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Author for correspondence:
Mario Giordano
e-mail: m.giordano@univpm.it

We dedicate this paper to the late Craig Sandgren who made major contributions to our understanding of the Chrysophyceae and Syurophyceae.

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Acquisition and metabolism of carbon in the Ochrophyta other than diatoms

John A. Raven[1,2] and Mario Giordano[3,4]

[1]Division of Plant Sciences, University of Dundee at the James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK
[2]Climate Change Cluster, University of Technology Sydney, Ultimo, New South Wales 2007, Australia
[3]Dipartimento di Scienze della Vita e dell'Ambiente, Università Politecnica delle Marche, Ancona 60131, Italy
[4]Institute of Microbiology, Academy of Sciences of the Czech Republic, Třeboň 37901, Czech Republic

JAR, 0000-0002-2789-3297; MG, 0000-0001-7754-0521

The acquisition and assimilation of inorganic C have been investigated in several of the 15 clades of the Ochrophyta other than diatoms, with biochemical, physiological and genomic data indicating significant mechanistic variation. Form ID Rubiscos in the Ochrophyta are characterized by a broad range of kinetics values. In spite of relatively high $K_{0.5}CO_2$ and low $CO_2 : O_2$ selectivity, diffusive entry of CO_2 occurs in the Chrysophyceae and Synurophyceae. Eustigmatophyceae and Phaeophyceae, on the contrary, have CO_2 concentrating mechanisms, usually involving the direct or indirect use of HCO_3^-. This variability is possibly due to the ecological contexts of the organism. In brown algae, C fixation generally takes place through a classical C3 metabolism, but there are some hints of the occurrence of C4 metabolism and low amplitude CAM in a few members of the Fucales. Genomic data show the presence of a number of potential C4 and CAM genes in Ochrophyta other than diatoms, but the other core functions of many of these genes give a very limited diagnostic value to their presence and are insufficient to conclude that C4 photosynthesis is present in these algae.

This article is part of the themed issue 'The peculiar carbon metabolism in diatoms'.

1. Introduction

Stramenopile eukaryotes comprise a diverse clade with a great range of nutritional modes [1] (table 1). Ochrophyta (i.e. Ochrista) is a diverse phylum of stramenopiles; most of the ochrophytan clades (16 in [5]) are photosynthetic or have photosynthetic members. The rest of the stramenopiles, the ecologically and economically very significant oomycetes and some others clades (a total of 8 in [2]), are all non-photosynthetic. The phylogenetic relations among these organisms are discussed by Baurain *et al*. [6], Brown & Sorhannus [7], Beakes *et al*. [2], Schmidt *et al*. [8] and Yang *et al*. [5].

The Bacillariophyceae is the best-investigated class of ochrophytes with respect to inorganic C acquisition and assimilation, showing the widespread occurrence of CO_2 concentrating mechanisms (CCMs) and of pyrenoids, energized transport of HCO_3^- and limited support for C_4 or C_4-like photosynthetic metabolism [9–12]. The emphasis on diatoms is reasonable in view of their phylogenetic and ecological diversity [13] and their contribution to global biogeochemical cycles (e.g. a marine diatom net primary productivity of about 20 Pg C per year [14]). However, there are 15 (possibly more) other ochrophyte classes with photosynthetic members [5–8,15]. The benthic marine macroalgal Phaeophyceae play a major role in primary productivity and as ecosystem engineers on rocky shores, especially in temperate and polar regions, with several species exceeding 10 m in length [16]. The Chrysophyceae and Synurophyceae are significant in the phytoplankton of acidic and often CO_2-enriched freshwaters [3,17–19], the microalga *Nannochloropsis* (Eustigmatophyceae) is being assessed for its biotechnological potential [20–22] and members of the Pelagophyceae and

Table 1. Examples of modes of nutrition in the Ochrophyta. See Raven *et al*. [1] for definition of modes of nutrition.

nutritional mode	example	references
free-living osmoorganotrophy	most Saprolegniales, e.g. *Saprolegnia* (oomycetes)	[2]
symbiotic (all parasitic)[a] osmoorganotrophy	perenosporalean clades, e.g. *Phytophthora*[b] (oomycetes); bicoecids; *Cafeteria*	[2]
phagoorganotrophy	some Chrysophyceae, e.g. *Paraphysomonas*	[2]
photolithotrophy	examples: Bacillariophyceae, Eustigmatophyceae, Phaeophyceae, Synurophyceae	[3]
phagophotomixotrophy	many Chrysophyceae	[3,4]

[a]Symbiosis is used in the broad sense, including mutualism and parasitism.
[b]Major parasite of flowering plants.

Raphidophyceae can form harmful algal blooms [23]. Here we synthesize what is known about inorganic carbon acquisition and assimilation in these non-diatom ochrophytes for comparison with what is known for diatoms.

2. Mechanisms of inorganic C influx: CO_2 diffusion and biophysical CO_2 concentrating mechanisms

Diffusive CO_2 entry from the bulk phase to ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) is driven by a concentration difference produced by the photosynthetic consumption of CO_2 that produces a lower CO_2 concentration in the proximity of Rubisco active sites than in the bulk phase supplying the CO_2. Here the energization of the flux is indirect, coming from the use of absorbed photons for the reduction of $NADP^+$ to NADPH and the phosphorylation of ADP to ATP; the NADPH and ATP are then used in CO_2 assimilation producing, in the first instance, carbohydrate. Under these circumstances, the CO_2 concentration and the CO_2: O_2 ratio at the Rubisco site typically allow significant Rubisco oxygenase activity and a corresponding operation of a photorespiratory C oxidation cycle that converts the 2-P-glycolate from Rubisco oxygenase into triose phosphate, with input of energy, directly or indirectly, from the thylakoid reactions of photosynthesis [24].

The functioning of CCMs, by definition, involves a higher steady state concentration of CO_2 at the site of Rubisco than occurs in the bulk medium. This transport of CO_2 against the free energy (concentration) gradient is energized independently of the energy input to the conversion of CO_2 to carbohydrate. This is the case for both the biophysical (energized, uphill transport of HCO_3^- or CO_2 or H^+ across a cell membrane) and the biochemical (C4 photosynthesis or Crassulacean acid metabolism, CAM) CCMs. This parallel input of energy comes ultimately from the thylakoid reactions of photosynthesis, but sometimes indirectly via photosynthate production and its catabolism through glycolysis and the mitochondrial reactions [24]. Generally, despite the occurrence of CCMs, the CO_2: O_2 ratio at the Rubisco active site is not higher enough to prevent a small flux of organic C through Rubisco oxygenase and a photorespiratory C oxidation cycle that adds to the energy cost [25], as does the inevitable leakage of some of the CO_2 accumulated at the Rubisco site [24,26,27].

3. Inorganic C acquisition in Ochrophyta other than diatoms

(a) Introduction

There is substantial physiological evidence on the mechanism of inorganic carbon acquisition for a number of classes of the Ochrophyta, i.e. Chrysophyceae, Eustigmatophyceae, Phaeophyceae and Synurophyceae, with a little information available for a few other classes; genomic data are also available for the Eustigmatophyceae and the Phaeophyceae. We also discuss the occurrence of pyrenoids, showing that pyrenoids are not always required for stramenopile CCMs [9,28], and there can be pyrenoids but no obvious CCM activity [3].

A common rationale for the occurrence of diffusive CO_2 entry or of a CCM is the natural external CO_2 and O_2 concentrations and the kinetics and intracellular content of Rubisco. There is considerable variation in the kinetics, e.g. $K_{0.5}$ for CO_2, $K_{0.5}$ for O_2 and CO_2: O_2 selectivity, among the form ID Rubiscos in the Ochrophyta (table 2), as well as variations in the CO_2-saturated rate of CO_2 fixation per gram of Rubisco protein, with high CO_2-saturated specific reaction rates being generally associated with high $K_{0.5}$ for CO_2 and a low CO_2 : O_2 selectivity (= Srel or τ) [35]. However, significant variations of $K_{0.5}(CO_2)$ and CO_2 : O_2 selectivity may occur within the mechanistic possibilities suggested by Tcherkez *et al*. [35]; this is the case for diatoms [29], in which there is evidence of positive selection of Rubisco [36]. Diffusive CO_2 entry is more likely in organisms whose Rubiscos have a relatively low $K_{0.5}(CO_2)$ and a high CO_2: O_2 selectivity; diffusive CO_2 entry would be particularly favoured if there is a CO_2 subsidy from the terrestrial catchment groundwater supply to rivers and some small lakes [37,38]. By contrast, Rubiscos with a high $K_{0.5}(CO_2)$ and a low CO_2: O_2 selectivity combined with no CO_2 subsidy and approximately air-equilibrium CO_2 and O_2 concentrations would be compatible with the occurrence of a CCM. The data in table 2 show that this expectation is not always met, with diffusive entry of CO_2 in the Chrysophyceae and Synurophyceae, and CCMs in the Eustigmatophyceae and Phaeophyceae (see below). The occurrence of diffusive CO_2 entry in organisms with relatively high Rubisco $K_{0.5}(CO_2)$ could be rationalized by the availability of sufficient energy, N and P (for the RNA needed for protein synthesis) to produce more Rubisco per cell and/or a large CO_2 subsidy.

Table 2. *In vitro* kinetics of ochrophytan Rubiscos. n.d., not determined.

species	class	CCM	$K_{0.5}CO_2$ (mmol m^{-3})	$K_{0.5}O_2$ (mmol m^{-3})	CO_2/O_2 selectivity	references
11 species	Bacillariophyceae	+	23–68	413–2032	57–116	[29]
Nannochlopsis sp.	Eustigmatophyceae	+	7–10	about 1000	27	[30]
Mallomonas papillosa	Synurophyceae	−	18.2	n.d.	n.d.	[31]
Synura petersenii	Synurophyceae	−	28.4	n.d.	n.d.	[31]
Synura uvella	Synurophyceae	−	41.8	n.d.	n.d.	[31]
4 species	Phaeophyceae	+	12–43	n.d.	n.d.	[32]
Olisthodiscus luteus	Raphidophyceae	?	45–59	692	101	[9,33,34]

(b) Chrysophyceae and Synurophyceae

The sister classes Chrysophyceae and Synurophyceae both have diffusive CO_2 transport from the bulk medium to Rubisco. Most of the data relate to the Synurophyceae [3,17,31,39–42], with some for the Chrysophyceae [3,41], which, as indicated in table 1, are frequently phagomixotrophic [43].

