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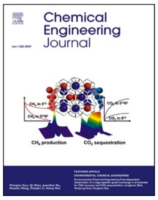

Review

Ion-driven communication and acclimation strategies in microalgae

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ABSTRACT

Similar to all living organisms since their appearance on Earth, microalgae have continuously been exposed to natural selection and consequently evolved by adapting to different or changing ecological niches and occupying ecosystems worldwide, with concomitant genetic mutations. This has to be distinguished from acclimation, a response to environmental stressors which can occur over several generations without genetic changes, and for which phenotypic plasticity can be critical. Pertaining to both mechanisms, microalgae can sense, cooperate and propagate a timely warning message upon changes in the environment. This is key to maintain communities thriving and sustain primary production and mineralization in most ecosystems, but also for sustainability in large scale production of microalgae such as for biofuels production. Nevertheless, the nature of ubiquitous cell–cell interactions and communications, mostly prompted by stress-induced alterations, remains poorly understood, especially due to the lack of technologies suited to decipher cohort signalling and communication. Here, we have critically reviewed microalgae literature, unravelling important cues in microalgae populations that co-ordinate responses to changes in light, temperature, reproduction, grazers and osmotic stress, most likely through a mass diffusion process over a "handover distance". We concluded that bioelectricity mediated through paracrine signalling, mostly involving Ca^{2+}, plays a key role in microalgae cell sensing and communication, being the mediator for timely perception and concomitant collective stress acclimation. This critical analysis and the hypothesis proposed pave the way for quantitative electrogenic assessments of Ca^{2+} signalling and also of the acclimation potential and exquisite evolutionary perseverance of numerous microalgal lineages.

1. Introduction

Photosynthetic microorganisms appeared on Earth before O_2 began to accumulate in the atmosphere 2.4 billion years ago [1]. Their evolutionary radiation changed the structure of the ecosystems and biogeochemical cycles in the oceans [2] and they are presently responsible for up to half of the planetary net primary production [3]. They contribute largely to species diversity globally, covering both prokaryotic and eukaryotic branches of the tree of life.

Microalgae are unicellular eukaryotic algae which thrive in

Abbreviations: IC, ion channels; VAC, voltage activated ion channels; EukCat, diatom voltage activated ion channel; AP, action potential; MEAs, multielectrode arrays; PSI and PSII, photosystem I and photosystem II; HAB, harmful algal bloom; NPQ, nonphotochemical quenching; cGMP, Guanosine 3′,5′-cyclic monophosphate; SIP, sex inducing hormones; ER, endoplasmic reticulum; TRP, transient receptor potential channel..

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freshwater and marine ecosystems as well as soil. Some species tolerate inhospitable habitats such as glaciers, thermal fountains, and saline ponds, as well as some unusual sites such as polar bear and sloth's fur. Their distribution is ubiquitous in the biosphere [4].

A major concern for a population of unicellular microorganisms including microalgae is how to communicate events to their peers and coordinate community responses, especially in large aquatic bodies. Mainly documented in animals, the existence of communication systems has also been demonstrated in fungi, in plants and more recently in microalgae, for the latter mostly related with sexual reproduction and partner attraction (for recent reviews, see [5,6] and references herein). Communication in microalgae seems to occur between individuals of different species as well as individuals of the same species. Communication mechanisms mainly rely on the production and the release in the medium of chemicals that convey information (infochemicals) from an emitter to a receiver, which responds to the chemical cues. Furthermore, many communication mechanisms depend on calcium, a key element of cell signalling in all forms of life [7], in particular microalgae [5].

For an applied prospect, the communication efficiency between a population of conspecifics (same-species cells) is important for the design of successful photobioreactors [6]. For instance, acclimation of microalgae due to changing environmental conditions, here themed as stress, such as nutrients, light (see section 3.3), or temperature (section 3.4) can thus trigger accumulation of high value products lipids and carotenoids, which has been explored for commercial use [8]. Stress-induced strategies utilized by biotechnological laboratories for improving lipid production and concomitant biomass concentration, such as the use of phytohormone, change of cultivation strategies, combined chemical additives and abiotic stresses including nutrient stress and metal ions are therefore becoming critical for microalgal-aided energy production [9,10]. The fundamental problem, however, is that stressful conditions often adversely affect microalgal growth and cause oxidative damage to the cells, lowering the yield of the desired products. The limitation to comprehend stress and communication in microalgae communities is therefore a critical aspect for biotechnological industries that remains unsolved.

The communication strategies mostly triggered by stress among same-species and inter-species neighbouring cells are intrinsic to the dynamic community actions. It starts with sensing the condition threatening the survival of the cells followed by signalling to the rest of the community occupying that particular ecosystem. There is compiling evidence that microorganisms acclimate to sudden environmental changes by activating cell defence mechanisms and their ability to timely reorganize as a group, survive and reproduce [11,12]. The processes through which microalgae communicate with conspecifics remain largely unknown [13], yet taking inspiration from communication in *e.g.* bacterial cells with quorum sensing or eukaryotic cells with paracrine and electrical signalling, we have hypothesised that microalgae communication is mediated through paracrine signaling in which ions act as primary messengers.

Most microalgae express a range of ion channels that serve important physiological purposes, including intracellular signalling which refers to signalling within a single cell and intercellular communication, or simply communication, which mainly refers to signalling between conspecific neighbouring cells. Ion channels are the main players of intracellular signalling and intercellular communication. Progressive studies on ion channels and their functions revealed their existence in all three domains of life e.g., Archea, Bacteria and Eukaria [14]. Most of the fundamental biological macromolecules in cells are negatively charged at physiological pH, primarily due to the phosphate group in DNA, RNA and proteins. The structural stability and function of these macromolecules therefore require positively charged counter-ions, to provide charge balance of cells [15].

Ion channels (IC) are protein forming pores in the cell membrane that control ion permeation and fluxes, which are the basis of many cell functions. They allow specific inorganic ions, namely Na^+, K^+, Ca^{2+} and Cl^-, to diffuse rapidly down their electrochemical gradients across the membrane. Ion channels are gated in order to open and close according to the metabolic situation. A stimulus is needed to open the gate, such as a change in the voltage across the membrane (voltage-gated channels), a mechanical stress (mechanically-gated channels) or the binding of a ligand either extracellular or intracellular (ligand-gated channels) [16].

There is a larger diversity of channels in unicellular eukaryotes compared to plants and animals [14] although the majority of studies focus on vertebrates and large invertebrates. Among eukaryotes there is evidence of this richness from the number of IC-coding genes present. The ciliate *Paramecium tetraurelia*, the green flagellate *Chlamydomonas reinhardtii* and the diatom *Thallassiosira pseudonana*, have all been known to express multiple K^+ channel genes [17] and to exhibit ionic currents [14]. The ion channels may assist the generation of electrochemical signals which promote communication among cells [18].

Diatoms are a large and highly diverse eukaryotic group of microalgae, mostly oceanic, which generate as much organic carbon per year as all terrestrial rainforests combined. A proportion of carbon generated by diatoms is rapidly consumed at the base of marine food webs [19]. Due to their silica cell wall, they play a pivotal role in both biogeochemical cycles of carbon and silicates [20]. Phylogenomic analyses suggest that diatoms belong to the eukaryotic supergroup of Stramenopiles (Chromalveolata), whose common ancestor is thought to derive from a secondary endosymbiotic event between a heterotrophic host, a red alga and possibly a green alga [21]. This original evolutionary history conferred diatoms with a unique genetic makeup and metabolism which may contribute to their ecological success in the contemporary oceans [19]. The presence of intracellular signaling and intercellular communication mechanisms in diatoms with roles in the acclimatory response to (a)biotic stressors has been attributed to grazing, hypo or hyperosmotic stress, light alterations, temperature changes [18,22–27].

Herein, we critically review for the first time the role of bioelectricity, governed by ion channels, in the translation of communication mechanisms in cohorts of microalgae. We discuss communication cues for microalgae when acclimating to different conditions, such as grazing, osmotic stress, light, temperature, and sexual reproduction. We propose a novel mechanism used by microalgae to sense, communicate, and acclimate to changes in their environments, with particular focus on the use of bioelectricity. We will demonstrate the importance of using sensitive electrophysiology, photophysiology and microscopy analytical tools to translate and ascertain the significance of paracrine communication processes in the perception and acclimation strategies in microalgal communities.

2. Fundamentals of communication in microalgae

2.1. Role of paracrine signalling and definition of "handover distance"

Marine phytoplankton in general and diatoms in particular are able to perceive and respond to chemical changes in their environment [12]. In diatoms, the stress-sensing is likely triggered by ion movements through channels in a cell membrane, measurable using the patch clamp technique, as action potential-like events [12,24]. The transmission of the information, from cell to cell, involves more than one receptor cell and cannot be measured with conventional single-cell patch clamp recordings. Probing the communication within microorganisms requires methods capable of monitoring cell populations. Thus, recent developments were made with highly sensitive multielectrode arrays (MEAs) [18,28,29], which enabled, as opposite to single cell measurements, the recording of whole diatom cohorts [18]. MEAs are used for non-invasive electrical recordings in electrogenic cells [30], over a time span of days or even months.

MEAs comprise planar electrodes placed on a substrate in contact with the cells cultured in a medium [28]. MEAs monitor the extracellular field potential, which typically comprises the contribution from voltage or ligand gated ion channels, and inherent voltage fluctuations across the cell membrane. Critical for decoding communication in microorganisms are the large electrode MEAs, which exhibit low impedance and optimize the signal-to-noise-ratio for low frequency measurements. In large MEAs, the measured signal originates from all single cell contributions adherent to the electrode (see Fig. 1A), which means that when cells cooperate, the signal emerges as synchronized electrical spikes, and when cells do not cooperate, the signal emerges as uncorrelated noise. It is therefore suggested that synchronized, or periodic electrical spikes across an electrode covered by hundreds of cells, represent co-ordinated communication events between conspecifics among a cell cohort. One example is the quasi-periodic electrical signal among a population of diatoms as seen in Fig. 1B. The histogram in Fig. 1C shows the interspike intervals distribution, extracted from a long-term recording of 20 h and reveals a Gaussian dispersion with a mean value of 80 s.

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Fig. 1A shows a population of diatoms adhered to an Au electrode, separated apart, on average, by a "handover distance" of 120 µm, as indicated by the blue dashed circle. Here we introduce the term handover distance for the diffusion-limited distance between neighboring cells, which allows them to communicate via release and uptake of ions. If cells are too far apart, beyond handover distance, they are unable to significantly influence the medium around neighbouring cells. In more detail, the diffusion of a small particle in its surroundings is driven by thermal agitation of the molecules on that particle according to Brownian movement. The particle movement arising from the continual and pseudo-random bombardment by the molecules of the surrounding liquid has been explained by Einstein in 1905 [31,32]. As described in the following sections, the Brownian particle can be Ca^{2+}, K^+ or Na^+. We consider the particle concentration, p and the concentration without living cells, p_0 - the latter is considered as the 'thermal equilibrium' concentration in the cell surrounding or extracellular space. The diffusion coefficient, D indicates the efficiency of the random walk. The Einstein relation describes the diffusion coefficient to the particle mass and its friction coefficient in the medium at a given temperature, and is given by:

$$\frac{D}{\mu} = \frac{\mathrm{kT}}{\mathrm{q}} \tag{1}$$

with μ the mobility, k is the Boltzmann constant, T temperature in Kelvin and q the elementary charge in coulombs. The mobility is proportional to the average collision time, τ_c and is inversely proportional to the mass of the particle as:

$$\mu = q\frac{\tau_c}{\mathrm{m}} \tag{2}$$

This work reviews a number of microalgal stress acclimation processes which primordially rely on ion efflux to the extracellular space. The injection of ions changes the equilibrium concentration and likely triggers a cascade of communication events is reviewed in detail in section 4.

To derive the handover distance or diffusion length L_p, we need first to introduce the concept of average lifetime, the continuity equation, and the steady state condition [33,34]. The average lifetime of a particle (ion), τ_p indicates how long the ion remains in the cell vicinity before it recombines or is recruited by a neighboring cell.

$$\frac{d\Delta p}{dt} = -\frac{\Delta p}{\tau_\mathrm{p}} \rightarrow \Delta p = \Delta p_0 e^{-t/\tau_p} \tag{3}$$

The continuity equation can be written considering mainly the diffusion transport in one dimension as

$$\frac{\partial \Delta p}{\partial t} = -\frac{\Delta p}{\tau_\mathrm{p}} + D\frac{\partial^2 \Delta p}{\partial x^2} \tag{4}$$

In steady state in which $\partial \Delta p / \partial t = 0$, we have

$$\mathrm{D}\frac{\partial^2 \Delta p}{\partial x^2} = \frac{\Delta p}{\tau_\mathrm{p}} \rightarrow \Delta p = \Delta p_0 e^{-x/L_p} \tag{5}$$

Which means that the diffusion length L_p, can be written as

$$L_p = \sqrt{D\tau_p} \tag{6}$$

In Fig. 1B, Ca^{2+} is suggested as the diffusion ion species responsible for the electrical signal from a cohort of cells within handover distance. The diffusion coefficient of Ca^{2+} in sea water is 7.5×10^{-6} cm^2/s [35]. Also seen in Fig. 1B, the majority of inter-spike intervals occur at 80 s. Hence, with $\tau_p = 80$ s as shown in Fig. 1C, this yields a diffusion length of ~250 µm. This distance is an approximation, and we note that different types of ions can have different diffusion recombination lengths, because the mass, diffusion coefficient and the recombination time differ. The diffusion length is of the same order of magnitude as the average distance between the diatoms (Fig. 1A) which further suggests that the intercellular waves are due to paracrine cell–cell communication. In fact, diatoms collectively engage into low frequency quasi-periodic oscillation patterns or intercellular waves (Fig. 1B) when responding to light and temperature stress [18]. The reported paracrine communication in the planktonic pennate HAB-forming diatom *Pseudo-nitzschia fraudulenta* is suggested to be mediated by Ca^{2+} ions as the intracellular messenger [18]. Furthermore, the detected electrical signal is interpreted as a communication strategy to acclimate to darkness. This electrical signal could thus reflect cohort communication between individuals, which may explain the exceptionally fast acclimation to physiological or chemical changes in the environment of microalgae, which is reviewed in section 3.

