, $DF = 4$, $p = .24$) (Fig. 1). *A. flos-aquae* was excluded from the analysis of biomass because this strain permanently formed fascicles visible in the beakers to the naked eye (Suppl. material 5). Some fascicles reached a length of ~2 cm.

In the Biosecurity(+) treatment, chlorophyll-*a* and cyanobacterial trichomes of each species were not detected in samples at both sampling points.

Dispersal of the wild plankton via a net and the fate of hitch-hikers

Chlorophyll-a concentration and phytoplankton

Chlorophyll-*a* concentration in the Gopło Lake was 79.1 ± 6.89 μg L^{-1} (mean ± SD). Wild phytoplankton successfully infiltrated the new environment and withstood there regardless of the presence or absence of the resident alga *T. obliquus*. Chlorophyll-*a* concentration in beakers without the resident *T. obliquus* increased over five times during the incubation time ($t_y = -4.2$, $DF = 2$, $p = .052$) (Fig. 2). In the case of beakers containing resident *T. obliquus*, the concentration of chlorophyll-*a* did not markedly differ between the start- and endpoint of the incubation ($t = 1.44$, $DF = 4$, $p = .22$).

Gopło's phytoplankton and hitch-hikers

Phytoplankton in the Gopło lake was represented by eight groups, including Chlorophyta (44 taxa), Bacillariophyceae (31 taxa), Cyanobacteria (10 taxa), Cryptophyceae (7 taxa), Euglenophyceae (5 taxa), Dinophyceae (3 taxa), Chrysophyceae (1 taxon), and Haptophyceae (1 taxon) (Fig. 3). Cyanobacterium *P. agardhii* was a dominant species, which accounted for 63% of the total phytoplankton density in Gopło.

Individuals of thirty-three phytoplankton taxa infiltrated the beakers with the Kierskie lake water via plankton nets (Fig. 3). Their identification resulted in the detection of Bacillariophyceae (13 taxa), Chlorophyta (11 taxa), Cyanobacteria (7 taxa), Cryptophyceae (1 taxon), and Euglenophyceae (1 taxon). Among all hitch-hikers,

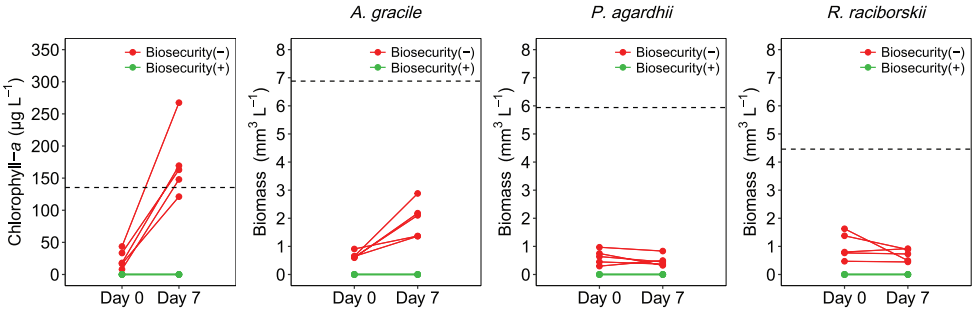

Figure 1. Chlorophyll-*a* concentration and biomass of *A. gracile*, *P. agardhii* and *R. raciborskii* at the start and endpoint of the incubation in Biosecurity(–) and Biosecurity(+) treatments. A dashed line indicates the mean value of a given variable in the container with a simulated bloom of cyanobacteria in which plankton nets were immersed before the experiment.

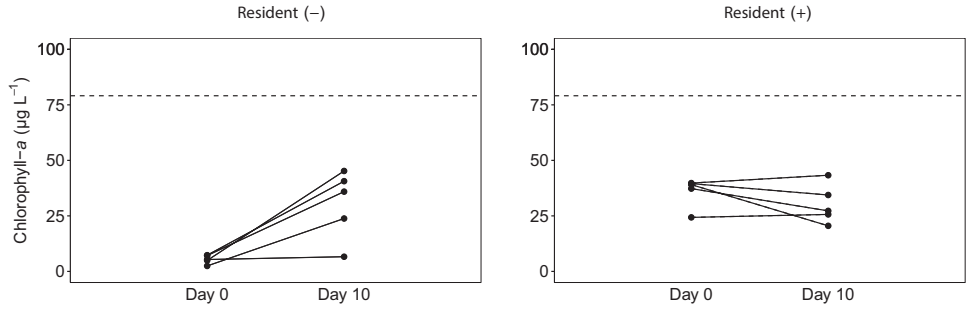

Figure 2. Chlorophyll-*a* concentration in beakers with Gopło's plankton net hitch-hikers at the beginning and the end of the incubation without or with resident *T. obliquus*. Horizontal line indicates chlorophyll-*a* concentration in the Gopło Lake.

