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Studies of bloom-forming dinoflagellates *Prorocentrum minimum* in fluctuating environment: contribution to aquatic ecology, cell biology and invasion theory

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| Submitted June 23, 2018 | Accepted August 27, 2018 |

Summary

The article reviews modern concepts of the protistan diversity patterns within the environmental gradients in the pelagic coastal ecosystems and the role of microplankton communities therein, with special emphasis on dinoflagellates. We revise the knowledge on biology of the bloom-forming, potentially toxic, mixotrophic dinoflagellates *Prorocentrum minimum* in gradually fluctuating environment of the brackishwater Baltic Sea, their reaction to abrupt external stresses in the experiment, metabolism and population heterogeneity, cellular and molecular adaptation strategies and broad ecological niche dimensions that empower this globally distributed species with substantial competitive advantages and pronounced invasive potential. Topicality of such synthesis is defined by high ecological and socio-economic importance of these potentially harmful organisms for humans and their environment: ecosystem health, fisheries, aquaculture, recreation and tourism, as well as for resolving a number of urgent issues in biomedicine and biotechnology.

Key words: adaptation to stress, Baltic Sea, dinoflagellates, ecological niche, estuaries, harmful algal blooms, invasive species, ion channels, mixotrophic metabolism, *Prorocentrum minimum*, protistan species maximum, salinity gradient

1. Introduction

The microscopically small unicellular eukaryotic microorganisms, or the protists, form the essential components of pelagic biota occupying the base of trophic networks in the aquatic ecosystems worldwide. They are highly diverse and mostly cosmopolitan although vulnerable to environmental alterations due to anthropogenic ecosystem modifications and climate change. Alternatively, protists can cause serious repercussions for the aquatic environment by affecting the biogeochemical cycles. These fast-developing and rapidly evolving unicellular plankton organisms benefit from the relative vacancy of brackishwater ecological niches and the impaired competitiveness within the pelagic communities at the intermediate (critical) salinities 5–8¹. These important considerations have serious though so far poorly disclosed implications in the invasion biology, as they qualify for the high rate of the protistan alien species introductions in brackish water bodies (Skarlato and Telesh, 2017).

Diversity of microorganisms in a broad sense, i.e. a variety of both prokaryotic and unicellular eukaryotic representatives, and their functional role in aquatic ecosystems form a vast field of knowledge which is traditionally underestimated unless the focused studies are performed. The researchers have just recently started to elucidate how environmental factors influence microbial communities at different scales (Azovsky and Mazei, 2013), and estimate the traits of poorly known microbial species which number in the millions (Naeem et al., 2012). Moreover, new data and re-analyses of historical knowledge on diversity of microorganisms in large brackish water bodies and estuarine ecosystems are transforming the traditional views of biodiversity in certain environments, thus underpinning novel biodiversity concepts and generating paradigm shifts (Elliott and Whitfield, 2011; Telesh et al., 2011a).

Alterations in the environment, including the gradually changing salinity regime and other chemical and physical conditions, along with nutrient input and grazing pressure of higher trophic levels strongly affect the diversity, community structure and temporal dynamics of microorganisms in plankton as well as in benthos (Telesh et al., 2013;

Tikhonenkov and Mazei, 2013). Meanwhile, cellular and molecular mechanisms that are responsible for the fitness, including metabolism, environmental preferences and effective adaptation strategies of protists, are largely understudied.

Mixotrophy remains one of the most enigmatic features of many protists (Matantseva and Skarlato, 2013), including the large and ecologically influential (in terms of species number, densities and environmental impact) group of planktonic dinoflagellates (Stoecker, 1999). Mixotrophic dinoflagellate species are numerous; many of those are cosmopolitan, inhabiting a variety of biotopes in different aquatic ecosystems. In plankton, dinoflagellate species generally shape biodiversity and back up the protistan species maximum in the horohalanicum (Telesh et al., 2011a, 2011b), thus influencing vulnerability of pelagic coastal ecosystems to alien species invasions.

Many of these protists are toxic or potentially toxic: they produce various secondary metabolites of different chemical structure that are hazardous to multicellular organisms, including plants, animals and human health (Okolodkov, 2011). Photo- and mixotrophic dinoflagellates are often responsible for the harmful algal blooms (HABs), or the so called red (mahogany) tides, that may occur even in the oligotrophic waters, but mostly develop in the eutrophic near-shore areas, thus deteriorating the quality of sea coastal waters and impacting negatively their flora, fauna and major ecosystem services: aquaculture, fisheries and recreational value of this environment for humans (Glibert et al., 2014).

In this review we analyze the recent knowledge of protistan diversity patterns within the environmental gradients in aquatic ecosystems, their spatial distribution and long-term population dynamics. We also revise the data on biology and modern morphology of a common planktonic, potentially harmful dinoflagellate, *Prorocentrum minimum* (Pavillard) Schiller, 1933, which is also known as *Prorocentrum cordatum*² (Ostenfeld) Dodge, 1975 – a model protistan species which is distributed globally and is still expanding its

¹ 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.

² Although the Latin name *Prorocentrum cordatum* (Ostenfeld) Dodge, 1975 should be preferably used for the indication of this dinoflagellate species according to the priority rule, in the present article we use the name *Prorocentrum minimum* to better relate our data to the results of the previous findings.

geographical range. The article is focused on adaptation strategies, invasion potential, cellular and molecular organization, population heterogeneity, metabolism and response of these potentially toxic, bloom-forming, mixotrophic dinoflagellates to various external stresses in the experiment and in the fluctuating environment of the brackishwater coastal ecosystems.

2. Environmental instability, gradients and the protistan species maximum

2.1. GRADIENTS IN FLUCTUATING ENVIRONMENT

The sea coastal regions are usually characterized by highly dynamic instability and excessive (compared to the open ocean) variability of abiotic conditions that shape aquatic communities. The combined effects of terrestrial runoff and tidal water movements cause fluctuations of salinity, nutrient concentrations, irradiance, temperature and many other physical and chemical parameters; in the shallowest parts, even water availability in the habitat may vary in tidal as well as wind-induced rhythms. As a consequence, distinct patterns of diversity and community structure of aquatic biota can be observed along the coastal gradients, reflecting the limits of specialization rates of their inhabitants (Whittaker, 1965; Nybakken, 1997; Castro and Huber, 2000). These patterns are well studied for benthic organisms and can be explained by the combined effect of osmotic, mechanical, temperature and irradiance stress, superimposed by grazing effects (Lüning and Yarish, 1990). However, relatively little is known about the effects of environmental gradients on the coastal plankton communities.

The abiotic environment of plankton communities in the coastal regions is different from that of the benthic ones in many aspects. Moving with the water masses, plankton are less impacted by water perturbations, including the tidal actions; moreover, temperature and irradiance variability which they encounter is less dependent from water level changes (Telesh et al., 2013). As a consequence, the relatively high importance of certain external factors like nutrient availability and salinity fluctuations for the short-living species contribute substantially to their pronounced seasonality (Sagert et al., 2008; Litchman et al., 2012; Olenina et al., 2016), which illustrates the results of complex interactions between the constraints of physical environment and the performance of biotic factors (Sommer

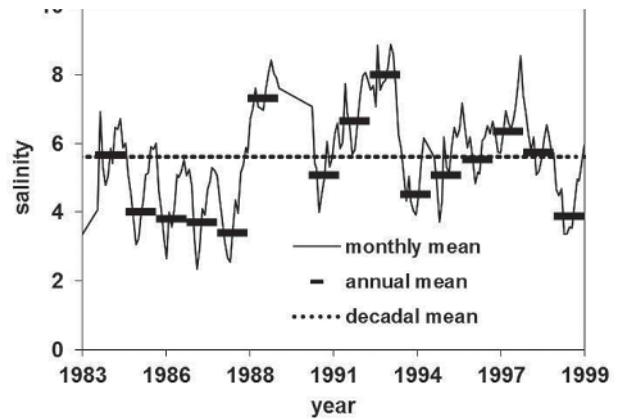


Fig. 1. Long-term salinity fluctuations around stable decadal mean value in the Zingster Strom (Darss-Zingst bodden chain, German Baltic coast). Based on data from Telesh et al. (2013).

et al., 1986). This situation becomes even more pronounced in estuaries, where nutrient-rich fresh waters mix with marine waters. The resulting steep gradient in nutrient availability, partly due to salinity gradient, is highly dynamic on small scale, too, impacting planktonic organisms irrespective of the peculiar viscosity conditions of their “life at low Reynolds number” (Purcell, 1977)

Fluctuating salinity is one of the major natural stress factors for aquatic biota, and its effects are also most striking in estuaries. As transition areas between rivers and seas, estuaries and other brackish coastal regions are generally characterized by a more or less pronounced salinity gradient, and it may even be postulated that an ‘estuarine’ ecosystem develops everywhere in the conditions of the salinity gradient (Telesh and Khlebovich, 2010).

Investigation of the effects of environmental variability and gradients on planktonic organisms, consequently, needs respecting both the different levels of variability as well as the biological specificity of the organisms as essential parts of the system. The long-term mean values of the abiotic parameters may demonstrate a surprising constancy irrespective of large (yearly, monthly, diurnally etc.) fluctuations that may be of high relevance with regard to the life-time of the organisms in focus. Consequently, sometimes it is hardly possible to register any statistically significant long-lasting changes in the abiotic parameters on the long run, even within the 30-years’ time span (Telesh et al., 2016).

This phenomenon can be well illustrated by the comparable amplitudes of long-term and short-term salinity fluctuations around a stable decadal mean

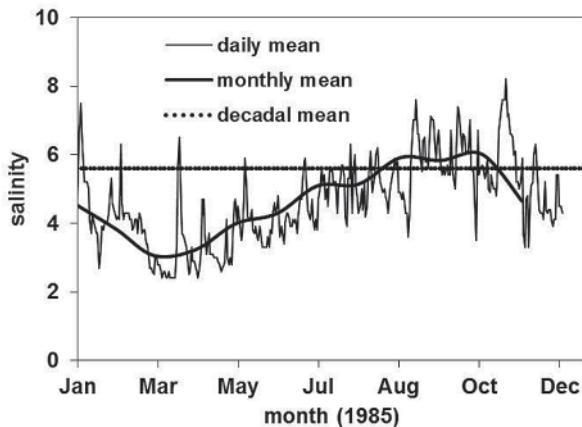


Fig. 2. Short-term salinity fluctuations around stable decadal mean value in the Zingster Strom (Darss-Zingst bodden chain, German Baltic coast). Based on data from Telesh et al. (2013).

value in the Zingster Strom of the Darss-Zingst bodden chain, German Baltic coast (Telesh et al., 2013). Indeed, the large-scale salinity gradient in the Baltic Sea is surprisingly stable both in space and time, and this can be proved by the astonishingly constant average salinity within the 17-years long cycle (Fig. 1). However, the inter-annual variations as well as the average salinity fluctuations at smaller time scales (months and days) demonstrated remarkable amplitudes. Specifically, the data for the Zingster Strom showed that the daily average water salinity values may fluctuate by up to $\pm 100\%$ within a day (Fig. 2).

2.2. LINKING ENVIRONMENTAL VARIABILITY TO ORGANISMAL TRAITS

Among the effects of many inter-related and pronouncedly fluctuating environmental characteristics, concentration of nutrients, level of illuminance and water temperature are known to affect community structure and size of planktonic organisms most strongly, especially when the unicellular plankton is concerned (Litchman et al., 2012; Olenina et al., 2016). Meanwhile, for unraveling the impact of variability of external stress factors on a community or ecosystem it is important to relate not only the amplitude, but also the periodicity of the stressor to the generation time of organisms, as postulated by the Intermediate Disturbance Hypothesis (Connell, 1978; Reynolds et al., 1993). This requirement matters particularly in the cases when the environmental factors fluctuate more frequently during the organisms' life time and, therefore, the period of time during which an

organism lives under the optimum conditions may not be sufficient enough to support a population's maintenance during the sub-optimum conditions (Telesh et al., 2013). Thus, high variability of salinity values and the subsequently high frequency of the stress events occur at a far more short time scales than the generation times of bottom dwellers (benthic organisms and macrophytes) that are measured in months. However, this frequency of salinity stress is quite comparable to the generation turnover times of planktonic organisms that account for several weeks (e.g., pelagic crustaceans) or days (e.g., rotifers), and especially of the protists whose cycles can be measured sometimes even in hours.

Short-living single-celled eukaryotes in general and their planktonic representatives in particular are known to be extremely sensitive to both local and global changes in the environment, to which they respond by not only changing the overall biomass, but also by structural transformations of their communities' composition (Li et al., 2009). Even high tolerance for salinity fluctuations might still provide sub-optimal conditions under salinity stress for them, and this reduces the strength of resource competition, allowing co-existence of species which otherwise might have been out-competed. Moreover, the short generation times of planktonic organisms, especially the smallest of them – the protists, might have allowed evolution of more brackishwater specialists, or rather provide benefits to the organisms using a subsidy of just being cosmopolitans, as most protists are known to be (Fenchel and Finlay, 2004). However, the large-scale (compared to the lifetime of organisms) gradients offer relatively stable environment for plankton drifting within water masses.

Consequently, assuming similar physiological salinity tolerance ranges for freshwater and marine plankton, the higher stability of the salinity regime for the planktonic organisms allows for less sharp decline of species number with changing salinity from both sides: from fresh to brackish waters, as well as from marine to brackish environment, if compared to the same trend for macrobenthos (Telesh et al., 2013). As a result of this differentiated reaction to salinity fluctuations, the sum curve for benthic species demonstrates the minimum at salinities 5–8, while the sum curve for plankton species numbers peaks in the same salinity zone (Fig. 3).

Thus, the higher stability of environmental regime for plankton provides a simple mechanistic explanation of the recently discovered protistan

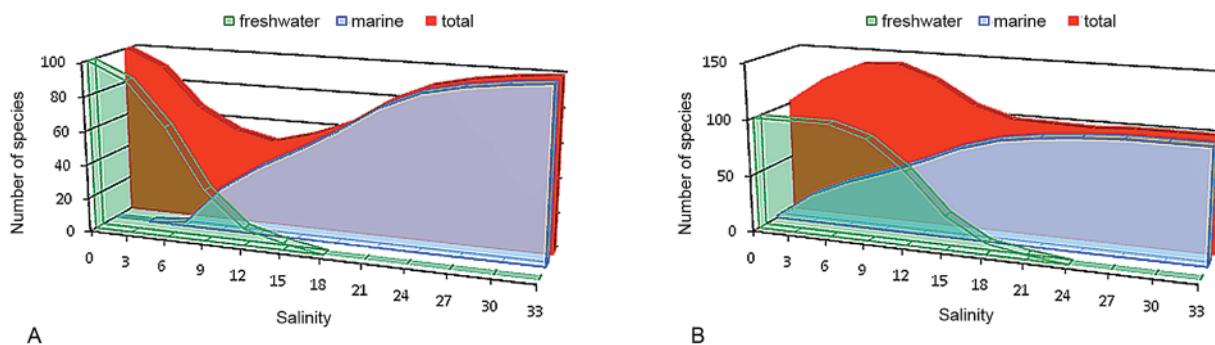


Fig. 3. Mechanistic explanation of the planktonic protistan species maximum and the benthic species minimum in the horohalimum. A sharper decline of benthic species number with changing salinity from freshwater and marine sites towards the horohalimum result in species minimum (A), while a less sharp decline of plankton species numbers is responsible for the sum curve with maximum species number in the same zone (B), due to different salinity tolerance ranges of benthic and planktonic organisms.

species maximum in the horohalimum (Telesh et al., 2011a). Consequently, the high plankton (protistan) species diversity may have buffering impact on the ecosystem's resistance to and recovery from external anthropogenic or natural stresses. Such resilience generates the ecosystem insurance value which is worth being incorporated into economic assessments, ecosystem health evaluations and management decisions (Worm et al., 2006), particularly concerning large brackish water ecosystems with pronounced environmental gradients.

2.3. LARGE-SCALE SALINITY GRADIENTS PROVIDE SUBSIDY RATHER THAN STRESS TO PLANKTONIC PROTISTS

Salinity gradient in coastal waters is usually unpredictable on a small scale: it depends on weather conditions, freshwater runoff and tidal regime. However, large brackish water bodies like the Baltic and Caspian Seas, San Francisco Bay or Chesapeake Bay can exhibit stable large-scale salinity gradients. As hypothesized by M. Elliott and V. Quintino (2007), the high natural variability in estuaries may confer an ability to withstand stress, both natural and anthropogenic – a supposition which logically supports the idea that in estuaries salinity decrease may not be a stress with only negative effect on biota but rather a subsidy, that is a sort of perturbation with a positive effect on the system (Costanza et al., 1992).

To check this hypothesis, we considered an example of the semi-enclosed Baltic Sea, which exhibits a remarkable salinity gradient: from fully marine values near its connection to the North Sea in

the west to almost freshwater conditions of the Gulf of Bothnia in the north and the Neva Bay of the Gulf of Finland in the north-east, both together receiving more than 50% of the total freshwater runoff to the Baltic Sea (Biological Oceanography, 2017).

