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Review

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Molecular and cellular mechanisms of diatom response to environmental changes

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ABSTRACT. The review is devoted adaptations of diatom under environmental changes and stress factors. Life cycle of diatoms is influenced by various environmental changes, including changes in light intensity, temperature, nutrient availability, and exposure to infochemicals, algicidal bacteria, and viruses. During evolution diatoms have evolved many molecular pathways to respond to stress factors: altering their metabolism to react on abiotic stressors, or producing secondary metabolites to protect themselves from competitors and predators. Prolonged exposure to a stress factor leads to a variety of disorders in cells – oxidative stress, disruption of photosynthesis, mitochondrial function, protein folding, resulting in cell death. It is assumed that these mechanisms have allowed diatoms to adapt to a variety of conditions and successfully exist in ecosystems for many millions of years.

Keywords: phytoplankton, diatoms, programmed cell death, molecular response, acclimation

1. Introduction

Diatoms are unicellular microalgae with a silica shell, often dominating in the composition of phytoplankton in water bodies (Round et al., 1990; Armbrust 2009). They play an important ecological role, providing up to 40% of the primary productivity of the marine environment (Sarhou et al., 2005). These microalgae are distinguished both huge morphological diversity and its ability to survive in various environments. Diatoms often dominate in abundance and biomass in freshwater and in the marine environment, and also live in various extreme conditions – in a wide range of salinity (Ayache et al., 2020), extreme cold water (Popovskaya, 2000) and thermal springs (Delgado et al., 2020). Living in the aquatic environment, diatoms encounter with various environmental changes, to which they need to adapt by changing their metabolism. The key factor to adapt to different conditions is a flexible metabolism that provides a sufficient response to stressful conditions (Fig. 1).

This review considers response mechanisms of diatoms on various environmental influences, including abiotic (changes in lighting, temperature, lack of essential nutrients) and biotic factors (interactions with other diatoms, bacteria and viruses). Special attention is paid to the molecular mechanisms and cellular manifestations of the reaction to these factors, and evidence of programmed cell death in diatoms and the activation of its members (in particular, metacaspases and death specific protein) are considered.

2. Effects of nutrient deficiency on diatoms

The presence of nutrients in the environment is the most common factor that can limit the development of a diatom population in nature. Diatoms require macro-, micronutrients, and vitamins (B1, B7, and B12) to grow (Orefice et al., 2019). Most of the research is related to the availability of silicon, nitrogen, iron and phosphorus, so they are covered in this review. When a necessary element is deficient, diatoms try to compensate for the lack of an element, for example, by replacing proteins that require it. However, if the substance is not supplied in the required amount, serious disorders occur, often associated with a violation of photosynthesis and the respiratory chain, oxidative stress develops and cell death processes are activated.

Silicon

Silicon in the form of silicic acid is a key limiting factor in the growth of diatoms in water bodies and in laboratory culture, as it is necessary for the silica shell formation (Dugdale et al., 1995; Martin-Jézéquel et al., 2000; Wang et al., 2017). At high concentrations of silicic acid, as an uncharged molecule, it can freely diffuse through membranes (Thamatrakoln and Hildebrand, 2008). However, at low concentrations, silicon as silicic acid is transported into the diatom cell from water using silicon transporter proteins (SIT)

