Nuisance algae *Gonyostomum semen* (Raphidophyta) in water bodies of protected natural areas in Middle Volga region (Russia)

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**Abstract.** The distribution of nuisance raphidophyte *Gonyostomum semen* in water bodies of protected natural areas of the Middle Volga region is analyzed. The abundance of the species was determined quantitatively in eight lakes using the method of fixation with mercuric chloride. In four more lakes, it was found by qualitative inspection of live samples. It develops mainly in lakes with high water color, reaching the cell number of $7.5 \times 10^6$ cells L$^{-1}$, but also occurs in clear-water lakes in minor abundance ($10^{-1600}$ cells L$^{-1}$). It has been demonstrated that some ciliate species e.g. *Frontonia leucas* can consume *G. semen* cells and therefore partially control its abundance. Taking into account the presented data, this species was recorded in the plankton of lakes in all regions of the forest part of the Middle Volga basin. Thus, these results significantly expand the geography of the planktonic habitats of *G. semen* in the whole Volga region.

1. Introduction

In the last century, changes of areas and intensity of development of many species of aquatic organisms are evident. This phenomenon, usually referred to as the spread of “alien species”, is associated mainly with growth of the human population, a sharp increase in its activity, and the expansion of its transport connections between different territories [1]. All these factors have a relatively small impact on protected areas, and especially on nature reserves. However, global climate changes are beginning to impact significantly on habitats transformation in last years [2, 3]. Along with direct climate change (i.e. increase in average temperatures, change in precipitation, lakes’ drying and salinization), they lead to indirect changes in key hydrological and hydrochemical conditions in inland water bodies, and primarily to an increase in surface runoff of nutrients and organic substances from catchment areas. This is reflected primarily in the accelerating of eutrophication and brownification (an increase of water color) of freshwater water bodies [4].

One of the species that has significantly increased its distribution and abundance in temperate waters is the freshwater raphydophyte *Gonyostomum semen* (Ehr.) Diesing. It has actively mobile teardrop-shaped cells with two unequal flagella, highly variable in size (30-100 µm in length). It develops mainly in summer, both in polymeric and stratified lakes having high water color and low water mineralization [5-7]. Due to its specific environmental requirements, separate codon Q was assigned in the morphofunctional classification for this species [8]. Presumable reasons for its expansion, along with an increase in the concentration of colored DOC, include effects of elevated
nutrients [9-10] and temperature [11] and its resistance to grazing [12] or low abundance of grazing species [13]. Some authors explained the expansion of this species by the anthropogenic acidification of inland water bodies [5, 14], but subsequent studies challenged this assumption [11, 13].

The species was first described in 1853 from a shallow pond or swamp near Berlin. In the 1930s, its mass development was described in polyhumic water body (the Cedar Pond) on the Atlantic coast of the United States [15]. Later, it was found in Scandinavia, Central Europe, North and South America, Asia and Africa [16]. In Russia, mass development of *G. semen* in plankton was first registered in 1985, in small low mineralized rivers with high water color of the left-Bank Volga in the Nizhny Novgorod region [17]. Then it was found in plankton in the Vologda, Vladimir, Leningrad, and Samara regions and in the Republic of Karelia [5, 18-22]. Thus, the geography of the findings of this species suggested its presence in the center of the Middle Volga region, in the Republics of Mari El and Tatarstan. However, no data on its presence in this region were available. Therefore, the purpose of this work was to assess the extent of distribution and discuss the development of *G. semen* in different types of lakes in this region, and first of all – in the protected nature areas.

2. Materials and methods

The research was carried out in lakes located on the territory of the Raifa section of the Volga-Kama State Nature Biosphere Reserve (Republic of Tatarstan), the Mari-Chodra National Park (Republic Mari-El), the protection zone of the State Nature Reserve "Prisursky" (Chuvash Republic) and the projected, as an expansion of the Bolshaja Kokchaga Nature Reserve, Kugu Kakshan Biosphere Reserve. Water samples were taken by a Ruttner bottle or by a pump thin-layer sampler in July-August of 2006, 2007, 2014, 2018 and 2019. During sampling, the main physical and chemical parameters were determined, including water color and specific electrical conductivity. In total, about 20 lakes were studied that differ in origin, level of mineralization, water color and some other ecological parameters.

The presence of *Gonyostomum semen* was evaluated qualitatively by microscopy of the live samples. Then its abundance was determined on fixed samples as proposed for the quantitative determination of ciliates [23]. Briefly, 150-300 mL of water sample was concentrated by filtering by gravity through membrane filters with 4-8 μm pores to the 10 ml final volume. The concentrate was transferred to the test tubes containing 1 mL of saturated HgCl₂ solution and stored until analysis. In the lab, fixed cells were washed with 1 M CaCl₂ solution by centrifugation [23, 24], transferred into water-glycerol slides, and counted by microscopy.

