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Bloom-forming potentially toxic dinoflagellates *Prorocentrum cordatum* in marine plankton food webs

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Summary

The article reviews the up-to-date concepts of an ambiguous status of the widely spread, bloom-forming, potentially toxic mixotrophic dinoflagellates *Prorocentrum cordatum* in marine planktonic food webs, with special emphasis on the ecological prerequisites of their proliferation and possible scenarios underlying the development of harmful algal blooms and their top-down control in marine coastal waters.

Key words: Baltic Sea, Black Sea, copepods, dinoflagellates, grazing, growth rate, harmful blooms, invasive species, mixotrophy, *Prorocentrum cordatum*, toxicity, trophic network

Contents

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1. Introduction

The armored dinoflagellate *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, 1975 (Alveolata: Dinophyceae: Prorocentrales) is a free-living cosmopolitan planktonic species with global distribution which causes harmful algal blooms (HABs, or red tides) in the sea coastal waters. Although *P. cordatum* is distributed worldwide, it is still expanding its geographic range and invading new environments. Currently, it is most commonly found in marine and brackish waters of the temperate climate zone and in subtropics, less seldom in tropical regions (Heil et al., 2005; Skarlato et al., 2018b; Skarlato and Telesh, 2018).

Prorocentrum cordatum, a taxonomically accepted name according to the World Register of Marine Species (WoRMS) (Guiry and Guiry, 2019), is also known in the scientific literature by its most commonly used synonym, *Prorocentrum minimum* (Pavillard) Schiller, 1933 (more than 12 000 citations in the Google Academia statistics during the recent two decades). Some other (historical) synonyms of *P. cordatum* are: *Exuviaella cordata* Ostenfeld, 1902; *Prorocentrum triangulatum* Martin, 1929; *Exuviaella minima* Schiller, 1933; *Exuviaella marie-lebouriae* Parke et Ballantine, 1957; *Prorocentrum cordiformis* A.S. Bursa, 1959, and *Prorocentrum marielebouriae* (Parke et Ballantine) A.R. Loeblich III, 1970.

The most detailed revision of the history of *P. cordatum* taxonomy and synonymy was first published by Velikova and Larsen (1999) and later by Heil with co-authors (2005). These authors provided the excessive information on the considerable confusion that has long existed with regard to the identification of *P. cordatum* since its initial description, largely due to its extremely variable cell shape (Hulburt, 1965) and the presence of a small anterior spine which is not always visible with light microscopy. This remarkable variability in cell shape has led to the description of a number of local forms as 'new species' (Fukuyo et al., 1990; Marasović et al., 1990), producing a variety of synonyms for *P. cordatum* in the literature (see Table 1 in Heil et al., 2005).

Specifically, until the late 1990-s there existed two similar species: *Prorocentrum cordatum* (Ostenfeld) Dodge (or *Exuviaella cordata* Ostenfeld, 1901; originally identified in the samples from the Caspian Sea collected in 1898–1899, and later registered in the Aral, Azov and Black seas; Velikova and Larsen, 1999), and *Prorocentrum minimum* (Pavillard) Schiller (or *Exuviaella minima* Pavillard, 1916; originally described from the Gulf of Lion, the Mediterranean Sea; Pavillard, 1916). These two species differed morphologically only by the apparent complete absence of an apical spine in *P. cordatum* when examined by light microscopy.

Meanwhile, processing of numerous samples from the red tides in the Western part of the Black Sea that were collected along the Bulgarian, Romanian and Ukrainian coasts in 1991 through 1997, and usage of the scanning electron microscopy (SEM) finally provided the solution of the abovementioned dilemma. It was proved that the apical spine which could not be seen under the light microscopy was found in the cells of *P. cordatum* in both the original Caspian isolates and those from the Black Sea; this finding allowed demonstrating the con-specificity of *Prorocentrum minimum* and *P. cordatum* (Velikova and Larsen, 1999).

Thus, despite some doubts about which name of the species to use in the current review: *P. minimum* – the most commonly used but unaccepted by WoRMS, or *P. cordatum* – the accepted by WoRMS yet still rarely used, in order to overcome the confusion and ensure the adequate citation of the previous publications, it was decided to use the taxonomically accepted, valid according to the priority rule Latin name *Prorocentrum cordatum* (Ostenfeld) J.D. Dodge, 1975 (http://www.marinespecies.org/aphia.php?p=taxdetails&id=232376; Guiry and Guiry, 2019) throughout this article, irrespective of the synonyms (in some cases given in brackets) used by the authors in the original publications.

Additionally, in the general discussions within this review concerning various strains of the species, the abbreviation PRORO was also employed, which covers a variety of names and strains of *P. cordatum* used by different authors. This approach made it possible to not only include large amount of modern research results into this review but also cite at least part of the scientific data from the 'grey literature'

published in the Russian language since the start of the XX century. The latter results remain virtually unavailable to date and thus unknown to broad scientific audience, not only to the English-speaking readers but also to the Russian-speaking researchers, because those publications as a rule do not appear in the electronic form online or anywhere else but exist only in the rare libraries as hard copies of the old journals and books.

Meanwhile, not only has the synonymy of *P. cordatum* long remained confusing. The mysterious flexibility of metabolism and tolerance of wide range of salinity and temperature enable this dinoflagellate species to adapt to various physico-chemical conditions, disperse, and invade various ecosystems, apparently due to synergetic effect of human nature-transforming activities and global warming of the World Ocean. To date, multiple results of research activities, from field observations to modern studies of molecular genetics, cell physiology, and fine structure of PRORO are accessible in the scientific literature (Skarlato et al., 2018a, 2018b, and references therein). However, the exact mechanisms allowing populations of these protists to dominate at very high densities during periodic red tide blooms and to suppress congeneric species when invading new environments still remain unresolved (Telesh et al., 2016, and references therein).

Some contradictory data exist also concerning high growth rates of PRORO leading to extraordinary blooms and toxicity of secondary metabolites of these dinoflagellates to aquatic organisms and humans, as well as their effects on various aspects of biology of marine zooplankton. Although *P. cordatum* at bloom concentrations were shown to be non-toxic for numerous zooplankters in the experiments (Petipa, 1965; Kovaleva, 1969, 1977, 1983; Tsuda and Nemoto, 1984; Ianora and Poulet, 1993; Uye, 1996; Khanaychenko, 1999; Besiktepe and Dam, 2002; Litaker et al., 2002; Johnson et al., 2003; Rosetta and McManus, 2003; Kim and Jeong, 2004; Dam and Colin, 2005; Jeong et al., 2010, 2018; Aganesova, 2011a, 2011b; Khanaychenko et al., 2016), still their blooms in coastal environments at certain times are accompanied by mortalities of marine fauna (Al-Hashmi et al., 2015, and references therein). As a permanent nanoplanktonic component of manifold marine coastal ecosystems, *P. cordatum* is likely exploiting the intricate (and largely underscored so far) interactions in marine pelagic food webs and impacts the higher trophic levels through the zooplankton grazers.

This review aims at the analysis and revision of the available knowledge of performance of the widely spread, ubiquitous, bloom-forming, potentially toxic mixotrophic dinoflagellates *Prorocentrum cordatum* in the marine plankton trophic networks, with a special emphasis on their distribution and characteristics in the Black and Baltic seas, the invasion potential of these protists, and possible scenarios of the top-down control of their harmful blooming in the marine coastal ecosystems.

2. Some remarkable features of *Prorocentrum cordatum*

2.1. A common component of phytoplankton

From the historical records stating that *P. cordatum* (*Exuviaella cordata*) was first found and described in the Krasnovodsky Bay of the Caspian Sea in April 1898 (Ostenfeld, 1901) and later – in the Aral Sea in 1899-1902 (Ostenfeld, 1908), it can be supposed that this species most likely originated in the Ponto-Caspian-Aral basin (Velikova and Larsen, 1999). However, this supposition cannot be proved basing on the morphological characteristics solely since only the genetic data can provide the precise and reliable information on the species' origin.

To date, *P. cordatum* (*E. cordata*) has been included in numerous lists of the Black Sea phytoplankton (Ryabushko, 2003; Gymez and Boicenco, 2004) as a perennial regular component of the phytoplankton community (Stelmakh and Georgieva, 2014). It is usually increasing its abundance during seasonal phytoplankton succession after the spring diatom bloom, attains the highest numbers in summer time (Sukhanova et al., 1988; Georgieva and Senichkina, 1996), and is considered among the 'core' dinoflagellate species in the Black Sea coastal waters (Bryantseva et al., 2016).

In the Baltic Sea, *P. cordatum* (*P. minimum*) is one of the most remarkable planktonic protists and the only one nonindigenous phytoplankton species (Skarlato and Telesh, 2018). Due to its high invasion potential, *P. cordatum* is one of just two dinoflagellate species (along with *P. balticum*) for which the reliable ecological niche was statistically defined (Telesh et al., 2016). According to published records, *P. cordatum* colonized the Baltic Sea more than three decades ago (Kimor et al., 1985; Olenina et al., 2010). This invasion process was rather effective, although the naturalization of the newcomer was

relatively slow (Telesh et al., 2016). After its bloom in the Skagerrak area in 1979, *P. cordatum* was first recorded in the Baltic waters in 1981 (Edler et al., 1982), and by 1999 this eurytopic marine species had already expanded its range to almost the entire brackishwater Baltic Sea, except for the Gulf of Bothnia, reaching (although at very low densities) as far to the north-east as the oligohaline Gulf of Finland (Hajdu et al., 2000, 2005; Witek and Pliński, 2000; Pertola, 2006).

The current distribution of *P. cordatum* in Eurasia is presented in Fig. 1. On the global scale, these dinoflagellates are known to be widely spread, eurytherm and euryhaline perennial protists living in different areas of the World Ocean in various marine environments and geographical areas, including southern and northern American coastal Pacific waters, the Atlantic coasts, European coastal areas from Finland and Norway, to the Baltic and Mediterranean seas, and the Indo-Pacific coastal areas (Heil et al., 2005). It is distributed in the cold-water White Sea in the Arctic region (Ilyash et al., 2003, 2015). This species is also found in the Russian Far East: in the Bering and Okhotsk seas, the Sea of Japan (Orlova et al., 2014) and down to Australia and New Zealand (Skarlato and Telesh, 2018, and references therein). It is common in the tropical coastal waters off Pakistan, in the Bay of Oman, the Arabian Sea (Al-Hashmi et al., 2015). Considering the confirmed global distribution of *P. cordatum*, the relatively small number of its records along the coasts of Africa and Southern America to date can be most likely explained by the limited number of the sea coastal monitoring and research programs in those regions (Heil et al., 2005).

2.2. Broad range of growth rates in the fluctuating environment

The shallow brackishwater coastal sea regions are usually characterized by high fluctuations of many abiotic parameters, including temperature, salinity, irradiance, nutrient concentrations etc. This environmental variability can be registered at many temporal scales: diurnally, monthly, yearly etc.; however, the long-term mean values of those parameters usually remain surprisingly stable, as exemplified by the data from the Baltic Sea (Telesh et al., 2016; Schubert and Telesh, 2017). High instability of the coastal environment impacts the structure of aquatic communities where small and fast-reproducing planktonic species experience lower stress effects compared to large bottom dwellers (Telesh et al., 2011a, 2011b, 2013, 2015; Schubert et al., 2011, 2017). Ecological niche of the generalist *P. cordatum* overlaps with the niches of some of its close congeners; but this does not prevent its coexistence with the other species due to ability of forming blooms and high population densities in a broader range of environmental parameters (Hajdo et al., 2000; Tango et al., 2005; Telesh et al., 2016).

One of the unique characteristics of *P. cordatum* is the large range of growth rates reported for these protists: from 0.12 to 3.54[1] day^{-1} (Smayda, 1996). Growth of microalgae population could be assessed basing on different parameters calculated from the increase of cell numbers during the certain period of time. The highest values of microalgal growth rate are achieved during the exponential phase of the population growth. During the exponential growth, the rate of increase in cell numbers per time unit is proportional to the number of cells present in the culture at the beginning of any unit of time (Wood et al., 2005). Among the parameters used for the assessment of the growth of microalgae populations are: the population growth rate (r, day^{-1}), the cell division rate – doublings, or cell divisions per day (k, divisions, or doublings day^{-1}), and the doubling time (T_2); these parameters can be computed as indicated below (Wood et al., 2005).

The population growth rate (r) during the exponential phase of growth is equal to specific growth rate (μ) when mortality is considered zero (according to equation 1):

$$r = \mu = (\ln N_t - \ln N_0) / \Delta t, \qquad (1)$$

where N_0 is the population density (number of cells) at the beginning of a time interval, N_t is the number of cells at the end of the time interval, Δt is the length of the time interval ($t_t - t_0$), and r is the proportional rate of change, also called the intrinsic rate of the population increase.

