Chapter X "Extracellular polymeric substance (EPS) production by benthic pennate diatoms"

Graham J. C. Underwood,
gjcu@essex.ac.uk
ORCID 0000-0001-5605-0697

Professor, School of Life Sciences, University of Essex, Colchester, Essex. CO16 8QQ, U.K.
Senior Fellow, Zukunftskolleg, Universität Konstanz, Konstanz, Germany.

Abstract: Benthic pennate diatoms are found in a wide variety of marine, freshwater and terrestrial environments. The majority of species produce extracellular polymeric substances (EPS, primarily polysaccharides) that coat the outside of the cell, form complex external structures such as stalks and tubes, biofilm matrices, and are involved in gliding motility. Diatom EPS is a significant carbon source in aquatic food webs, influencing, and being influenced by, closely-associated heterotrophic bacterial assemblages. Diatoms produce many different forms of EPS, varying in chemistry, properties and rates of production. EPS production is linked to photosynthesis, but diatoms can also produce EPS in darkness, utilising the storage product chrysolaminarin as a source of glucose. Environmental factors, cell growth rates and nutrient stoichiometry all influence EPS production and composition, but the mechanisms by which diatoms regulate and alter their EPS production pathways are not known. Reprogramming of metabolic pathways involved in carbohydrate synthesis, determined by RNA transcriptomics, results in changed EPS composition and production in response to environmental cues in some benthic diatoms. Some key enzymes involved in carbohydrate cycling and EPS synthesis have been identified, but the molecular machinery for polysaccharide assembly and intracellular translocation is not understood in detail. Rhythms of diatom behaviour in responses to light intensity, circadian controls and stress-responses are linked to EPS production, but the controlling mechanisms and metabolic pathways and feedbacks are not known. The development of molecular biology approaches are providing tools that will enable researchers to understand how diatoms regulate the production of a range of complex EPS types, the production of which are fundamental to life of benthic diatoms.

Keywords epipelic; biofilms; carbohydrates; polysaccharides; metabolic pathways; chemical properties; diatom-bacterial interactions

Running head: Ch1. EPS production by benthic diatoms

Links

www.essex.ac.uk/bs/staff/profile.aspx?ID=1255

https://scholar.google.co.uk/citations?user=qqBoiBIAAAAJ

1.1 Ubiquity and ecological function of EPS production in diatoms

Almost all diatoms produce a diverse range of extracellular polymeric substances (EPS) consisting of polysaccharides with additional constituents such as uronic acids, sulphated sugars, amino-sugars and proteins [1.1] [1.5] [1.11] [1.25] [1.56] [1.103] [1.104] [1.127] [1.27]. Production of polysaccharide-rich EPS is ubiquitous by microorganisms in many environments; water, sediments, benign and pathogenic biofilms [1.37] [1.117], where EPS play important roles in cell adhesion, cell signalling, ligand binding, cell protection against resuspension and desiccation, and as a carbon source [1.51] [1.52] [1.120] [1.124]. This chapter is focussed on EPS production by benthic diatoms, predominantly pennate diatom taxa that live either in sediments or on surfaces, within an environment of secreted polysaccharides that provides a matrix forming a biofilm. Production of EPS is a characteristic feature of the diatoms that are dominant in autotrophic marine biofilms [1.15] [1.27] [1.87] [1.88] [1.104] [1.111] [1.120] [1.127], microbial mats and microbialites [1.114] [1.106] [1.124], and in sea ice microbial assemblages [1.8] [1.10] [1.11] [1.65] [1.69] [1.102] [1.125]. This chapter will review the chemical composition, properties and functions of EPS produced by benthic diatoms, the metabolic pathways for EPS production, and species-species interactions between diatoms and bacteria that influence the production and properties of EPS.

1.2 Types of EPS produced by benthic diatoms

Benthic diatoms produce many types of EPS [1.127]. The production of EPS by benthic diatom biofilms is related to cell primary production, as photosynthesis is the source of the carbohydrate components utilised in the synthesis of polysaccharides [1.11] [1.98] [1.109] . High rates of EPS production can correspond to high rates of photosynthesis (sometimes referred to as the “overflow hypotheses” [1.112]), but this is not a direct (obligate) pathway, as diatoms can produce EPS in the absence of light [1.109] [1.127] and even up to 10 day periods of darkness [1.110] [1.123].

The complex and variable nature of polysaccharide chemical composition, molecular size and biophysical properties, and the methodological challenges in isolating EPS components, makes definition of specific “types” of EPS problematic [1.117]. Benthic diatom EPS exhibits a continuum of size and solubility, determined in part by chemical composition of the molecules concerned, with aggregation and dis-aggregation between dissolved and

particulate forms influenced by factors such as salinity and ionic composition [1.35] [1.130]. Various fractionation processes based on concepts of molecular size and solubility have been used to isolate different diatom EPS fractions, with terms such as dissolved, soluble, colloidal, loose, bound, capsular, structural, being applied [1.1] [1.8] [1.9] [1.14] [1.138] [1.125]. In addition to extraction approaches, differential labelling and staining techniques [1.1] [1.71] [1.73] [1.135] [1.139], and tools such as atomic force microscopy (AFM), are used to characterise different forms of EPS [1.39] [1.55] [1.89] [1.104] [1.135] have been applied. The appropriateness of different methodological approaches is often constrained by the nature of the investigation (field-based, laboratory, mixed or single species, ecological, physiological or chemical focus, different laboratory foci). Each of these methodological approaches reveals different characteristics of EPS molecules, and a combination of approaches therefore provides a useful overview of the role of EPS in the physiology and ecology of the diatom species concerned.

1.2.1 Solubility and molecular size characterisation of different EPS

A single molecule of glucose (monosaccharide) has a molecular weight of approximately 180 daltons, but polysaccharides may consist of many thousands of constituent monosaccharide units [1.117], with molecular weights ranging into the hundreds and thousands of kilodaltons (kDa). Molecular size fractionation by membrane filtration allows for the separation and purification of particular size categories of diatom EPS which have different ecological functions, but requires sufficient sample volumes for reliable filtration. Approximately 32% of the dissolved carbohydrate present in Arctic sea ice diatom biofilms was retained by 8 kDa dialysis membrane filtration [1.10]. This represents a polysaccharide of approximately 40 monosaccharide units in length, which is a small molecular size compared to the polysaccharides (containing thousands to tens of thousands of monosaccharides) implicated in formation of gels and structures [1.56] [1.77] [1.130]. Many diatom EPS are clearly larger than 8 kDa in size. Size fractionation (100 kDa filter) of colloidal EPS produced in culture by the estuarine benthic diatom *Nitzschia tubicola* found that 60% of the extracted colloidal EPS consisted of polymers larger than 100 kDa. The most abundant monosaccharides were glucose, rhamnose, galactose, and mannose, collectively contributing 77% of the monosaccharide content [1.120]. The greatest increases in concentrations over the course of a tidal emersion period on mudflats were found in the >100 kDa and <10 kDa size classes of EPS fractions, with glucose content substantially increasing

after a period of emersion in four different EPS size classes (>100, 100-50, 50-10, <10 kDa size classes) [1.32]. Differential molecular size filtration has also been used to isolate different components of sea ice dissolved organic matter and EPS; a high molecular weight (HMW) dissolved organic carbon (DOM) fraction (rich in diatom EPS) retained on a 100 kDa filter; and a lower molecular weight (LMW) EPS-rich DOM fraction retained between 10 kDa and 100 kDa filters, which differed in chemical composition [1.126]. These HMW and LMW diatom-derived EPS fractions were preferentially utilised by a subset of sea ice bacterial taxa, resulting in a changed community composition [1.126]. Studies on the EPS trails deposited by moving cells of *Amphora coffeaeformis* (current accepted name, *Halamphora coffeaeformis* (C. Agardh) Levkov 2009) found two different size groups of adhesive proteins, one >250 kDa and a second, less than 30 KDa, in molecular size.[1.71].

Sequential extractions using different extraction media have been used to isolate different EPS fractions from benthic diatoms, both in culture and in natural, mixed field samples [1.14] [1.56] [1.86] [1.87] [1.88] [1.99] [1.100] [1.105] [1.113] [1.138] [1.127]. A frequently-used approach is to extract the soluble (colloidal) polysaccharides ("colloidal EPS", also sometimes termed "dissolved EPS", but probably incorrectly, as EPS may not technically be "dissolved" but present as colloids [1.130]) present as extracellular EPS in the media or environmental sample (using water or saline extractions). Some of this material is truly dissolved, and also includes mono- and oligosaccharide carbohydrates and other organic fractions, as well as EPS [1.127]. This is followed by a hot water extraction (HW), that primarily solubilises intracellular polysaccharide storage compounds (chrysolaminarin in diatoms [1.26] [1.70]), though potentially including EPS from any extracellular or loosely-bound matrix, followed by extractions in hot bicarbonate solution (HB), which solubilises capsular, stalk and other tightly-bound (or structurally complex) EPS. A final hot alkali extraction (HA) dissolves the silica diatom frustules and liberates EPS that are closely associated with the silica frustule [1.1] [1.14] [1.140]. Other approaches have used either an EDTA or Dowex resin extraction phase to isolate bound EPS [1.86] [1.99] [1.105] [1.111].

It is very important to understand that all these fractions are operationally defined by the extraction protocol used, and within each fraction a number of different EPS components may be present [1.123]. The solubility of polysaccharides is dependant on the chemical structure of the polysaccharide, but also on chemical (salinity, pH, ionic composition) and physical (temperature) conditions [1.130]. Diatoms also change the composition of the EPS

they produced, depending on their physiological state, or in response to environmental cues (see Section 1.3). This means it is not possible to (a) identify specific cut off boundaries between one "fraction" and another, and (b) compare different extraction approaches, unless they have been carried out on the sample material. However, it is clear that there is a gradient of solubilities across the spectrum of EPS produced by diatoms, and increasing the temperature or chemistry of extraction approaches (*e.g.* bicarbonate, EDTA, other chelators etc.) will solubilise the more structural or "bound" EPS from cultures or field material [1.119]. While there is potentially significant overlap in the fractions thus obtained, these sequential extractions do contain component elements that have significant differences in chemical compositions and turnover times (e.g. identified using ^{14}C and ^{13}C labelling [1.15] [1.88] [1.109]), and different ecological dynamics under field situations [1.10] [1.96] [1.100] [1.105] [1.120]. It is important to characterise the system being studied using a variety of techniques, and limit the number of changes of approach within a programme of research to allow for intercomparisons.

The dissolved polysaccharides in aqueous solutions (termed colloidal EPS) are routinely precipitated in 70% (v.v) alcohol–water solution at 4 °C for 24 h [1.35] [1.126] [1.8] [1.10]. Reducing or increasing the proportion of alcohol used in this precipitation approach can isolate "less water-soluble", or "more water-soluble" polysaccharides respectively, and this reveals that there can be several solubility-defined different EPS present within a colloidal EPS extract. *Cylindrotheca closterium* produces a range of different EPS within the colloidal fraction, with the EPS precipitated at 20% and 40% v/v ethanol having differing monsaccharide and uronic acid composition profiles, compared to EPS precipitated at 60% and 80% alcohol [1.123]. The production of these different EPS components varied under nutrient-replete or nutrient-limited growth, and in light or darkness [1.123]. The sea ice diatom *Fragilariopsis cylindrus* increases the production of more complex and less-soluble EPS components (those EPS isolated by precipitation in 30% v/v alcohol) when exposed to salinity stress compared to controls at normal polar seawater conditions [1.11].

