Knowl. Manag. Aquat. Ecosyst. 2017, 418, 15
© W. Pęczuła *et al.*, Published by EDP Sciences 2017
DOI: 10.1051/kmae/2017006

www.kmae-journal.org

Knowledge & Management of Aquatic Ecosystems

Journal fully supported by Onema

RESEARCH PAPER OPEN ACCESS

An experimental study on the influence of the bloom-forming alga *Gonyostomum semen* (*Raphidophyceae*) on cladoceran species *Daphnia magna*

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Abstract – The effect of the unicellular, bloom-forming alga *Gonyostomum semen* (*Raphidiophyceae*) on the survival rate and body size of *Daphnia magna* was tested under experimental laboratory conditions. Using samples from four humic lakes with a long history of *Gonyostomum* blooms, we exposed *D. magna* for 72 h to various *Gonyostomum* treatments which included homogenized biomass (frozen and fresh), live cell populations as well as lake water separated from the concentrated biomass of live cells. Filtered lake water and the chlorophycean alga *Stichococcus bacillaris* population (homogenized biomass or live cells) we used as controls. Our study revealed that (1) frozen homogenized *G. semen* biomass in the concentrations typical for blooms was not harmful for *Daphnia* and appeared to have a nutritive effect because it supported its growth; however, *Daphnia* mortality occurred after exposure to fresh and highly concentrated cell homogenate containing high amount of mucilage; (2) it is unlikely that living *Gonyostomum* cells excrete extracellular substances harmful for *Daphnia*; however, dense live *Gonyostomum* population that formed mucilaginous aggregates immobilized *Daphnia* and increased its mortality. The results suggest that various interactions between *G. semen* and *D. magna* take place and may play an essential role in natural freshwater ecosystems.

Keywords: algal blooms / mucilage / crustaceans / toxicity

Résumé – Une étude expérimentale de l'influence de l'algue formant des efflorescences *Gonyostomum semen* (*Raphidophyceae*) sur l'espèce de cladocère *Daphnia magna*. L'effet de l'algue unicellulaire, formant des efflorescences, *Gonyostomum semen* (*Raphidiophyceae*) sur le taux de survie et la taille corporelle de *Daphnia magna* a été testé dans des conditions expérimentales de laboratoire. En utilisant des échantillons de quatre lacs humiques avec un long passé d'efflorescences de *Gonyostomum*, nous avons exposé *D. magna* pendant 72 h à différents traitements de *Gonyostomum* qui incluent : la biomasse homogénéisée (congelée et fraîche), des populations de cellules vivantes ainsi que l'eau du lac séparée de la biomasse concentrée de cellules vivantes. L'eau filtrée du lac et une population d'algues chlorophycées *Stichococcus bacillaris* (biomasse homogénéisée ou cellules vivantes) ont été utilisées comme témoins. Notre étude a révélé que : (1) la biomasse congelée homogénéisée de *G. semen* dans les concentrations typiques d'efflorescences n'était pas nocif pour *Daphnia* et semblait avoir un effet nutritif car elle a soutenu sa croissance, cependant, la mortalité de *Daphnia* a eu lieu après une exposition à un homogénat frais et hautement concentré de cellules contenant une grande quantité de mucilage ; (2) il est peu probable que les cellules vivantes de *Gonyostomum* excrètent des substances extracellulaires nocives pour *Daphnia*, cependant, une population vivante dense de *Gonyostomum* vivante qui a formé des agrégats mucilagineux a immobilisé *Daphnia* et a augmenté sa mortalité. Les résultats suggèrent que diverses interactions entre *G. semen* et *D. magna* ont lieu et peuvent jouer un rôle essentiel dans les écosystèmes naturels d'eau douce.

Mots-clés : bloom à *Gonyostomum* / mucilage / *Daphnia magna* / toxicité

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1 Introduction

Interactions between planktonic algae (including cyanobacteria) and zooplankton have been intensively studied for decades in terms of the regulation of plankton communities by top-down and bottom-up control mechanisms (Agrawal, 1998; Ger *et al.*, 2014). Generally, research is primarily focused on direct trophic interactions covering zooplankton grazing as well as phytoplankton defence strategies (Lampert, 1987; Tillmann, 2004).

Phytoplankton has developed various defence mechanisms against zooplankton grazing pressure. These include, among others, increase of cell size (Yoshida *et al.*, 2004; Kampe *et al.*, 2007), change of cell morphology (Van Donk *et al.*, 1997), motility, which enables active escape (Strom and Buskey, 1993; Buskey, 1997), agglomerating into aggregates (Hessen and Van Donk, 1993), as well as production of various chemical substances for repelling or toxic action towards zooplankton (Wolfe, 2000; Pohnert *et al.*, 2007). Some algae have developed very "active" modes of defence by ejecting threads of mucilage from trichocysts which act as a repellent for grazers. This phenomenon is known in dinoflagellates (like *Oxyrrhis marina*, Martel and Flynn, 2008) as well as in raphidophytes (like *Fibrocapsa japonica*, Tillmann and Reckermann, 2002). Some marine raphidophytes are also known to produce toxic compounds, which may be lethal or sublethal for fish, crustaceans or ciliates (Yan *et al.*, 2003; Fu *et al.*, 2004; Okaichi, 2004; Clough and Strom, 2005; Mohamed and Al-Shehri, 2012).

