



Concepción Iñiguez Moreno

DOCTORAL THESIS

**Effects of the Increase in CO₂ and
Temperature on the Carbon Acquisition
and Assimilation Mechanisms
in Polar Macroalgae**

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DE MÁLAGA

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FACULTAD DE CIENCIAS

DEPARTAMENTO DE ECOLOGÍA Y GEOLOGÍA

Área de Ecología

Effects of the Increase in CO₂ and Temperature on
the Carbon Acquisition and Assimilation Mechanisms
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Memoria presentada para optar al

grado de Doctor en Ciencias Ambientales

por Concepción Iñiguez Moreno

Dirigida por Francisco J. López Gordillo y F. Xavier Niell Castanera





UNIVERSIDAD DE MÁLAGA
FACULTAD DE CIENCIAS
DEPARTAMENTO DE ECOLOGÍA Y GEOLOGÍA
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Visado en Málaga a 10 de Octubre de 2016

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Memoria presentada para optar al Grado de Doctor en Ciencias Ambientales

Fdo. Concepción Iñiguez Moreno



UNIVERSIDAD DE MÁLAGA

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Área de Ecología

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CERTIFICAN:

Que la presente memoria titulada "Effects of the Increase in CO₂ and Temperature on the Carbon Acquisition and Assimilation Mechanisms in Polar Macroalgae" presentada por la Licenciada en Ciencias Ambientales, Concepción Iñiguez Moreno, ha sido realizada bajo nuestra dirección y el trabajo presentado y las publicaciones que lo avalan no han sido utilizado en tesis anteriores. Y considerando que representa trabajo de Tesis Doctoral, autorizamos su exposición y defensa para optar al Grado de Doctor en Ciencias Ambientales.

Y para que así conste, a los efectos oportunos, firma el presente en Málaga a 10 de Octubre de 2016.

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Diseño y maquetación: La Hacedora Comunicación | Ana Gámiz Jiménez

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Chapter

01

General Introduction



General introduction

1.1 The coastal polar ecosystems

1.1.1 Similarities and differences between Arctic and Antarctic coastal environments

Polar regions are extreme environments characterized by constant low temperatures and a strong seasonality of light conditions, differentiating them from all other regions of the world.

Despite these harsh conditions, coastal hardbottom zones of polar regions are dominated by dense macroalgal communities producing high amounts of biomass, which represents a major trophic contribution to these systems and have a dominant role in C fluxes at regional scale. Moreover, they provide food, shelter and substrate for an elevated number of animals and other algae (Dunton and Schell 1987; Iken et al. 1998).

Arctic and Antarctic coastal regions are exposed to long polar nights and sea-ice cover in winter that prolong periods of darkness. At the northern and southern distribution limits of seaweeds in the Arctic (80°N) and Antarctica (77°S), the polar night lasts for about 4 months. Within the northern border of the Antarctic region, at King George Island (South Shetland Islands; 62°S), daylength varies between 20 h in summer and 5 h in winter (Wiencke 1990). After the sea-ice break-up in spring, the light field changes drastically (Aguilera et al. 2002). In this moment water is clear and sunlight penetrates deeply into the water column. However, the development of phytoplankton blooms along with the inflow of turbid water coming from inland ice melting in summer through autumn progressively reduce light in the sublittoral zones (Drew and Hastings 1992). This results in short periods of favourable light conditions for seaweed photosynthesis of about 2-3 months per year.

Despite their similarities, Arctic and Antarctic regions present substantial differences. Much of the Arctic region is a land-surrounded ocean, covered by pack ice that can persist for several years. The Arctic has large areas of tundra

and permafrost and several very large river systems. In contrast, the Antarctic is made up of a land mass almost entirely covered by the huge East and West Antarctic ice sheets (Thomas et al. 2008). Whereas the Arctic has land connections with other climate zones, the Antarctic is effectively isolated from the rest of the world due to the barrier of the Southern Ocean (see Fig. 1.1). This separation is amplified by the Antarctic Circumpolar Current (which probably developed 25 million years ago), that thermally isolates seawaters around the Antarctic continent (Lüning 1990). Contrastingly, there is a considerable inflow of warm North Atlantic waters in Arctic regions through the Fram Strait, where the West Spitsbergen Current mixes with Arctic waters (Zacher et al. 2011).

Ocean temperature has a narrow range along the western Antarctic Peninsula, between -1.8°C in winter and 2.5°C in summer (Drew and Hastings 1992), while the range of the seawater surface temperature in Arctic coastal environments is from -1°C in winter to around 6°C in summer (Cottier et al. 2005). Within the limit between polar and cold-temperate regions, summer maximum air temperatures at the surface can reach 5°C in the Antarctic and $8\text{-}10^{\circ}\text{C}$ in the Arctic (Müller et al. 2009), being this boundary between 48 and 62°S in Antarctica, while in the Arctic it is located between 52 and 70°N .

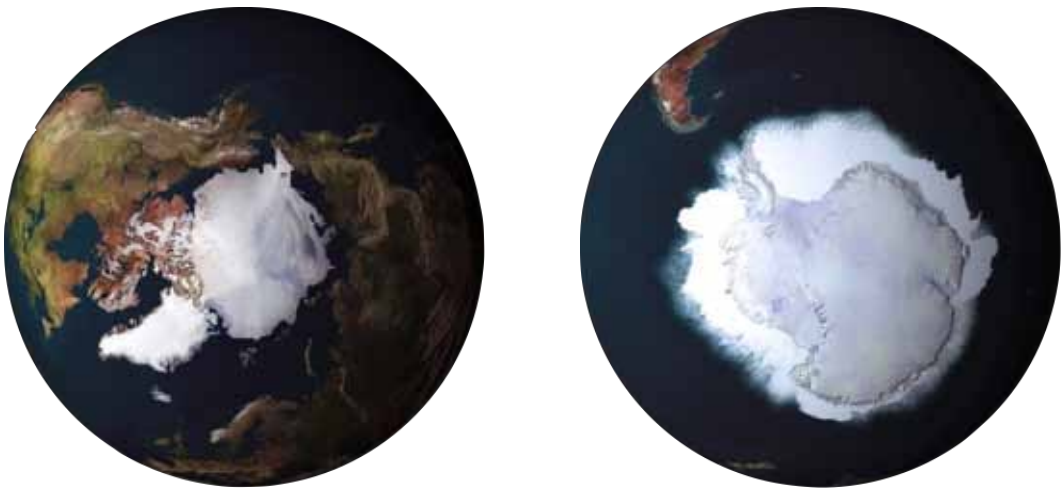


Figure 1.1. Satellite pictures of the North Pole (left) and South Pole (right). Taken from ESA/AOES Medialab.

Polar Oceans also differ considerably in their cold-water history and their genesis. Whereas the water temperatures in the Southern Ocean have been low for 14 million years, glaciation and a winter sea-ice cover did not develop until two million years ago in the Arctic (Crame 1993; Zachos et al. 2001). This strong difference in time exposure to permanent low temperatures must be responsible for the high percentage of endemisms in Antarctic waters compared to the Arctic.

A critical difference for algal development in these systems is the concentration of macronutrients. Nitrate and phosphate concentrations are high throughout the year in the Southern Ocean (Drew and Hastings 1992), while there is a strong seasonal variation of these nutrients in the Arctic. In the latter, nitrogen and phosphorus levels are relatively high during the winter months but both macronutrients drop below detection limits during spring due mainly to phytoplankton blooms, and remain low until autumn (Aguilera et al. 2002). Only for a few weeks in early spring in the Arctic, seaweeds encounter an increasing availability of light, an increase in water temperature and relatively high levels of nutrients before phytoplankton use them up. The timing of sea-ice melting and the depth of vertical mixing are important factors for the onset of the spring phytoplankton bloom (Hodal et al. 2012).