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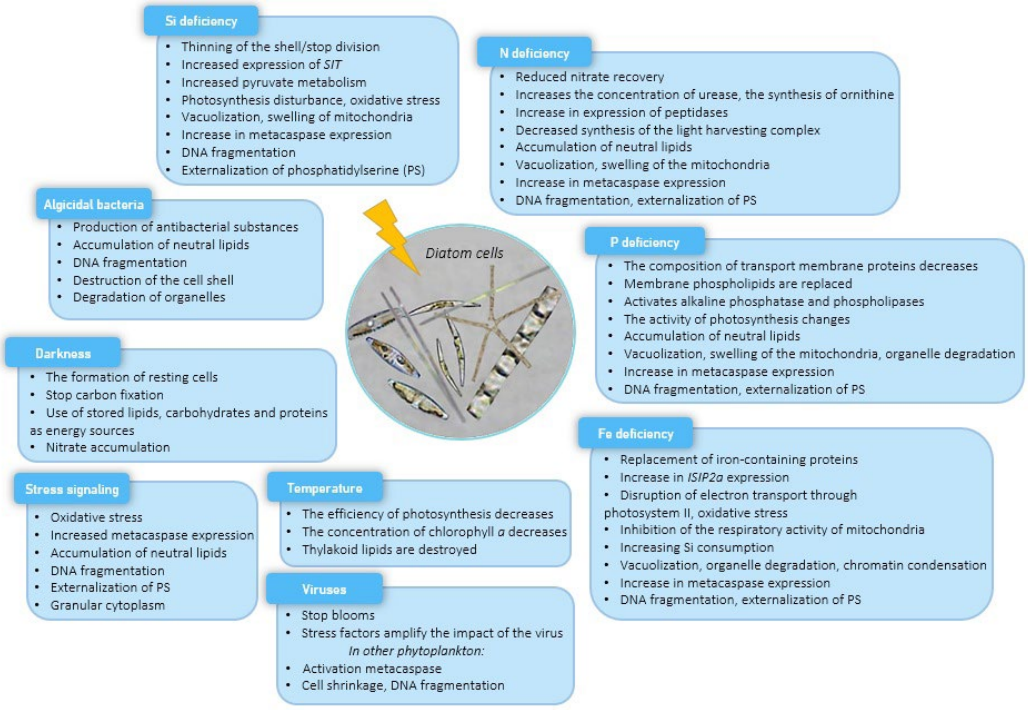

Fig.1. Molecular and cellular changes observed under the influence of various stress factors.

(Hildebrand et al., 1997; Durkin et al., 2016; Knight et al., 2016).

It has been shown that under silicon limitation the diatom cell cycle arrests (Vaulot et al., 1987). When silicon is deficient in diatoms, there are two possible cell cycle arrest points: the G1/S phase (Si is required for DNA replication) and the G2/M phase (Si is required for the formation of new valves) (Vaulot et al., 1987).

First of all, diatom cells adapt to the conditions of silicon deprivation the transport of silicon into the cell. As shown for *Skeletonema marinoi* and *Thalassiosira pseudonana* under these conditions, there is an increased expression of the silicon transporter gene *SIT* (Mock et al., 2008; Wang et al., 2017). Already on the second day of silicon deficiency, the expression of genes involved in antioxidant protection increases (Wang et al., 2017). It is suggested that ROS generation in silicon deficiency may occur due to disturbances in electron transport in the photosynthetic chain of chloroplasts and the respiratory chain of mitochondria (Thangaraj et al., 2019; Bucciarelli and Sunda, 2003) which may be related to various consequences of silicon deficiency. It has been shown that diatoms have extracellular carbonic anhydrase, which catalyzes the formation of CO_2 from HCO_3^- on their surface. It is likely that silica on the diatom cell surface is a buffer for carbonic anhydrase activity, and silicon restriction also entails CO_2 restriction (Milligan and Morel, 2002). Transcriptome analysis during silicon starvation showed an increase in the expression of genes associated with carbon uptake, the glyoxylate cycle, light perception, and pigment metabolism (Sapriel et al. 2009). Also, with silicon deficiency, there is an increase of the conversion of pyruvate to acetyl-CoA, which is used in the Krebs cycle, or for the synthesis of fatty acids (Thangaraj et al., 2019). In addition, researchers note a link between silicic acid and iron metabolism, which is very important for the photosynthetic apparatus (Mock et al., 2008; Sapriel et al. 2009).

Metacaspase expression was shown to increase on the second day of silicon fasting (Wang et al., 2017). Metacaspases are proteases that are analogs of animal caspases (Klemenčič and Funk, 2018). It is known that in diatoms, metacaspases are often associated with cell death, but it is assumed that they can also participate in the processes of adaptation to stressful conditions (Wang et al., 2017, van Creveld et al., 2021, Bidle and Bender 2008).

At the same time, the expression of DSP (death specific protein) in *S. marinoi* also increases on eighth day of silicon deprivation (Orefice et al., 2015). DSP is a diatom-specific protein, the expression of which, on the one hand, increases with cell death, and on the other hand, it seems to be involved in increasing the efficiency of photosynthesis, which is the process of acclimatization (Chung, 2008; Thamatrakoln, 2013).