1.2.2 Chemical composition and structures of EPS

It is well established that for many benthic diatom species, the chemical composition of EPS produced during the active growth phase differs to that of EPS produced in nutrient-

limited stationary phase [1.1] [1.8] [1.110] [1.123] [1.127]. Rheological properties of polysaccharides (influencing gel formation) are dependent on physico-chemical, structural (chain length, branching patterns) and environmental conditions [1.24] [1.34] [1.119] [1.130]. Altered chemical composition and different branching patterns and side groups change the properties of the EPS. In the sea ice biofilm diatoms *Fragilariopsis cylindrus* and *F. curta*, yields of colloidal carbohydrates (which also had a reduced glucose content) declined as cells entered stationary phase, while the content of structural monosaccharide components (xylose, mannose) increased; changes that could contribute to increase the gelling properties of *Fragilariopsis* polysaccharides in brine channels [1.8] [1.68]. The sea ice diatom *Synedropsis* sp. increased the glucose and galactose content of colloidal polysaccharides produced in stationary phase (components low in gelling potential, [1.141] [1.45]) and decreased the mannose content. *Synedropsis* sp. produced high yields of extracellular dissolved carbohydrates compared to *Fragilariopsis* species, but only 20% of this material were polysaccharides greater in size than 8 kDa. The colloidal polysaccharides of *Synedropsis* sp. were mainly highly soluble EPS (precipitating in 50 to 70% alcohol) and non-EPS carbohydrates. This contrasted to the high proportion of high molecular weight EPS in the *Fragilariopsis* species; adaptations that are probably related to the different ecologies of these three species within the sea ice environment [1.8]. *Stauroneis amphyioxys* produces charged and sulphated heteropolysaccharides which change in composition between exponential growth and stationary phase conditions [1.79]. *Fragilariopsis curta* produces colloidal carbohydrates with a high proportion of amino sugars [1.8]. Amino sugars (constituents of the chitin spines of many centric diatoms) have rarely been detected in the soluble EPS from pennate diatom species [1.25], though gene expression for chitin synthase was upregulated in *F. cylindrus* under conditions of high salinity (salinity 52) and low temperatures (- 8 °C) [1.11], and similar genes are present in *Phaeodactylum* [1.140]. Amino sugars have been detected in soluble polysaccharides produced by bacteria [1.84] [1.95] and fungi [1.107].

Hot-water (HW) extracts from diatoms cultures and field samples are dominated by glucose, present mainly as ß-1,3 linked glucan (chrysolaminarin), the major storage carbohydrate for many diatom species [1.127] [1.123] [1.26] [1.70] [1.8] [1.60]. Other monosaccharides mainly galactose, mannose, xylose and rhamnose can be detected in HW fractions. These sugars probably have an extracellular location [1.1] [1.26], co-extracted with intracellular storage compounds [1.15] [1.125], as there are no reports of large quantities of

any intracellular carbohydrate polymers other than chrysolaminarin in diatoms [1.60] [1.70]. Declines in yield and in glucose content of HW carbohydrate fractions are associated with lower rates of photosynthesis [1.15] [1.33] [1.50], and occur when the cells entered stationary phase. Declines in glucan-chrysolaminarin concentrations have also been found in natural Antarctic sea ice assemblages during nutrient limitation [1.78].

Conducting hot-bicarbonate extractions (HB) on cell pellets or environmental samples after extraction of the colloidal and HW fractions, solubilises complex EPS structures (mucilage pads, stalks, cell coatings etc. [1.14] [1.138], including more recalcitrant gel material termed transparent exopolymer particles (TEP) [1.36]. HB-extracted EPS generally has an increased content of the monosaccharides mannose, rhamnose, fucose, xylose and arabinose, consistent with the structural role these monosaccharide moieties can play in EPS [1.8] [1.75] [1.141]. *Fragilariopsis cylindrus*, *F. curta* and *Synedropsis* sp., produce heterogenous monosaccharide profiles in HB-extracted mucilages, dominated by xylose, mannose and galactose, similar to the HB fractions of other benthic diatom species [1.1] [1.25] [1.27] [1.78] [1.79] [1.123]. HB-extracted EPS forms mucilage pads, stalks and gels, that stain strongly with alcian blue in culture [1.1] [1.138], indicating a mainly acidic polysaccharide content [1.41] and probably represent much of the particulate EPS, TEP and 'mucilage sheets' observed in other culture and field studies [1.2] [1.36] [1.65] [1.69] [1.79].

The hot alkali extraction approach (HA) dissolves the silica frustules of diatoms and liberates the polysaccharides intimately associated with the frustule [1.27]. This includes the diatotepum layer on the inner surface of the frustule, and polysaccharides and proteoglycans within the silica matrix [1.49]. Substituted mannans with high concentrations of uronic acids (glucuronomannans) are the most abundant polysaccharides extracted with hot alkali [1.1] [1.27] [1.79] [1.138], and are present in the thin organic casing attached to the siliceous wall [1.131]. Culture studies of various diatom species have found the HA- EPS fraction of diatom cultures to be highly enriched with mannose (often glucuronomannan composites) [1.8] [1.27] [1.138], and similar mannose-rich profiles are found in sea ice HA –extracted carbohydrate environmental samples [1.10] and HA-extracts from sediment biofilms [1.15]. Although these EPS are frustule-associated and are therefore presumably deposited as frustules are formed in the silica deposition vesicle during cell growth and division, the HA fraction does exhibit changes in chemical composition during the shift into stationary phase, including substantial increases in amino-sugars, particularly in *Fragilariopsis cylindrus* [1.8].

The HA fraction of *Phaeodactylum tricornutum* also changes (with increased sulphate and uronic acid content) during both phosphorus limitation and salinity stress [1.1]. The role that amino sugars may play in EPS structural properties is unclear. Amino sugars are present in diatom cell-wall associated polysaccharides [1.78] [1.40] and chitin is a main component within the silica cell walls of *Thalassiosira pseudonana* [1.121].

1.3 Functions of EPS in benthic diatoms in relation to chemical composition

EPS capsules and biofilm matrices are important adaptations to protect cells from desiccation and other stresses. This can be particularly important for cells living in intertidal biofilms that are exposed to rapidly changing temperature and salinity conditions during periods of tidal exposure [1.81] [1.98] [1.122] [1.124], and for diatoms living in sea ice, where the environmental conditions within sea ice brine channels change over a period of days and weeks during sea ice formation and maturation [1.69]. Physico-chemical properties play a major role in determining the solubility and structural potential of EPS [1.24] [1.119] [1.130]. Pennate diatoms and other microorganisms alter the rate of production and the chemical composition of the different EPS secreted in response to inorganic nutrient limitation, salinity and low temperature stress [1.1] [1.5] [1.8] [1.13] [1.31] [1.76] [1.85] [1.92] [1.110] [1.123]. Nutrient limitation, either nitrogen or phosphorus generally increases EPS production, both of material "colloidal" in the culture media (e.g. with *Halamphora luciae* [1.31]), but also the production of stalks, e.g. in *Licmophora* [1.13]. There are clear indications that multiple interacting environmental conditions result in changes in EPS production, e.g. temperature and nutrients, temperature and salinity, light and salinity [1.8] [1.10] [1.13] [1.67]. This adds further complexity in understanding which cues diatoms detect in order to switch their EPS production pathways in response.

The benefits to a diatom cell of altering the composition and properties of EPS include cryo-protection, production of salinity barriers [1.67] [1.8] [1.42] and creation of localised microclimates for cells [1.68] [1.69] [1.115]. In sea ice, EPS and dissolved carbohydrates modify the physical structure of the ice-water matrix, altering the structure of brine channels as seawater freezes [1.65] [1.67] [1.68] [1.69]. Increases in the mannose, rhamnose, fucose, xylose and arabinose content of EPS increases the structural diversity of EPS [1.141] [1.8] [1.75], affording ice crystal-influencing properties [1.67] [1.69], the formation of sticky brine channel plugs [1.65] [1.68] and protective mucilages surrounding diatom cells [1.115]. The

solubility characteristics, and monosaccharide and uronic acid composition of dissolved EPS and HB-extracted EPS components isolated from first year arctic sea ice [1.10] corresponds to those properties of mucilage EPS that have been shown to affect ice crystal and brine channel formation [1.67] [1.69].

There is now good experimental, culture, and field evidence that polar diatoms change both the quantity and composition of their EPS due to external salinity and temperature stress [1.8] [1.10] [1.11], which corresponds to the presence of more chemically diverse EPS (including a higher uronic acid content) at lower temperatures and higher salinities in both Arctic and Antarctic sea ice [1.10] [1.11] [1.125]. Temperate and tropical biofilms can also be mucilage rich, which probably serves to protect cells from desiccation due to the water-retaining properties of an EPS gel [1.14] [1.81] [1.112] [1.72]. Compared to sea ice diatoms [1.8], benthic temperate-zone diatoms produce lower proportions of uronic acids within their dissolved EPS (<5 % contribution, [1.1]), with higher proportions (10 – 25 %) in their cell associated carbohydrate fractions [1.1] [1.103], but also increase the uronic acid content when salinity and nutrient stressed [1.1]. *Phaeodactylum tricornutum* and *Cylindrotheca closterium* also show similar plasticity in carbohydrate and EPS physiology, producing more carbohydrate (especially in HB and in EDTA-extracted fraction) under altered salinity conditions, and show altered motility, increased aggregation and altered polymer chemistry [1.1] [1.5]. Experimental approaches using additions of complex polysaccharides (xanthan gum) show that the presence of EPS can lower the freezing point temperature, in addition to the impact of the diatoms and their own secretions (including ice-binding proteins and other anti-freeze components as well as polysaccharides [1.7] [1.61]. These effects expand the temperature 'window' wherein media remains liquid from -8 to -12 ^{o}C and from -12 to -20 ^{o}C in 34 and 52 salinity cultures respectively [1.8]. Growth of *Cylindrotheca closterium* in a xanthan gum matrix increased cell viability (determined by SYTOX-Green staining) and resulted in increased growth rates and increased population densities (by up to 300, 2,300 and 200% for cultures grown at salinities of 50, 70 and 90, respectively), compared to cells grown at these salinities without the presence of additional polysaccharide matrix [1.115]. Under acute salinity shock treatments (at salinities of 17.5, 50, 70 and 90), *C. closterium* in a xanthan gum matrix maintained photosynthetic capacity, Fq′/Fm′, within 4% of pre-shock values, whereas Fq′/Fm′ in cells grown without xanthan gum declined by up to 64% with hypersaline shock [1.115].