Being a large microtidal brackish habitat, the Baltic Sea is characterized by the pronounced gradients of climatic and hydrological factors, both affecting the occurrence and distribution of aquatic plant and animal communities. The large-scale salinity gradient in the Baltic Sea is uniquely smooth and the zone of intermediate salinities occupies the major area of the Baltic proper as well as a great part of the vast coastal zone of the sea (Schiewer, 2008; Schubert and Telesh, 2017). Most of the Baltic Sea exhibits surface salinities of 5–8 (Feistel et al., 2010) that correspond to critical salinity zone where sharp changes in the ionic composition of seawater diluted with freshwater occur (Khlebovich, 1968).

Khlebovich (1969) argued that these ionic changes constitute a physical-chemical barrier between marine and freshwater faunas, and Kinne (1971) proposed the term 'horohalimum' (from the Greek 'horos': boundary line) for this salinity range. It is generally accepted that the horohalimum provides unfavorable osmotic conditions for aquatic organisms, impeding high species diversity and causing the *Artenminimum* (species minimum) effect (Remane, 1934), since considerable hypo- and hyperosmotic adjustments are required within this region (Telesh and Khlebovich, 2010).

Meanwhile, long-term biodiversity data from the Baltic Sea allowed distinguishing between groups of aquatic organisms for which the salinity gradient within the horohalimum can act either as

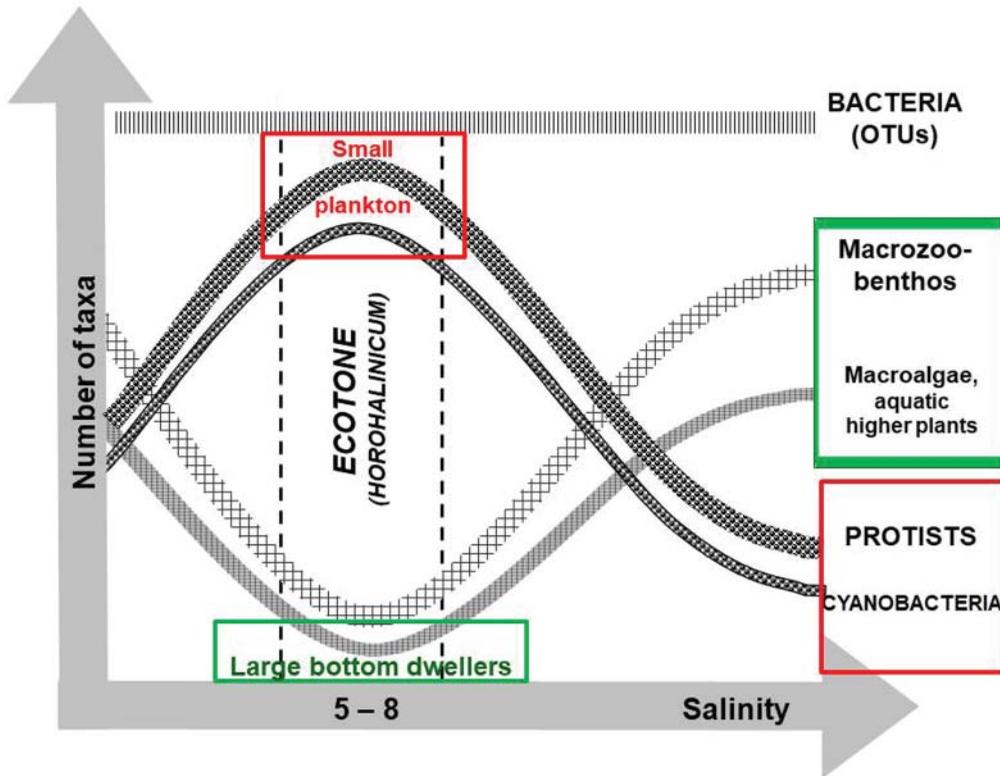


Fig. 4. Conceptual model of distribution of the micro- and macroorganisms' diversity in the salinity gradient (see text for the explanations).

stressor or as subsidy, as illustrated by Fig. 3. Recent investigations documented the difference in species richness' distribution mode of macrozoobenthos, macrophytes, eukaryotic plankton and bacteria within the salinity gradient of the Baltic Sea, with an emphasis on horohaliniacum where the salinity changes and biotic alterations were the sharpest (Bleich et al., 2011; Herlemann et al., 2011, 2014; Schubert et al., 2011; Telesh et al., 2011a).

Specifically, it was shown that in the case of variable and/or abrupt salinity gradient, the adverse environmental conditions provided significant stress to bottom-dwelling aquatic organisms which effect was the greatest for sessile macrofauna and macrophytes; meanwhile plankton, especially their smallest fractions, reacted differently to salinity fluctuations (Telesh et al., 2011a, b; Schubert et al., 2011). Contrary to bottom-dwellers, small-sized motile plankton organisms, presumably prokaryotes and eukaryotic microbes, or protists, regardless of whether they are heterotrophs (protozoa), phototrophs (protophytes), or mixotrophs demonstrated the opposite distribution mode, with maximum species richness in the horohaliniacum, as shown for the brackish Baltic Sea (Fig. 4). These findings served the basics for the "protistan species-

maximum concept" for the zone of critical salinities (Telesh et al., 2011a).

Moreover, the smallest protists (<50 μm) contributed the greater part to the phytoplankton species number in the horohaliniacum (Fig. 5).

Thus, it is now the established knowledge that the general diversity of plankton in the Baltic Sea is very high, exceeding 4056 species (the bacteria not included), and this fact challenges the previous viewpoint that the Baltic Sea is "poor in species" (see the discussion in Telesh et al., 2011a, and references therein). The species richness of major plankton groups (Fig. 6A) and their contributions to the overall pelagic diversity in the Baltic Sea (Fig. 6B) account for dominance of protists: Heterokontophyta and Ciliophora.

Consequently, the horohaliniacum in the Baltic Sea most likely provides subsidy rather than stressful environment for planktonic protists, most probably due to their high physiological adaptability to fluctuating salinity which allows them prosper in the conditions of variable environment within the salinity gradient (Skarlato et al., 2018). This positive effect can be visualized by maximum species richness of the eukaryotic microorganisms and the prokaryotes (cyanobacteria) in the horohaliniacum

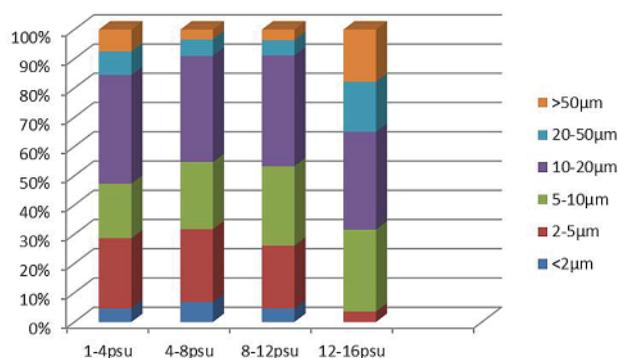


Fig. 5. Percentage of phytoplankton size classes at different salinities in the Baltic Sea. A dataset of approximately 3000 samples collected during the long-term monitoring studies along the German Baltic coast, including the inner coastal water bodies, was analyzed for the relative distribution of algae of different size classes within a salinity gradient. Each salinity class is represented by data from at least 200 samples. The total number of data analyzed exceeds 10,000 data points with respect to cell size information (for details about the database see Sagert et al., 2008).

of the Baltic Sea that most likely capitalize on the lack of inter-specific competition; thus, they not only achieve high population densities (Herlemann et al., 2011), but also reach the exceptionally high taxonomic diversity (Telesh et al., 2011a). This diversity ensures the ecosystem is maintained, providing benefits for the species adapted to the inherently variable conditions (Elliott and Quintino, 2007).

2.4. HOROHALINICUM AS AN ECOTONE

The high protistan species richness and its specific distribution with maximum in the horohalinicum at salinities 5-8 in the Baltic Sea (Telesh et al., 2011a) is in accordance with one of the classical patterns: the increased biodiversity within an ecotone due to mixing of biotic components from two adjacent systems, marine and freshwater. Paradoxically, until recently this phenomenon has never been documented in estuarine ecotones (Attrill, 2002; Elliott and Whitfield, 2011). Meanwhile, the horohalinicum zone of the Baltic Sea is evidently an example of the exceptionally large-scale pelagic ecotone system where the microplankton communities demonstrate the increased biodiversity (Telesh et al., 2013), as it would be expected in a classical ecotone, due to high degree of cosmopolitanism of planktonic protists and their

effective physiological adaptations to subsidy of brackish environment. The unidirectional transport of water masses towards the sea is a major difference between estuaries and “typical” ecotones; however, it is of minor importance for the Baltic Sea.

Not actually supporting the extreme idea of considering the entire Baltic Sea as a giant estuary, which had been repeatedly debated in the literature decades ago (e.g., Schubel and Pritchard, 1990; McLusky and Elliott, 2004), we nevertheless cannot ignore the mere fact that the protistan species maximum and high overall plankton diversity in the Baltic Sea are among the major characteristic features of an ecotone pointing indirectly at the optional estuarine quality of this peculiar sea (Telesh and Khlebovich, 2010).

The mechanisms behind the phenomenon of the protistan species maximum in the Baltic Sea horohalinicum as an ecotone are still to be thoroughly investigated. On the one hand, the impact of salinity gradient on different aquatic communities is not yet fully understood; similarly, the net effect of environmental fluctuations on the overall biological diversity is still largely unknown (Huisman and Weissing, 1999; Roelke et al., 2003; de Jonge, 2007; Benincà et al., 2008). On the other hand, Remane’s minimum of macrozoobenthic species within the horohalinicum (Remane, 1934) and the similar pattern of macroalgal diversity change confront undoubtedly the peculiarity of spatial dynamics of plankton diversity in the salinity gradient of the Baltic Sea (Telesh et al., 2011a). This contradiction denotes significant differences in reaction to salinity fluctuations of large sessile or attached organisms versus small motile plankton-dwellers that are driven to considerable distances within large water masses (Telesh et al., 2013). It is also important to mention here that the average salinity ranges taken as a measure in Remane’s conceptual model for the pooled data on macrozoobenthos’ species numbers – the famous “species-minimum curve” (Remane, 1934) – imply large variability of this stress factor which reaches the lethal limits of individual salinity tolerance of many benthic invertebrates in the critical zone within the horohalinicum (Telesh et al., 2013).

Using the Baltic Sea case studies, the differences mentioned above were described by the mathematical formulae (Table 1) and a conceptual model for spatial dynamics of the species number of micro- and macroorganisms in the salinity gradient of a large estuary-like brackish water body was proposed (Telesh et al., 2013).

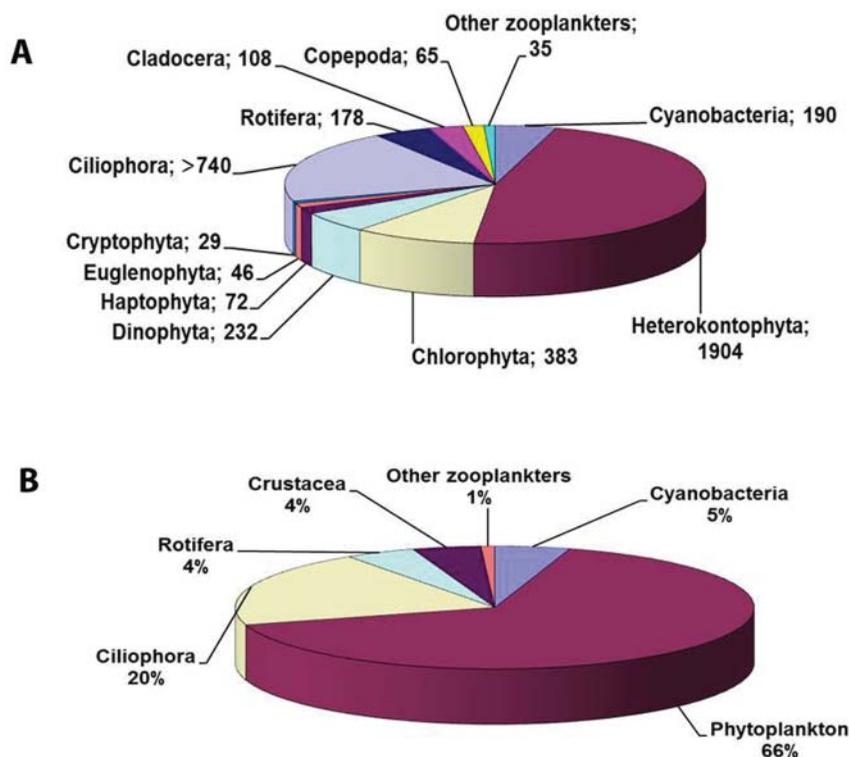


Fig. 6. Species numbers (A) and contribution, % (B) of different phyto- and zooplankton groups to the total plankton species richness in the Baltic Sea. Based on the data from Telesh et al. (2011a) and Mironova et al. (2014).

Namely, the diversity of planktonic bacteria, measured in operational taxonomic units (OTUs) and therefore reflecting the molecular diversity, differs notably from other trend lines which reflect the dynamics of morphological species' diversity, and demonstrates a steady line for bacteria within the studied salinity range, with variation around the relatively constant average values of ca. 350 OTUs per sampling site, as shown by Herlemann and co-authors (2011). Measured for the first time within the entire 2000 km long Baltic Sea salinity gradient, the remarkable molecular bacterial diversity accounts for a large variety of favorable environmental conditions for these microbes in the study area; their distribution trend line reflects only little impact of salinity fluctuations on the number of bacterial OTUs. Reduced bacterial diversity at brackish conditions was not established, possibly due to the rapid adaptation rate of the bacteria which has enabled a variety of lineages to fill in the relatively free ecological niches (Herlemann et al., 2011).

Alternatively, dynamics of species number of macrozoobenthos, macrophytes, cyanobacteria and planktonic protists in the salinity gradient can be all approximated by the polynomial (binomial)

trend lines (Table 1). However, the curves for protists (unicellular eukaryotes) and cyanobacteria (unicellular prokaryotes) mirror the trend lines for macroorganisms, thus demonstrating maximum species numbers in the horohalinicum, contrary to the minimum of benthic species numbers in this critical salinity zone (Fig. 4).

Explanations for this amazing integrity, and at the same time discrepancy, are not evident; they require further analyses, experimenting, and certain theoretical generalizations 3) that might contribute to understanding why species numbers of fast-reproducing, small motile protists and cyanobacteria peak in the horohalinicum contrary to slow-moving, sedentary or rooted, relatively large bottom inhabitants that experience dramatic salinity stress in the same environment (Skarlato and Telesh, 2017; Whitfield et al., 2012).

2.5. PROTISTAN DIVERSITY IN THE ECOTONE AND THE ECOLOGICAL NICHE CONCEPT

Additionally to the ecotonal features of the critical salinity zone, which evidently subsidizes the protistan species maximum, several other solid

Table 1. Diversity patterns of macro- and microorganisms in the salinity gradient of the Baltic Sea. (N_{sp} – number of species; S – salinity; a , b , c – coefficients; modified from Telesh et al., 2013).

Organisms	Equation	Pattern description	Comments and references
Macrozoobenthos	$N_{sp} = a_1S^2 - b_1S + c_1$	Minimum number of species in horohalinićum	The data for analysis are taken from Remane (1934).
Macrophytes	$N_{sp} = a_2S^2 - b_2S + c_2$	Minimum number of species in horohalinićum	Macrophytes here are macroalgae and higher plants; for data see: Schubert et al. (2011).
Protists	$N_{sp} = -a_3S^2 + b_3S + c_3$	Maximum number of species in horohalinićum	Protists are all eukaryotic unicellular photo-, mixo- and heterotrophic organisms; for data see: Telesh et al. (2011a).
Cyanobacteria	$N_{sp} = -a_4S^2 + b_4S + c_4$	Maximum number of species in horohalinićum	Prokaryotic unicellular organisms, commonly considered as part of phytoplankton; for data see: Telesh et al. (2011a).
Bacteria	$N_{sp} = \text{const.}$	Number of species varies around relatively stable mean value.	Very high molecular diversity; "taxa" – operational taxonomic units (OTUs); for data see: Herlemann et al. (2011).

theoretical explanations can be suggested for the discovered diversity distribution trends and patterns. Among those, Hutchinson's Ecological Niche Concept (Hutchinson, 1957), the species-area relationships (Kratochwil, 1999), especially those developed for protists (Gaston, 2000; Fenchel and Finlay, 2004; Fuhrman, 2009), and the Intermediate Disturbance Hypothesis (Connell, 1978) are providing the most convincing arguments in favor of the new biodiversity concept for protists (Telesh et al., 2011a).

According to Hutchinson (1957), the ecological niche can be defined as the n -dimensional hyper-volume where dimensions are the environmental conditions and the resources that allow a population to exist. Considering this viewpoint in combination with Gause's competitive exclusion principle (Hardin, 1960), which states that no two species can occupy the same niche in the same environment (habitat) for the long time, provides understanding that the number of species in a given habitat depends on the number of distinct niches. If not taking salinity effects into account, this generally means that we have no arguments to expect existence of different number of niches in freshwater and marine habitats; consequently, the number of species should also be similar in freshwater and marine habitats within one water body. This logic naturally admits that potential niches can be still left open in those cases where evolution has not yet delivered the respective specialists – e.g., in the geologically young brackish waters – and, therefore, the eurytopic species in those habitats are still without specialized competitors that might have been outcompeting them and thus disjointing the niche (Telesh et al., 2013).