The evidence on inorganic C entry includes pH drift. Through this method, it was possible to show that the photosynthetic activity of these algae can increase the pH and deplete CO_2 to values attributable to a diffusive CO_2 entry. More direct estimates of relatively high CO_2 compensation concentrations from net photosynthetic rates as a function of inorganic C concentration, as well as estimates of photorespiration, support the view of inorganic carbon acquisition in Chrysophyceae and Synurophyceae as mostly due to CO_2 diffusive entry driven by consumption of CO_2 in the chloroplasts. For the two cases investigated (one Chrysophyceae and one Synurophyceae), there is no external carbonic anhydrase activity [3]. Vegetative cells of the Synurophyceae and cysts of the Chrysophyceae and Synurophyceae have extraprotoplasmic silicification, in common with several other clades of the Ochrophyta, especially the Bacillariophyceae and Bolidophyceae [44,45]. Among other roles of silicification is that as a pH buffer facilitating H^+ movement related to extracellular carbonic anhydrase activity [46]; this cannot be the case in the Chrysophyceae and Synurophyceae with no extracellular carbonic anhydrase. Despite the lack of CCMs, pyrenoids occur in some Chrysophyceae (*Chromulina*, but not *Ochromonas*) [3,9] and Synurophyceae (e.g. *Synura*) [3,47].

For the Synurophyceae, there are also comparisons of the *in vitro* $K_{0.5}$ of Rubisco and *in vivo* photosynthesis. The form ID Rubiscos of the Synurophyceae have $K_{0.5}$ values (mmol m^{-3}) *in vitro* of 18.2 (*Mallomonas papulosa*), 28.4 (*Synura petersenii*) and 41.8 (*Synura uvella*) [31]. The photosynthetic $K_{0.5}(CO_2)$ are 92.0–440.5 mol m^{-3} for *M. papillosa* (varying with the buffer used to maintain the pH at 7.0), 40.4–43.7 mol m^{-3} (varying with pH 6–7) for *S. petersenii* and 44.9–209 mol m^{-3} for *S. uvella* (varying with pH 6–7) [31]. The *in vivo* photosynthetic $K_{0.5}$ values can be accommodated by the *in vitro* Rubisco $K_{0.5}$ with diffusive CO_2 entry, granted a Rubisco content that gives a V_{max} for Rubisco equal to the V_{max} for *in vivo* photosynthesis, and allowing for the necessary decrease in CO_2 concentration along the diffusion pathway from the bulk medium to Rubisco [41]. The Rubisco assays used unpurified cell extracts [31], so the CO_2-saturated Rubisco specific reaction rate cannot be calculated.

(c) Eustigmatophyceae

Eustigmatophyceae appear to all have a CCM, when cells are grown in culture media in equilibrium with the present atmosphere. The freshwater *Eustigmatos vischeri* and *Vischeria stellata* can take up both CO_2 and HCO_3^- [48–50], while the soil-dwelling *Monodus subterraneus* can only use CO_2 [48,49]. Most of the data are available for the marine *Nannochloropsis* spp. (*N. gaditana*, *N. oceanica* and *N. salina*) and *Monallatus* sp., where mass spectrometric and other evidence show HCO_3^- influx powered by mitochondrial respiration, with significant simultaneous efflux of CO_2 [48,49,51–58]. Merrett *et al*. [53] showed that HCO_3^- influx was inhibited by DIDS (4′4′-diisothiocyantostilbene-2,2-disulfonic acid), an inhibitor of anion exchangers, and by the absence of Cl^-, consistent with the occurrence of a $HCO_3^-:Cl^-$ antiport of unknown stoichiometry. The energized HCO_3^- influx is downregulated when cells are grown at high CO_2 [58], consistent with decreased CCM expression at high CO_2 when diffusive CO_2 entry can provide as high a CO_2 concentration at the active site of Rubisco as does the CCM at lower external CO_2 concentrations. However, Merrett *et al*. [53] were unable to demonstrate a higher internal than external inorganic C concentration using silicone oil centrifugation, just as Huertas *et al*. [48,49] were unable to do from CO_2 efflux kinetics just after cessation of illumination. Huertas *et al*. [48] suggest that the intracellular pool occupies a small fraction of the cell volume and/or that *Nannochloropsis* Rubisco had, like red algal Rubiscos, a low $K_{0.5}$ for CO_2. This latter suggestion was verified by Tchernov *et al*. [30], who found a $K_{0.5}$ for CO_2 of extracted *Nannochloropsis* sp. Rubisco of 7–10 mol m^{-3}, the lowest values in table 2. This notwithstanding, the need for a CCM exists in *Nannochloropsis* due to a very low $CO_2:O_2$ selectivity of 27 [30] and incomplete suppression of Rubisco oxygenase activity *in vivo* [59]. Expressing the photosynthetic $K_{0.5}$ for HCO_3^- in terms of CO_2 gives $K_{0.5}$ values of 0.27 mmol m^{-3} for *Monallantus* sp. and 0.63 mmol m^{-3} for *Nanochloropsis gaditana* [52], i.e. an order of magnitude higher affinity than for the Rubisco of *Nannochloropsis* sp. [30], indicating the involvement of a CCM. Furthermore, the occurrence of a net CO_2 efflux in the light with all inorganic C entering as HCO_3^- and high affinities for inorganic C, a close approximation to inorganic C saturation at seawater inorganic C concentration, a low (zero)

and O_2-insensitive CO_2 compensation point, a high ability to photosynthesize at high pH and no inhibition of photosynthesis by 21 kPa O_2 relative to 2 kPa O_2 means that there has to be a higher internal than external CO_2 concentration. Pyrenoids are apparently universal in the Eustigmatophyceae (but see [60]), in parallel with the occurrence of CCMs [61,62].

The genomic data show probable HCO_3^- transporters of the anion exchanger family at the plasmalemma (Nga00165.01) and the chloroplast envelope (Nga06584) of *Nannochloropsis gaditana* CCMP526 [21]; see www.nannochloropsis.org/gene/Naga_10007g30 and www.nannochloropsis.org/gene/Naga_10007g124. The *Nannochloropsis oceanica* CCMP1779 genome has two genes that resemble the LCIA 1595 in *Chlamydomonas* [63,64]: one is a chloroplast envelope carrier protein (CCP), which is induced by low CO_2 (CCMP1779_7325-mRNA-1); the other is the LCIA protein (CCMP1779_6536-mRNA-1), which belongs to the formate/nitrite transporter family, has an unknown location and is also induced under low CO_2 [22]. Using the same strain of *N. oceanica*, Poliner *et al*. [65] found two SL4 HCO_3^- transporters. The SLC4 family from metazoans catalyses a variety of HCO_3^- transport processes, i.e. 1 HCO_3^- : 1 Cl^- exchanger, 1 HCO_3^- : 1 Na^+ cotransporter, 2 HCO_3^- : 1 Na^+ cotransporter, 3 HCO_3^- : 1 Na^+ cotransporter and a (2 HCO_3^- + 1 Na^+) : 1 Cl^- exchanger [66]. Of these, only the 1 HCO_3^- : 1 Na^+ cotransporter could lead to inorganic carbon accumulation in the cytosol, granted the probable gradients of Na^+ and Cl^- across the plasmalemma [67,68]. SLC4 HCO_3^- transporters are also known from the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* and the brown alga *Ectocarpus siliculosus* [65]. The occurrence of SL4 HCO_3^- transporters is not diagnostic of CCMs since they are found in the C_3 terrestrial flowering plant *Arabidopsis thaliana* [65] and metazoans [66]. Li *et al*. [69] found two low CO_2-induced putative formate/nitrite transporters in the plastid envelope of *N. oceanica* IMET1.

(d) Phaeophyceae

The largest ochristan algae occur in the Phaeophyceae [16]. The final pH and corresponding equilibrium CO_2 concentration achieved in pH drift experiments with Phaeophyceae show HCO_3^- use or, less likely, energized accumulation of CO_2 in almost all cases [70–78], with the proviso that the final pH may be restricted by pH *per se* rather than by limitations on the removal of inorganic C from seawater [41,79]. Similar conclusions arise from the meta-analysis by Stepien [80] of $\delta^{13}C$ natural abundance of organic C of Phaeophyceae collected from their natural habitats, with the proviso that the $^{13}CO_2$: $^{12}CO_2$ discrimination of the isolated Rubisco of the only ochristan tested, the diatom *Skeletonema costatum* [81], differs from that assumed by Stepien [80] and the authors that she cites.

Of the intertidal and subtidal Phaeophyceae from the northeast Atlantic examined by Johnston & Raven [82–84], Surif & Raven [85,86] and Johnston [32], the intertidal species (Fucales) show high affinities for inorganic C, a close approximation to inorganic C saturation at seawater inorganic C concentration, a low (zero) and O_2-insensitive CO_2 compensation concentration, a high ability to photosynthesize at high pH and no inhibition of photosynthesis by 21 kPa O_2 relative to 2 kPa O_2. By contrast, the subtidal Laminariales have a lower affinity for inorganic C, lack of inorganic C saturation at seawater inorganic C concentration, a higher and O_2-sensitive CO_2 compensation concentration, a smaller ability to photosynthesize at high pH and some inhibition of photosynthesis by 21 kPa O_2 relative to 2 kPa O_2. These characteristics indicate the occurrence of a CCM in all species; the CCM of the Laminariales appears to be less developed in terms of inorganic C affinity and O_2 insensitivity of photosynthesis than that of the Fucales. The work of Johnston & Raven [82–84], Surif & Raven [85,86] and Johnston [32] involved experiments under submersed and under emersed conditions with (where identical treatments were used) closely similar results, although the external availability of both HCO_3^- and CO_2 in submersed experiments and external availability of only CO_2 in emersed experiments leaves unanswered questions about the mechanism of inorganic C entry to cells.