P. agardhii was the most numerous in the beakers: it constituted on average 89% of the total phytoplankton density.

After ten days of hitch-hikers' incubation without resident *T. obliquus* (Resident(–)), thirty-seven phytoplankton taxa were found in beakers (Fig. 3): Chlorophyta (20 taxa), Bacillariophyceae (10 taxa), Cyanobacteria (5 taxa), Cryptophyceae (1 taxon), Chrysophyceae (1 taxon). *P. agardhii* was the dominant species in these beakers. Its density ranged from .42 to 5.78 million individuals L^{-1} and constituted on average 66% of the total phytoplankton density. Chlorophyte *Mougeotia* sp. was the second most abundant taxon: its density constituted almost 12% of the total phytoplankton density.

After ten days of hitch-hikers' incubation with resident *T. obliquus* (Resident(+)), fourteen phytoplankton taxa were found in beakers (Fig. 3): Bacillariophyceae (6 taxa), Chlorophyceae (4 taxa), Cyanobacteria (3 taxa), and Euglenophyceae (1 taxon). Chlorophyte *T. obliquus* (the resident strain plus conspecifics transferred from the Gopło lake) was the dominant species in these beakers: its density ranged from 5.5 to

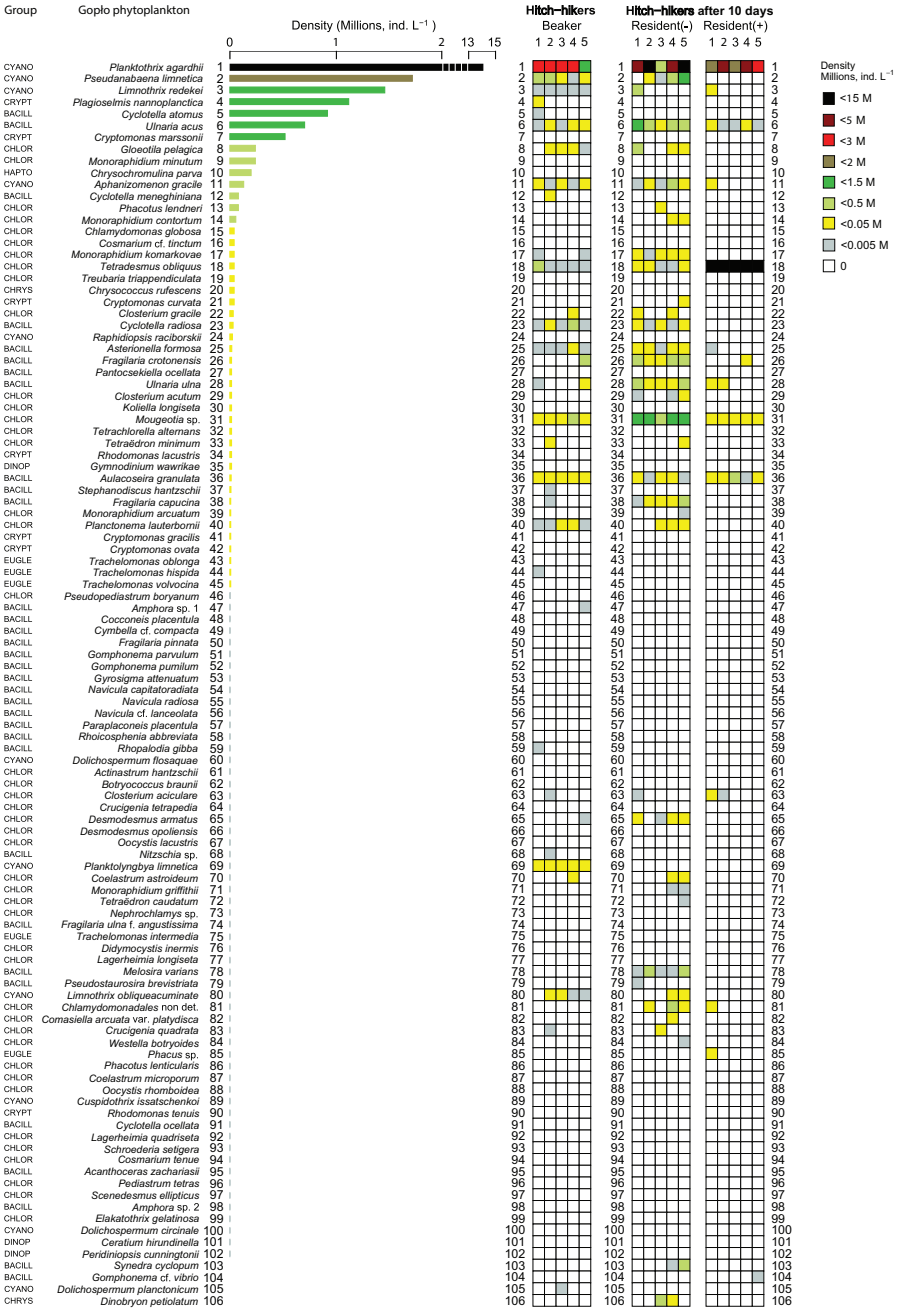

Figure 3. Phytoplankton community structure in the Gopło lake, phytoplankton hitch-hikers infiltrated into beakers through plankton nets and their fate after ten days of incubation in filtered lake water from the Kierskie lake without and with resident *T. obliquus* (Resident(–), Resident(+); respectively). Abbreviations: CYANO – Cyanobacteria, CRYPT – Cryptophyceae, BACILL – Bacillariophyceae, CHLOR – Chlorophyta, DINOP – Dinophyceae, HAPTO – Haptophyceae, EUGLE – Euglenophyceae, CHRYS – Chrysophyceae.

10.6 million individuals L^{-1} and constituted 73% of the total phytoplankton density. *P. agardhii* was the second most abundant taxon in the beakers: its density ranged from 1.68 to 4.08 million individuals L^{-1} (26% on average of the total phytoplankton density).

Effect of resident on Gopło's dominant hitch-hiker

At the start of the incubation, *P. agardhii* biomass in Resident(–) and Resident(+) was, respectively, .99 ± .16 and 1.09 ± .19 mm^3 L^{-1} (mean ± SD). After 10 days of incubation, *P. agardhii* biomass in the Resident(–) treatment increased up to 7.42 ± 3.55 mm^3 L^{-1} and was over a half higher than in the Resident(+) treatment (3.15 ± 1.8 mm^3 L^{-1}) (t_y = 4.14, DF = 8, p = .01) (Fig. 4).