However, freshwater as well as marine habitats are known as relatively stable ones (Remmert, 1969), allowing for long evolutionary histories of organisms which are filling the niches within these habitats. Therefore, applying the above-given assumptions to the data on macrozoobenthos' diversity provided by Remane (1934), we can conclude that both marine and freshwater ranges of the Baltic Sea ecosystem are filled in with species, but there is still a biodiversity gap in between – i.e., in the brackish environment. This allows presuming that the brackishwater biodiversity gap has not yet been filled with the respective species (Telesh et al., 2013), because of the relatively short evolutionary time during which this geologically young brackishwater sea has been existing (Schiewer, 2008). Therefore, the vacant brackishwater niches here are first invaded by the fastest, the smallest, rapidly evolving and the most highly adaptable organisms: the bacteria and the protists. New data on planktonic ciliates in the Neva Estuary (eastern Baltic Sea) supports this conclusion by showing, for example, that among the 111 ciliate species discovered in the area during 2007 through 2009, 12% of species were freshwater, 14% known from only marine and/or brackish waters, while the majority of species were small ciliates of 20–30 μm in length with a broad range of salinity tolerance (Mironova et al., 2012).

Moreover, the relative vacancy of brackishwater pelagic niches in the Baltic Sea can be proved by the intensively on-going niche-occupation process which can be well illustrated by the high rate of unintentional biological invasions of planktonic alien species through different natural and human-mediated pathways from other marine and freshwater basins (Telesh et al., 2011a, and references

therein). Among the most recent invaders in plankton are the ponto-caspian species: onychopod crustaceans *Cercopagis pengoi* and ctenophores *Mnemiopsis leydii* that have successfully established permanent populations which cause significant impact on the Baltic Sea ecosystem (Ojaveer et al., 2010).

Another supportive argument is the species-area relationship, which on a global scale restricts the number of species within a habitat to the size of a given habitat (Kratohwil, 1999). In the case of brackish water habitats (e.g., in estuaries), that are relatively narrow and usually not interconnected, the taxonomic diversity is generally low, limiting the evolution of specialized forms, as shown earlier for some other fragmented ecosystems (Templeton et al., 2001). Moreover, the size of a habitat must also be considered in relation to the individual size of organisms – the larger an average organism, the smaller the relative size of a habitat and, consequently, the slower the evolution rate because of lower generation frequency within a population of larger organisms if compared to smaller ones (Schaefer, 1999). However, the dimensions of the horohaliniacum in the Baltic Sea are very spacious, accounting for the major part of the entire area of the sea (Telesh et al., 2011a; Biological Oceanography, 2017). Therefore, high protistan diversity is fairly concordant with the species–area relationship established earlier for protists (Gaston, 2000; Fenchel and Finlay, 2004; Fuhrman, 2009).

3. Planktonic dinoflagellates: a brief overview of major biological traits

3.1. DINOFLAGELLATES AND THEIR ROLE IN HARMFUL ALGAL BLOOMS

Dinoflagellates are a large group of unicellular flagellate eukaryotes that belong to the super-group Alveolata (Adl et al., 2012, 2018). Most of them are marine plankton, but also can be benthic and often common in freshwater habitats, being distributed in water bodies depending on nutrients concentration, water temperature, salinity or depth (Okolodkov, 2011). In terms of species numbers, dinoflagellates are one of the largest groups of marine eukaryotes, although it is substantially smaller than diatoms (Guiry, 2012). Many dinoflagellates are photosynthetic, but a large fraction of those are mixotrophic (Stoecker, 1999). About half of the dinoflagellate species are exclusively heterotrophic

and feed on bacteria, flagellates, diatoms, and other dinoflagellates (Hansen, 1991). Some species are endosymbionts of marine animals and play an important role in the biology of coral reefs; others are unpigmented predators on other protozoa, and a few are parasitic (Okolodkov, 2011). Some dinoflagellates produce resting stages called dinoflagellate cysts (or dinocysts) as part of their life-cycles (Matsuoka and Fukuyo, 2000).

Many dinoflagellates, mainly benthic but also some planktonic representatives, are toxic *sensu stricta*: they produce paralytic and haemolytic toxins, hepatotoxins etc.; those are, for example, *Pfiesteria*, *Pseudopfiesteria* and a number of other Pfiesteriaceae, as well as representatives of the genera *Dinophysis*, *Alexandrium* and others that cause fish kills in the estuaries of the eastern coast of the USA, northern Europe and New Zealand (Burkholder et al., 2001).

Dinoflagellates are capable of exhibiting bioluminescence – primarily emitting blue-green light (Haddock et al., 2010). There is an opinion that dinoflagellates are responsible for most of the bioluminescence observed in the surface ocean waters (Tett, 1971). Within this group, bioluminescence is present in a number of ecologically important species, many of which form blooms (Valiadi and Iglesias-Rodriguez, 2013, and references therein).

Blooms (high population densities) of dinoflagellates develop mainly in the sea coastal waters (Zingone and Wyatt, 2005). These blooms can be either nontoxic or toxic, depending on the species which causes the bloom, its physiological and molecular features, as well as the environmental conditions; meanwhile, the factors initiating such blooms are largely unknown (Okolodkov, 2011). Interestingly, blooms can occur in different types of coastal ecosystems: in eutrophic and strongly polluted to clean oligotrophic waters, in isolated enclosed water bodies to regions with intensive mesoscale oceanographic processes (Zingone and Wyatt, 2005).

While some algae produce toxins that can be accumulated by filter-feeding organisms making them hazardous for humans, blooms of the other (nontoxic) species can result in high fish mortalities caused by development of low oxygen conditions (Al-Hashmi et al., 2015, and references therein). A bloom of certain non-toxic or potentially toxic dinoflagellates can result in a visible coloration of the water known as red (or mahogany) tide. Those blooms are harmful since the organisms excrete secondary metabolites that at high concentrations

in water can produce negative effects on aquatic biota (cause fish and shellfish poisoning) and human health if humans eat contaminated seafood, or cause harm to fish farming, recreation etc.; therefore, such events were coined “harmful algal blooms” (HABs).

Cell abundances that characterize bloom conditions vary greatly with regions and species (Smayda, 1997). For instance, the toxic species *Dinophysis acuminata* and *Alexandrium* spp. are considered at bloom conditions in Danish waters when their abundances are 500 cells mL⁻¹; meanwhile, 200 cells mL⁻¹ is enough to consider *Dinophysis acuta* Ehrenberg, 1839 at bloom condition off Portugal (Andersen, 1996). Some species are harmful even at very low cell abundances (Hansen et al., 2001). For example, off the British Isles, the mere presence of *Prorocentrum lima* (Ehrenberg) Dodge, 1975 was sufficient to incite restrictions on fisheries (Andersen, 1996).

Interestingly, in the eutrophic Chesapeake Bay the criterion for HAB caused by *Prorocentrum minimum* is its population density >3000 cells mL⁻¹ (Tango et al., 2005), while in the mesotrophic SW Baltic Sea bloom events can be considered at three times lower densities of the dinoflagellate cells: >1000 cells mL⁻¹ (Telesh et al., 2016).

Although harmful species can be found in many taxonomic groups of algae, dinoflagellates represent the major pool of HAB-inducing microorganisms: nearly 50 species of dinoflagellates are considered to be harmful (Hallegraeff et al., 2004). They can produce monospecific, or almost monospecific, blooms that occur below or at the water surface of marine (presumably coastal) regions worldwide. Usually, dinoflagellates tend to dominate phytoplankton communities under high temperatures and relatively low nutrient concentrations (Lalli and Parsons, 1997). The latter effect is considered to be largely due to mixotrophy of many dinoflagellates which supports their ability to consume both organic and inorganic nutrient substances (Stoecker, 1999; Fan et al., 2003; Hajdo et al., 2005; Glibert et al., 2012, 2013, 2014, 2016; Matantseva and Skarlato, 2013; Matantseva et al., 2016).

In recent decades, algal blooms have been increasing in frequency and magnitude in many oceanic and coastal regions of the world (GEOHAB, 2001; Anderson et al., 2002; Gomes et al., 2014). Although HABs were initially recorded in tropical regions, until recently major blooms were believed to be restricted mostly to temperate waters; however, since the 1990s a trend to increasing algal blooms has been observed also in tropical and subtropical regi-

ons (Hallegraeff et al., 2004; Al-Hashmi et al., 2015). Coastal ecosystems are becoming more vulnerable to HABs, especially in the enclosed seas and coastal embayments, largely as a result of increased nutrient enrichment caused by urbanization, tourism, industrial wastes, desalination plants and agricultural activities (Anderson et al., 2002; Sellner et al., 2003; Heil et al., 2005). Natural processes, such as circulation of water masses, upwelling and cyst formation are considered important factors contributing to formation of algal blooms (Levinton, 2001; Sellner et al., 2003). Selected regional studies of HABs due to dinoflagellates are briefly referred to below.

For example, in the Chesapeake Bay, blooms of dinoflagellates represent a major portion of local phytoplankton biomass and production; they usually occur in late spring to early fall in response to nutrient inflow from terrestrial run-off, and at certain times the dinoflagellate abundances are high enough to visibly discolor the water, causing “red tides” (Li et al., 2000). Several species are reported to be involved in the formation of blooms in the Chesapeake Bay: *Prorocentrum minimum*, *Gymnodinium sanguineum*, *Gyrodinium uncatenum* and *Ceratium furca*. Although these species are generally not toxic in the mid-Atlantic region, their dense blooms can, nevertheless, have harmful impacts on the ecosystem since they inhibit feeding of zooplankton and invertebrate larvae, thus modifying coastal food webs (Turner and Tester, 1997). Sedimentation and decomposition of senescent blooms can lead to low oxygen concentration in near-bottom waters, causing fish kills and losses of benthic invertebrates (Hallegraeff, 1993; Turner and Tester, 1997).

The Arabian Sea (including the Sea of Oman) provides another example of the region where the increased occurrence of coastal HABs caused by dinoflagellates *Ceratium* spp., *Karenia* spp. and *Noctiluca scintillans* has been recorded for a long time – since 1976 (Al-Gheilani et al., 2011). Since then, *Noctiluca scintillans* appeared responsible for >50% of HABs, causing fish kills due to oxygen depletion (Al-Azri et al., 2012; Al-Gheilani et al., 2011). In Muscat coastal waters, blooms of *N. scintillans* are usually seasonal events (Al-Azri et al., 2012). Before 1997, blooms of *N. scintillans* and cyanobacteria (*Trichodesmium* sp.) in the Bay of Bandar Khayran were accompanied by coral bleaching, fish mortalities and development of cancerous growths on corals (Coles and Seapy, 1998). *Cochlodinium polykrikoides* blooms in the Sea of Oman and along the eastern coast of the

Arabian (Persian) Gulf have caused massive fish mortalities, limited traditional fishery operations, impacted coastal tourism, and forced the closure of desalination plants (Matsuoka et al., 2010; Richlen et al., 2010). A continuous occurrence of *Prorocentrum minimum* was reported in the Sea of Oman for the first time in 2015, although phytoplankton studies in the Muscat region started in 1995; presumably, this species was overlooked during the earlier studies due to its small size (Al-Hashmi et al., 2015). In view of these phenomena, regional studies of HAB species' dynamics and investigation of relationships between their abundance and environmental factors are of the utmost importance.

As shown recently, harmful dinoflagellates were abundant and dominated the HAB assemblages in the Sea of Oman most of the time (Al-Azri et al., 2012; Al-Hashmi et al., 2012). Moreover, many authors believe that on the inter-annual scale the contribution of dinoflagellates to algal blooms is increasing in the Arabian Sea basin, when compared to diatom contributions (Subba-Rao and Al-Yamani, 1998; Gomes et al., 2009; Piontkovski et al., 2011). This decline in diatom biomass is the result of a decline in the availability of nitrate caused by increased thermohaline stratification associated with rising temperatures (Smayda, 1997). Most of the HAB events in Oman's coastal waters have been dominated by dinoflagellates, with *N. scintillans* occurring most commonly (Al-Gheilani et al., 2011; Al-Azri et al., 2012).

In the Russian coastal waters, the dinoflagellate HAB phenomena reach the largest scale in the Far Eastern seas. The investigations have revealed that among almost 30 species causing phytoplankton blooms in this region, 24 species are known to be harmful; among those species, the majority belong to planktonic dinoflagellates, diatoms, and raphidophytes (Orlova et al., 2014). The greatest number of HABs in the Far Eastern Seas of Russia is caused by dinoflagellates; several of those provoke "red tides", among them *Noctiluca scintillans* and *Prorocentrum minimum*. *N. scintillans* is one of the most common species in the region and its distribution is restricted to the southern part of the Pacific coast of Russia (Orlova et al., 2014).

The three examples mentioned above provide just a quick look into the overall HAB problems and highlight the crucial role of dinoflagellates in this deteriorating phenomenon in different regions of the world. However, apart from being harmful, toxic or potentially toxic, some dinoflagellates also

demonstrate remarkable invasive potential which backs up their broad, global-scale distribution and cosmopolitanism. For instance, planktonic dinoflagellates from the genus *Prorocentrum* are among the most commonly recognized, world-widely distributed harmful algae that are increasing in frequency, duration, and magnitude globally (Heil et al., 2005; Glibert et al., 2008). For example, although 11 potentially harmful dinoflagellates were detected in the Bay of Bandar Khayran (the Arabian Sea), only three species: *Prorocentrum minimum*, *Scrippsiella trochoidea* Balech ex Loeblich III, 1965, and *N. scintillans* dominated regularly in the water column (Al-Hashmi et al., 2015). The dinoflagellates *P. minimum* and *S. trochoidea* were observed throughout the sampling period with higher abundances; this trend was only interrupted from December to January, when the massive blooms of *Cochlodinium polykrikoides* occurred. For example, *P. minimum* began increasing in abundance during the *Cochlodinium* bloom from 530 cells L⁻¹ in October 2008 (prior to the bloom) to 2800 and 5500 cells L⁻¹ during the bloom in November and December 2008, respectively; the highest recorded abundance of *P. minimum* was 8000 cells L⁻¹ in September 2009 (Al-Hashmi et al., 2015). Principal component analysis indicated a clear correlation between increases in *P. minimum* abundance, nitrate plus nitrite increase, and lower temperatures (Al-Hashmi et al., 2015). Therefore, since the latter species is often encountered as one of the most common bloom-forming dinoflagellates worldwide, we have concentrated much of our attention and research efforts on these particular protists.

3.2. BLOOM-FORMING, POTENTIALLY TOXIC DINOFLAGELLATE *PROROCENTRUM MINIMUM*

The dinoflagellate species *Prorocentrum minimum* (Pavillard) Schiller, 1933 is also known as *Prorocentrum cordatum* (Ostenfeld) Dodge, 1975; its other synonyms are *Prorocentrum triangulatum* Martin, 1929; *Exuviaella minima* Schiller, 1933; *Exuviaella marie-lebouriae* Parke and Ballantine, 1957; *Prorocentrum cordiformis* A.S. Bursa 1959, and *Prorocentrum marielebouriae* (Parke and Ballantine) A.R. Loeblich III, 1970 (Fig. 7).

Prorocentrum minimum is a free living planktonic dinoflagellate; it is commonly found in marine and brackish waters of the temperate climate zone and in subtropics, less seldom in tropical regions. This cosmopolitan species was originally described from

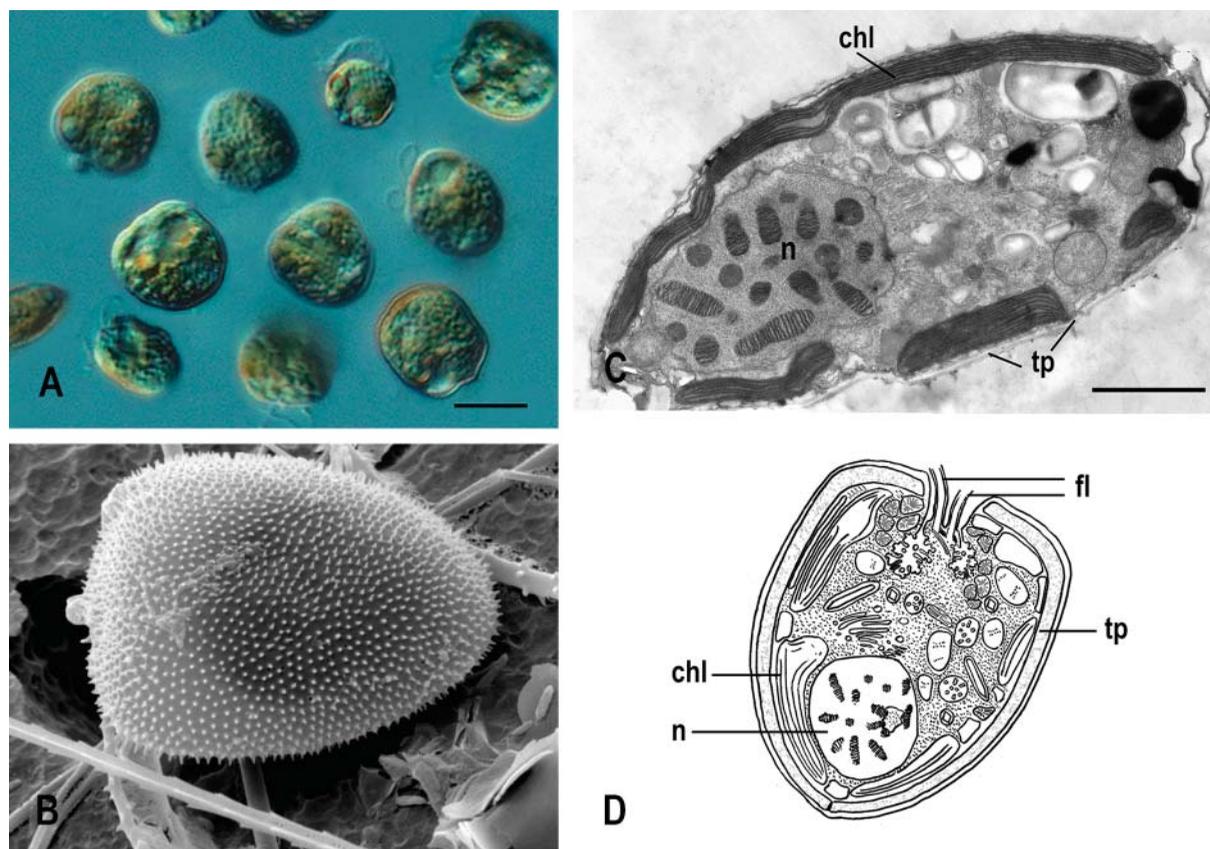


Fig. 7. *Prorocentrum minimum* (Pavillard) Schiller, 1933. A – Live cells in culture (photo M.A. Berdieva); B – SEM photo of a cell (photo M.A. Faust; <http://www.vieraslajit.fi/lajit/MX.52909/show>); C – electron-microscopy photo, and D – scheme of a cell (modified from: Berdieva et al., 2016). *Abbreviations:* chl – chloroplasts, fl – flagella, n – nucleus, tp – thecal plates. Scale bars: A – 10 μm , C – 2 μm .

the Mediterranean Sea (the Gulf of Lyon, France) and is currently expanding its range having pan-global distribution (Heil et al., 2005) (Fig. 8).