Why, in ecological and evolutionary terms, is there this difference between intertidal and subtidal brown algae in the northeast Atlantic? We need to look at other possible determinants of zonation, such as tolerance of desiccation, especially for high-intertidal algae, which can be emersed for long periods during neap tides [16]. One would expect that desiccation tolerance would be important for high-intertidal algae, especially when emersed for long periods at spring tides. Surif & Raven [86] suggest that a high affinity for inorganic C of intertidal brown algae in the NE Atlantic increases the photosynthetic C gain per emersion period. However, there are several intertidal (some very high intertidal) red algae lacking CCMs [41], e.g. *Bostrychia arbuscula* (formerly *Stictosiphonia arbuscula*) in New Zealand in the high-intertidal zone occupied by *Pelvetia canaliculata*, a fucoid with a CCM, on NE Atlantic coasts [16,87].

The extent of suppression of Rubisco oxygenase and hence of photorespiration by CCMs of Phaeophyceae has been investigated by Surif & Raven [86] using a comparison of photosynthetic rates as a function of CO_2 from 50 to 950 $\mu mol\ mol^{-1}$ total gas with O_2 at 20 or 210 $mmol\ mol^{-1}$ total gas. The photosynthetic rates of the five intertidal species of the Fucaceae (Fucales) were unaffected by the different O_2 levels [84] while those of the normally submerged Laminariales and the normally submerged member of the Cystoseiraceae (Fucales) were lower in 210 than in 20 $mmol\ mol^{-1}$ total gas at all the CO_2 levels tested [86]. The O_2 inhibition of the normally submerged algae differs from that of typical C3 physiology plants by the absence of CO_2–O_2 competition.

Other data on O_2 inhibition of photosynthesis in the Phaeophyceae [88–92] show increased glycine and serine production (as markers for the photorespiratory carbon oxidation cycle) as a fraction of total inorganic C assimilated with increasing O_2 [90]. The presence of the photorespiratory carbon oxidation cycle in the Phaeophyceae has been shown by enzyme assays [93,94].

Much of the information on other members of the Phaeophyceae show characteristics more like those of normally submerged algae than the intertidal algae studied by Johnston & Raven [82–84], Surif & Raven [85,86] and Johnston [32]. Examples of the inorganic C affinity and O_2 sensitivity of photosynthesis by other members of the Phaeophyceae are provided by Black *et al*. [88], Downton *et al*. [89], Burris [90], Dromgoole [91,92] and Raven *et al*. [71–73]: see review by Raven & Hurd [95].

The general assumption is that the CCM is based on the direct or indirect use of external HCO_3^- rather than on active influx of CO_2. HCO_3^- use could involve direct HCO_3^- transport using an anion transport protein in the

plasmalemma that is usually detected in macroalgae by inhibition of photosynthesis by DIDS and/or SITS (4′-acetamido-4′-isothiocyantostilbene-2,2-disulfonic acid). Such transport is known for the gametophyte, but not for the sporophyte phase of the kelp *Undaria pinnatifida* [96] and for the sporophyte phase (gametophyte not examined) of the giant kelp *Macrocystis pyrifera* over a wide range of pH values [28]. No inhibition of photosynthesis by DIDS was found for the members of the Desmarestiales, Fucales, Laminariales, Sphacelariales or Scytosiphonales studied by Larsson & Axelsson [97] or Zou & Gao [74]. Evidence of HCO_3^- entry in *E. siliculosus* (Ectocarpales) comes from experiments at an external pH of 9.5, where CO_2 is less than 0.23 mmol m^{-3} [98]. In these experiments, the photosynthetic rate at pH 9.5 was still 30% of that at pH 7.9 and was insensitive to buffering of the medium with 50 mol m^{-3} of either cationic or anionic pH buffers, or to inhibition of external carbonic anhydrase, thus ruling out the mechanism of HCO_3^- use discussed in the two following paragraphs [98]. HCO_3^- entry could account for part of the photosynthesis of *E. siliculosus* at an external pH of 7.9–8.2 ([98]; cf. [99]). At an external pH of 8 in photosynthesis-saturating red light, *E. siliculosus* accumulates 4–5 mol m^{-3} inorganic C, i.e. about twice the external concentration; this internal pool is decreased to about 2 mol m^{-3} when blue light is added, with photosynthetic consumption of the released inorganic C [100].

The alternative mechanism is based on localized acidification of the surface of the organism using energized H^+ efflux, although the diffusion boundary layer of photosynthesizing cells is at a higher pH than the bulk medium [101]. The acid zones have an equilibrium CO_2: HCO_3^- ratio that increases by an order of magnitude with each pH unit decrease, and there is also an order of magnitude increase in the rate of uncatalysed HCO_3^- conversion to CO_2 [101]. The higher concentration of CO_2 at the cell surface has two fates; one is to leak back to the bulk phase, the other is a biologically useful diffusion into the cell to Rubisco. This mechanism is commonly supplemented by the presence of extracellular carbonic anhydrase [102]. The generation of surface acid areas to facilitate CO_2 diffusion towards Rubisco is well characterized in freshwater Charales and some submerged freshwater flowering plants, where each acid zone has an area of more than 1 mm^2. Price & Badger [103] showed that this mechanism of HCO_3^- use is inhibited by higher concentrations (20–50 mol m^{-3}) of pH buffers of an appropriate pK_a. Such attempts that have been made to identify acid zones on the surface of the Phaeophyceae during photosynthesis have not identified areas in the square millimetres [95], although the techniques used were not suited to identifying smaller acid areas. Transient surface acidification occurring with blue light added to photosynthesis-saturating red light in a range of brown algae [104] shows that surface acidification can occur during photosynthesis in the Phaeophyceae.

In the absence of direct measurements of sustained localized surface acidification, the occurrence of a CCM of the kind proposed by Walker *et al.* [101] has been suggested (see discussion above) on the basis of inhibition of photosynthesis by pH buffers and by inhibitors of extracellular carbonic anhydrases. Sometimes the absence of inhibition by the HCO_3^- transporter inhibitors DIDS and SITS has also been used as negative evidence for acid zone-dependent CCMs. Support for a Walker *et al.* style [101] CCM by the use of purported inhibitors of H^+ efflux catalysed by plasmalemma H^+-ATPase is equivocal because the H^+ gradient generated could also be used to indirectly energize a Na^+–HCO_3^- symport influx of HCO_3^-. With these provisos, the mechanism that occurs in the Laminariales other than gametophytes of *U. pinnatifida* and sporophytes of *M. pyrifera* is consistent with a mechanism based on that described by Walker *et al.* [101] for *Chara* ([105–107]; see also [108,109], as is some evidence for this mechanism in a member of the Scytosiphonales [74], but not for a member of the Fucales [77]).

Despite the occurrence of CCMs, most brown algae lack pyrenoids although these structures have evolved independently in several clades of the Phaeophyceae [9,28,78,110,111].

The genome of *Ectocarpus siliculosa* contains a putative Na^+–HCO_3^- transporter targeted to the chloroplast, and a putative Cl^-–HCO_3^- transporter with no clear targeting [112,113]. Nakajima *et al.* [11] and Poliner *et al.* [65] found two SLC4 HCO_3^- transporters in the genome of *E. siliculosa*; other Ochrophyta have two (in the eustigmatophycean *N. oceanica* and the centric diatom *T. pseudonana*) and four (in the pennate diatom *P. tricornutum*) SLC4 genes. The *P. tricornutum* PtSLC4-2 is targeted to the plasmalemma, is inhibited by the anion transporter inhibitor DIDS and is Na^+-dependent. Ye *et al.* [114] report the genome sequence of the kelp (Laminariales) *Saccharina japonica*; the analysis by Bi & Zhou [115], however, does not mention HCO_3^- transporters or H^+-ATPases, but does show a range of carbonic anhydrases. Genomic data indicate the occurrence of many of the enzymes of the photorespiratory carbon oxidation cycle [112].

(e) Raphidophyceae and Tribophyceae

Little is known of the inorganic C acquisition by the Raphidophyceae, other than Rubisco kinetics [33,116]. The *in vitro* $K_{0.5}$ for CO_2 of Rubisco in the marine *Olisthodiscus luteus* is 45 mmol m^{-3} at 23°C (table 2); for the marine *Heterosigma carterae*, the $K_{0.5}$ for inorganic C dependence of the rate of photosynthesis *in vivo*, expressed in terms of CO_2, is 3 mmol m^{-3} at 16°C [117]. This difference suggests that a CCM is operative in the algae of this class. The pH drift work of Nimer *et al.* [118] indicated low photosynthetic rates for the marine *Heterosigma akashiwo*; little can be deduced from these data about the mode of inorganic C acquisition. Pyrenoids seem to be universal in the Raphidophyceae [119]. For the Tribophyceae (i.e. Xanthophyceae), the only data are those of Beardall and Entwisle [120] on the terrestrial–freshwater *Botrydiopsis intercedens*. In this species, internal inorganic C accumulation occurs to a greater extent than it would for diffusive CO_2 entry, in accord with the pH gradient; this is suggestive of the operation of a CCM. Several members of the Tribophyceae have pyrenoids [9] though there seem to be no data on *B. intercedens*. There are no data on inorganic C acquisition by the sister clade to the diatoms, the Bolidiophyceae, now known to have the Parmales as the silicified cyst phase [121].

(f) Pyrenoids and CO_2 concentrating mechanisms

As indicated above for individual taxa, pyrenoids seem to be essential features of some CCMs based on active transport across membranes [9,113]. However, pyrenoids occur in some species of the Chrysophyceae and Synurophyceae, classes that uniformly lack CCMs, while the Phaeophyceae, apparently with CCMs in all taxa, generally lack pyrenoids, with several independent origins of pyrenoids. In the Eustigmatophyceae, all members investigated have CCMs and

pyrenoids. In the Synchromophyceae, a condensed pyrenoid is often present in the chloroplast stroma [8], although there is no evidence on the occurrence of CCMs in this class. This is also the case for the Pelagophyceae, in which *Aureaumbra* (at least) has pyrenoids [122].