TEM observations showed that the cells under low silicate conditions were characterized by vacuolization with intact cell membranes and swollen already on day 2 of silicon deficiency and he cells appeared empty on day 7. Staining for externalization of phosphatidylserine (FITC-Annexin-V) and caspase activity (FITC-z-VAD-FMK) was positive after nine days of cultivation, indicating PCD processes (Wang et al., 2017).

Iron

It is known that presence of iron in the environment is critical important for marine diatom survival. Iron is required at relatively higher concentrations per cell than all other metals due to its ability to catalyze redox reactions, transfer electrons, and reversibly bind and thus transfer ligands such as oxygen dioxide. As for autotrophs diatoms needs iron for a number of metabolic processes, including

photosynthesis (photosystems, ferredoxin, cytochrome b6f), the Krebs cycle (cofactor for aconitase, fumarase), and nitrate assimilation (nitrate and nitrite reductase) (Milligan and Harrison, 2000; Behrenfeld and Milligan, 2013). Diatoms have several enzymes to absorb iron – ferric reductase dissociates from organic Fe^{3+} ligands, ferroxidase, which oxidizes Fe^{2+} to Fe^{3+}, and iron permease, which is able to transport Fe^{3+} across the membrane (Gao et al., 2021).

It was shown that cells under the iron deficiency stress use compensatory mechanisms in order to survive. These processes include the replacement of Fe-containing proteins with Fe-independent ones to reduce the need for Fe in cells. For example, Cyt c 553 (iron-containing) is replaced by copper-dependent plastocyanin, and ferredoxin is partially replaced by flavodoxin (non-iron-containing) (Ferreira and Straus, 1994; Mckay et al., 1999). In addition, in the first days of iron limitation, the expression of the ISIP2a protein (Iron Starvation Induced Protein 2a), which accumulates Fe^{3+} on the cell surface, was shown (Morrissey et al., 2015).

Diatoms are able to use the system of iron storage while centric and pennate species differ in ways of iron storage. It was shown that for pennate diatoms (*Pseudo-nitzschia australis*) ferritin is involved in the iron accumulation (Marchetti et al., 2009) and for centric diatom (*Thalassiosira weissflogii)* it was shown the iron accumulation in the vacuoles (Nuester et al., 2012). Excess iron is no less harmful to cells, since it has a good ability to accept and donate electrons. Due to this reactive hydroxyl radical are formed (Graff van Creveld et al., 2016). It is assumed that under normal conditions, ferritin, or vacuoles, serve to safely store iron in cells.

The continuation of this stress leads to disruption of electron transport through photosystem II, and, as a consequence, the formation of high concentrations of reactive oxygen species (ROS) – an increase in the concentration of manganese-dependent superoxide dismutase, an antioxidant defense enzyme, has been shown (Peers and Price, 2004). In addition, the concentration of powerful antioxidants, such as tocopherol, is increased, as is the expression of the gene encoding 2-phosphoglycolate phosphatase (GPH), which is involved in the repair of a class of DNA damage caused by oxidative stress. The cytochrome level also decreases with iron deficiency and ROS production increases. At the same time, the activity and expression of mitochondrial alternative oxidase increases in cells, thereby reducing the formation of ROS (Allen et al., 2008).

Differential expression of *T. pseudonana* metacaspases was shown during iron starvation - some of them were activated in the first days of cultivation, and some after five days, which correlates with the cytological manifestations of PCD. This points to possible different roles of metacaspases. At the same time the addition of the caspase inhibitor z-VAD-FMK to Fe-starved cells reduced cellular caspase activity and increased cell survival (Bidle and Bender, 2008). It has been found that DSP *T.pseudonana* enhances growth during acute Fe limitation at subsaturating light by increasing the photosynthetic efficiency of carbon fixation (Thamatrakoln et al., 2013). It has been shown that under iron limitation most cells *T. pseudonana* lacked the most recognizable organelles by day 4 and appeared empty by day 6 while maintaining cell membrane integrity. Annexin V-FITC and FITC-z-VAD-FMK staining demonstrates PCD processes under the influence of iron starvation (Bidle and Bender, 2008; Luo et al., 2014).