Gopło's zooplankton and hitch-hikers

Zooplankton community in Gopło was represented mainly by rotifers (Fig. 5). Rotifer *Keratella cochlearis* was a dominant species, which comprised 69% of total Gopło's zooplankton density. There were two forms of *K. cochlearis*, f. typica and tecta, and the density of both forms ranged between 200–300 individuals L^{-1} with spineless f. tecta being slightly more numerous. The density of other taxa was lower than 100 individuals L^{-1}. The total density of zooplankton in the Gopło Lake was 718 individuals L^{-1}.

Three Rotifer taxa, four Cladocera taxa, one Copepoda taxon, and larval and juvenile forms of Copepoda (Calanoida and Cyclopoida) infiltrated the beakers with the lake water through plankton nets in quantities large enough to be detected upon first sampling (Fig. 5). *Keratella quadrata*, Cyclopoida nauplii and *Keratella cochlearis* f. typica were the most abundant in the beakers and constituted, respectively, 41.99%, 29.51%, and 12.5% of the total zooplankton density. The total density of zooplankton was on average 217.6 ± 110.6 individuals L^{-1}. After ten days of Gopło's hitch-hikers' incubation without resident *T. obliquus* (Resident(–)), six zooplankton taxa were found in beakers: Rotifera (4 taxa), Cladocera (2 taxa). Nauplii and copepodites of Cyclopoida and copepodites of Calanoida copepods were also detected. The total density of zooplankton in Resident(–) was 731 ± 1289.5 individuals L^{-1}. Nine zooplankton taxa were found on day 10 in beakers with resident *T. obliquus* (Resident(+)): Rotifera (4 taxa), Cladocera (3 taxa), Copepoda (2 taxa). There were also nauplii and copepodites of Calanoida and Cyclopoida. Four Rotifer taxa, *K. quadrata*, *Lecane* sp., *K. cochlearis* f. typica, and *Brachionus* sp. were most abundant in the community, and they constituted, respectively, 46.59%, 26.27%, 17.28%, and 8.49% of the total zooplankton density. The total density of zooplankton in Resident(+) was 1378 ± 481.39 individuals L^{-1}.