The growth rate (r) can be converted to doublings, or cell divisions per day (k), by dividing r by the natural logarithm of 2.0, according to the equation:

$$k = r / \ln 2, \text{ or } k = r / 0.693 \qquad (2)$$

The doubling time (T_2) for the culture, expressed in the same units of time as r, can be calculated from an estimate of r using the equation:

[1] Overestimated value; see the explanations below.

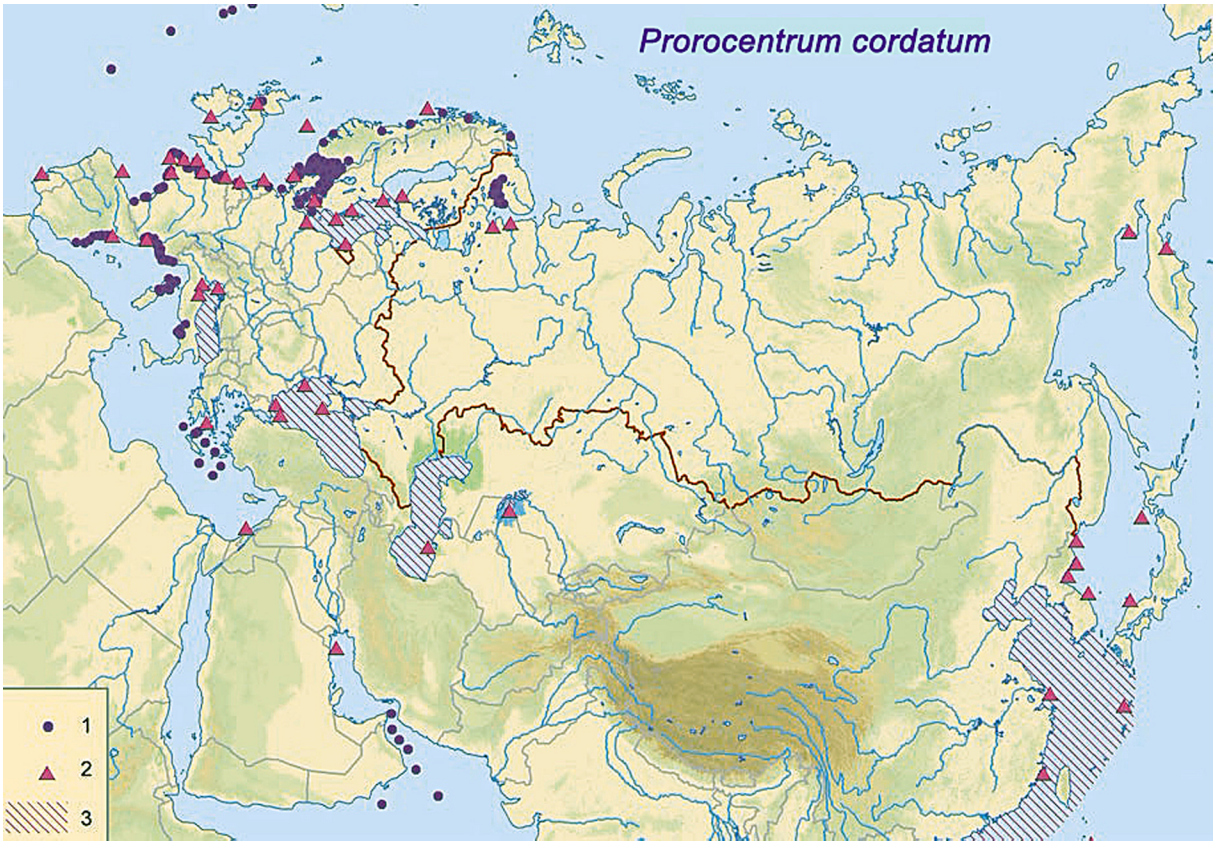

Fig. 1. Distribution of *Prorocentrum cordatum* in Eurasia: 1 – from GBIF.org (21st October 2018) GBIF Occurrence Download https://doi.org/10.15468/dl.ctsyya); 2 – according to published data (see text); 3 – present in the seas. From Skarlato and Telesh, 2018, with modifications and additions.

$$T_2 = 0.693 / r \qquad (3)$$

For the valid comparison of different results from variable experimental and field conditions provided in manifold published papers, it is necessary to know which parameters exactly were used by the authors when they were describing the population growth, in order to assess the microalgae growth rates using the same parameter. Therefore, for correctness of comparison of the data on the growth rates of dinoflagellates cited after different authors in this review, we standardized those results by using three abovementioned parameters (equations 1–3).

The results showed that the conditions for the growth optimum of *P. cordatum* vary depending on the origin of the strain and the combination of different physical factors. The first Black Sea strain of *P. cordatum* (*E. cordata*) which was isolated into the culture of the collection of alive phytoplankton at the Institute of Biology of Southern Seas (Sevastopol) by L.A. Lanskaya and maintained since the early 1960-s (Stelmakh et al., 2014), was cultured on mineral medium within the temperature range 10–28 °C, and achieved its maximum growth rate at 26 °C (Lanskaya, 1971). This strain grown in the range of salinities[2] 10–38 demonstrated the optimum growth at salinity 20; it reduced the growth rate by a half of the maximum at salinity 38, and the cells division ceased completely at salinity 5. Meanwhile, the maximum growth rate of the Red Sea isolate of *P. cordatum* (*E. cordata*) was recorded at salinities >30 (Finenko and Lanskaya, 1971). The optimum growth of *P. cordatum* in the Black Sea *in situ* was registered within the temperatures 20–24 °C and salinities 10–16 (Sukhanova et al., 1988). Growth of the Black Sea isolate of *P. cordatum* at salinity 18 was observed in vitro in the range of temperatures 10–30 °C, and optimum growth was revealed at 23

[2] Salinity is reported using the Practical Salinity Scale approved by the Joint Panel of Oceanographic Tables and Standards, according to which salinity is defined as a pure ratio, and has no dimensions or units.

°C (Stelmakh et al., 2014). The Mediterranean strain of *P. cordatum* (*P. minimum*) isolated from the Gulf of Marseille after the adaptation period survived salinities from 5 to 37 and temperatures from 4 to 31 °C, but the optimum growth was attained in a rather narrow temperature interval of 18–26.5 °C and salinity range of 15–35 at irradiance higher than 46 μE m^{-2} sec^{-1}; however, even at higher irradiance (up to 500 μE m^{-2} sec^{-1}) no photoinhibition of growth occurred (Grzebyk and Berland, 1996). In the Chesapeake Bay and the inflowing rivers, *P. cordatum* (*P. minimum*) was registered year-round over a broad range of habitats restricted by salinities 4.5–12.8 and temperature 12–28 °C (Tango et al., 2005). In the Baltic Sea, the species was registered in the salinity range of 4.7–17.0, dominating in the phytoplankton community whenever at high (e.g. 15.7 in the Bay of Mecklenburg) or relatively low salinity (4.8 in the Gulf of Finland) (Heil et al., 2005). Still, major blooms of these non-indigenous dinoflagellates in the Baltic Sea occurred at salinity 7.9±1.8 and temperature 20.8±3.7 °C (Telesh et al., 2016). Interestingly, red tides of *P. cordatum* (*P. minimum*) in the Lake Nakanoumi (Japan) were observed *in situ* in the cold waters (3–12 °C) at salinities 9–15, in the conditions that are not optimal *in vitro* for the strain isolated from the same waters (Kondo et al., 1990).

Thus, in different environments and experiments *in vitro* the maximum growth rates of *P. cordatum* under high irradiance varied in the range of 0.6–2.4 cell div. day^{-1}. Potential growth rate of the Black Sea strain of *P. cordatum* at 20 °C increased from μ=0.41 day^{-1} at low light intensity (17.2 μE m^{-2} sec^{-1}) to μ_{max}=0.66 day^{-1} at high light intensity of 206 μE m^{-2} sec^{-1} (Solomonova and Mukhanov, 2015), corresponding to cell division rate k=0.59 div. day^{-1} and doubling time T_2=41 h at low light, and k=0.95 day^{-1} and T_2=25 h at high light intensity. At 26 °C and light intensity 120 μE m^{-2} sec^{-1} (7000 lx), the Black Sea isolate of *P. cordatum* grew at the rate of k=1.4 cell div. day^{-1} (corresponding to population growth rate μ=0.97 day^{-1} and T_2=17 h); but they attained even higher growth rates of k=2.4 cell div. day^{-1} (μ=1.66 day^{-1} and T_2=10 h) under the natural summer light conditions at >10000 lux (>172 μE m^{-2} sec^{-1}) (Lanskaya, 1971). Similar growth rate of *P. cordatum*, k=2.2 div. day^{-1} (corresponding to μ=1.52 day^{-1} and T_2=11 h), was registered in patches at culmination of its bloom in June 1984 in the Burgas Bay (Bulgarian sector of the North-Western part of the Black Sea) (Sukhanova et al., 1988).

Meanwhile, the maximum achieved population growth rate (μ_{max}) of *P. cordatum* under the optimal temperature (23 °C) at the natural Black Sea salinity of 18 and optimum irradiance of 100 μE m^{-2} sec^{-1} was found to depend significantly on the physiological state of the dinoflagellate population, and varied in the range of $0.76 \le \mu_{max} \le 1.36$ day^{-1}, corresponding to $0.76 \le 1.36$ $k_{max} \le 1.96$ div. day^{-1} and $22 \ge T_2 \ge 12$ h (Stelmakh et al., 2014). The highest growth rate of *P. cordatum* isolate from the brackish-water lake Nakanoumi (Japan), μ=0.94 day^{-1} (corresponding to k_{max}=1.36 div. day^{-1} and T_2=18 h), was also achieved at 23 °C, salinity 18, and continuous light (Kondo et al., 1990). The Mediterranean isolate of *P. cordatum* reached the maximum growth of k_{max}=1.15 div. day^{-1} (μ_{max}=0.8 day^{-1} and T_2=21 h) at salinity 17, continuous light ≥250 μE m^{-2} sec^{-1} and temperature >20 °C (Grzebyk and Berland, 1996). Thus, the optimum for maximum cell division rate for the most of the strains of PRORO lays within a combination of salinities 10–20, temperatures >20–26 °C, and irradiance from 100 up to 250 (or even up to 500) μE m^{-2} sec^{-1}.

The highest ever reported growth rate for *P. cordatum* (*P. minimum* clone EX) *in vitro*, up to 3.84 day^{-1} (Smayda, 1996), actually appears to be rather doubtful. The graphical data on the increase of the dinoflagellate cells abundance provided by Smayda (1996) allowed recalculating the growth parameters of the experimental dinoflagellate populations. The only one formula for calculation of the growth parameter k (that is "doublings per day") was presented in the Methods-section of the abovementioned article. However, the daily growth rates (μ, day^{-1}), or maximum growth rates (μ_{max}), and data on "generation time" (in hours) were provided for the dinoflagellates in Table 2 (without formulas for calculation) and in description of the dinoflagellate growth in the Results-section (Smayda, 1996). Growth curves of *P. minimum* were not presented in the paper at all but the author noted that they "were similar" to those of *Amphidinium carterae* (Smayda, 1996). According to Smayda (1996), the maximum growth rate (2.89 day^{-1}) during the exponential growth of *A. carterae* was obtained when cell density increased from 50 cells mL^{-1} at day 0 to final maximal cell yield 24775 cells mL^{-1} at day 7 (text and Figure 1 in Smayda, 1996). However, if we use these data for recalculation of three main parameters characterizing growth of *A. carterae* population (during exponential phase of growth prior to the culture's transition

to the stationary phase), we obtain the maximum population growth rate of μ=1.05 day^{-1} which is corresponding to the maximum division rate of k=1.5 div. day^{-1} and population doubling time (generation time) T_2=0.66 days, or 16 h, but not the population growth rate μ=2.89 day^{-1} which corresponds to generation time 8 h, as given in Table 1 and Figure 1 in Smayda (1996).

Given in Table 1 in Smayda (1996) the maximum growth rate of *P. cordatum* (*P. minimum*) of μ_{max}=3.54±0.21 day^{-1} (that is maximum level of μ_{max}=3.75 day^{-1}) should correspond to the incredibly high value of k_{max}=5.1 div. day^{-1} and the shortest doubling time of T_2=4.7 h. Given in the text of Results (Smayda, 1996) the maximum growth rate of *P. cordatum* μ=3.84 day^{-1} corresponds to division rate k_{max}=5.5 div. day^{-1} and doubling time T_2=4.3 h. Such rates of cell division are inconceivable for any armored dinoflagellate cells of similar dimensions, and were never registered by any other researcher in whatever experimental conditions, or during the monitored natural blooms of these species. Anyway, irrespective of what was a misconception of the author (Smayda, 1996): either the erroneous use of the parameters characterizing the growth curves of dinoflagellates, or the mistake in formulas used for their computation – it is clear that the maximum growth rates of whatever armored dinoflagellate species presented in the paper (Smayda, 1996) were significantly overestimated.