1.1.2 Species composition, depth distribution and productivity of marine macroalgae at polar coastal areas

Although macroalgae are common elements to rocky littoral areas from both polar regions, providing structure for the community, there are substantial differences in seaweed species composition from the Arctic and Antarctic.

The majority of Arctic macroalgae have an Atlantic and Pacific origin due to the connection of Arctic waters with other climate zones, with few cosmopolitan or endemic species (Lüning 1990). In contrast, Antarctic regions possess 35% of endemisms of macroalgal species, many of them belonging to the phylum Heterokontophyta (Wiencke and Clayton 2002). Most seaweed species from both polar regions are found in the sublittoral and very few species inhabit the eulittoral, although there are some specialized species with a bipolar distribution

that occur exclusively in the supralittoral and eulittoral zones, such as *Prasiola crispa*, *Urospora penicilliformis* and *Acrosiphonia arcta*.

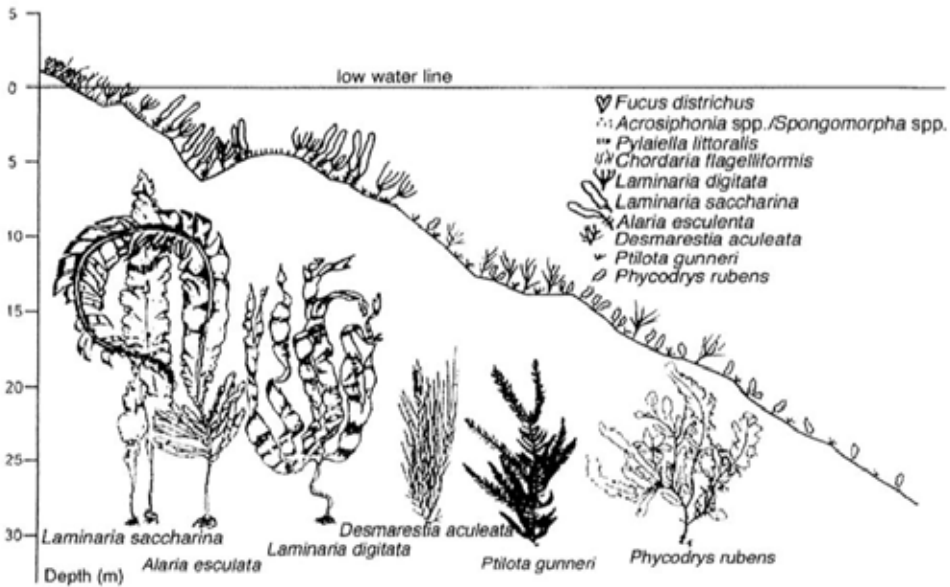


Figure 1.2. Vertical zonation of dominant macroalgae at Kongsfjorden (Spitsbergen, Svalbard). Taken from Wiencke et al. (2004).

One of the areas from the Arctic Ocean with a greater diversity of species is the well-studied Kongsfjorden in Spitsbergen (Svalbard). The hard-bottom sublittoral areas of Kongsfjorden are dominated by high seaweed biomass, with up to 21 kg FW m⁻² (Hop et al. 2002). The upper sublittoral is characterized by the brown algae *Fucus distichus*, *Pylaiella littoralis*, *Chordaria flagelliformis*, the kelp *Saccorhiza dermatodea*, the endemic red alga *Devaleraea ramentacea* and green algae of the genus *Acrosiphonia* (see Fig. 1.2). The mid sublittoral is dominated by the kelps *Alaria esculenta*, *Laminaria digitata* and *Saccharina latissima*. The brown algae *Desmarestia viridis* and *Desmarestia aculeata* occur as undergrowth species in this zone, the latter sometimes forming a separate belt between the mid- and low sublittoral. Characteristic for the lower sublittoral are the red algae *Phycodrys rubens* and *Ptilota gunneri*, which grow down to 30 m depth. Both species also appear as under-growth species in the mid sublittoral. The endemic Arctic kelp *Laminaria solidungula* is retreating and becoming less abundant,

occurring predominantly in the inner zones of the fjord (Hop et al. 2002; Wiencke et al. 2004). However, in the Alaskan and Canadian High Arctic, *L. solidungula* is the dominant kelp species along with *A. esculenta* and *S. latissima* (Dunton and Schonberg 2000), with productivity estimates of 6–20 g C m⁻² year⁻¹ (Dunton and Schell 1986; Chapman and Lindley 1980).

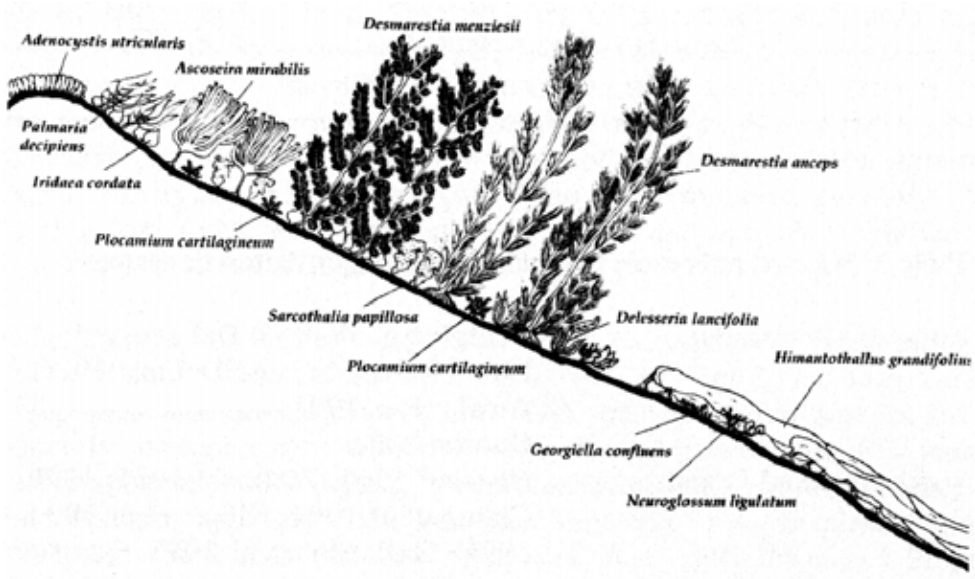


Figure 1.3. Vertical zonation of dominant species on rocky substrates down to 40 m at King George Island (South Shetland Islands). Taken from Wiencke and Clayton (2002).

The rocky shores along the northern portion of the western Antarctic Peninsula and adjacent islands are dominated by extensive subtidal macroalgal assemblages, often covering over 80% of the bottom with standing biomass areas in the range of 5–10 kg FW m⁻² (Amsler et al. 1995; Brouwer et al. 1995). One of the best described depth zonation patterns correspond to rocky shores of King George Island (see Fig. 1.3). The upper sublittoral is often colonized by species such as the red algae *Palmaria decipiens*, *Porphyra endivifolium* and *Iridaea cordata*, and the brown *Adenocystis utricularis*. Below this zone, canopies of highly productive large brown algae dominate the sublittoral. These large seaweeds are perennial and belong to the order Desmarestiales, which ecologically replace Laminariales forests from temperate and Arctic waters (Clayton 1994). These species are *Desmarestia menziesii*, which occurs in the

upper sublittoral down to 10 m depth; *Desmarestia anceps*, which grows in the mid sublittoral down to 15-20 m depth, and *Himantothallus grandifolius*, which is common in the lower sublittoral down to depths of at least 35-40 m (Amsler et al. 1995; Quartino et al. 2001). The latter develops sporophytes that can reach > 30 m in length with productivity estimates in the northern part of its distribution of 16–56 g C m⁻² year⁻¹ (Drew and Hastings 1992).