3. Results and Discussion

Lacking rigid cell wall, *G. semen* cells are very sensitive to various influences. As an example, figure 1(e-h) illustrates the stages of destruction of *G. semen* cells by an excess of light. There are numerous references to the destruction of its cells under the influence of traditional phytoplankton fixatives, e.g. Lugol’s solution or unbuffered formalin [25]. We have found that mercuric chloride (sublimate), which we used to quantify the number of ciliates and some other protists, effectively protect *G. semen* cells from destruction and keeps its cellular structure unchanged (figure 1a). The use of sublimate fixation allowed us to determine this species at abundance <10 cells L⁻¹. Although we cannot recommend this method for routine phytoplankton monitoring due to the high toxicity of mercury salts, there is a clear need for specific fixation techniques to investigate this and similar phytoplankton species with delicate cell envelopes.

The development of *G. semen* was found in 12 of the water bodies we have studied (table 1). Its abundance in different lakes varied from 10 cells L⁻¹ to 7.46×10⁶ cells L⁻¹ (table 2). In four lakes, no quantitative analysis was performed, and *G. semen* was observed only in live samples. The range of ecological conditions (pH, water color, conductivity) in the lakes where the development of *G. semen* was detected is quite wide (table 1-3).
Figure 1. (a) Cells of Gonyostomum semen at the HgCl$_2$-fixed slide; (b) aggregating of G. semen in the water sample from the Lake Izyar after 1 day storage; (c) intact G. semen cell; (d) cell of the ciliate Frontonia leucas with G. semen cells in the digestive vacuoles; (e-h) the stages of G. semen destruction under microscope illumination (bar – 10 µm); (i) – G. semen cell broken by filtration

G. semen predominantly developed and formed higher abundance in polyhumic lakes with a water color over 100°Pt (table 2). In a very small amount, less than 100 cells L$^{-1}$, it was also found in less colored lakes (50-60°Pt), including high-sulfate, meromictic Lake Shungaldan with surface conductivity of 780 µSm cm$^{-1}$ (table 1). Its presence in the clear-water Lake Raifskoe in July 2019 (table 1, 2, figure 2c) was also unexpected. Although the water color in the surface layer of the lake is 30°Pt, it has a dark-water tributary, the Ser-Bulak River. We propose that in 2019, the water from the tributary containing G. semen entered the thermocline zone of the lake, more precisely, into the water layer at 4 m depth, where the water color raised to 65°Pt and a narrow maximum of G. semen was found (figure 2). Since we failed to detect it previously, in 2006 and 2007, it is unclear whether its presence in 2019 is a short-term episode of regular seasonal processes or a single event caused by a rare combination of environmental factors.

G. semen cells are motile and therefore capable of significant vertical migrations in the water column under the influence of environmental factors, e.g. [26, 27]. In our study, it always formed maxima in the aerobic or microaerobic layers of the metalimnion of stratified lakes, and was sporadically detected in the aphotic anaerobic water layers (figure 2). As a rule, the area of its maximum was relatively wide, ~3 m; however, under certain conditions it formed narrow “plate”, no more than 0.25 m thick, as, for example, in the lakes Izyar and Raifskoe (figure 2).
Table 1. Some characteristics of studied lakes with *G. semen* development

| Lake            | Coordinates                  | Length (m) | Width (m) | Max depth (m) | Cond. (µSm cm⁻¹) | Transp. (m) | Year of first finding |
|-----------------|------------------------------|------------|-----------|---------------|------------------|-------------|----------------------|
| Beloe           | 55.9239 N, 48.7640 E        | 520        | 180       | 3.5           | 380-465          | 1.4         | 2006                 |
| Gniloe          | 55.9179 N, 48.7780 E        | 220        | 60        | 5             | 30-138           | 0.5-0.6     | 2006                 |
| Dolgoe          | 55.9016 N, 48.8388 E        | 120        | 50        | 12.5          | 40-66            | 0.8-1.5     | 2006                 |
| Raifskoe        | 55.9048 N, 48.7269 E        | 1300       | 300       | 19            | 270-383          | 1.25        | 2019                 |
| Ilantovo        | 55.9215 N, 48.7853 E        | 900        | 200       | 2.4           | 75-95            | 1.4         | 2006                 |
| Karasikha       | 55.9065 N, 48.7478 E        | 65         | 55        | 10.8          | 98-164           | 0.5         | 2006                 |
| Shungaldan      | 56.1488 N, 48.4420 E        | 160        | 100       | 11.5          | 900-1650         | 2.5         | 2007                 |
| Big Kichier     | 56.0704 N, 48.3459 E        | 1250       | 550       | 16.5          | 113-190          | 0.6         | 2006                 |
| Bezukladovskoe  | 56.6104 N, 47.0523 E        | 630        | 440       | 8.5           | 76-130           | 0.6         | 2014                 |
| Izyar           | 56.4268 N, 47.0015 E        | 300        | 230       | 10.5          | 23-84.5          | 1.6         | 2014                 |
| Shirshyar       | 56.4412 N, 46.9557 E        | 270        | 420       | 12.5          | 45-53            | 1.8         | 2014                 |
| Chebak          | 55.0133 N, 46.5812 E        | 1200       | 75        | 145           | 0.35             | 2018        |                      |