Thus, our recalculations allowed to question the validity of the maximum growth rate of PRORO (μ_{max}>2.5 day^{-1}) presented by Smayda (1996), which is often referred to in literature as a possible highest growth rate of dinoflagellates during the blooms.

The above example shows that comparing the data from different sources without disclosure of the formulas used for the assessment of microalgae growth rates can lead to the confusing and biased results on the feasible growth rates of the dinoflagellate populations in nature.

2.3. Variable bloom threshold abundances in different water bodies

Phytoplankton blooms have been a hot issue of marine ecology for a long time since the end of the XIX century. In particular, the seasonally alternating diatom and dinoflagellate blooms attracted maximum attention of researchers. First registrations of dinoflagellate blooms in the Black Sea coastal areas can be attributed to the beginning of the XX century (Zernov, 1913; Ussachev, 1928). During the period of time between 1973 and 2005, nearly 158 phytoplankton blooms dominated by 50 species and varieties of algae including 25 species of diatoms and 7 species of dinoflagellates were registered in the Black Sea (Terenko and Terenko, 2008; Nesterova et al., 2008).

The term 'bloom' indicates different abundances of algal cells in different environments (Skarlato et al., 2018). For example, blooms of *P. cordatum* (*P. minimum*) as a native species in the eutrophic Chesapeake Bay occur at threshold densities > 3×10^3 cells mL^{-1} (Tango et al., 2005). However, the 3 times lower abundance of >103 cells mL^{-1} characterizes the bloom of this species as an alien in the mesotrophic waters of the Baltic Sea (Telesh et al., 2016). Moreover, the population density of *P. cordatum* equal to just 82 cells mL^{-1} (82×10^3 cells m^{-3}) at a 5 m depth above pycnocline at 14 °C and salinity 24.5 in the Onega and Kandalaksha bays of the White Sea (Ilyash et al., 2015) also can be considered as significant blooms for this northern geographical area (Table 1).

Taking into consideration the enormous data on PRORO blooms in different geographical regions of the world, we decided to focus just on two semi-closed brackish water basins of the Atlantic Ocean system: the Black Sea and the Baltic Sea.

The Black Sea, where *P. cordatum* is considered as a native species since the beginning of the XX century (Ostenfeld, 1901), is a basin where the fresh water input dilutes the surface waters to salinities 17–18.5, on average fluctuating in the range of 18±2 (in the North-Western part) due to seasonally substantial river discharge from the vast drainage area and rainfalls, with coastal water temperatures changing seasonally from 6 to 28 °C.

P. cordatum occurs perennially in the coastal waters of the Black Sea where it has been registered during phytoplankton monitoring studies carried out on the regular basis in the Sevastopol Bay in 1938–1939, since 1948 until present (Morozova-Vodjanickaja, 1948, 1954; Lanskaya, 1971; Senicheva, 1983, 1990; Lopukhina and Manzhos, 2005), and since the 1960-s – in the North-Western Black Sea coastal area (NWBSCA), in the Odessa Bay (Terenko and Terenko, 2008).

In 1973 through the 1980-s, blooms of *P. cordatum* reached the highest red tide level at 2.2×10^5 cells mL^{-1} and had been regularly covering over more than a half of the NWBSCA area (Nesterova, 1979, 1985). One of the remarkable red tide blooms of *P. cordatum* in the NWBSCA was initiated at the sea surface in September 1973, and the highest

Table 1. Bloom threshold abundances (cells mL^{-1}) of *Prorocentrum cordatum* in different water bodies and background environmental characteristics.

Water body	Bloom threshold abundance, *P. cordatum* (cells mL^{-1})	Environmental characteristics			Reference
		Water temperature (T, °C)	Salinity (S)	Total nitrogen (TN; μmol L^{-1})	
White Sea	82	14.0	24.5	ND*	Ilyash et al., 2015
Baltic Sea	1 x 10^3	17.1–24.5	8.1–9.7	28.3 ± 8.7	Telesh et al., 2016
Sea of Marmara, Golden Horn Estuary	1 x 10^3	20.4–23.1	17.0–18.5	0.71–1.03	Taş et al., 2011
Black Sea, NWBSCA	3 x 10^3	16.0–23.0	13–15	ND	Nesterova, 1979; Sukhanova et al., 1988
Chesapeake Bay	3 x 10^3	12.3–28.0	4.5–12.8	ND	Tango et al., 2005
Lake Nakanoumi	ND	3.0–12.0	9–15	ND	Kondo et al., 1990

***** ND – no data

concentration of dinoflagellate cells (up to 1.4×10^5 cells mL^{-1}) was observed in a thin neuston layer (0–5 cm), whilst in the lower layers (to 10–20 m) their concentration was two orders of magnitude lower (Nesterova, 1979).

During the same period of time, in August 1973, in the Sevastopol Bay at calm weather conditions and during sharp decrease of water temperature from 24 to 22°C after a heavy storm, the typical red tide patches of *P. cordatum* were observed for 2 days with the highest concentration of 2×10^4 cells mL^{-1}, which decreased abruptly 10 times on the 3rd day and 100 times on the 4th day (Senicheva, 1990). Significant number of empty shells of *P. cordatum* observed during this phenomenon undoubtedly implies ecdysis of the dinoflagellate cells which is usually induced by stress (Pozdnyakov and Skarlato, 2012; Berdieva et al., 2016, 2018; Skarlato et al., 2018a). In the abovementioned case, ecdysis was caused by storm and upwelling; the latter also may possibly involve dinoflagellate cysts in bloom development.

Concentration of PRORO during blooms in the Black Sea (NWBSCA) oscillated from the moderate level of 3.5×10^3 cells mL^{-1} in 1954–1960 to the highest abundance of 2.2×10^5 cells mL^{-1} in 1973–1980; it slightly decreased to 10^5 cells mL^{-1} in 1981–1994, and then leveled off to moderate values in 1995–2005 (Nesterova, 2001;Terenko and Terenko, 2008). After the decrease of PRORO blooms, since the first decade of the XXI century, dinoflagellates *Heterocapsa triquetra, Scripsiella trochoidea* and *Akashiwo sanguinea* (syn. *Gymnodinium sanguineum*) that were registered since 1960-s regularly but at low densities, also formed bloom patches (8–25.8×10^3 cells mL^{-1}) at reduced salinity in the NWBSCA – near the Romanian coasts and in the Odessa Bay (Terenko and Terenko, 2008). In the North-Eastern Black Sea coastal area, including the Sevastopol Bay, at present no blooms of these dinoflagellates have been observed, and the summer maximum of *P. cordatum* never exceeds 20 cells mL^{-1}.

Several typical successive events of phytoplankton bloom cascade were stimulated in the polluted Turkish waters (the Golden Horn Estuary) during the water rehabilitation project in 2000–2001 by the extreme changes of the initially calm water conditions (lack of adequate upper layer circulation, presence of extreme pollution and light limitation) to rapid renewal by turbulent waters followed by significant fluctuations in salinity between 2.5 and 21.6 with a sudden interim decrease to salinity 0.5 in the surface water layer due to a heavy rainfall. As a consequence of these extreme physical changes, the bloom peaks of cryptophytes in April–June reached 7.8×10^3 cells mL^{-1}, and that of *Skeletonema* cf. *marinoi* – 4.6×10^4 cells mL^{-1}; those blooms were followed by the increase of *P. cordatum* concentration up to 3.6×10^4 cells mL^{-1} (Taş and Yilmaz, 2015).

Blooms of *P. cordatum* in the Black Sea developed very rapidly, with 100 to 2000-fold increase of cell density – from a background concentration of ca. 5×10^2 cells mL^{-1} up to the maximum attainable concentration of ca. 8×10^5 cells mL^{-1} observed in patches in the upper layers 0.5–1.5 m within 3–10 days, mainly at water temperatures above 20 °C, reduced salinities of 11–15, and high irradiance at calm weather providing the glassy sea surface (Sukhanova et al., 1988; Nesterova, 2001).

The dinoflagellate blooms registered during 1970–2005 in the Black Sea (NWBSCA) in June–July, were dominated mainly by *P. cordatum* (making ca. 85% of the total phytoplankton bio-

mass and reaching the highest abundances of 1.5×10^4–2.2×10^5 cells mL^{-1}), usually appearing as successive seasonal blooms after the preceding spring diatom blooms dominated by *Skeletonema costatum* (Nesterova, 1979, 2001; Petrova-Karadjova, 1985; Sukhanova et al., 1988; Bodeanu, 1995; Nesterova et al., 2008; Terenko and Terenko, 2008). Similar bloom concentrations of *P. cordatum* (2×10^3–2×10^4 cells mL^{-1}) making up >90% of the total nanophytoplankton community occurred during Australian springs in 2003–2016 in a 15-meter deep site of the Berowra Creek (Australia); they also succeeded the *Skeletonema* sp. bloom (Ajani et al., 2018). Between the diatom and dinoflagellate blooms, the intermediate bloom of cryptophytes (ca. 2×10^3 cells mL^{-1}) often occur; however, they are not always registered in the preserved samples.

Abrupt increase of *P. cordatum* cell abundance was most often registered when other phytoplankton groups declined (Taş and Yilmaz, 2015; Ajani et al., 2018), and thereafter the maximum concentration of dinoflagellates is achieved rather quickly, mainly in a thin superficial/upper layer (0–5 cm) in a coastal area. The Black Sea blooms dominated by *P. cordatum* were often paired with other dinoflagellate species (the so-called mixed dinoflagellate blooms) where the minor component could be either *Heterocapsa triquetra*, or the congeneric *Prorocentrum micans* (Nesterova, 2001). The latter one, being the native species in the Baltic Sea, coexists with the alien *P. cordatum* since 1998 (Telesh et al., 2016).

The Baltic Sea, whereto *P. cordatum* was introduced nearly 40 years ago, presumably through ballast waters discharge (Heil et al., 2005), is known for its large-scale, significantly variable environmental gradients, when salinity is gradually decreasing from 20 at its southwestern entrance to as low as 2 in its inner north-eastern parts, and seasonal range of coastal water temperatures fluctuates between 2–5 °C in winter and 24.5 °C above picnocline in the end of July through the start of September (Telesh et al., 2016).

Introduction of the alien *P. cordatum* to the Baltic Sea started after its bloom at the unbelievable density of 1.8×10^6 cells mL^{-1} registered in the outer Oslo fjord (Skagerrak) on September 11, 1979 (Tangen, 1980). Blooms of this dinoflagellate with abundances up to 3.5×10^5–5.8×10^5 cells mL^{-1} developed in the 1980-s in different localities of the Baltic Sea, and since the middle of the 1990-s *P. cordatum* was already registered as a regular component of the summer–autumn (from July to October) phytoplankton assemblage, occasionally reaching the maximum densities of 50 cells mL^{-1} and dominating the overall phytoplankton biomass (>90%) at salinities 4.8–15 and temperatures 2.7–26.4 °C (Hajdu et al., 2005). The extraordinarily extensive bloom of *P. cordatum* (up to 3.5×10^5 cells mL^{-1}) in the Baltic Sea at 17–22 °C was recorded in the Gulf of Gdańsk (at the mouth of the Chylonka river, exactly in one of Gdynia harbor basins); in this case, a 400 m^2 area suddenly turned brownish-red and remained as such from 22 August until 9 September 1997; this bloom disappeared as suddenly as it had appeared (Witek and Pliński, 2000). Importantly, during that year these dinoflagellates dominated in the phytoplankton of the entire Baltic Sea in late August and early September (Witek and Pliński, 2000). In 2002, the abundance of *P. cordatum* in the northern Baltic Proper reached ~10^3 cells mL^{-1} and this corresponded to 90% of the total phytoplankton biomass, whilst it colored the surface waters along the southwest coast of Finland reaching the abundance of ca. 10^5 cells mL^{-1} and penetrated further to the North along the Finnish coast (Hajdu et al., 2005). During 2000–2004, its abundance also increased significantly in the Southern Baltic (Olenina et al., 2010). A delay in naturalization of the *P. cordatum* populations in different areas of the Baltic Sea can be explained by its more narrow (compared to the Chesapeake Bay) tolerance range in relation to salinity and temperature (Table 2), high overall protistan diversity in the Baltic Sea (Telesh et al., 2009; Mironova et al., 2013, 2014), and the presence of several congeneric competitors among the diverse phytoplankton community in this temperate brackishwater environment (Telesh et al., 2016).