Both Arctic Laminariales and Antarctic Desmarestiales-based communities have been shown to be as productive as temperate kelp forests (Amsler et al. 1995; Quartino and Boraso de Zaixso 2008).

1.1.3 Adaptation to polar environments

Local regimes of temperature, light and nutrients trigger the major biological processes of polar algae (Wiencke et al. 2007). Polar seaweeds are generally well adapted to live in cold waters, but Antarctic seaweeds are more strongly adapted than Arctic species due to the longer cold-water history of the Southern Ocean. Sporophytes of most endemic Antarctic seaweeds grow up to 5°C, some of them showing optimum growth rates at 0°C, and possess upper survival temperatures (USTs) of 7-13°C (Bischoff-Bäsmann and Wiencke 1996). Contrastingly, sporophytes of the endemic Arctic kelp *L. solidungula* have optimum growth rates at 5-10°C, but can grow up to 15°C, and its UST is around 16°C (tom Dieck 1992); while the Arctic red alga *D. ramentacea*, with a strong distributional centre in the Arctic, grows at temperatures up to 10°C, with an optimum at 0°C and exhibits USTs of 18-20°C (Bischoff and Wiencke 1993). Other non-endemic Antarctic seaweeds can grow up to 5-10°C and have USTs of 19°C (Bischoff-Bäsmann and Wiencke 1996), whereas Arctic populations of species with a cold-temperate distribution can grow up to 15-20°C, with optima between 5 and 15°C, and exhibit USTs between 17 and 23°C. The adaptation of polar seaweeds to low temperatures is also reflected in their photosynthetic rates. Maximum photosynthetic rates of a number of polar seaweeds are comparable to those reported for temperate seaweeds at their respective environmental temperatures (Wiencke et al. 1993; Eggert and Wiencke 2000).

Moreover, the temperature optima for photosynthesis, at least in some species from the Antarctic, are well below values determined in temperate species.

Until now, the amount of data on temperature responses of growth and photosynthesis in Arctic and Antarctic macroalgae is not paralleled by the amount of research on the biochemical mechanisms that constrain the rates of photosynthetic carbon fixation at low temperatures.

As observed in cold-adapted photosynthetic microorganisms, metabolic features influencing growth and photosynthesis at low temperatures might include the maintenance of membrane fluidity by the incorporation of polyunsaturated fatty acids, molecular adaptation of enzymes that compensate for the reduction in chemical reaction rates, and adaptations of the photosynthetic electron transport chain that balance the transduction of light energy to chemical energy at low temperatures (Morgan-Kiss et al. 2006).

In general, it has been observed that a 10°C reduction in temperature causes a decline in biochemical reaction rates of two to three times (Q_{10} 2-3). A feature that compensates the reduction in reaction rates could be the increase in enzyme contents. This has been observed in some Antarctic fish, specifically in enzymes involved in aerobic respiration (Eastman 1993). Davison (1987) showed that acclimation of photosynthesis to low temperature of cold-temperate *S. latissima* produced high concentrations of Calvin cycle enzymes. However, this increase in enzyme contents would be energetically inefficient for cold-adapted algae in some situations, and it has been proposed that a major adaptive trait that compensates for reduced reaction rates in photosynthetic microorganisms might be an increase in the catalytic efficiency (k_{cat}/K_M) of cold-adapted enzymes at low temperatures (Gerday et al. 1997). This can be accomplished by either an increase in the catalytic turnover rate (k_{cat}) over the substrate concentration at half-maximum activity (K_M) and/or a reduced thermal dependence of catalysis (lower activation energy).

Adaptation to low polar light conditions includes the survival of sublittoral seaweeds during dark periods for as long as a year and low light requirements for the completion of their life cycle (Wiencke and Amsler 2012). Some endemic

Antarctic seaweeds require annual light doses only half of what is commonly needed by temperate kelp species (Wiencke 1990). Moreover, species from both polar regions showed high photosynthetic efficiencies, low respiratory rates, low saturation points for photosynthesis, and low compensation points for photosynthesis (Gómez et al. 2011). Nevertheless, just after the sea-ice break-up in spring, high irradiances up to $300 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ can reach 10 m depth (Gómez et al. 1997). This situation of low temperature combined with high irradiance could lead to an imbalance between the temperature insensitive photochemical reactions and the temperature sensitive enzymatic reactions, as light absorption may exceed the metabolic energy requirements of the organism. Thus, subtidal polar algae are not only strongly shade-adapted but can also cope with high light conditions due to different photoprotective mechanisms. These mechanisms can include a reduction in the light-harvesting antenna size and an increase in non-photochemical energy dissipation as heat, termed dynamic photoinhibition (Krause and Weis 1991; Osmond 1994), which also structures seaweed depth distribution (Hanelt 1998). Furthermore, an important level of cyclic electron flow around PS I has been observed in Antarctic phytoplankton (Goldman et al. 2015), that acts as an additional photoprotective mechanism.

In relation to the adaptation to the strong seasonality of the light regime, some polar seaweeds begin growing under short-day conditions in late winter/early spring, often under the sea-ice. Their growth is probably based on circannual rhythms and photoperiodism, synchronized or triggered by daylength, as shown for some Antarctic (Wiencke 1990; Wiencke et al. 1996) and cold-temperate seaweeds (Lüning 1988; Lüning and Kadel 1993). This kick start does not restrict the growth period to the short summer months, when light conditions are more favourable. These species are termed season anticipators, in contrast to the rest of the species that do not start growth before a favourable light condition is present (late spring and summer), which are termed season responders, being mainly opportunistic species from the littoral zone (Kain 1989). Typical Antarctic season anticipators are the brown algae *D. menziesii*, *D. anceps*, *H. grandifolius* and the red alga *P. decipiens*, while examples of typical Antarctic season

responders are the brown alga *A. utricularis*, the red alga *I. cordata* and the green alga *A. arcta* (Wiencke 1990; Gómez and Wiencke 1997; Weykam et al. 1997).

In the Arctic there is not only a strong seasonal variation of the light regime, but also of the levels of nitrate and phosphate, which are high in winter and low in summer. Thus, Arctic season anticipators show accumulation of photosynthates during summer which support new growth during N-replete winter (Chapman and Lindley 1980; Wiencke et al. 2007). The best example is the endemic kelp *L. solidungula*, in which the mobilization of photosynthates occurs during the 9 months of darkness under the ice, when the alga completes over 90% of its annual growth. During this time, up to 30% of its total carbon content is depleted before photosynthetic production begins next spring. During summer, *L. solidungula* shows high photosynthetic rates but a very low biomass formation (Dunton and Schell 1986). In comparison, *S. latissima* grows mostly during a brief period in late spring, when the first light penetrates into the water during sea-ice break-up (Wiencke and Amsler 2012).

1.2 Global Change in polar regions

Rapid changes in the global climate system due to the increase of greenhouse gases emissions over the past and present century have been reported over the world, and many of those changes are unprecedented over decades to millennia. It is unequivocal that the atmosphere and ocean have warmed, the amounts of snow and ice have diminished and sea level has risen (IPCC 2013). The oceans have absorbed about 30% of the emitted anthropogenic carbon dioxide (CO_2) since the pre-industrial era, leading to ocean acidification (OA) (Doney et al. 2009). OA occurs by the dissolution of atmospheric CO_2 in seawater and the subsequent formation of carbonic acid from CO_2 and H_2O , which dissociates into HCO_3^- and H^+ , causing a final reduction in the pH and increasing dissolved CO_2 , as well as diminishing CO_3^{2-} concentrations. In the past century, atmospheric CO_2 concentrations and average Earth's surface temperatures have risen by approximately 120 ppmV and 0.8°C , respectively, while pH of ocean surface water has decreased by 0.1 units (a 30% increase in acidity) since the industrial revolution (IPCC 2013).