Nitrogen

Nitrogen is a constituent of proteins and nucleic acids. It has been shown that nitrogen starvation reduces the translation of proteins involved in the reduction of nitrate to ammonium. However, the concentration of enzymes involved in ammonium assimilation does not decrease, which means that this process is important under conditions of nitrogen deprivation (Hockin et al., 2012).

For *T. pseudonana* and *Phaeodactylum tricornutum* it was shown that diatoms have several transport proteins for the absorption of inorganic (for example, nitrate, NO^{3-}, ammonium, NH^{4+}) and organic nitrogen (for example, urea, amino acids) (Rogato et al., 2015). Nitrate entering the cell is reduced to nitrite by cytosolic NADH-dependent nitrate reductase (Allen et al., 2005), then nitrite is transported to the chloroplast and reduced to ammonium by cyanobacterial-like ferredoxin-dependent nitrite reductase (Hockin et al., 2012). After that, ammonium is assimilated by glutamate synthase and glutamine synthetase into amino acids and other nitrogenous compounds (Rogato et al., 2015).

Whole genome data (*T. pseudonana*) showed that diatoms have a complete urea cycle (Armbrust et al., 2004) and can also use alternative sources of nitrogen. Thus, an increase in the level of expression of the urea transporter and amino acids, as well as some peptidases, was shown in the presence of nitrate deficiency in the medium (Hockin et al., 2012). Under conditions of nitrogen restriction, an increase in the urease content, enzymes involved in the synthesis of ornithine was found. It is suggested that ornithine can serve as a reservoir for the storage of reduced nitrogen. In addition, acetyl aminotransferase catalyses the transamination of ornithine (Allen et al., 2011).

The destruction of pigment-protein complexes, a violation in the conversion of energy in photosystem II and lipid content increasing in the cell were shown under a nitrogen lack for the diatoms of the Antarctic ice (Mock and Kroon, 2002). Chlorophyll is a nitrogenous macromolecule, and a decrease in its synthesis reduces the need for cells in nitrogen, and also reduces the ability to capture light and the formation of reactive oxygen species. It has been shown that, under conditions of nitrogen starvation, the concentration of proteins involved in the synthesis of the light harvesting complex in *T. pseudonana* and *P. tricornutum* cells decreases (Hockin et al., 2012; Yang et al., 2014).

Under nitrogen starvation, markers of programmed cell death in diatoms have also been found (Berges and Falkowski, 1998; Lin et al., 2017). The nitrogen-limited cells *S. marinoi* after day 4 displayed

cytoplasmic vacuolization and swollen mitochondria when the membrane and nuclei were intact. At the same time an increase in the expression of metacaspases, DSP, as well as externalization of phosphatidylserine has been shown (Wang et al., 2020).

Phosphorus

The growth of phytoplankton is also affected by the phosphorus content in the reservoir. Phosphorus is a part of nucleic acids, plays a central role in the energy processes of the cell, as part of ATP, as well as phospholipids that make up the cell membrane, and many enzyme cofactors (NAD, FAD). Phosphorus enters water bodies through the weathering of phosphorus-containing rocks and atmospheric precipitation. Currently, various anthropogenic sources of phosphorus are also widespread, which lead to the esterification of water bodies (Paytan and McLaughlin, 2007).

Phosphorus available to diatoms is present in water bodies as dissolved inorganic (mineral) phosphate (primarily orthophosphate, Pi) or in the form of a variety of dissolved organic phosphorus (DOP) compounds, including NA, phospholipids, phosphorylated proteins, and carbohydrates. (Lin et al., 2016). The most common soluble inorganic form of phosphorus taken up by phytoplankton is orthophosphate (Pi) anions (Alipanah et al., 2018). At the same time, the absorption of phosphorus is an energy-consuming process, ATP molecules are wasted (Lin et al., 2016). When the mineral phosphate concentration is depleted, phytoplankton growth often depends on the ability to use the much more abundant DOP by enzymatic hydrolysis with alkaline and acid phosphatase to Pi (Lin et al., 2013).

It was shown for *P. tricornutum* that diatoms limit phosphorus uptake under its lack, primarily by reducing the transport protein composition in the membrane (for example, permease) and by activating alkaline phosphatase, which breaks down organic phosphate on the cell surface. In addition, cell division occurs for two generations, but the concentration of phosphate in cells decreases in the next generation (Feng et al., 2015).