Blooms of *P. minimum* are most common in the coastal waters of the temperate and subtropical regions of the Northern hemisphere: in the northern part of the Pacific and Atlantic oceans along the coasts of Russia, China, Japan and Canada; along the eastern and southern coasts of the USA (in the Chesapeake Bay, Gulf of Mexico), and the Caribbean Sea. Its blooms were also registered in the NE Atlantic close to the British Isles (Parke and Dixon, 1976; Dodge, 1982). In northern Europe, first bloom of this species was observed in the English Channel in 1976 (Smayda, 1990). In the North Sea, *P. minimum* was first recorded near the coast of The Netherlands in 1976 (Kat, 1979), and later – close to Norway (Tangen, 1980) and in the Norwegian fiords (Kimor et al., 1985). It appeared in the Danish Straits in 1979, and in the Baltic Sea – in 1981 (Edler

et al., 1982). In the Arctic region, it is a common phytoplankton component in the major gulfs of the White Sea (Ilyash et al., 2003, 2014).

Blooms of *P. minimum* are often recorded along the Atlantic coasts of France and Portugal (Moita and Vilarinho, 1999). In the Black Sea, *P. minimum* is known from the Romanian coast since 1950-s, and in the Adriatic Sea – since 1983 (Marazović, 1986). It also inhabits the Caspian, Azov and Aral seas (Marazović et al., 1990). In the Russian Far East, *P. minimum* was registered in the Bering Sea near Kamchatka and in the Okhotsk Sea; it also blooms in the Amursky Bay of the Sea of Japan and along the coasts of Japan (Stonik, 1995; Orlova et al., 2014); is common in the South China Sea along the coasts of Taiwan and Philippines (Azanza et al., 2005), in the tropical waters near Pakistan (Rabbani et al., 1990), in the Sea of Oman, the Arabian Sea (Al-Hashmi et al., 2015), near Australia and New Zealand (Heil et al., 2005) (Fig. 8).

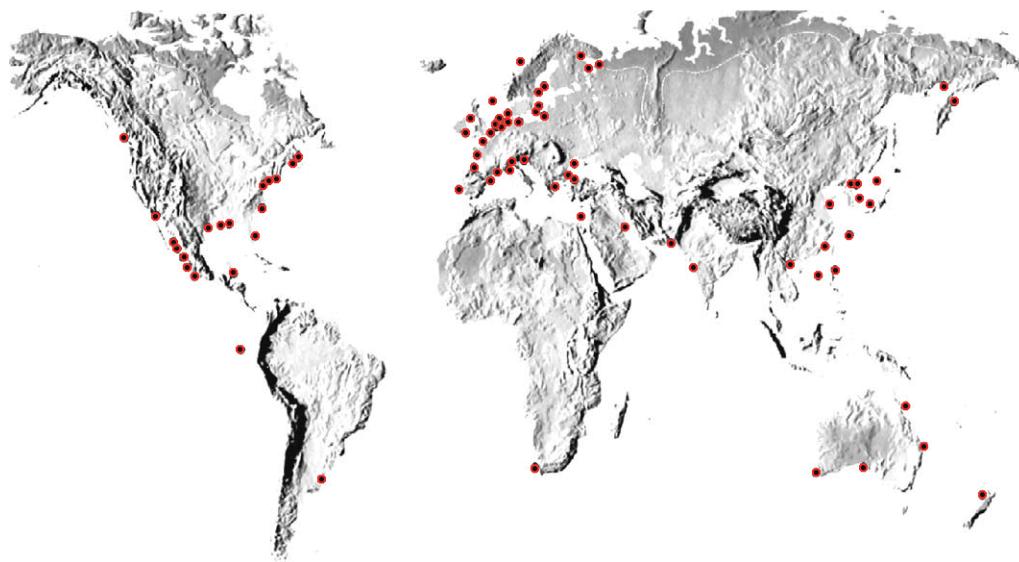


Fig. 8. Distribution of *Prorocentrum minimum* (modified from: Heil et al., 2005, with additions).

Considering the global distribution of *P. minimum*, the relatively small representation of this species near the coasts of Africa and South America can be most likely explained by a lack of regular monitoring observations in those regions (Heil et al., 2005).

P. minimum can be present in plankton during all seasons; it blooms mainly in sea coastal waters, estuaries and river mouths, bays and fiords that are usually impacted by nutrients input from drainage basins due to eutrophication, but also in oligotrophic waters of the marine pelagic areas (Steidinger and Tangen, 1996). This species has wide ecological plasticity: in nature, it can live at temperatures 3–31 °C and salinities 2–37 (Berland and Grzebyk, 1991; Telesh et al., 2016); in the experiments, these ranges are even wider (Olenina et al., 2016; Knyazev et al., 2018). Blooms of *P. minimum* are known to correlate with the input of organic nitrogen (Hajdo et al., 2005; Glibert et al., 2008).

The dinoflagellates *P. minimum* are characterized by a number of peculiar features that are briefly mentioned below. Their cells are small (length 14–22 µm, width 10–15 µm), flattened and armored, with 2 flagella. The complex cell coverings (amphiesma) of these dinoflagellates include plasma membrane and flattened amphiesmal vesicles with thecal cellulose plates. Two largest thecal plates embrace the cell like shell valves, and there are also 8 small apical thecal plates in the zone of the flagella canal (Pozdnyakov and Skarlato, 2012; Berdieva et al., 2016).

Pronounced morphological variability is a characteristic feature of *P. minimum*, which is best

expressed in highly variable shape of the cells: from nearly round or oval to heart-shaped or triangular (Olenina et al., 2016). Another unique trait of *P. minimum* is the large range of growth rates: from 0.12 to 3.54 day⁻¹ (Heil et al., 2005). Cells of these dinoflagellates usually undergo simple binary division; however, recently the ability of *P. minimum* to switch to sexual process during the life cycle in the conditions of nutrients deficit was discovered (Berdieva and Kalinina, 2018).

These dinoflagellates are presumably phototrophic organisms but they can also act as mixotrophs, consuming both inorganic (e.g. dissolved nitrogen) and organic substrates — e.g. urea which is a common component of fertilizers and inflows to the coastal sea areas from the drainage basin (Stoecker et al., 1997; Burkholder et al., 2008; Matantseva et al., 2016).

Tyler and Seliger (1981) demonstrated that *P. minimum* can survive between 20 and 35 days in total darkness, depending upon temperature. This species also has been shown as photosynthetically flexible, increasing pigment concentrations and altering photosynthetic physiology to survive extremely low light levels for extended periods (Tyler and Seliger, 1981; Harding et al., 1983; Harding, 1988). This suggests that *P. minimum* would be capable of surviving in ballast water tanks of ships for rather long periods of time (Heil et al., 2005)

P. minimum is a bloom-forming dinoflagellate which is considered potentially toxic; however, some of its clones that were isolated from certain areas (e.g., French Mediterranean coast) have got

demonstrative toxic components (see the debate in Heil et al., 2005, and references therein).

P. minimum is widely distributed in the coastal waters of the World Ocean (Fig. 8), and its expanding geographical distribution is indicative of a strong relationship between both dissolved inorganic nitrogen (DIN) export and dissolved organic nitrogen (DON) export into coastal waters (Glibert et al., 2008; Li et al., 2011). It is important that blooms of *P. minimum* induce fish and shellfish mortalities (Steidinger, 1993; Tango et al., 2005; Li et al., 2012), cause harm to aquaculture (Alonso-Rodríguez and Páez-Osuna, 2003), and are therefore dangerous to humans who consume mussels and fish poisoned by the secondary metabolites of these dinoflagellates (Kat, 1979). Thus, *P. minimum* blooms are seriously deteriorating coastal ecosystems' health and their environmental, recreational and socio-economic services (Heil et al., 2005; Olenina et al., 2010).

The currently on-going range expansion of this harmful species witnesses for its powerful invasive potential and high competitive advantages that allow this alien species conquering new environments after being introduced there by the common vectors: with the ballast waters of cargo ships, or due to the intensified aquaculture in the sea coastal regions (GEOHAB, 2001).

3.3. INVASION HISTORY OF *PROROCENTRUM MINIMUM* IN THE BALTIC SEA

In Europe, invasion of the Baltic Sea by harmful dinoflagellates *Prorocentrum minimum* represents one of the most outstanding examples of HABs development history in a large, semi-closed brackishwater sea of the utmost ecological and economic importance for the human population of 9 countries located on its shores (Telesh et al., 2016). *P. minimum* is one of the most curious non-indigenous species in the Baltic Sea. According to published records, this alien colonized the Baltic Sea more than three decades ago (Edler et al., 1982; Kimor et al., 1985; Olenina et al., 2010). This invasion process was rather slow though amazingly effective. After its massive bloom in the Skagerrak area in 1979, *P. minimum* in 1981 reached the Kattegat and was first recorded in the western Baltic waters (Edler et al., 1982). It subsequently entered the Baltic Sea: in 1982 – to the Belt Sea area (Edler et al., 1982), in 1983 – in Kiel fjord (Kimor et al., 1985), and in 1989 – in the southern part of the Baltic Sea and the Gulf of Gdańsk (Mackiewicz, 1995). In 1989–1993, the species was found in the

central and northern parts of the Baltic Proper, while in 1997–1999 it extended its range to the Gulfs of Finland and of Riga (Hajdu et al., 2000). Thus, by 1999 this eurytopic marine species has expanded its range to almost the entire brackishwater Baltic Sea (except for the Gulf of Bothnia) reaching as far to the north-east as the oligohaline Gulf of Finland (Hajdu et al., 2000, 2005; Witek and Pliński, 2000; Pertola, 2006).

Currently, *P. minimum* is one of the five dinoflagellate species from the genus *Prorocentrum* that inhabit the Baltic Sea; the other four species are: *P. balticum* (Lohmann) Loeblich, 1970, *P. micans* Ehrenberg, 1833, *P. compressum* (Bailey) Abé ex Dodge, 1975, and *P. triestinum* Schiller, 1918, among more than 2000 other phytoplankton species (Hällfors, 2004; Telesh et al., 2011a, 2016).

The dinoflagellate *P. minimum* is the only one phytoplankton species in the Baltic Sea which can be considered truly invasive (Olenina et al., 2010), because the dynamics and ecological importance of only this unicellular alien meets the major established requirements of the “invader” (IUCN, 1999; Occhipinti-Ambrogi and Galil, 2004; Ojaveer et al., 2010). This implies that, firstly, the population of *P. minimum* is growing exponentially, rapidly expanding its range and, secondly, this potentially toxic species is widely known to be able to cause environmental damage, economic loss or harm to human health being implicated in the elevated fish and shellfish mortalities and human poisoning during blooms (Wickfors and Smolowitz, 1995; Denardou-Queneherve et al., 1999; Heil et al., 2005; Tango et al., 2005; Olenina et al., 2010; Glibert et al., 2012; Al-Hashmi et al., 2015), and seriously affecting aquaculture (Alonso-Rodríguez and Páez-Osuna, 2003; Azanza et al., 2005). The pan-global distribution of this species indicates its high and increasing invasibility, negative ecosystem impacts and growing economic importance (Heil et al., 2005).

Like other invasive ballast-transported protists that are harmless in native habitats but may modify or trigger changes in plankton assemblages of the recipient water bodies after invasion (Hülsmann and Galil, 2002), *P. minimum* can affect pelagic (and also benthic, via the benthic-pelagic coupling) communities by complete outcompeting or partially displacing native planktonic taxa (Fig. 9).

It is the accepted knowledge that the invaders usually conquer new environments and extend their range fast due to lack of competitors, predators and parasites, which allows them to quickly achieve high

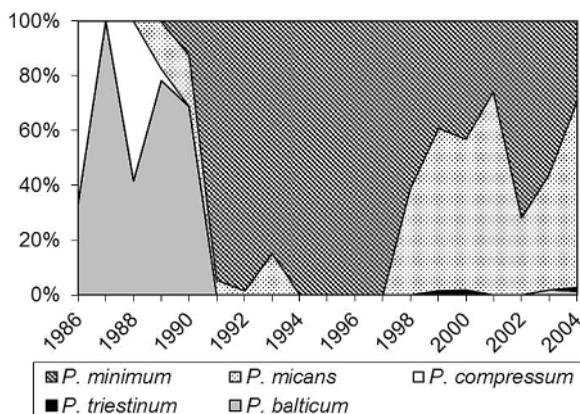


Fig. 9. Average annual representation (percent of total sample numbers) of the five *Prorocentrum* species (*P. minimum*, *P. balticum*, *P. micans*, *P. compressum* and *P. triestinum*) in the database from the Baltic Sea in 1986–2005. Based on data from Telesh et al. (2016).

population densities (Carlton, 1996, 2002). One such example of a very rapid colonization process among aquatic metazoans is the invasion of the Lake Ontario (the Great Laurentian Lakes, USA) by the planktonic predatory water flea *Cercopagis pengoi* (Ostroumov, 1891), which reached its maximum population density already within the first year after the invasion (Makarewicz et al., 2001; Laxson et al., 2003). This scenario is soundly expected to be particularly true also for the unicellular eukaryotes as well as the prokaryotes (Telesh et al., 2016), due to their exceptionally rapid reproduction and very short generation times (Hülsmann and Galil, 2002).

Meanwhile, the uncommonly long (for the unicellular organisms) period of time – nearly a decade – passed between *P. minimum* first appeared in the Baltic waters until it has become an established species in this brackishwater sea, with the population ability to reach pronounced abundances exceeding 100 cells mL⁻¹ (Olenina et al., 2010). Moreover, the first real bloom of *P. minimum* with densities >3000 cells mL⁻¹, according to the definition of a bloom proposed for the Chesapeake Bay (EPA, 2003; Tango et al., 2005), was registered in the Baltic Sea only in the late 1990s, i.e. nearly two decades after the initial invasion (see Table 5 in Olenina et al., 2010). Recently, the possible reasons of this peculiarly long though successful invasion history have been investigated, analyzed and debated (Telesh et al., 2016).

3.4. BROAD ECOLOGICAL NICHE — A PREREQUISITE TO SUCCESSFUL INVASION

A focused research allowed unveiling the ecological mechanisms behind the specific invasion history, spatial distribution and the bloom-forming potential of the planktonic dinoflagellates *P. minimum* in the Baltic Sea (Telesh et al., 2016). In this study, the ecological niche concept (Hutchinson, 1957; Leibold, 1995; Chesson, 2000; Litchman et al., 2007, 2012; Chesson and Kuang, 2008) was applied to a large, long-term phytoplankton database from the SW Baltic Sea, and the obtained data allowed analysing the possible reasons of the Baltic Sea invasion by *P. minimum* which, however, was characterised by a remarkable delay in the species ability to form blooms in the recipient sea.

The timeline of *P. minimum* colonization of the SW Baltic Sea coastal waters was analyzed in details using the data on phytoplankton composition, abundance and biomass collected in 1972–2005 (Sagert et al., 2008). The ecological niche dimensions of *P. minimum* and its congeners were identified as the optimum environmental conditions for the species based on evaluations of water temperature, salinity, pH, concentration of nutrients (PO₄³⁻; total phosphorus, TP; total nitrogen, TN; SiO₄⁴⁻), TN/TP-ratio and habitat types during the bloom events (Telesh et al., 2016). The frequency of those optimum environmental conditions that were most likely backing up the massive population development of *P. minimum* in the SW Baltic Sea was determined, and the possible competitors of *P. minimum* were identified. A research hypothesis was tested arguing that the lag-phase preceding the first bloom of *P. minimum* in the Baltic Sea was caused by the biotic restrictions such as competition and resource partitioning with one or several congeneric species whose ecological niches overlapped (at least partly), thus hampering the invader's range expansion. This phenomenon was supposed to be backed up by the high overall protistan diversity in the brackish SW Baltic coastal waters.