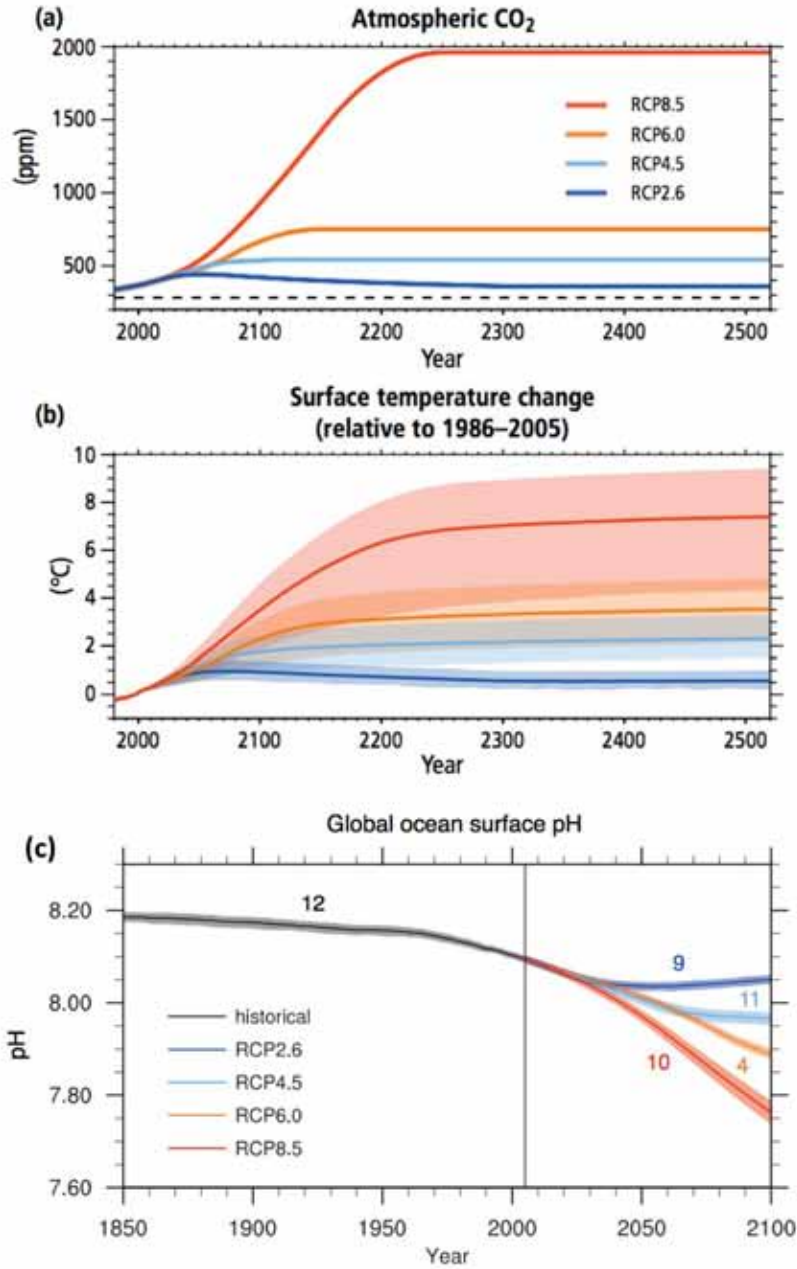


Figure 1.4. Atmospheric CO₂ (a) and projected global mean surface temperature change (b) as simulated by Earth System Models of Intermediate Complexity (EMICs) for the four Representative Concentration Pathways (RCPs) scenarios up to the year 2300 (relative to 1986–2005) followed by a constant (year 2300 level) radiative forcing. The dashed line on (a) indicates the pre-industrial CO₂ concentration. Time series (model averages and minimum to maximum ranges) of multi-model surface ocean pH (c) for the four RCPs scenarios for the 1850–2100 period (relative to 1986–2005). Taken from the Fifth IPCC Assessment Report (2013).

The worst-case scenario of the fifth Intergovernmental Panel on Climate Change (IPCC) report (RCP8.5, Fig. 1.4) projects an increase in the atmospheric CO₂ concentration of more than 900 ppmV along with a maximum decrease in seawater pH of 0.32 by the year 2100. This model also predicts a rise in the atmospheric surface temperature in the range of 2.6-4.8°C, which could lead to an increase of more than 2°C in the upper ocean by the end of the century.

Polar regions, particularly the Arctic and the Antarctic Peninsula, are already showing the strongest and most rapid impact of Climate Change of any region on Earth (ACIA 2005; Larsen et al. 2014). Warming in the Arctic region over the last three decades has been three-times faster than the global average, as expected from ice-albedo feedback effects, with an increase of ~0.6°C per decade in the Arctic compared to an increase of ~0.17°C per decade globally (Comiso and Hall 2014). Moreover, climate models project that the Arctic ecosystems will experience the fastest and most extreme warming this century, probably exceeding more than twice the global average temperature (IPCC 2013). In addition to the increase in temperature in the Arctic, other changes have also been observed, such as reduced extents of sea ice, glacier retreat, thawing of permafrost and a shortening of the duration of the snow season in many regions. Sea ice extent at the summer minimum has decreased significantly in recent decades, and the Arctic Ocean is projected to become nearly ice-free in summer within this century (Larsen et al. 2014). The lack of light-attenuating sea-ice cover will certainly result in increased irradiance levels, that will possibly lead to a higher primary production in nutrient-replete conditions, particularly in the littoral zones, now freed from ice scouring. However, the increase in river run-off and ice melting could enhance turbidity, producing a decrease in irradiance levels. Furthermore, an increased nutrient supply by higher river run-off rates in the Arctic may support an increase in the primary productivity of opportunistic species, whereas a decrease in salinity due to freshening may counteract this effect and could alter vertical stratification patterns (Pivovarov et al. 2003).

Regarding the Antarctic, temperatures on the western side of the Antarctic Peninsula have risen more than anywhere else in the Southern Hemisphere

(between 0.2°C and 0.3°C per decade), with the resultant massive retreat of glaciers and changes in permafrost and biota (Larsen et al. 2014). Some locations of the West Antarctic Peninsula have been warmed up to 7°C over the past 50 years and sea-ice duration has declined by almost 100 days a year since 1978, causing a decrease in phytoplankton productivity in the northern peninsula region (Ducklow et al. 2013), which may result in an increase in subtidal irradiance in areas of low turbidity. Significant warming averaging 2.38°C over 80 years in the upper 150 m has been recorded for some regions of the Southern Ocean (Whitehouse et al. 2008), and models predict that sea surface temperatures in summer South of 60°S are likely to be 0.5-1°C warmer at the end of the century (Convey et al. 2009). On the eastern side of the continent, the largest increases in temperature have been recorded during summer, and large parts of the Larsen Ice Shelf have disintegrated, although the rest of the Antarctic continent shows less prominent changes (Turner et al. 2007).

Polar waters are particularly vulnerable to OA due to the increased solubility of CO₂ at low temperatures; an effect that is further amplified by large quantities of freshwater coming from ice melting, and increased CO₂ uptake in response to sea-ice retreat (Steinacher et al. 2009). Decreases in seawater pH of about 0.02 per decade have been observed since the late 1960s in the Iceland and Barents Seas. Notable chemical effects related to OA have also been reported in surface waters of the Bering Strait and the Canada Basin of the central Arctic Ocean (AMAP 2013 and references therein). Moreover, cold polar waters will become corrosive to calcium carbonate earlier than warmer waters at lower latitudes, and there is a general consensus that both, the Arctic and the Southern Ocean, will become undersaturated with respect to aragonite when atmospheric CO₂ reaches 450 ppmV (McNeil and Matear 2008; Steinacher et al. 2009), with potentially large implications for calcifying organisms and the whole marine ecosystem. All the described effects will presumably give rise to large ecosystem changes. Thus, polar regions are considered a harbinger of global change.