Diatom EPS production is closely related to diatom motility and behaviour, with EPS being a component of the unique gliding motility mechanisms possessed by benthic diatoms [1.74] [1.103] [1.104]. The actual nature of the "motility polymer", one of the EPS types produced by diatoms, is still debated: isolation and biochemical analysis of diatom trail material from *Amphora coffeaeformis* and *Craspedostauros australis* found a predominance of carbohydrates over proteins (~70:30%), with the carbohydrates dominated by uronic acids [1.103], and also different molecular sizes of EPS within trails [1.71]. This, and other work (e.g. AFM studies to characterise properties of individual EPS, [1.55] [1.39] [1.89] reveals there is a protein component in "motility-EPS", with evidence for the presence of hydrophilic amino acids in adhesive trails [1.103]. The current stage of knowledge in this area has been reviewed by Poulsen et al. [1.104].

An important consequence of EPS production in non-cohesive and cohesive sediments (sands, silts, muds), particularly due to the movement of diatoms through the sediment matrix or adhesion to multiple sand grains, is the binding of sediment particles and production of a surface matrix of extracellular material that creates a biofilm [1.112] [1.113] [1.127]. Such biostabilisation has important consequences for sediment geomorphology [1.58], such that diatoms are sometimes referred to "ecosystem engineers" [1.97]. This chapter will not address this topic, but for recent reviews of this subject, and the importance of diatom biofilms in coastal habitats, see [1.97] [1.101] [1.124] [1.58].

1.4. Metabolic pathways of EPS production and regulation in diatoms

A series of elegant cytological, microscopic and staining studies have revealed that the main site of synthesis of EPS in diatoms is in the Golgi, where EPS is packaged in vesicles and translocated to the cell membrane [1.12] [[1.104] [1.134]. Photosynthesis is the primary source of the carbohydrates and other organic constituents of diatom EPS. Investigations of carbon flow in intact estuarine diatom biofilms, using ^{14}C-bicarbonate [1.109] and ^{13}C-bicarbonate [1.83] pulse-chase approaches, found that up to 60% of photoassimilated carbon was secreted as colloidal EPS within 3 to 4 h. Cook et al. [1.30] measured a transfer of approximately 50% of microphytobenthos (MPB)-fixed carbon to bacteria within 24 h in experimental sandy-sediment mesocosms. Bellinger et al. [1.15] conducted an *in situ* ^{13}C bicarbonate-labelling experiment and found intense ^{13}C-isotopic labelling, with the HW fraction (extracting storage compounds, mainly glucans) having the largest initial labelling (3 μmol ^{13}C g^{-1} per dry weight of biofilm) at 4 h, representing ~75%

of the total amount of ^{13}C measured in all neutral sugar fractions. Enrichment of extracellular colloidal and HB-soluble EPS fractions with ^{13}C followed this peak. The HW, HB and colloidal carbohydrate fractions showed large losses in ^{13}C content between 4 h and 12 h, with a decline to <1 μmol ^{13}C g^{-1} dry weight biofilm after 48 h, with the HB fraction containing ~33% of the ^{13}C measured in the neutral saccharides at this time [1.15]. The maximum isotopic enrichment for glycosyl residues occurred at 4 h and declined dramatically by 12 h, while the opposite was true for xylose and other monosaccharide residues, that were more ^{13}C-enriched at 24 h [1.15].

Benthic diatoms continue to secrete EPS when placed in darkness [1.98] [1.109] [1.110] with ^{14}C-labelling showing that this EPS is produced by utilising intracellular reserves of photosynthate. Glucans (chrysolaminarin) are the major intracellular storage carbohydrate of diatoms [1.60] [1.70] [1.90] and glucan reserves are utilized when cells are not illuminated, with rapid catabolism of glucan by exo-(β-1,3)-D-glucanase [1.70] [1.90] [1.129]. Using a combination of ^{14}C-labelling and a glucan synthase inhibitor (2,6-Dichlorobenzonitrile (DCB)) and glucanase activity inhibitor (P-nitrophenyl b-D-glucopyranoside (PNGP)) with the diatom *Cylindrotheca closterium* in the light, Underwood et al. [1.123] showed that 200 μM DCB reduced the production rates of colloidal carbohydrate, glucan, and EPS after 3, 6, and 8 h respectively while not affecting photosynthesis. A concentration of 10 mM PNGP inhibited glucanase activity while having no effect on overall photosynthesis, but resulted in accumulation of ^{14}C-glucan and reduced ^{14}C-EPS production. *Phaeodactylum tricornutum* possesses a single gene encoding a putative β-1,3-glucan synthase. In experiments using genetic mutants, when the activity of this gene was reduced, chrysolaminarin production decreased, and photoassimilates accumulated in lipids and soluble sugars instead [1.60]. These studies indicate that there is a set of metabolic pathways linking photosynthetic production of sugars, intracellular storage of chrysolaminarin, and then the production and secretion of the different EPS that diatoms secrete. While the evidence that glucans are involved in the production of EPS, especially during periods of darkness is clear, the compositional mismatch between the intracellular glucan fraction (consisting of 90% glucose) and EPS produced (containing a number of other sugars and uronic acids [1.123], and also differential ^{13}C labelling patterns in natural biofilms [1.15], indicates that though glucose from glucan may be directly incorporated into EPS, other sugars also need to be synthesized and incorporated.

Little is known about patterns of gene expression and activity of enzymes involved in the biosynthesis pathway of EPS in diatoms [1.19] [1.37] [1.49] [1.60]. Differential patterns of gene expression are part of a set of regulatory steps, including protein abundance, enzyme activation and presence of co-factors, that result in changes to cell metabolism and will control EPS production ([1.48] [1.63] [1.132]. Polysaccharide production pathways are conserved across the prokaryote and eukaryote domains [1.37] [1.82], with monosaccharides converted to nucleotide sugars and assembled into polysaccharides by the action of glycotransferases [1.49] [1.51]. This has allowed the main pathways of photosynthesis and carbohydrate metabolism in diatoms to be reconstructed [1.43] [1.49] [1.70]. Aslam et al. [1.11], in a study combining RNA transcriptomics, EPS chemistry and cell physiology, produced the first evidence for differential gene expression linked to a pathway for EPS synthesis in *Fragilariopsis cylindrus*, providing a model for the production of EPS by other diatoms (e.g. *Phaeodactylum* [1.140]). *Fragilariopsis cylindrus* responded to external conditions (temperature and salinity) by reprogramming gene expression in metabolic pathways that converted the primary products of photosynthesis, and storage compounds, into different nucleotide sugars, and changed activity of glycosyl-transferases in the Golgi. These changes corresponded to changes in the rates of EPS production and EPS chemistry under different conditions [1.11].

In actively growing and photosynthesising *F. cylindrus*, the main genes upregulated were components of the pathways for glucose and fructose activation, and conversion to the nucleotide sugars GDP-mannose (GDP-Man) and GDP-fructose (GDP-Fru). Glucose and fructose are products of the pentose-phosphate pathways [1.70], and are utilised for ATP production, storage compounds (chrysolaminarin), or activated to make other sugars and derivatives. Induction of the enzymes Fructokinase and Mannose-6 phosphate isomerase is indicative of activation of the fructose-mannose pathway, generating Mannose-6-Phosphate [1.43] [1.82] which is utilised to produce the nucleotide sugars GDP-Mannose and GDP-Fucose. These changes corresponded to the inclusion of mannose and fucose in EPS produced in those conditions, a response to colder temperatures, altering the rheological properties of the EPS produced to provide protective cell coatings [1.8] [1.141] [1.118]. Mannose is an important constituent of diatom EPS, particular in structural EPS [1.1] [1.25].

Lowering temperature to -4 °C for 8 d caused a significant change in the transcriptome of *F. cylindrus*, with up-regulation of phosphoglucomutase and UDP-N-acetylglucosamine diphosphorylase, leading to UDP-Glucose, and to uronic acid precursors (UDP-glucuronic acid and UDP-galacturonic acid), and down-regulation of many glycosyltransferases [1.11]. Glycosyltransferases play a role in the Golgi body where polysaccharides are constructed stepwise on the endoplasmic reticulum membranes or in the lumen of the Golgi [1.4]. Low-temperature and salinity stresses significantly reduced diatom photosynthesis and growth [1.11], and induction of the pathway for synthesizing UDP-N-acetylglucosamine. This compound is used for the production of glycoproteins which contribute to folding and adhesion properties in diatom EPS [1.135], and homologs of two genes linked to cell adhesion molecules in *P. tricornutum* [1.135] were upregulated in *F. cylindrus*. The strong induction of almost all glycosyltransferases encoded in the *F. cylindrus* genome, and several ABC transporter and flippases under the lowest temperatures and highest salinities, may reflect the need of the cells to produce protective EPS at this time. Increased expression of UDP-N-acetylglucosamine transferases and alpha-N-acetylglucosaminidases as temperatures declined and salinity increased, and high gene expression of chitin synthase indicate potential for chitin secretion in *F. cylindrus* EPS. Amino-sugars are present in all the different EPS fractions produced by *F. cylindrus*, including in the HA fraction, which is closely associated with the silica frustule [1.8] but their functional role is unclear.

The diatom *Thalassiosira weisflogii* also alters its transcriptome to maintain rates of carbon metabolism and growth between salinities of 21 to 35 [1.22], and increased EPS production in response to salinity occurs in *Phaeodactylum tricornutum* and *Cylindrotheca closterium* [1.1] [1.5]; *F. cylindrus* [1.8]; and to a variable extent in *T. weisfloggi* [1.22]. Increased transcriptional responses of genes for chryoslaminarin degradation have been observed in *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* when under P limitation [1.19], with this effect also associated with increased sulpholipid pathway expression. There is further evidence for diatoms reprogramming their EPS production pathways in response to environmental conditions. Zhang et al., [1.140] showed increased production (using colloidal, EPS, HW, HB, and HA- EPS extractions) of intra- and extracellular carbohydrates and EPS in *Phaeodactylum tricornutum* grown under higher pCO2 conditions for over 200 generations. The carbohydrate-EPS synthesis pathway (*sensu*

[1.11]) was upregulated under increased pCO_2 conditions, with 74.0% of the genes having up-regulated expression profiles under high pCO_2 conditions, including increased expression of pathways leading to uronic acid synthesis. These changed expression patterns correlated with the physiological and biochemical properties of the cells [1.140]. The types of NDP-sugar synthases differentially expressed in response to high carbon conditions in *Phaeodactylum* were the same as those responding to temperature and salinity stresses in *Fragilariopsis* [1.11], for example, the production of the NDP-sugars GDP-Mannose, GDP-Fucose, UDP-Glucose, UDP-Galactose, and UDP-Glucuronic Acid, as well as genes encoding the enzymes involved in UDP-Xylose and UDP-Arabinose. These studies suggest that the flexibility of these metabolic pathways are key adaptations allowing diatoms to modify the chemistry and production of different EPS fractions in response to environmental cues.