Assessment of the ecological niche dimensions of the alien dinoflagellate *P. minimum* and its major native congeners in the SW Baltic Sea has led to the conclusion that *P. minimum* is a generalist species, because it occupies a rather broad ecological niche (Table 2), which covers the niches of *P. balticum*, *P. compressum* and *P. micans*. The latter one, however, stretches its ecological niche beyond the edges of the *P. minimum*' niche, and this allows *P. micans* to

Table 2. Major ecological niche dimensions of the dinoflagellates *Prorocentrum minimum* defined for the bloom events with the abundances >1000 cells mL⁻¹. Outliers with >2SD from the mean value were excluded from the analysis (based on the data from Telesh et al., 2016).

Parameter	<i>Prorocentrum minimum</i>	
	Median value ± SD	n
Day of the year	223 ± 21	19
Water temperature above pycnocline; T °C	20.8 ± 3.7	19
Salinity above pycnocline	7.9 ± 1.8	19
PO ₄ ³⁻ (μmol L ⁻¹)	0.6 ± 0.7	17
Total phosphorus (TP; μmol L ⁻¹)	2.0 ± 1.4	17
Total nitrogen (TN; μmol L ⁻¹)	28.3 ± 8.7	17
Dissolved inorganic nitrogen (DIN, calculated; μmol L ⁻¹)	1.7 ± 0.7	19
TN/TP Ratio	13.7 ± 10.3	17
pH	8.4 ± 0.2	18
SiO ₄ ⁴⁻ (μmol L ⁻¹)	27.1 ± 14.1	18

succeed in competition with *P. minimum* as well as with the other planktonic dinoflagellates in the SW Baltic Sea. This fact explains why since 1998 these two close congeners, *P. minimum* and *P. micans*, have been the most highly abundant, coexisting planktonic dinoflagellates in the Baltic Sea (Fig. 9).

Additionally, long-term fluctuations of the major environmental characteristics in the Baltic coastal regions did not reveal any specific changes during 1985-1998 (Feistel et al., 2008; Schiewer, 2008) – the critical post-invasion period of time for *P. minimum*, and this fact might support the invader's range expansion in the brackish SW Baltic coastal waters. However, it cannot explain the delayed blooms of this species after the invasion. The long-term data, therefore, supported the research hypothesis which argued that the lag-phase in the invasive *P. minimum* population development prior to its first bloom in the SW Baltic Sea was most likely caused by the complexity of biotic interactions in the highly diverse phytoplankton community (Telesh et al., 2011a). Specifically, competition with several congeneric dinoflagellate species occupying at least part of the same ecological niche and, therefore, hampering the fast spread and explosive development of the invader, was considered as the major biotic factor hampering bloom formation by *P. minimum* (Telesh et al., 2016).

The accepted invasibility criterion for successful species coexistence requires population of each species to be able to grow in the presence of other

species in the community (Chesson, 2000), while close congeners are known to be the strongest competitors (Chesson and Kuang, 2008). Moreover, correlation between biodiversity and vulnerability to invasions as an example of ecosystem disturbance is generally well established (Shea and Chesson, 2002) and largely depends on the environmental characteristics, salinity being one of the main variables in the coastal environments.

In the Baltic Sea, the major part of coastal waters are usually characterized by the average critical salinity of 5-8 (Feistel et al., 2008; Schiewer et al., 2008) at which sharp changes in the ionic composition usually occur (Khlebovich, 1968). It is generally accepted that this boundary zone provides unfavorable osmotic conditions for aquatic organisms, and for this reason the Baltic coastal regions have long been considered as species-poor habitats – since Remane (1934) published his conceptual model showing species minimum of macrozoobenthos in the horohalinicum of the Baltic Sea. However, in our previous studies we revisited Remane's species-minimum concept and performed meta-analysis of several large, long-term databases providing convincing evidence for the high plankton diversity and the protistan species maximum in the critical salinity zone of the Baltic Sea (Telesh et al., 2011a, b, 2013, 2015; Shubert et al., 2011). Results of the recent study, exemplified by the delayed first bloom of the invasive dinoflagellate *P. minimum* in the species-rich phytoplankton community with four other congeneric species, demonstrated close linkage between high overall protistan diversity at critical salinities and resilience of pelagic communities to alien species invasions in coastal waters (Telesh et al., 2016).

It is common knowledge that interactions between close congeners and the species within one trophic level are usually the most complex (de Jonge, 2007). Currently, five planktonic *Prorocentrum* congeners coexist in the SW Baltic Sea, and this simple fact advocates the intensive interspecific competition due to considerable niche overlap (Telesh et al., 2016). Among those 5 species, however, only *P. minimum* and *P. balticum* most often dominate the *Prorocentrum*-containing phytoplankton communities. Theoretical research supports the concept that stable coexistence necessarily involves important ecological differences between species that may be thought of as distinguishing their niches (Chesson, 2000). However, the overlapping ecological niche of the generalist *P. minimum*

and its congeners does not prevent its coexistence with the other species due to its ability of forming blooms and population success in a broader range of environmental variations (Hajdo et al., 2000; Tango et al., 2005).

First evaluation of the ecological niche of *P. minimum* in the SW Baltic Sea (Telesh et al., 2016) revealed that its major blooms usually occur at salinities 7.9 ± 1.8 and temperature 20.8 ± 3.7 °C (Table 2). To compare, in the Chesapeake Bay (Western Atlantic Ocean) high density blooms (>3000 cells mL⁻¹) of these dinoflagellates are reached at temperatures between 12–22 °C and salinity 5–10 (Tango et al., 2005), and these habitat ranges are much broader than in the SW Baltic Sea. On the one hand, the relatively narrow Baltic ecological niche of *P. minimum* (compared to the Chesapeake Bay), if coupled with the presence of four congeners competing for the same resources, might have been responsible for a delay in *P. minimum* population development prior to the first bloom of this invader in the Baltic Sea. On the other hand, the differences in morphology, ecophysiological characteristics and niche dimensions of *P. minimum* in these two water basins may be attributed to specific hydrological and hydrochemical regimes, various concentrations of nutrients and differences in trophic status of both water bodies (Telesh et al., 2016).

Moreover, recent experimental studies showed that cell size of *P. minimum* was inversely proportional to both salinity and nutrient content; the larger cells were growing slower than the smaller ones but their abundance was increasing (Olenina et al., 2016). Larger cells usually possess higher ability to nitrogen uptake related to their requirements and larger storage capacity, if compared with the smaller cells (Maracyn et al., 2013). Additionally, *P. minimum* is known to be favored by runoff from land and the increased organic nutrient loads as its abundance reacts positively to elevated concentrations of phosphorus and nitrogen (Granéli et al., 1985; Berland and Grzebyk, 1991; Hajdu et al., 2000; Pertola, 2006; Ou et al., 2014). Thus, ecological niche dimensions of these dinoflagellates depend on environmental fluctuations and correlate with eutrophication, particularly in the coastal regions where salinity declines due to freshwater inputs. Consequently, significant ecosystem effects of *P. minimum* blooms are expected in the future with the forecasted decreasing salinity and increasing eutrophication of the Baltic coastal waters (Vuorinen et al., 2015). The potential ecosystem changes may include alterations in pH, oxygen depletion,

and pronounced light attenuation in the bloom-impacted regions (Heil et al., 2005).

4. Competitive advantages of *Prorocentrum minimum* in the changing environment

4.1. ADAPTATION STRATEGIES

Many physiological features and ecological requirements of planktonic protists are characterized by clearly pronounced flexibility; their different properties often vary in broad ranges, and the responses exhibited by planktonic organisms towards fluctuating environment change non-linearly (Skarlato and Telesh, 2017, and references therein). Like many other dinoflagellates, *P. minimum* – a unicellular, photosynthetic, free-swimming biflagellate species – represents a significant component of many brackish and marine phytoplankton communities and forms an important link in the trophic chains (Fan et al., 2003b; Heil et al., 2005). Meanwhile, adaptation strategies and molecular mechanisms that back up high adaptive potential of these organisms are understudied, largely due to insufficient knowledge about some cellular features of these protists. Elucidation of these traits and prerequisites to their roles in the life cycle is crucial for understanding the processes, leading to massive proliferation or harmful algal blooms caused by many dinoflagellates (Dapena et al., 2015), and also for development of management tools for combatting HABs. These issues require clear understanding of how these microorganisms adapt to harsh environment; they are of particular importance when the environment represents a large water body, which plays a key role in the economy of coastal areas of several densely populated countries – for example, the Baltic Sea (Telesh et al., 2013).

A number of environmental characteristics fluctuate in natural habitats. Among those, water temperature and salinity are among the major stress factors for aquatic biota in the brackishwater seas, their coastal regions, estuaries and lagoons (Schubert and Telesh, 2017). Large areas in these relatively shallow and, therefore, ecologically variable water bodies are represented by the critical salinity (5–8) zones, or “barrier zones”, featured by the unstable physical and chemical characteristics (Khlebovich, 1968; Khlebovich and Abramova, 2000; Telesh and Khlebovich, 2010). However, unlike the effects of water temperature, illumination, or turbulence of water masses on the organisms, the impact of

variations in salinity of natural waters on plankton communities is insufficiently studied, both at regional and global scales (de Jonge et al., 2002, 2014; Elliott and Whitfield, 2011; Vuorinen et al., 2015; Schubert et al., 2017; Skarlato and Telesh, 2017).

Response of cells viability to short-term salinity stress

Planktonic mode of life allows microorganisms drifting within large water masses that are characterized by relatively low environmental fluctuations at small scales. At the same time, their fast reproduction, ability to form temporary cysts and small body sizes can help the unicellular organisms not only to survive but to prosper and even reach maximum species diversity within the zone of sharp salinity variations at larger scales (Telesh et al., 2015; Skarlato and Telesh, 2017). However, the cellular and molecular mechanisms that maintain homeostasis of planktonic micro-eukaryotes in the fluctuating brackishwater environment are not well understood yet. Therefore, a number of recent studies aimed at demonstrating the impact of different levels of abrupt salinity stress on cell viability/mortality, cell cycle pattern, RNA synthesis, DNA replication, and ultrastructure of *P. minimum* – a species with broad geographical distribution range, which has currently received additional attention of scientists researching into cell biology and investigating environmental alterations in estuaries and sea coastal regions, due to high invasive potential of these dinoflagellates and their pronounced bloom-forming capacity (Skarlato et al., 2018).

Specifically, the series of laboratory experiments with short-term stress by salinities 4, 8 and 35 allowed discovering that deteriorating effect was the greatest in the oligohaline conditions at salinity 4, where the highest mortality of *P. minimum* cells was observed (Fig. 10). Meanwhile, the lowest mortality was registered at salinity 8, suggesting that salinity stress response of the cells was most effective in the critical salinity conditions. These findings imply that during sharp alterations of the environmental conditions, such as in the case of ballast water release (Leppäkoski et al., 2002), these protists most likely will not just survive but even become more competitive in a brackish environment, if compared with oligohaline waters or high-salinity regions (Skarlato et al., 2018).

These results support the previous findings of the broad ecological niche of *P. minimum* (Telesh

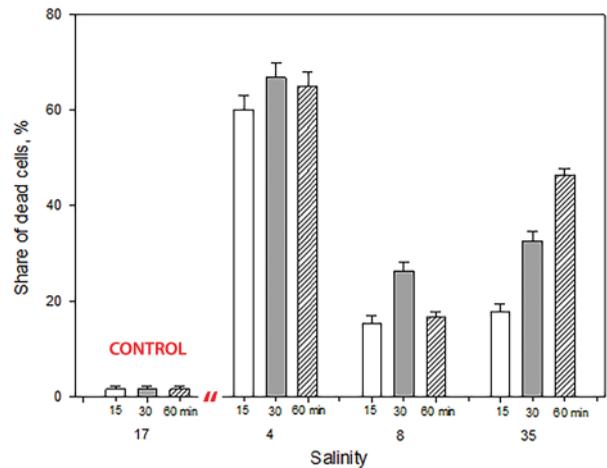


Fig. 10. Share of dead cells (% , \pm SD) of *Prorocentrum minimum* at different salinities (17-control; 4, 8 and 35) and varying exposure time (15, 30 and 60 min). Based on data from Skarlato et al. (2018).

et al., 2016). It was also shown earlier that these dinoflagellates preferably inhabit coastal brackish waters of temperate and tropical regions (Smayda and Reynolds, 2001; Heil et al., 2005); however, they can survive in nature in the very wide range of salinity (5-37) within the broad range of temperatures: 4-31 °C (Berland and Grzebyk, 1991). The Alg@line Project team in 1998-1999 registered high densities of *P. minimum* in the western Gulf of Finland at salinity 4.5 (see Fig. 2 in Pertola et al., 2005), while the newest experimental results provided support to the viewpoint that in nature salinity 4, most likely, is beyond the optimum range of environmental conditions for this species (Skarlato et al., 2018).

Salinity impact on cell cycle

Despite the long discussion, it is currently accepted knowledge that the cell cycle of some dinoflagellates can be described as a typical eukaryotic G_0/G_1 -S- G_2 -M-phase model, with a discrete short phase S (Triemer and Fritz, 1984; Soyer-Gobillard, 1996; Bhaud et al., 2000). Skarlato et al. (2018) revealed that salinity stress caused only moderate impact on the cell cycle pattern of *P. minimum* by showing that the share of cells at phase G_0/G_1 , in which the growing protists exhibited high metabolic activity, did not change much at salinities 17, 8 and 35, being above 89.3% in all cases. After stress, the share of cells at phase S was increasing at salinities 8 and 35, while the percentage of cells at the G_2 /M phase was decreasing, if compared with

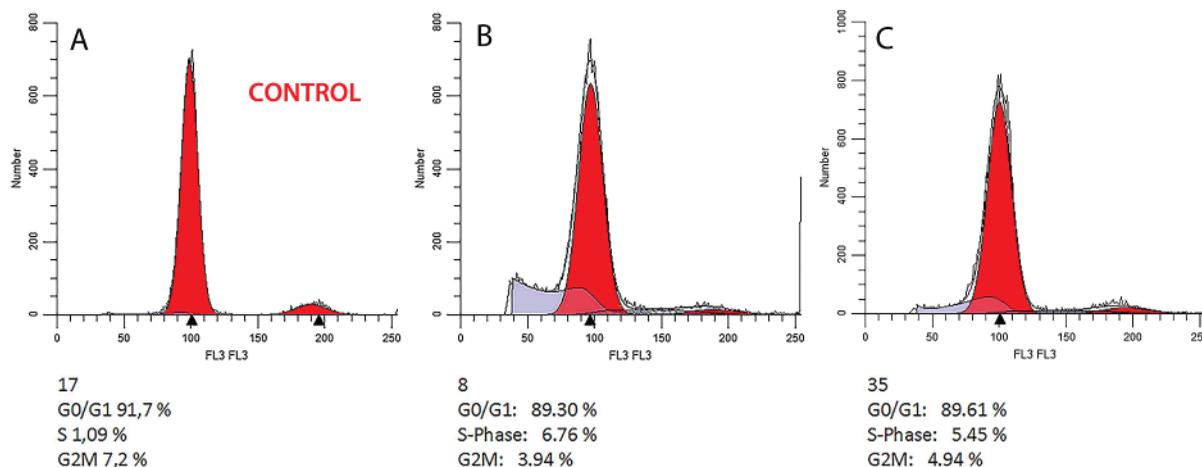


Fig. 11. Flow cytometry analysis of *P. minimum* culture at salinities 17 (A, control), 8 (B) and 35 (C); temperature 25 °C; shown are number of cells at different phases of the cell cycle: G0/G1, S and G2/M. Based on data from Skarlato et al. (2018).

the control (Fig. 11). The growing share of cells at the S phase in the population stressed by salinity 8 witnessed for the elevated DNA replication which can be considered as the dinoflagellates' response to stress. The authors believe that the prolonged phase G₁ and very short phase S that are typical for the *P. minimum* mode of life are backing up the fitness of these protists in the changeable environment of the horohalinicum/ecotone region (sensu Basset et al., 2013), since *P. minimum* cells responded to stress by the increased DNA replication in order to survive the critical salinity conditions (Skarlato et al., 2018).

Enhanced DNA and RNA synthesis as reaction to salinity stress

The cells of *P. minimum* stressed by salinity in the above mentioned study demonstrated the largest increase of ³H-thymidine and ³H-uridine incorporation at the horohalinicum conditions (salinity 8), when compared to the control (Fig. 12).

A three-fold increase of ³H-thymidine incorporation by cells stressed by salinity 8 can be viewed as a sign of the elevated DNA synthesis; this result is in agreement with the flow-cytometry data (Fig. 12). These findings were also supported by the data from the experiments with the longer (60 min) exposure to salinity 8, where share of dead cells decreased if compared to the tests with 30 min exposure, most likely being the outcome of the effective cells' response to short-term salinity 8 stress by the elevated DNA synthesis which increased viability of the dinoflagellate population.

The total RNA content in living organisms, including marine unicellular plankton, depends primarily on the ribosome number which is known to correlate with protein synthesis potential and growth rate (Nomura et al., 1984). It has been mentioned elsewhere that RNA concentration in a cell is an indicator of cell functioning and adaptability (Dahlhoff, 2004; Chicharo and Chicharo, 2008; Blazewicz et al., 2013). Thus, a 2-fold increase in ³H-uridine uptake by *P. minimum* stressed by salinity 8, as demonstrated in our study, can be a result of the increased RNA synthesis which, in turn, reflects transcription of genes involved in the maintenance of osmotic homeostasis in the cells, namely: synthesis of osmolites, membrane transport of ions, etc. (Skarlato et al., 2018).