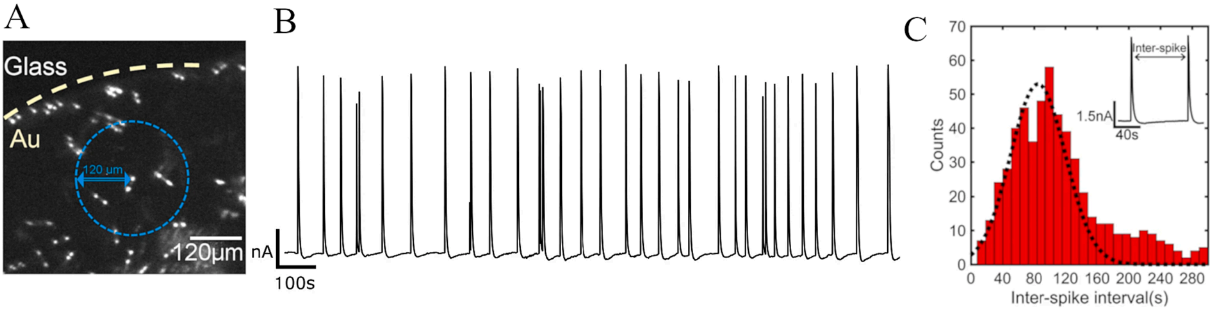
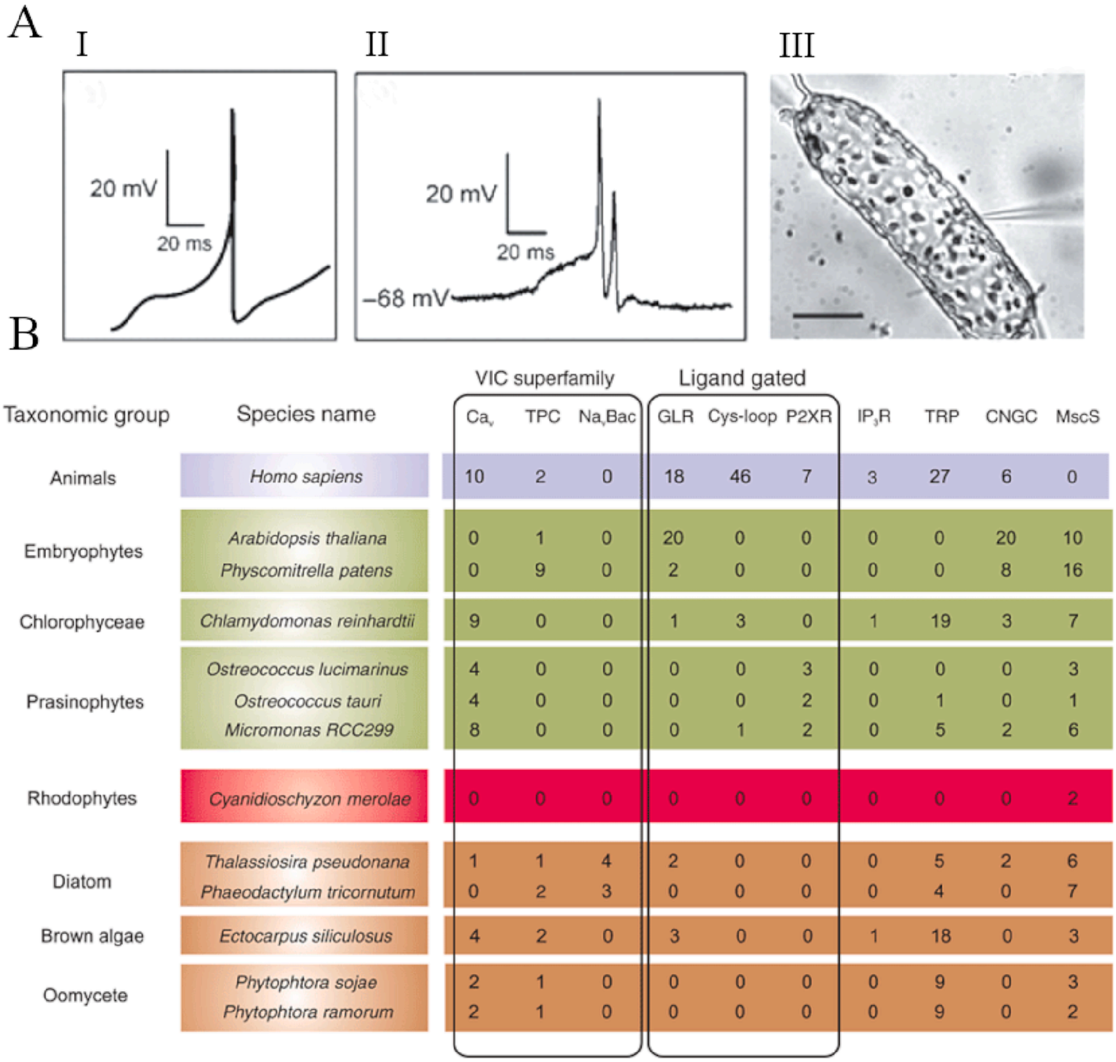


Fig. 1. Sensing current pulses from a cohort of *Pseudo-nitzschia fraudulenta* diatoms. (A) Bright field optical micrograph taken 24 h after depositing cells on the transducer. The dotted line separates the Au electrode and the glass substrate. The blue circle is centered at a single diatom and represents an area with a radius of 120 µm. (B) Quasi-periodic electrical recording in cells under dark stress for 72 h. (C) Histogram showing the inter-spike intervals with Gaussian distribution, the mean interval is 80 s. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Electrogenic microalgae

Electrophysiological measurements of the plasma membrane potential in the diatom species *Coscinodiscus radiatus* indicated a resting potential of −40 to −80 mV and even up to more negative values at ∼ −140 mV suggesting the presence of an electrogenic pump [36]. In the same study, spontaneous and light/dark transition-induced oscillations of plasma membrane potentials were recorded suggesting the presence of controlled ion influx/efflux involved in cellular osmoregulation, given their ability to regulate their sinking dynamics in response to irradiance [36].

Subsequent electrophysiological studies conducted in the diatom species *Odontella sinensis* provided a step forward in our understanding of diatom plasma membrane electrical properties. By using single electrode voltage clamp experiments, this study showed the unanticipated presence of an endogenous Ca^{2+}/Na^{+} based fast action potential with biophysical and pharmacological characteristics similar to that found in animals (Fig. 2A) [24,37]. Fast Ca^{2+}/Na^{+} based plasma membrane action potential is a well-known property of skeletal/cardiac and neuronal cells triggered by the activity of a specific class of voltage activated ion channels (VACs) presenting four repeated transmembrane domains (4D-VACs). Compiling electrophysiological studies and genomic information supported a model in which Ca^{2+}/Na^{+} based action potential and 4D-VACs evolved with the appearance of the first neuromuscular system, hence being considered specific to animal cells [14,38,39]. Comprehensive phylogenetic analysis of the ion channel repertoires of the two main model diatom species, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum,* with available sequenced genomes revealed the presence of diverse families of voltage and ligand activated ion channels [40–42], (Fig. 2B). While a Ca^{2+}/Na^{+} 4D-VAC homologue was identified in the centric species *T. pseudonana,* no such homologue was found to date in the pennate species *P. tricornutum*. Both diatom species, however, present several homologues of one domain VAC, a class of Na^{+} permeable VAC previously identified in bacteria and playing a role in cell motility, chemotaxis and pH homeostasis [43,44]. Due to the small size of *T. pseudonana*, the electrical



Taxonomic group	Species name	VIC superfamily			Ligand gated						
		Ca_v	TPC	Na_vBac	GLR	Cys-loop	P2XR	IP_3R	TRP	CNGC	MscS
Animals	*Homo sapiens*	10	2	0	18	46	7	3	27	6	0
Embryophytes	*Arabidopsis thaliana*	0	1	0	20	0	0	0	0	20	10
	Physcomitrella patens	0	9	0	2	0	0	0	0	8	16
Chlorophyceae	*Chlamydomonas reinhardtii*	9	0	0	1	3	0	1	19	3	7
Prasinophytes	*Ostreococcus lucimarinus*	4	0	0	0	0	3	0	0	0	3
	Ostreococcus tauri	4	0	0	0	0	2	0	1	0	1
	Micromonas RCC299	8	0	0	0	1	2	0	5	2	6
Rhodophytes	*Cyanidioschyzon merolae*	0	0	0	0	0	0	0	0	0	2
Diatom	*Thalassiosira pseudonana*	1	1	4	2	0	0	0	5	2	6
	Phaeodactylum tricornutum	0	2	3	0	0	0	0	4	0	7
Brown algae	*Ectocarpus siliculosus*	4	2	0	3	0	0	1	18	0	3
Oomycete	*Phytophtora sojae*	2	1	0	0	0	0	0	9	0	3
	Phytophtora ramorum	2	1	0	0	0	0	0	9	0	2

Fig. 2. Electroactive microalgae (A) Action potentials in squid and in diatom species. (I) Representative example of an action potential recorded from the giant squid. Reproduced with permission from [42] and [37], copyright Wiley & Sons; (II) A spontaneous action potential recorded in the diatom *Odontella sinensis*. Reproduced from [20] Copyright PLOS under CC BY 4.0 license (https://creativecommons.org/licenses /by/4.0/); (III) A single cell of *O. sinensis* with intracellular recording electrode in place. Scale bar represents 20 μm. Reproduced from [24] copyright PLOS under CC BY 4.0 licence (https://creativecommons.org/licenses/by/4.0/). (B) Distribution of the major classes of Ca^{2+}-permeable channels identified in photosynthetic eukaryote genomes. Ca_v, voltage-dependent Ca^{2+} channel; CNGC, cyclic nucleotide-gated channel; Cys-loop, cysteine loop; GLR, glutamate receptor; IP_3R, inositol-1,4,5-trisphosphate receptor; MscS, mechanosensitive ion channel; Na_vBac, single-domain, bacterial-type channel; P2XR, purinergic P2X receptor; TPC, two-pore Ca^{2+} channel; TRP, transient receptor potential; VIC, voltage-gated ion channel. Reproduced with permission from [42], copyright Wiley & Sons.

properties of the plasma membrane could not be investigated using microelectrodes, highlighting the need for electrophysiology tools capable of investigating electrogenic microalgae, with multiple scales. The public release of several diatom transcriptomes, including of *O. sinensis*¸ sequenced within the frame of The Marine Microbial Eukaryote Transcriptome Sequencing Project provided the means to better characterize the VAC repertoire present in diatoms [45]. Surprisingly, no 4D-VAC homologue was found in *O. sinensis* transcriptome as well as in the vast majority of the other sequenced diatoms [23]. This finding raised the intriguing hypothesis that another class of VAC may be responsible for the fast Ca^{2+}/Na^{+} based action potential (AP) found in *O. sinensis* (and other diatom species lacking 4D-VAC including the genetically tractable diatom species *P. tricornutum*). Detailed phylogenetic analysis indicated that 1D-VACs were conserved in diatoms. Diatom 1D-VACs clustered away from their bacterial counterparts forming a novel class of diatom specific VAC named EukCatA, with *O. sinensis* and *P. tricornutum* both presenting three EukCatA orthologues [23]. Similar to *T. pseudonana,* however, the small cell size of *P. tricornutum* precluded a direct investigation of its plasma membrane electrical properties with microelectrodes. Cloning and ectopic expression of the *P. tricornutum* and *O. sinensis* EukCat orthologues *PtEUKCATA1* and *OsEUKCATA*1 in HEK293 cells followed by electrophysiological analysis demonstrated that these channels could trigger rapid community depolarization-induced Ca^{2+}/Na^{+} based AP [23]. In addition, epifluorescence microscopy imaging in live cells of *P. tricornutum* transgenic lines expressing the intracellular Ca^{2+} sensor R-GECO supported the presence of endogenous depolarization-induced Ca^{2+} elevations in this species. By employing CRISPR-Cas9 directed gene knock-out method readily available in *P. tricornutum*, *PtEUKCATA1* is required to trigger community depolarization-induced Ca^{2+} elevations and to sustain cell gliding motility or translocation ability. Further studies employing *P. tricornutum* R-GECO expressing lines provided mounting evidence for a role of intracellular Ca^{2+} signaling, with propagating speeds of ~ 37 μm/s, in the acclimatory response to hypo-osmotic stress [46]. In addition, the authors showed that extracellular Ca^{2+} is necessary for mediating K^{+} efflux through Ca^{2+} regulated K^{+} channels, in response to hypo-osmotic shock, suggesting the existence of a warning message through the neighboring community mediated by Ca^{2+}.