Under natural conditions, it might be expected that there would be a rhythmicity of gene expression within EPS-production pathways, corresponding to the behavioural rhythms of photosynthesis and motility in benthic marine diatoms that are themselves aligned to circadian, diurnal and tidal cycles [1.52] [1.59] [1.108] [1.124]. Gene expression in the marine benthic diatom *Seminavis robusta* has been found to be strongly phased with diel or semidiurnal cycles in culture [1.16]. Genes involved in chrysolaminarin synthesis were phased to be most active at dawn, corresponding to patterns of daylight accumulation of chrysolaminarin and depletion of chrysolaminarin at night. Genes putatively involved in silica frustule biosynthesis were upregulated during the middle "night" period, which would also require the production of the frustule-associated EPS (HA fractions). Interestingly, many genes encoding for glycosyltransferases, sulfotransferases, sugar phosphate transporters and fasciclin-like adhesive proteins were enriched in 12-h periods, phased to early morning, and late evening. Bilck et al., [1.16] hypothesise that such phasing could align with the semi-diurnal phasing of natural biofilms [1.52] [1.124] influenced by tides, though the culture conditions prevented this from being experimentally confirmed. Comparison of gene expression patterns in algal groups from across the Archaeplastida has found that many of the major carbohydrate metabolism genes are rhythmically expressed to an 8 h cycle [1.44].

There are still major questions concerning the nature of the chemical, conformational and structural changes that EPS molecules undergo when excreted into the external environment and interact with aquatic or saline conditions [1.47] [1.71]. The EPS stalks of some diatoms,

for example *Achnanthes longipes*, undergo a set of self-assembly reactions outside of the frustule wall, resulting in the formation of a structural EPS stalk [1.56] [1.133] [1.139]. Similar reactions probably take place in the formation of all EPS structures that form outside of the frustule. The EPS used directly in motility contains protein/ proteoglycan components, and remains in connection via the raphe slit with the intracellular force-generation machinery (actin-myosin) within the cell [1.71] [1.104]. These EPS molecules are eventually detached from the cell during motility, and subsequently left as EPS trails, or exchange into the colloidal phase as cells move through their environment [1.47] [1.71] [1.104]. Various mechanisms to prevent EPS sticking inside the raphe slit have been proposed [1.47], but this is an area that requires further research. Other types of EPS appear to be secreted more generally over the frustule surface, providing a protective outer capsule (e.g. HB-extracted EPS) to the cell [1.135] [1.138] [1.139], though the exact routes of such extracellular secretion are not well-described, nor is it understood how these different EPS interact, or do not interact, with each other in the external environment.

1.5. Interactions between diatoms, EPS and bacteria

EPS produced by benthic diatoms provide a carbon source for heterotrophic bacteria, and thus contributes to sediment organic carbon biogeochemistry and also sediment nutrient cycling (see [1.91] [1.124]). The degradation of organic matter is influenced by chemical composition, size and reactivity, and the ability of microorganisms to synthesize extracellular enzymes for hydrolysis of larger compounds, and to take up smaller molecules into their cells [1.6] [1.17] [1.57]. The freshly-produced lower molecular weight exudates in diatom-dominated assemblages (colloidal carbohydrates, colloidal EPS) are labile, and support a particular subset of the bacterial assemblages; for example major utilizers of diatom EPS in aerobic sediments are Alphaproteobacteria, Gammaproteobacteria and Bacteriodetes, and in anaerobic conditions Deltaproteobacteria [1.17] [1.53] [1.57] [1.80] [1.86] [1.120] [1.126].

Diatom-derived dissolved organic carbon compounds contribute up to 50% of the total organic matter in some sediments, though the importance of this carbon source varies in different habitats (sandy to muddy; temperate to tropical, inter- to subtidal) [1.15] [1.30] [1.93] [1.94] [1.101]. Benthic diatom-fixed carbon has a characteristic $\delta^{13}C$ signal allowing tracking through food webs, and studies indicate that EPS carbon is a major contribution to the food web of coastal sediment communities [1.28]. ^{13}C-carbon-EPS has been tracked into the phospholipid fatty acids (PLFA) and RNA of various bacterial groups [1.15] [1.45] [1.83]

[1.120]. Different bacterial groups (for example Sphingobacteria and *Tenacibaculum* (Bacteroidetes), two classes of Verrucomicrobia (Verrucomicrobiae and Opitutae)) grow preferentially on labile (colloidal) and refractory (HB-extracted) diatom EPS [1.17] [1.88] [1.120] [1.126]. Turnover rates of these different EPS fractions vary under aerobic and anaerobic conditions, with anaerobic conditions causing the preferential breakdown of HB-extracted EPS, with the growth of Firmicutes and sulfate-reducing Deltaproteobacteria (Desulfobacteraceae and Desulfobulbaceae) [1.80][1.88].

Though diatoms in culture often grow well and can even be dependent on bacteria [1.3], the presence of bacteria can also decreases the productivity of monocultures of common benthic diatoms (*Cylindrotheca closterium*, *Navicula phyllepta*, and *Seminavis robusta)* [1.66]. This effect on overall productivity was not present for diatoms grown in co-diatom cultures but there were species-specific effects, as each diatom species developed a bacterial community that differed in composition. Experiments on the effect of high and low densities of bacteria on the growth and EPS production of *Halamphora coffeaeformis* and *Entomoneis paludosa* revealed different responses by each diatom species, with enhanced carbohydrate concentrations (per cell) present with high bacterial abundance, but induced lower extracellular carbon (EPS fraction) per diatom cell [1.62]. Bacteria however did not affect *E. paludosa* final carbon biomass and no major change was observed in both diatoms for cellular carbohydrates, C/N ratio, and photosynthetic pigments [1.62]. The presence of a wide range of bacteria strains from the Alphaproteobacteria, Gammaproteobacteria, Flavobacteriia, and Actinobacteria had either negative or neutral effects on the growth of *Cylindrotheca closterium*, *C. fusiformis* and *Seminavis robusta* cultures, with the most negative impacts produced by bacteria strains unfamiliar to the diatoms in culture [1.116].

In contrast, for diatoms that are primarily stalked or live a permanently-attached lifestyle in periphytic biofilms, EPS production and biofilm formation by the diatoms can be enhanced by the presence of certain bacterial taxa. Bruckner et al. [1.20] found that growth of the freshwater diatom *Cymbella microcephala* was enhanced in the presence of six associated bacterial strains and soluble polysaccharide secretion was increased in the presence of Proteobacteria in particular. All the bacterial strains could grow in *C. microcephala* cultures without the addition of organic co-substrates. Alphaproteobacteria appear to utilize cell-bound polysaccharides as in co-cultures, the content of cell-associated carbohydrates decreased and soluble sugars started to accumulate. A strain of Bacteroidetes strongly

stimulated the amount of bound carbohydrate secretion by the alga, via a dissolved compound present in the culture medium. This conclusion was further supported by experiments co-cultivating 12 different diatom strains isolated from epilithic biofilms with 4 different taxa of bacteria, which showed that (generally, but not exclusively) both direct presence and the presence of spent bacterial-media (containing a putative protein signal) enhanced the production of EPS by these diatom taxa [1.21]. Bacteria have a strong impact on the biofilm formation of the pennate diatom *Achnanthidium minutissimum* [1.136] [1.73]. Capsules of insoluble EPS, mostly consisting of carbohydrate, were produced only in the presence of bacteria. In axenic cultures of *A. minutissimum*, no capsules were formed, and the cells remained suspended in the growth media, and produced large amounts of soluble carbohydrate. This response to move to an attached growth form was achieved even when the diatom was treated with sterile spent media, or solid phase extracts from *Dyadobacter* sp. 32 (Bacteroidetes) monocultures. Similar interactions have also been found with the marine diatom *Phaeodactylum tricornutum*, where a marine alphaproteobacterium, *Roseovarius* sp. strain 217, induced the formation of the oval phase and strong attachment [1.23]. The production of highly glycosylated extracellular diatom proteins involved in the adhesion of the diatoms to surfaces was dependent on the presence of bacteria or of bacterial factors present in spent bacterial culture supernatant [1.23]. Similar responses have also been reported for the stalk-forming freshwater diatom *Didymosphenia geminata* where co-culturing with bacteria enhanced the survival, attachment and production of stalks by the diatom compared to axenic cultures [1.18].

The strong positive role of bacteria in inducing the formation of structures (EPS stalks, pads) is intriguing. It has long been known that periphytic diatoms will only settle and grow on submerged surfaces after a period of bacterial conditioning and colonisation of surfaces [1.128]. Windler et al., [1.137] proposed that *A. minutissimum* capsules might be part of a mutualistic relationship between the diatom and its associated bacteria, with diatom cells surrounded by a bacteria-free capsular space, colonised on its surface by a layer of densely aggregated bacteria cells [1.136]. There appears to be a strong synergy between bacteria and EPS stalk production in some diatom taxa (not for all species: the marine diatom, *Achnanthes longipes* produces stalks in axenic conditions) [1.133] [1.139]. The research on epilithic biofilms (op.cit) demonstrates that bacteria have the potential to influence diatom growth, EPS secretion, and biofilm formation, through the involvement of a chemical signal, with the diatoms being the source of the organic matter necessary for bacterial growth. The

genome of one of the bacteria involved in these interactions, *Dyadobacter*, possesses a set of genes enabling it to utilise diatom carbohydrates as a sole carbon source [1.38]. There does not appear to be such strong positive interaction effects on EPS production between epipelic benthic diatom taxa and specific bacteria. Epipelic diatoms tend not to produce EPS structures, being continuously mobile in their environment, and perhaps this continuous relocation prevents the formation of spatially-structured interactions? However, there are a number of sediment-inhabiting diatoms that manufacture EPS tubes (e.g. *Gyrosigma balticum* [1.64], *Berkelaya* spp.) where similar diatom-bacteria interactions may be occurring. Further research into diatom and bacteria interactions and the chemical communication pathways linked to EPS production is needed.

1.6. Future directions

The main features of production of EPS by benthic diatoms are summarized in Figure 1. Benthic diatoms produce numerous types of EPS, differing in chemical composition and properties, and cells secrete these EPS into specific compartments either within the cell, or as coating on the external surface of the frustule, or to produce mucilage stalks, tubes or other structures, and in locomotion (see Section 1.2.1 and Section 1.2.2). Some of this external material significantly alters to local microenvironment of the cells, providing a biofilm environment which can aid cell survival (see Section 1.3). Though it is possible to isolate some broadly defined fractions based on different extraction techniques, or other methods to measure the location of the EPS in the cell (see section 1.2), there is still significant scope to develop approaches for identifying and isolating particular EPS types present (particularly the relative role of carbohydrate and glycoprotein elements within different EPS, e.g. [1.71] [1.103], and to understand how these specific forms are recognised and transported intracellularly [1.48]. Recent transcriptomic studies have revealed reprogramming of EPS production pathways in response to environmental variables within a number of diatom taxa (see Section 1.4; [1.11] [1.16] [1.140]). This discovery supports the extensive environmental and experimental data on changes in EPS content and chemistry in population of diatoms in biofilms in different environments (see Section 1.3). The potential role of amino-sugars in EPS, and the presence and location of chitin production in benthic diatom EPS requires further investigation.