The results described above provided experimental evidence that the effective acclimation expressed in salinity stress-induced RNA and DNA synthesis alterations in *P. minimum* might increase the chances of its adaptation to the environmental fluctuations thus enhancing fitness and competitiveness of these micro-eukaryotes. As shown recently, the ecological niche of *P. minimum* is rather large, and the advanced ecological strategies of this invasive species allowed it outcompeting one of the native congeners, *Prorocentrum balticum*, from the assemblage of the dinoflagellate dominants in the species-rich phytoplankton community of the Baltic Sea during quite a reasonable time span (Telesh et al., 2016). These effective molecular adaptations may play a positive role for *P. minimum* in the highly variable Baltic coastal environment, particularly

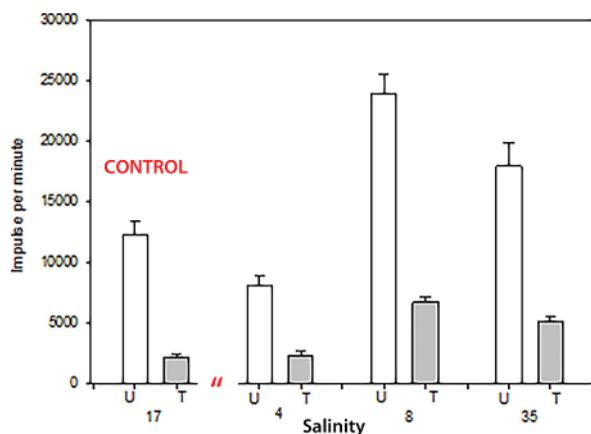


Fig. 12. Uptake (impulse per min, \pm SD) of ^3H -uridine (U) and ^3H -thymidine (T) by *P. minimum* cells stressed by different salinities: 4, 8 and 35 (salinity 17 – control); exposure time 30 min. Based on data from Skarlato et al. (2018).

considering the differentiated reaction of various phytoplankton species to the impacts of physical and chemical parameters, which is best exemplified by the coastal upwelling and/or downwelling events (Lips and Lips, 2010).

It is most likely that the increased RNA and DNA synthesis in the salinity-stressed *P. minimum* cells represents the advanced molecular mechanism to overcome the osmotic stress in the highly unstable critical-salinity environment which is characteristic of the vast coastal and open areas of the Baltic Sea (Schiewer, 2008; Biological Oceanography, 2017). We infer that the effective salinity stress response of *P. minimum*, as demonstrated experimentally (Skarlato et al., 2018), would provide these bloom-forming protists with pronounced ecological benefits in the future decades, in the conditions of the forecasted desalination of the Baltic surface waters (Vuorinen et al., 2015). Salinity decrease, which is often resulting in the increasing nutrient concentrations, is known to be a crucial factor stimulating *P. minimum* blooms (Grzebyk and Berland, 1996). Recent studies have shown that these dinoflagellates are well adjusted to coping with the changeable salinity conditions in the sea coastal waters. This remarkable plasticity, therefore, inevitably yields substantial advantages to the successive generations of these dinoflagellates that can form harmful mahogany tides in the conditions of pronounced anthropogenic impact on the water bodies, in both native and newly colonized aquatic ecosystems.

Temperature stress response

For better understanding of complex processes that define the response of marine planktonic microorganisms to environmental stress, biomarkers of temperature as important characteristics were studied using the dinoflagellates *P. minimum* as a model object (Knyazev et al., 2018). The impact of temperature stress on viability, cell cycle, RNA synthesis and DNA replication in these flagellates was evaluated. It was shown by the flow cytometry method that temperature increase from 25 °C (control) to 37 °C or 42 °C for the time span of 15 to 60 min did not cause any substantial alterations in the cell cycle of these dinoflagellates; meanwhile, their cell mortality increased from $\leq 1\%$ (in the control) to 2-12% at 37 °C and 4-22% at 42 °C. Along with low cell mortality, after temperature was increased to 37 °C and (or) 42 °C, *P. minimum* demonstrated the ability to elevated synthesis of DNA (1.7-1.9 and 1.2-1.6 times higher, respectively) and especially RNA (3.1-2.5 and 2.8-1.7 times higher, respectively) during the first 15-30 min after stress. At certain stages of the life cycle, this effect can be crucial for maintaining the viability and normal development of the *P. minimum* population (Knyazev et al., 2018). The obtained results clearly demonstrated that the significantly elevated synthesis of the nucleic acids can serve as an indicator (biomarker) of the environmental stress.

4.2. MIXOTROPHIC METABOLISM

The relationship between increasing total nutrient loads and increased abundance of harmful algae is now well recognized as an expression of eutrophication (Anderson, 1989; Anderson et al., 2002; Glibert et al., 2005, 2010; Glibert and Burkholder, 2006; Heisler et al., 2008). However, species-specific response is still enigmatic, depending on the physiology of the particular organisms present in the community, environmental conditions and the form of nutrient supplied (Glibert and Burkholder, 2006; Burkholder et al., 2008; Glibert et al., 2012). Global nutrient loads are not only disproportional spatially, but are also changing in stoichiometry and form as land use changes, agricultural and aquacultural practices change, and as human population and its effects increase (e.g., Smil, 2001; Galloway and Cowling, 2002; Galloway et al., 2002; Howarth et al., 2002; Glibert et al., 2010). In particular, elevated levels of dissolved

organic matter (DOM), e.g. organic nitrogenous compounds as part of manure or fertilizers, become more and more common in coastal waters of highly populated regions (Glibert et al., 2005; Harrison et al., 2005; Glibert and Burford, 2017). Such shifts in water chemistry can favor certain groups of phytoplankton, among which dinoflagellates are probably the brightest example.

Many plastid-containing dinoflagellates are in fact mixotrophs, i.e. they are able to utilize not only inorganic but also organic sources of nutrients (Stoecker, 1999; Fan et al., 2003a, 2003b; Burkholder et al., 2008; Jeong et al., 2010; Solomon et al., 2010; Hansen, 2011; Matantseva and Skarlato, 2013). The organic sources available to dinoflagellates are both particulate and dissolved organic matter. The former can be represented by detrital particles and prey cells; in this case, dinoflagellates acquire organics via the process of phagotrophy. There are numerous publications describing phagotrophy in various photosynthetic dinoflagellates, namely the range of prey organisms, mechanisms, and environmental conditions favoring it (Hansen and Calado, 1999; Jeong et al., 1999, 2004, 2005; Kang et al., 2011; Johnson, 2015; Lee et al., 2016). Nevertheless, phagotrophy by primary producers is still poorly accounted in environmental studies, since our current knowledge does not allow the accurate quantification of this process *in situ*, especially under quickly changing environmental conditions (Flynn et al., 2018).

Uptake and utilization of dissolved organic substrates is referred to as osmotrophy, and this strategy seems to be common among different phytoplankton species, including dinoflagellates (Berman and Chava, 1999; Berman and Bronk, 2003; Glibert and Legrand, 2006; Solomon and Glibert, 2008; Sinclair et al., 2009; Jauzein et al., 2011). Often osmotrophy serves to supply nitrogen, an element essential to all living organisms and limiting in many marine habitats; therefore, nitrogenous organic compounds has been under scrutiny for a long time (Bronk et al., 2007). In multiple studies, it was demonstrated that dinoflagellates readily consume such nitrogen-containing substances as urea and dissolved free amino acids, often in preference to inorganic nitrate and ammonium (John and Flynn, 1999; Kudela and Cochlan, 2000; Collos et al., 2004; Li et al., 2009, 2010; Solomon et al., 2010; Jing et al., 2017).

The dinoflagellate *P. minimum* causing harmful algal blooms worldwide is an ecologically successful

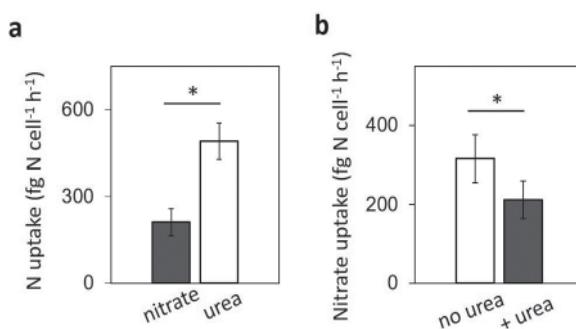


Fig. 13. Urea and nitrate uptake by *P. minimum*. a – Concurrent uptake of nitrate and urea; b – nitrate uptake in the absence and presence of urea. Asterisks indicate significant difference ($p < 0.05$, $n = 6$). Based on data from Matantseva et al. (2016).

mixotroph capable of both phago- and osmotrophy (Stoecker et al., 1997; Heil et al., 2005). According to a recent investigation, phagotrophy in this organism can be an important source of carbon, nitrogen and phosphorus, is induced by phosphorus limitation and characterized by prey species selectivity (Johnson, 2015). Osmotrophy in *P. minimum* is more extensively studied. Both field and laboratory populations of this species possess molecular mechanisms to consume urea (Fan et al., 2003a, b; Fan and Glibert, 2005; Solomon and Glibert, 2008; Ou et al., 2014) and amino acids (Fan and Glibert, 2005; Glibert et al., 2012). It was shown that *P. minimum* cells took up urea at the rate two folds exceeding that of the concurrent nitrate uptake when both nutrients were supplied at the same high concentrations ($200 \mu\text{M N}$). Interestingly, in the presence of urea the nitrate uptake by this species was 30–40% suppressed (Matantseva et al., 2016) (Fig. 13). A similar result was obtained for the concurrent uptake of nitrate and glycine. The rate of glycine uptake by the dinoflagellates was two times higher than the rate of nitrate uptake, but glycine did not have an inhibitory effect on the latter (Matantseva et al., 2018) (Fig. 14).

Both urea and glycine potentially can be used as carbon sources. However, in the laboratory experiments with *P. minimum* cultured under nitrogen replete conditions, consumption of carbon from these compounds was disproportionately low compared to the consumption of nitrogen from the same sources and did not exceed 1.5% of the total carbon uptake (mainly in the form of inorganic carbon) by dinoflagellates. Such minute levels of organic carbon assimilation were explained by the metabolic processing of urea and glycine in a cell (Matantseva et al., 2018).

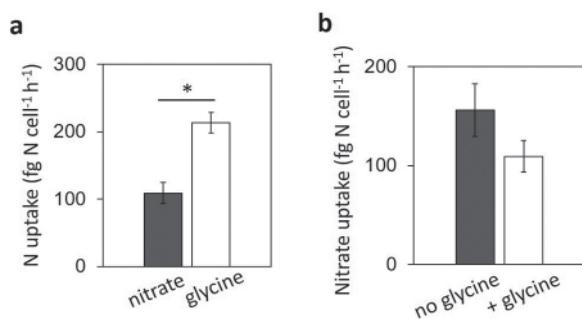


Fig. 14. Glycine and nitrate uptake by *P. minimum*. a – Concurrent uptake of nitrate and glycine; b – nitrate uptake in the absence and presence of glycine. Asterisk indicates significant difference ($p < 0.05$, $n = 3$). Based on data from Matantseva et al. (2018).

The analysis of the *P. minimum* transcriptomes demonstrated that this species has genes encoding urease and the proteins of glycine decarboxylation complex. The activity of these enzymes leads to a release of carbon originating from urea and glycine in the form of carbon dioxide (bicarbonate) which can easily escape from the cell and mix with the huge pool of inorganic carbon in the environment (Matantseva et al., 2018). On the one hand, the described observations are supported by the field data of Fan and Glibert (2005) who demonstrated that carbon uptake from urea was less than 1% of the inorganic carbon uptake by the dinoflagellates *P. minimum* during their bloom. On the other hand, the recent study of natural dinoflagellate blooms showed that assimilation of organic carbon from urea and dissolved free amino acids was much more substantial and contributed a lot to the *P. minimum* carbon demand (Mulholland et al., 2018). Apparently, environmental conditions, e.g. light and nutrient availability, affect the level of organic carbon utilization by dinoflagellates, and more experimental data are needed to understand these interrelationships.

Previously it was assumed that mixotrophy was induced under conditions impeding effective photosynthesis, for example, in response to low inorganic nutrient concentrations. However, there are evidences for mixotrophy in eutrophied, nutrient-rich habitats (Adolf et al., 2008; Burkholder et al., 2008). In such habitats, versatility of their metabolism allows mixotrophs to obtain nutrients from plenty of alternative sources, store nutrients available in excess, or balance the elemental stoichiometry of nutrient uptake and biomass; this can result in the prolongation of blooming events

(Glibert et al., 2018). The dinoflagellate *P. minimum* has a great potential for mixotrophy, which is a key to understand its success in eutrophied coastal regions experiencing drastic quantitative and qualitative fluctuations of nutrient loads (Cecchi et al., 2016).

4.3. INTRAPOPULATION HETEROGENEITY AND ITS RELEVANCE TO ECOLOGICAL MODELLING

In the last decades, newly developed research techniques allowing studies at the single-cell level were applied in different fields of microbial ecology and ecophysiology (Brehm-Stecher and Johnson, 2004; Popa et al., 2007; Musat et al., 2008, 2012; Matantseva and Skarlato, 2015). One of the most enlightening results of this methodological revolution was the finding that a cellular population can be substantially heterogeneous, with distinct cells expressing various traits (Altschuler and Wu, 2010; Kreft et al., 2013). Most published single-cell studies were focused on the physiological variability within populations of bacteria and classical model organisms, such as *Saccharomyces cerevisiae* (Acar et al., 2008; Ackermann, 2015; Gao et al., 2016), while we still have very limited information about this phenomenon in planktonic protists. In particular, it is not clear whether such intrapopulation heterogeneity is typical for dinoflagellates. Nevertheless, scarce data available to date imply that it can be functionally important for their populations.

It was shown that nutrient status, i.e. cell quota of nitrogen, phosphorus and carbon, varied among individual cells of natural population of the dinoflagellate *Dinophysis norvegica* from the Baltic Sea (Gisselson et al., 2001). Furthermore, by means of the nanoscale secondary ion mass spectrometry (NanoSIMS) laboratory populations of the dinoflagellate *P. minimum* were found to be heterogeneous in terms of nitrogen and carbon uptake rates (Matantseva et al., 2016). The rates of uptake of the same nutrient (nitrate, urea, or bicarbonate) by distinct cells significantly differed, and the range of the revealed variability was up to 10-folds. Remarkably, whereas most cells in the population used nitrogen of both nitrate and urea present in the medium, a substantial fraction of individual dinoflagellates consumed exclusively urea-N. These data indicated the existence of functionally diverse sub-populations exploiting different nutritional strategies (Matantseva et al., 2016).

The revealed cell-to-cell variability likely provides a serious competitive advantage for dinoflagellates. Variability represents an effective mechanism to maintain microbial populations under changing environmental conditions and ensures their survival even following the exposure to severe stress (Booth, 2002; Kussel and Leiber, 2005). In the recent experiments, it was demonstrated that the level of heterogeneity within populations of the bacteria *Klebsiella oxytoca* and green algae *Chlamydomonas reinhardtii* increased in response to nitrogen limitation, which implies that heterogeneity may represent an adaptive mechanism (Krismer et al., 2016; Schreiber et al., 2016). Intrapopulation heterogeneity allows the more effective use of available resources and alleviates intrapopulation competition, as shown for *P. minimum* (Matantseva et al., 2016). Moreover, theoretical investigations provide evidences for the importance of individual variability for the interspecific competition and niche differentiation, especially in the case of asexually reproducing organisms (Menden-Deuer and Rowlett, 2014, 2018). Thus, cell-to-cell nutritional variability of *P. minimum* populations is another proof for the enhanced competitive ability of this species, and it can back up its rather large ecological niche dimensions and the advanced invasive potential in the variable marine coastal environments (Telesh et al., 2016).

Moreover, intrapopulation heterogeneity is likely very important from the standpoint of ecological modelling. A population response to a certain environmental factor is not a sum of equal responses of individual cells, but rather represents a sum of various individual responses. This fact should not be ignored during simulation of the population growth of microorganisms in modelling. Under the same conditions homogeneous and heterogeneous populations (Fig. 15) will probably have different dynamics, because each of the functional sub-populations will be characterized by its own, specific growth rate.

The need in individual-based modelling in microbial research has already been discussed in the literature (Kreft et al., 2013; Hellweger et al., 2016), but it has not yet been widely applied in practice, especially in modelling of dinoflagellate populations and harmful algal blooms (Glibert et al., 2010; Flynn and McGillicuddy, 2018). More single-cell data describing heterogeneity of natural and laboratory microbial populations are required to ensure reliable ecological modelling in a changing

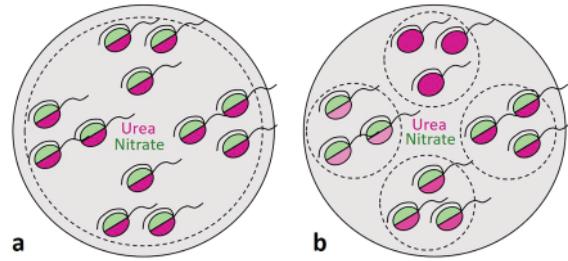


Fig. 15. Homogeneous and heterogeneous populations of *P. minimum* in respect to urea and nitrate uptake (according to Matantseva et al., 2016). In a homogeneous population (a), all cells are functionally equal. In a heterogeneous population (b), several functional sub-populations characterized by various nutritional strategies are present. Growth rate of the entire population should represent a sum of the growth rates of distinct functional sub-populations encircled by dashed lines. Magenta colour represents urea uptake, green – nitrate uptake. Lighter shades reflect lower uptake rates, darker shades – higher uptake rates.

world, which is one of the main research directions of the near future.