Biosecurity measures

After data filtration (see "Plankton sampling – survey" subsection), responses from 388 respondents were used in the analysis of the survey data, including 340 (88%) broadly understood biologists at a university or freelancer biologists, 12 (3%) employees at

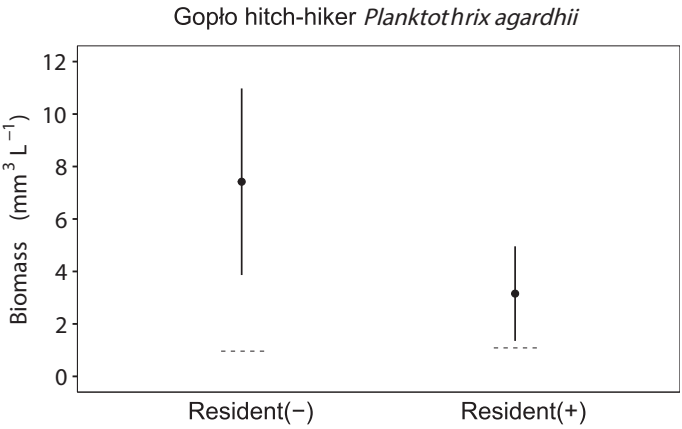

Figure 4. Average biomass (± SD) of Gopło's hitch-hiker *P. agardhii* at the endpoint of the incubation in the Resident(–) and Resident(+) treatments. Dashed horizontal lines indicate the average biomass of Gopło's hitch-hiker *P. agardhii* at the start point of the incubation.

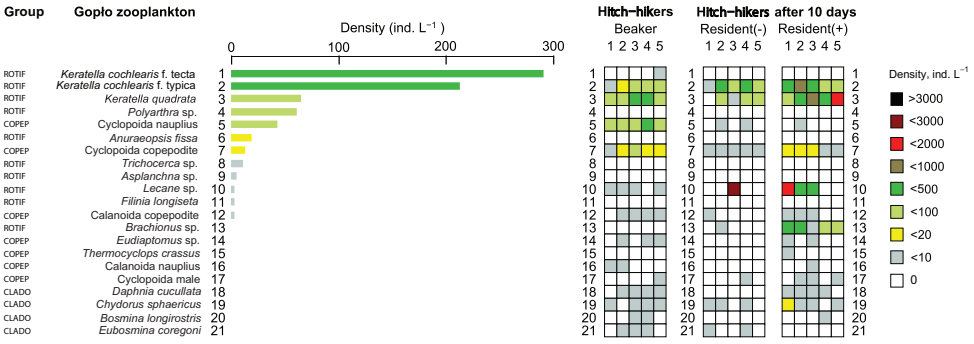

Figure 5. Zooplankton community structure in the Gopło lake, zooplankton hitch-hikers infiltrated into beakers through plankton nets and their fates after ten days of the incubation in filtered lake water from the Kierskie lake without and with resident *T. obliquus* (Resident(–), Resident(+); respectively). Abbreviations: ROTIF – Rotifera, COPEP – Copepoda, CLADO – Cladocera.

water quality monitoring service and 36 (9%) people of another profession. The responses came from 58 countries (Suppl. material 6). Our pool included professors (158 respondents, 41%), naturalists with a doctoral degree (184, 47%), PhD students (21, 5%), naturalists with a Master's degree (22, 6%) and a Bachelor's degree (3, 1%).