Cells replace membrane phospholipids with lipids that do not require phosphate (for example, *P. tricornutum* – sulfolipids), the production of phospholipases increases (Feng et al., 2015), which allows the use of phospholipids as a phosphate source (Martin et al., 2011; Zhang et al., 2016).

At an early stage of phosphate deficiency, the cell maintains the required level of photosynthesis and carbon fixation, however, the longer the restriction lasted, the more disturbances were found (Feng et al., 2015). The efficiency of photosynthesis decreases, while in *P. tricornutum* the expression of proteins important for this process (proteins of photosystem I, cytochrome c550 of photosystem II, subunits of ATP synthase beta and gamma) decreased. Interestingly, in *Skeletonema costatum*, on the contrary, an increase of the expression of proteins important for photosynthesis under phosphorus limitation was observed (Zhang et al., 2016).

After 48 hours of phosphate-deficient exposure, diatom cells also exhibited accumulation of neutral lipids (Feng et al., 2015), which is often seen under the other stressors described above.

For *S. marinoi*, morphological changes were shown on the 4th day after cultivation of cells with a deficiency of phosphorus – vacuolization, internal degradation of organelles, externalization of phosphatidylserine, as a sign of PCD. Increased expression of the *DSP* gene, which is believed to be associated with PCD processes, as well as the *TSG101* (tumor susceptibility gene 101) gene, which controls cell division, has been shown. Expression *ALDH* (aldehyde dehydrogenase), *GSHS* (glutathione synthase) decreased as stress conditions developed (as the authors suggest, in this way cells implement an energy-saving strategy without spending resources on extra proteins), while the expression of *GOX* (glycolate oxidase), an enzyme that oxidizes glycolate, which important for photorespiration – was increased. The expression of metacaspase genes was also increased (Wang et al., 2020). For *T. pseudonana*, the researchers did not find significant generation of ROS, as well as signs of PCD when cultivated in a medium with a low amount of mineral phosphorus (Lin et al., 2017).

3. Stress signaling triggers PCD in diatoms

Infochemicals are signaling chemical compounds by which cells interact in a population. In the diatom arsenal there are several biologically active compounds belonging to the family of oxylipins. They include short chain polyunsaturated aldehydes (PUAs) and derivatives of hydroxy, keto, and epoxy hydroxy fatty acids (Fontana et al., 2007). It is assumed that these fatty acid oxidation products serve as a chemical reaction of diatoms to stress factors. The most common PUAs are decadienal, decatrienal, octodienal, octotrienal, and heptadienal (Wichard et al., 2005; Cutignano et al., 2006).

This system is initiated by phospholipases and glycolipases after damage to the cell or chloroplast lipid membrane, resulting in the release of polyunsaturated fatty acids (FA), which are oxidized and cleaved to form polyunsaturated aldehydes (Pohnert, 2002; Cutignano et al., 2006). This is often observed when copepods feed on phytoplankton, and it is assumed that the secretion of these aldehydes serves to protect the population from further predator attacks, disrupting the development and reproduction of copepods (Caldwell et al., 2002), and also initiate the splitting of sea urchin, polychaete, and ascidian embryos (Caldwell et al., 2002; Lettieri et al., 2015; Ruocco et al., 2019).

The production of PUAs increases with increasing culture age, with limited nutrients and Si (Ribalet et al., 2007b; Vidoudez et al., 2008). It has been shown that oxidative stress occurs in diatom cells under the influence of decadienal, the expression of metacaspase genes increases, and morphological changes characteristic of programmed cell death occur (Vardi et al., 2008; Graff van Creveld et al., 2021). This is due to the fact that this aldehyde triggered a dose-dependent calcium transient that has derived from intracellular store and subsequently, Ca^{2+} increase led

to nitric oxide what causes oxidative stress (Vardi et al., 2006).

It has been shown *in vitro* that diatom PUAs are able to suppress the growth of other phytoplankton species (Ribalet et al., 2007a). However, the question remains whether decadienal is indeed capable of transmitting signals in a natural population of diatoms, since the concentrations of aldehyde in vitro, which cause programmed cell death, significantly exceed the possible concentrations in the natural population, according to the calculations of the authors (Dolch et al., 2017).