4. Inorganic C assimilation in Ochrophyta other than diatoms

(a) Introduction

All photosynthetic, eukaryotic C assimilation pathways have the photosynthetic carbon reduction cycle (PCRC, or Calvin–Benson cycle), with Rubisco as the carboxylase, at their core. Raven *et al*. [25] discussed alternative inorganic C assimilation pathways found in some autotrophic Archaea and Bacteria, and energetic, inorganic C affinity and O_2 damage as reasons why many of them are not appropriate for functioning in the present atmosphere. In some organisms, the PCRC is downstream of diffusive entry, in others it is downstream of a 'biophysical' CCM based on active transport of HCO_3^-, CO_2 or H^+ to produce C_3 photosynthetic biochemistry. In some oxygenic photosynthetic organisms, the PCRC is downstream of a C_3–C_4 cycle in C_4 photosynthesis; this is a 'biochemical' CCM, although it occurs downstream of a 'biophysical' CCM in some aquatic flowering plants. In C_4 photosynthesis, there is close temporal coupling of the C_3–C_4 and PCRC cycles with small pool sizes of the C_4 and C_3 intermediates. Another upstream C_3–C_4 cycle contributes to crassulacean acid metabolism (CAM); here there is a temporal lag of about 12 h between the scotophase acidication with CO_2 fixation into malate, which is stored as malic acid in vacuoles, and deacidification in the photophase, where CO_2 is released by decarboxylation of malic acid, and refixed by Rubisco and the PCRC, with the C_3 moiety from malate decarboxylation stored until the next scotophase as mono- or polysaccharide.

The biochemistry of autotrophic CO_2 assimilation was determined in pre-molecular biology by two main methods. One was short-time (seconds) exposure of the organism to $^{14}CO_2$ (terrestrial organisms) or ^{14}C inorganic C (aquatic organisms) in the light, followed by rapidly killing the organism and quantification of the water- or ethanol-soluble organic compounds labelled with ^{14}C. C_3 biochemistry is characterized by 3-phosphoglycerate (PGA) as the initial labelled compound, followed by PCRC sugar phosphates and then compounds derived from the PCRC; labelling of C_4 dicarboxylic acids in anaplerotic processes is only a few percent of that through the PCRC. C_4 biochemistry has C_4 dicarboxylic acids as the initial labelled products, followed by PGA and other PCRC compounds; a few seconds labelling (pulse) followed by a change back to unlabelled inorganic C (chase) shows a decrease in label in PGA and an increase in label in C_4 dicarboxylic acids. This was the method used in the 1950s and 1960s to establish the C_3 pathway of autotrophic inorganic C assimilation in green microalgae (*Chlorella*, *Scenedesmus*) and the terrestrial flowering plant *Hordeum*, and the C_4 pathway in the terrestrial flowering plants *Saccharum* and *Zea*. The other, much less decisive, method is determination of the activity of carboxylase enzymes in cell extracts; a high Rubisco : PEPC (phosphoenolpyruvate carboxylase-oxygenase) activity ratio has been taken to indicate C_3 biochemistry, and a low ratio (less than 1) is suggestive of C_4 biochemistry.

(b) Phaeophyceae

Both the kinetics of labelling of organic compounds after addition of ^{14}C inorganic C and the *in vitro* activity of carboxylases methods have been applied to the Phaeophyceae [32,123–128]. The kinetics of the ^{14}C-inorganic C labelling method shows C_3 biochemistry in a range of brown algae [32,123,127–129]. In the brown algae examined by Küppers & Kremer [130], Kremer [129] and Hillrichs & Schmid [127] there is significant labelling of C_4 acids (aspartate, malate) in the light, and the time course of the label position within aspartate shows that the PEP comes from photosynthetic 3-PGA, with slower incorporation of ^{14}C-inorganic C into the β-carboxyl of aspartate by phosphoenolpyruvate carboxykinase (PEPCK) or, more likely, PEPC. The ratio of ^{14}C-inorganic C labelling of organic C (initially mainly into aspartate and malate) by carboxylation of PEP to that carried out by Rubisco is lowest in mature tissue and highest in growing tissue, consistent with an anaplerotic role of PEP carboxylation, although the rates may be higher than the anaplerotic requirement [129,130]. ^{14}C-inorganic C labelling of organic C (mainly aspartate and malate) occurs in the dark at a rate higher than that of green and red marine macroalgae and is also probably higher than the anaplerotic requirement [129]. As indicated below, in some fucoids, a part of this dark ^{14}C–inorganic C assimilation can be attributed to low amplitude CAM.

Possibly related to the labelling of C_4 acids mentioned above is the finding of a 'buffer system' taking up (at high pH in the dark) and releasing (at normal seawater pH in the light) inorganic C, as indicated by stimulation of O_2 production in the light in North Atlantic intertidal members of the Fucales (species of *Ascophyllum*, *Fucus* and *Pelvetia*; all Fucaceae) [131,132]. This 'buffer system' is associated with H^+ exchange between seawater and the algae, is not found in other subtidal brown algae examined, i.e. *Halidrys*, Fucales (Cystosieraceae), *Desmarestia* (Desmarestiales) and *Laminaria* (Laminariales), and is paralleled by a particular spatial organization of organelles in the outer cell layer (meristoderm) of the thallus [131,132]. A more widespread phenomenon among brown algae is the stimulation of photosynthesis by additional blue light to already saturating red light [133,134], which involves release in blue light of at least some of the inorganic C from an intracellular pool accumulated in red light [100]. The blue light effect was not found in the only diatom examined, *P. tricornutum* [133]. Possibly related to the two preceding sets of data, but probably not, is the interesting finding in the work of Johnston [32] on ^{14}C inorganic C pulse-chase incorporation in the North Atlantic intertidal *Ascophyllum nodosum* of a continued increase in label incorporation in organic compounds in the chase period, in algae collected in July 1998 but not in January 1988. After a 5 s pulse in July 1988, the increase in label of organic C seems to be saturated at the longest time period tested (300 s) at about eight times the organic C label at the end of the pulse period. This requires a very substantial accumulation of an acid labile pool of either inorganic C or of an acid-labile organic C compound in the 5 s pulse, with incorporation into a range of acid-stable compounds in the chase period. Kawamitsu and Boyer [135] used the North Atlantic intertidal *Fucus vesiculosus* and showed that, after exposure to light in seawater, photosynthetic O_2 production continued for a decreasing rate over 2 h in CO_2 free air. A small fraction of the C store on which this O_2 production was based was inorganic C; most of it was organic, presumably (an)

organic acid(s). Unlike CAM, and the work of Axelsson *et al*. [131,132], this C store was not filled during the scotophase [135], although there is evidence of minor CAM inorganic C assimilation in several fucoid brown algae including *F. vesiculosus* [136]. Axelsson *et al*. [131,132] comment that their 'photosynthetic buffer' may be related to the very low CAM activity found in the species showing the 'photosynthetic buffer'.

The enzyme analyses showed significant activity of PEPCK, but higher activities of Rubisco [32,123,126]. PEPC activity could not be detected in these investigations, including that of Busch & Schmid [126] on *E. siliculosus*, although genomic evidence indicates that PEPC occurs in this alga [112].

The most recent tool for distinguishing between C_3 and C_4 (and CAM) photosynthetic biochemistry is genomics and transcriptomics. However, the presence of genes encoding enzymes used in C_4 (and CAM) photosynthesis such as PEPC, PEPCK, PPDK (phosphate pyruvate dikinase, sometimes considered for diagnostic C_4 photosynthesis), MDH (malate dehydrogenase), NADP-ME (NADP malic enzyme) and NAD-ME (NAD malic enzyme), is not of itself sufficient to show that C_4 photosynthesis occurs. One reason is that the enzymes have other functions and occur in photosynthetic organisms known to use C_3 biochemistry [137,138]. Another is that the enzyme might not be expressed in an intracellular location compatible with C_4 photosynthesis [139]. Bi & Zhou [115] have produced a generalized diagram (their fig. 1) including C_4 photosynthesis and list genes [114] compatible with the PEPCK C_4 mechanism (granted appropriate intracellular localization of the enzymes), but this is not evidence demonstrating that C_4 photosynthesis occurs. The conclusion of Gravot *et al*. [112] is that there is not clear evidence from genome analysis as to the occurrence of C_4 photosynthesis in the model brown alga *E. siliculosus*.

The other inorganic C assimilation pathway found in the Phaeophyceae is very low amplitude CAM in some members of the Fucales, but not in other orders of the brown algae [112,136].

(c) Raphidophyceae and Eustigmatophyceae

For other photosynthetic Ochrophyta, Descolas-Gros & Oriol [140] found activity of PEPCK but not PEPC in *H. akashiwo* (Raphidophyceae). However, PEPC was found in the proteome of another raphidophycean, *Aureococcus anophagefferens* [141].

Genomic data on *N. gaditana* (Eustigmatophyceae) shows PEPC, MDH (NAD(P) malic dehydrogenase) and PPDK in the cytosol, and NAD(P)-ME in the chloroplast, that could be part of C_4 photosynthetic biochemistry, as well as pyruvate carboxylase (PC) in the mitochondria (fig. 6 of [21]).

Genomic data on *N. oceanica* CCMP1779 (Eustigmatophyceae) show the presence of PEPC, MDH, PPDK and NAD(P)-ME, but the intracellular location is not indicated (table S10 of [22]). Vieler *et al*. [22] (their table S10) show that expression of probable inorganic C transporters is increased at low CO_2 concentrations, but make no comment about the effects of CO_2 on expression of the enzymes that could be involved in C_4 photosynthesis. Li *et al*. [69] used transcriptomic data on *N. oceanica* IMET1 to predict the location of enzymes that could be part of a C_4 photosynthesis and found that enzyme location is not entirely as required for an effective C_4 pathway.