Regarding the frequency of plankton sampling, most respondents reported that they sample/have sampled plankton with a plankton net irregularly, including years without sampling (33%). Regular plankton sampling, either usually more than twenty times a year or usually up to ten times a year was reported, respectively, by 21% and 20% of respondents. Regular sampling, usually from eleven to twenty times a year,

was reported by 14% of the respondents. Irregular sampling of up to ten times a year in some years and more often in other years was declared by 11% of the respondents. There were also 1% of respondents that marked the response "None of the above".

Regarding the number of water bodies sampled per day, 39% of respondents declared to sample usually one water body per day and rarely more than one, 38% of the respondents reported to sample usually more than one but not more than five water bodies per day, 17% indicated that they always sample one water body per day. Another 6% of respondents usually sample more than five water bodies per day.

Among those who sample more than one lake a day, only 22.5% of respondents declared to use a different plankton net for each lake, while 77.5% use the same net in all sampled lakes.

A little over a half of the respondents declared to always rinse/have rinsed an open plankton net after sampling with distilled water/tap water and let it dry (Fig. 6).

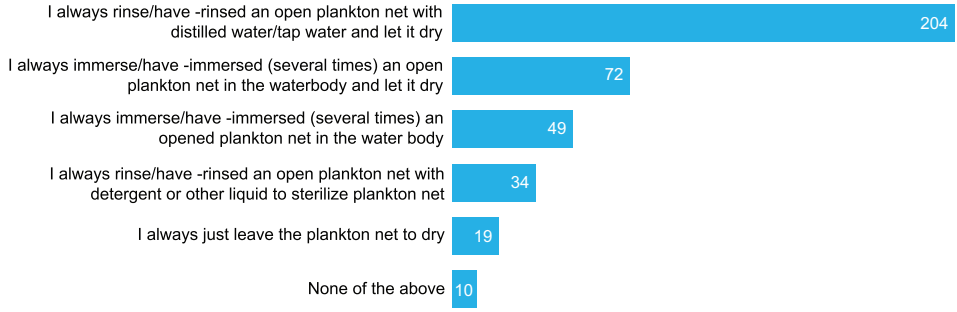

Figure 6. Biosecurity procedure(s) used by plankton samplers worldwide with the plankton net after sampling. Numbers inside the bars indicate the number of respondents who marked a given response.

Table 2. The detailed explanations that were provided by the 3% of respondents who chose the answer "None of the above" when asked what they do with plankton net after sampling.

No.	*Original answers*
1	"Rinse with distilled water if sampling in the same water body, wash with detergent if using in different water bodies."
2	"We use separate plankton nets for each lake - one lake, one plankton net. But we rinse it 10 times after sampling and 10 times before sampling next month."
3	"It depends, if there is a risk of spreading invasive species, I sterilise and then dry the net. Otherwise I take it back to the lab and rinse it with distilled water before letting it dry."
4	"After sampling I take the plankton net to the next sampling place and rinse there the net several times before sampling."
5	"Rinse and preserve in formalin."
6	"The sample should be concentrated and the net should be washed and dried."
7	"I don´t know it so precisely."
8	"Samples after draining are fixed with formalin or alcohol."
9	"I added formaldeyhde to a 4% concentration and buffer with hexamethiltetramine until a pH of 8.2."
10	"i."

The second most popular method used by 19% of the respondents is to always immerse (several times) an open plankton net in the water body and let it dry, while 13% of respondents admitted to always immerse (several times) an open plankton net in the water body. Only 9% of respondents indicated that they always rinse/have rinsed an open plankton net with a detergent or other liquid to disinfect the plankton net. There was also 5% of the respondents who always just leave the plankton net to dry, and 3% of the respondents that marked the "None of the above" response. These last 3% of the respondents were additionally asked to specify what they do with the plankton net after sampling and all the answers are outlined in Table 2.