Many descriptions of the phenomenon of dinoflagellate blooms (red tides) indicate the following physical conditions as bloom prerequisites (besides the reduced salinity): clear sky, direct hot sun, high irradiance, high temperatures >20 °C, absence of wind, calm sea, smooth water surface or "dead calm weather with glassy sea surface", while the consequences can be as follows: very sharp and distinct lines of demarcation between the blooming and the clear waters, huge differences between the upper blooming layer and the underlying water masses, as well as the fact that "bloom disappeared as suddenly as it had appeared" (Nesterova, 1979; Sukhanova et al., 1988; Witek and Pliński, 2000). It is likely that the highest concentrations of dinoflagellates observed during the red tide events are not only linked to the increase of the division rate of the cells in the conditions that are favorable for

Table 2. Temperature and salinity tolerance ranges of *Prorocentrum cordatum* in nature (the Baltic Sea and Chesapeake Bay) and in the laboratory experiments.

Features	Water temperature (°C)	Salinity	References
Survives in nature	4.0–31.0	5.0–37.0	Berland and Grzebyk, 1991
Occurs in the SW Baltic Sea	3.0–24.0	2.0–22.0	Telesh et al., 2016
Occurs in the Baltic Sea	2.7–26.4	4.8–15.0	Hajdu et al., 2005
Blooms in the Baltic Sea (>1000 cells mL^{-1})	17.1–24.5	6.1–9.7	Telesh et al., 2016
Blooms in the Chesapeake Bay (> 3000 cells mL^{-1})	12.3–28.0	4.5–12.8	Tango et al., 2005
Lowest tolerance limit in the laboratory experiments	ND*	1.8–3.6	Olenina et al., 2016

* ND – no data

the species growth. Evidently, this also could be connected with excystation, cell ecdysis after stress, besides the specific ability of the actively swimming dinoflagellates to swarm in localities of the most favorable nutrient and light conditions forming the highest concentrations in the upper layers of the water column in patches and stripes in the absence of water mixing (Nesterova, 1979; Senicheva, 1990; Sukhanova et al., 1988; Witek and Pliński, 2000; Taş and Yilmaz, 2015; Ajani et al., 2018).

2.4. High competitiveness due to effective mixotrophic metabolism

The grounds for high competitiveness, strong invasion potential, and fast bloom formation by the dinoflagellates were analyzed by various researchers over the range of eutrophication conditions and key environmental factors including physical parameters (salinity, temperature, turbulence, oxygen concentration, etc.) and chemical nutrients (inorganic and organic dissolved matters) consumed by these microalgae (Fan et al., 2003; Glibert et al., 2008, 2018; Matantseva et al., 2016, 2018; Telesh et al., 2016; Knyazev et al., 2018; Skarlato et al., 2018b, and references therein).

In contrast to diatoms, dinoflagellates have a lower affinity for nutrients, considerable nutritional diversity involving mixotrophic nutrition, and motility (Smayda, 1997). However, the nutrients availability governing cell division in dinoflagellates is affected by a complex combination of biotic and abiotic factors. In the conditions of replete inorganic nutrients, under natural light, photosynthesis *in situ* takes place at day time while cell division of *P. cordatum* mainly occurs at night (Lanskaya, 1971). Meanwhile, different cases of experimental conditions *in vitro* or disturbances in natural environment can modify these processes. For example, during diurnal vertical migration typical for marine planktonic dinoflagellates, *P. cordatum* were shown to be the fastest both to descend and ascend in comparison with *Prorocentrum micans* and *Ceratium furca*, and were capable of assimilating nitrogen both in the light and dark (Olsson and Granéli, 1991). In darkness, *P. cordatum* absorbed up to 12.2% of cell's dry weight (DW) of the dissolved organic matter (DOM) originated from hydrolisate of other microalgae labelled by ^{14}C isotope thus proving involvement of organic substances in metabolism (Bourlakova et al., 1971). Triacylglycerides and glycine were utilized by this dinoflagellate under the prolonged dark cultivation conditions as the alternative carbon sources (Manoharan et al., 1999). Moreover, in the laboratory experiments it was shown that glycine uptake by *P. cordatum* exceeded the uptake of nitrate when both nutrients were present in equal nitrogen amounts (Matantseva et al., 2018). The uncoupling of organic nitrogen and carbon assimilation was observed as a result of urea and glycine metabolic processing by urease and the glycine decarboxylation complex. The authors argue that such uncoupling reduces the net dissolved inorganic carbon (DIC) removal by dinoflagellates since the acquisition of nitrogen from urea and glycine leads to DIC release (Matantseva et al., 2018). Nevertheless, massive blooms of *P. cordatum* with high primary production (ca. 30 mg C L^{-1} day^{-1} at culmination) along the Bulgarian Black Sea coast during late spring-summer were supposed to advance in nitrogen deficiency conditions, due to active uptake of DOM (amino acids and glucose) (Hiebaum and Karamfilov, 1992). Dinoflagellates' rapid growth in the culture was also stimulated by addition of the humic acid (Heil et al., 2005).

Among the diversity of nitrogen sources and the variety of uptake mechanisms employed by *P. cordatum* are the negative response to NO_3^- in the

presence of other nitrogen sources; strong affinity for NH_4^+ and amino acids; affinity for urea not affected by temperature; and contribution of all of those substances as the primary nitrogen (N) sources; all those are supposed to aid the development and maintenance of blooms (Fan et al., 2003; Matantseva et al., 2016, 2018). Additionally, the evaluation of expression levels of urea transporter gene *dur3* and nitrate transporter gene *nrt2* in *P. cordatum* by RT-qPCR revealed their variable sensitivity to input of different N sources (Pechkovskaya et al., 2017, 2020). Thus, *dur3* expression levels were downregulated after the supplementation of additional N sources and were 1.7 to 2.6-fold lower than in the nitrate-grown culture, and the *nrt2* expression levels decreased 1.9-fold in the presence of NH_4^+. Although the addition of N compounds did not affect the DNA synthesis rates of these protists, the transcription levels increased up to 12.5-fold after the N supplementation in the urea-limited treatments (Pechkovskaya et al., 2020).

Possessing the immense diversity of mechanisms of acquiring and storing nitrogen relative to phosphorus, *P. cordatum* is distinguished by multiple nutrient pathways (Glibert et al., 2012) and flexible metabolism using various sources of inorganic and organic nutrient substrates (Matantseva and Skarlato, 2013; Matantseva et al., 2016, 2018). High intra-population heterogeneity of cells enables *P. cordatum* to expose various affinity for manifold nitrogen sources exploiting different nutritional strategies: some types of cells in the presence of multiple nitrogen sources uptake dissolved organic source of nitrogen (urea) at higher rates in comparison with inorganic source (nitrate), and thereafter, an increase in urea concentration leads to suppression of nitrate uptake (Matantseva et al., 2016). In the presence of both urea and nitrate, the absorption rate of nitrogen from urea by experimental laboratory populations of *P. cordatum* was twice as fast as the uptake of nitrogen from nitrate sources (Matantseva et al., 2016), and glycine uptake occurred twice as fast as nitrate uptake (Matantseva et al., 2018). In contrast to high nitrogen assimilation, carbon assimilation from the same organic sources is very low, revealing specific processing of urea and glycine by decarboxylation complex and significant release of the dissolved organic carbon (Matantseva et al., 2018). The contribution of nitrogen from urea to the total nitrogen absorbed by the cells of these protists was approximately 70%, while carbon from urea constituted only 0.4% of the total carbon uptake by the cells (Matantseva et al., 2018).

Besides their remarkable plasticity to chemical and physical factors, cells of *P. cordatum* after the adaptation period displayed considerable plasticity also to photosynthetic parameters, and this resulted in three- to four-fold increases in chlorophyll *a* concentration per cell within temperature range of 10–20°C (Heil et al., 2005, and references therein). Increase of light intensity by an order of magnitude from 17 to 172 μE m^{-2} sec^{-1} resulted in decrease of photosynthetic pigment content in *P. cordatum* from 4.9 to 1.8 pg chlorophyll *a* $cell^{-1}$ concurrent with the increase of cell volume from 1.3 to 2×10^{-2} μm^3 and of C/N cell ratio from 4.7 to 6.4 (Mansurova, 2016). The highest growth rates of PRORO were found under blue and especially under red light (available in estuaries where *P. cordatum* blooms); and thereafter, its high photosynthetic activity was found both in the visible light with chlorophyll absorption (430–460 nm and 660–680 nm), and in the low-light adapted cells in the 500–560 nm region via the carotenoid peridinin which is typical for dinoflagellates (Heil et al., 2005, and references therein). The active synthesis and accumulation of mycosporine-like amino acids and xanthophyll pigments stimulated by the sharp increase in the levels of solar UVB radiation (UVB index ca. 9.0), especially in the ozone-hole affected areas, were postulated recently as extra competitive advantages for *P. cordatum*, favoring its massive bloom development (Carreto et al., 2018).

No inhibition of other microalgae species, neither auto-inhibition was observed for external metabolites of the Black Sea isolate of *P. cordatum* in contrast to external metabolites produced by the diatom *Skeletonema costatum* inhibiting growth not only of other microalgae but its own cells as well (Finenko and Lanskaya, 1971). Quite on the contrary, exometabolites of the dinoflagellate *Glenodinium foliaceum* inhibited *in vitro* growth of *P. cordatum* during high level of inorganic nutrients in 2-species mixed cultures, and the pronounced growth of *P. cordatum* was restored only during the decline of *G. foliaceum* when inorganic nutrients were depleted but DOM increased (Ilyash and Fedorov, 1985). When cultivated in the 2-species mixed cultures with *Prorocentrum micans, P. cordatum* also significantly increased its growth rate when inorganic resources were already exhausted. In both variants of the 2-species mixed cultures, *P. cordatum* reached maximum abundance during the depletion of inorganic nutrients and decrease of the partner species' growth; and its growth and abundance even overtopped those achieved in the

single-species cultures (Ilyash and Fedorov, 1985). The results of this experiment are consistent with the ability of *P. cordatum* to coexist with a variety of phytoplankton species in the sea, or even successfully outcompete the congeners, as shown for *P. balticum* in the Baltic Sea (Telesh et al., 2016). These effects can be explained by multiple ways of *P. cordatum* metabolism as the basics for its adaptability.

Basing on the current data on distribution and blooms intensity (Olenina et al., 2010), variability of cells morphology (Olenina et al., 2016), sustainability of ultrastructure (Berdieva et al., 2016), high intrapopulation heterogeneity (Matantseva et al., 2016) and the extremely flexible ecophysiological potential of these protists under the varying environmental conditions (salinity and temperature stresses; amounts, proportions and sources of nutrients, etc.), the dinoflagellate *P. cordatum* was qualified as a generalist occupying a broad ecological niche (Telesh et al., 2016; Skarlato et al., 2018b). Mixotrophy, assumed to underlie one of the mechanisms of the outbreak persistence (Jeong et al., 2010), provides this species with significant advantages to increase its competitiveness under variable environmental conditions as it is clear that this dinoflagellate is characterized by labile transition from phototrophy to mixotrophy. Being a constitutive mixotroph (Johnson, 2015), in the nutrient replete conditions *P. cordatum* exploits its own plastids to perform photosynthesis using dissolved nutrients and CO_2 during the light phase, while during nutrient deficiency, especially of phosphorus, or at elevated ratio of dissolved inorganic nitrogen to PO_4^-, it may switch over to osmotrophy, or phagotrophy (Li et al., 2011; Johnson, 2015).

This protist can cover its needs for energy and nutrients by feeding on manifold prey including heterotrophic bacteria, cyanobacteria, haptophytes, cryptophytes, dinoflagellates, diatoms, and even ciliates (Li et al., 1996; Stoecker et al., 1997; Jeong et al., 2010). Captured in the flagellar canal, the prey is engulfed by *Prorocentrum* using receptor-mediated endocytosis or micropinocytosis (Kalinina et al., 2018). *P. cordatum* can also utilize organic phosphorus (Heil et al., 2005) by producing extracellular and intracellular alkaline phosphatase (Dyhrman and Palenik, 1999), thus being particularly effective at gaining phosphorus from the ingested prey, although it is very prey-specific to cryptophytes, especially to *Teleaulax amphioxeia* (Johnson, 2015).