The accelerating development of gene-knock out and other molecular approaches, and the publication of further diatom genomes and transcriptomes, will allow research on the

regulatory mechanisms controlling EPS production. Given that various external signals are known to change EPS production (see Fig. 1.), there must be recognition and signalling pathways located within the cells that result in the observed changes in EPS production. The specifics of regulating controls are unknown. How EPS production is linked to the endogenous motility behaviour of natural biofilms, and mediated by individual diatom cell responses to light intensity and spectral quality [1.105] [1.108] [1.29] [1.124], and the importance of circadian and other clock-systems needs further research. Continuing developments of –omic approaches to diatom cell biology provides tools to address these questions [1.16] [1.48] [1.60]. Are there key or conserved metabolic pathways and regulators, or do different species, with differing ecologies, have specific abilities? Understanding how diatoms control the formation of specific EPS types, and developing tools and approaches to manipulate and modify diatom EPS properties and production rates may lead to future interesting biotechnological applications.

1.7. Acknowledgements

GJCU acknowledges the many contributions of colleagues, Ph.D. students, postdoctoral researchers, and technical staff who have contributed to this research over the years and whose work is cited in this chapter. GJCU is grateful for the constructive suggestions from two anonymous reviewers of the manuscript. The following funding bodies and awards are gratefully acknowledged: U.K. Natural Environment Research Council (NERC) EPStromNet (NE/V00834X/1); NERC SSB programme, module Blue Carbon, (NE/K001914/1) and the UK Royal Society, (IES\R1\201260). GJCU gratefully acknowledges the support provided by the award of a Senior Fellowship at the Zukunftskolleg, Universität Konstanz, Germany from June 2021 to May 2022, funded by the Federal Ministry of Education and Research (BMBF) and the Baden-Württemberg Ministry of Science as part of the Excellence Strategy of the German Federal and State Governments..

1.8. References

[1.1] Abdullahi, A.S., Underwood, G.J.C. and Gretz, M.R. (2006) Extracellular matrix assembly in diatoms (Bacillariophyceae). V. Environmental effects on polysaccharide synthesis in the model diatom, *Phaeodactylum tricornutum*. *Journal of Phycology* 42, 363-378.

[1.2] Aletsee, L. and Jahnke, J. (1992) Growth and productivity of the psychrophilic marine diatoms *Thalassiosira antarctica* comber and *Nitzschia frigida* grunow in batch cultures at temperatures below the freezing point of seawater. *Polar Biology* 11, 643-47.

[1.3] Amin, S.A., Parker, M.S. and Armbrust, E.V. (2012) Interactions between diatoms and bacteria. *Microbiology and Molecular Biology Reviews* 76, 667-684.

[1.4] Andreeva. A.V., Kutuzov, M.A., Evans, D.E. and Hawes, C.R. (1998) The structure and function of the Golgi apparatus: a hundred years of questions. *Journal of Experimental Botany* 49, 1281-1291.

[1.5] Apoya-Horton, M.D., Yin, L., Underwood, G.J.C. and Gretz, M.R. (2006) Movement modalities and responses to environmental changes of the mudflat diatom *Cylindrotheca closterium* (Bacillariophyceae). *Journal of Phycology* 42, 379–390.

[1.6] Arnosti, C. (2011) Microbial extracellular enzymes in the marine carbon cycle. *Annual Review Marine Science* 3, 401-425.

[1.7] Arrigo, K.R., Mock, T. and Lizotte, M.P. (2010) Primary producers in sea ice. In: *Sea ice 2nd Edition*, Thomas, D.N. and Dieckmann G.S. (eds.) Wiley-Blackwell : 283-325.

[1.8] Aslam, S.N., Cresswell-Maynard, T., Thomas, D.N. and Underwood, G.J.C. (2012a) Production and characterisation of the intra-and extracellular carbohydrates and polymeric substances (EPS) of three sea-ice diatom species, and evidence for a cryoprotective role for EPS. *Journal of Phycology* 48, 1494–1509.

[1.9] Aslam, S.N., Underwood, G.J.C., Kaartokallio, H., Norman, L., Autio, R., Fischer, M., Kuosa, H., Dieckmann, G.S. and Thomas, D.N. (2012b) Dissolved extracellular polymeric substance (dEPS) dynamics and bacterial growth during sea ice formation in an ice tank study. *Polar Biology* 35, 661–676.

[1.10] Aslam, S.N., Michel, C., Niemi, A. and Underwood, G.J.C. (2016) Patterns and drivers of carbohydrate budgets in ice algal assemblages from first year Arctic sea ice. *Limnology and Oceanography* 61, 919-937.

[1.11] Aslam S.N., Strauss, J., Thomas, D.N., Mock, T. and Underwood, G.J.C. (2018) Identifying metabolic pathways for production of extracellular polymeric substances by the diatom *Fragilariopsis cylindrus* inhabiting sea ice, *The ISME Journal* 12, 1237–1251.

[1.12] Bedoshvili, Y.D. and Likhoshway Y.V. (2021) Cellular mechanisms of raphid diatom gliding. In: *Diatom Gliding Motility* Cohn S., Manoylov, K., and Gordon R. (eds.) Scrivener Publishing LLC. J. Wiley and Sons, Inc. Hoboken NJ: 65-76.

[1.13] Belando, M.D., Martínez, P.M. and Aboal, M. (2019) Interactive effects of warming and eutrophication on population dynamics and stalk production of epiphytic diatoms in transitional waters. *Estuarine, Coastal and Shelf Science* 229, #106413.

[1.14] Bellinger, B.J., Abdullahi, A.S., Gretz, M.R. and Underwood, G.J.C. (2005) Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms. *Aquatic Microbial Ecology* 38, 169–180.

[1.15] Bellinger, B.J., Underwood, G.J.C., Ziegler, S.E. and Gretz, M.R. (2009) Significance of diatom-derived polymers in carbon flow dynamics within estuarine biofilms determined through isotopic enrichment. *Aquatic Microbial Ecology* 55, 169–187.

[1.16] Bilcke, G., Osuna-Cruz, C.M., Silva, M.S., Poulsen, N., D'hondt, S., Bulankova, P., Vyverman, W., De Veylder, L. and Vandepoele, K. (2021) Diurnal transcript profiling of the diatom *Seminavis robusta* reveals adaptations to a benthic lifestyle. *The Plant Journal* 107, 315–336.

[1.17] Bohórquez J, McGenity, T.J., Papaspyrou, S., García-Robledo, E., Corzo, A. and Underwood G.J.C. (2017) Different Types of Diatom-Derived Extracellular Polymeric

Substances Drive Changes in Heterotrophic Bacterial Communities from Intertidal Sediments. *Frontiers in Microbiology* 8, #245.

[1.18] Brandes, J., Kuhajek, J.M., Goodwin, E. and Wood, S.A. (2016) Molecular characterisation and co-cultivation of bacterial biofilm communities associated with the mat-forming diatom *Didymosphenia geminata*. *Microbial Ecology* 72, 514–525.

[1.19] Brembu, T., Mühlroth, A., Alipanah, L. and Bones, A.M. (2017) The effects of phosphorus limitation on carbon metabolism in diatoms. *Philosophical Transactions of the Royal Society B* 372, #20160406.

[1.20] Bruckner, C.G., Bahulikar, R., Rahalkar, M., Schink, B. and Kroth, P.G. (2008) Bacteria associated with benthic diatoms from Lake Constance: Phylogeny and influences on diatom growth and secretion of extracellular polymeric substances. *Applied and Environmental Microbiology* 74, 7740–7749.

[1.21] Bruckner, C.G., Rehm, C., Grossart. H-P. and Kroth, P.G. (2011) Growth and release of extracellular organic compounds by benthic diatoms depend on interactions with bacteria. *Environmental Microbiology* 13, 1052-1063.

[1.22] Brussard, A., Corre, E., Hubas, C., Duvernois-Berthet, E., Le Corguill, G., Jourdren, L., Coulpier, F., Claquin, P. and Lopez, P.J. (2017) Physiological adjustments and transcriptome reprogramming are involved in the acclimation to salinity gradients in diatoms. *Environmental Microbiology* 19, 909-925.

[1.23] Buhmann, M.T., Schulze, B., Förderer, A., Schleheck, D., and Kroth, P.G. (2016) Bacteria may induce the secretion of mucin-like proteins by the diatom *Phaeodactylum tricornutum*. *Journal of Phycology* 52, 463–474.

[1.24] Chen, G. and Hoffman, A.S. (1995) Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH. *Nature* 373,49-52.

[1.25] Chiovitti, A., Higgins, M.J., Harper, R.E., Wetherbee, R. and Bacic, A. (2003) The complex polysaccharides of the raphid diatom *Pinnularia viridis* (Bacillariophyceae). *Journal of Phycology* 39, 543–554.

[1.26] Chiovitti, A., Molino, P., Crawford, S.A., Teng, R.W., Spurck, T. and Wetherbee, R. (2004) The glucans extracted with warm water from diatoms are mainly derived from intracellular chrysolaminaran and not extracellular polysaccharides. *European Journal of Phycology* 39, 117-28.

[1.27] Chiovitti, A., Harper, R.E., Willis, A., Bacic, A., Mulvaney, P. and Wetherbee, R. (2005) Variations in the substituted 3-linked mannans closely associated with the silicified walls of diatoms. *Journal of Phycology* 41, 1154-61.

[1.28] Christianen, M.J.A., Middleburg, J.J., Holthusijsen, S.J., Jouta, J., Compton, T.J., van der Heide, T., Piersma, T., Sinninghe Damste, J.S., van Derveer, H.W., Schouten, S. and Olff, H. (2017) Benthic primary producers are key to sustain the Wadden Sea food web: stable carbon isotope analysis at landscape scale. *Ecology* 98, 1498–1512.

[1.29] Cohn S.A., Warnick, L. and Timmerman, B. (2021) Photophobic responses of diatoms – motility and interspecies modulation, In: *Diatom Gliding Motility* Cohn, S., Manoylov, K., and Gordon, R. (eds.), Scrivener Publishing LLC. J. Wiley and Sons, Inc. Hoboken, NJ: 111-134.

[1.30] Cook, P.L.M., Veuger, B., Boer, S. and Middelburg, J.J. (2007) Effect of nutrient availability on carbon and nitrogen incorporation and flows through benthic algae and bacteria in near-shore sandy sediment. *Aquatic Microbial Ecology* 49, 165–180.

[1.31] Daglio, Y., Maidana, N.I., Matulewicz, M.C. and Rodríguez, M.C. (2016) Changes in motility and induction of enzymatic activity by nitrogen and phosphate deficiency in benthic *Halamphora luciae* (Bacillariophyceae) from Argentina. *Phycologia* 55, 493-505.

[1.32] de Brouwer, J.F.C., and Stal, L.J. (2001) Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. *Marine Ecology Progress Series* 218, 33–44.

[1.33] de Brouwer, J.F.C. and Stal, L.J. (2002) Daily fluctuations of exopolymers in cultures of the benthic diatoms *Cylindrotheca closterium* and *Nitzschia* sp. (Bacillariophyceae). *Journal of Phycology* 38, 464-72.

[1.34] Decho, A.W. (1994) Molecular-scale events influencing the macroscale cohesiveness of exopolymers. In: *Biostabilization of Sediments.* Krumbein, W.E., Paterson, D.M., and Stal, L.J. (eds.). Bibliotheks und Informationssystem der Universitat Oldenburg: 135–148.

[1.35] Decho, A.W. (2000) Microbial biofilms in intertidal systems: an overview. *Continental Shelf Research* 20, 1257-1273.