The freshwater raphidophyte group consists of several species (Fott, 1968), from which *Gonyostomum semen* is the best known and intensively studied over the last decade due to the increase in its abundance and common occurrence in European aquatic ecosystems (Cronberg *et al.*, 1988; Rengefors *et al.*, 2012; Pęczuła, 2013; Karosiene *et al.*, 2014; Hagman *et al.*, 2015). The species was previously considered inedible (Havens, 1989) due to its large dimensions which are above the preferred size range for many filter-feeding zooplankton. In addition, its cells lack a cell wall and contain mucilaginous trichocysts (South and Whittick, 1987) which make them very fragile and results in the cell disintegration under the influence of chemical or physical stimulus. In this case, they eject threads of mucilage from trichocysts which could be a repellent (or possibly toxin) for zooplankton. This mode of action was proposed by Cronberg *et al.* (1988) to be a defence mechanism against grazers. Nevertheless, recent experimental studies revealed that *G. semen* cells may be grazed by some rotifers (*Asplanchna priodonta*), cladocerans (*Daphnia magna*, *D. pulicaria*, *Holopedium gibberum*) or copepods (*Diaptomus oregonensis*, *Eudiaptomus gracilis*) (Williamson *et al.*, 1996; Lebret *et al.*, 2012; Johansson *et al.*, 2013; Björnerås, 2014). As reported by Hagman *et al.* (2015), in lakes with high *Gonyostomum* biomass, bathers often suffered from skin irritation and itching after contact with lake water. This has had social consequences in Scandinavia where several bathing places lost their recreational attractiveness, and the Swedish Environmental Protection Agency recognizes this alga as a noxious species (Angeler and Johnson, 2013). In many papers (Cronberg *et al.*, 1988; Findlay *et al.*, 2005; Figueroa and Rengefors, 2006; Trigal *et al.*, 2011), this species is described as a "noxious" or a "nuisance". Rengefors *et al.* (2008) reported that *G. semen* caused cell lysis of small phytoplankton flagellates, what suggests that it can be harmful for other freshwater organisms. Some field studies focused on the impact of *G. semen* blooms on phytoplankton, zooplankton, benthic invertebrates and fish (Trigal *et al.*, 2011; Angeler and Johnson, 2013; Johansson *et al.*, 2013), but there is no evidence that this species may negatively affect zooplankton in a direct way. To fulfill this gap, we designed an experimental study with the aim to test the influence of *G. semen* biomass on zooplankton using *D. magna* as a model organism.

2 Materials and methods

2.1 Sampling and extraction of *Gonyostomum* biomasses

Algal samples were collected in the end of July and the beginning of August 2014 from four humic lakes which have had a long history of *G. semen* blooms (Pęczuła *et al.*, 2013; Karosiene *et al.*, 2014). Two lakes are located in Eastern Poland (Jelino and Płotycze) and two in Lithuania (Slabada and Natalka). *G. semen* was a dominating species in phytoplankton of all lakes composing from 91 to 96% of the total phytoplankton biomass with a low proportion of the other algae, mainly small phytoflagellates (cryptophytes, chlorophytes and euglenophytes). Lake water (18–25 L) was sampled by means of the Ruttner sampler in the central part of each lake: from 0.5 m in shallow lakes and 3 m in deeper lakes, *i.e.* at depths of expected high *Gonyostomum* abundance (Pęczuła *et al.*, 2013; Karosiene *et al.*, 2014). All samples were kept in the dark and protected against heating during transportation to the laboratory.

The following *G. semen* preparations were made to test their effects on *D. magna*: (a) *G. semen* cell homogenates obtained from the four sampled lakes (marked as "H"); (b) water from concentrated live *G. semen* biomass sampled from the same lakes, after removal of algal cells by filtration (marked as "W"); (c) homogenized fresh *G. semen* biomass with four- to five-fold higher density compared to the afore mentioned treatments, obtained from Lake Jelino (marked as "FH"); (d) dense biomass of live *G. semen* cells from Lake Jelino (marked as "L").