Albeit much data have been accumulated about nutrient sources that can be utilized by *P. cordatum* and the dinoflagellates in general, the knowledge about cellular and molecular aspects of nutrient acquisition in these protists is still limited. Dinoflagellates are capable of endocytosis and probably possess highly diverse proteins mediating transmembrane transport of the dissolved nutrients, but it is not clear so far how this nutrition potential is realized at the cellular level. The existence of the very complex cell covering (amphiesma) in dinoflagellates (Pozdnyakov and Skarlato, 2012; Pozdnyakov et al., 2014, 2018; Berdieva et al., 2016, 2018) impose limitations on both endocytosis and the membrane transport of dissolved nutrients. Future investigations will provide the solutions of such topical questions as how the systems responsible for the nutrient uptake are distributed in the amphiesmal membranes, and do the morphological features like the flagellar canal and the pusule represent the major regions of the uptake and engulfment of various nutrient sources by dinoflagellates (Kalinina et al., 2018).

Meanwhile, the ability of conquering the new environment by the invader, as was the case of the recent invasion of *P. cordatum* into the Baltic Sea, where it step-by-step suppressed, driven out and replaced the native con-generic species *Prorocentrum balticum* (Telesh et al., 2016), means not only adaptation to physical conditions and competitiveness with native species for nutrients on the basis of flexible metabolism of different nitrogen sources (Matantseva et al., 2016) and changes in photosynthesis on molecular level of transport genes (Pechkovskaya et al., 2017, 2020). It also implies the competitiveness as a prey and a predator, which means "to eat more and not being eaten up". Both tendencies, and especially the ability of "not being eaten", are very important for bloom development, since they mean that either there is a lack of grazers of this dinoflagellate in certain location prior to the start of the bloom, or those grazers are too few.

To develop the bloom, PRORO might, besides competing for dissolved nutrients, use manifold mechanisms of uptake of various nutrient sources including effective grazing on the concurrent protozoa species outcompeting predominately phototrophic species, whereas concomitantly present numerous protists and metazoan species grazing on PRORO might delay its bloom development. Extensively, and intermittently intensively, distributed in different marine ecosystem environments this planktonic dinoflagellate undoubtedly should play significant role in marine pelagic food webs.

And indeed, being ubiquitous, widely distributed and forming high densities during seasonal blooms in the coastal waters of the World Ocean, mixotrophic dinoflagellates *P. cordatum* interact in the complicated marine trophic webs with the amazingly large number of diverse organisms, and the means of these interactions also vary broadly.

3. Controversial data on toxicity of *P. cordatum*

3.1. Toxicity to shellfish

Various toxic effects of different *P. cordatum* strains were reviewed elsewhere (Heil et al., 2005, and references therein); however, the data on toxicity of this bloom-forming dinoflagellate in marine pelagic food webs are controversial, and their blooms can develop according to various scenarios.

Although a bloom of *P. cordatum* was reported as potentially toxic to different life stages of the oyster *Crassostrea virginica* (Wikfors and Smolowitz, 1995), no negative effects on the embryos and larvae of the same oyster species were observed in the experimental bloom of *P. cordatum* at concentration as high as 10^4 cells mL^{-1} during 2-days exposures; meanwhile, negative effect of the toxic *Karlodinium veneficum* was registered in the same conditions (Stoecker et al., 2008). Saba and co-authors (2011) tested for relative toxicity between the two growth phases of the *P. cordatum* (*P. minimum*) culture by conducting an oyster exposure experiment. The results indicated that *P. minimum* at late stationary phase was toxic to oysters and the log phase culture was not (Saba et al., 2011). Moreover, high survival of oysters' larvae in the presence of *P. cordatum* at high cell density was commonly observed during blooms, and positive effects on growth of the eastern oyster spat in the 12-day laboratory experiments were reported (Brownlee et al., 2005). The latter could be compared with the effect of other dinoflagellates (*Amphidinium fusiforme* and *Gymnodinium splendens*) blooming in the Delaware Bay: "those areas in which red water is most frequently encountered have been found by long experience to provide the best fattening grounds for oysters in the entire district" (Martin and Nelson, 1929).

Attempts to find toxicity of the Baltic Sea dinoflagellates in mouse tests (Kimor et al., 1985) and in the Black Sea mussels from dinoflagellates bloom area also revealed only minor symptoms of toxicity (Moncheva, 1991). However, methanolic extracts from the cultures of several isolates of *P. cordatum* from various French Mediterranean and English Channel sites provoked neurotoxic activity in mouse tests (Grzebyk et al., 1997). The deaths (including liver damage) of more than hundred people consuming oysters and clams (*Venerupis semidecussata*) from a coastal lagoon (Lake Hamana) in Japan from which a toxin venerupin was isolated, were attributed to *P. cordatum* (*E. mariae-lebouriae*) abundant during this period of time in the seawater (Akiba and Hattori, 1949). Episodes of human poisoning symptoms characteristic of paralytic shellfish poisoning (PSP) occurred after consumption of the shellfish from the Obidos Lagoon (Portugal) (Silva and Sousa, 1981), and nausea and late gastrointestinal disorders similar in their nature and development to those of VSP (though less dramatic) after consumption of mussels from Norway (Tangen, 1983) were also attributed to the presence of *P. cordatum* in the harvesting areas. Still, no negative effects on humans or benthic fauna were reported to be associated with seasonal blooms of *P. cordatum* (up to 2×10^4 cells mL^{-1}) in the oyster-growing river/estuary system of the Berowra Creek (Australia) during 2003-2016 (Ajani et al., 2018).

At high concentration of *P. cordatum*, no mortalities of scallops were observed; however, production of their pseudofeces increased (Wikfors, 2005). The mussels *Mytilus galloprovincialis* that were cultivated in Sevastopol area ingested dinoflagellates (20–69% of the total phytoplankton abun-dance) year-round, and in November *Prorocentrum micans, P. compressum, P. cordatum* can account for ≥50% of food mass in their stomachs (Pospelova et al., 2016). Still, most of *Prorocentrum* spp. in stomachs and feces of the mussels were observed alive indicating indirectly that the mollusks do not have enough enzymes to digest these dinoflagellates; however, no toxic effect on the mussels was registered. Similarly, juveniles of the eastern oyster during short-time experiments filtered *P. cordatum* cells but did not digest them since those cells were egested as pseudofeces; still after a refractory period of about 2 weeks the oysters developed the ability to digest dinoflagellate cells (Wikfors and Smolowitz, 1995).

None of the direct studies had verified the toxicity of *P. cordatum* itself. Despite extracts of the mussels from the area of a large mixed bloom of two dinoflagellates (*P. cordatum* and *P. micans*) on the French Mediterranean coast revealed a rapid neurological effect in the mouse tests, it was not clearly

demonstrated whether exactly dinoflagellates or another unrevealed indirect factor connected with their blooms was the source of toxicity (Grzebyk et al., 1997; Wikfors and Fernandez, 2013). Hence, most likely, the biochemical composition of the PRORO cells is challenging to mollusks' digestive system, but those cells are not toxic to mussels.

3.2. Toxicity to fish

Nanophytoplankton organisms including various dinoflagellates are often registered in the guts of viable early larvae of different fish species. A positive correlation between *P. cordatum* (*P. minimum*) and presence of the planktivorous fish, *Brevoortia tyrannus*, was noted by Friedland et al. (1989), while ingestion of only few cells of the toxic *Alexandrium tamarense* was found to be lethal to the first-feeding capelin (*Mallotus villolus*) and herring (*Clupea harengus harengus*) larvae (Gosselin et al., 1989). Seasonal blooms of dinoflagellates, including *Prorocentrum*, coincide temporarily with the development of planktonic fish larvae and high reproduction rates of their preys – copepods. *Prorocentrum* spp. in general and *P. cordatum* (*E. cordata*) in particular were minor but usual components among the food items in the gut content of the early larvae of different Black Sea coastal fish species (*Callyonimus* spp., *Trachurus trachurus, Engraulis encrasicolus* and different gobies) (Duka, 1969, 1971). Guts of the small turbot larvae off the northeast coast of England also contained the remains of phytoplankton, particularly of dinoflagellates (Last, 1979).

Furthermore, since prominent effect of PRORO was observed on reproduction of the copepods *Acartia* spp., these dinoflagellates were added to the rearing system of the Black Sea turbot during the larval stage in order to co-feed their zooplankton prey (rotifers and copepods) (Bityukova et al., 1990; Khanaichenko et al., 1994). Moreover, turbot larvae that were grazing zooplankton feeding on PRORO at a high rate were shown to develop normally (Khanaychenko and Bityukova, 2007).

3.3. Toxicity to planktonic protists and crustaceans

Experimental and field data reported in the previous chapters confirm that *P. cordatum* itself does not decimate planktonic organisms (protists and calanoid copepods) that are ingesting these protists. Besides the numerous evidences of positive effects of feeding of various species of marine calanoid copepods on *P. cordatum*, high survival of the *Artemia* metanauplii, which are often used for toxicity bioassays, fed *ad libitum P. cordatum* in the experimental conditions (95% during 3 days of rearing) was recorded (Smirnov et al., 2019). For comparison, the excreted metabolites of some diatoms were reported toxic to *Artemi*a nauplii (Caldwell et al., 2003).

Moreover, in the middle of the 10 days-long bloom of *P. cordatum* in NWBSCA in the Burgas Bay in June 1986, numerous mesozooplankton species were observed vigorous, including the larvae of *Polychaeta* (170 ind. L^{-1}), rotifers (140 ind. L^{-1}), and different stages of *Acartia* spp.; and even during the decline of the dinoflagellates bloom followed by the rapid cascade development of bacteria, zooflagellates and populations of other nanoheterotrophs, no pronounced toxic effects of metabolites and degradation products of dinoflagellates on components of the planktonic community were registered (Sukhanova et al., 1988). Thereby, proliferation of various protists and copepods grazing on these dinoflagellates even at bloom concentrations confirms the absence of toxic effects, and thus generally *P. cordatum per se* should be considered nontoxic to most of planktonic grazers (Wikfors, 2005).

3.4. The overall toxicity of *P. cordatum* blooms

The data on toxicity of *Prorocentrum* species are rather controversial; they imply that toxicity depends on species, strain, and/or certain conditions of strain development or cells' concentration, or microbial environment developing in these conditions, and target organism to which it may be considered toxic. Apparently, PRORO in nature are toxic only sporadically, and mainly during the decline or late stationary phase of bloom development that takes place in the oxygen-deficient or 'dead' zones of the coastal waters. The data providing the reliable proof that *P. cordatum* is toxic *per se*, at least to zooplankton, are so far unavailable.

Meanwhile anoxia often develops during various phytoplankton blooms, including those of non-toxic species. For example, anoxia caused by the bloom of non-toxic *Lingulodinium polyedrum* (0.9×10^6 cells L^{-1}) in October 1999 in the Black Sea coastal waters (the Dniepro-Bug Estuary) was accompanied by fish mortality (Terenko and Terenko, 2008). The animals' death was observed after a reduction in dissolved oxygen concentration (DOC) in the

area during the bloom decline (Rabbani et al., 1990). The elevated phytoplankton activity in the dinoflagellate-bloom areas initially increases DOC to super-saturation levels (20.4 mg L^{-1}), while the decomposition of dinoflagellate stock at the end of the bloom in absence of water mixing ends up with anoxic conditions (Taş et al., 2011). Dissolved oxygen can decrease drastically during blooms reaching as low level as 0.2 mg L^{-1} near bottom (Tango et al., 2005).

Anoxia in coastal shallow waters is likely one of the main reasons for mortality of fish, mollusks and crustaceans that are suffocated during the collapse of dinoflagellate bloom. Oxygen depletion to the level below 1 mg L^{-1} drastically reduces metabolism of copepods and their eggs and causes their quick death, especially of embryos and nauplii (Roman et al., 1993). Thus, detrimental indirect impacts associated with PRORO blooms may be attributed to the direct effect of low dissolved oxygen concentration.