3. Evidence of communication in microalgal cohorts - Acclimation to sudden changes in the environment

A stressor can be defined as pressure that provokes a response or acclimation from an organism, which in turn can have indirect impacts on every related organism. This universal phenomenon applies to macro- and microorganisms in all ecosystems. The stress response or acclimation to a sudden change in the environment in microorganisms remains a poorly understood phenomenon [47]. Single-cell studies on microalgae have shown their ability to detect these environmental changes and trigger a stressor-specific response [12,40,41]. Yet, the stress response seen from the actual microalgae cohort remains unknown. The intercellular communication occurring after stress sensing is less straightforward to measure but it is essential to understand how the stress response is timely propagated from its initial stimuli point throughout the whole microalgal population. Considering communication-acclimation to stressors at a community level, the behavioral or physiological response of phytoplankton can contribute to explain how these organisms are so resilient and successful in survival, acclimation and on a longer-term adaptation in all ecological niches worldwide, to the point that they can overtake entire ecosystems.

Hence, in section 3 we discuss how the community response to the presence of grazing organisms, mating partners, changes in water temperature, light variations and osmotic stress, provokes a metabolic response from microalgae for acclimation and sometimes survival, as illustrated in Fig. 3.

3.1. Grazing pressure

Microalgae are valuable food resources for zooplankton grazers. Upon zooplankton grazers proximity, microalgal cells are often observed to respond collectively to the grazing pressure. An experiment where cenobia-forming *Scenedesmus acutus* and *Desmodesmus subspicatus* cells were cultivated with filtered culture medium where *Daphnia* has been previously cultivated, aimed at finding the defence response in *S. acuta* unicells. When not stressed by grazers, *Scenedesmus/Desmodesmus* may either be in 4-celled cenobia or they may be unicells, as a result of cenobium disintegration or as a preferred planktonic morphotype at the euphotic zone (Fig. 4A). However, the results showed that when

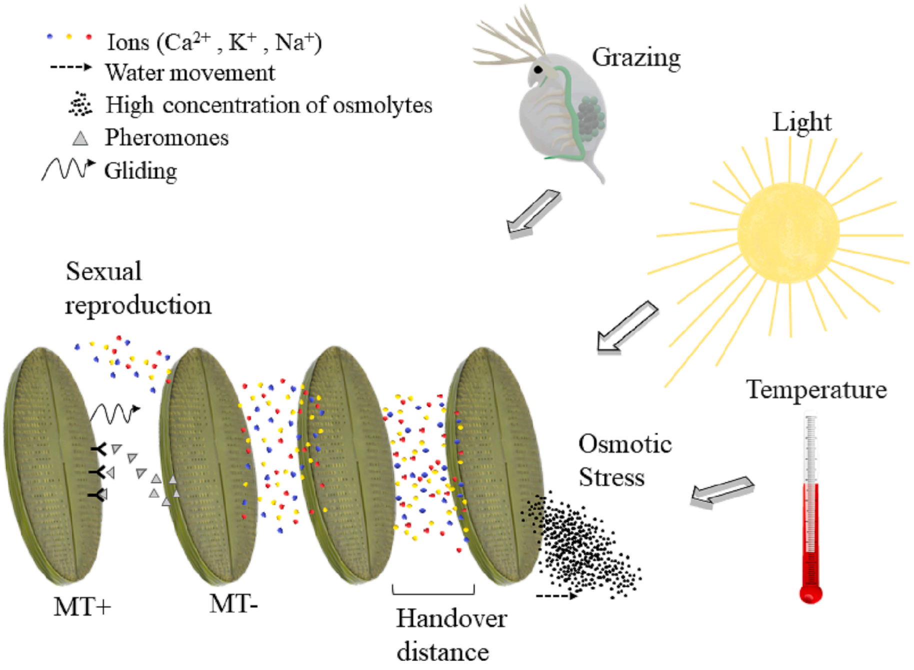


Fig. 3. Triggers of stress-induced communication. Environmental stressors activating sensing mechanisms in marine microorganisms, here represented by diatom cells separated by a handover distance. Stressors here represented are the grazing pressure, light intensity variations, temperature changes, sudden osmotic alterations, and sexual reproduction which involves chemical signalling via pheromone release from a cell (MT-) to its mating counterpart (MT+), resulting in gliding to a closer distance. The stress is sensed by each cell, causing metabolic response such as the synthesis of molecules and peer communication by cation-based influx/efflux. These cell reactions originate an initial signal which is then propagated at a community level.

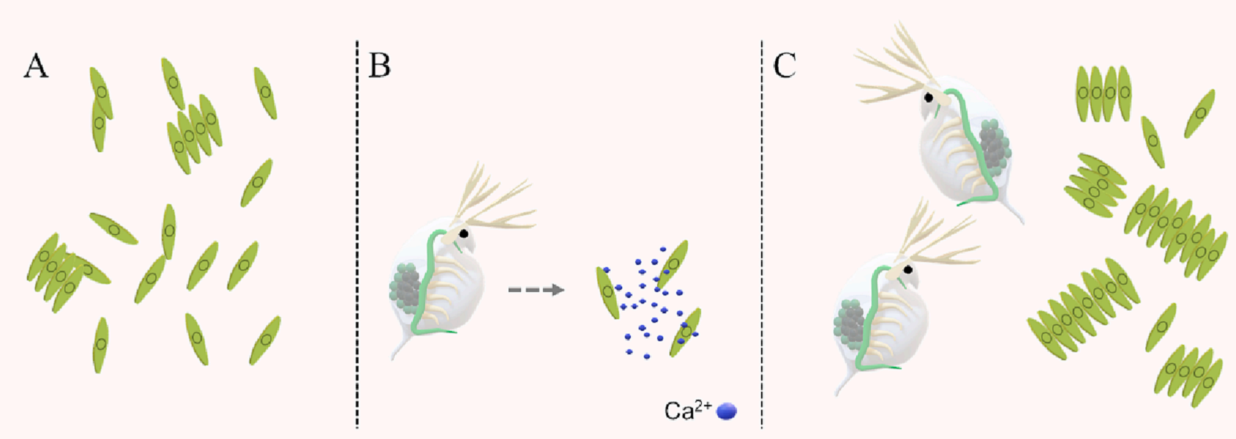


Fig. 4. Adaptive response of *Scenedesmus acutus* to grazing pressure of *Daphnia magna* (based on [48]) suggesting a diffusion-limited ion flow process. (A) *Scenedesmus* unicells or 4-celled cenobia in absence of the grazer *Daphnia magna* (B) *Daphnia* start to feed on *Scenedesmus* unicells which very likely triggers communication events of *Scenedesmus* unicells in handover distance to other unicells. (C) *Scenedesmus* cells systematically aggregate to form large cenobia as a defence response to grazing.

Scenedesmus cells were exposed to filtered *Daphnia* medium, the otherwise abundant unicellular morphotype was replaced by 4 or 8 celled cenobia [48]. (Fig. 4B and 4C). Lürling [48] suggested that the response was triggered by grazing-associated chemicals, released in handover distance. The molecules released by the grazers may have a role in mediating protective actions in the cellular cohort by producing 8-celled cenobia, which hamper ingestion due to their large size. An additional anti-grazing defense in species of *Desmodesmus* are bristles and spines throughout the cenobium, which may discourage even larger grazers [48]. Furthermore, *Daphnia* needed to feed first to induce colony formation, suggesting the first few degraded cells as the triggering agents for infochemicals release by *Daphnia*. In contrast, the presence of starved grazers did not induce colony formation. Eight-celled cenobia is therefore considered as an indicator of grazing pressure [49] and it is very likely that communication between unicell conspecifics happens prior to cenobia formation. Lürling hypothesized that the phenotypic plasticity exhibited by *Scenedesmus* may be a result of herbivorous zooplankton grazing-associated infochemicals and it also may contribute to a morphology cycle in which the heavier cenobia sink and disintegrate into single cells which then resuspend and inoculate another blooming site, therefore having a role in the phenotypic plasticity displayed by *Scenedesmus* and *Desmodesmus*.

These studies support the hypothesis that phytoplankton species can detect grazers in handover distance through a chemical cue. Although there is no direct evidence for Ca^{2+} mediated signaling, it is likely that this cue triggers a communication event on a community level of phytoplankton, causing the adaptation response to occur collectively and rapidly, due to the imminent danger of ingestion.

The *Daphnia* animals are stimulated by nearby dead algal remains and release kairomones to the water. These infochemicals seem to diffuse and reach the nearest algal cells which initiate the perception of danger in the neighbouring microalgal cohort. The perception is possibly followed by the emission of a message from the stressed cells to inform the cells positioned away from the grazer perimeter that danger is imminent. One may hypothesize that an electrical signal caused by ionic currents is started by the grazing-affected cells and acts as a warning signal to the unicells which are close enough, or within handover distance (Fig. 4B). These cells perceive the ions released by a neighboring cell, then the membrane potential is altered and cascades (Fig. 4C).

3.2. Osmotic regulation

Pennate diatoms have bilateral symmetry, and some are able to migrate on a substrate, a process called gliding, using actin-myosin cytoskeletal structures [50]. Since the cell is surrounded by a rigid silica frustule, the movement is possible by the existence of openings in the cell wall, a system of grooves called a raphe which allow the secretion of adhesive mucilage molecules, on which the cell pulls using the actin-myosin system [51]. The raphid pennate diatom *Phaeodactylum tricornutum* glides in the challenging environment of agar plates and the movement is dependent on EukCats activity and Ca^{2+} availability in culture. Ca^{2+} entering in the cell during action potential-like events is critical for intracellular signalling and gliding motility [23,25].

The mechanisms by which diatoms sense their environment may depend on the concentration and type of salt they live in. In seawater (where the Na^+ concentration is ~ 600 mM) diatoms, during an action potential the Na^+ channels open and Na^+ enters through EukCats channels and depolarizes the membrane. In freshwater (Na^+ ~1 mM), diatoms, Ca^{2+} selectively enters through the EukCats and activates Ca^{2+} -dependent anion channels and Cl^- is released from the cell thus depolarizing it. In both cases, repolarization occurs after closure of EukCats followed by K^+ outflow [25].

Diatoms exist not only in marine and freshwater habitats but also in coastal and estuarine regions where they may need to respond to rapid changes in osmolarity. Changes in osmolarity are often assisted by ion channels. Recent studies using single-cell imaging show that the cells of *P. tricornutum* display intracellular Ca^{2+} elevations in sensing hypo-osmotic stress [46]. The authors observed that nearly all fusiform cells exposed to 30 s treatments with diluted culture medium in the absence of Ca^{2+} (medium prepared without $CaCl_2$ and with Ca^{2+} chelator EGTA) would burst due to hypo-osmotic stress. On the contrary, when extracellular Ca^{2+} was available, the exposure to the hypo-osmotic treatments did not trigger cell bursting in most cells. This evidences that the removal of extracellular Ca^{2+} inhibited its elevation in the cytoplasm and hampered the ability of the cells to survive hypo-osmotic stress through osmotic regulation. The authors determined that the two large vacuoles positioned in the tips of the fusiform cells have a role on regulation processes by rearranging their structure and fragmenting into smaller vacuoles as a response to hypo-osmotic stress. Also, this species displays morphological plasticity, where the cells may be triradiate, fusiform or oval morphology and may change morphology in response to stress. Upon mild osmotic stress, the apical tips of fusiform cells were shown to have a sensing role, with Ca^{2+} elevations detected by fluorescence microscopy first in the apical tips and then spread to the central part of the cell within 2–3 s. The sensing and regulating response may differ according to the time required for the acclimation. The cells may activate these Ca^{2+} dependent processes for a short-term response, in the order of seconds to prevent cell swelling or by extruding K^+ outside the cell membrane in order to regulate cell volume. Medium to long-term responses, within minutes to days involve efflux of organic osmolytes and changes in gene expression, and ultimately originate a change from the triradiate or fusiform into the benthic oval morphotype, which is more stress resilient [46].

3.3. Light

Light is an important environmental factor for photosynthetic organisms because they use light to produce energy through the photosynthetic electron transport chain. Briefly, there are two light reactions occurring in photosystems I and II (PSI and PSII) of the chloroplast membranes. These operate in series, whereby electrons are extracted from water molecules by PSII then transferred through a transport chain of transporters and redox-active proteins to PSI and ultimately producing the reducing agent nicotinamide adenine dinucleotide phosphate (NADPH). This complex process is accompanied by proton pumping to the thylakoid lumen, creating a proton gradient used to synthesise ATP using the proton-pump ion channel ATPase. Both ATP and NADPH are the reducing agents required for carbon fixation and many other metabolic processes [52].