*Volgo-Kama State Nature Biosphere Reserve; National Park Mary-Chodra; Projected Biosphere Reserve "Kugu Kakshan"; buffer zone of State Nature Reserve "Prisursky"*

Table 2. Quantitative parameters of *G. semen* development in the lakes of the forest region of Volga basin

| Water body            | Date       | pH       | Water color (°Pt) | Maximal abundance (10⁵ cells L⁻¹) | Maximal biomass (mg L⁻¹) | Source       |
|-----------------------|------------|----------|-------------------|-----------------------------------|--------------------------|--------------|
| Rivers Krutets, Kerzhzenets and Linda | 1985       | 6.6-6.8  | <300              | 770                               | 13.6                     | [17]         |
| Lake Krivoe           | 1989-1997  | 4.4-7.3  | 400               | 581                               | 15.1                     | [5]          |
| Lake Svetlinsk’koe    | 6.2-6.5    | 13-14    | –                 | 0.15                              | [20]                     |
| Lake Sankhar          | 7.6        | 30       | –                 | 0.30                              | [20]                     |
| Lake Bolshie Garavy   | 2002-2004  | 5.6-5.7  | 65-70             | –                                 | 0.18                     | [20]         |
| Lake Bolshoe Poridovo | 6.4-6.5    | 290-324  | –                 | 0.96                              | [20]                     |
| Lake Dolgoe           | 17.07.2006 | 5.6-6.6  | 220               | 35.0                              | 0.81                     | This work    |
|                        | 05.08.2007 |          | 210               | 79.4                              | 1.83                     |              |
|                        | 03.07.2019 |          | 185               | 124                               | 2.85                     |              |
| Lake Gnileoe          | 18.07.2006 | 5.3-7.5  | 400               | 187                               | 4.30                     | This work    |
| Lake Ilantovo         | 13.07.2006 | 6.4-6.9  | –                 | 13.5                              | 0.31                     | This work    |
|                        | 02.07.2019 |          | 165               | 61.7                              | 1.42                     |              |
| Lake Izyar            | 03.07.2014 | 5.8-7.7  | 143               | 7463                              | 171.65                   | This work    |
| Lake Raifskoe         | 09.07.2019 | 6.9-9.3  | 65                | 1.57                              | 0.03                     | This work    |
| Lake Shungaldan       | 08.08.2007 | 7.0-7.9  | 60                | 0.10                              | 0.002                    | This work    |
| Lake Big Kichier      | 20.07.2006 | 6.0-6.9  | 50                | 0.03                              | 0.001                    | This work    |
| Lake Chebak           | July 2018  | 7.1-7.7  | –                 | 24.0                              | 0.55                     | This work    |

Statistical analysis of environmental factors in localities of *G. semen* development (table 3) shows that it preferred elevated water color and low mineralization, tolerate oxygen deficit and even low sulfide concentrations, and therefore often met together with anoxygenic phototrophic bacteria (as evident from *Bchl a* concentrations).

At the end of the 20th century, the expansion of this species was frequently explained by anthropogenic acidification of water bodies; however, this was apparently due to the correlation
between color and low mineralization, from one side and sensitivity to acidification, from the other, since it is currently successfully developing in circumneutral water bodies. According to our results, its pH optimum lay around 6.5, and in ~25% cases, it developed at pH > 7.5.

This species contains numerous extrusomes, which are defined as trichocysts or mucocysts in various sources. Under mechanical influences or when attacked by predators, they “shoot out” releasing slimy fibers [12], which can interfere with the movement of predators and immobilize or even kill other phytoplankton species. An excretion of extrusomes may occur even when water samples are stored under unfavorable conditions, and G. semen cells are combined into loose aggregates (figure 1b).