1.3 Effects of Global Change on the physiological performance of algae

1.3.1 Carbon acquisition and assimilation mechanisms in algae

The vast majority of aquatic photosynthetic organisms assimilate inorganic carbon using the Calvin Cycle (C_3 pathway) via ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco, EC 4.1.1.39). This enzyme is probably the most abundant on Earth (Ellis 1979) and it is highly conserved through evolution among the different photosynthetic groups in terms of its primary structure (Whitney et al. 2011). It catalyses the addition of CO_2 to ribulose 1,5-bisphosphate (RuBP), producing two molecules of 3-phosphoglycerate. Despite its importance in the carbon cycle, Rubisco possesses inherent catalytic inefficiencies such as a relatively low affinity for CO_2 (high semi-saturation constant for CO_2 , K_c), and a slow carboxylation turnover rate (k_{cat}^c) of about 1-10 reactions per second. The former means that, for most species, Rubisco is not saturated under current CO_2 levels (Giordano et al. 2005). In addition, this enzyme does not only catalyse the carboxylation of RuBP, but also can catalyse the oxygenation, leading to a competition between O_2 and CO_2 for the active sites of Rubisco. The reaction with O_2 produces one molecule of 3-phosphoglycerate and one molecule of 2-phosphoglycolate, the latter being processed through the photorespiratory pathway, that requires an extra energy investment and produces a reduction in the net photosynthetic rate (Beardall et al. 2003). The proportion between both reactions in a photoautotrophic cell will depend on the concentrations of CO_2 and O_2 in the active sites of Rubisco and the specific kinetic properties for this enzyme in a given organism. Although all Rubiscos have a highly conserved primary structure, it often shows wide differences in its catalytic properties and distinct kinetic behaviour between different photosynthetic groups (Tabita 1999).

In nature, Rubisco is found in a variety of oligomeric forms. All plants, most algae and many photosynthetic bacteria possess the Form I Rubisco, consisting of eight large and eight small subunits (Whitney et al. 2011). The large subunit has a molecular weight of about 50 kD and possesses the active site, whereas the small subunit has a molecular weight of approximately 13–17 kD and, despite not

being strictly required for CO₂ fixation, it is essential for maximal activity and provide structural stability (Andersson and Backlund 2008). Form I Rubisco is subdivided into Forms IA-ID depending on its sequence and lineage (Tabita et al. 2008). Green algae, higher plants and most cyanobacteria have Form IB, while rhodophytes, cryptophytes, heterokontophytes and haptophytes have Form ID (Raven and Beardall 2003).

The general intrinsic catalytic inefficiencies of Rubisco mean that marine autotrophs relying on diffusive CO₂ entry should exhibit a significant inhibition of CO₂ fixation by O₂, high CO₂ compensation points, and low affinities for external CO₂, having into account the low dissolved CO₂ levels and its slow diffusion rate in seawater. The concentration of dissolved inorganic carbon (DIC) in seawater is approximately 2.2 mM and, at pH 8.1, only 1% of DIC is in the form of CO₂ in air-equilibrated waters which represents approximately 12 μM (at 20°C and a salinity of 35‰, Park 1969), while 93% of DIC is in the form of HCO₃⁻. However, most marine algae examined up to date possess carbon concentrating mechanisms (CCMs) that overcome the inefficiencies of Rubisco operating in a low CO₂ environment. These CCMs have a high energetic cost and are mainly based on biophysical processes involving either localized enhancement of external CO₂ concentration by acidification of the external medium, and/or the active transport of DIC across one or more cellular membranes, finally increasing CO₂ concentration around Rubisco (Raven and Beardall 2003). CCM components of eukaryotic algae consist of CO₂/HCO₃⁻ transporters at one or more cellular membranes, and/or proton pumps, which contribute to the creation of acid zones in the cell wall region, coupled with carbonic anhydrases (CAs) located in different cellular compartments (periplasmic space, chloroplast stroma and thylakoid lumen), that catalyse the interconversion of HCO₃⁻ to CO₂ (see Fig. 1.5) (Maberly 1990; Maberly et al. 1992; Larsson and Axelsson 1999; Sherlock and Raven 2001; Raven et al. 2002a). The existence of CCMs has been elucidated in macroalgae through a number of approaches: (a) pH drift experiments, which indicate the extent of the algal ability to scavenge DIC from seawater (Maberly 1990), (b) the photosynthetic response after the inhibition of different CCM components, like sulphonamides (CA inhibitors), substituted stilbene inhibitors

(e.g. DIDS) of anion exchangers, and buffers (e.g. Tris) for dissipation of proton gradients (Haglund et al. 1992; Axelsson et al. 2000; Mercado et al. 2006), (c) stable isotope measurements of the $^{13}\text{C}/^{12}\text{C}$ ratio of organic cellular material relative to source DIC ($\delta^{13}\text{C}_{\text{alga}}$), that can be used as a proxy for the presence of CCMs in algae (Maberly et al. 1992; Raven et al. 2002a), (d) photosynthetic measurements at different external DIC concentrations for the calculation of the photosynthetic affinity and compensation point for CO_2 (Surif and Raven 1989), and (e) photosynthesis inhibition at increasing O_2 concentrations (Raven and Beardall 2003). Nevertheless, direct measurements of intracellular accumulation of inorganic carbon are scarce in seaweeds (but see Beer et al. 1990).

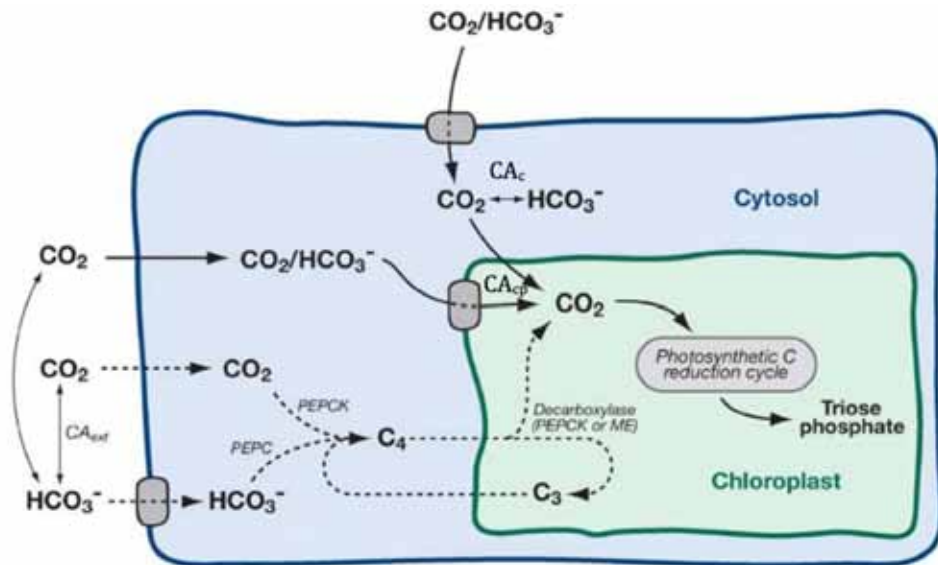


Figure 1.5. Schematic model for the different inorganic carbon transport and CO_2 accumulation processes that can be found in eukaryotic algal cells. The model incorporates the possibilities for DIC transport at the plasmalemma and/or chloroplast envelope as well as a putative C_4 -like mechanism. CO_2 crosses membranes by diffusion, whereas active transport (shown by the shaded boxes) can be of CO_2 or HCO_3^- . There are multiple CA isoenzymes that could be present in the different cellular compartments. Modified from Giordano et al. (2005).