Optimal light conditions for photosynthesis are not always possible. Therefore, sensing for preventing damage in the case of high light intensity is vital. Microalgae have developed various ways to sense and acclimate to changing light conditions. Considering a wider perspective of change, global warming events indirectly impact the amount of light reaching aquatic ecosystems, with higher sunlight intensity and/or dose reaching the ocean surface which, together with higher temperatures, can lead to increased rates of chemical and biological processes, causing increased algal proliferation and Harmful Algal Bloom (HAB) formation, lower dissolved oxygen, among other pernicious impacts [53].

In corals, elevated light dose and temperature interact in a negative manner to result in stress-induced breakdown of the electron transport chain and reactive oxygen production in endosymbiotic algae, zooxanthellae. If this occurs over prolonged periods in scleractinian corals, the corals respond to the oxygen radicals by expelling the algae (which may provide the colour to coral) resulting in the commonly known process of coral-bleaching [54]).

At a shorter timescale, daily, hourly or even shorter (e.g., sunlight flecking) fluctuations of light intensity can have detrimental impact to photosynthesis. The photosynthesis impact affects microalgal relative pigment composition such as the protective de-epoxidation of xanthophyll pigments (violaxanthin, zeaxanthin and diadinoxanthin) and nutrient uptake to a point that the cells start high light mitigation processes including non-photochemical quenching (NPQ). NPQ is the process by which algae and plants exhibit down regulation through xanthophyll cycling, induced by a *trans*-thylakoid proton gradient resulting from light induced electron transport. Excess excitation energy is then dissipated as heat resulting in photo-protection ([55] and references therein).

Fluorescence studies of microalgae have elucidated different forms of photoprotection which can broadly be defined as either physiological or behavioural [56]. Physiological photoprotection is largely in the form of a cohort of cells responding to changes in light level, principally photodose, by either increasing or decreasing NPQ. NPQ in turn can be regulated or unregulated [57] and can be observed as photoacclimation. With increasing light dose, photoacclimation occurs such that apparent upregulation of photochemistry is observed (Fig. 5A) [58] e.g. with an increase in electron transport rate in response to the increasing photodose. In this scenario it is the physiological down regulation of excitation energy that is responsible for the form of the xanthophyll cycle (Fig. 5B). However, cell movement can also result in photoprotection, with cells actively moving away from unfavourably high light given the organism's limited assimilation capacity, which can result in oxidative stress and ultimately cell death. Critically, cells may actively move to be shaded by other cells or in the case of benthic diatoms, actively move down into a muddy sediment where light is attenuated to a lower level (Fig. 5C, D) [59], suggesting the existence of an underlying communication at the cohort level, leading to a concerted reorganization and acclimation. In the case of the latter, this has been referred to as microcycling [60], a turnover of cells at the light exposed surface of a sediment, as well as bulk movements of the community down into the sediment, likely due to combined stimuli involving light and tidal/diel stimuli. This results in a community response to move upwards towards light when intensity is lower than optimum for photochemistry, and to move away from photo-inhibitory or photodamaging light levels that exceed the optimum. It would seem highly likely that, when considering the response to other stressors discussed above, communication between conspecifics is an active component of these community responses.

Other microalgae are known to possess sophisticated sensing mechanisms, such as in the case of *Chlamydomonas*, a large genus of unicellular bi-flagellates occurring in widespread environments including seawater, freshwater, soil and even snow. The swimming cells of *Chlamydomonas* are phototactic and can re-orient their movement in relation to the light source [61]. The cell has a red eyespot, which is an organelle containing a photoreceptor chemically similar to retinal. A retinal-binding protein called chlamyopsin, very similar to animal opsins, is present and interacts with signal transduction proteins [62]. The absorption of light by chlamyopsin triggers the opening of Ca^{2+} channels

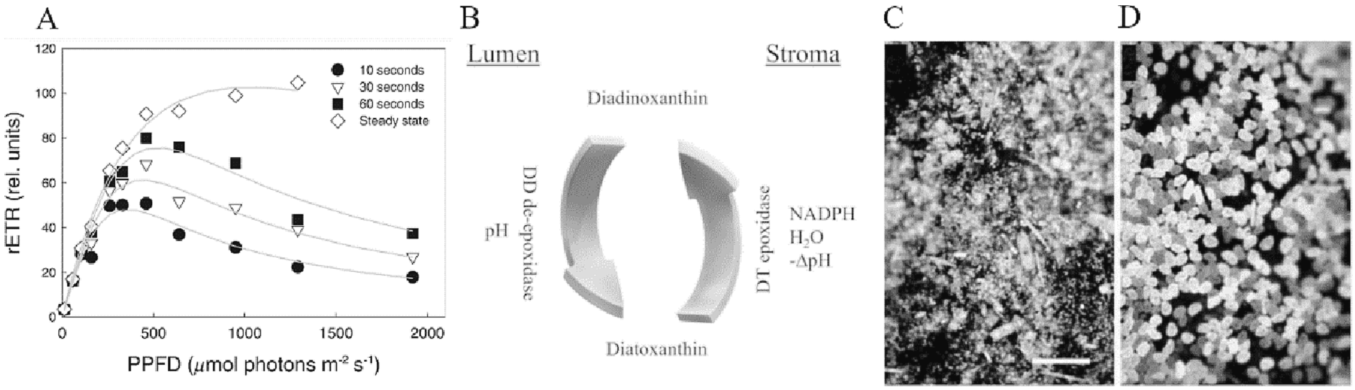


Fig. 5. Photoacclimation states in microalgae in response to light exposure. (A) photoacclimation increasing relative electron transport rate in response to increased photodose during light curve measurements, reproduced with permission from [58], copyright Springer Nature BV; (B) physiological down regulation utilising the xanthophyll cycle in diatoms; (C) and (D) behavioural down regulation using cell movement as cells microcycle through the surface of a benthic microalgal community, with diatom cells moving downward to protect from excessive light (C) and euglenoids moving up to biofilm surface to expose to light (D), C and D scale bar 100 μm, reproduced with permission from [59], copyright Inter-Research 2002.

which originates a fast inward cation current [63]. The first evidence that photoinduction of electric potentials dependent on Ca^{2+} (and in response to phototaxis) was in the flagellated cells of the microalga *Haematococcus pluvialis* in a non-invasive approach where a single cell was immobilized inside the tip of a pipette and the extracellular potential difference was measured. The authors also determined that the photoinduced polarization of the cell is due to the asymmetrical location of the photoreceptor in the cell membrane. Its location is at the anterior end of the cell, and it generates the positive potential inside the cell with respect to the outer space with incident light. Also, the resting potentials of the anterior and posterior membranes compensate for one another in the dark. When illuminated, the photoreceptor in the anterior part of the cell membrane generates an electrical potential. The blue-green region of the spectrum is responsible for the generation of the described potentials [63].

3.4. Temperature

Microorganisms have developed mechanisms for surviving temperature changes. Recent studies concerning microorganisms' acclimation to temperature are linked with industrial biomass production and the low productivity caused by temperature rise inside the culture ponds due to high levels of sunlight in bioreactors [64]. These studies are useful for predicting the impact of temperature shock on microorganisms living in ponds and tidal environments. Cold stress is known to reduce growth in many taxa, by decreasing enzyme activity and membrane fluidity which is compensated by unsaturation of membrane lipids. Heat stress conversely causes high fluidization of the membranes, which is compensated by the synthesis of membrane-stabilizing proteins and the replacement of unsaturated fatty acids by *de novo* synthetized saturated fatty acids [65].

The effects of temperature increase, or decrease are known to affect the metabolism in many species of microalgae. The underlying process that controls these responses as well as other physiological responses to temperature stress is slowly being elucidated. A study by Kobayashi and colleagues (2014) [66] showed that *Cyanidioschyzon merolae,* which is a primitive red alga inhabiting acidic hotsprings, is a species with heat-tolerant evolutive traits, including a simpler cell architecture and the expression of unique small heat shock proteins (HSPs). Similar HSPs were found in the freshwater *C. reinhardtii*, which inhabits non-thermal water bodies. The authors found that despite the fact that both organisms express these heat tolerant molecules when exposed to heat shock, the temperature threshold differs and more interestingly, *C. merolae* senses exact temperature rather than a temperature change, prior to HSP (heat shock protein) encoding gene expression response. These results indicate the possible existence of an absolute temperature sensing mechanism which leads to heat shock protection response [66]. Nearly a decade later, it has been shown using transcriptome analysis that ion channels, particularly those transporting Ca^{2+}, play an important role in timely perceiving heat stress, in *Auxenochlorella protothecoides* [67]. The authors showed increasing levels of extracellular Ca^{2+} entering in the cell and an increasing Ca^{2+} influx from chloroplasts into the cytoplasm, under heat stress suggesting a direct link.

An ion-driven temperature sensing mechanism has been also found in ensembles of *Pseudonitzschia fraudulenta* diatoms. Under a thermal gradient from 19 °C to 35 °C, a cohort of *P. fraudulenta* diatoms exhibited a quasi-periodic electrical signal. The measured electrical current oscillations changed as a function of temperature by nearly 2 orders of magnitude and enable the extraction of an activation energy of at least 1 eV [18] a number considerably higher than the activation energy for the diffusion coefficient of ions in water, 0.2 eV [68]. The thermal analysis revealed an increasing ion flux dependent with temperature, highlighting again that intercellular communication is a feedback strategy that informs variations in the physicochemical environment, which is critical for diatom acclimation and survival.

Recently, cytoplasmic Ca^{2+} elevations were observed in diatoms in response to fast cooling [27]. The authors found those elevations in *P. tricornutum* upon fast cooling but not as a response to rapid warming. The cells expressing R-GECO1 showed that the Ca^{2+} elevations initiate at the tips of the cell and then spread toward the central region. Also, a concomitant Ca^{2+}-dependent K^{+} efflux occurs. Furthermore, the authors found a correlation between the kinetics of the rising concentration of Ca^{2+} in the cytoplasm and the decreasing rate of temperature employed. These ionic flux responses are related to cell survival to hypo-osmotic shocks. The authors propose that the Ca^{2+} and K^{+} ionic currents provide cold-acclimation communication which helps the cell withstand simultaneous hypo-osmotic shock. These findings provide insight on the ion fluxes-based communication processes in sensing multiple stresses.

The thermal acclimation process must account for sensing and communication strategies occurring in the cell cohort. A behavioural response to temperature changes and consequent acclimation response on a community level can contribute to explaining how phytoplankton species are so successful in acclimating and surviving to environmental variation.

3.5. Sexual reproduction

Sexual reproduction has long been known in different groups of microalgae. In diatoms, it is a crucial mechanism known to restore the size of the cells, after progressive cell size reduction occurring over time due to mitotic cell division. A diatom frustule is made of two valves that are held together by silicified bands (the girdle), the epivalve and the hypovalve that is slightly smaller. After mitosis, each daughter cell inherits one valve of the mother cell and synthesizes a new hypovalve. As a consequence, one daughter cell is the same size as the mother cell, the other is smaller, which contributes to the decrease with time of the mean cell size of a population. The pennate diatom *Haslea* genus has first been shown to reproduce homothallically (self-fertility) when in clonal cultures [69], but it has been proved *in vitro* to also have heterothallic sexual reproduction (requiring two compatible individuals for sexual reproduction) [70]. This process is well described, and it starts with a girdle-to-girdle pairing of compatible mating cells and then immediately the chloroplasts start to reposition from the girdle to beneath the valves in premeiotic configuration (Fig. 6A), after which a series of reproductive events follow, gametogenesis, fecundation, formation of zygote and auxospore. This auxospore will expand and generate an initial cell that will restore the maximum cell length in a new linage of somatic cells. Contrary to other pennate diatoms, *H. ostrearia* does not have any visible mucilage surrounding the cells or at the contact area. The same is apparent in the case of *Haslea karadagensis* [71], *Haslea provincialis* [72] and *Haslea silbo* [73], the other blue *Haslea* species in which heterothallic reproduction has been observed. The process is quite similar, and the authors refer that no mucilage was visible. This evidences that other factors than mucilage contribute to the positioning and maintaining of the mating cells in heterothallic diatoms.

Recently, independent transcriptomic datasets have been analysed in the pennate diatom *Seminavis robusta* to prioritize genes responding to sex inducing pheromones (SIPs), suggesting a significant role of Guanosine 3′,5′-cyclic monophosphate (cGMP) signalling during the early stages of sexual reproduction, more specifically the production of the attraction pheromone diproline in mating type – and mate-searching behavior in mating type + [74]. It is worthwhile noting that cGMP is a well-known secondary messenger molecule that modulates bacterial cell growth and behaviour including motility, virulence, biofilm formation and cell cycle progression via activation and alteration of specific ion channels, including Ca^{2+} [75]. In fact, the existence of a handover distance in which the identification and attraction of a compatible mating cell can occur is likely to facilitate intercellular coordination between partners. This coordination could be mediated by ion gradients prompted by pheromones, which triggers heterothallic reproduction and possibly homothallic reproduction as well.