[1.36] Decho, A.W. and Gutierrez, T. (2017) Microbial Extracellular Polymeric Substances (EPSs) in Ocean Systems. *Frontiers in Microbiology* 8, #922.

[1.37] Donot, F., Fontana, A., Baccou, J.C. and Schorr-Galindo, S. (2012) Microbial exopolysaccharides: Main examples of synthesis, excretion, genetics and extraction. *Carbohydrate Polymers* 87, 951-962.

[1.38] Dow, L., Morrissey, K.L., Willems, A. and Kroth, P.G. (2020) Complete genome sequence of *Dyadobacter* sp. 32, isolated from a culture of the freshwater diatom *Cymbella microcephala. Marine Genomics* 52, #100720.

[1.39] Dugdale, T.M., Dagastine, R., Chiovitti, A. and Wetherbee, R. (2006) Diatom adhesive mucilage contains distinct supramolecular assemblies of a single modular protein. *Biophysical Journal* 90, 2987–2993.

[1.40] Durkin, C.A., Mock, T. and Armbrust, E.V. (2009) Chitin in diatoms and its association with the cell wall. *Eukaryotic Cell* 8, 1038-1050.

[1.41] Engel. A., Endres, S., Galgani, L. and Schartau, M. (2020) Marvelous Marine Microgels: On the Distribution and Impact of Gel-Like Particles in the Oceanic Water-Column. *Frontiers in Marine Science* 7, #405

[1.42] Ewert, M. and Deming, J. W. (2013) Sea Ice Microorganisms: Environmental constraints and extracellular responses. *Biology*, 2, 603-628.

[1.43] Fabris, M., Matthijs, M., Rombauts, S., Vyverman, W., Goossens, A. and Baart, G.J. (2012) The metabolic blueprint of *Phaeodactylum tricornutum* reveals a eukaryotic Entner–Doudoroff glycolytic pathway. *The Plant Journal* 70, 1004-1014.

[1.44] Ferrari C., Proost, S., Janowski, M., Becker, J., Nikoloski, Z., Bhattacharya, D., Price, D., Tohge T., Bar-Even, A., Fernie, A., Stitt, M. and Mutwil1, M. (2019) Kingdom-wide comparison reveals the evolution of diurnal gene expression in Archaeplastida. *Nature Communications* 10, #737

[1.45] Gihring, T.M., Humphrys, M., Mills, H.J., Huettel, M. and Kostka, J.E. (2009) Identification of phytodetritus-degrading microbial communities in sublittoral Gulf of Mexico sands. *Limnology and Oceanography* 54, 1073–1083.

[1.46] Giroldo, D., Viera, A.A.H. and Paulsen, B.S. (2003) Relative increase in deoxy sugars during microbial degradation of an extracellular polysaccharide release by a tropical freshwater *Thalassiosira* sp. (Bacillariophyceae). *Journal of Phycology* 39, 1109-1115.

[1.47] Gordon, R. (2021) The whimsical history of proposed motors for diatom motility. In: *Diatom Gliding Motility*. Cohn S., Manoylov, K., and Gordon R. (eds.), Scrivener Publishing LLC. J. Wiley and Sons, Inc. Hoboken NJ: 335-420.

[1.48] Gruber, A and Kroth, P.G. (2017) Intracellular metabolic pathway distribution in diatoms and tools for genome-enabled experimental diatom research. *Philosophical Transactions of the Royal Society B* 372, #20160402.

[1.49] Gügi, B., Le Costaouec, T., Burel, C., Lerouge, P., Helbert, W. and Bardor, M. (2015) Diatom-specific oligosaccharide and polysaccharide structures help to unravel biosynthetic capabilities in diatoms. *Marine Drugs* 13, 5993-6018.

[1.50] Hanlon, A.R.M., Bellinger, B., Haynes, K., Xiao, G., Hofmann, T.A., Gretz, M.R., Ball, A.S., Osborn, A.M. and Underwood, G.J.C. (2006) Dynamics of extracellular polymeric substance (EPS) production and loss in an estuarine, diatom-dominated, microalgal biofilm over a tidal emersion-immersion period. *Limnology and Oceanography* 51, 79–93.

[1.51] Hao, Y. and Lam, J.S. (2011) Pathways for the biosynthesis of NDP sugars. In *Bacterial lipopolysaccharides*, Knirel, A. and Valvano, M.A. (eds), Springer; Vienna: 195–235.

[1.52] Haro, S., Bohórquez, J., Lara, M., González, C.J., Crespo, J.M., García-Robledo, E., Papaspyrou, S. and Corzo, A. (2019) Diel patterns of microphytobenthic primary production in intertidal sediments: circadian photosynthetic rhythm and migration. *Scientific Reports* 9, #13376.

[1.53] Haynes, K., Hofmann, T.A., Smith, C.J., Ball, A.S., Underwood, G.J.C. and Osborn, A.M. (2007) Diatom-derived carbohydrates as factors affecting bacterial community composition in estuarine sediments. *Applied and Environmental Microbiology* 73, 6112–6124.

[1.54] Hidalgo-Cantabrana, C.,Sánchez, B., Milani, C., Ventura, M., Margolles, A. and Ruas-Madiedo, P. (2014) Genomic overview and biological functions of exopolysaccharide biosynthesis in *Bifidobacterium* spp. *Applied and Environmental Microbiology* 80, 9-18.

[1.55] Higgins, M.J., Molino, P., Mulvaney, P. and Wetherbee, R. (2003) The structure and nanomechanical properties of the adhesive mucilage that mediates diatom-substratum adhesion and motility. *Journal of Phycology* 39, 1181–1193.

[1.56] Hoagland, K.D., Rosowski, J.R., Gretz, M.R. and Roemer, S.C. (1993) Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *Journal of Phycology* 29, 537-566.

[1.57] Hofmann, T., Hanlon, A.R.M., Taylor, J.D., Ball, A.S., Osborn, A.M. and Underwood, G.J.C. (2009) Dynamics and compositional changes in extracellular

carbohydrates in estuarine sediments during degradation. *Marine Ecology Progress Series* 379, 45–58.

[1.58] Hope, J.A., Paterson, D.M. and Thrush, S.F. (2020) The role of microphytobenthos in soft-sediment ecological networks and their contribution to the delivery of multiple ecosystem services. *Journal of Ecology* 108, 815–830.

[1.59] Hörnlein, C., Confurius-Guns, V., Stal, L.J. and Bolhuis, H. (2018) Daily rhythmicity in coastal microbial mats. *NPJ Biofilms Microbiomes* 4, #11.

[1.60] Huang, W., Haferkamp, I., Lepetit, B., Molchanova, M., Hou, S., Jeblick, W., Bártulos, C. R. and Kroth, P.G. (2018) Reduced vacuolar β-1,3-glucan synthesis affects carbohydrate metabolism as well as plastid homeostasis and structure in *Phaeodactylum tricornutum*. *Proceedings of the National Academy of Sciences of the United States of America* 115, 4791–4796.

[1.61] Janech, M.G., Krell, A., Mock, T., Kang, J.S. and Raymond, J.A. (2006) Ice-binding proteins from sea ice diatoms (Bacillariophyceae). *Journal of Phycology* 42, 410–416.

[1.62] Jauffrais, T., Agogué, H., Gemin M.-P., Beaugeard, L. and Martin-Jézéquel, V. (2017) Effect of bacteria on growth and biochemical composition of two benthic diatoms *Halamphora coffeaeformis* and *Entomoneis paludosa*. *Journal of Experimental Marine Biology and Ecology* 495, 65–74.

[1.63] Jensen, E., Clément, R., Maberly, S.C. and Gontero, B. (2017) Regulation of the Calvin–Benson–Bassham cycle in the enigmatic diatoms: biochemical and evolutionary variations on an original theme. *Philosophical Transactions of the Royal Society B* 372, #20160401.

[1.64] Jönnson, B., Sundbäck, K., and Nilsson, C. (1994) An upright life-form of an epipelic motile diatoms: on the behaviour of *Gyrosigma balticum*. *European Journal of Phycology*, 29, 11-15.

[1.65] Juhl, A.R., Krembs, C., Meiners, K.M. (2011) Seasonal development and differential retention of ice algae and other organic fractions in first year Arctic sea ice. *Marine Ecology Progress Series* 436, 1-16.

[1.66] Koedooder, C., Stock, W., Willems, A., Mangelinckx, S., De Troch, M., Vyverman, W. and Sabbe, K. (2019) Diatom-bacteria interactions modulate the composition and productivity of benthic diatom biofilms. *Frontiers in Microbiology* 10, #1255.

[1.67] Krembs, C. and Deming, J. (2008) The role of exopolymers in microbial adaptation to sea-ice. In: *Psychrophiles: from biodiversity to biotechnology* Margesin, R., Schinner, F., Marx, J-C. and Gerday, C. (eds.) Verlag Berlin: 247-264.

[1.68] Krembs, C., Eicken, H., Junge, K. and Deming, J.W. (2002) High concentration of exopolymeric substances in Arctic winter seawater: implication for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep-Sea Research I* 49, 2163-2181.

[1.69] Krembs, C., Eicken, H. and Deming, J.W. (2011) Exopolymer alteration of physical properties of sea ice and implications for ice habitability and biogeochemistry in a warmer Arctic. *Proceedings of the National Academy of Sciences of the United States of America* 108, 3653-3658.

[1.70] Kroth, P.G., Chiovitti, A., Gruber, A., Martin-Jezequel, V., Mock, T., Parker, M.S., Stanley, M.S., Kaplan, A., Caron, L., Weber, T., Mahaswari, U., Armbrust, E.A and

Bowler, C. (2008) A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from comparative whole genome analysis. PLoS ONE 3:e1426,

[1.71] Lachnit, M., Buhmann, M.T., Klemm, J., Kröger, N. and Poulsen, N. (2019) Identification of proteins in the adhesive trails of the diatom *Amphora coffeaeformis*. *Philosophical Transactions of the Royal Society B* 374, #20190196.

[1.72] Laut, L., Figueiredo, M. S. L., Lorini, M. L., Belart, P., Clemente, I., Martins, M.V. A., Mendonça Filho, J. G. and Laut, V. (2019) Diatoms from the most hypersaline lagoon in Brazil: Vermelha lagoon. *Continental Shelf Research* 181, 111–123.

[1.73] Leinweber, K. and Kroth, P.G. (2015) Capsules of the diatom *Achnanthidium minutissimum* arise from fibrillar precursors and foster attachment of bacteria *Peer Journal* 3:#e858.

[1.74] Lind, J.L., Heimann, K., Miller, E.A., van Vliet, C., Hoogenraad, N.J., and Wetherbee R. (1997) Substratum adhesion and gliding in a diatom are mediated by extracellular proteoglycans. *Planta* 203, 213–221.

[1.75] Liu, S.B., Chen, X.L., He, H.L., Zhang, X.Y., Xie, B.B., Yu, Y., Chen, B., Zhou, B-C. and Zhang, Y-Z. (2013) Structure and ecological roles of a novel exopolysaccharide from the Arctic sea ice bacterium *Pseudoalteromonas* sp. Strain SM20310. *Applied and Environmental Microbiology* 79, 224–230.