Koch et al. (2013) reviewed that most seaweeds analysed up to date possess the ability of utilising HCO_3^- , but many of them are not saturated at current DIC concentrations. Thus, CCM operation in those species could improve carbon

fixation rates without completely saturating Rubisco activity. Still, it is necessary to take into account that many photosynthesis-DIC curves used for the determination of the apparent photosynthetic affinity for CO₂ of those species were measured under the presence of buffers that could dissipate external proton fluxes, inhibiting this component of CCMs.

With respect to the operation of a biochemical CCM (C₄ metabolism) in algae, it has been suggested in several instances, although in most cases the evidence of its existence is weak (Giordano et al. 2005). C₄ metabolism in algae are based in a cytosolic carboxylase, that could be phosphoenolpyruvate carboxylase (PEPc) or phosphoenolpyruvate carboxykinase (PEPck), and a chloroplastic decarboxylase that could be PEPck or NAD⁺(or NADP⁺) malic enzyme (ME) (see Fig. 1.5). However, the only convincing data up to date of C₄ photosynthesis have been obtained for the green ulvophycean macroalga *Udotea flabellum* (Reiskind and Bowes 1991). The presence of C₄-related enzymes in other species is suspected to be related to their participation in other biological processes different from C₄ metabolism (John Beardall, pers. comm.).

Only few marine macroalgal species are supposed to possess photosynthesis that relies only on CO₂ passive diffusion. These are mainly red algae from the class Florideophyceae, most of them inhabiting lower subtidal habitats with low light levels where only a smaller CO₂ flux is required to satisfy light-limited cellular assimilatory requirements (Sherlock and Raven 2001; Raven et al. 2002a, b).

It has been predicted that algal species without CCMs should have Rubiscos with higher affinities for CO₂ and higher specificity factors of CO₂ over O₂ (S_{C/O}), as the presence of a CCM may relax selection for high CO₂ affinities and for high S_{C/O} (Tcherkez et al. 2006), similar to what is found in C₃ and C₄ plants (form IB Rubiscos). Yet, there is no empiric evidence of that assumption up to date for the form ID Rubiscos (non-green algae) when comparing CCM and CCM-lacking organisms.

It has been previously suggested that polar seaweeds should have a lower requirement of CCMs mainly due to the higher levels of dissolved CO₂ in cold waters and the suspected increase in Rubisco affinity for CO₂ and in S_{C/O} at lower

temperatures, as observed in Rubiscos from other photosynthetic organisms (Jordan and Ogren 1984; Raven et al. 2002b). Despite these assumptions, some studies have revealed that most of the analysed polar species possess the ability to use HCO_3^- for photosynthesis (Maberly et al. 1992; Fisher and Wiencke 1992; Wiencke and Fisher 1990) and exhibited rates of DIC-dependent O_2 evolution greater than calculated rates of uncatalysed CO_2 supply from the dehydration of HCO_3^- , when measured (Beardall and Roberts 1999). In addition, all Arctic seaweeds analysed until now have shown very high external CAs activities (Gordillo et al. 2006), which might be part of CCMs in many of those species. High activities were also found in some species lacking CCMs, such as *P. rubens* and *Ptilota plumosa*, pointing to a higher demand of continuous supply for CO_2 to the cells at low temperatures. This occurs because the enzyme only acts to accelerate the carbonate equilibrium shift from HCO_3^- to CO_2 or vice versa, which depends on the external pH. Thus, an increase in external CO_2 with respect to the concentration found in equilibrium will only occur when external CA activity is coupled to an acidification of some external regions of the plasma membrane. Nevertheless, it might be anticipated that external CA activity should enhance CO_2 diffusion by catalysing the conversion of HCO_3^- to CO_2 in the boundary layer (Koch et al. 2013).

1.3.2 Ocean acidification effects

Increased dissolved CO_2 concentrations are expected to have a fertilization effect on marine autotrophs by increasing gross primary production (Hein and Sand-Jensen 1997), as CO_2 is the only inorganic carbon substrate of the enzyme Rubisco, which in most cases is not carbon saturated under current CO_2 levels (Giordano et al. 2005). However, the impact of high CO_2 on photosynthesis will depend on the occurrence and activity of CCMs, the Rubisco kinetics and its quantity and the cellular energetic and nutritional demands of the organism in a specific environmental condition.

Photosynthesis of macroalgae with CCMs is supposed not to be affected by the increase in CO_2 when CCM operation already saturates the capacity of Rubisco carbon fixation at current conditions. This absence of response has been

observed in several Mediterranean seaweeds (Israel and Hophy 2002), which are known to possess CCMs, whereas a positive effect has been found for species lacking CCMs, such as *Lomentaria articulata* (Kübler et al. 1999). Still, these mechanisms are energetically expensive and their partial down-regulation at higher concentration of CO₂ could decrease the cellular energetic requirements of the organism, as it has been shown for *Fucus serratus* (Johnston and Raven 1990), *Ulva lactuca* (Magnusson et al. 1996), *Corallina officinalis* and *Undaria pinnatifida* (Cornwall et al. 2012). Furthermore, a decreased activity of external or internal CAs has been often reported (Björk et al. 1993; Mercado et al. 1997; Klenell et al. 2004). In some circumstances, the energy saved due to CCM down-regulation could lead to an increase in growth rate (Gordillo et al. 2001a). Contrarily, other studies carried out with the species *Ulva rigida* (Rautenberger et al. 2015), *Gracilaria lemaneiformis* (Zou and Gao 2009) and *Hizikia fusiformis* (Zou et al. 2011a) did not show any deactivation of CCMs in response to increased CO₂. This response might be highly determined by the ability of the species to increase inorganic carbon concentration around Rubisco, as replacement of the CCMs by diffusive CO₂ entry would require an external CO₂ concentration in excess of what is achieved internally by the CCMs (Hepburn et al. 2011; Raven et al. 2012). Consequently, an increase in dissolved CO₂ could be positive for photosynthesis in algae that are under-saturated with respect to current DIC levels, regardless of their capacity to utilize HCO₃⁻.

Physiological processes other than photosynthesis might be also directly or indirectly affected by OA. It has been reported that algae apparently possess sensory systems that respond to environmental CO₂ concentrations and control the CO₂ acquisition efficiency (Matsuda et al. 2001; Burkhardt et al. 2001), and a number of differentially expressed genes codifying for proteins involved in different processes like ion-homeostasis, redox balance, nitrogen metabolism, respiration and carbon acquisition has been observed in algae after acclimation to increased CO₂ (Benner et al. 2013; Rokitta et al. 2012; Li et al. 2015; Crawford et al. 2011; Hennon et al. 2015). These studies indicate that algae go through a metabolic readjustment in response to external CO₂ concentrations, leading to

differential effects on photosynthesis, redox state, biochemical composition and growth in some species when exposed to increased CO₂ levels.

Little attention has been paid to the effects on the main cellular carbon losses, i.e. respiration and dissolved organic carbon (DOC) release, that can explain this uncoupling between photosynthesis and growth. Previous evidence of a decrease in respiration rate by high CO₂ concentrations has been reported in vascular plants (Bunce and Caulfield 1991; Azcón-Bieto et al. 1994) and in *U. rigida* (Gordillo et al. 2001a), whereas an increase in the respiration rate was recorded in some vascular plants (Davey et al. 2004) and in the diatom *Thalassiosira pseudonana* (Yang and Gao 2012). DOC release is a common physiological process observed in many algal species that would maintain the metabolic integrity of the cell and would protect the photosynthetic apparatus from an overload of products that cannot be used in growth or stored (Wood and Van Valen 1990). The release of DOC under high CO₂ levels increased in the unicellular green algae *Dunaliella salina* (Giordano et al. 1994) and *Dunaliella viridis* (Gordillo et al. 2003b), in the cyanobacterium *Spirulina platensis* (Gordillo et al. 1999) and in the coccolithophorid *Emiliania huxleyi* (Borchard and Engel 2012), while Hopkinson et al. (2010) and Sobrino et al. (2014) found that high CO₂ concentrations reduced cellular carbon loss in natural phytoplanktonic communities.