Lake water samples were three times gently filtered through a plankton net (25 μm mesh) to the volume of 1 L and gently centrifuged (4000 rpm for 10 min at 20 °C). After centrifugation, the pellets were collected, and the concentrated samples were combined, giving a total of 100 mL sample from each lake. *Gonyostomum* cells were live in all concentrated samples, as noted by examination under light microscope. The dense samples were kept frozen at −20 °C for 2 or 3 weeks (depending on the lake). On the day of the experiment setup, samples were thawed out and part of the sample was filtered through GF/C Whatman glass fiber filters to be used in the experiment as "W" treatments (filtered water). The rest of the biomass samples (10 mL) were ultrasonicated (two-times for 5 min at 50 W in Sonoplus, Bandelin ultrasonic homogenizer), and after centrifugation (14 000 rpm for 10 min 20 °C) supernatants were collected and used in the tests as "H" treatments (homogenates). In addition, we collected extra samples from Lake Jelino to

prepare much higher concentrated homogenates according to the same procedure as above with the exception that extracts were used directly without freezing and thawing ("FH" treatments). For experiments with live cells of *Gonyostomum* (marked as "L"), the algal samples were collected and concentrated as described before and were kept for three days under light:dark cycle – 14:10 h, light intensity 10 μmol $m^{-2} s^{-1}$, at 20 °C (to mimic light and temperature conditions in sampled lake).

2.2 Experimental procedures

Although *D. magna* is a crustacean rarely occurring in humic lakes, we decided to use this species as it is the most often tested zooplankter in aquatic toxicology. We used juvenile *D. magna* hatched from eggs (Daphtoxkit FTM MAGNA) delivered by MicroBioTests Inc. (Belgium). The incubation and bioassays were conducted according to the producer protocols, compatible with OECD Standard Guideline 202 (1996). After 72 h incubation in standard medium (light 6000 lx, temperature 20 °C) and hatching, daphnids were fed with lyophilized *Spirulina*. For preparation of the standard medium (ISO 6341, 2012), up to 2 L of deionized water and following concentrated salts: $NaHCO_3$ (67.75 mg L^{-1}), $CaCl_2 \times 2H_2O$ (294 mg L^{-1}), $MgSO_4 \times 7 H_2O$ (123.25 mg L^{-1}), KCl (5.75 mg L^{-1}) were used. Then, five individuals were flushed with the medium solution and transferred to 5 mL experimental chambers. Increasing concentrations of *G. semen* homogenates (expressed as chl-*a*: 85–3576 mg m^{-3}, which corresponded to *G. semen* biomass range of 9–386 g of fresh weight [FW] m^{-3}) or increasing biomass of live cells (72–773 g m^{-3}, respectively) were used in three replicate experiments. As controls, we always used filtered (GF/C Whatman) lake water, marked in experiments as "C(W)". In the bioassays with *G. semen* extracts ("H") and alive cells ("L"), we used additional controls: (a) a homogenized biomass of the chlorophycean microalga *Stichococcus bacillaris*, marked as "C(Sb)" (chl-*a*: 4202 mg m^{-3}), prepared in the same way as that of *G. semen*; (b) alive cells of *S. bacillaris* (72 g FW m^{-3}), respectively. Multiwell plates with *D. magna* and extracts were kept in a growth chamber at 20 °C in darkness, whereas plates with alive *G. semen* were kept at the light:dark cycle (14:10 h), light intensity of 10 μmol $m^{-2} s^{-1}$, and 20 °C.

The experiments were carried out for 72 h. Every 24 h we checked the viability and general condition of *Daphnia* specimens under a microscope and classified those into "live" or "dead" categories. During *L* tests in which *G. semen* cells formed mucilaginous aggregations, we classified *D. magna* to three classes: "live swimming", "live entrapped" or "dead entrapped". The test end-point was a death or/and entrapment of the organisms. Data obtained from the bioassays were expressed as the percentage (%) of the organisms' survival or entrapment compared to the respective controls (in which the survival rate was always 100% in all experimental variants).

G. semen abundance, fresh weight and/or chlorophyll-*a* concentration (ISO 10260, 1992) were determined before the set-up of the experiments. The abundance and fresh weight were determined using an inverted microscope according to Utermöhl's method (Vollenweider, 1969). As there is no generally accepted formula for *Gonyostomum* biovolume calculation in the published data, we have chosen a prolate spheroid as a geometric model (Hillebrand *et al.*, 1999). We recalculated the biovolume to fresh weight with an assumption that the density of algal cells equals 1 g cm^{-3}. At the end of the experiments, we measured also the body length of the survived *D. magna*. Prior to the measurements, *Daphnia* specimens were fixed in formaldehyde.

2.3 Statistical analysis

In order to determine the significance of differences in daphnids length between controls and various treatments, a nonparametric Mann–Whitney *U* test was performed according to the fact that there were significant difference between the variances in population analysed (Levene's test, mean, $P < 0.01$). In the case of two series of data (*H* tests in lakes Natalka and Slabada: treatments against "CSb" control), where variances were homogenous, a Student *t*-test was used. Pearson correlation was used to determine the relationship between the amount of *G. semen* homogenate added and daphnids body length. The test of Shapiro–Wilk was performed to verify the normal distribution of the analysed data. All analyses were carried out using XLSTAT add-in for MS Excel (Addinsoft).