[1.76] Marx, J.G., Carpenter, S.D. and Deming, J.W. (2009) Production of cryoprotectant extracellular polysaccharide substances (EPS) by the marine psychrophilic bacterium *Colwellia psychreythraea* strain 34H under extreme conditions, *Canadian Journal of Microbiology* 55, 63-72.

[1.77] McConville, M.J. (1985) Chemical composition and biochemistry of sea ice microalgae. In: *Sea Ice biota.* Horner, R.A. (ed.) CRC Press. Boca Raton, FL: 105-129.

[1.78] McConville, M.J., Mitchell, C. and Wetherbee, R. (1985) Patterns of carbon assimilation in a microalgal community from annual sea ice, East Antarctica. *Polar Biology*. 4, 135-41.

[1.79] McConville, M.J., Wetherbee, R. and Bacic, A. (1999) Subcellular location and composition of the wall and secreted extracellular sulphated polysaccharides/proteoglycans of the diatom *Stauroneis amphioxys* Gregory. *Protoplasma* 206, 188-200.

[1.80] McKew, B.A., Dumbrell, A., Taylor, J.D., McGenity, T.J. and Underwood, G.J.C. (2013) Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments. *FEMS Microbiology Ecology* 84, 495–509.

[1.81] McKew, B.A., Taylor, J.D., McGenity, T.J. and Underwood, G.J.C. (2011) Resistance and resilience of benthic biofilm communities from a temperate saltmarsh to desiccation and rewetting. *The ISME Journal* 5, 30–41.

[1.82] Michel, G., Tonon, T., Scornet, D., Cock, J.M. and Kloareg, B. (2010) The cell wall polysaccharide metabolism of the brown alga *Ectocarpus siliculosus*. Insights into the evolution of extracellular matrix polysaccharides in Eukaryotes. *New Phytologist* 188, 82-97.

[1.83] Middelburg, J.J., Barranguet, C., Boschker, H.T.S., Herman, P.M.J., Moens, T. and Heip, C.H.R. (2000) The fate of intertidal microphytobenthos carbon: An *in situ* ^{13}C-labelling study. *Limnology and Oceanography* 45, 1224-1334.

[1.84] Mirelman, D., Lotan, R., Bernsten, Y., Flowers, H. M. and Sharon, N. (1973) Purification and properties of an extracellular polysaccharide containing amino-sugars formed by *Bacillus cereus*. *Journal of General Microbiology* 77, 5-10.

[1.85] Mishra, A. and Jha, B. (2009) Isolation and characterization of extracellular polymeric substances from micro algae *Dunaliella salina* under salt stress. *Bioresources Technology* 100, 3382-86.

[1.86] Miyatake, T., Moerdijk-Poortvliet, T.C. W., Stal, L.J. and Boschker, H.T.S. (2014) Tracing carbon flow from microphytobenthos to major bacterial groups in an intertidal marine sediment by using an *in situ* ^{13}C pulse-chase method. *Limnology and Oceanography* 59, 1275-1287.

[1.87] Moerdijk-Poortvliet, T.C.W., van Breugel, P., Sabbe, K., Beauchard, O., Stal, L.J. and Boschker, H.T.S. (2018a) Seasonal changes in the biochemical fate of carbon fixed by benthic diatoms in intertidal sediments. *Limnology and Oceanography* 63, 550–569.

[1.88] Moerdijk-Poortvliet, T.C.W., Beauchard, O., Stal, L.J. and Boschker, H.T.S. (2018b) Production and consumption of extracellular polymeric substances in an intertidal diatom mat. *Marine Ecology Progress Series* 592, 77–95.

[1.89] Molino, P.J., Hodson, O.M., Quinn, J.F. and Wetherbee, R. (2006) Utilizing QCM-D to characterize the adhesive mucilage secreted by two marine diatom species *in-situ* and in real-time. *Biomacromolecules* 7, 3276–3282.

[1.90] Myklestad, S. and Haug, A. (1972) Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *willei* (Gran) Hustedt. I. Effect of the concentration of nutrients in the culture medium. *Journal of Experimental Marine Biology and Ecology* 9,125-136.

[1.91] Nedwell, D.B., Underwood, G.J.C., McGenity, T.J., Whitby, C. and Dumbrell, A.J. (2016) The Colne Estuary: A Long-Term Microbial Ecology Observatory, *Advances in Ecological Research* 55, 227-281.

[1.92] Nevot, M., Deroncele, V., Montes, M. J. and Mercade, E. (2008). Effect of incubation temperature on growth parameters of *Pseudoalteromonas antarctica* NF (3) and its production of extracellular polymeric substances. *Journal of Applied Microbiology* 105, 255-63.

[1.93] Oakes, J.M., Eyre, B.D., Middelburg, J.J. and Boschker, H.T.S. (2010) Composition, production, and loss of carbohydrates in subtropical shallow subtidal sandy sediments: Rapid processing and long-term retention revealed by ^{13}C-labeling. *Limnology and Oceanography* 55, 2126–2138.

[1.94] Oakes, J.M., Eyre, B.D. and Middelburg, J.J. (2012). Transformation and fate of microphytobenthos carbon in subtropical shallow subtidal sands: A ^{13}C-labeling study. *Limnology and Oceanography* 57, 1846-1856.

[1.95] Orgambide, G., Montrozier, H., Servin, P., Roussel, J., Trigaletdemery, D. and Trigalet, A. (1991) High heterogeneity of the exopolysaccharides of *Pseudomonas solanacearum* strain gmi 1000 and the complete structure of the major polysaccharide. *Journal of Biological Chemistry* 266, 8312-8321.

[1.96] Passarelli, C., Cui, X., Valsami-Jones, E. and Underwood, G.J.C. (2020) Environmental context determines the impact of titanium oxide and silver nanoparticles on the functioning of intertidal microalgal biofilms. *Environmental Science: Nano* 7, 3020-3035.

[1.97] Paterson, D.M, and Hope, J.A. (2021) Diatom biofilms: ecosystem engineering and niche construction. In: *Diatom Gliding Motility* Cohn S., Manoylov, K., and Gordon R. (eds.). Scrivener Publishing LLC. J. Wiley and Sons, Inc. Hoboken NJ: 135-158.

[1.98] Perkins R.G., Underwood, G.J.C., Brotas, V., Snow, G.C., Jesus, B. and Ribeiro, L. (2001) Responses of microphytobenthos to light: primary production and carbohydrate allocation over an emersion period, *Marine Ecology Progress Series* 223, 101-112.

[1.99] Pierre, G., Graber, M., Rafiliposon, B.A., Dupuy, C., Orvain, F., de Crignis, M. and Maugard, T. (2012) Biochemical composition and changes of extracellular polysaccharides (ECPS) produced during microphytobenthic biofilm development (Marennes–Oléron, France). *Microbial Ecology* 63, 157–169.

[1.100] Pierre, G., Zhao, J.M., Orvain, F., Dupuy, C., Klein, G., Graber, M. and Maugard, T. (2014) Seasonal dynamics of extracellular polymeric substances (EPS) in surface sediments of a diatom-dominated intertidal mudflat (Marennes-Oléron, France). *Journal of Sea Research* 92, 26-35.

[1.101] Pinckney, J.L. (2018) A mini-review of the contribution of benthic microalgae to the ecology of the continental shelf in the South Atlantic Bight. *Estuaries and Coasts* 41, 2070-2078

[1.102] Poulin, M., Daugbjerg, N., Gradinger, R., Ilyash, L., Ratkova,T. and von Quillfeldt, C. (2011) The pan-Arctic biodiversity of marine pelagic and sea-ice unicellular eukaryotes: a first-attempt assessment. *Marine Biodiversity* 41, 13–28.

[1.103] Poulsen, N., Kröger, N., Harrington M.J., Brunner, E., Paasch, S. and Buhmann, M.T. (2014) Isolation and biochemical characterization of underwater adhesives from diatoms. *Biofouling* 30, 513-523.

[1.104] Poulsen, N., Davutoglu. M. G. and Suchanova, J. Z. (in press) Diatom adhesion and motility In: *The Molecular Life of Diatoms*. Falciatore A, and Mock, T. (eds.), Springer, Cham. 367 - 393

[1.105] Prins, A., Deleris, P., Hubas, C. and Jesus, B. (2020) Effect of light intensity and light quality on diatom behavioral and physiological photoprotection. *Frontiers in Marine Science* 7, #203

[1.106] Rishworth, G.M., Dodd, C., Perissinotto, R., Bornman, T.G., Adams, J.B., Anderson, C.R., Cawthra, H.C., Dorrington, R.A., du Toit, H., Edworthy, C., Gibb, R-LA., Human, L.R.D., Isemonger, E.W., Lemley, D.A., Miranda, N.A.F., Peer, N., Raw, J.L., Smith, A.M., Steyn, P-P., Strydom, N.A., Teske, P.R.and Welman, S. (2020) Modern supratidal microbialites fed by groundwater: functional drivers, value and trajectories. *Earth Science Reviews* 210, #103364.

[1.107] Sandford, P.A., Pittsley, J.E., Watson, P.R., Burton, K.A., Cadmus, M.C. and Jeanes, A. (1978) Rheological and other physical characteristics of polysaccharides from 2 black yeast like fungi. *Journal of Applied Polymer Science* 22,701-10.

[1.108] Serôdio, J. (2021) Diatom motility: mechanisms, controls and adaptive value, In: *Diatom Gliding Motility.* Cohn S., Manoylov, K., and Gordon R. (eds.) Scrivener Publishing LLC. J. Wiley and Sons, Inc. Hoboken NJ:159-184.
[1.109] Smith, D.J. and Underwood, G.J.C. (1998) Exopolymer production by intertidal epipelic diatoms. *Limnology and Oceanography* 43, 1578–1591.
[1.110] Smith, D.J. and Underwood, G.J.C. (2000) The production of extracellular carbohydrate exopolymers (EPS) by estuarine benthic diatoms: the effects of growth phase and light and dark treatment. *Journal of Phycology* 36, 321-333.
[1.111] Staats, N., de Winder, B., Stal, L.J. and Mur, L.R. (1999) Isolation and characterisation of extracellular polysaccharides from the epipelic diatoms *Cylindrotheca closterium* and *Navicula salinarum*. *European Journal of Phycology* 34, 161-169.
[1.112] Stal, L.J. (2003). Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments. *Geomicrobiology Journal* 20, 463-478.
[1.113] Stal, L.J. (2010) Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization. *Ecological Engineering* 36, 236-245.
[1.114] Stal, L.J., Bolhuis, H. and Cretoiu, M.S. (2019) Phototrophic marine benthic microbiomes: the ecophysiology of these biological entities. *Environmental Microbiology* 21, 1529–1551.
[1.115] Steele D.J., Franklin, D. J. and Underwood, G.J.C. (2014) Protection of cells from salinity stress by extracellular polymeric substances in diatom biofilms. *Biofouling*, 30, 987-998.
[1.116] Stock, W., Blommaert, L., De Troch, M., Mangelinckx, S., Willems, A., Vyverman, W. and Sabbe, K. (2019) Host specificity in diatom–bacteria interactions alleviates antagonistic effects, *FEMS Microbiology Ecology* 95: #fiz171.
[1.117] Sutherland, I. (2001) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 147: 3-9.
[1.118] Suzuki, A. and Tanaka, T. (1990) Phase transition in polymer gels induced by visible light. *Nature* 346, 345-347.
[1.119] Takahashi, E., Ledauphin, J., Goux, D. and Orvain, F. (2009) Optimising extraction of extracellular polymeric substances (EPS) from benthic diatoms: comparison of the efficiency of six EPS extraction methods *Marine and Freshwater Research* 60, 1201-1210.
[1.120] Taylor, J.D., McKew, B.A., Kuhl, A., McGenity, T.J. and Underwood, G.J.C. (2013). Microphytobenthic extracellular polymeric substances (EPS) in intertidal sediments fuel both generalist and specialist EPS-degrading bacteria. *Limnology and Oceanography* 58, 1463-1480.
[1.121] Tesson, B., Masse, S., Laurent, G., Maquet, J., Livage, J., Martin-Jezequel, V. and Coradin, T. (2008) Contribution of multi-nuclear solid state NMR to the characterization of the *Thalassiosira pseudonana* diatom cell wall. *Analytical and Bioanalytical Chemistry* 390, 1889-1898.
[1.122] Underwood, G.J.C. (2002) Adaptations of tropical marine microphytobenthic assemblages along a gradient of light and nutrient availability in Suva Lagoon, Fiji. *European Journal of Phycology* 37, 449–462.