Table 3. Summary of mechanisms of inorganic carbon acquisition and assimilation among classes in the Ochrophyta. No data are available for the Aureanophyceae, Bolidophyceae, Chrysomeridophyceae, Dictyochophyceae, Pelagophyceae, Phaeothamniophyceae, Pinguiophyceae, Schizocladiophyceae and Synchromophyceae. See the main text for details.

class	Rubisco kinetics	diffusive CO_2 entry	CCM	C_3 biochemistry	C_4 biochemistry	CAM	phagotrophy
Bacillariophyceae	yes	no	yes	yes	C_3–C_4 in some?	no	no
Chrysophyceae	no data	yes	no	assumed C_3	no data	unlikely	no
Eustigmatophyceae	yes	no	yes	assumed C_3	?	unlikely	no
Phaeophyceae	yes	no	yes	yes	some production of C_4 acids in the light	low amplitude in some species	low amplitude in some species
Raphidophyceae	yes	probably not	probably yes	no data	no data	unlikely	no
Synurophyceae	yes	yes	no	no data	no data	unlikely	no
Tribophyceae	no data	no	yes	no data	no data	unlikely	no

5. Conclusion

We only have a significant body of information on inorganic C acquisition and assimilation for four non-diatom classes of Ochrophyta, i.e. the Chrysophyceae, Eustigmatophyceae, Phaeophyceae and Synurophyceae, with some information on the Raphidophyceae and Tribophyceae (table 3). Diffusive CO_2 entry from the bulk medium to Rubisco occurs in the Chrysophyceae and Synurophyceae, while the Eustigmatophyceae and Phaeophyceae have CCMs involving influx of HCO_3^- and/or CO_2 (table 3).

The Phaeophyceae is the only class for which there is biochemical evidence on the pathway of inorganic C assimilation, showing that there is predominantly C_3 biochemistry, but with occasional elements of C_4 biochemistry and low-amplitude CAM in some members of the Fucales.

The energization of CCMs involves active influx of HCO_3^- or active efflux of H^+ at the plasmalemma, with little or no role for C_4 biochemistry, and no net inorganic C entry in the dark in the few brown algae with low amplitude CAM. The distribution of pyrenoids does not completely parallel that of CCMs based on active transport across membranes.

Molecular genetic investigation has not provided definitive evidence as to the occurrence of C_4 photosynthesis and has indicated possible membrane transporters involved in CCMs. Some members of the Fucaceae, and *Ectocarpus*, have incompletely explained inorganic C reservoirs, as inorganic C and/or as (presumably) carboxylate C that can be readily converted to inorganic C.

Returning to the topic of the other papers in this thematic issue, i.e. C metabolism in the Bacillariophyceae, the other classes of Ochrophyta for which information is available show a greater diversity of mechanisms of inorganic C acquisition and biochemistry of autotrophic CO_2 assimilation. The occurrence of CCMs is less widespread among other ochrophytes than in diatoms, as is the correlation between the occurrence of CCMs and the presence of pyrenoids. While C_4 or C_4-like photosynthetic metabolism is a possibility in both diatoms and other ochrophytes, no diatom is known to have the low-amplitude CAM found in some fucalean brown algae.

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References

1. Raven JA, Beardall J, Larkum AWD, Sánchez-Baracaldo P. 2013 Interactions of photosynthesis with genome size and function. *Phil. Trans. R. Soc. B* **368**, 20120264. (doi:10.1098/rstb.2012.0264)
2. Beakes GW, Glockling SL, Sekimoto S. 2012 The evolutionary phylogeny of the oomycete 'fungi'. *Protoplasma* **249**, 3–19. (doi:10.1007/s00709-011-0269-2)
3. Maberly SC, Ball LA, Raven JA, Sültmeyer D. 2009 Inorganic carbon acquisition by chrysophytes. *J. Phycol.* **49**, 1052–1061. (doi:10.1111/j.1529-8817.2009.00734.x)
4. Wilken S, Schuurmans JM, Matthijs HCP. 2014 Do mixotrophs grow as photoheterotrophs? Photophysiological acclimation of the chrysophyte *Ochromonas danica* after feeding. *New. Phytol.* **204**, 882–889. (doi:10.1111/nph.12975)
5. Yang EC, Boo GH, Kim HJ, Cho SM, Boo SH, Anderson RA, Yoon HW. 2012 Supermatrix data highlight the phylogenetic relationships of photosynthetic stramenopiles. *Protist* **163**, 217–231. (doi:10.1016/j.protis.2011.08.001)
6. Baurain D *et al.* 2010 Phylogenetic evidence for separate acquisition of plastids in cryptophytes, haptophytes and stramenopiles. *Mol. Biol. Evol.* **22**, 1698–1709. (doi:10.1093/molbev/msq059)
7. Brown JW, Sorhannus U. 2010 A molecular genetic timescale for the diversification of autotrophic stramenopiles (Ochrophyta): substantive underestimation of putative fossil ages. *PLoS ONE* **5**, e12759. (doi:10.1371/journal.pone.0012759)
8. Schmidt M, Horn S, Flieger K, Ehlers K, Wilhelm C, Schnetter R. 2012 *Synchroma pusillum* sp. nov. and other new algal isolates with chloroplast complexes confirm the Synchromophyceae (Ochrophyta) as a widely distributed group of amoeboid algae. *Protist* **163**, 544–559. (doi:10.1016/j.protis.2011.11.009)
9. Badger MR, Andrews TJ, Whitney SM, Ludwig M, Yellowlees DC, Leggat, W, Price DG. 1998 The diversity and co-evolution of Rubisco, plastids, pyrenoids and chloroplast-based CO_2-concentrating mechanisms in algae. *Can. J. Bot.* **76**, 1052–1071. (doi:10.1139/b98-074)
10. Roberts K, Granum E, Leegood RC, Raven JA. 2007 Carbon acquisition by diatoms. *Photosynth. Res.* **93**, 79–88. (doi:10.1007/s11120-007-9172-2)
11. Nakajima K, Tanaka A, Matsuda Y. 2013 SLC4 family transporters in a marine diatom directly pump bicarbonate from seawater. *Proc. Natl Acad. Sci. USA* **110**, 1767–1772. (doi:10.1073/pnas.1216234110)
12. Clement R, Dimnot L, Maberly SC, Gontero B. 2016 The nature of the CO_2-concentrating mechanisms in a marine diatom, *Thalassiosira pseudonana*. *New Phytol.* **209**, 1417–1427. (doi:10.1111/nph.13728)
13. Round FE, Crawford RM, Mann DG. 1990 *Diatoms: biology and morphology of the genera.* Cambridge, UK: Cambridge University Press.
14. Rousseaux CS, Gregg WW. 2014 Interannual variation in phytoplankton primary production at a global scale. *Remote Sens.* **6**, 1–19. (doi:10.3390/rs6010001)
15. Silberfeld T, Leigh JW, Verguggen H, Cruaud C, de Reviers B, Rousseau F. 2014 A multi-locus time-calibrated phylogeny of the brown algae (Heterokonta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the 'brown algal crown radiation'. *Mol. Phylogenet. Evol.* **56**, 659–674. (doi:10.1016/j.ympev.2010.04.020)
16. Hurd CL, Harrison PJ, Bischof K, Lobban CS. 2014 *Seaweed ecology and physiology*, 2nd edn. Cambridge, UK: Cambridge University Press.
17. Saxby-Rouen KJ, Leadbeater BS, Reynolds CS. 1997 The relationship between the growth of *Synura petersenii* (Synurophyceae) to photon flux density, temperature and pH. *Phycologia* **36**, 233–243. (doi:10.2216/i0031-8884-36-3-233.1)
18. Nixdorf B, Mischke U. 1998 Chrysophytes and chlamydomonads: pioneer colonists in extremely acid mining lakes (pH $<$ 3) in Lusatia (Germany). *Hydrobiologia* **369/370**, 315–327. (doi:10.1023/A:1017010229136)
19. Wolfe AP, Siver PA. 2013 A hypothesis linking chrysophyte microfossils to lake carbon dynamics on ecological and evolutionary time scales. *Glob. Planet. Chang.* **111**, 189–198. (doi:10.1016/j.gloplacha.2013.09.014)
20. Sukenik A, Beardall J, Kromkamp J, Kopecký J, Macojídek J, Bergegeijk S, Gabon S, Shahan E, Yamishon A. 2009 Photosynthetic performance of outdoor *Nannochloropsis* mass cultures under a wide range of environmental conditions. *Aquat. Microb. Ecol.* **56**, 297–308. (doi:10.3354/ame01309)