3 Results

3.1 The effect of homogenized *G. semen* biomass on *Daphnia* survival rate

Generally, addition of increased amounts of homogenized *G. semen* biomass (frozen and thawed out, "H" treatments) in the range of 84–1530 mg m^{-3} (corresponding to 9–161 g FW m^{-3}) did not affect *Daphnia* survival rate (Fig. 1). However, in the experimental variant with the most concentrated *G. semen* cell homogenate (Lake Jelino; chl-*a* = 3503 mg m^{-3}, biomass = 378 g FW m^{-3}) the survival rate of daphnids decreased to 60% after 24 h and 40% after 48 h of exposure in comparison with controls (Fig. 1). Fresh cell homogenate of *G. semen* collected from Lake Jelino ("FH" treatments) with chl-*a* range: 894–3576 mg m^{-3} had a distinct negative effect on *Daphnia* survival (Fig. 2). This negative effect increased with increasing biomass of alga and time of exposure. At lower chl-*a* concentration (894 and 1778 mg m^{-3}), the survival rate averaged 40–70% after 24 h and 20–30% after 48 and 72 h of exposure. However, the addition of the most concentrated and fresh *G. semen* homogenized biomass (chl-*a*: 3576 mg m^{-3}) caused 100% *Daphnia* mortality after 24 h (Fig. 2). When the water separated by filtration ("W" treatments) from the concentrated *G. semen* biomass was added to experimental chambers with *Daphnia* no mortality of the organisms was observed.

3.2 The effect of homogenized *G. semen* biomass on *Daphnia* body length

The addition of frozen and then thawed out homogenized *G. semen* biomass from all lakes ("H" treatments) considerably influenced daphnids body length measured after 72 h

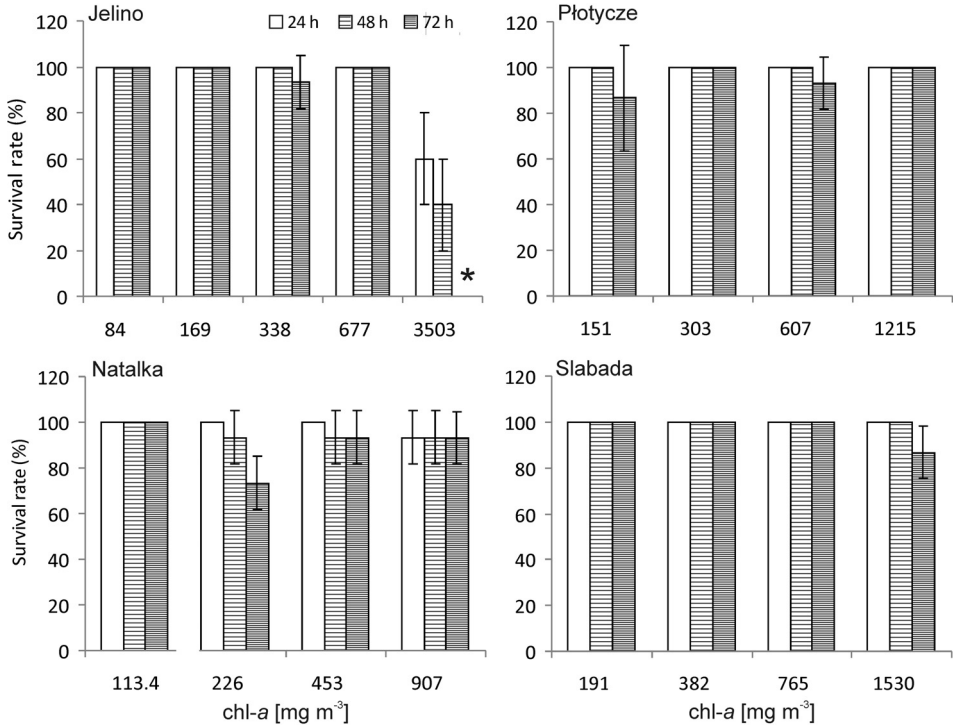

Fig. 1. The survival rate of daphnids' treated by increasing amounts of homogenized biomass of GS expressed as chl-*a* concentrations. The survival rate in controls was set as 100%.

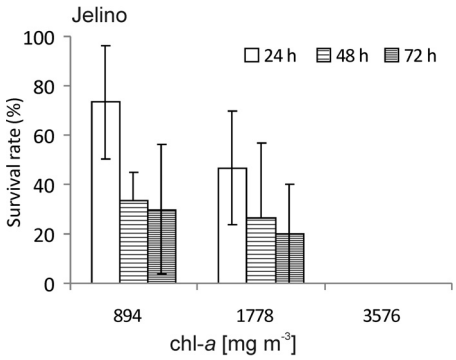

Fig. 2. The survival rate of daphnids' treated by fresh homogenized biomass of GS from lake Jelino. The survival rate in controls was set as 100%.

exposure (Fig. 3). At increasing homogenate concentrations (chl-*a*: 84–1530 mg m^{-3}) body length of *Daphnia* was always significantly greater compared with filtered water controls ($P < 0.01$, Mann–Whitney *U* test) and in some cases (at chl-$a \geq 453$ mg m^{-3}) when compared with *S. bacillaris* homogenate controls ($P < 0.01$, Mann–Whitney *U* test or Student's *t*-test). Generally (Fig. 4), *Daphnia* body lengths positively correlated with the algal homogenate concentration ($r^2 = 0.57$, $P < 0.01$, Fig. 4a).