Discussion

The results of our experiments demonstrate that a plankton net is an efficient vector for dispersion of plankton organisms. In the first experiment, all examined strains of cyanobacteria were able to disperse from the source into the new medium through the plankton net. They were able to survive three hours on the plankton nets out of the water and inhabit the new medium. There was one dominant species (*A. gracile*) that increased markedly in its biomass at the endpoint of the incubation; others (*P. agardhii*, *R. raciborskii*) maintained a relatively stable biomass during the experiment. There were also visible numerous enlarged *A. flos-aquae* fascicles at the endpoint of the incubation. All of these species often co-occur and compete for dominance in eutrophic waters of the temperate zone (Kobos et al. 2013). One of them, *R. raciborskii* has a tropical and subtropical origin and exhibits a great invasive potential (Antunes et al. 2015), and its presence in temperate waters was distinctly noticed in recent years (Wilk-Woźniak et al. 2016; Kokociński et al. 2017; Rzymski et al. 2018). It was predicted that cyanobacteria without a biosecurity treatment could be successfully spread by a plankton net and establish stable populations in the simulated new environment. After all, this experiment was conducted on laboratory strains and we used the same medium that the examined strains were customarily cultured in. Moreover, cyanobacterial trichomes had a high variability in length (since ~20 to ~1700 μm) which can make some of them easy to entangle and anchor in the nylon grid of the plankton net, even if the mesh size was 100 μm. The ability of cyanobacteria to secrete exopolysaccharides (EPS) that have adhesive and gluing properties (Cruz et al. 2020) might additionally help them to stick to the nylon threads of a net and temporarily protect cyanobacterial cells against desiccation as well (Costa et al. 2018). Although the ability of the examined strains to produce EPS has not yet been checked, literature data indicate that some members of the *Aphanizomenon*, *Planktothrix*, and *Raphidiopsis* genera possess this feature (respectively: Xue et al. 2015; Meccheri 2010; Zarantonello et al. 2018). Hence, a lack of proper biosecurity methods implemented during plankton sampling can facilitate spread of nuisance bloom-forming species and those of a high invasive potential. Toxicity of hitch-hikers seems to have no impact on their efficiency to spread between the water bodies through a plankton net. All of the tested strains had relatively stable populations at the endpoint of the incubation. *A. flos-aquae* was free of the most common cyanotoxins

(anabaenopeptins, anatoxin-a, β-N-methylamino-L-alanine, cylindrospermopsin, microcystins, nodularin, saxitoxin), *A. gracile* and *R. raciborskii* produced anabaenopeptins only and *P. agardhii* produced anabaenopeptins and some microcystins.

Keeping the plankton net in 30% ethanol after sampling seems to be a promising method to prevent the spread of cyanobacteria between different water bodies via the plankton net. After seven days of incubation, neither chlorophyll-*a* nor cyanobacterial trichomes/fascicles were detected in the medium. This result additionally points to the superiority of the biosecurity method based on disinfection with ethanol over only flushing the net in the water body and letting it dry. The biosecurity method using ethanol does not require a lot of work, time, and space in the vehicle, and is also relatively cheap (it only takes a bucket with a lid + ethanol + distilled/tap water). There are also other possible disinfectant liquids that could be used, but we believed that due to rapid evaporation, ethanol would have negligible negative effects on the environment. The advantage of biosecurity methods based on disinfectant liquids is that their power can be maximized by using a higher concentration of a disinfectant to get an assurance that other planktonic organisms and their propagules will also become irreversibly inactive when transferred into a new water body.

Identifying which phyto- and zooplankton species are effective or ineffective plankton net hitch-hikers was enabled in the second experiment, which was based on plankton from the Gopło Lake. Out of one-hundred and two Gopło's phytoplankton taxa, more than thirty of them were transferred into beakers with filtered lake water from another lake and survived ten days after the inoculation. A few zooplankton taxa from the Gopło Lake turned out to be successful intruders as well, some of them infiltrated the new simulated water body quite abundantly. There were numerous phytoplankton and only a couple of zooplankton hitch-hikers that expanded their abundance in the lake filtrate within ten days. The higher number of phytoplankton taxa found at the end point of the incubation in comparison to the start means that "no one is excluded from the game" and even those hitch-hikers that were initially easy to overlook due to low abundance can quickly proliferate in the new environment and succeed there. However, organisms with spines, bristles, and other protruding elements can have a greater chance of hitchhiking. For example, rotifer *K. cochlearis* f. typica with a caudal spine infiltrated the water more effectively than *K. cochlearis* f. tecta without the spine. This raises the question as to whether such spines provide a better chance of spreading on some of the natural vectors like bird feathers. The dominance of *P. agardhii* in the Gopło Lake and in the filtrates of the Kierskie Lake at the start- and endpoint of the experiment confirm that bloom-forming cyanobacteria can be easily spread by plankton samplers if there are no efficient biosecurity methods used. Because eutrophic waters are subjected to more frequent biomonitoring (due to e.g. phytoplankton bloom events, ongoing ecosystem restoration) and they often offer good conditions for the growth of nuisance plankton, efficient net hitch-hikers might have consequently a greater chance to spread and expand their distribution range. This might explain to some extent why numerous nuisance plankton taxa are cosmo- or at least subcosmopolitan. In the light of the 1) paradigm "everything is everywhere, but, the environment selects" (Baas-Becking 1934), 2) the significance of environmental heterogeneity for organismal distribution ("everything is