5. Cell and molecular biology of dinoflagellates: implications for biotechnology and environmental management

5.1. STRESS-INDUCED GENE EXPRESSION

Albeit dinoflagellates are a group of extremely high ecological relevance, information concerning their cell biology and physiology is still limited. Compared to cell biology of the animal cells, or cells of some parasitic protists of high medical importance, the knowledge about functioning of the dinoflagellate cells is rather modest. However, data obtained using the approaches of cell and molecular biology can significantly improve the understanding of fundamental aspects of the dinoflagellate ecology and provide new instruments for biomedicine, biotechnology and environmental management.

One of the topical questions related to unraveling of cell physiology is the studies of changes at the level of expression of various genes in response to environmental stimuli. The widely applied approaches to investigate gene expression at the transcriptional level are high-throughput RNA

sequencing and RT-qPCR (reverse transcription quantitative PCR). These approaches allowed determination of genes regulated by light, nutrient limitation, growth stage, etc. in different dinoflagellate species (reviewed by Morse et al., 2018). Compared to the analysis of transcriptomic information, RT-qPCR remains a method of choice when fine regulation of certain metabolic or signaling pathways has to be elucidated. For instance, by means of this technique it was demonstrated that expression of genes involved in nutrient acquisition is regulated by nutrient availability. The transcription level of the enzyme alkaline phosphatase was higher under phosphate-depleted conditions and repressed following phosphate resupply in the dinoflagellate *Amphidinium carterae* (Lin et al., 2011). Genes encoding the nitrate transporter, urease and nickel transporters in *Prorocentrum donghaiense* were up-regulated in both nitrate- and urea-limited cultures, whereas transcription of the urea transporter mainly depended on the availability of urea (Jing et al., 2017).

Some further steps towards understanding of differential gene expression in *P. minimum* have been made recently. Several reference housekeeping genes required for normalization of the RT-qPCR data were tested, and the genes encoding α -tubulin and malate dehydrogenase were validated as most reliable for this purpose (Guo and Ki, 2012a). Subsequently, it was shown that expression of heat shock proteins HSP90 and HSP70 increased under thermal stress and high concentration of copper in the environment (Guo and Ki, 2012b; Guo et al., 2012). Furthermore, Guo and co-authors (2016) used microarray analysis to identify 515 genes involved in the cellular response to the copper stress. Moreover, recently primers targeting urea and nitrate transporters of *P. minimum* suitable for RT-qPCR were developed (Pechkovskaya et al., 2017) and can be now used to clarify regulation of their expression in this organism as it was done for other dinoflagellate species.

Gene expression can be studied not only at the transcriptional level, but also at the translational (protein) level, and the latter investigations are especially important since they better reflect the actual physiological activity of cells. Such methods as protein electrophoresis, immunoblotting, two-dimensional protein electrophoresis and even protein sequencing have been successfully applied to dinoflagellates (Wang et al., 2014; Morse et al., 2018). Akimoto et al. (2004) analyzed protein

expression profiles of *Lingulodinium polyedrum* and found 28 proteins controlled by the circadian rhythms, some of which were identified. Eleven years later Zhang and colleagues (2015b) applied high-throughput proteomic approach and were able to identify over 3000 proteins; 185 of those were differentially displayed in toxin-producing and non-toxic cultures of *Alexandrium catenella*. In *Prorocentrum donghaiense*, proteins participating in nitrogen transport and metabolism were shown to be up-regulated in nitrogen-deplete medium, whereas proteins responsible for carbon fixation, protein and lipid biosynthesis were down-regulated under the same conditions (Zhang et al., 2015a). Remarkably, the nitrate transporter NRT2.1 appeared to be constitutively expressed in *Lingulodinium polyedrum*: its level did not depend on the nitrogen availability, which provided additional evidence for the species-specific gene expression patterns among dinoflagellates (Bellefeuille and Morse, 2016).

5.2. CELL COVERINGS AND CYTOSKELETON

It should be highlighted that not only the amount of certain proteins, but also their spatial distribution in a cell is functionally important. This is especially relevant in the case of dinoflagellates that possess very complex cell covering (Pozdnyakov and Skarlato, 2012). Dinoflagellate cell covering (amphiesma) places restrictions on physiology of these microorganisms, e.g. on their nutrition (Schnepf and Elbrächter, 1992). This idea was further developed by Kalinina et al. (2018), who suggested several potential scenarios for nutrient transporter localization in the amphiesma, including their presence in the regions of sutures between amphiesmal vesicles, the region of flagellar canal and pusule, as well as duplication of the nutrient transporter set in the plasma membrane and membranes of amphiesmal vesicles (see Fig. 1 in Kalinina et al., 2018). The actual distribution of proteins in a cell can be elucidated by immunocytochemistry (protein labeling with specific antibodies), which will definitely represent one of the key directions of dinoflagellate research in the coming years.

Usage of fluorescent staining and immunocytochemical methods for studying the dinoflagellate cells opens a number of pathways for not only elucidating their cell organization but also unveiling mechanisms underlying different cellular processes. However, in this regard intricacy of dinoflagellates

as an object for cell biology studies should be mentioned. Their complex cell coverings act as a barrier to penetration of reagents. Besides, most dinoflagellates demonstrate highly intense pigment autofluorescence (Tang and Dobbs, 2007). To ensure the effective application of microscopical methods, special protocols should be applied (e.g., Lin and Carpenter, 1996; Soyer-Gobillard, 1996; Tang and Dobbs, 2007; Heimann et al., 2009; Berdieva et al., 2018).

Cytoskeleton is dynamic complex machinery that maintains cell shape and integrity and provides numerous vital functions. In the case of dinoflagellates, for a long time the main attention of researchers has been focused on the exploration of the microtubular systems (Brown et al., 1988; Roberts et al., 1988, 1992; Roberts and Roberts, 1991; Perret et al., 1993; Okamoto and Keeling, 2014). These protists possess two flagella – transverse and longitudinal – with associated roots. The microtubules beneath amphiesmal vesicles form the cortical cytoskeleton in the cells of most dinoflagellates (Morrill and Loeblich, 1983; Roberts et al., 1992).

Modern studies that aim at exploring the organization and functioning of actin cytoskeleton emphasize its involvement in various processes in the dinoflagellate cells. Application of immunocytochemical and fluorescent staining approaches has allowed showing that filamentous actin forms dense network in the cytoplasm of some species from different groups of these protists (Soyer-Gobillard et al., 1996; Heimann et al., 2009; Villanueva et al., 2014; Stires and Latz, 2017; Berdieva et al., 2018). This network could be more diffuse and loose, as in the cases of *Lingulodinium polyedra*, *Symbiodinium kawagutii*, *Crypthecodinium cohnii*, *Prorocentrum micans* and *P. minimum*, or well-defined as in *Pyrocystis lunula* cells.

The species of the genus *Prorocentrum* are characterized by the peculiar cell architecture, which is determined by their cell cover that distinguishes them from the other “core” dinoflagellate groups. They have no cortical microtubules, as has been demonstrated for *P. micans* and *P. minimum* by using immunocytochemical and electron-microscopical methods (Schnepf et al., 1990; Berdieva et al., 2018). Actin cytoskeleton, by contrast, is well developed in their cells (Soyer-Gobillard et al., 1996; Berdieva et al., 2018). In *P. minimum*, F-actin forms a dense layer in the cortical cytoplasm and concentrates in the region adjacent to the nucleus (Fig. 16) (Ber-

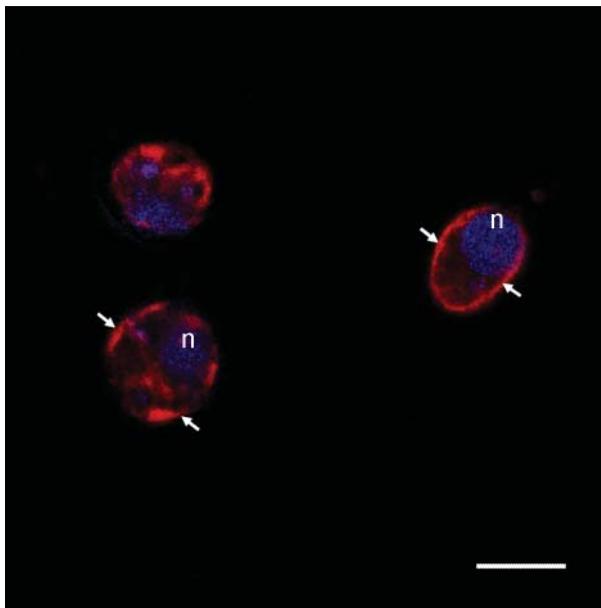


Fig. 16. Filamentous actin distribution in *Prorocentrum minimum* cells. Cortical actin layer is indicated by arrows. n – nucleus (DAPI staining). Scale bar: 10 μ m. Based on data from Berdieva et al. (2018).

dieva et al., 2018). It can be assumed that actin cytoskeleton likely performs some functions of the cortical microtubular system in their cells.

Among the basic actin functions in the dinoflagellate cells, researchers define its participation in completion of cytokinesis and involvement in the morphogenesis of amphiesma in dividing cells, positioning of the nucleus and possible participation in the orientation of the nucleus division (Schnepf, 1988; Soyer-Gobillard et al., 1996). Moreover, recent studies have revealed importance of actin cytoskeleton for the cells ‘proper’ response to stress. For example, intensity of bioluminescence, which occurs as rapid reaction to mechanical stress, decreased significantly after microtubular and actin cytoskeleton disruption was induced by pharmacological treatment in *Pyrocystis lunula* and *Lingulodinium polyedra* cells (Heimann et al., 2009; Stires and Latz, 2017). In the latter case, the leading role in this process was attributed to actin (Stires and Latz, 2017). The authors considered different variants of actin participation in providing the mechanosensitivity of a cell: from translocation of bioluminescence-specialized organelles to interacting with mechanotransducers in the plasma membrane (Stires and Latz, 2017).

For *Prorocentrum minimum* cells, mechanical impact was demonstrated to induce ecdysis, which is a process of cell cover shedding (Pozdnyakov et al., 2014; Berdieva et al., 2016). Ecdysis is a type of the dinoflagellate response to stress: a cell discards components of the old amphiesma, thus entering a stage of temporal (or “ecdysal”, “pellicle”) cyst, and then forms a new amphiesma, reverting to normal state (Bravo and Figueroa, 2014). Filamentous actin turns out to be involved in the ecdysis process.

Studies of the cytoskeleton in *P. minimum* cells revealed that pharmacological treatment with latrunculin B decreased the level of stress-induced ecdysis to the minimum (Berdieva et al., 2018). The possible mechanisms whereby actin may participate in this process are (1) the direct contractile action, which sheds cell cover, or (2) the involvement in the formation of a new amphiesma (Berdieva et al., 2018).

In addition, actin polymerization may affect ecdysis by regulation of calcium release from endoplasmic reticulum stores in cells. Formation of a pellicle cyst in response to elevation of intracellular calcium ion concentration was demonstrated for the dinoflagellates *Alexandrium catenella* and *C. cohnii* (Tsim et al., 1997). Besides, taking into account the above data on actin involvement in the cell mechanosensitivity, it can be hypothesized that interaction of actin with the membrane mechanotransducers may be essential for the signal transduction leading to ecdysis.

5.3. CHROMOSOME STRUCTURE

Another key method to explore cellular reactions to environmental factors is electron microscopy. In the study of *P. minimum* cells stressed by salinity their fine structure was investigated and curious alterations in the chromosomes ultrastructure were revealed (Skarlato et al., 2018). In the control series and experiments after salinity increase, several patterns of the chromosome fine structure in *P. minimum* cells were observed; they differed within the normal variation range. As shown earlier, alterations in the overall chromosome structure and the degree of condensation may occur throughout the cell cycle as a result of their activity (Sigeo, 1986; Bhaud et al., 2000). However, short-term salinity stress during 30 min caused evident and unusual but reversible changes of the chromosome fine structure at salinities 4 and 8: chromosome banding pattern was disrupted and electron-transparent spaces

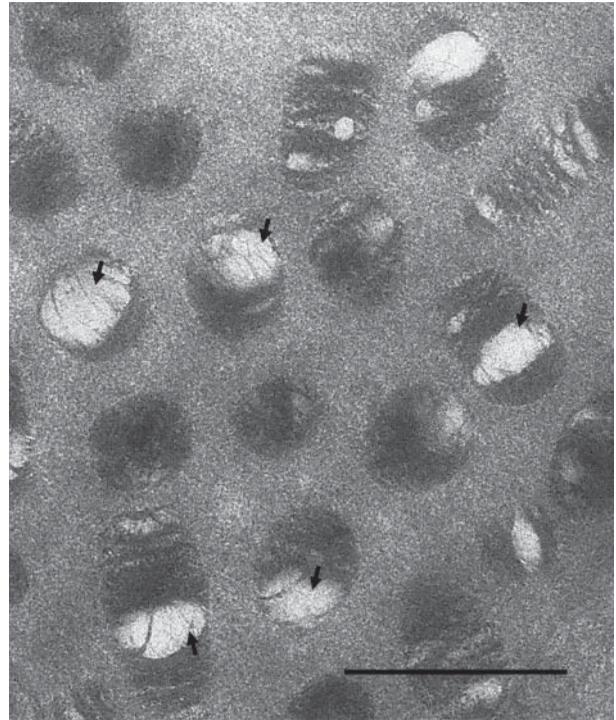


Fig. 17. Alterations in *Prorocentrum minimum* chromosome ultrastructure after low salinity treatment. Chromosome banding pattern is disrupted and electron-transparent spaces (arrows) between fibrils arise locally. Scale bar: 1 μ m. Based on data in Skarlato et al. (2018).

between fibrils arose locally (Fig. 17). Transfer of cells back into the original conditions ensured recovering of chromosome organization patterns (see Fig. 5 in Skarlato et al., 2018).

Revealing correlation between alterations in the chromosome organization and the subsequent surge in replicative and transcriptional activity can be a promising tool for future research. Meanwhile, the direct impact of changing ion concentration in the medium on the pattern of chromosome organization should not be ignored. The obtained results appeared to be similar to the effects of hypotonic treatment on the mammalian mitotic chromosomes (Zatsepin et al., 1989). However, strict differentiation in decondensation/condensation pattern was not revealed so far, and the character of decondensation was absolutely peculiar, which is apparently due to the uniqueness of the dinoflagellate chromosome structure.

Interestingly enough, the dinoflagellate chromosomal apparatus is still a hot topic for research. They have no the “beads-on-string” level of DNA

compaction; DNA fibers are packaged in a special way (Rizzo and Burghardt, 1980; Fukuda and Suzaki, 2015). Several models of the chromosome architecture have been proposed for dinoflagellates – from the toroidal bundle of chromatin filaments to the cholesteric liquid crystal model that is currently more (though not completely) accepted by researchers (Fukuda and Suzaki, 2015). Obviously, the molecular composition of the dinoflagellate chromosome affects its structural characteristics. Dinoflagellates have no typical core histones; the latter were replaced by special proteins (Dinoflagellate/Viral NucleoProteins, or DVNPs, and Histone-Like Proteins, or HLPs) in the course of evolution (Taroncher-Oldenburg and Anderson, 2000; Chudnovsky et al., 2002; Gornik et al., 2012; Irwin et al., 2018). Despite the detection of the core histones and histone-modifying proteins' sequences in the transcriptome of the dinoflagellate *Lingulodinium* sp., their proposed roles might only include a contribution to the DNA repair pathway and transcriptional regulation (Fukuda and Suzaki, 2015). New results of molecular studies in combination with modern electron-microscopic investigations should resolve the organization, functioning and possible evolution of this unique chromosomal apparatus. These data will serve a foundation for the future molecular investigations of dinoflagellates.

5.4. ION CHANNELS

Most physiological reactions of a cell directly or indirectly lead to the opening or closure of membrane protein pore complexes, ion channels. These channels are involved in signal transduction, cell motility and proliferation, gene expression, apoptosis, etc. (Hille, 2001; Schubert et al., 2017). The obvious biomedical significance of these molecular complexes has led to a strong prevalence of studies on the ion channels in multicellular organisms (animals). Nevertheless, evidence has recently emerged that the study of ion channels of the ecologically relevant protists is also of great importance for understanding the behavior of these organisms in a changing world (Taylor and Brownlee, 2003; Taylor et al., 2011; McLachlan et al., 2012; Echevarria et al., 2016). However, despite dinoflagellates represent one of the most productive groups of phototrophs in the ocean and many of them cause harm to aquatic biota, aquaculture, fisheries and human health due to production of

toxins or harmful algal blooms, their ion channels still remain virtually unexplored.