When fresh *G. semen* homogenate ("FH" treatments) was used, we also observed the slight increase of daphnids body length after 72 h exposure (Fig. 5), but it was not statistically significant ($P = 0.108$–0.231, Mann–Whitney *U* test).

3.3 The effect of live *G. semen* cells on daphnids survival rate and body length

The effect of dense live *Gonyostomum* population on daphnids survival rate was tested with the use of the samples from lake Jelino ("L" treatments) (Fig. 6). The addition of increasing amounts of algal cells had a dose- and time-dependent negative effect on daphnids survival rate. Increased *Daphnia* mortality was observed at two highest *G. semen* biomass additions (386 and 773 g FW m^{-3}), where the survival rate decreased to 40–60% of the control after 72 h of the exposure (Fig. 6a). Simultaneously, we observed that after 24 h of experiment (Fig 6b) most daphnids (from 40 to 100%, depending on the treatment) were entrapped in mucilaginous macroscopic aggregations of *G. semen* cells (of a few mm in a diameter). After increased time (72 h), all *Daphnia* specimens were entrapped in the mucilaginous aggregations – live or dead. Only a few free-swimming specimens of *Daphnia* were found at densities of *G. semen* cells of 96–193 g FW m^{-3} and any at the two highest densities (Fig. 6c) at the end of the experiment. Neither *G. semen* ingestion by daphnids nor a presence of algal cells inside *Daphnia* guts were observed during the experiment. Moreover, we did not observe any positive effect on daphnids' body length during any of the tested quantities of live *G. semen* cells (Fig. 7).

4 Discussion

In the present study, we aimed to evaluate the effect of very high *G. semen* biomass on the survival rate of the common member of freshwater zooplankton *D. magna*. The analysis of

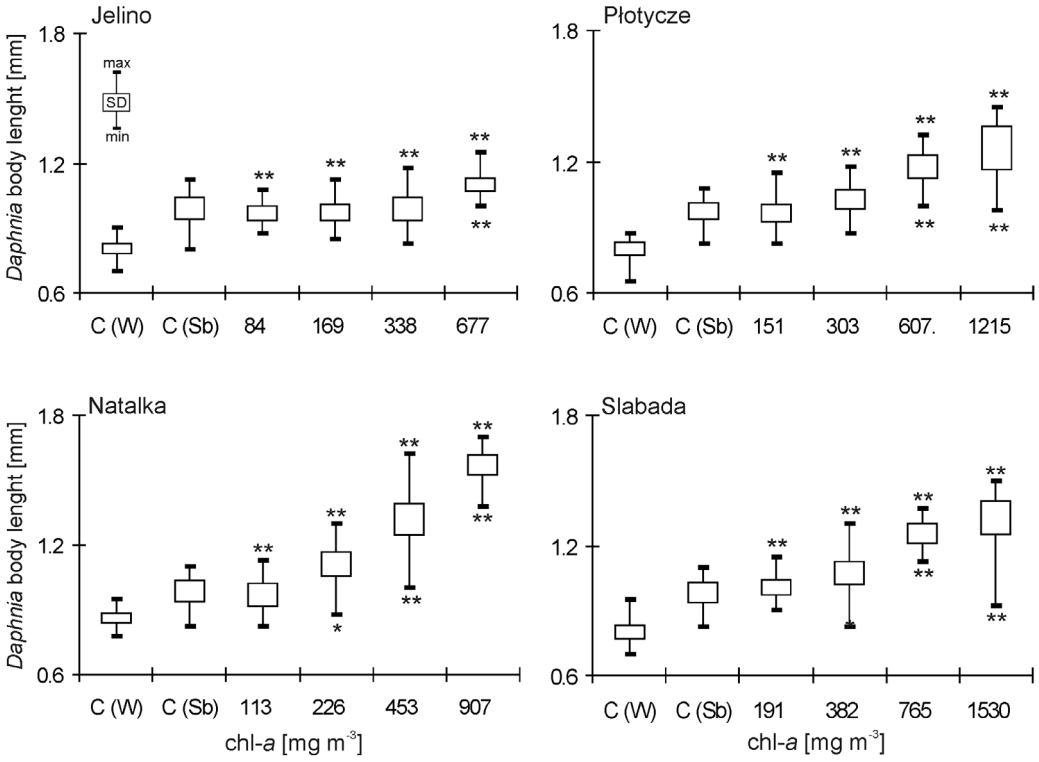

Fig. 3. Body length of *Daphnia* (DL) exposed for 72 h to homogenized GS biomasses from various lakes (C(W) – lake water control, C (Sb) – homogenized *Stichococcus bacillaris* biomass control; asterix above bars = significant difference against C(W) control, asterix below bars = significant difference against C(Sb) control, * $P < 0.05$, ** $P < 0.01$, Mann–Whitney test or Student's *t*-test).