It can be hypothesized that PUAs may act to deter various groups of organisms, such as copepods and bacteria, as well as act as signals mediating interactions between phytoplankton, including a signal to stop flowering and start PCD.

4. Effects of bacteria on diatoms

Bacteria are present in the ecosystem along with phytoplankton, and their interactions occur in a wide range, from mutualism to competition and parasitism. Aquatic bacteria as heterotrophs obtain most of the carbon they need directly from phytoplankton (Fouilland et al., 2014). Coexistence led to the development of a mechanism that ensures the interaction of bacteria and diatoms with the help of chemicals. Diatoms that are able to recognize signals from bacteria are likely to have a greater competitive advantage than those that are not; the same applies to bacteria (Amin et al., 2012).

Gram-negative bacteria produce acyl homoserine lactone (AHL) – hydrophobic molecules that can penetrate the membrane of diatoms and accumulate in their cells (Cuadrado-Silva et al., 2013). In turn, diatoms release extracellular organic biomolecules, often referred to as transparent exopolymer particles (TEP), either actively or as a product of cell lysis. It is assumed that diatoms can use TEP to attract certain types of bacteria (Fukao et al., 2010).

Bacteria can not only release chemicals into the environment in which diatoms live, but also actively attach to algae through several mechanisms. First, with the help of TEP, bacteria recognize the presence of diatoms and initiate attachment to TEP, which can also serve as a source of nutrients for bacteria. Second, bacteria also release exopolysaccharides (EPS) in response to the presence of phytoplankton, which can initiate attachment (Rinta-Kanto et al., 2012). In this case, a symbiosis can form between bacteria and diatoms. For example, bacteria produce vitamin B12, which diatoms need, and their co-cultivation significantly increases the viability of diatom cells (Croft et al., 2005).

Most of the iron in water bodies is represented by hard-to-digest ferric iron. Diatoms absorb ferrous iron best of all, but it is rarely available to them in this form. One of the iron uptake strategies for diatoms is the use of sideraphores, chelate compounds produced by bacteria (Kazamia et al., 2018). To effectively obtain iron from siderophores, diatoms must respond quickly to the appearance of bacteria in the environment. If diatoms can sense AHL produced by a siderophore producing bacterium, they can quickly mobilize iron assimilation mechanisms, thereby increasing their life advantage (Amin et al., 2012).

In addition, bacteria are able to protect diatoms by detoxifying the by-products produced during diatom metabolism. For example, epiphytic bacteria on the Antarctic diatom *Amphiprora kufferathii* help the diatom cope with oxidative stress caused by the production of hydrogen peroxide as cells enter the stationary phase. Hydrogen peroxide is involved in the formation of highly reactive hydroxide radicals, which can inhibit CO_2 fixation in bacteria. Therefore, bacteria use their catalases to neutralize hydrogen peroxide (Hünken et al., 2008).

However, the algicidal effect of bacteria is no less extensive. Bacteria can release chemicals that, among other things, have a devastating effect on diatom cells. These compounds have been shown to lead to the death of eukaryotic cells, resulting in the release of nutrients for bacteria, while the algicidal substance can be either secreted into the environment or produced after bacteria attach to algae (Kang et al., 2005; Paul and Pohnert, 2011; Wang et al., 2016). It was showed (Wang et al., 2016) that the impact on *Skeletonema* sp. algicidal bacteria leads to vacuolization, degradation of organelles, destruction of chloroplasts and mitochondria, and destruction of the cell shell. Study of the effect of *Bacillus mycoides* on *Ulnaria acus* found the destruction of the cell shell, DNA fragmentation, the accumulation of neutral lipids in diatom cells, the destruction of the nuclear membrane, but intact chloroplasts and mitochondria (Bedoshvili et al., 2021). Diatoms, in turn, are also able to produce antibacterial substances. These substances are FA derivatives and may be species specific (Desbois et al., 2008). These are polyunsaturated aldehydes, which also have antibacterial activity, apparently accumulating in bacterial membranes (Ribalet et al., 2008; Pepi et al., 2017). It has been shown that *Chaetoceros didymus* is resistant to algicidal bacteria, since it produces specific proteases together with oxylipins (Paul and Pohnert, 2013; Meyer et al., 2018).