Figure 2. Vertical distribution of G. semen in lakes Gniloe (a), Dolgoe (b), Raifskoe (c) and Izyar (d).

Table 3. The range of selected ecological parameters of G. semen development in the studied lakes. Statistical characteristics are weighted using log-transformed G. semen abundance.

|                | Min  | Q1   | Median | Q3   | Max  | Mean  | SD  |
|----------------|------|------|--------|------|------|-------|-----|
| Depth (m)      | 0    | 1,0  | 2,0    | 4,0  | 15   | 3,18  | 3,31|
| T (°C)         | 3,6  | 8,8  | 17,3   | 20   | 27,5 | 15,0  | 7,2 |
| O₂ (mg L⁻¹)    | 0    | 0    | 1,47   | 6,45 | 11,58| 3,23  | 3,95|
| S² (mg L⁻¹)    | 0    | 0    | 0,04   | 1,38 | 0,05 | 0,15  |     |
| pH             | 5,5  | 5,7  | 6,4    | 7,54 | 8,79 | 6,59  | 0,87|
| Conductivity (µSm cm⁻¹) | 25  | 43   | 62     | 119  | 980  | 120   | 153 |
| Pₘ (mg L⁻¹)    | 0    | 0    | 0,011  | 0,058| 0,183| 1,669 | 0,160| 0,274|
| NH₄ (mg L⁻¹)   | 0,007| 0,024| 0,251  | 0,414| 1,743| 0,285 | 0,278|
| Color (°Pt)    | 32   | 143  | 220    | 368  | 400  | 216   | 122 |
| Bchl a (µg L⁻¹) | 0    | 0    | 0,4    | 0,9  | 7,6  | 0,9   | 1,7 |

In cases of mass development of this species, its mucus may cause skin irritation in humans [28], thus justifying G. semen definition as “nuisance species”; however, unlike the marine raphydophytes, it is apparently unable to produce any toxin. Such a “nuisance” is not too significant for water bodies of specially protected natural areas. A much greater danger is a sharp decrease in the biodiversity of the plankton community during the mass development of this species [29]. Since only a few species of zooplankton are able to consume its cells, its development is often poorly controlled by grazing. However, we have found that at least some species of ciliates developing together with G. semen are able to feed on them (figure 1d), and therefore at least partially control its abundance.

Although G. semen has often been considered an invasive species, there is no clear evidence that it is in all cases expanding its range, settling in new water bodies, instead of just increasing its abundance in already inhabited ones. The low genetic diversity of the Scandinavian strains [30] suggests recent colonization; however, paleolimnological data indicate that it was present in the lakes
of Scandinavia as early as in the middle of the 19th century [31]. The problem is further complicated by the fact that, due to the fragility of its cells, this species can be easily overlooked or lost during routine analysis. Thus, the literature data do not allow one to draw an unambiguous conclusion about the G. semen historical area and its recent changes.

4. Conclusion
The results presented here significantly expand the geography of the planktonic habitats of G. semen in Volga region. Now this species has been found in the plankton of lakes in all regions of the forest-steppe part of the Middle Volga basin, and the absence of its locations in the Ulyanovsk region and the forest-steppe part of the Tatarstan Republic may well be a consequence of the poor knowledge of the phytoplankton of the lakes of this region.

The spread of G. semen is commonly attributed to the global climate change. During the Little Ice Age, before 20th century, ecological conditions in the water bodies of the temperate zone were far from optimum of this species either due to low summer temperatures or due to low content of colored organic matter (cDOC). Thus, it developed in certain brown-water refugia either in littoral habitats or among moss vegetation, and, generally, never appeared in the pelagic zones. With the onset of global warming, however, the lower limit of its temperature requirements shifts northward; at the same time, brownification has led to the southward expansion of lakes that meet its cDOC requirements. The area of intersection of the zones where both these requirements met is therefore expanding, and the combination of temperature and cDOC conditions is getting more and more close to ecological optimum of G. semen, allowing it to (re)colonize euplanktonic habitats.

However, the development of G. semen in the clear-water lakes of France, Spain and Russia (in some karst lakes in Vladimir region and highly mineralized meromictic Lake Shungaldan in Rep. Mari El) indicate much higher ecological plasticity of this species than assumed in the scheme proposed above, indicating its oversimplification.

Although the water ecosystems of protected areas are more or less protected from local anthropogenic impact, they are subject to global changes. Therefore, rearrangements of plankton communities similar to the mass development of G. semen are probably inevitable in water bodies of protected areas. In these circumstances, careful monitoring of protected water bodies is urgently needed.

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