[1.123] Underwood, G.J.C., Boulcott, M., Raines, C.A. and Waldron, K. (2004) Environmental effects on exopolymer production by marine benthic diatoms: Dynamics, changes in composition, and pathways of production. *Journal of Phycology* 40, 293-304.
[1.124] Underwood, G.J.C., Dumbrell, A.J., McGenity, T.J., McKew B. A., Whitby C. (2022). The Microbiome of Coastal Sediments. In: *The Marine Microbiome.* Stal, L.J. and Cretoiu, S. (eds.), Springer Nature Switzerland AG: 479-534.
[1.125] Underwood, G.J.C., Fietz, S., Papadimitriou, S., Thomas, D.N. and Dieckmann, G. S. (2010) Distribution and composition of dissolved extracellular polymeric substances (EPS) in Antarctic sea ice. *Marine Ecology Progress Series* 404, 1-19.
[1.126] Underwood, G.J.C., Michel, C., Meisterhans, G., Niemi, A., Belzile, C., Witt, M., Dumbrell, A.J. and Koch, B.P. (2019) Organic matter from Arctic sea ice loss alters bacterial community structure and function. *Nature Climate Change* 9, 170–176.
[1.127] Underwood, G.J.C. and Paterson, D.M. (2003) The importance of extracellular carbohydrate production by marine epipelic diatoms. *Advances in Botanical Research* 40, 184-240.
[1.128] Underwood, G.J.C. and Thomas, J. D. (1990) Grazing interactions between pulmonate snails and epiphytic algae and bacteria. *Freshwater Biology* 23, 505-522.
[1.129] Varum, K.M., and S. Myklestad (1984) Effects of light, salinity and nutrient limitation on the production of β-1, 3-D-Glucan and Exo-D-Glucanase activity in *Skeletonema costatum* (Grev.) Cleve. *Journal of Experimental Marine Biology and Ecology* 83, 13-25.
[1.130] Verdugo, P. (2012) Marine microgels. *Annual Review of Marine Science* 4, 9.1–9.25.
[1.131] Volcani, B.E. (1981) Role of silicon in diatom metabolism and silicification. In: *Silicon and siliceous structures in biological systems.* Simpson, T.L. and Volcani, B.E. (eds.) Springer, New York, USA: 157-200.
[1.132] Wagner H., Jakob, T., Fanesi, A. and Wilhelm C. (2017) Towards an understanding of the molecular regulation of carbon allocation in diatoms: the interaction of energy and carbon allocation. *Philosophical Transactions of the Royal Society B.* 372, #20160410.
[1.133] Wang, Y., Chen, Y., Lavin, C., and Gretz, M.R. (2000) Extracellular matrix assembly in diatoms (Bacillariophyceae). IV. Ultrastructure of *Achnanthes longipes* and *Cymbella cistula* as revealed by high-pressure freezing / freeze substitution and cryo-field emission scanning electron microscopy. *Journal of Phycology* 36, 367–378.
[1.134] Wetherbee, R., Lind, J.L., Burke, J., and Quatrano, R.S. (1998) The first kiss: establishment and control of initial adhesion by raphid diatoms. *Journal of Phycology* 34, 9–15.
[1.135] Willis, A., Eason-Hubbard, M., Hodson, O., Maheswari, U., Bowler, C. and Wetherbee, R. (2014) Adhesion molecules from the diatom *Phaeodactylum tricornutum* (Bacillariophyceae): genomic identification by amino-acid profiling and *in vivo* analysis. *Journal of Phycology* 50, 837-849.
[1.136] Windler, M, Gruber, A., Kroth, P.G. (2012) Purification of benthic diatoms from associated bacteria using the antibiotic imipenem. *Journal of Endocytobiosis and Cell Research* 22, 62–65.

[1.137] Windler, M., Leinweber, K., Rio Bartulos, C., Philipp, B. and Kroth, P.G. (2015) Biofilm and capsule formation of the diatom *Achnanthidium minutissimum* are affected by a bacterium. *Journal of Phycology* 51, 343-55.
[1.138] Wustman, B.A., Gretz, M.R. and Hoagland, K. D. (1997) Extracellular matrix assembly in diatoms (Bacillariophyceae). I. A model of adhesives based on chemical characterization and localization of polysaccharides from the marine diatom *Achnanthes longipes* and other diatoms. *Plant Physiology*, 113, 1059-69.
[1.139] Wustman, B.A., Lind, J., Wetherbee, R. and Gretz, M.R. (1998) Extracellular matrix assembly in diatoms (Bacillariophyceae) III. Organization of fucoglucuronogalactans within the adhesive stalks of *Achnanthes longipes*. *Plant Physiology* 116, 1431–1441.
[1.140] Zhang, W., Tang, X., Yang, Y., Zhang, X. and Zhang, X. (2020) Elevated pCO2 level affects the extracellular polymer metabolism of *Phaeodactylum tricornutum*. *Frontiers in Microbiology* 11, #339.
[1.141] Zhou, J., Mopper, K. and Passow, U. (1998) The role of surface active carbohydrates in the formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnology and Oceanography* 43, 1860-1871.

1.9 Figures.

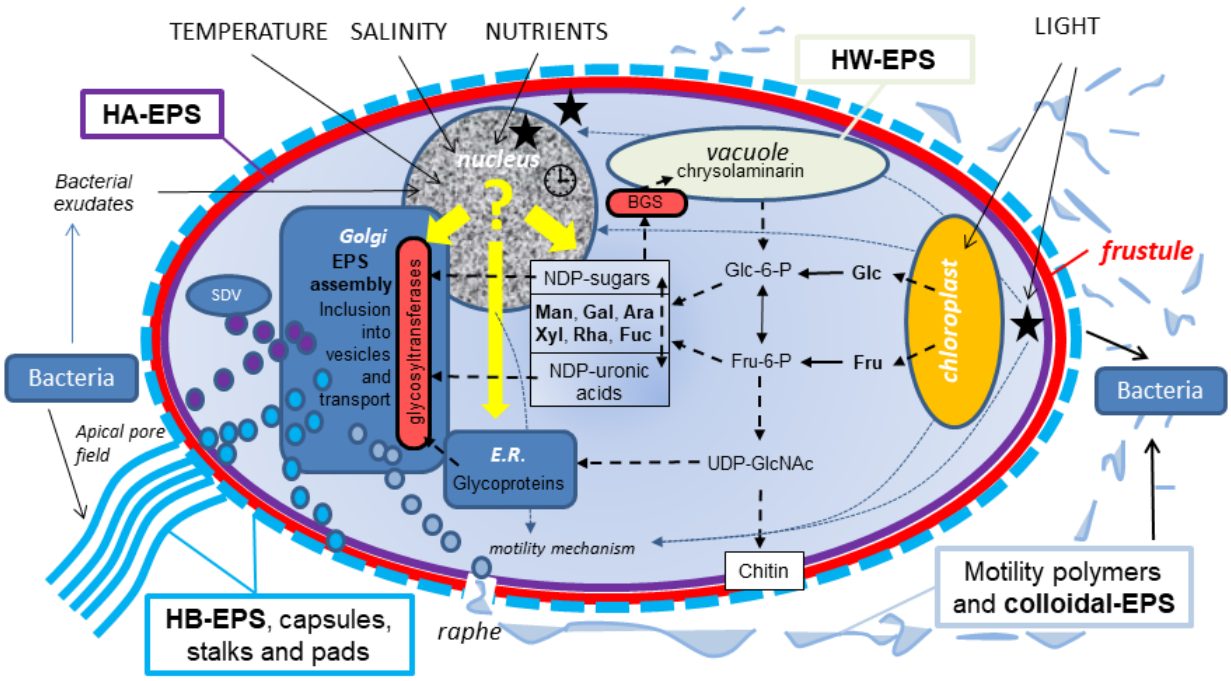

Figure 1. Schematic of production pathways and influences on EPS production in benthic diatoms. Glucose (**Glc**) and Fructose (**Fru**) generated by photosynthetic activity is phosphorylated (Glc-6-P and Fru-6-P) and undergoes various transformations to produce NDP-sugars and NDP-uronic acid derivatives of Glc and other monosaccharides (mannose **Man**, galactose **Gal**, arabinose **Ara**, xylose **Xyl**, rhamnose **Rha** and fucose **Fuc**). These are transported into the Golgi. Fru-6-P can be converted to UDP-N-acetylglucosamine (UDP-GlcNAc) which contributes to glycoprotein formation in the endoplasmic reticulum (*E.R.*). UDP-GlcNAc is also a precursor to chitin synthesis. EPS is assembled into polysaccharides by the activity of glycosyltransferases, packaged into vesicles and transported to the cell membrane for secretion. The control mechanisms for determining EPS chemistry, transport of constituent molecules, packaging and transport of EPS-filled vesicles to specific locations, and the degree of extracellular assembly, are only tentatively known for some EPS. Four extractable fractions of EPS are indicated: hot-water extracted (**HW-EPS**), which is primarily

chrysolaminarin, produced by β-glucan synthase (BGS) located in the vacuole membrane, and stored in the vacuole. Chrysolaminarin is utilised as a carbon source for EPS production in the dark. Hot-alkali extracted EPS (**HA-EPS**) is associated with the silica frustule; hot-bicarbonate extracted EPS (**HB-EPS**) is present in stalks and coating on the outside of the frustule; and **colloidal-EPS** is present in the environment surrounding the cells, and also includes motility polymers and smaller polysaccharide moieties. Light, temperature, salinity and nutrients, photoreceptors (★), circadian regulators (🕒) and bacteria signals all influence EPS production, but the mechanisms are unresolved. Dotted lines indicate a sequence of transformations regulated by various enzymes (Figure based on information from [1.11] [1.16] [1.48] [1.56] [1.60] [1.71] [1.104] [1.105] [1.123] [1.140]).

END