Another example is sexual reproduction in *Volvox* (Fig. 6B, 6C and

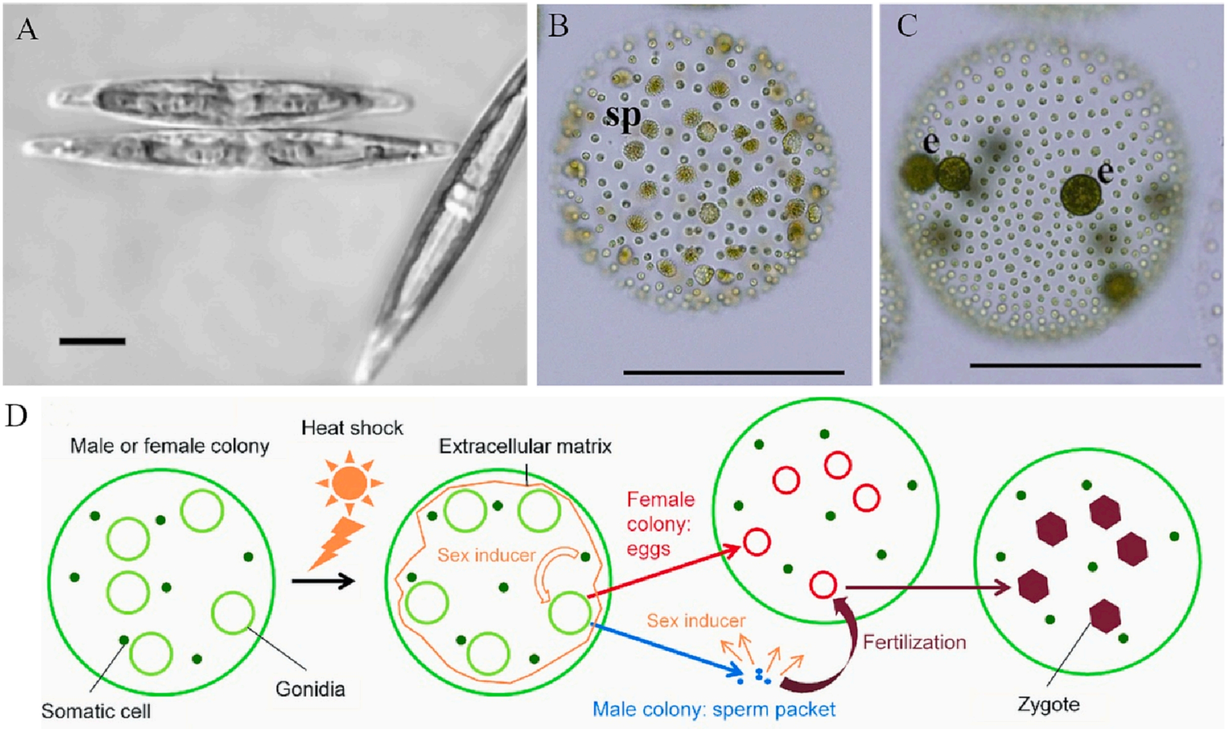


Fig. 6. Sexual reproduction of microalgae as a cue to communication. (A) Girdle-girdle pairing of two gametangia cells in heterothallic formation of *Haslea ostrearia*. Reproduced with permissions from [70], copyright Taylor & Francis, scale bar 10 μm. (B, C, D) *Volvox* sexual reproduction; (B) Adult male *Volvox africanus* with sperm packets (sp), scale bar 200 μm; (C) Adult female colony with eggs (e), scale bar 200 μm; (D) Vegetative *Volvox* colonies are hollow spheres of cells and display a degree of specialization, the somatic *Chlamydomonas*-like cells form a network bound by extracellular matrix, positioned with the flagella outward. Inside the colony there are specialized reproductive gonidia cells. (D) Sexual reproduction in *Volvox* triggered by heat stress. The somatic cells start producing sex-inducing pheromones which induce alterations of the extracellular matrix and cause sperm and egg production in male and female colonies, respectively. After release from male colony, the sperm pack finds a female colony and then the sperm enters and finds an egg, the resulting zygote is drought resistant. We speculate that in parallel with the pheromones and chemical alterations of the process, the sperm cells sense and communicate the location of the mating counterparts through ion fluxes within handover distance (B) and (C) Reproduced from [79], copyright Springer Nature under CCA4 licence (https://creativecommons.org/licenses/by/4.0/); (D) Reproduced from [78], copyright John Wiley & Sons.

6D). *Volvox* species are the most recent in the volvocine lineage of evolution, which starts with *Chlamydomonas* unicell and evolved through the line of succession into more complex and grazing-resistant species consisting of more *Chlamydomonas*-like cells organized in colonies such as *Volvox* [76]. *Volvox* is thus a well-studied algal genus because it is considered as an evolutionary transition to multicellularity [77]. The reason is that unlike other colonial organisms consisting of organized homogenous cell aggregates, the colonies of *Volvox* exhibit a degree of specialization. Vegetative spheroids possess a fixed number of somatic cells, in the order of ~ 2000, which resemble *Chlamydomonas* unicells. They are precisely positioned and oriented in a monolayer bound by a glycoprotein-rich extracellular matrix at the surface of the colony, forming a hollow sphere. These cells possess two flagella positioned outwards of the spheroid, which are responsible for the movements of the colony, and their eyespot guides the colony according to light requirements. The other specialized cells form the asexual germline of *Volvox* and are called gonidia which are larger, aflagellar cells positioned in tiers in the inner face of the spheroid. They undergo a program of embryonic differentiation to produce miniature juvenile spheroids residing inside the mother colony. Eventually these grow up to a point that they break out and start a new round of vegetative colonies [77].

Regarding sexual reproduction, studies with male and female spheroids of *Volvox africanus* (Fig. 6B, 6C) shed some light on the complex sexual systems of this species and the signalling involved in mating colonies. Around 10 eggs form in the female spheroid and sperm packets form and are distributed through the surface of the male spheroid, each discharge 100–200 male gametes. It is still unknown how exactly the sperm reaches and is accepted by the female spheroid. We speculate that synchronized steps which involve handover distance of reproductive cells, possibly has an ionic flux intervenient for the identification of a compatible mating cell [78]. Interestingly, in *V. carteri*, sexual reproduction is triggered as a response to drought, since only dormant zygotes survive in dry ponds during the summer. Species-specific sexual hormones (glycoproteins) are released by somatic cells after heat shock and induce gametogenesis in the gonidia of male and female individuals. Then, the initial sperm cells also produce these proteins, which now act as pheromones which induce male conversion throughout the spheroid and also cause molecular remodelling of the extracellular matrix to become sticky and to be able to facilitate transport of the positively charged SIP. The release of the sperm packets is followed by the necessary finding of a female spheroid, entering the colony and then each sperm must find an egg to fertilize. The formed zygote initially grows inside the mother colony (Fig. 6D) [78]. This complex process involves two moments when flagellated sperm cells must find their female counterparts and besides the contribution of pheromones in the process, there is also a possibility that ion fluxes may intervein in the sensing and detection of a mate, within handover distance.

Another evidence for cohort communication is the extreme case of the volvocine species *Chlamydomonas reinhardtii* which lacks pheromones but is able to obtain synchronization of sexual reproduction events from the start. In summary, the identification and pairing of mating cells seems to involve more than pheromone attraction, and it can be hypothesized they are co-mediated by ion-driven channels [80].

4. Mechanisms of communication in microalgae

The evidence cited above indicates that ion channels are important for sensing and transmitting acclimation responses and signals throughout microalgal communities. Ca^{2+} ions are also necessary in the transduction of spatial and temporal signals due to their rapid reversible binding ability to molecules. The generation of Ca^{2+} signals in microalgae has similar features to animal cells [42]. Algal cells show cytosolic Ca^{2+} elevations in response to environmental stress, such as those observed in the marine diatom *P. tricornutum* upon mechanical stimuli [26] or hypo-osmotic stress [46] and in the marine diatom *P. fraudulenta* after light stress [18]. The rise of cytosolic Ca^{2+} was also observed in the freshwater *C. reinhardtii* after osmotic shock exposure [81]. The observation of Ca^{2+} elevations in the cytosol after environmental stress in microalgae supports the view that Ca^{2+} ion fluxes participate in spatial and temporal communication in algal cells as a response to stress. A key question therefore is how do microalgae increase Ca^{2+} ions in the cytoplasm?

Cells actively maintain low Ca^{2+} levels in the cytosol *ca.* less than 1 mmol/m^3 [82], in order to prevent the toxic effects of excessive Ca^{2+} cytosolic concentration. Much of the cell's Ca^{2+} is in storage organelles, therefore enabling the cells to finely regulate its cytosolic concentration. In animal cells the largest Ca^{2+} storage compartment is the endoplasmic reticulum (ER), where it reaches 1 mM, comparing to the cytosolic concentration of about 100 nM. Mitochondria and lysosomes are also known Ca^{2+} storage organelles in animal cells. Upon physiological stimuli, Ca^{2+} is released from the ER to the cytoplasm which in turn stimulates Ca^{2+} influx through the plasma membrane, therefore creating a transient cytosolic Ca^{2+} concentration rise which is used by the cell for Ca^{2+} dependent functions [83]. The Ca^{2+} flux from the storage organelle to the cytoplasm is through ligand-gated Ca^{2+} channels in the organelle membrane, called inositol 1,4,5-trisphosphate receptors (IP3R) [84]. There are a few studies indicating the presence of IP3-mediated signalling in few non-animal eukaryotes such as the macroalga *Ulva compressa* [85], but they are mostly absent [42]. Recent studies in the cellular membrane of *U. compressa* show that intracellular calcium release is due to the initial extracellular calcium entry through activated transient receptor potential (TRP) channels which provokes depolarization and activation of voltage-gated calcium channels allowing additional calcium entry. In *U. compressa*, this increase of intracellular calcium triggers the release of IP3 from the cell membrane and this leads to the release of calcium stored in the endoplasmic reticulum to the cytoplasm [85]. TRP genes are absent in plant genomes but present in macroalgae [85] and recent studies revealed the structure of TRP channels in *Chlamydomonas reinhardtii* involved in temperature stress response [83] but more studies are needed to understand the importance and distribution of these channels in ion-driven response to stress in microalgae.

Regarding the storage of Ca^{2+} in microalgal cells, there is evidence that chloroplasts are storage organelles in diatoms [18], but other organelles may also be such reservoirs, such as the nucleus [46]. The role of the vacuole as storage organelle in Ca^{2+} communication is still unclear, but very likely that it serves the role of osmotic regulation in the cytoplasm. The fusiform morphotypes of *P. tricornutum* exhibit two large vacuoles in each side of the cell. Upon hypo-osmotic stress, vacuole integrity and osmotic regulation functions rely on the Ca^{2+} signalling because Ca^{2+} independent efflux of organic osmolytes is not sufficient to overcome osmotic stress to the cell [46]. Further research is therefore necessary to dissect the cellular mechanisms involved in increased calcium in the cytoplasm.

There is some evidence of the involvement of the vacuole in algal communication from early studies on freshwater microalgae. The first studies of action potential in plant cells were conducted in the freshwater microalgal family *Characeae*, due to the realization that their large internodal cells were excitable and generated a signal, which was considered as an action potential [86]. These early studies focused mostly in species of *Chara* [87,88], *Nitella* [89,90] and *Nitellopsis* [91]. Since the vacuole occupies most of the cell volume in these internodal cells, Findlay and Hope [87] used *Chara australis* to demonstrate that Ca^{2+} mediates an "action potential" across the tonoplast (the vacuole membrane). The role of Cl^- in the intracellular signalling was evidenced, with these early studies showing that Ca^{2+} mediate an increase in the permeability of the plasmalemma to anions including chloride, following a stimulus [88].

The Ca^{2+} diffusion into and out of the cell is possible through membrane channels and pumps. When open, the gated channels let selected ions diffuse rapidly down electrical and osmotic gradients, whereas ion pumps are constantly active by consuming energy to slowly move ions against the ion gradients [92]. In animal cells the increases in intracellular Ca^{2+} concentration may be propagated to directly contacting neighboring cells, thus originating intercellular Ca^{2+} waves as a mechanism for coordinating multicellular responses. The onset of such waves is triggered by a variety of stimuli and involves the release of Ca^{2+} from internal stores [93]. In animal cells the gap junction pannexins play a significant role in paracrine signalling by releasing ATP and they modulate the range of intercellular Ca^{2+} waves transmitted between astrocytes [94]. In contrast, their existence in microalgae is unknown. The recurrently reported ion efflux to extracellular space is further discussed in detail in section 4.1.