The other possible indirect detrimental effect could be the development of microflora associated with high concentration of dinoflagellate cells. After *P. cordatum* reached its highest abundance of 10^5 cells mL^{-1} in the Burgas Bay (Bulgaria), the increase in density of bacteria mainly aggregated and attached to dinoflagellate senescent cells was observed (Sukhanova et al., 1988). The processes of sloppy feeding by zooplankters during high density of the blooming dinoflagellates and viral lysis of phytoplankton cells can contribute a significant amount of the phytoplankton DOM released into seawater that is quickly utilized by the bacteria that are increasing their numbers rapidly (Møller, 2007). Microflora belonging to *Roseobacter* clade (i.e. *Dinoroseobacter shibae*) related to dinoflagellate blooms was isolated from the decaying *P. cordatum* cells and is known to have mutualistic interactions with the latter. However, it is also associated with and was isolated from a prymnesiophyte *Isochrysis galbana* which had never been considered toxic, but is used widely in aquaculture since these bacteria synthesize essential vitamins B_{12} and provide them to the auxotrophic *P. cordatum* that, in turn, supply the bacteria with carbon sources of the dissolved organic matter (Wagner-Döbler et al., 2010). At *P. cordatum* density of 2.5×10^6 cells mL^{-1} (in the experimental culture), numerous *Roseobacter* isolates colonized the dinoflagellate cells' surfaces and produced tropodithietic acid which negatively impacted *Vibrios* (Wagner-Döbler et al., 2010). However, the bacteria from *Roseobacter* clade are also known to produce ocadaic acid toxins (lipophilic marine biotoxin produced by *Dinophysis*), and hence can contaminate shellfish, thus poisoning humans that consume them.

4. *Prorocentrum cordatum* as an essential food web component

4.1. Cell size and carbon content of *P. cordatum*

P. cordatum occupy a rather broad ecological niche compared to many other dinoflagellates (Telesh et al., 2016). The cells of *P. cordatum* vary in size and shape (from triangular to oval and to heart-shaped), as well as in carbon content under the range of salinity, temperature and irradiance conditions. The mean size of *P. cordatum* isolated from the Black Sea waters (mean salinity 18) is 16 μm long, 12 μm wide (Lanskaya, 1971). Cell dimensions of the species from the Caspian Sea decrease from 16–20 μm long, 13.3–16.5 μm wide at higher salinities (12–13) to 6.5–16.5 μm long, 5–13.8 μm wide at lower salinities (1.2–7.0) (Velikova and Larsen, 1999). *P. cordatum* cells from the Sea of Azov increase their dimensions with higher salinity, varying from 9 to 19 μm in length and from 7 to 16 μm in width (Krakhmalny et al., 2004).

Cell size and morphology of *P. cordatum* from the Atlantic waters also differ with strain origin, culture medium, salinity, and growth rate. The French RSC strain of *P. cordatum* (*P. minimum*) has the length 14–22 μm and width 10–15 μm (Ianora and Poulet, 1993); the American strain's length is 14.0±1.4 μm, the Adriatic – 17.0±1.2 μm, the Baltic – 11.6±1.7 μm (Monti et al., 2005); the mean ESD of the clone Exuv is 13 μm (Dam and Colin, 2005). The average length of the Baltic strain KAC72 varied from 13 μm at low salinity (3.6) to 22 μm at brackish salinity of 7.2 (Olenina et al., 2016). Length of the cells sampled in different Baltic Sea sites varied from 17 to 25 μm (Hajdu et al., 2005).

The carbon content per cell of *P. cordatum* according to different authors (either estimated or measured directly) falls in the range between 0.129 ng C $cell^{-1}$ (Cohen et al., 2007) and 0.596 ng C $cell^{-1}$ (Laabir et al., 1999) (Table 3).

Cell dimensions of *P. cordatum* are within the limits of the optimal prey for its detection (≥10 μm) and capture (≤25 μm) by numerous planktonic calanoid copepods, especially the coastal and estuarine species, e.g. representatives of the family Acartiidae, since these protists exceed the distances

Table 3. Carbon content (ng C cell^{-1}) of *Prorocentrum cordatum* (*P. minimum*).

Region, strain, cultivation conditions	Carbon content (ng C cell^{-1})	References
Culture was grown in filtered Gulf Stream seawater diluted with distilled water to a salinity of 30, enriched with f/2 nutrients, at 22°C.	0.133 ± 0.004	Cohen et al., 2007
Culture was grown in 0.22-µm filtered sea water enriched with K medium at 20°C and on a 12-h dark/12-h light cycle	0.177	Carotenuto et al., 2002
Culture was grown on sterilized Black Sea water at salinity 18, enriched f/2 at 19-22 °C. Illumination was performed by luminescent PHILIPS TL RS 20W/54-765 (light intensity 17-172 µE m^{-2} sec^{-1}).	0.195–0.276	Mansurova, 2015
Laboratory-cultured dinoflagellates *P. minimum* (length, 14-22 µm; width, 10-15 µm), in excess concentrations (4.8-8.7 x10^4 cells mL^{-1}).	0.274	Ianora and Poulet, 1993
Culture was grown at 20±1°C. No data on salinity (water from Long Island Sound, USA). Exponential growth in f/2 medium.	0.280	Besiktepe and Dam, 2002
P. minimum was cultured in two separate batches: (1) a non-toxic batch in which the culture was kept in exponential growth phase with fresh additions of nutrient-replete L1 media every 2–3 days, and (2) a toxic batch where the culture was grown in low-nutrient media (L1/20) to late stationary phase before being transferred into new L1/20 media.	0.290–0.313	Saba et al., 2011
The cultures were maintained in a temperature-controlled incubator at 20 °C with a 12:12 light–dark cycle, in the exponential growth phase maintained by dilution every 2 days with f/2 medium.	0.293	Dam and Colin, 2005
Dinoflagellates *P. minimum* were grown in K-media, at 17 °C and on a 12 h light (200 µE m^{-2} s^{-1}) : 12 h dark cycle. Cell density: 2.3x10^3 cells mL^{-1}; equivalent carbon conc.: 1370 µg C L^{-1}.	0.596	Laabir et al., 1999

between the setulae that act as filters in the filtering apparatus of the adult copepods and even their juveniles. Interestingly, the latter are decreasing abruptly the ingestion rate of the cells that are larger than 15 µm in diameter (Gruzov, 1985).

4.2. Grazers of *P. cordatum*

The ubiquitous dinoflagellates *P. cordatum* are involved in a variety of trophic interactions in plankton food webs. These interactions and major grazers of *P. cordatum* in the Black Sea trophic network are schematically represented in Fig. 2.

Experimental data revealed that among protists the main pelagic grazers of *P. cordatum* are mixotrophic dinoflagellates *Heterocapsa triquetra* (Litaker et al., 2002); heterotrophic dinoflagellates *Gyrodinium* spp. (Johnson et al., 2003); tintinnids (*Favella ehrenbergii, F. taraikaensi, Eutintinnus pectinis, Metacylis angulata*), and the aloricate ciliates *Strombidinopsis* spp., *Strombidium* spp., and *Pelagostrobilidium* sp. (Jeong et al., 1999, 2010, 2018; Rosetta and McManus, 2003). Heterotrophic dinoflagellates *Polykrikos kofoidii* feed at low rate but not select *P. cordatum* (Jeong et al., 2001); *Aduncodinium grandula* feed on *P. cordatum* but not at maximum possible ingestion rate (Jang et al., 2016). Among ciliates, the maximum daily ingestion rate on *P. cordatum* (1300 cells ind.$^{-1}$ day^{-1}) was registered for *Strombidinopsis* spp. (Jeong et al., 1999), while the maximum grazing rates of heterotrophic dinoflagellates *Gyrodinium spirale* and *Gyrodinium dominans* on *P. cordatum* was limited to 91 and 8 cells ind.$^{-1}$ day^{-1}, correspondingly (Kim and Jeong, 2004) (Table 4).

Among metazoans, the main grazers of dinoflagellates (besides other phytoplankton) are herbivorous and omnivorous calanoid copepods (Table 4), the most numerous and abundant component of marine plankton which plays one of the key roles in the pelagic food webs (Fig. 2). Being the major link between the lower (phytoplankton) and the higher trophic levels (fish) in marine pelagic food networks and constituting the bulk of the diet of planktonic fish larvae as highly efficient natural food, planktonic calanoid copepods thus effectively regulate fish recruitment (Poulet and Williams, 1991; Thor and Wendt, 2010). While the copepod trophic linkage is only a part of the complicated planktonic food web which involves PRORO (Jeong et al., 2010), the substantial, or in some cases even preferential predation of copepods on dinoflagellates, besides other protists, should be taken into account mandatorily (Paffenhöfer et al., 2005, and references therein).

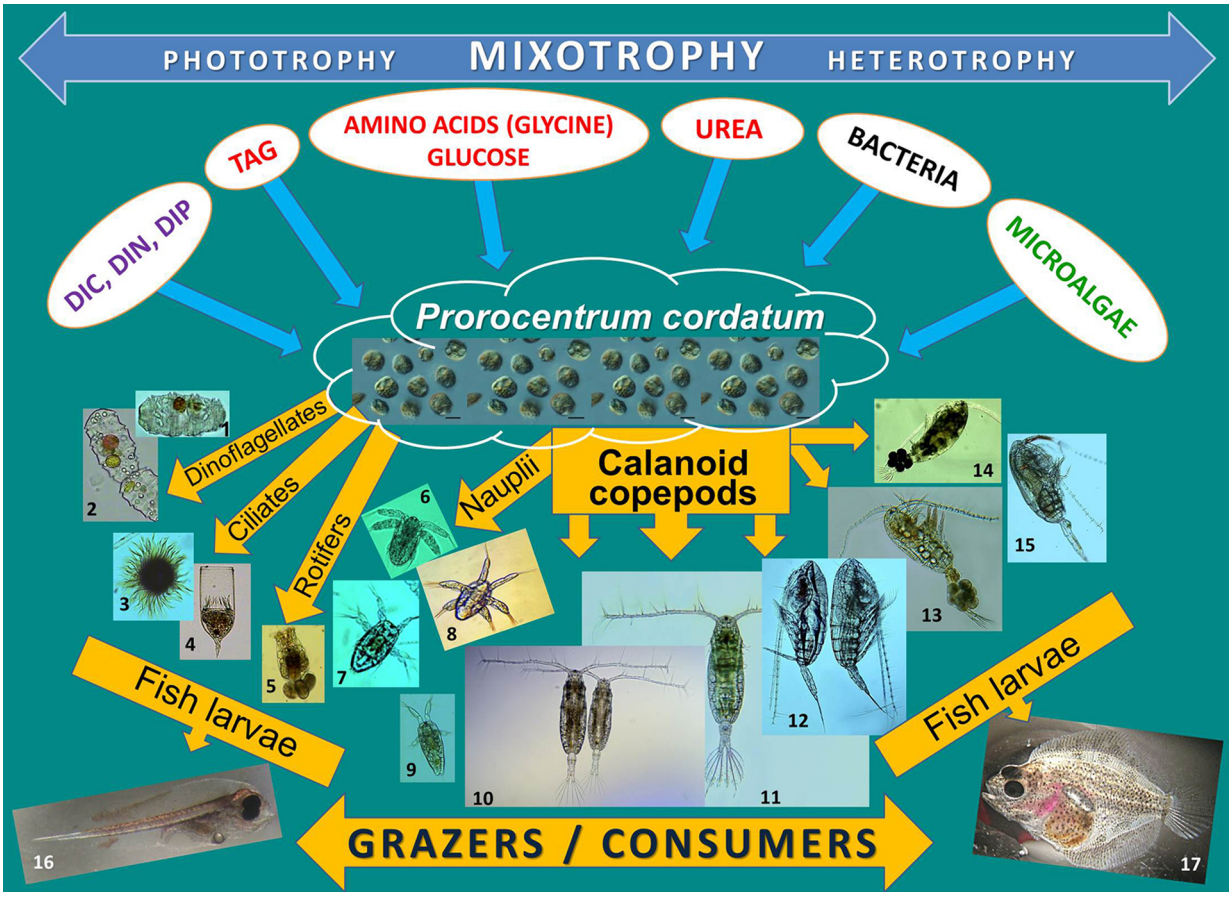

Fig. 2. Mixotrophic dinoflagellates *Prorocentrum cordatum* in the Black Sea pelagic trophic web: their food substrates/sources and major grazers/consumers. Heterotrophic dinoflagellates: 1, 2, *Polykrikos kofoidii*. Ciliates: 3, *Strombidium* sp.; 4, *Favella ehrenbergii*. Rotifers: 5, *Brachionus plicatilis*. Larvae of calanoid copepods: 6, 7, *Acartia tonsa*, nauplii; 8, *Calanus helgolandicus*, nauplius; 9, *Calanipeda aquaedulcis*, nauplius. Adult calanoid copepods: 10, *Acartia clausi* (left – female, right – male); 11, *Acartia tonsa*, female; 12, *Calanus helgolandicus* (left – male, right – female); 13, *Calanipeda aquaedulcis*, female with egg sac; 14, *Arctodiaptomus salinus*, female with egg sac; 15, *Pseudocalanus elongatus*, female. Fish larvae: 16, *Scophthalmus maximus* var. *maeoticus*, early larvae (4 days); 17, *Scophthalmus maximus* var. *maeoticus*, metamorphosing larvae. Photo courtesy of N.A. Gavrilova (1, 2, 4), T.V. Rauen (5), and L.S. Svetlichny (7, 10-14). Photo A.N. Khanaychenko (3, 6, 8, 9, 15-17). *Prorocentrum cordatum*, live cells in culture (photo courtesy of M.A. Berdieva). DIC, dissolved inorganic carbon; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; TAG, triacylglycerides.