Changes in carbon assimilation and losses at elevated CO₂ would produce changes in the partitioning of absorbed energy between heat dissipation and photochemistry in the thylakoid membrane and in the way photochemical energy is used by the metabolism (Drake et al. 1997), which might be reflected in pigment contents alterations after acclimation to OA (Johnston and Raven 1990; Andría et al. 2001; Gordillo et al. 2003a).

In addition to providing carbon for photosynthesis, increased dissolved CO₂ concentrations also produce a concomitant reduction in seawater pH and the saturation state of calcium carbonate, which could make calcification more difficult for marine calcifying seaweeds (Gao et al. 1993; Price et al. 2011). It has been shown that rocky shore communities in natural high CO₂/low pH zones

(CO₂ vents) changed to the benefit of non-calcified algae (Hall-Spencer et al. 2008). Reduced pH associated with the increase in dissolved CO₂ may have negative consequences for some non-calcifying species as well (Mercado et al. 1999; Israel and Hophy 2002; Gutow et al. 2014). This could be related to an influence of the extracellular pH changes in the membrane electrochemical potential, enzyme activities and proton or ion channels by altering the structure of periplasmic proteins (Sültemeyer 1998; Kramer et al. 2003; Beardall et al. 2009; Lü et al. 2011).

Studies of OA effects on polar macroalgae are certainly lacking. Recently, Gordillo et al. (2016) observed few physiological responses on six Arctic macroalgal species to increased CO₂, with no changes in chlorophyll *a* (Chl *a*) content and no significant decrease in external CA activity in any of those species. Young et al. (2015a) also found no response to increased CO₂ levels in the growth rates of natural Antarctic phytoplankton communities, despite the observed CCM down-regulation, and explained it by a very small energetic requirement to concentrate CO₂ and saturate Rubisco at low temperatures. Conversely, Trimborn et al. (2013) revealed the operation of very efficient CCMs (i.e., high inorganic C affinities) in four different Antarctic phytoplankton species that were not down-regulated after acclimation to elevated CO₂ levels.

1.3.3 Global warming effects

From a physiological point of view, increased temperature, inside the thermal range of each species for growth, generally results in higher metabolic activity. Perennial seaweeds, being sessile organisms, are acclimated to the wide annual thermal range of their habitat. However, seasonal temperature changes in the medium are accompanied by changes in irradiance, daylength and nutrients availability, which drive different metabolic patterns in these organisms, and thus, a change in the environmental temperature outside the corresponding range for a determined season could lead to a metabolic disturbance until acclimatization takes place. Also, the effects of temperature on the metabolism of seaweeds are different after a short-term or a long-term exposure; the latter producing a readjustment of cellular and metabolic processes, such as photosynthesis and

respiration, as well as changes in membrane lipid composition and pigment content, among others (Raven and Geider 1988; Staehr and Wernberg 2009).

Acclimation to temperature stress in seaweeds has been mostly studied at the level of photosynthesis or growth (Davison et al. 1991; Kübler and Davison 1995; Eggert et al. 2006), but not at the genomic or molecular level. Locally, the acclimation potential of thermal traits can shape the vertical or seasonal distribution pattern of species (Davison and Pearson 1996; Zardi et al. 2011) but it is unknown whether species with a broad acclimation potential (eurithermal relative to stenothermal species) will have a faster genetic adaptation to global warming. This is a main issue of concern since endemic species are expected to face competition from invading cosmopolitan species from cold-temperate areas, which may result in drastic community shifts (Jueterbock et al. 2013).

The increase in temperature associated with global change could alter the geographical distribution boundaries of macroalgae, as these are determined by the temperature requirements for growth, reproduction and survival of the species (Lüning 1990; Müller et al. 2009). A poleward shift in the geographic range of major subtidal kelp species has been suggested for future climate scenarios in marine coastal ecosystems (Merzouk and Johnson 2011).

The expansion of boreal and sub-Arctic species onto the Arctic Ocean has been predicted by Weslawski et al. (2011) and Krause-Jensen and Duarte (2014). The disappearing of coastal ice cover decreases the physical abrasion and is accompanied by an increase in the duration of the available light period for photosynthesis and growth of algae. Moreover, Kongsfjorden (Svalbard) is considered to be undergoing a transition from typically Arctic to mainly Atlantic seaweed composition due to a higher influence of the West Spitsbergen current and Arctic warming (Müller et al. 2009; Walczowski et al. 2012). Biomass and seaweed cover already increased between 1988 and 2008 in the rocky littoral of Sorkapland (Svalbard; Weslawski et al. 2010) in the Arctic accompanied by an increase in air and surface seawater temperatures and a marked decrease in the duration and extent of sea-ice cover. However, no new species were detected until now but are expected in the future along with a northward distribution of

Arctic species, as their summer isotherm boundary move northwards (Müller et al. 2009). In contrast, there will be almost no change in the northern limit of the Antarctic region (Bartch et al. 2012). Indirect effects via changes in sea-ice extent and duration, particularly along the southern portion of the western Antarctic Peninsula, could modify the southern limit of Antarctic macroalgae, as it seems likely that seaweed communities typical of the northern portion of the western Antarctic Peninsula are expanding southward. Nevertheless, this area is still very poorly studied (Wiencke and Amster 2012).

1.3.4 Interaction of ocean acidification and global warming with other stressors

The physiological response of macroalgae to ocean acidification and warming separately might be modified by the interaction of both stressors, producing antagonistic or synergistic effects, and the relation with other stressors associated with global change, such as light availability or nutrient concentrations, could complicate this prediction. Only a few studies have elucidated the interactive effects of increased CO₂ and other abiotic factors on non-calcified marine macroalgae, mostly with a focus on the combined effects of increased CO₂ and nutrients (Gordillo et al. 2001a, 2003a; Russell et al. 2009; Xu et al. 2010; Zou et al. 2011a). These studies suggest that nutrient availability in seawater may strongly affect the response of marine algae to elevated CO₂.

The growth rates of two Arctic kelps, *A. esculenta* and *S. latissima*, were favoured by increased CO₂ concentrations but only when it was in combination with a higher ultraviolet radiation (UVR; Gordillo et al. 2015). The physiology of the kelp *Macrocystis pirifera* was not affected by increased CO₂ and negatively affected by elevated temperature, but a positive response was observed when the alga was grown under both elevated temperature and CO₂ (Brown et al. 2014). Connell and Russell (2010) reported positive synergistic effects of CO₂ and temperature on the growth of turf-forming algae, that negatively affected the development of kelp beds. Moreover, a transplant experiment along CO₂ vents in the Mediterranean Sea showed that some calcifying species were more

vulnerable to the effects of ocean acidification at warmer seawater temperatures (Rodolfo-Metalpa et al. 2011).