4.1. Model of paracrine communication in diatoms

To account for the many observation in algae, we propose a model which explains how communication through Ca^{2+} may occur. We hypothesize that a stressor-specific physiological response triggers communication at a community level, which starts a collective acclimation response. The process happens in two distinctive phases, the signal generation (Fig. 7A) and the signal transmission (Fig. 7B). *Signal generation* likely occurs not in a single cell, but in a particular group of cells, which are simultaneously exposed to an environmental stress event, considered as a trigger. The cues explained in the previous sections indicate that possible environmental triggers are variations of the ideal conditions such as nutrient availability, light intensity, temperature and grazing pressure. In our model, we consider light stress response on a marine diatom.

We argue that it is not the amount of high background level of dissolved Ca^{2+} but rather the capacity to disturb the resting membrane potential. The resting membrane potential is maintained, with the cytoplasm charged negatively, by mechanisms of maintaining positively charged ions outside, namely through K^+ outward leakage channels and also Ca^{2+} storage in organelles such as the chloroplast. When a stimulus occurs e.g., an environmental trigger, Ca^{2+} is released from the storage organelles by Ca^{2+} channels (element (i) in Fig. 7A), causing elevation of Ca^{2+} concentration in the cytoplasm, followed by intracellular diffusion. Although the Ca^{2+} channels involved in the release of stored Ca^{2+} from the chloroplast is not yet determined, it may function in a similar way to animal ER membrane-bound Ca^{2+} channel IR3R. The identification and characterization of the storage sites and storage/release mechanisms involved in intracellular Ca^{2+} in microalgae is still not well understood.

The immediate consequence of the positively charged ionic loading of the cytosol (the aqueous content of the cytoplasm, sometimes generalized in the term cytoplasm) is an altered membrane potential, which opens membrane voltage-gated Ca^{2+} channels (EukCats) and Ca^{2+} enters the cell (element (ii) in Fig. 7A). If the environmental trigger is a molecule such as allelochemicals or hormones, the Ca^{2+} intake possibly occurs through ligand-gated membrane channels, which potentiates the initial rise in intracellular Ca^{2+}. At this point, the cell is loaded with elevated Ca^{2+} concentration, affecting the cell osmolarity and electric balance. The population of cells which are under the direct influence of the environmental trigger are represented by cell A in Fig. 7A.

The *signal propagation* (Fig. 7B) starts after the stress-sensing cell cohort activates mechanisms to expel the elevated Ca^{2+} concentration

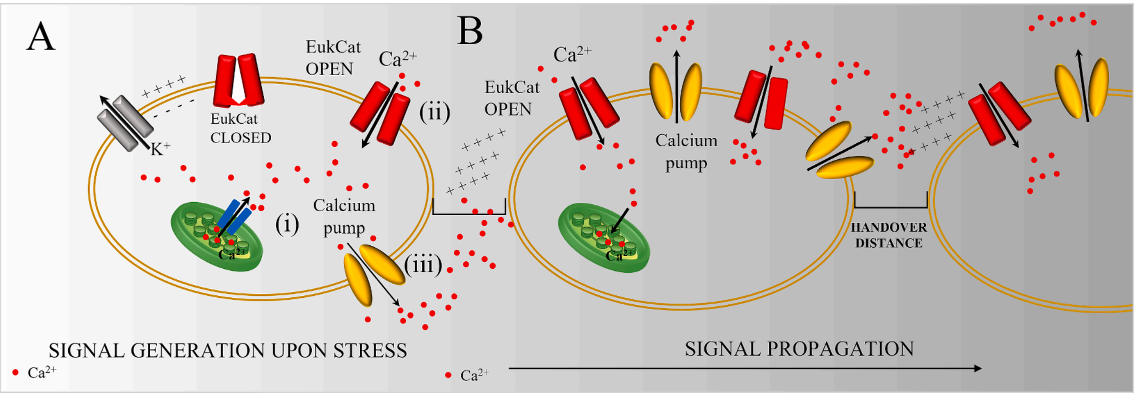


Fig. 7. Model of paracrine communication in diatoms upon stress, based on Ca^{2+} diffusion fluxes. The algal cell wall is not represented, for simplicity. The mechanism of signal transduction has two distinct phases. Signal generation (A), part of the community of algal cells suffers an environmental trigger, which originates the signal generation. The Ca^{2+} ions are recruited from storage organelles causing excessive positive charging of the cytosol (i). Then the voltage-gated channels open allowing the intake of Ca^{2+} to the cytosol, which causes the aggravation of positive charge and osmotic imbalance (ii). The cell activates the Ca^{2+} pumps to exclude Ca^{2+} ions (iii) and also some are recruited back to storage. Signal propagation (B), when the neighboring cells out of the stressor range zone are in a handover distance, the Ca^{2+} diffusion causes a positively charged environment in the vicinity, leading to the opening of voltage-gated channels and the Ca^{2+} diffusion into the cell, starting Ca^{2+} diffusion waves along the population of cells.

within the cytoplasm into the surrounding extracellular environment. We hypothesize that most Ca^{2+} ions stored in the cytoplasm are expelled outward, to the extracellular vicinity, via Ca^{2+} pumps (element (iii) in Fig. 7A). To ascertain cell osmotic regulation, a small proportion of Ca^{2+} ions are likely restored in the internal storage sites of the cells, such as chloroplasts.

The Ca^{2+} concentration elevates in the extracellular environment and reaches the neighboring cells, which haven't been affected by the stress-triggering event and are located within the so-called handover distance (Fig. 7B). The increase in Ca^{2+} concentration creates a significative rise of positively charged ions, above a certain threshold, causing their membrane voltage-gated channels to open and intake Ca^{2+} ions. The communication signal is augmented, which, in turn, starts an orchestrated mechanisms of Ca^{2+} diffusion outward by the ion pumps and into the storage organelles. These diffusion waves cause the propagation of the signal to neighboring cells in the form of oscillations occurring at spatial locations that reflect Ca^{2+} outflux. The signal length is possibly determined by the ionic diffusion rate in that particular habitat and also the cell proximity, which must be within tens of micrometers. This mechanism provides intercellular communication to cells outside of stressor range. In this way these peripheral cells receive the signal through electrically driven propagation of the Ca^{2+} wave. The cohort communication process falls into the concept of paracrine communication, where extracellular Ca^{2+} may function as a paracrine messenger by triggering the opening of plasma membrane-bound Ca^{2+} channels. It does not exclude the possibility that other ions may be involved, such as Na$^+$, widely available in the case of marine diatoms (also transported through EukCats) and Cl$^-$, as previously mentioned. There is also a possibility that other molecules may act as a paracrine messenger, by acting on Ca^{2+} sensing receptors (not yet described for most microalgae) or on ligand-sensing receptors (channels).

Our model may be expanded to include studies with osmotic stress, which triggers Ca^{2+} communication with specific spatial–temporal patterns. Osmotic variations cause several adaptive responses in diatoms, such as the morphotype interchange of the pleomorphic *P. tricornutum*, with the oval form considered as the most hypo-osmotic stress tolerant [95]. The cells acclimate to repetitive hypo-osmotic shock. Hypo-osmotic treatment leads to cell swelling and triggers Ca^{2+} elevations in the cytosol. Upon mild hypo-osmotic treatment, the Ca^{2+} diffusion starts at the apical tips of the cell and then diffuses to the rest of the cytosol whereas a half-strength hypo-osmotic treatment originated rapid and transient Ca^{2+} elevation along the cell simultaneously [46]. The role of the apical tip in *P. tricornutum* fusiform cells is therefore important in sensing subtle osmotic stress. The benthic oval morphotype does not display this lateral diffusion and it has been proven to be less sensitive to subtle hypo-osmotic stress and more resilient to fluctuating osmotic conditions [46]. In the freshwater *C. reinhardtii*, salt stress induces single Ca^{2+} elevation in the cells, the rise of Ca^{2+} concentration being modulated by the strength of the stimulus. The rise of Ca^{2+} originates in the apex of the cell and then spreads as a fast Ca^{2+} wave. In contrast, hypo-osmotic stress induces a series of repetitive and uniform Ca^{2+} elevations in the cytosol and also independently in the flagella [81].

5. Conclusions and perspectives

Intercellular communication in microalgal communities was reviewed for short term acclimation to stress in the aquatic ecosystem and long-term prevalence and evolution. Ion channels enable the regulated ion flow across membranes and mediate cell-to-cell communication through a mass diffusion process. Ion channels and their electrogenic activity are thus hypothesized to be a key component of the acclimation capacity of microalgae against environmental stressors, which paves the way for future research in omic methods and phytoplankton ecophysiology.

Marine phytoplankton in general and diatoms in particular are also able to communicate between conspecifics and acclimate to sudden physiological or chemical changes in their environment. Recent studies suggest that diatoms are capable of paracrine communication with the intercellular messenger, Ca^{2+}. A chemical signal is released, recognized, and transmitted by neighbouring cells within handover distance, herewith propagating a message throughout the entire community based on paracrine signalling of Ca^{2+}.

A spectrum of cues and evidence highlighted communication as one of the most ancient and yet robust acclimation strategies for survival and adaptation. Patterns of intercellular interactions are currently overlooked and need to be addressed with advanced sensing technology, including (but not limited to) functional multielectrode arrays coupled with electrochemical noise and impedance spectroscopy analytical tools, fluorescence microscopy, pulse-amplitude-modulation fluorometry and saturation pulse methods. Future studies aiming at identifying and functionally characterizing diatom ion channels are guaranteed to help advance the understanding of the role of membrane excitability, intercellular Ca^{2+} communication and ion fluxes in the acclimatory collective response and change in diatom population fitness with fluctuating environments. Ion-driven synchronization of microalgal cohorts could contribute to obtaining more efficient bioelectricity from microalgal-aided systems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] R.E. Blankenship, Early evolution of photosynthesis, Plant Physiol. 154 (2) (2010) 434–438.

[2] P.G. Falkowski, M.E. Katz, A.H. Knoll, A. Quigg, J.A. Raven, O. Schofield, F.J. R. Taylor, The evolution of modern eukaryotic phytoplankton, Science 305 (5682) (2004) 354–360.

[3] C.B. Field, M.J. Behrenfeld, J.T. Randerson, P. Falkowski, Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components, Science (80-) 281 (1998) 237–240, https://doi.org/10.1042/bst0040954.

[4] J. Singh, R.C. Saxena, An Introduction to Microalgae: Diversity and Significance, in: S.-.-K. Kim (Ed.), Handbook of Marine Microalgae: Biotechnology Advances, Elsevier, 2015, pp. 11–24, https://doi.org/10.1016/B978-0-12-800776-1.00002-9.

[5] G. Wheeler, K. Helliwell, C. Brownlee, Calcium signalling in algae, Perspect. Phycol. 6 (1–2) (2019) 1–10, https://doi.org/10.1127/pip/2018/0082.

[6] S. Mugnai, N. Derossi, Y. Hendlin, Algae communication, conspecific and interspecific: the concepts of phycosphere and algal-bacteria consortia in a photobioreactor (PBR), Plant Signal. Behav. 18 (1) (2023) pp, https://doi.org/10.1080/15592324.2022.2148371.

[7] S. Luan, C. Wang, Calcium signaling mechanisms across kingdoms, Annu. Rev. Cell Dev. Biol. 37 (1) (2021) 311–340.

[8] R.J. Wicker, E.K. Kwon, V. Kumar, A. Bhatnagar, The potential of mixed-species biofilms to address remaining challenges for economically-feasible microalgal biorefineries: A review, Annu. Rev. Cell Dev. Biol. 37 (2021) 311–340, https://doi.org/10.1146/annurev-cellbio-120219-035210.

[9] M. Sharma, E.S. Salama, N. Thakur, H. Alghamdi, B.H. Jeon, X. Li, Advances in the biomass valorization in bioelectrochemical systems: A sustainable approach for microbial-aided electricity and hydrogen production, Chem. Eng. J. 465 (March) (2023) 142546, https://doi.org/10.1016/j.cej.2023.142546.

[10] K. Behl, M. Joshi, M. Sharma, S. Tandon, A.K. Chaurasia, A. Bhatnagar, S. Nigam, Performance evaluation of isolated electrogenic microalga coupled with graphene oxide for decolorization of textile dye wastewater and subsequent lipid production, Chem. Eng. J. 375 (2019) 121950.

[11] C. Bleuven, C.R. Landry, Molecular and cellular bases of adaptation to a changing environment in microorganisms, Proc. R. Soc. B Biol. Sci. 283 (1841) 2016, https://doi.org/10.1098/rspb.2016.1458.

[12] A. Vardi, F. Formiggini, R. Casotti, A. De Martino, F. Ribalet, A. Miralto, C. Bowler, J. Dangl, A stress surveillance system based on calcium and nitric oxide in marine diatoms, PLOS Biol. 4 (3) (Feb. 2006) e60.

[13] M. Venuleo, J.A. Raven, M. Giordano, Intraspecific chemical communication in microalgae, New Phytol. 215 (2) (2017) 516–530, https://doi.org/10.1111/nph.14524.

[14] B. Martinac, Y. Saimi, C. Kung, Ion channels in microbes, Physiol. Rev. 88 (4) (Oct. 2008) 1449–1490, https://doi.org/10.1152/physrev.00005.2008.