not everywhere", Ribeiro et al. 2018), and 3) the increasing number and importance of human vectors and human-mediated natural vectors of organism dispersal, it seems that nowadays vectors are so powerful that they mainly decide where everything is, but the environment still selects and decides when everything has its own five minutes.

Predicting the fate of hitch-hikers is a great challenge as it depends on the resultant force of environmental factors in the new environment which these organisms and/or their propagules have to face. Among the factors determining winners and losers among newcomers, competitive abilities of native residents and biotic resistance of the whole native communities are often one of the most crucial (Dzialowski et al. 2007; Weithoff et al. 2017; Buchberger and Stockenreiter 2018). Our second experiment was too simple to get a deep insight into this problem; after all, in the treatment with "Resident(+)", we examined the effect of only one resident (*T. obliquus*) on the fate of plankton net hitch-hiker horde. Nevertheless, even the presence of one resident species strongly shaped the success of the hitch-hikers. The presence of laboratory strain of *T. obliquus* exacerbated the competition between all phytoplankters in the new environment. Consequently, the number of phytoplankton-hitch-hiker taxa at the endpoint of the incubation with resident *T. obliquus* was markedly reduced. Moreover, their abundance was generally lower than in the Resident(–) treatment and this referred as well to the dominant Gopło hitch-hiker *P. agardhii*. Some previous laboratory experiments (Mur et al. 1978; Ji et al. 2017) revealed that green algae are often strong competitors of bloom-forming cyanobacteria when there are no limiting factors for them, while our second experiment lasted only ten days and e.g. resources were generally not exhausted at the end point of the incubation (Suppl. material 7). Completely unlike phytoplankton, zooplankton hitch-hikers took advantage of the presence of resident *T. obliquus* which was manifested in the increased number of zooplankton taxa at the endpoint of the incubation. Moreover, some rotifer taxa increased in their biomass in comparison to the Resident(–) treatment. The high nutritious value and easy manageability of green algal cells or colonies in comparison e.g. to cyanobacteria (Bednarska et al. 2014; Sikora et al. 2014) possibly explain why the presence of the resident triggered positive effects on zooplankton newcomers. Moreover, zooplankton-hitch-hikers were composed of small-bodied taxa, mainly rotifers that can easily avoid less edible phytoplankton and its negative effects (Ger et al. 2016).

Getting insight into how aquatic ecologists worldwide prevent nowadays the spread of the plankton via the sampling net during their field works was also our "must-know" in this study. As revealed by the survey, less than a tenth of plankton samplers implement a biosecurity treatment based on disinfectant liquids (rinsing the net after sampling with detergent or other disinfectant liquid). The overwhelming majority of the respondents use other ways: 1) rinsing the net with open buckets after sampling with distilled/tap water, 2) immersing the net several times in the water body and letting it dry, 3) immersing the net in the waterbody without taking care about its dryness or 4) just leaving the net to dry. Based on the results of our two experiments, we already know that biosecurity treatment relying on flushing the net with an open bucket after sampling in the waterbody and letting it dry does not prevent plankton spread between the water bodies. This especially refers to the situation when sampling of several water bodies a day is planned, and considering the answers of our respondents on – "How many water bodies (e.g. lakes, reservoirs,