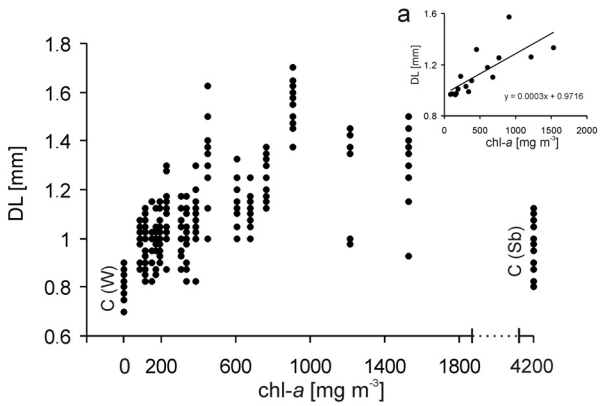

Fig. 4. Daphnids' body length (DL) related to the density of homogenized GS biomass addition; a – relationship between mean body length and chl-*a* concentrations (C (W) – lakes water control, C (Sb) – homogenized *Stichococcus bacillaris* biomass control).

the results leads to the conclusion that: (a) *G. semen* biomass frozen prior to the experiment and then homogenized even in high typical bloom concentrations were not harmful for *D. magna*. Moreover, it appeared to be nutritive for the crustaceans as it supported their body growth; (b) daphnids' mortality occurred only after addition of very concentrated fresh homogenate (much higher than this occurring during blooms in lakes); (c) it is unlikely that live *G. semen* exudate or extra-cellular substances were harmful for *Daphnia* as the filtered water did not affect them; (d) dense live *Gonyostomum* cells population that formed mucilaginous aggregates immobilized *Daphnia* physically and increased its mortality.

Harmful action of fresh *G. semen* homogenates was detected at the biomass concentrations considerably exceeding the values found commonly in natural lakes. Reports from Scandinavia and Canada show that during *Gonyostomum* blooms the alga forms biomass between 1 and 20 g fresh biomass m^{-3} (Lepistö *et al.*, 1994; Hongve *et al.*, 1988; Findlay *et al.*, 2005; Angeler *et al.*, 2010; Trigal *et al.*, 2011) and very rarely as high as 45 g fresh biomass m^{-3} (Cronberg *et al.*, 1988; Hagman *et al.*, 2015). Nevertheless, *G. semen* can occasionally be found in extremely high densities. A few studies from countries situated south of Scandinavia reported one order of magnitude higher maximum biomass of the alga which ranged 143–841 g fresh biomass m^{-3} (Pithart *et al.*, 1997; Hehmann *et al.*, 2001; Pęczuła *et al.*, 2013; Karosiene *et al.*, 2014) and which more or less correspond to densities used in our experiments. *Gonyostomum* density, even during blooms, in lakes of non-Scandinavian part of Europe is usually not higher than 50 mg FW L^{-1} (Le Cohu *et al.*, 1989; Vetrova and Okhapkin, 1990; Negro *et al.*, 2000; Hutorowicz *et al.*, 2006; Pęczuła, 2013; Karosiene *et al.*, 2014); therefore, the previously noted extremely high and incidental biomass values may be an exception rather than the rule. The results obtained here indicated that the high *G. semen* biomass typical of lakes is generally not toxic for daphinds. The lower survival rates of daphnids observed at extremely high concentrations of fresh homogenate in our experiment may be thus explained by the mechanical trapping and immobilization through the high

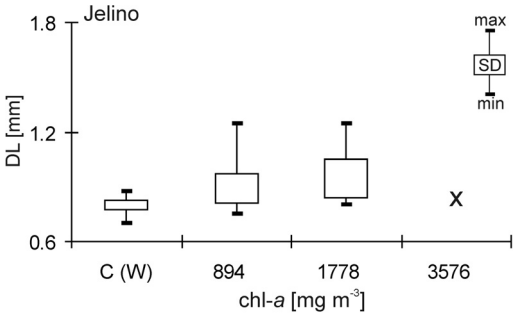

Fig. 5. Daphnids' body length (DL) after 72-h exposure on fresh homogenized biomass of GS from Lake Jelino (C(W) – lake water control). x – no live daphnids occurred and their body length was not estimated.