4.3. Copepods feeding on *P. cordatum* and other dinoflagellates

Copepods' diet is diverse, and in natural environment it is rather advantageous since it includes microalgae of various taxa and for omnivorous species – different microzooplankton organisms. Feeding of copepods is governed by the availability and quality of food, while size, shape, species and biochemical composition of the prey are the important aspects of food 'quality'. The diet of most species of marine calanoid copepods consists mainly of diatoms, dinoflagellates and cryptophytes, and varies with seasonal succession of different microalgae groups and species. Nutritional requirements of calanoid copepods are thought to be fully covered by mixed diet (Broglio et al., 2003). Indeed, typical neritic ubiquitous calanoid copepods *Acartia* spp. are usually described as omnivorous: late copepodites and adults of *A. clausi* and *A. tonsa* prey on organisms ranging in size from 4 up to 700 μm, including more than 45 species of phytoplankton (dinoflagellates, diatoms, cryptomonads) and various small microzooplankton (aloricate ciliates, tintinnids, small rotifers, and copepod eggs and nauplii (Petipa, 1959; Kovaleva, 1983; Stoecker and Egl-

Table 4. Maximum grazing rates of some major consumers of *Prorocentrum cordatum*.

Grazers	Maximum grazing rates (cells ind.$^{-1}$ day^{-1})	References
Ciliates		
Strombidinopsis sp.	1300	Jeong et al., 1999
Heterotrophic dinoflagellates		
Gyrodinium spirale	91	Kim and Jeong, 2004
Gyrodinium dominans	8	Kim and Jeong, 2004
Calanoid copepods		
Acartia clausi	49979	Kovaleva, 1977
Acartia tonsa	17880	Khanaychenko et al., 2016
Acartia tonsa	28800	Besiktepe and Dam, 2002
Acartia erythraea	27730	Liu et al., 2006
Pseudocalanus elongatus	13500	Kovaleva, 1983
Calanipeda aquaedulcis	19 000	Khanaychenko et al., 2016
Arctodiaptomus salinus	17 000	Khanaychenko et al., 2016

off, 1987; Khanaychenko, 1989; Kleppel, 1993; Khanaychenko et al., 2016).

Significance of dinoflagellates as food for marine calanoid copepods was first questioned and established basing on the field and experimental data from the 1950-s through 1970-s. It was found that in the Black Sea during late warm season (in September) about 66% of the daily biomass of *P. cordatum* per night (8 h of darkness) was consumed by zooplankton that consisted mainly of copepods (Morozova-Vodjanickaja, 1954). Whereas diatoms that dominated in the Black Sea phytoplankton (from 0.23 up to 10^6 cells m^{-3}) during cold seasons contributed up to 65% of the total numbers and 48% of phytoplankton biomass to copepods' diet, dinoflagellates added to their diet only 12–18% in terms of numbers and 9–23% of total phytoplankton biomass during the same periods of time. However, from the start of warm season, in late spring and later on during the copepods' high reproduction period, the contribution of dinoflagellates (with *P. cordatum* among the dominant species) to their diet increased by 2-3 times: up to 24-54% by numbers and 18-69% by biomass (Kovaleva et al., 1969; Kovaleva, 1983).

Measurements of different pigments in *A. tonsa* guts and comparison of those results with pigments of the ambient microplankton in estuarine, shelf and open waters off the southern USA coasts also revealed a preferential feeding of copepods on dinoflagellates (besides microzooplankton) relative to diatoms (Kleppel et al., 1991). These data were supported by observations of Swadling and Marcus (1994) who compared carotenoid pigments in water column in Los Angeles Harbor (California, USA) and in the guts of copepods, and concluded that the diet of the adult *A. tonsa* was dominated by peridinin, the main carotenoid pigment of dinoflagellates which constituted from 20% up to 60% of their diet in March, despite the low concentration of dinoflagellates in the phytoplankton community.

Undoubtedly, contribution of dinoflagellates to the copepods' diet is season-specific and species--specific: dinoflagellates may contribute a largely variable portion, e.g. from <0.2% to >60% (on average 33%) of the carbon in the diets of common estuarine copepods in Los Angeles Harbor, California (USA) (Kleppel, 1992). In natural environment, some copepods (*Calanus helgolandicus* and *Temora longicornis*) feed on but do not select dinoflagellates; by contrast, *Centropages chierchiae* consumed dinoflagellates at a significant clearance rate of 4.9 mL copepod^{-1} h^{-1}, despite the established fact that within a given prey category (ciliates or dinoflagellates) copepods often select larger prey (>40 μm) over smaller ones (≤40 μm) (Vincent and Hartmann, 2001).

Possessing the ability to distinguish between edible and inedible food particles, copepods reduce their feeding, or totally reject some bad-tasted microalgae, even those suitable in terms of their preferable prey size range. Despite being considered omnivorous, planktonic copepods such as *Acartia* spp. and *Pseudodiaptomus salinus* rejected some phytoplankton species including several red-tide dinoflagellates (Uye and Takamatsu, 1990, and references therein). Copepods are known to

discriminate between toxic and non-toxic *Alexandrium* spp. cells (Teegarden, 1999). Copepods *Calanus pacificus* and *Paracalanus parvus* avoided grazing on dinoflagellates *Gonyaulax tamarensis*, *G. tamarensis, Ptychodiscus brevis, Protoceratium reticulatum* (Huntley et al., 1986), and *T. longicornis* and *A. tonsa* reduced significantly the ingestion rate in the presence of toxic dinoflagellates *Alexandrium tamarense* (Xu et al., 2018).

Although avoiding toxic dinoflagellates *Karenia brevis*, copepods *A. tonsa, T. turbinata* and *Centropages typicus*, however, grazed actively on *P. cordatum* (*P. minimum*) at bloom concentrations (10^3–10^4 cells mL^{-1}) (Cohen et al., 2007). Adults of *A. clausi* exhibited high feeding selectivity of dinoflagellates, including *P. cordatum*, versus various diatoms from microalgal mixture (Petipa, 1959; Kovaleva, 1977). Determined by the radioactive ^{14}C method, daily ration of the adult copepods *A. clausi* on dinoflagellates (including *P. cordatum*) exceeded 40–97% of copepod's wet weight at highest among other microalgae' assimilation rates (Petipa et al., 1970a, 1970b).

Numerous calanoid copepods graze on *Prorocentrum* during different stages of their lifespan. High electivity index (E_i=+0.74) for *P. cordatum* (*E. cordata*) was obtained for the nauplii of *Calanus helgolandicus* (Petipa, 1965). When fed exclusively *P. cordatum* from hatching onwards, both *C. helgolandicus* (Khanaychenko, pers. com.) and *Acartia* spp. (Khanaychenko, 1999) terminated the post-embryonic development. Among several mono-diets, *P. cordatum* at concentration 4.5×10^5 cells mL^{-1} was the best food object utilized by females of *C. helgolandicus*, as indicated by the mean pellet production of above 45 pellets $female^{-1}$ day^{-1} (Lacoste et al., 2001). In a mixture of edible non-toxic microalgae (*P. cordatum*, two species of cryptophytes, prymnesiopytes *Isochrysis galbana* and green microalgae *Tetraselmis suecica*), copepods *A. tonsa* tended to select (electivity index E_i=+0.16) *P. cordatum* while the green microalgae *Tetraselmis suecica* was actively avoided demonstrating high negative electivity index, E_i=-0.7 (Khanaychenko et al., 2016).

Active grazing on *P. cordatum* at concentration similar to bloom conditions (4×10^3–4.5×10^5 cells mL^{-1}) was observed for copepods *A. clausi* (Kovaleva, 1977), *C. pacificus* (Uye, 1996), *Temora longiremis* and *T. stylifera* (Ianora and Poulet, 1993), *A. tonsa* (Khanaychenko et al., 2016), *A. omorii* (Tsuda and Nemoto, 1984), *Calanipeda aquaedulcis* and *Arctodiaptomus salinus* (Aganesova, 2011a, 2011b; Khanaychenko et al., 2016).

Grazing rate of *A. clausi* on *P. cordatum* increased from ca. 4×10^3 cells cop^{-1} day^{-1} at a threshold concentration of about 100 cells mL^{-1} to maximum 5×10^4 cells cop^{-1} day^{-1} at 7.5×10^3 cells mL^{-1} (Kovaleva, 1977). Adult females of con-generic ubiquitous copepod *A. tonsa* consumed daily ca. $2.5–4.6\times10^4$ cells of *P. cordatum* (equal to ca. 150–250% of body C $ind.^{-1}$ day^{-1}) at *P. cordatum* concentrations of 1×10^3–2.2×10^3 cells mL^{-1} (Besiktepe and Dam, 2002; Dam and Colin, 2005). Similar feeding activity of calanoid copepods *A. tonsa, C. aquaedulcis* and *A. salinus* on *P. cordatum* at concentration of ca. 6×10^3 cells mL^{-1} in microalgae mixtures was observed: 18×10^3, 19×10^3 and 17×10^3 cells $ind.^{-1}$ day^{-1}, correspondingly (Khanaychenko et al., 2016).

Thus, the typical representatives of both graspers and filtrators among calanoid copepods graze on *P. cordatum* either selectively or non-selectively. Importantly, the copepods do not avoid these dinoflagellates in the mixture with other food particles, perceiving them as non-toxic edible microalgae.

4.4. Assimilation of *P. cordatum* by calanoid copepods

Calanoid copepods rapidly respond to favorable trophic conditions and can fully recover from food depletion within the 24 h feeding at elevated rates (ca. 120 to 250% body carbon d^{-1}) when the conditions change (Kovaleva, 1983; Tiselius, 1998; Besiktepe and Dam, 2002; Dam and Colin, 2005; Khanaychenko et al., 2016). In the guts of copepods, food transmission at 18 °C takes approximately 5.7–6.5 h (Kleppel, 1992; Thor and Wendt, 2010). The digested food quickly undergoes gut absorption, transportation across cell membranes, and transformation of biomolecules. During intensive feeding of *Acartia* sp. in excess of food, already in several hours the stored lipids emerged in the visible oil drops dispersed in the body of copepods colored depending on the presence of the algal taxon-specific carotenoids: light yellow indicating that copepods ingested diatoms, and bright orange indicating that the animal ingested dinoflagellates; the total volume of oil drops during intensive feeding of *A. clausi* could exceed 7.10^{-5} mm^3 (Kovaleva, 1983; Khanaychenko, pers. com.). Similar oil drops are visible in the other copepods (Fig. 3), especially prior to the diapausing period (Telesh et al., 2009).

'Sloppy' feeding (with decrease of digestion

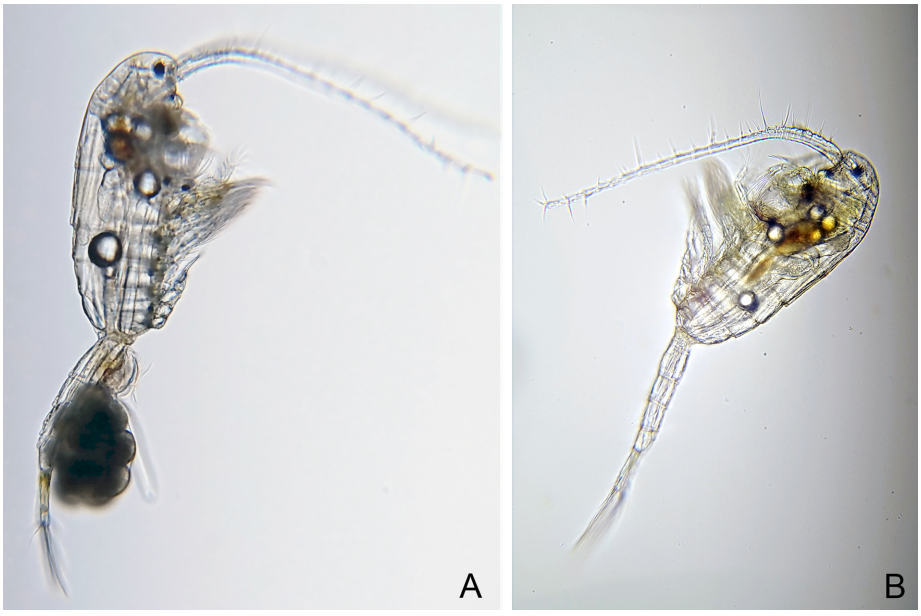

Fig. 3. *Calanipeda aquaedulcis* from the Black Sea. A – Female, B – male; oil drops with stocked lipids (presumably originating from dinoflagellates) are well seen. Photo courtesy of L.S. Svetlichny.

efficiency) may occur when copepods feed at high ingestion rates. However, when *A. tonsa* ingested up to 28800 cells day^{-1} (ca. 10 mg C ind.$^{-1}$) of *P. cordatum*, its assimilation efficiency (AE) was the highest (75–85%) among other tested diets; meanwhile, it was the lowest compared to diatoms (15–50%), green microalgae, heterotrophic dinoflagellates and ciliates (Besiktepe and Dam, 2002).