[15] L. Galera-Laporta, C.J. Comerci, J. Garcia-Ojalvo, G.M. Süel, IonoBiology: The functional dynamics of the intracellular metallome, with lessons from bacteria, Cell Syst. 12 (6) (2021) 497–508, https://doi.org/10.1016/j.cels.2021.04.011.

[16] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, "Ion Channels and the Electrical Properties of Membranes", in *Molecular Biology of the Cell*, 4th ed.,, Garland Science, New York, 2002.

[17] S.H. Loukin, M.M.C. Kuo, X.L. Zhou, W.J. Haynes, C. Kung, Y. Saimi, Microbial K+ channels, J. Gen. Physiol. 125 (6) (2005) 521–527, https://doi.org/10.1085/jgp.200509261.

[18] P.R.F. Rocha, A.D. Silva, L. Godinho, W. Dane, P. Estrela, L.K.J. Vandamme, J. B. Pereira-Leal, D.M. de Leeuw, R.B. Leite, Collective electrical oscillations of a diatom population induced by dark stress, Sci. Rep. 8 (1) (Dec. 2018), https://doi.org/10.1038/s41598-018-23928-9.

[19] E.V. Armbrust, The life of diatoms in the world's oceans, Nature 459 (7244) (May 14 2009) 185–192, https://doi.org/10.1038/nature08057.

[20] V. Smetacek, Diatoms and the silicate factor, Nature 391 (6664) (1998) 224–225.

[21] A. Moustafa, B. Beszteri, U.G. Maier, C. Bowler, K. Valentin, D. Bhattacharya, Genomic footprints of a cryptic plastid endosymbiosis in diatoms, Science (80-) 324 (5935) (2009) 1724–1726, https://doi.org/10.1126/science.1172983.

[22] K.E. Helliwell, E.L. Harrison, J.A. Christie-Oleza, A.P. Rees, F.H. Kleiner, T. Gaikwad, J. Downe, M.M. Aguilo-Ferretjans, L. Al-Moosawi, C. Brownlee, G. L. Wheeler, A Novel Ca2+ Signaling Pathway Coordinates Environmental Phosphorus Sensing and Nitrogen Metabolism in Marine Diatoms, Curr. Biol. 31 (5) (2021) 978–989.e4.

[23] K.E. Helliwell, A. Chrachri, J.A. Koester, S. Wharam, F. Verret, A.R. Taylor, G. L. Wheeler, C. Brownlee, Alternative Mechanisms for Fast Na + /Ca 2+ Signaling in Eukaryotes via a Novel Class of Single-Domain Voltage-Gated Channels, Curr. Biol. 29 (9) (2019) 1503–1511.e6.

[24] A.R. Taylor, F. Berger, A fast Na+/Ca2+-based action potential in a marine diatom, PLoS One 4 (3) (Mar. 2009) e4966.

[25] R. Hedrich, Diatom signaling: a novel channel type identified, Curr. Biol. 29 (9) (2019) R319–R321, https://doi.org/10.1016/j.cub.2019.03.038.

[26] A. Falciatore, M.R. D'Alcalà, P. Croot, C. Bowler, Perception of environmental signals by a marine diatom, Science (80-) 288 (5475) (2000) 2363–2366, https://doi.org/10.1126/science.288.5475.2363.

[27] F.H. Kleiner, K.E. Helliwell, A. Chrachri, A. Hopes, H. Parry-Wilson, T. Gaikwad, N. Mieszkowska, T. Mock, G.L. Wheeler, C. Brownlee, Cold-induced [Ca2 +]cyt elevations function to support osmoregulation in marine diatoms, Plant Physiol. 190 (2) (2022) 1384–1399.

[28] P.R.F. Rocha, P. Schlett, U. Kintzel, V. Mailänder, L.K.J. Vandamme, G. Zeck, H. L. Gomes, F. Biscarini, D.M. de Leeuw, Electrochemical noise and impedance of Au electrode/electrolyte interfaces enabling extracellular detection of glioma cell populations, Sci. Rep. 6 (1) (2016), https://doi.org/10.1038/srep34843.

[29] P.R.F. Rocha, M.C.R. Medeiros, U. Kintzel, J. Vogt, I.M. Araújo, A.L.G. Mestre, V. Mailänder, P. Schlett, M. Dröge, L. Schneider, F. Biscarini, D.M. de Leeuw, H. L. Gomes, Extracellular electrical recording of pH-triggered bursts in C6 glioma cell populations, Sci. Adv. 2 (12) (2016), https://doi.org/10.1126/sciadv.1600516.

[30] D. a Borkholder, Cell Based Biosensors using Microelectrodes, PhD thesis, no. September, pp. 1–253, 1998.

[31] A. Einstein, Uber die von der molekularkinetischen Theorie der Warme geforderte Bewegung vow in ruhenden Flussigkeiten suspendierten Teilchen; von A. Einstein, Ann. Phys. (N. Y)., 322(8) (1905) 549–560 [Online]. Available: http://onlinelibrary.wiley.com/doi/10.1002/andp.19053220806/abstract.

[32] D.K.C. MacDonald, Noise and Fluctuations, Dover edit, Dover, New York, 1962.

[33] R.A. Smith, Semiconductors, Cambridge, Cambridge, New York, 1978.

[34] S.O. Kasap, Principles of Electronic Materials and Devices, 4th ed., McGraw-Hill Education, New York, 2002.

[35] Y.H. Li, S. Gregory, Diffusion of ions in sea water and in deep-sea sediments, Geochim. Cosmochim. Acta 38(2) (1974) 703–714. [Online]. Available: https://ac.els-cdn.com/0016703774901458/1-s2.0-0016703774901458-main.pdf?_tid=b35975da-7bec-4114-bab2-ef1ca3163da1&acdnat=1542644091_a41d580f1d3f24f07fa2219e6d73d4b4.

[36] D. Gradmann, C.M. Boyd, Membrane voltage of marine phytoplankton, measured in the diatom Coscinodiscus radiatus, Mar. Biol. 123 (4) (1995) 645–650, https://doi.org/10.1007/BF00349107.

[37] A.L. Hodgkin, A.F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve, J. Physiol. 117 (4) (1952) 500–544.

[38] R. Llinás, Y. Yarom, Properties and distribution of ionic conductances generating electroresponsiveness of mammalian inferior olivary neurones in vitro, J. Physiol. 315 (1) (1981) 569–584, https://doi.org/10.1113/jphysiol.1981.sp013764.

[39] A.L. Goldin, Evolution of voltage-gated Na+ channels, J. Exp. Biol. 205 (2002) 575–584.

[40] E.V. Armbrust, J.A. Berges, C. Bowler, B.R. Green, D. Martinez, N.H. Putnam, S. Zhou, A.E. Allen, K.E. Apt, M. Bechner, M.A. Brzezinski, B.K. Chaal, A. Chiovitti, A.K. Davis, M.S. Demarest, J.C. Detter, T. Glavina, D. Goodstein, M.Z. Hadi, U. Hellsten, M. Hildebrand, B.D. Jenkins, J. Jurka, V.V. Kapitonov, N. Kröger, W. W.Y. Lau, T.W. Lane, F.W. Larimer, J.C. Lippmeier, S. Lucas, Mónica Medina, A. Montsant, M. Obornik, M.S. Parker, B. Palenik, G.J. Pazour, P.M. Richardson, T. A. Rynearson, M.A. Saito, D.C. Schwartz, K. Thamatrakoln, K. Valentin, A. Vardi, F. P. Wilkerson, D.S. Rokhsar, The genome of the diatom Thalassiosira Pseudonana: Ecology, evolution, and metabolism, Science (80-.) 306 (5693) (2004) 79–86.

[41] C. Bowler, A.E. Allen, J.H. Badger, J. Grimwood, K. Jabbari, A. Kuo, U. Maheswari, C. Martens, F. Maumus, R.P. Otillar, E. Rayko, A. Salamov, K. Vandepoele, B. Beszteri, A. Gruber, M. Heijde, M. Katinka, T. Mock, K. Valentin, F. Verret, J. A. Berges, C. Brownlee, J.-P. Cadoret, A. Chiovitti, C.J. Choi, S. Coesel, A. De Martino, J.C. Detter, C. Durkin, A. Falciatore, J. Fournet, M. Haruta, M.J. J. Huysman, B.D. Jenkins, K. Jiroutova, R.E. Jorgensen, Y. Joubert, A. Kaplan, N. Kröger, P.G. Kroth, J. La Roche, E. Lindquist, M. Lommer, V. Martin–Jézéquel, P. J. Lopez, S. Lucas, M. Mangogna, K. McGinnis, L.K. Medlin, A. Montsant, M.-P. Secq, C. Napoli, M. Obornik, M.S. Parker, J.-L. Petit, B.M. Porcel, N. Poulsen, M. Robison, L. Rychlewski, T.A. Rynearson, J. Schmutz, H. Shapiro, M. Siaut, M. Stanley, M.R. Sussman, A.R. Taylor, A. Vardi, P. von Dassow, W. Vyverman, A. Willis, L.S. Wyrwicz, D.S. Rokhsar, J. Weissenbach, E.V. Armbrust, B.R. Green,

Y. Van de Peer, I.V. Grigoriev, The Phaeodactylum genome reveals the evolutionary history of diatom genomes, Nature 456 (7219) (2008) 239–244.

[42] F. Verret, G. Wheeler, A.R. Taylor, G. Farnham, C. Brownlee, Calcium channels in photosynthetic eukaryotes: Implications for evolution of calcium-based signalling, New Phytol. 187 (1) (2010) 23–43, https://doi.org/10.1111/j.1469-8137.2010.03271.x.

[43] M. Ito, H. Xu, A.A. Guffanti, Y.i. Wei, L. Zvi, D.E. Clapham, T.A. Krulwich, The voltage-gated Na+ channel NavBP has a role in motility, chemotaxis, and pH homeostasis of an alkaliphilic Bacillus, Proc. Natl. Acad. Sci. U. S. A. 101 (29) (2004) 10566–10571.

[44] E. Padan, E. Bibi, M. Ito, T.A. Krulwich, Alkaline pH homeostasis in bacteria: New insights, Biochim. Biophys. Acta - Biomembr. 1717 (2) (2005) 67–88, https://doi.org/10.1016/j.bbamem.2005.09.010.

[45] P.J. Keeling, F. Burki, H.M. Wilcox, B. Allam, E.E. Allen, L.A. Amaral-Zettler, E. V. Armbrust, J.M. Archibald, A.K. Bharti, C.J. Bell, B. Beszteri, K.D. Bidle, C. T. Cameron, L. Campbell, D.A. Caron, R.A. Cattolico, J.L. Collier, K. Coyne, S. K. Davy, P. Deschamps, S.T. Dyhrman, B. Edvardsen, R.D. Gates, C.J. Gobler, S. J. Greenwood, S.M. Guida, J.L. Jacobi, K.S. Jakobsen, E.R. James, B. Jenkins, U. John, M.D. Johnson, A.R. Juhl, A. Kamp, L.A. Katz, R. Kiene, A. Kudryavtsev, B. S. Leander, S. Lin, C. Lovejoy, D. Lynn, A. Marchetti, G. McManus, A.M. Nedelcu, S. Menden-Deuer, C. Miceli, T. Mock, M. Montresor, M.A. Moran, S. Murray, G. Nadathur, S. Nagai, P.B. Ngam, B. Palenik, J. Pawlowski, G. Petroni, G. Piganeau, M.C. Posewitz, K. Rengefors, G. Romano, M.E. Rumpho, T. Rynearson, K.B. Schilling, D.C. Schroeder, A.G.B. Simpson, C.H. Slamovits, D.R. Smith, G. J. Smith, S.R. Smith, H.M. Sosik, P. Stief, E. Theriot, S.N. Twary, P.E. Umale, D. Vaulot, B. Wawrik, G.L. Wheeler, W.H. Wilson, Y. Xu, A. Zingone, A.Z. Worden, R.G. Roberts, The Marine Microbial Eukaryote Transcriptome Sequencing Project (MMETSP): Illuminating the Functional Diversity of Eukaryotic Life in the Oceans through Transcriptome Sequencing, PLoS Biol. 12 (6) (2014) e1001889.

[46] K.E. Helliwell, F.H. Kleiner, H. Hardstaff, A. Chrachri, T. Gaikwad, D. Salmon, N. Smirnoff, G.L. Wheeler, C. Brownlee, Spatiotemporal patterns of intracellular Ca2+ signalling govern hypo-osmotic stress resilience in marine diatoms, New Phytol. 230 (1) (2021) 155–170.

[47] L.J. Rothschild, R.L. Mancinelli, Life in extreme environments, Nature 409 (2001) 1092–1101.

[48] M. Lürling, Phenotypic plasticity in the green algae Desmodesmus and Scenedesmus with special reference to the induction of defensive morphology, Ann. Limnol. 39 (2) (2003) 85–101, https://doi.org/10.1051/limn/2003014.

[49] M. Lurling, Effect of grazing-associated infochemicals on growth and morphological development in Scenedesmus acutus (Chlorophyceae), J. Phycol. 34 (4) (1998) 578–586.