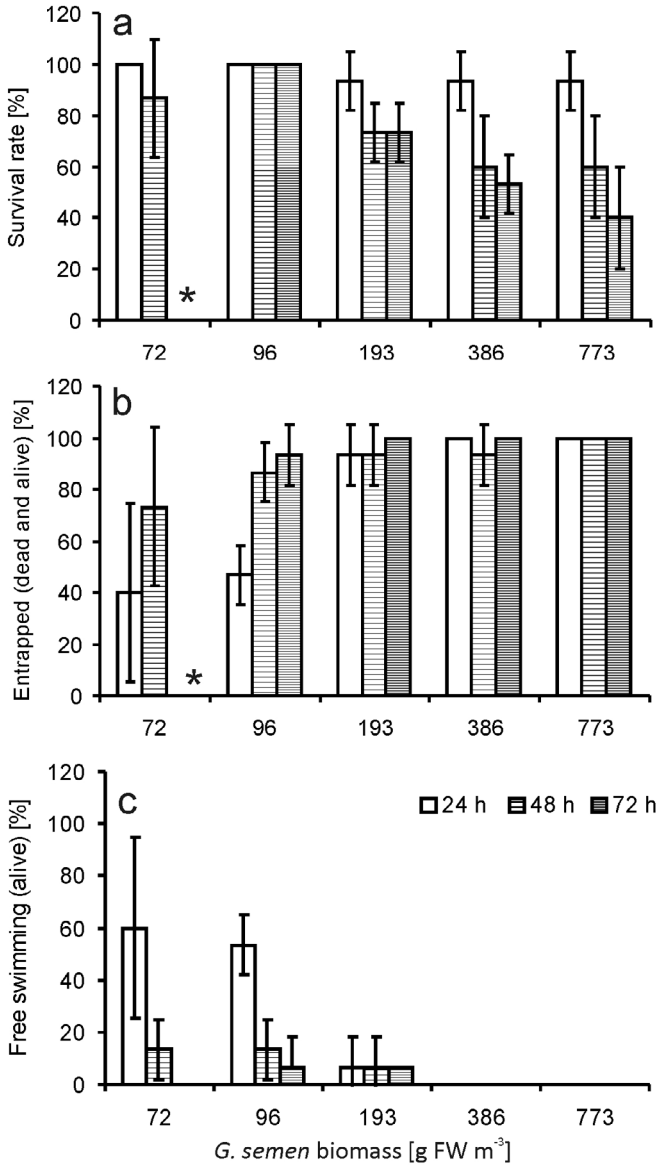

Fig. 6. Daphinds' response to the addition of GS live cells from Lake JEL (100% = control). * – 72-h exposure not tested.

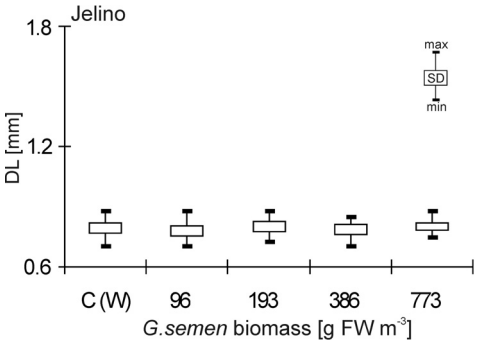

Fig. 7. Daphnids' body length (DL) after 72-h exposure to GS live cells collected from Lake Jelino (C(W) – lake water control).

mucilage amount released by *G. semen* biomass. Nevertheless, the fact that the fresh homogenate used had a more negative influence on *Daphnia* survival rate (in comparison to frozen one) may suggest that *G. semen* cells might contain some intracellular compounds with potentially harmful action which may be unstable and could be reduced by freezing and thawing. Some harmful algal secondary metabolites, *e.g.* various cyanotoxins (anatoxin-a, microcystins) produced by cyanobacteria, are known to have different stability dependent on various factors like temperature or light (Tsuji *et al.*, 1995; Hardy, 2008). However, our conclusion should be treated with caution as the concentrations of fresh and frozen homogenates in the experiment were not the same, but only to some extent comparable.

Our results do not support the hypothesis suggested by Cronberg *et al.* (1988) that the phenomenon of expelling threads of mucilage during cell disintegration, serves as a repellent or even as a toxicant for grazers. It seems to be unlikely that very small amount of mucilage from one cell may affect *Daphnia* specimen as we did not observe any negative effects on a *Daphnia* survival in the presence of high concentrations of homogenate biomass. However, such effect cannot be excluded in the case of small zooplankton species. Some earlier reports showed that *G. semen* presence caused cell lysis of *Rhodomonas lacustris*, probably *via* expelled mucilage from trichocysts, but this evidence concerned a small (10 μm) algal flagellate lacking a cell wall (Rengefors *et al.*, 2008) which may not correspond to the effect on large crustaceans. Nevertheless, recent studies revealed that *G. semen* cells with significantly reduced trichocysts were more vulnerable to grazing by rotifers and copepods, which indicates that these organelles may still play some defensive role in this raphidophyte alga (Björnerås, 2014).