5. Viruses trigger PCD in diatom phytoplankton

In 1989, it was found that water contains a large number of viruses – 2.8×10^8 viral particles (including bacteriophages) per milliliter of water (Bergh et al., 1989), suggesting that viruses play a role in the ecology of aquatic organisms. Work gradually began to appear showing that viruses can cause death of phytoplankton cells (Bratbak et al., 1991; Cottrell and Suttle, 1991) and diatoms in particular (Tomaru et al., 2009). Like bacteria, viruses can limit phytoplankton blooms (Bratbak et al., 1991; Tomaru et al., 2009). In 2004, a lytic virus was first isolated that infects the diatom *Rhizosolenia setigera* (RsetRNAV) (Nagasaki et al., 2004).

It is assumed that during viral infection, processes are triggered that lead to PCD in order to

limit the spread of the virus in the population. Virus-infected phytoplankton have been shown to have typical features for PCD, including cell shrinkage, DNA fragmentation, and metacaspase activation (Bidle et al., 2007; Vardi et al., 2009; Liu et al., 2018). However, the molecular responses of diatoms to viral infection have not yet been studied.

It has been shown that cells under stress (under silicon limitation etc.) undergo more active lytic stages of viral infection (Kranzler et al., 2019). It was reported the appearance of viruses infecting *Chaetoceros* sp. in the Chesapeake Bay, USA (Bettarel et al., 2005). They found the maximum viral abundance a month after the winter-spring flowering of *Chaetoceros*. They hypothesized that a viral infection, due to an increase in the virus population in the water column, was responsible for stopping the flowering of the *Chaetoceros* host.

6. The response of diatoms to changes in the physical parameters

Lighting

Diatoms are passively transported by currents and turbulent mixing. They are brought to the surface of the water column where they must cope with intense light that can cause photo damage, and also descend to depths below the photic zone where solar energy is too low for oxygenic photosynthesis. In addition, in freezing water bodies, diatom cells are under a layer of ice and snow, where the penetration of sunlight is difficult.

The protein complexes of diatom pigments are called fucoxanthin-chlorophyll protein (FCP) complexes. Fucoxanthin allows diatoms to absorb more of the electromagnetic spectrum, especially the blue-green wavelengths that dominate in the aquatic environment (Bertrand, 2010). The main pigments involved in photoprotection are xanthophyll cycle pigments diadinoxanthin and diatoxanthin (Kuczynska et al., 2015).

PGR5 (Proton Gradient Regulation 5) has been found to play an important role in the adaptation of diatoms to changing light. PGR5 prevents excessive PSI recovery on the acceptor side by increasing the ratio of ATP/NADPH production through ATP generation and electron transport for Cyt b6f or PQ. Indeed, diatoms always experience frequent fluctuations in light intensity with high cell concentration, wind swell, and the surface lens effect (Zhou et al., 2021).

It is known that several species of diatoms survive for weeks and months and in some cases for several years in total darkness as vegetative dormant cells (Veuger and van Oevelen, 2011; Schaub et al., 2017). Common features of resting diatom cells are a low metabolic rate and a condensed cytoplasm with chloroplasts localized in the center of the cell. Within a few hours or days after returning to favorable growth conditions, the normal internal structure of cells is restored and cell division resumes (Nymark et al., 2013).

Surviving an extended period of darkness requires the maintenance of a photosynthetic apparatus vital for efficient photosynthesis when returning to favorable light conditions. During *P. tricornutum* study it was found that after 48 hours of darkness, the expression of light-harvesting complex genes and photosynthetic ability increased already within 30 min after the illumination restoration (Nymark et al., 2013). Also, it was found that by supporting important metabolic processes in the dark, the cells *Fragilariopsis cylindrus* retain the functionality of the photosynthetic apparatus, providing a quick recovery when the light returns. Proteomic analysis showed that in the dark, the concentration of enzymes of metabolic pathways involved in the processes of respiration, the tricarboxylic acid cycle, glycolysis, the Entner-Doudoroff pathway, the urea cycle, and the mitochondrial electron transport chain increased. Inside the plastid, carbon fixation ceased, and the upper sections of the glycolysis, gluconeogenesis, and pentose phosphate pathways became less active (Kennedy et al., 2019).