Two main reasons explain a small amount of data on the ion channels of dinoflagellates. First, there are almost no genomic data available. Due to the huge genome sizes in many dinoflagellate species, the fully sequenced genomes of free-living dinoflagellates are still negligible. Nowadays, the genome of the endosymbiotic algae *Symbiodinium kawagutii* is so far the only one sequenced dinoflagellate genome (Lin et al., 2015). Second, many electrophysiological techniques, such as single- or two-electrode voltage-clamp as well as patch-clamp, are difficult to apply to study ion channels in these organisms due to their complex cell covering and motility.

The first obstacle can be partially overcome with the help of transcriptomic data. The recently published Marine Microbial Eukaryote Transcriptome Sequencing Project (Keeling et al., 2014) contains 30 transcriptomes of about 20 dinoflagellate species. Analysis of *P. minimum* transcriptomes revealed homologs of ion channels from voltage-gated cation channel superfamily, i.e. voltage-gated potassium channels, inwardly rectifying potassium channels, calcium-activated potassium channels, cyclic nucleotide-gated cation channels, TRP channels, two-pore calcium channels, four-domain channels, and voltage-gated proton channels (Pozdnyakov and Skarlato, 2015). The transcriptome analysis of many dinoflagellate species demonstrated a wide distribution of voltage-gated proton channels among dinoflagellates (Kigundu et al., 2018). Bioinformatic and phylogenetic analysis of the dinoflagellate four-domain channels showed that (1) dinoflagellates possess their own clades of these channels, that cluster with neither voltage-gated sodium nor voltage-gated calcium channels of animals, and (2) these channels are probably calcium-permeable and calmodulin-regulated ones (Pozdnyakov and Skarlato, 2016; Pozdnyakov et al., 2018).

The second methodological problem can be solved in three different ways. One way is to use an appropriate model organism. For example, *Noctiluca miliaris* has the characters of an appropriate model object for such studies since it is: (1) naked, i.e. does not contain the cellulosic thecal plates in its cell covering; (2) almost non-motile; (3) large: its cells can reach ca. 2 mm in diameter. It is not surprising therefore that two-electrode voltage-clamp was successfully applied to this dinoflagellate (Eckert and Sibaoka, 1967, 1968; Nawata and

Sibaoka, 1979; Oami et al., 1988, 1990, 1995). The main results of these studies were the discovery of two species of action potentials regulating tentacle contraction and bioluminescence activation, as well as a description of depolarization-activated proton currents, Ca²⁺-dependent depolarization-activated sodium currents, and hyperpolarization-activated chlorine currents.

Nevertheless, most of the dinoflagellate species do not possess such features as *Noctiluca*: they are motile and/or armored, i.e. contain rigid thecal plates in cortical alveoli (Pozdnyakov and Skarlato, 2012). Hence, special methods are needed to modify the cell surface which is initially unsuitable for electrophysiological techniques. The common way to study ion channels of a cell with rigid cell covering such as cells of plants, algae, fungi, and bacteria, is obtaining spheroplasts (Martinac et al., 2008).

Recently, a new approach to obtain spheroplasts of armored dinoflagellate *P. minimum* has been proposed (Pozdnyakov et al., 2014). This approach is based on the action of 2,6-dichlorobenzonitrile, a cellulose synthesis inhibitor, on a dinoflagellate cell. It was shown that this compound not only inhibits synthesis of new thecal plates, but also induces discarding of the already existing theca (Fig. 18). The patch-clamp experiments with such *P. minimum* spheroplasts for the first time demonstrated single-channel activity of the dinoflagellate ion channels (Pozdnyakov et al., 2014).

Since the investigation of dinoflagellate ion channels *in situ* remains tricky, there is still another way for electrophysiological studies of these organisms – an expression of the known ion channel gene in a heterologous system such as frog oocytes or cell cultures of vertebrates. Recently, such an approach was applied to study voltage-gated proton channels of *Karlodinium veneficum* (Smith et al., 2011) and *Lingulodinium polyedra* (Rodriguez et al., 2017), by means of patch-clamp technique expressing them in HEK293 or COS-7 cells. Moreover, Smith and colleges (2011) using mutagenesis analysis identified the selectivity filter of *K. veneficum* proton channel as Asp⁵¹.

Overall, the use of bioinformatics and experimental approaches to study ion channels of dinoflagellates has provided new information about their structural and functional features. The increased interest in the dinoflagellate ion channels in the last decade suggests further data accumulation in the nearest coming years.

5.5. PRACTICAL USE OF THE CELLULAR AND MOLECULAR DATA

Studies of cellular and molecular mechanisms underlying the physiology of the dinoflagellate cells are necessary to understand their ecological success. Dinoflagellates are constantly experiencing different (often stressful) effects in natural conditions – fluctuating temperature, shifts in nutrient concentration, mechanical impact (wind-induced disturbances of the upper water layer, such as vertical mixing and turbulence), etc. The efficiency of their response to environmental disturbances is defined by changes in gene expression that ensure adequate adaptation and survival of the cells in the unfavorable conditions. In addition to survival in the active state, some species appear to use such cellular response as formation of temporal (ecdysal) cysts. Besides, in some cases temporal cysts can be considered as a promising mechanism to control dinoflagellate propagation. It was also suggested that accumulation of cysts facilitates bloom development (Bravo and Figueroa, 2014).

Moreover, the data concerning cell and molecular biology of dinoflagellates open new horizons in the ecological modelling aimed at forecasting and management of the environmental processes, as well as in biotechnology. Dinoflagellates represent the highest number of toxin-producing species among the marine eukaryotic phytoplankton (Cembella, 2003). Many of their toxins are considered as main components of therapeutic drugs used to treat serious diseases such as schizophrenia, drug addiction, mycosis and cancer (Camacho et al., 2007). Results of physiological studies of dinoflagellates can shed light on cellular and molecular mechanisms controlling production of bioactive compounds and therefore promote the use of dinoflagellates in biomedicine and biotechnology in the nearest perspective.

6. Outlook: Future challenges and perspectives

Despite the world-wide distribution of the potentially toxic, mixotrophic dinoflagellates *Prorocentrum minimum* and the multiple repeated surveys of their harmful blooms in different regions of the globe, the accurate forecast of the timing and intensity of these events remain problematic. The bloom-forming capacities of *P. minimum* in the brackish waters and its potential toxicity in the

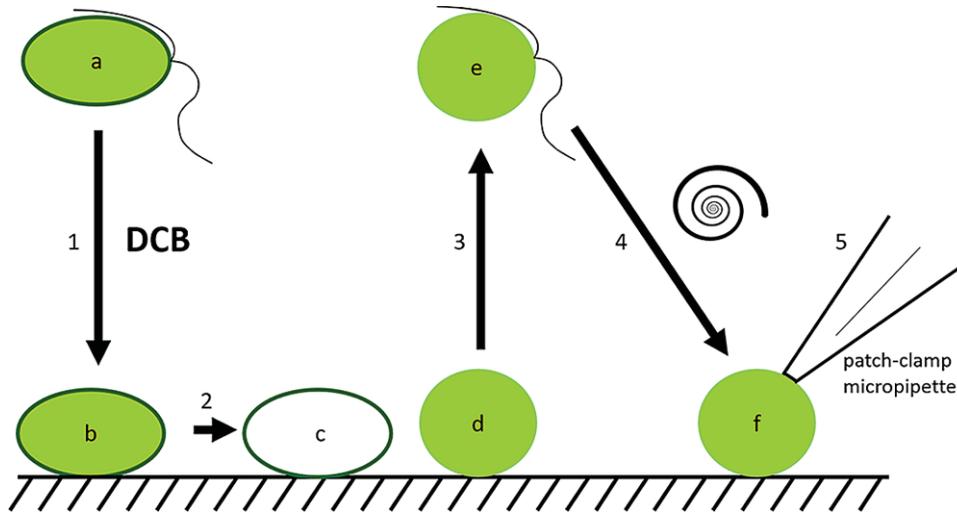


Fig. 18. Obtaining spheroplasts of *Prorocentrum minimum*. 1. Application of 2,6-dichlorobenzonitrile (DCB) induces ecdysis of a vegetative motile cell (a) which is started from discarding of flagella and formation of a temporary cyst (b). 2. The second stage of ecdysis – discarding of theca (c) and formation of a spheroplast (d), in which synthesis of new thecal plates is inhibited by DCB. 3. DCB-induced spheroplast forms new flagella and becomes motile (e). 4. After centrifugation motile spheroplast becomes non-motile (f). 5. Such spheroplasts can be used in patch-clamp experiments. According to Pozdnyakov et al., 2014.

relatively unstable environment, as exemplified by the Baltic coastal regions, are largely understudied. Meanwhile, the projected decrease of the surface water salinity and increase of temperature and nutrient concentrations in this sea (Rajasilta et al., 2014; Vuorinen et al., 2015) will highly likely result in the shifts in species distribution, abundance and overall diversity (Telesh et al., 2015; Vuorinen et al., 2015), increased intensity and frequency of algal (including harmful algae) blooms and the consequent reduction of the diatoms/dinoflagellates biomass ratio during the blooms (Wasmund et al., 2008; Olenina et al., 2010). A niche modelling example based on nutrients, temperature and salinity conditions forecast significant increase in potential habitat suitability for the harmful algal blooms of the *Prorocentrum* species under climate change (Glibert et al., 2014). Moreover, as suggested recently, in the future the decline of salinity and the elevated nutrient loads in the Baltic Sea may result in the prolonged duration of *P. minimum* presence in the plankton, thus causing longer periods of algal blooms (Olenina et al., 2016). These perspectives appeal to the necessity of better understanding the reasons of the peculiar invasive history and population dynamics of the potentially harmful dinoflagellates *P. minimum* in the conditions of varying plankton diversity and altered pelagic community structure in the sea under the environmental change.

The ecosystem resistance to disturbances, including biological invasions, is known to be dependent on species richness (Shea and Chesson, 2002). In this respect, microplankton diversity will play a role in niche formation by *P. minimum* and impact the frequency of its bloom events. The geologically young brackishwater Baltic Sea, commonly named “the sea of invaders” (Leppäkoski and Olenin, 2000), has long been considered a species-poor ecosystem where the general biodiversity is low due to prevailing intermediate salinities. Therefore, the currently ongoing biological invasions of species with a wide range of environmental adaptability are often successful in this waterbody (Olenin, 2005). It can be even hypothesized that the effective range expansion of *P. minimum* to nearly the entire Baltic Sea might have been a result of its colonization of a relatively “empty” niche, as until recently this water basin has been considered a “species-unsaturated ecosystem”. However, our results on the determination of the ecological niche dimensions of *P. minimum* and its niche overlap with the five congeners did not support these suppositions (Telesh et al., 2016). Moreover, the recent findings have shown that the Baltic Sea is not a “species-poor” basin, as it was thought earlier: on the contrary, the overall taxonomic diversity of eukaryotic microplankton and the bacteria in the brackish Baltic waters is unexpectedly high (Mironova et al., 2008, 2009,

2012, 2013, 2014; Herlemann et al., 2011, 2014; Telesh and Heerkloss, 2002, 2004; Telesh et al., 2008, 2009, 2011a, 2011b).

The data on distribution, population dynamics and environmental preferences of *P. minimum* in the Baltic Sea are helpful for understanding the mechanisms underlying the protistan species maximum in the critical salinity zone. At the same time, the high overall microplankton diversity at critical salinities can be considered as a possible reason for the exceptionally prolonged delay in the *P. minimum* population development preceding the first bloom formation after its invasion into the taxonomically diverse and variable brackishwater environment – the Baltic Sea.

For the invasive species, recent niche theory clarifies the prediction that low niche opportunities (i.e. invasion resistance) result from high species diversity (Shea and Chesson, 2002), although linear relationships between biodiversity, ecosystem functioning and stability are highly unlikely (Pinto et al., 2014). However, despite the recently evolved ‘Environmental Knowledge Paradox’, which argues that more detailed knowledge will not necessarily reduce major uncertainties in the environmental decision-making (de Jonge and Giebels, 2015), the exact mechanisms that trigger harmful algal blooms still need to be evaluated and used in prognostic models aimed at assessing ecosystem susceptibility to human pressures. Future progress in defining the mechanisms behind the species invasibility and bloom-forming potential of harmful algae such as some dinoflagellates would benefit from the analytical, experimental and theoretical research into niche dimensions defined by a species’ response and effect at each point in niche space (Leibold, 1995; Chesson, 2000). Coupled with the analyses of the competitive resource utilisation and elimination by grazers, both processes may be viewed as diversity-dependant and equally able to either limit or promote biodiversity of natural communities and thus impact ecosystems’ stability and functioning. Recent studies reviewed here provide valuable contribution to understanding the ecological mechanisms responsible for ecosystem invasibility and frequency of HABs in highly diverse coastal environments worldwide.

The importance of regular validation of the accepted paradigms along with the development of new concepts based on the recent knowledge and/or meta-analyses of the long-term databases, as shown for the Baltic Sea plankton studies (Telesh et al., 2011a, 2011b, 2013, 2015, 2016), is defined not

only by the fundamental value of these concepts for understanding the functions of aquatic ecosystems in the unsteady environmental conditions, but also by their prognostic value in the conditions of the changing climate. For instance, the Baltic Sea surface salinity below 7 is currently recorded from almost 65% of the area of the sea surface (Feistel et al., 2010). However, in the coming decades, according to the model-based predictions, this salinity most likely will be typical for all surface waters in the Baltic Sea if the current climate change trend persists. In this case, the changes in biodiversity, structure, and productivity of the aquatic communities in the Baltic Sea are inevitable due to shifts in distribution ranges of abundant commercial invertebrates and fish species, which may have a negative impact on the economic development in the region (Rajasilta et al., 2014; Vuorinen et al., 2015).

The theoretical and model-based studies have shown that significant spatial heterogeneity of natural communities contributes to a reduction of the probability of large-scale catastrophic alterations in the state of ecosystems, if the local indicators of environmental conditions vary within a smooth gradient of their respective values (van Nes and Scheffer, 2005). Thus, patterns of spatial distribution of the size structure of major primary producers in the communities exposed to the current conditions and variations in the salinity gradient as a result of climate change provide essential information that is required for the accurate predictions of productivity and biodiversity of marine ecosystems, as well as for the effective monitoring of the water quality and the status of aquatic resources (Zingone et al., 2010).

Moreover, the protistan plankton assemblages have a substantial regulatory effect on the global climate change by participating in the biogeochemical cycles that directly depend on the composition of pelagic communities (Winder and Sommer, 2012). In the case of a large-scale variation in climate conditions, the characteristics of the environment will differentially influence various groups of organisms, and the effect of the same factors on organisms of different sizes can even be the opposite. For example, it was found that small-sized species of phytoplankton in the Arctic basin are flourishing, while larger cells are in a depressed state (Li et al., 2009). A reduction of the mean size of an individual in a community due to the increase in the number of individuals in small-sized species as the water temperature rises may be regarded as one of such universal responses to global warming (Daufresne et al., 2009). If the currently observed climatic

trends persist in the future, we may expect larger transformations of food webs due to modifications of the size structure of pelagic communities and the associated redistribution of the carbon flux in aquatic ecosystems (Winder and Sommer, 2012).

In view of these findings, concepts and theories, understanding of the factors and processes that regulate the structure and dynamics of communities of microscopic plankton organisms is essential for predicting the changes in the status and functions of aquatic ecosystems under the conditions of varying external stressors, including the global climate. New discoveries facilitate reassessment of the outdated paradigms and simultaneous development of novel scientific concepts, thus contributing to the formation of modern views in aquatic ecology and development of invasion theory, biodiversity conservation, and effective ecological management aimed at maintaining the sustainable environmental balance.

To resolve the abovementioned uncertainties and develop the reliable methods for predicting HABs of *P. minimum*, control its population density and forecasting the possible future invasions to new recipient water bodies, fundamental investigations of cell biology and physiology of these organisms must be carried out using modern techniques and methodologies, and several preventive environmental policy-making measures should be taken to task. Those are:

(1) Investigating the cellular and molecular adaptation strategies of these protists, including stress-induced gene expression and enhanced DNA/RNA synthesis, mixotrophic metabolism and cell-to-cell variability that enable these dinoflagellates to withstand external stressors and prosper in the fluctuating environment.

(2) Revealing, mapping and modeling the environmental conditions that will favor blooms of *P. minimum*, to be able of forecasting its range expansion.

(3) Maintaining permanent professional monitoring of phytoplankton species composition, nutrient concentrations and physical/chemical parameters in the coastal waters of seas that may be under the risk of potential *P. minimum* invasion, such as the Russian Arctic seas.

To conclude, the reasonable synthesis of modern knowledge of population heterogeneity, invasion potential, cellular and molecular organization, metabolism, and adaptation strategies of the harmful mixotrophic dinoflagellates as response to various

external stresses will contribute substantially to resolving the hottest environmental problems and providing the effective tools for prognostic ecological modeling. Usage of the newest, advanced methodologies, such as single-cell studies, in combination with the classical research techniques and close linkage of cellular biology, molecular ecology and bioinformatics with the ecological theories and practices is a promising perspective for the future studies. This approach is in the mainstream of the forthcoming developments in the Translational Aquatic Ecology – one of the recently emerged scientific disciplines that capitalize on the prompt and smart conversion of the newest basic findings and hot-spot discoveries to their actual practical use in the essential human life activities: fisheries, aquaculture, biotechnology, environmental management and nature protection.

Acknowledgments

The research was funded by the Russian Science Foundation (project 16-14-10116 at the Institute of Cytology RAS). The English language check was made by the “Effective Language Tutoring Services”, Canada.

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