The nutritional value of *Gonyostomum* as prey for zooplankton was firstly suggested by Williamson *et al.* (1996). It was revealed that *Daphnia* reproduction rate was higher when fed on metalimnetic *Gonyostomum*-rich seston as compared to *Gonyostomum*-poor epilimnetic seston. Further research indicated that cells of this raphidophyte contain significant amounts of ω-3 polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic and linolenic acid (Gutseit *et al.*, 2007; Johansson *et al.*, 2016), previously found also in marine raphidophytes (Marshall *et al.*, 2002). PUFAs are one of the main (besides phosphorus) limiting

components in daphnids food (Gulati and Demott, 1997) and play an essential role in the growth of *D. magna* (Becker and Boersma, 2005; Martin-Creuzburg and von Elert, 2009). Therefore, it became clear that the homogenized *Gonyostomum* biomass, besides not being harmful, may serves as a nutritive factor supporting body growth of *Daphnia*, as directly indicated in our experiments. Earlier findings revealed that only large daphinds are able to ingest this raphidophyte cells while smaller species do not due to the large size of the algal cells (Lebret *et al.*, 2012; Johansson *et al.*, 2013); however, a recent study showed that also small-sized rotifers may ingest their cells (680 cells day^{-1}) and grow on *Gonyostomum* (Björnerås, 2014). Our findings suggest that daphnids may also benefit from disintegrated *G. semen* biomass, available during or after the algal bloom. Very similar observations were reported for *Daphnia* feeding on potentially inedible cyanobacteria. Experimental research revealed that decaying biomass of *Planktothrix limnetica* or *Microcystis* sp. was more suitable food source for *Daphnia* spp. than fresh cyanobacterial cells (Repka *et al.*, 1998; Gulati *et al.*, 2001; Luo *et al.*, 2015). A similar mechanism may have occurred in our experiment where frozen and thawed *G. semen* homogenate from disintegrated cells could be easily ingested and became a satisfactory food source for *Daphnia*. A decaying *G. semen* biomass supporting an increase of benthic invertebrate biomass was also suggested by Trigal *et al.* (2011) but the authors did not present any direct evidence. A more possible explanation could be related to the extended bacterial growth, which may occur on the disintegrated algal biomass, thus creating an additional food source for *Daphnia* and enhancing its growth. The increase of bacterial abundance at the end of algal blooms is a known phenomenon (Van Boekel *et al.*, 1992). Kamiyama *et al.* (2000) observed the increase of heterotrophic bacteria numbers around the end of raphidophyte *Heterosigma akashiwo* bloom as well as the increase in phosphates produced as the bacteria mineralized organic phosphorus originating from decaying algae. Johansson *et al.* (2016) found that in lakes with higher *G. semen* biomass, cladoceran species contained more bacterial fatty acids than those of algal origin which revealed that *Gonyostomum* bloom results in increased utilization of bacterial resources by zooplankton.

Very interesting results were obtained when *D. magna* was exposed to high densities of live *Gonyostomum* cells, where we observed a formation of macroscopic mucilaginous algal aggregations. After 24-h exposure, great proportion of crustaceans was entrapped within them, even at not extremely high and environmentally relevant algal biomass (72 g FW m^{-3}), and the number of free-swimming *Daphnia* specimens decreased with increasing algal biomass. *Daphnia* exposure to the most concentrated biomass (773 g FW m^{-3}) resulted in a significant decrease in its survival rate. Thus, it supports our previous observation that highly concentrated biomass homogenates containing cell mucilage might mechanically immobilize daphnids resulting in their higher mortality, probably due to starvation. The formation of algal aggregations in lakes ("lake snow") is known, but rarely documented, due to the fragile nature of this phenomenon (Grossart and Simon, 1993). In experimental studies, aggregations formation was observed in various *Scenedesmus* species which occurred in the presence of chemicals released by *Daphnia* to the water (Hessen and Van Donk, 1993; Luring, 1999). *Daphnia*-induced increased mucilage production, which resulted in more sticky trichomes in the formation of the large aggregates, was observed in *Aphanizomenon flos-aquae* (Kiorboe and Hansen, 1993). However, it seems unlikely that *Gonyostomum* formed aggregations under the influence of zooplankton presence. *G. semen* aggregation is a known phenomenon that sometimes be seen in lakes during blooms, in dense laboratory cultures or when *Gonyostomum* net samples are mechanically disturbed during transportation (Koreiviene and Pęczuła, own observations). The mechanism of daphnids immobilization in *G. semen* aggregations observed in our experiment cannot be excluded as a phenomenon that may exist during mass development of this alga.

The present study enhanced the understanding of the ecology of the expanding flagellate alga *G. semen*, particularly in terms of its interactions with zooplankton grazers. Our results showed, contrary to previous beliefs, that disintegrated *G. semen* biomass is not harmful for crustaceans when it occurs in concentrations even higher than blooms in lakes. The evidence from this study suggests also that disintegrated *Gonyostomum* cells may serve as nutritive component enhancing daphnids' body growth. However, one of the more interesting findings emerging from this study, is that a dense *G. semen* population forms mucilaginous aggregates which may serve as mechanical traps for daphnids and in this way may enhance organisms mortality. Further, field and experimental investigations are needed to estimate the significance of our findings in the food web of freshwater ecosystems facing mass development of *G. semen*.

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Cite this article as: Pęczuła W, Toporowska M, Pawlik-Skowrońska B, Koreiviene J. 2017. An experimental study on the influence of the bloom-forming alga *Gonyostomum semen* (*Raphidophyceae*) on cladoceran species *Daphnia magna*. *Knowl. Manag. Aquat. Ecosyst.*, 418, 15.