21. Radakovits R, Jinkerson RE, Fuerstenberg SI, Tae H, Sattlage RE, Boore JL, Rosewitz MC. 2011 Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. *Nat. Commun.* **3**, 686. (doi:10.1038/ncomms1688)
22. Vieler A *et al.* 2012 Genome, functional gene annotation, and nuclear transformation of the heterokont oleaginous alga *Nannochloropsis oceanica* CCMP1779. *PLoS ONE* **8**, e1003064. (doi:10.1371/journal.pgen.1003064))
23. Tang YZ, Koch F, Gobler CJ. 2010 Most harmful algae are vitamin B1 and B12 auxotrophs. *Proc. Natl Acad. Sci. USA* **107**, 20 756–20 761. (doi:10.1073/pnas.1009566107)
24. Raven JA, Beardall J, Giordano M. 2014 Energy costs of concentrating mechanisms in aquatic organisms. *Photosynth. Res.* **121**, 111–124. (doi:10.1007/s11120-013-9962-7)
25. Raven JA, Giordano M, Beardall J, Maberly SC. 2012 Algal evolution in relation to atmospheric CO_2: carboxylases, carbon-concentrating mechanisms and carbon oxidation cycles. *Phil. Trans. R Soc. B* **367**, 493–507. (doi:10.1098/rstb.2011.0212)
26. Raven JA, Beardall J. 2014 CO_2 concentrating mechanisms and environmental change. *Aquat. Bot.* **118**, 24–37. (doi:10.1016/j.aquabot.2014.05.008)
27. Raven JA, Beardall J. 2016 The ins and outs of carbon dioxide. *J. Exp. Bot.* **67**, 1–13. (doi:10.1093/jxb/erv451)
28. Fernández PA, Hurd CL, Roleda MY. 2014 Bicarbonate uptake via an anion exchange protein is the main mechanism of inorganic carbon acquisition by the giant kelp *Macrocystis pyrifera* (Laminariales, Phaeophyceae) under variable pH. *J. Phycol.* **50**, 998–1008. (doi:10.1111/jpy.12247)
29. Young JN, Heureux AMC, Sharwood RE, Rickaby REM, Morel FMM, Whitney SM. 2016 Large variations in the Rubisco kinetics of diatoms reveals diversity among their carbon concentrating mechanisms. *J. Exp. Bot.* **67**, 3445–3456. (doi:10.1093/jxb/erw163)
30. Tchernov D, Livne A, Kaplan A, Sukenik A. 2008 The kinetic properties of ribulose-1,5-bisphosphate carboxylase/oxygenase may explain the high apparent photosynthetic affinity of *Nannochloropsis* sp. to ambient inorganic carbon. *Isr. J. Plant Sci.* **56**, 37–44. (doi:10.1560/IJPS.56.1-2.37)
31. Bhatti S, Colman B. 2008 Inorganic carbon acquisition in some synurophyte algae. *Physiol. Plant.* **133**, 33–40. (doi:10.1111/j.1399-3054.2008.01061.x)
32. Johnston AM. 1991 The acquisition of inorganic carbon by marine macroalgae. *Can. J. Bot.* **69**, 1123–1132. (doi:10.1139/b91-144)
33. Newman SM, Cattolico RA. 1987 Structural, functional and evolutionary analysis of Ribulose-1,5-bisphosphate carboxylase from the chromophyte alga *Olisthodiscus luteus*. *Plant Physiol.* **84**, 483–490. (doi:10.1104/pp.84.2.483)
34. Newman SM, Derocher J, Cattolico RA. 1989 Analysis of chromophytic and rhodophytic ribulose-1.5-bisphosphonate carboxylase indicated extensive structural and functional similarities among evolutionarily diverse algae. *Plant Physiol.* **91**, 939–946. (doi:10.1104/pp.91.3.939)
35. Tcherkez GGB, Farquhar GD, Andrews TJ. 2006 Despite slow catalysis and confused kinetics, nearly all ribulose bisphosphate carboxylases may be nearly perfectly optimised. *Proc. Natl Acad. Sci. USA* **103**, 7246–7251. (doi:10.1073/pnas.0600605103)
36. Young JN, Rickaby REM, Kapralov MV, Filatov DA. 2012 Adaptive signals in ancient Rubisco reveal a history of ancient atmospheric carbon dioxide. *Phil. Trans. R. Soc. B* **367**, 483–492. (doi:10.1098/rstb.2011.0145)
37. Maberly SC. 1996 Diel, episodic and seasonal changes in pH and inorganic carbon in a productive lake. *Freshwater Biol.* **35**, 579–598. (doi:10.1111/j.1365-2427.1996.tb01770.x)
38. Casper P, Maberly SC, Hall GH, Finlay BJ. 2000 Fluxes of methane and carbon dioxide from a small productive lake to the atmosphere. *Biogeochemistry* **49**, 1–10. (doi:10.1023/A:1006269900174)
39. Saxby-Rouen KJ, Leadbeater BS, Reynolds CS. 1998 The growth of *Synura petersenii* (Synurophyceae) and components of the dissolved inorganic carbon system. *Phycologia* **37**, 467–477. (doi:10.2216/i0031-8884-37-6-467.1)
40. Bhatti S, Colman B. 2005 Inorganic carbon acquisition in the chrysophyte alga *Mallomonas papillosa*. *Can. J. Bot.* **83**, 891–897. (doi:10.1139/b05-075)
41. Raven JA, Ball LA, Beardall J, Giordano M, Maberly SC. 2005 Algae lacking carbon-concentrating mechanisms. *Can. J. Bot.* **83**, 879–890. (doi:10.1139/b05-074)
42. Bhatti S, Colman B. 2011 Evidence of the occurrence of photorespiration in synurophyte algae. *Photosynth. Res.* **109**, 251–256. (doi:10.1007/s11120-011-9639-z)
43. Wilken S, Huisman J, Naus-Wiezer S, van Donk E. 2013 Mixotrophic organisms became more heterotrophic with rising temperatures. *Ecol. Lett.* **16**, 225–233. (doi:10.1111/ele.12033)
44. Yamada K, Yoshikawa S, Inchinomiya M, Kuwata A, Kamiya M, Ohki K. 2014 Effects of silicon-limitation on growth and morphology of *Triparma laevis* NIES-2565 (Parmales, Heterokontophyta). *PLoS ONE* **9**, e103289. (doi:10.137q/journal.pone.0103289)
45. Finkel ZV. 2016 Silicification in Microalgae. In *The physiology of microalgae. Developments in applied phycology 6* (eds MA Borowitzka, J Beardall, JA Raven), pp. 289–300. Cham, Switzerland: Springer International Publishing.
46. Milligan AJ, Morel FMM. 2002 A proton buffering role for silica in diatoms. *Science* **297**, 1848–1850. (doi:10.1126/science.1074958)
47. Hibberd DJ. 1978 The fine structure of *Synura sphagnicola* (Korsch.) Korsch (Chrysophyceae). *Brit. Phycol. J.* **13**, 403–412. (doi:10.1080/00071617800650451)
48. Huertas IE, Colman B, Espie GS. 2002 Mitochondrial-driven bicarbonate transport supports photosynthesis in a marine microalga. *Plant Physiol.* **130**, 284–291. (doi:10.1104/pp.004598)
49. Huertas IE, Colman B, Espie GS. 2002 Inorganic carbon acquisition and its energization in eustimatophyte algae. *Funct. Plant. Biol.* **29**, 271–277. (doi:10.1071/PP01181)
50. Huertas IE, Bhatti S, Colman B. 2005 Characterization of the CO_2-concentrating mechanism in the unicellular alga *Eustigmatos vischeri*. *Eur. J. Phycol.* **40**, 409–415. (doi:10.1080/09670260500342571)
51. Colman A, Huertas IE, Bhati S, Dason JS. 2002 The diversity of inorganic carbon acquisition mechanisms in eukaryotic algae. *Funct. Plant Biol.* **29**, 261–270. (doi:10.1071/PP01184)
52. Munoz J, Merrett MJ. 1989 Inorganic carbon transport in some marine eukaryotic microalgae. *Planta* **178**, 450–455. (doi:10.1007/BF00963814)
53. Merrett MJ, Nimer MJ, Dong LF. 1996 The ulilization of bicarbonate ions by the marine microalga *Nannochloropsis oculata* (Droop) Hibberd. *Plant Cell Environ.* **19**, 478–484. (doi:10.1111/j.1365-3040.1996.tb00340.x)
54. Sukenik A, Tchernov D, Kaplan A, Huertas E, Lubian LM, Livne A. 1997 Uptake, efflux and photosynthetic utilization of inorganic carbon by the eustigmatophyte *Nannochloropsis* sp. *J. Phycol.* **33**, 969–974. (doi:10.1111/j.0022-3646.1997.00969.x)
55. Tchernov D, Hassidim M, Luz B., Sukenik A, Reinhold L, Kaplan A. 1997 Sustained net CO_2 evolution during photosynthesis by marine microorganism. *Curr. Biol.* **7**, 723–738. (doi:10.1016/S0960-9822(06)00330-7)
56. Huertas IE, Lubian LM. 1998 Comparative study of inorganic carbon utilization and photosynthetic responses in *Nannochloris* (Chlorophyceae) and *Nannochloropsis* (Eustigmatophyceae) species. *Can. J. Bot.* **76**, 1104–1108. (doi:10.1139/b98-068)
57. Huertas IE, Espie GS, Colman B, Lubian LM. 2000 Light dependent bicarbonate transport and CO_2 efflux in the marine microalgae *Nannochloropsis gaditana*. *Planta* **211**, 43–49. (doi:10.1007/s004250000254)
58. Hanson DT, Collins AM, Jones HDT, Roesgen J, Lopez-Nieves S, Timlin JA. 2014 On-line stable isotope gas exchange reveals an inducible but leaky carbon concentrating mechanism in *Nannochloropsis salina*. *Photosynth. Res.* **121**, 311–322. (doi:10.1007/s11120-014-0001-0)
59. Raso S, van Genugten B, Vermuë M, Wijffels RH. 2012 Effect of oxygen concentration on the growth of *Nannochloropsis* sp. at low light intensity. *J. Appl. Phycol.* **24**, 863–871. (doi:10.1007/s10811-011-9706-z)
60. Maruyama I, Nakamura T, Matsubayashi T, Ando Y, Maeda T. 1986 Identification of the alga known as 'marine *Chlorella*' as a member of the Eustigmatophyceae. *Jap. J. Phycol.* **34**, 319–325.
61. Hibberd DJ, Leedale GF. 1972 Observations on the cytology and ultrastructure of the new algal class, Eustigmatophyceae. *Ann. Bot.* **36**, 49–71. (doi:10.1093/oxfordjournals.aob.a084577)
62. Suda S, Atsumi M, Miyashita M. 2002 Taxonomic characterization of a marine *Nannochloropsis* species