Considering that copepods mainly assimilate nutrient substrates from microalgal cytoplasm during digestion (Thor and Wendt, 2010), *P. cordatum* turns out to be the energetically costly food. It was shown that phosphorus (P) contributes 0.7% to the *P. cordatum* cells' volume, and 85.5% of P is stocked in the algal cytoplasm (Liu et al., 2006). Consequently, P is quickly incorporated into the organic molecules in copepods and is likely used in ATP.

Indeed, among 6 different algal diets (at equal biomass of 1.45 mg C L^{-1}), the assimilation efficiency of P by copepods *Acartia erythraea* ranged between 19 and 78%; the highest P assimilation efficiency (AE ca. 78%) was also registered at a very high ingestion rate of *P. cordatum*: 1.33 μg C ind.$^{-1}$ h^{-1} (Liu et al., 2006). Consequently, the cells of *P. cordatum* are not only ingested actively but also digested at a high rate by calanoid copepods, being quickly absorbed and assimilated effectively, including highly energetic components incorporated into copepods' metabolism.

5. Role of copepods in top-down control of *P. cordatum* blooms

Trophic structure of plankton communities plays a crucial role in the functioning of pelagic ecosystems. However, plankton dynamics are difficult to predict; therefore, very often they are not easily reconciled with the conventional cause-effect relationships, concepts and paradigms that contribute to our understanding of the aquatic ecosystems' functioning (Fussmann and Heber, 2002; Telesh et al., 2011a, 2011b, 2013, 2015; Skarlato and Telesh, 2017; Hillebrand et al., 2018; Pennekamp et al., 2018; Telesh et al., 2019). The trophic structure of plankton likely determines the HABs development and duration due to the complex interplay of external triggers and internal driving forces of plankton dynamics within the communities (Telesh et al., 2019). Indeed, one should expect that in the cases when the grazing pressure on the blooming dinoflagellates is high enough to restrict their excessive growth rate, the bloom either doesn't develop or, once started, it terminates quickly. However, there exist some conflicting evidences of the role of grazers in controlling the dynamics of PRORO blooms. For example, the development and persistence of PRORO blooms are expected by some studies to be affected and controlled by the grazing pressure of zooplankton (Sukhanova et al., 1988; Dam and Colin, 2005). Meanwhile, the

other authors argue a minor impact of grazers on the dinoflagellate blooms (Glibert et al., 2012, and references therein).

As we have already mentioned earlier (see Chapter 4 of this review), successful feeding of calanoid copepods on *Prorocentrum* and effective reproduction of these copepods in the laboratory experiments occur at concentrations of PRORO ranging 10^2–10^4 cells mL^{-1} that are close to their concentrations in nature during blooms (ca. 10^5–10^7 cells L^{-1}). Consequently, these experimental results can be useful (to certain degree!) for identification of the possible trends in the development of the dinoflagellate blooms in the coastal areas in the presence of certain mesozooplankton grazers.

Although the ingestion rates of the copepods *Acartia tonsa* on PRORO could exceed 5×10^4 cells cop^{-1} day^{-1}, in nature the abundance of these copepods vary greatly. For example, the maximum abundance of *A. tonsa* in the coastal environments of the Black Sea is not always high: when averaged for August in the 1990-s, it reached 1.2 ind. L^{-1} (Gubanova, 2000), and in the Baltic Sea coastal waters in August-September (>17 °C) it can increase up to 2.5 ind. L^{-1} (Polunina, 2018). In such cases, the highest predicted grazing impact of *A. tonsa* population on PRORO can be presumably restricted to the maximum of 150 cells mL^{-1} day^{-1}.

However, the copepods *Acartia* spp. are known for their behavioral trends to swarm to favored conditions of light and food, and in patches it can congregate up to 1 ind. mL^{-1}; thereafter, the grazing impact of *Acartia* spp. in the bloom patches can possibly approach ca. 5×10^4 cells mL^{-1} day^{-1}. Thus, assuming the highest possible patchy distribution of *A. tonsa*, we suggest that theoretically it is able to control the increase of PRORO concentration in patches up to 5×10^4 cells mL^{-1}, in case the dinoflagellate cell proliferation does not exceed 1.2 divisions per day.

Meanwhile, earlier the potential grazing impact of copepods *Acartia* spp. on *P. cordatum* (*P. minimum*) was evaluated as 20 times lower than that of the ciliates *Strombidinopsis* sp. (Glibert et al., 2012). This confusing result was most likely obtained due to misinterpretation of the reference data since the authors indicated that in their calculations they used the maximum values of *A. tonsa* grazing rate on *P. minimum* as provided by Besiktepe and Dam (2002). However, in the Table 7 by Glibert et al. (2012) the authors mistakenly provided the unbelievably small value of the maximum grazing rate of *A. tonsa* on *P. minimum* which was equal to 10 ng C $ind.^{-1}$ d^{-1}, whereas in the reference paper (Besiktepe and Dam, 2002) and in the other studies (Khanaychenko et al., 2016) this parameter was evaluated as three orders of magnitude higher and accounted for 10 μg C $ind.^{-1}$ d^{-1}. Moreover, even higher maximum grazing rate of *A. tonsa* on *P. minimum* was reported reaching 32 μg C $ind.^{-1}$ d^{-1} at certain times (Liu et al., 2006), which should be undoubtedly considered as the sloppy feeding; however, it is still feasible. Thus, the conclusion about the weak top-down control of *P. minimum* blooms by copepods (Glibert et al., 2012) was based on the erroneously used reference data on the maximum grazing rate of *A. tonsa* on *P. minimum*.

Our attempt to evaluate the possible top-down effect of *Acartia* spp. on the blooming population of *P. cordatum* in the Black Sea provided a different result. During the extraordinarily strong bloom of *P. cordatum* (*E. cordata*) in 1975 in the NWBSCA, the Romanian coastal area (Petran, 1976), in the water layer 0–10 m where the dinoflagellate maximum was observed, the abundance of *Acartia* spp. was registered to be as high as 92600 ind. m^{-3} (>92 ind. L^{-1}), reaching at maximum 114000 ind. m^{-3} (114 ind. L^{-1}). Using the equation (4) from Kim and Jeong (2004) for calculation of the grazing coefficient (g, h^{-1}) of *Acartia* spp. at the bloom concentration of *P. minimum* cells ca. 10^4 cells mL^{-1}, and assuming that the ingestion rate of *Acartia* spp. is similar to that obtained in the separate other experiments (Kovaleva, 1977; Besiktepe and Dam, 2002; Khanaychenko et al., 2016) and exceeds 5×10^4 cells cop^{-1} day^{-1}, we obtained the grazing coefficient value of $g=0.61$ h^{-1}.

Earlier, Dam and Colin (2005) assumed that *A. tonsa* population solely is able to yield a total grazing impact of the order of 33% of the daily growth rate of *P. minimum* during bloom. Additionally, the maximum ingestion rate of the heterotrophic dinoflagellate *Gyrodinium spirale* on unialgal diet of *P. minimum* was shown to exceed 90 cells $ind.^{-1}$ d^{-1}, and its grazing rate even at the highest density of 60 $ind.^{-1}$ mL^{-1} exceeded 0.64 h^{-1} (Kim and Jeong, 2004).

The abundance of some tintinnid ciliate species in the Black Sea coastal waters in late summer can reach 20 ind. mL^{-1} (Gavrilova and Dovgal, 2016), while the population densities of aloricate ciliates in this season are also high. Thus, assuming that all ciliates are grazing on PRORO, they can possibly impose the similar grazing pressure on the dinoflagellate population as the copepods *A. tonsa* do.

Therefore, the top-down control of PRORO blooms may be exerted by the complex of its planktonic grazers (protists and copepods) which do not

experience harmful effects of this dinoflagellate but proliferate while grazing on it. However, they can contribute to suppression or decline of the bloom only in the case of the coupling of the highest abundance of grazers with the increasing population of the dinoflagellates. This assumption highlights the importance of knowledge on the linkage in time and space between the dinoflagellate bloom development and the sufficiently high population density of its grazers.

6. Conclusions

The focused studies of the role and performance of *P. cordatum* in the marine plankton food webs are not numerous. Still, the vast field of information on biology of these globally distributed, ecologically, socially and economically important protists allows concluding that these dinoflagellates form a key trophic link in the pelagic food webs, especially in the coastal and estuarine ecosystems.

P. cordatum is distinguished by multiple nutrient pathways and flexible mixotrophic metabolism using various sources of inorganic and organic nutrient substrates. High intra-population heterogeneity of cells enables *P. cordatum* to expose different affinity for manifold nitrogen sources exploiting various nutritional strategies. It is a phototrophic protist, but it is also able to consume heterotrophic bacteria, cyanobacteria, haptophytes, cryptophytes, other dinoflagellates, diatoms, and even ciliates. However, the existence of a very complicated cell covering (amphiesma) imposes limitations on both endocytosis and the membrane transport of dissolved nutrients in *P. cordatum*. Therefore, it is not clear so far how exactly this powerful nutrition potential is realized at the cellular level, and whether or not such features as the flagellar canal and the pusule represent the principal regions of the uptake and engulfment of various nutrient sources.

Experimental data revealed that *P. cordatum* can be consumed by a large variety of unicellular and multicellular planktonic grazers, including other mixotrophic dinoflagellates, tintinnids and the aloricate ciliates, herbivorous and omnivorous calanoid copepods. Being the major link between the lower (phytoplankton) and the higher trophic levels (fish) in marine pelagic food webs and constituting the bulk of the diet of planktonic fish larvae as highly efficient natural food, planktonic calanoid copepods are thus the major regulators of fish recruitment. Meanwhile, dinoflagellates form the substantial, or in some cases even preferential items in the diet of copepods, and this fact involves these ubiquitous protists in the regulation of fish populations as well.

In certain environmental conditions, including low pressure of grazers, *P. cordatum* is able to form harmful algal blooms. However, the exact mechanisms allowing populations of these protists to dominate at very high densities during periodic red tide blooms still remain unresolved. Similarly, the reasons of fast disappearance of blooms remain enigmatic. The attempts to evaluate the possible top-down control of the blooming population of *P. cordatum* by the calanoid copepods (e.g., *Acartia* spp.) provided controversial results. Nevertheless, in general it can be concluded that copepods which do not experience harmful effects of this particular dinoflagellate species can provide the grazer-mediated suppression of *P. cordatum* blooms, but only at the nexus of the highest abundance of grazers and the moderately increasing dinoflagellate population coupled in time and space.

Although the direct toxic effects of the metabolites of *P. cordatum* were not confirmed, the overall impact of its massive proliferation which can result in the red tides has a substantial negative influence on the coastal ecosystems worldwide, especially in the semi-closed brackishwater seas. *P. cordatum* at bloom concentrations were shown to be non-toxic for numerous zooplankters in the experiments; still their blooms in coastal environments at certain times are accompanied by mortalities of marine fauna. The devastating effects of these red tides are most crucial in the vicinity of large cities, industrial and cultural centers, recreational areas etc. since HABs negatively impact water quality, aquaculture, fisheries, tourism, and human health.

Currently it is well demonstrated that despite *P. cordatum* has the global distribution, the strong invasion potential allows it to further expand the geographical range and occupy new ecological niches that are not yet fully inhabited, or even replace some other species that possess lower competitive abilities. Due to its effective mixotrophic metabolism and advanced adaptation strategies, *P. cordatum* proliferates broadly. Recently this species has become a good model object for the experimental investigations of mixotrophy on both population and single-cell levels. Particularly, remarkable progress has been achieved in the studies of the molecular mechanisms of nutrients' uptake by the dinoflagellate cells.

Further studies of cellular and molecular organization, physiology and mixotrophic metabolism of the bloom-forming dinoflagellates *P. cordatum*, a permanent nanoplanktonic component of various marine coastal ecosystems, will advance the understanding of the intricate, largely underscored so far, complex interactions in marine pelagic food webs that control the emergence of HABs, drive their decay, and regulate the overall ecosystems' functioning and sustainability

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