It has been shown that different diatom species use different survival strategies in the dark, which may affect their competitive advantage (Peters and Thomas, 1996). The arctic benthic diatom *Navicula perminuta* used stored lipids, carbohydrates, and proteins as energy sources during 8 weeks of darkness, while phospho- and glycolipids of photosynthetic membranes remained unchanged (Schaub et al., 2017). The vegetative stages of temperate benthic diatoms survived one year of darkness, coinciding with an exponential decline in photosynthetic pigments (Veuger and van Oevelen, 2011).

Diatom cells have been shown to survive in the dark by accumulating NO_3^- and reducing it to ammonium, which is a process of anaerobic respiration (Kamp et al., 2011).

During the dark period, POC (particulate organic carbon) and PON (particulate organic nitrogen) decreased slightly, indicating that cellular metabolism (e.g. respiration) was reduced to a minimum rate. The content of POC in cultures decreased markedly only in the first week, which was probably due to the final division before growth stopped. There was a need for energy to maintain respiration in order to maintain vitality and, in particular, the ability to resume rapid growth as soon as light appeared (Peters and Thomas, 1996).

Temperature

Different temperature ranges and temperature dependences of growth among species are thought to play an important role in algal competition (Kudo et al., 2008). Warming within a certain optimum range increases phytoplankton growth until reaching a maximum, and then, when the temperature becomes above the optimum, growth slows down sharply (Eppley, 1972). With an increase in temperature, diatom cells increase metabolic costs (for example, mitochondrial respiration) and the rate of enzymatic reaction increases (Kudo et al., 2008).

High temperatures can cause disruption of photosynthetic electron transport and carbon fixation mechanisms, leading to a decrease in the productivity and efficiency of photosynthesis (Falk et al., 1996).

An analysis of the fatty acid profiles of *P. tricornutum* also showed that thermal exposure negatively affects thylakoid lipids, consistent with the observed decrease in photosynthesis (Feijão et al., 2018).

The rate of protein synthesis increases significantly at high temperatures, although the number of ribosomes and their associated rRNA decreases, with the opposite effect with decreasing temperature (Toseland et al., 2013).

The rate of the enzymatic reaction is reduced by 2-3 times with a decrease in temperature by 10˚C. To overcome slow enzymatic rates at low temperatures, phytoplankton cells use a combination of two strategies: the evolution of cold-adapted enzymes, which have a thermal optimum much lower than their mesophilic counterparts, or an increase in the concentration of enzymes in the cell (Morgan-Kiss et al., 2006).

RUBISCO is believed to be the limiting factor for diatom blooms at low temperatures (Young et al., 2015). A decrease in the concentration of chlorophyll a by 30% per cell volume was revealed when the cultivation temperature was reduced from 20 to 10˚C for *P. tricornutum* (Kudo et al., 2008).

During cultivation of *F. cylindrus* cells at -1˚C chlorophyll fluorescence and electron transport significantly decreased, while the concentrations of fucoxanthin, chlorophyll *a* and *c* increased. Within a few days, the cells acclimatized, and when cultured for several months, a higher electron transfer rate was observed compared to higher temperatures, and a decrease in pigment content was also observed. Exposure to this low temperature led to the suppression of genes encoding PSII proteins (psbA, psbC) and carbon binding (rbcL), but the expression of genes encoding chaperones (hsp70) and genes for the synthesis and turnover of plastid proteins (elongation factor EfTs, ribosomal protein rpS4, protease ftsH) increased (Mock and Hoch, 2005).

7. Conclusions

The successful evolutionary fate of diatoms depends on their ability to adapt to changes in environmental parameters. A quick response to stress factors is most often a change in the expression level of metabolic genes, the replacement of one enzyme system by another, the activation of the antioxidant system, and the production of chemicals both for signaling and for attacking consumers and bacteria. However, under prolonged exposure to stressful conditions, the cells lack resources for adaptation and PCD can be triggered (Supp. Table). The study of the reactions of diatoms to stress factors will reveal their methods of regulation, including the population size in adverse natural conditions, which may be PCD, as well as other molecular and cellular adaptive mechanisms.

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Conflict of interest

The authors declare no conflict of interest.

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