N. oceanica sp. nov. (Eustigmatophyceae). *Phycologia* **4**, 273–279. (doi:10.2216/i0031-8884-41-3-273.1)

63. Chen Z-Y, Lavigne LL, Mason CB, Moroney JV. 1997 Cloning and overexpression of two cDNAs encoding the low-CO_2 inducible chloroplast envelope protein LIP-36 from *Chlamydomonas reinhardtii*. *Plant Physiol.* **114**, 265–273. (doi:10.1104/pp.114.1.265)
64. Miura K *et al.* 2004 Expression profiling-based identification of CO_2-responsive genes regulated by CCM1 controlling a carbon-concentrating mechanism in *Chlamydomonas reinhardtii*. *Plant Physiol.* **135**, 1595–1607. (doi:10.1104/pp.104.041400)
65. Poliner E *et al.* 2015 Transcriptional coordination of physiological responses in *Nannochloropsis oceanica* CCM1779 under light/dark cycles. *Plant J.* **83**, 1097–1113. (doi:10.1111/tpj.12944)
66. Romero MF, Chen A-P, Parker MD, Boron WF. 2013 The SLC4 family of bicarbonate (HCO_3^-) transporters. *Mol. Aspects Med.* **34**, 159–182. (doi:10.1016/j.mam.2012.10.008)
67. Raven JA. 1984 *Energetics and transport in aquatic plants*. New York, NY: A.R. Liss.
68. Raven JA. 2016 Chloride: essential micronutrient and multifunctional beneficial ion. *J. Exp. Bot.* **68**, 359–367. (doi:10.1093/jxb/erw421)
69. Li J, Han D, Wang D, Ning K, Jia J, Jing X, Hunang S, Chen Q, Xu J. 2014 Choreography of transcriptomics and lipidomics of *Nannochloropsis* reveals the mechanisms of oil synthesis in microalgae. *Plant Cell* **26**, 1645–1665. (doi:10.1105/tpc.113.121418)
70. Maberly SC. 1990 Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. *J. Phycol.* **26**, 439–449. (doi:10.1111/j.0022-3646.1990.00439.x)
71. Raven JA, Beardall J, Roberts S. 1989 The ecophysiology of inorganic carbon assimilation by *Durvillaea potatorum* (Durvillaeales, Phaeophyta). *Phycologia* **28**, 429–437. (doi:10.2216/i0031-8884-28-4-429.1)
72. Raven JA, Beardall J, Johnston AM, Kübler JE, Geoghegan I. 1995 Inorganic carbon acquisition by *Hormosira banksii* (Phaeophyta: Fucales) and its epiphyte *Notheia anomala* (Phaeophyta: Fucales). *Phycologia* **34**, 267–277. (doi:10.2216/i0031-8884-34-4-267.1)
73. Raven JA, Beardall J, Johnston AM, Kübler JE, McInroy SG. 1996 Inorganic carbon acquisition by *Xiphophora chondrophylla* (Phaeophyta: Fucales). *Phycologia* **35**, 83–89. (doi:10.2216/i0031-8884-35-2-83.1)
74. Zou D, Gao K. 2010 Acquisition of inorganic carbon by *Endarachne binghamianum* (Scytosiphonales, Phaeophyta). *Eur. J. Phycol.* **45**, 117–126. (doi:10.1080/09670260903383909)
75. Hepburn CD, Pritchard DW, Cornwall CE, McLeod RJ, Beardall J, Raven JA, Hurd CL. 2011 Diversity of carbon use strategies in a kelp forest community: implications for a high CO_2 ocean. *Glob. Change Biol.* **17**, 2488–2497. (doi:10.1111/j.1365-2486.2011.02411.x)
76. Marconi M, Giordano M, Raven JA. 2011 Impact of taxonomy, geography and depth on $\delta^{13}C$ and $\delta^{15}N$ variation in a large collection of macroalgae. *J. Phycol.* **47**, 1023–1035. (doi:10.1111/j.1529-8817.2011.01045.x)
77. Zou D, Gao K, Chen W. 2011 Photosynthetic carbon acquisition in *Sargassum henslowianum* (Fucales, Phaeophyta), with special reference to the comparison between vegetative and reproductive tissue. *Photosynth. Res.* **107**, 159–168. (doi:10.1007/s11120-010-9612-2)
78. Fernández PA, Roleda MY, Hurd CL. 2015 Effects of ocean acidification on the photosynthetic performance, carbonic anhydrase activity and growth of the giant kelp *Macrocystis pyrifera*. *Photosynth. Res.* **124**, 293–304. (doi:10.1007/s11120-015-0138-5)
79. Middelboe AL, Hansen PJ. 2007 Direct effects of pH and inorganic carbon on macroalgal photosynthesis and growth. *Mar. Biol. Res.* **3**, 134–144. (doi:10.1080/17451000701320556)
80. Stepien CC. 2015 Impacts of geography, taxonomy and functional group on inorganic carbon use patterns in marine macrophytes. *J. Ecol.* **103**, 1372–1383. (doi:10.1111/1365-2745.12451)
81. Boller AR, Thomas PJ, Cavenaugh M, Scott KM. 2015 Isotopic discrimination and kinetic parameters of Rubisco from the marine bloom forming diatom *Skeletonema costatum*. *Geobiology* **13**, 33–43. (doi:10.1111/gbi.12112)
82. Johnston AM, Raven J.A. 1986 The utilization of bicarbonate by the macroalga *Ascophylum nodosum* (L.) Le Jol. *Plant Cell Environ.* **9**, 175–184.
83. Johnston AM, Raven JA. 1986 The analysis of photosynthesis in air and water by the *Ascophyllum nodosum* (L.) Le Jol. *Oeologia* **69**, 175–184. (doi:10.1007/bf00377636)
84. Johnston AM, Raven JA. 1987 The C_4-like characteristics of the intertidal macroalgae *Ascophyllum nodosum* (L.) Le Jolis (Fucales, Phaeophyta). *Phycologia* **26**, 159–166. (doi:10.2216/i0031-8884-26-2-159.1)
85. Surif MB, Raven JA. 1989 Exogenous inorganic carbon sources for photosynthesis in seawater by members of the Fucales and the Laminariales (Phaeophyta): ecological and taxonomic implications. *Oecologia* **78**, 97–105. (doi:10.1007/BF00377203)
86. Surif MB, Raven JA. 1990 Photosynthetic gas exchange in eulittoral and normally submersed members of the Fucales and Laminariales: interpretation in relation to C isotope ratio and N and water use efficiency. *Oecologia* **82**, 68–80. (doi:10.1007/BF00318535)
87. Lüning K. 1990 *Seaweeds: their environment, biogeography and physiology*. New York, NY: John Wiley.
88. Black CC, Burris JE, Everson RG. 1976 Influence of oxygen concentration of photosynthesis in marine plants. *Aust. J. Plant Physiol.* **3**, 81–86. (doi:10.1071/PP9760081)
89. Downton WJS, Bishop DG, Larkum AWD, Osmond CB. 1976 Oxygen inhibition of photosynthetic O_2 evolution in marine plants. *Aust. J. Plant Physiol.* **3**, 73–78. (doi:10.1071/PP9760073)
90. Burris JE. 1977 Photosynthesis, photorespiration, and dark respiration in eight species of algae. *Mar. Biol.* **39**, 371–379. (doi:10.1007/BF00391940)
91. Dromgoole FI. 1978 The effects of pH and inorganic carbon on photosynthesis and dark respiration of *Carpophyllum* (Fucales, Phaeophyceae). *Aquat. Bot.* **4**, 11–22. (doi:10.1016/0304-3770(78)90003-7)
92. Dromgoole FI. 1978 The effects of O_2 on dark respiration and apparent photosynthesis of marine macro-algae. *Aquat. Bot.* **4**, 281–297. (doi:10.1016/0304-3770(78)90025-6)
93. Gross W. 1990 Occurrence of glycolate oxidase and hydroxypyruvate reductase in *Egregia menziesii* (Phaeophyta). *J. Phycol.* **26**, 381–383. (doi:10.1111/j.0022-3646.1990.00381.x)
94. Iwamoto K, Ikawa T. 1997 Glycolate metabolism and subcellular distribution of glycolate oxidase in *Spatoglossum pacificum* (Phaeophyceae, Chromophyta). *Phycol. Res.* **45.** 77–83. (doi:10.1111/j.1440-1835.1997.tb00066.x)
95. Raven JA, Hurd CL. 2012 Ecophysiology of photosynthesis in macroalgae. *Photosynth. Res.* **113**, 105–125. (doi:10.1007/s11120-012-9768-z)
96. Zhang X, Hu H, Tan T. 2006 Photosynthetic organic carbon utilization by gametophytes and sporophytes of *Undaria pinnatifida* (Phaeophyceae). *Phycologia* **45**, 642–647. (doi:10.2216/05-28.1)
97. Larsson C, Axelsson L. 1999 Bicarbonate uptake and utilization in marine macroalgae. *Eur. J. Phycol.* **34**, 79–86. (doi:10.1080/09670269910001736112)
98. Schmid R. 1998 Photosynthesis by *Ectocarpus siliculosus* in red light and after pulses of blue light at high pH—evidence for bicarbonate uptake. *Plant Cell Environ.* **21**, 523–529. (doi:10.1046/j.1365-3040.1998.00297.x)
99. Schmid R, Forster R, Dring MJ. 1992 Circadian rhythm and fast responses in *Ectocarpus* (Phaeophyta, Ectocarpales): II. Light and CO_2 dependence of photosynthesis. *Planta* **187**, 60–66. (doi:10.1007/BF00201624)
100. Schmid R, Hillrichs S. 2001 Uptake and accumulation of inorganic carbon in *Ectocarpus sliculosus* and its relation to blue light stimulation of photosynthesis. *Eur. J. Phycol.* **36**, 257–264. (doi:10.1080/0967026110001735408)
101. Walker NA, Smith FA, Cathers IR. 1980 Bicarbonate assimilation by fresh-water charophytes and higher plants: I. Membrane transport of bicarbonate is not proven. *J. Membr. Biol.* **57**, 51–58. (doi:10.1007/BF01868985)
102. Price GD, Badger MR, Bassett ME, Whitecross MI. 1985 Involvement of plasmalemmasomes and carbonic anhydrase in photosynthetic utilization of bicarbonate in *Chara corallina*. *Aust. J. Plant Biol.* **12**, 241–256. (doi:10.1071/PP9850241)
103. Price GD, Badger MR. 1985 Inhibition by proton buffers of photosynthetic utilization of bicarbonate in *Chara corallina*. *Aust. J. Plant Biol.* **12**, 257–267. (doi:10.1071/PP9850257)
104. Schmid R, Dring MJ. 1993 Rapid, blue light-induced acidifications at the surface of *Ectocarpus* and other

marine macroalgae. *Plant Physiol.* **101**, 907–913. (doi:10.1104/pp.101.3.907)