ponds) biologists sample using plankton net per day?" – this practice is quite common in multiple sampling cases. Flushing the net after sampling in the water body and letting it dry fails as a biosecurity method also because it does not prevent the spread of plankton propagules (cysts, resting eggs and cells, spores etc.) that can well handle desiccation (Hori et al. 2003; Radzikowski 2013). This fact also puts the effectiveness of rinsing the net after sampling with distilled/tap water into question. Thus, it appears that, worldwide, the most commonly used biosecurity methods to prevent plankton spread between water bodies via the net are simply ineffective, and that the silent spreading and possible invasion can easily happen as a result of sampling. Our study has shown only a part of the problem. After all, aquatic naturalists use a variety of instruments and other necessary equipment during their work, and all these items, without proper biosecurity treatments, might also potentially be a vector for plankton spread between the water bodies. Moreover, we revealed this problem based on phyto- and zooplankton as a model. Whereas, considering that plankton organisms have their own "hitch-hikers" (bacteria, parasites, viruses; Grossart et al. 2010; Frada et al. 2014; Bass et al. 2021), it is likely that a lack of biosecurity treatments implemented by the naturalists might also facilitate the spread of pathogenic bacteria, parasitic and viral infections in the plankton communities between water bodies.

Conclusion

In summary, this study demonstrates that plankton net is an efficient vector for dispersion of plankton organisms. The fate of plankton net hitch-hikers in the new environment is strongly shaped by the native residents. A promising biosecurity method preventing the spread of plankton between water bodies is disinfection of the plankton net with an ethanol solution after sampling. Survey data indicate that the vast majority of people use either ineffective or questionable biosecurity treatments. No longer facilitating the plankton spread is the milestone that we, naturalists worldwide, should achieve together in the nearest future.

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CRediT authorship contribution statement: Wejnerowski Ł: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision, Project administration. Aykut TO: Methodology, Investigation, Writing - Review & Editing. Pełechata A: Methodology, Software, Investigation, Writing - Review & Editing. Rybak M: Methodology, Software, Investigation, Writing – Review & Editing. Dulić T: Methodology, Investigation, Writing - Review & Editing. Meriluoto J: Methodology, Software, Investigation, Writing - Review & Editing. Dziuba MK: Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Review & Editing.

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Supplementary material 1

Detailed methodology of toxicological analyses performed for the purpose of this study and the results in the graphical form

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: text + figures

Explanation note: Detailed methodology of toxicological analyses performed for the purpose of this study and the results in the graphical form. Abbreviations: APs – anabaenopeptins, ATX-a – anatoxin-a, BMAA – β-N-methylamino-L-alanine, CYN – cylindrospermopsin, MCs –microcystins, NOD – nodularin, STXs – saxitoxins.

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Link: https://doi.org/10.3897/neobiota.73.82636.suppl1

Supplementary material 2

Scheme of the handmade plankton nets used in the experiments and dimensions

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: figure

Explanation note: Scheme of the handmade plankton nets used in the experiments and dimensions.

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Supplementary material 3

Basic information about the Gopło Lake and some characteristics of the surface water measured during field campaigns

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: Table + text + figures

Explanation note: Basic information about the Gopło Lake and some characteristics of the surface water measured during field campaigns.

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Link: https://doi.org/10.3897/neobiota.73.82636.suppl3

Supplementary material 4

The list of questions and possible responses in the survey

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: text

Explanation note: The list of questions and possible responses in the survey.

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Supplementary material 5

Top view of the content of two randomly selected beakers of Biosecurity(–) treatment after seven days of incubation in a phytotron

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: image

Explanation note: Top view of the content of two randomly selected beakers of Biosecurity(–) treatment after seven days of incubation in a phytotron.

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Link: https://doi.org/10.3897/neobiota.73.82636.suppl5

Supplementary material 6

Survey responses by country

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: figure

Explanation note: Survey responses by country.

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Link: https://doi.org/10.3897/neobiota.73.82636.suppl6

Supplementary material 7

Concentration of ammonium, nitrate, orthophosphates in the Kierskie Lake filtrates (Resident(-), Resident(+)) at endpoint of the second experiment

Authors: Łukasz Wejnerowski, Tümer Orhun Aykut, Aleksandra Pełechata, Michał Rybak, Tamara Dulić, Jussi Meriluoto, Marcin Krzysztof Dziuba

Data type: figure

Explanation note: Concentration of ammonium (NH4+), nitrate (NO3-), orthophosphates (PO43-) in the Kierskie lake filtrates (Resident(-), Resident(+)) at endpoint of the second experiment.

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