[50] N.C. Poulsen, I. Spector, T.P. Spurck, T.F. Schultz, R. Wetherbee, Diatom gliding is the result of an actin-myosin motility system, Cell Motil. Cytoskeleton 44 (1) (1999) 23–33, https://doi.org/10.1002/(SICI)1097-0169(199909)44:1<23::AID-CM2>3.0.CO;2-D.

[51] K.G. Bondoc-Naumovitz, S.A. Cohn, Motility of Biofilm-Forming Benthic Diatoms, Diatom Glid. Motil. (2021) 77–109, https://doi.org/10.1002/9781119526483.ch4.

[52] J. Rochaix, Regulation of photosynthetic electron transport, BBA - Bioenerg. Biochimica et Biophysica Acta 1807 (3) (2011) 375–383, https://doi.org/10.1016/j.bbabio.2010.11.010.

[53] R.L. Wilby, et al., Science of the Total Environment Evidence needed to manage freshwater ecosystems in a changing climate: Turning adaptation principles into practice, Sci. Total Environ. 408 (19) (2010) 4150–4164, https://doi.org/10.1016/j.scitotenv.2010.05.014.

[54] S. Sinutok, P. Chotikarn, M. Saengsakda Pattaratumrong, P. Moungkeaw, P. Pramneechote, M. Yucharoen, Synergistic Effect of Elevated Temperature and Light Stresses on Physiology of Pocillopora acuta from Different Environments, J. Mar. Sci. Eng. 10 (6) (2022) 790, https://doi.org/10.3390/jmse10060790.

[55] W.K. Yong, Y.H. Tan, S.W. Poong, P.E. Lim, Response of Microalgae in a Changing Climate and Environment, Malaysian J. Sci. 35 (2) (2016) 169–191, https://doi.org/10.22452/MJS.VOL35NO2.7.

[56] R.G. Perkins, J.C. Kromkamp, J. Serôdio, J. Lavaud, B. Jesus, J.L. Mouget, S. Lefebvre, R.M. Forster, in: Chlorophyll a Fluorescence in Aquatic Sciences: Methods and Applications, Springer Netherlands, Dordrecht, 2010, pp. 237–275.

[57] C. Klughammer, U. Schreiber, Complementary PS II quantum yields calculated from simple fluorescence parameters measured by PAM fluorometry and the Saturation Pulse method, PAM Appl. Notes 1 (2008) 27–35.

[58] R.G. Perkins, J.-L. Mouget, S. Lefebvre, J. Lavaud, Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms, Mar. Biol. 149 (4) (2006) 703–712, https://doi.org/10.1007/s00227-005-0222-z.

[59] R.G. Perkins, K. Oxborough, A.R.M. Hanlon, G.J.C. Underwood, N.R. Baker, Can chlorophyll fluorescence be used to estimate the rate of photosynthetic electron transport within microphytobenthic biofilms? Mar. Ecol. Prog. Ser. 228 (Mar. 2002) 47–56, https://doi.org/10.3354/meps228047.

[60] J. Kromkamp, C. Barranguet, J. Peene, Determination of microphytobenthos PSII quantum efficiency and photosynthetic activity by means of variable chlorophyll fluorescence, Mar. Ecol. Prog. Ser. 162 (1998) 45–55.

[61] M.A. Borowitzka, in: Microalgae in Health and Disease Prevention, Elsevier, 2018, pp. 23–72.

[62] E.P. Spalding, Ion channels and the transduction of light signals, Plant Cell Environ. 23 (7) (2000) 665–674, https://doi.org/10.1046/j.1365-3040.2000.00594.x.

[63] F.F. Litvin, O.A. Sineshchekov, V.A. Sineshchekov, Photoreceptor electric potential in the phototaxis of the alga Haematococcus pluvialis, Nature 271 (5644) (1978) 476–478.

[64] R. Barten, Y. Djohan, W. Evers, R. Wijffels, M. Barbosa, Towards industrial production of microalgae without temperature control: The effect of diel temperature fluctuations on microalgal physiology, J. Biotechnol. 336 (February) (2021) 56–63, https://doi.org/10.1016/j.jbiotec.2021.06.017.

[65] D.A. Los, K.S. Mironov, S.I. Allakhverdiev, Regulatory role of membrane fluidity in gene expression and physiological functions, Photosynth. Res. 116 (2–3) (2013) 489–509, https://doi.org/10.1007/s11120-013-9823-4.

[66] Y. Kobayashi, et al., Algae Sense Exact Temperatures: Small Heat Shock Proteins Are Expressed at the Survival Threshold Temperature in Cyanidioschyzon merolae and Chlamydomonas reinhardtii, Genome Biol. Evol. 6 (10) (2014) 2731–2740.

[67] J. Yang, W. Li, C. Xing, G. Xing, Y. Guo, H. Yuan, Ca2+ participates in the regulation of microalgae triacylglycerol metabolism under heat stress, 112696, Environ. Res. 208 (2022), https://doi.org/10.1016/j.envres.2022.112696.

[68] H. Sato, M. Yui, H. Yoshikawa, Ionic Diffusion Coefficients of Cs+, Pb2+, Sm3+, Ni2+, SeO2- 4 and TcO− 4 in Free Water Determined from Conductivity Measurements, J. Nucl. Sci. Technol. 33 (12) (Dec. 1996) 950–955, https://doi.org/10.1080/18811248.1996.9732037.

[69] D. Neuville, P. Daste, Observations preliminaires concernant láuxosporulation chez la diatomee Navicula ostrearia (Gaillon) Bory en culture in vitro, Comptes rendus Hebd. des seances 281(Serie D) (1975) 1753–1756.

[70] N.A. Davidovich, J.L. Mouget, P. Gaudin, Heterothallism in the pennate diatom Haslea ostrearia (Bacillariophyta), Eur. J. Phycol. 44 (2) (May 2009) 251–261, https://doi.org/10.1080/09670260802710301.

[71] N.A. Davidovich, R. Gastineau, P. Gaudin, O.I. Davidovich, J.L. Mouget, Sexual reproduction in the newly-described blue diatom, Haslea karadagensis, Fottea 12 (2) (2012) 219–229, https://doi.org/10.5507/fot.2012.016.

[72] R. Gastineau, G. Hansen, N.A. Davidovich, O. Davidovich, J.-F. Bardeau, I. Kaczmarska, J.M. Ehrman, V. Leignel, Y. Hardivillier, B. Jacquette, M. Poulin, M. Morançais, J. Fleurence, J.-L. Mouget, A new blue-pigmented hasleoid diatom, Haslea provincialis, from the Mediterranean Sea, Eur. J. Phycol. 51 (2) (2016) 156–170.

[73] R. Gastineau, G. Hansen, M. Poulin, C. Lemieux, M. Turmel, J.-F. Bardeau, V. Leignel, Y. Hardivillier, M. Morançais, J. Fleurence, P. Gaudin, V. Méléder, E. J. Cox, N.A. Davidovich, O.I. Davidovich, A. Witkowski, I. Kaczmarska, J. M. Ehrman, E. Soler Onís, A.M. Quintana, M. Mucko, S. Mordret, D. Sarno, B. Jacquette, C. Falaise, J. Séveno, N.L. Lindquist, P.S. Kemp, E. Eker-Develi, M. Konucu, J.-L. Mouget, Haslea silbo, a novel cosmopolitan species of blue diatoms, Biology (Basel) 10 (4) (2021) 328.

[74] G. Bilcke, K. Van den Berge, S. De Decker, E. Bonneure, N. Poulsen, P. Bulankova, C.M. Osuna-Cruz, J. Dickenson, K. Sabbe, G. Pohnert, K. Vandepoele, S. Mangelinckx, L. Clement, L. De Veylder, W. Vyverman, Mating type specific transcriptomic response to sex inducing pheromone in the pennate diatom Seminavis robusta, ISME J. 15 (2) (2021) 562–576.

[75] U. Jenal, A. Reinders, C. Lori, Cyclic di-GMP: second messenger extraordinaire, Nat. Rev. Microbiol. 15 (5) (2017) 271–284, https://doi.org/10.1038/nrmicro.2016.190.

[76] D.H. Lankenau, The legacy of the germ line-maintaining sex and life in metazoans : Cognitive roots of the concept of hierarchical selection, Genome Dyn. Stab. 3 (2008) 289–339, https://doi.org/10.1007/7050_2007_030.

[77] G. Matt, J. Umen, Volvox: a simple algal model for embryogenesis, morphogenesis and cellular differenciation, Dev. Biol. 419 (1) (2016) 99–113, https://doi.org/10.1016/j.ydbio.2016.07.014.

[78] J. Frenkel, W. Vyverman, G. Pohnert, Pheromone signaling during sexual reproduction in algae, Plant J. 79 (4) (2014) 632–644, https://doi.org/10.1111/tpj.12496.

[79] H. Nozaki, W. Mahakham, W. Heman, R. Matsuzaki, M. Kawachi, Morphology, mating system and taxonomy of Volvox africanus (Volvocaceae, Chlorophyceae) from Thailand, Bot. Stud. 63 (1) (2022) 1–10, https://doi.org/10.1186/s40529-022-00332-1.

[80] R. Luxmi, C. Blaby-Haas, D. Kumar, N. Rauniyar, S.M. King, R.E. Mains, B. A. Eipper, Proteases shape the chlamydomonas secretome: Comparison to classical neuropeptide processing machinery, Proteomes 6 (4) (2018) 36.

[81] P. Bickerton, S. Sello, C. Brownlee, J.K. Pittman, G.L. Wheeler, Spatial and temporal specificity of Ca2+ signalling in Chlamydomonas reinhardtii in response to osmotic stress, New Phytol. 212 (4) (2016) 920–933, https://doi.org/10.1111/nph.14128.

[82] R.E. Williamson, C.C. Ashley, Free Ca2+ and cytoplasmic streaming in the alga Chara, Nature 296 (5858) (1982) 647–651, https://doi.org/10.1038/296647a0.

[83] A. Raffaello, C. Mammucari, G. Gherardi, R. Rizzuto, Calcium at the center of cell signaling: interplay between endoplasmic reticulum, mitochondria and lysosomes, Trends Biochem. Sci. 41 (12) (2016) 1035–1049.

[84] K. Mikoshiba, The lnsP3 receptor and intracellular Ca2+ signaling, Curr. Opin. Neurobiol. 7 (1997) 339–345.

[85] H. Osorio, P. Tapia-Reyes, D. Espinoza, D. Laporte, A. González, E. Castro-Nallar, A. Moenne, The Genome of the Marine Alga Ulva compressa (Chlorophyta) Reveals Protein-Coding Genes with Similarity to Plants and Green Microalgae, but Also to Animal, Bacterial, and Fungal Genes, IJMS 23 (13) (2022) 7279.

[86] M. Kikuyama, Role of Ca2+ in membrane excitation and cell motility in characean cells as a model system, Int. Rev. Cytol. 201 (2001) 85–114, https://doi.org/10.1016/s0074-7696(01)01002-6.

[87] G. Findlay, A. Hope, Ionic Relations of Cells of Chara Australis VII. The Separate Electrical Characteristics of the Plasmalemma and Tonoplast, Aust. J. Biol. Sci. 17 (1) (1964) 62–77, https://doi.org/10.1071/bi9640062.

[88] G. Findlay, A. Hope, Ionic Relations of Cells of Chara Australis IX. Analysis of Transient Membrane Currents, Aust. J. Biol. Sci. 17 (2) (1964) 400–411, https://doi.org/10.1071/bi9640400.

[89] A.E.S. Macklon, Calcium fluxes at plasmalemma and tonoplast, Plant Cell Environ. 7 (6) (1984) 407–413, https://doi.org/10.1111/j.1365-3040.1984.tb01430.x.

[90] M.H. Weisenseel, H.K. Ruppert, Phytochrome and Calcium Ions are Involved in Light-induced Membrane Depolarization in Nitella, Planta 137 (3) (1977) 225–229.

[91] V.Z. Lunevsky, O.M. Zherelova, I.Y. Vostrikov, G.N. Berestovsky, Excitation of Characeae cell membranes as a result of activation of calcium and chloride channels, J. Membr. Biol. 72 (1–2) (1983) 43–58, https://doi.org/10.1007/BF01870313.

[92] D.C. Gadsby, Ion channels versus ion pumps:the principal difference, in principle, Nat. Rev. Mol. Cell Biol. 10 (5) (2009) 344–352.

[93] L. Leybaert, M.J. Sanderson, Intercellular Ca2+ waves: Mechanisms and function, Physiol. Rev. 92 (3) (2012) 1359–1392, https://doi.org/10.1152/physrev.00029.2011.

[94] E. Scemes, S.O. Suadicani, G. Dahl, D.C. Spray, Connexin and pannexin mediated cell—cell communication, Neuron Glia Biol. 3 (3) (2007) 199–208, https://doi.org/10.1017/S1740925X08000069.Connexin.

[95] A. De Martino, A. Bartual, A. Willis, A. Meichenin, B. Villazán, U. Maheswari, C. Bowler, Physiological and Molecular Evidence that Environmental Changes Elicit Morphological Interconversion in the Model Diatom Phaeodactylum tricornutum, Ann. Anat. 162 (3) (2011) 462–481.