105. Axelsson L, Mercado JM, Figueroa FL. 2000 Utilization of HCO_3^- at high pH by the brown macroalga *Laminaria saccharina*. *Eur. J. Phycol.* **35**, 53–59. (doi:10.1080/09670260010001735621)
106. Klenell M, Snoejis P, Pedersén M. 2004 Active carbon uptake in *Laminaria digitata* and *L. saccharina* (Phaeophyta) is driven by a proton pump in the plasma membrane. *Hydrobiologia* **514**, 41–53. (doi:10.1023/B:hydr.0000018205.80186.3e)
107. Mercado J.M, Andria J.R, Pérez-Llorens JL, Vergara JJ, Axelsson L. 2006 Evidence for a plasmalemma-based CO_2 concentrating mechanism in *Laminaria saccharina*. *Photosynth. Res.* **88**, 259–268. (doi:10.1007/s11120-006-9039-y)
108. Giordano M, Maberly SC. 1989 Distribution of carbonic anhydrase in British marine macroalgae. *Oecologia* **81**, 534–539. (doi:10.1007/BF00378965)
109. Haglund K, Ramazanov Z, Mtolera M, Pedersén M. 1992 Role of external carbonic anhydrase in light-dependent alkalization by *Fucus serratus* L. and *Laminaria saccharina* (L.) Lamour. (Phaeophyta). *Planta* **188**, 1–6. (doi:10.1007/BF01160705)
110. Silberfeld T, Leigh JW, Verbruggen H, Cruaud C, De Reviers B. 2010 A multilocus time-calibrated phylogeny of the brown algae (Heterokonotophyta, Ochrophyta, Phaeophyceae): investigating the evolutionary nature of the 'brown algal crown radiation'. *Mol. Phylogenet. Evol.* **56**, 659–674. (doi:10.1016/j.ympev.2010.04.020)
111. Silberfeld T, Racault M-FLP, Fletcher RL, Couloux A, Rousseau F, de Reviers B. 2011 Systematics and evolutionary history of pyrenoid-bearing taxa in the brown algae (Phaeophyceae). *Eur. J. Phycol.* **46**, 361–377. (doi:10.1080/09670262.2011.628698)
112. Gravot A, Dittami SM, Rousvoal S, Lugan R, Eggert A, Collén J, Boyen C, Bouchereau A, Tonon T. 2010 Diurnal oscillations of metabolite abundances and gene analysis prove new insights into central carbon metabolic processes of the brown alga *Ecocarpus siliculosus*. *New Phytol.* **188**, 98–110. (doi:10.1111/j.1469-8137.2010.03400.x)
113. Meyer M, Griffiths H. 2013 Origins and diversity of eukaryotic CO_2-concentrating mechanisms: lessons for the future. *J. Exp. Bot.* **64**, 769–786. (doi:10.1093/jxb/ers390)
114. Ye N *et al.* 2015 Saccharina genomes provide novel insights into kelp biology. *Nat. Commun.* **6**, 6986. (doi:10.1038/ncomms7986)
115. Bi Y, Zhou Z. 2016 Absorption and transport of inorganic carbon in kelps with emphasis on *Saccharina japonica*. In *Applied photosynthesis—new progress* (ed. MM Najafpour), pp. 111–131. Rijeka, Croatia: InTech Open Publishers.
116. Newman S, Deracher TS, Cattolico RA. 1989 Analysis of chromophytic and rhodophyte ribulose-1,5-bisphosphate indicates extensive structural and functional similarities among evolutionarily diverse algae. *Plant Physiol.* **91**, 939–946. (doi:10.1104/pp.91.3.939)
117. Clark DR, Flynn KJ. 2000 The relationship between dissolved inorganic carbon concentration and growth rate in marine phytoplankton. *Proc. R. Soc. B.* **267**, 953–959. (doi:10.1098/rspb.2000.1096)
118. Nimer NA, Iglesias-Rodriguez MD, Merrett MJ. 1997 Bicarbonate utilization by marine phytoplankton species. *J. Phycol.* **33**, 625–631. (doi:10.1111/j.0022-3646.1997.00625.x)
119. Yamaguchi H, Nakayama T, Murakami A, Inouye I. 2010 Phylogeny and taxonomy of the Raphidophyceae (Heterokontophyta) and *Chlorinolomas sublosa* gen. et sp. nov., a new marine sand-dwelling raphidophyte. *J. Plant Res.* **123**, 333–342. (doi:10.1007/s10265-009-0281-1)
120. Beardall J, Entwisle L. 1984 Evidence for a CO_2 concentrating mechanism in *Botrydiopsis* (Tribophyceae). *Phycologia* **23**, 511–513. (doi:10.2216/i0031-8884-23-4-511.1)
121. Ichinomiya M *et al.* 2016 Diversity and oceanic distribution of the Parmales (Bolidiphyceae), a picoplanktonic group closely related to the diatoms. *ISME J.* **10**, 2419–2434. (doi:10.1038/ismej.2016.38)
122. DeYoe HR, Stockwell DA, Bidigare RR, Latasa M, Johnston PW, Hargreaves PE, Suttle CA. 1997 Description and characterization of the algal species *Aureoumbra lagunensis* gen. et sp. nov, and referral of *Aureoumbra* and *Aureococcus* to the Pelagophyceae. *J. Phycol.* **33**, 1042–1048. (doi:10.1111/j.0022-3646.1997.01042.x)
123. Kremer BP, Küppers U. 1977 Carboxylating enzymes and the pathway of photosynthetic carbon assimilation in different marine algae—evidence for the C_4 pathway? *Planta* **133**, 191–196. (doi:10.1007/BF00391918)
124. Kremer BP. 1980 Taxonomic implications of algal photoassimilate patterns. *Brit. Phycol. J.* **15**, 399–409. (doi:10.1080/00071618000650401)
125. Kremer BP. 1980 Photorespiration and β-carboxylation in brown macroalgae. *Planta* **150**, 189–190. (doi:10.1007/BF00582365)
126. Busch S, Schmid R. 2001 Enzymes associated with β-carboxylation in *Ectocarpus siliculosus* (Phaeophyceae): are they involved in net carbon acquisition? *Eur. J. Phycol.* **36**, 61–70. (doi:10.1017/s0967026201003067)
127. Hillrichs S, Schmid R. 2001 Activation by blue light of inorganic carbon acquisition for photosynthesis in *Ectocarpus siliculosus*: organic acid pools and short-term carbon fixation. *Eur. J. Phycol.* **36**, 71–79. (doi:10.1080/09670260110001735218)
128. Koch M, Bowes G, Ross C, Zhang X-H. 2013 Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Glob. Chang. Biol.* **19**, 103–132. (doi:10.1111/j.1365-2486.2012.02791.x)
129. Kremer BP. 1981 C_4-metabolism in marine brown macrophytic algae. *Z. Naturforsch.* **39C**, 840–847.
130. Küppers U, Kremer BP. 1978 Longitudinal profiles of carbon dioxide fixation capacities in marine macroalgae. *Plant Physiol.* **62**, 49–53. (doi:10.1104/pp.62.1.49)
131. Axelsson L, Carlberg S, Ryberg H. 1989 Adaptations by macroalgae to low carbon availability. I. A buffer system in *Ascophyllum nodosum*, associated with photosynthesis. *Plant Cell Environ.* **12**, 765–770. (doi:10.1111/j.1365-3040.1989.tb01637.x)
132. Axelsson L, Carlberg S, Ryberg H. 1989 Adaptations by macroalgae to low carbon availability. II. Ultrastructural specializations, related to the function of a photosynthetic buffer system in the Fucaceae. *Plant Cell Environ.* **12**, 771–778. (doi:10.1111/j.1365-3040.1989.tb01638.x)
133. Forster RM, Dring MJ. 1994 Influence of blue light on the photosynthetic capacity of marine plants from different taxonomic, ecological and morphological groups. *Eur. J. Phycol.* **29**, 21–27. (doi:10.1080/09670269400650441)
134. Dring MJ, Forster RM, Schmid R. 1994 Ecological significance of blue light stimulation of photosynthetic capacity in *Laminaria* spp. and other brown algae. *Mar. Ecol. Progr. Ser.* **113**, 271–277. (doi:10.3354/meps113271)
135. Kawamatsu Y, Boyer JS. 1999 Photosynthesis and carbon storage between tides in a brown alga, *Fucus vesiculosus*. *Mar. Biol.* **133**, 361–369. (doi:10.1007/s002270050475)
136. Keeley JE. 1998 CAM photosynthesis in submerged aquatic plants. *Bot. Rev.* **64**, 121–175. (doi:10.1007/BF02856581)
137. Aubry S, Brown NJ, Hibberd JM. 2011 The role of proteins in C_3 plants prior to their recruitment into the C_4 pathway. *J. Exp. Bot.* **62**, 3049–3059. (doi:10.1093/jxb/err012)
138. Chi S, Wu S, Yu J, Wang X, Tang X, Liu T. 2014 Phylogeny of C_4-photosynthesis enzymes based on algal transcriptomic and genomic data supports an archaeal/proteobacterial origin and multiple duplication for most C_4-related genes. *PLoS ONE* **9**, e110154. (doi:10.1371/journal.pone.0110154)
139. Kroth PG *et al.* 2008 A model for carbohydrate metabolism in the diatom *Phaeodactylum tricornutum* deduced from whole genome analysis. *PLoS ONE* **3**, e1426. (doi:10.1371/journal.pone.0001426)
140. Descolas-Gros C, Oriol L. 1992 Variations in carboxylase activity in marine phytoplankton cultures. *Mar. Ecol. Progr. Ser.* **85**, 163–169. (doi:10.3354/meps085163)
141. Wurch LL, Bertrand EH, Saito MA, van Mooy BAS, Dyhrman ST. 2011 Proteome changes driven by phosphorus deficiency in the brown tide-forming alga *Aureococcus anophagefferens*. *PLoS ONE* **6**, e28949. (doi:10.1371/journal.pone.0028949)