Carbon acquisition, and thus, the potential effects of OA, are highly dependent on light availability. Hepburn et al. (2011) found more negative $\delta^{13}\text{C}$ of macroalgae in deeper low light environments compared with the same species in shallower high light habitats, although pH drift experiments indicated that these macroalgae did possess CCMs and were able to remove similar amounts of DIC from seawater when supplied with saturating light for 24 h, independent of depth. This evidence suggests that the physiological machinery is available for operation of CCMs but that energetic constraints limit carbon uptake through this pathway *in situ*. The red macroalga *G. lemaneiformis* (Zou and Gao 2009) and the coccolithophorid *E. huxleyi* (Feng et al. 2008) showed a significant increase in growth rate at elevated pCO_2 but only at near-saturating irradiances and not at low irradiances, indicating that the energetic limitation in low light environments constrain the carbon fixation rates of those species. Global change could also affect the threshold at which irradiance becomes excessive, as shown for the chlorophyte *Dunaliella tertiolecta*, which exhibited a higher physiological tolerance for excessive irradiance conditions at elevated CO_2 compared to current CO_2 levels (García-Gómez et al. 2014). Since light availability changes drastically between different seasons in benthic polar environments, global change effects in macroalgae are supposed to be seasonally dependent.

Some studies on the physiological effects of the interaction between ocean acidification and global warming has been performed in polar phytoplankton but very few in polar seaweeds. The gross primary production of Arctic phytoplankton was enhanced by increased CO_2 , but only at low temperatures, indicating that the positive effects of OA are constrained by nutrient and light availability to the spring period (Holding et al. 2015). However, other studies have revealed that increased temperature, rather than elevated CO_2 , was responsible for the observed increase in growth rates of some polar diatoms at near-future conditions (Torstensson et al. 2012; Pancic et al. 2015). Gordillo et al. (2016) hypothesised that Arctic seaweed communities are more resilient than expected in comparison with their temperate counterparts (Connell et al. 2013), as

suggested by the lower significant percentage of interactions of increased CO₂ and temperature found in the physiological measurements on six Arctic macroalgal species.

All this information is especially relevant since adaptation and relocation of one species relative to another within the system may lead to fundamental changes in trophodynamic phasing, with major consequences on biogeochemical fluxes (Carmack and Wassmann 2006).

1.4 Thesis outline

Despite the high productivity and ecological relevance of canopy-forming Arctic Laminariales and Antarctic Desmarestiales-based macroalgal communities, and the fact that polar regions are experiencing the strongest and fastest effects of Climate Change of any region on Earth, studies on their physiological acclimation response to the projected environmental scenarios of increased CO₂ and temperature are very scarce. Most of the physiological studies on polar macroalgae have been carried out in relation to increased UVR (reviewed by Bischof and Steinhoff 2012; Karsten et al. 2011) or nutrients changes (Aguilera et al. 2002; Gordillo et al. 2006), but almost none of them was related to elevated dissolved CO₂, even though this is the only inorganic carbon substrate for Rubisco. The effects of increased temperatures have been mostly analysed in relation to distributional shifts (Müller et al. 2009, 2011) but not from a physiological perspective. Furthermore, studies about the molecular mechanisms underlying its physiological responses are almost non-existent in polar macroalgae. More knowledge about the carbon acquisition, assimilation and carbon losses mechanisms, as well as their alteration under near-future abiotic conditions are urgently needed for making predictions of macroalgal productivity, species composition, and ecosystem functioning as affected by global change. This information is especially relevant as it is expected that changes in polar macroalgal communities will propagate along the food web. Thus, the aim of this thesis is to characterise the physiological acclimation response of ecologically relevant polar seaweeds to an increase in temperature and CO₂ to the levels expected by the end of this century.

The **general hypothesis** is that polar seaweeds might reveal patterns of acclimation to increased CO₂ and temperature that differ from those of cold-temperate populations due to their specific adaptation to polar environments. The species-specific differences in their acclimation mechanisms to increased CO₂ and temperature will determine which species will benefit from the projected environmental scenario, and which others will be more negatively affected or not altered, enabling to identify winner and loser species as well as the degree of change expected in the community.

Therefore, the present study addresses the following major questions:

1. How will the cellular carbon balance of polar macroalgae be modified by increased CO₂? Does growth rate reflect the effect of increased CO₂ on photosynthesis?
2. Do CCMs become down-regulated at increased CO₂ in polar macroalgae? How dependent on external and internal CA is their photosynthesis and how is it affected by increased CO₂?
3. How susceptible are polar macroalgae to an increase in temperature? Are there interactions between temperature and CO₂ effects? Is this interaction dependent on irradiance levels?
4. Is there a different physiological and biochemical response to increased CO₂ and temperature between polar and cold-temperate populations?
5. Will Arctic populations of cold-temperate distributed species be more benefited than Arctic endemic species by near-future scenarios of increased CO₂ and temperature?
6. How is the transcriptomic response underlying the physiological performance altered by these factors of global change in polar macroalgae?
7. Do polar macroalgae exhibit less effective CCMs than cold-temperate counterparts? How are their Rubisco carboxylation kinetics at low temperature? Is Rubisco saturated at present CO₂ levels?

The results of this thesis provide novel and valuable data on the knowledge of cellular carbon fluxes, biochemical composition, physiology and photochemical performance, carbon acquisition and assimilation mechanisms and transcriptomic responses in polar seaweeds as they are affected by OA and warming. It includes a novel in-depth analysis of Rubisco kinetics that helps to understand the adaptation mechanisms to polar environments.

Chapter

02

**CO₂ and temperature as factors of
Global Change affecting natural
populations of Arctic brown seaweeds**



Chapter

2.1

Increased CO₂ modifies the carbon balance and the photosynthetic yield of two common Arctic brown seaweeds: *Desmarestia aculeata* and *Alaria esculenta*

Concepción Iñiguez, Raquel Carmona, M. Rosario Lorenzo, F. Xavier Niell, Christian Wiencke, Francisco J. L. Gordillo

Polar Biology (2015) doi:10.1007/s00300-015-1724-x



Increased CO₂ modifies the carbon balance and the photosynthetic yield of two common Arctic brown seaweeds: *Desmarestia aculeata* and *Alaria esculenta*

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Abstract

Ocean acidification affects with special intensity Arctic ecosystems, being marine photosynthetic organisms a primary target, although the consequences of this process in the carbon fluxes of Arctic algae are still unknown. The alteration of the cellular carbon balance due to physiological acclimation to an increased CO₂ concentration (1300 ppm) in the common Arctic brown seaweeds *Desmarestia aculeata* and *Alaria esculenta* from Kongsfjorden (Svalbard) was analysed. Growth rate of *D. aculeata* was negatively affected by CO₂ enrichment while *A. esculenta* was positively affected, as a result of a different reorganization of the cellular carbon budget in both species. *Desmarestia aculeata* showed increased respiration, enhanced accumulation of storage biomolecules and elevated release of dissolved organic carbon, whereas *A. esculenta* showed decreased respiration and lower accumulation of storage biomolecules. Gross photosynthesis (measured both as O₂-evolution and ¹⁴C-fixation) was not affected in any of them, suggesting that photosynthesis was already saturated at normal CO₂ conditions and did not participate in the acclimation response. However, electron transport rate changed in both species in opposite directions, indicating different energy requirements between treatments and species-specificity. High CO₂ levels also affected the N-metabolism, and ¹³C isotopic discrimination values from algal tissue pointed to a deactivation of carbon concentrating mechanisms. Since increased CO₂ has the potential to modify physiological mechanisms in different ways in the species studied, it is expected that this may lead to changes in the Arctic seaweed community, which may propagate to the rest of the food web.

Chapter

2.2

Increased temperature, rather than elevated CO₂, modulates the carbon assimilation of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula*

Concepción Iñiguez, Raquel Carmona, M. Rosario Lorenzo, F. Xavier Niell, Christian Wiencke, Francisco J. L. Gordillo

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Increased temperature, rather than elevated CO₂, modulates the carbon assimilation of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula*

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Abstract

Ocean acidification and warming are affecting with special intensity the Arctic Ocean. Arctic coastal ecosystems are dominated by kelp forests with a high biomass production, which are expected to be directly affected by the increases in CO₂ and temperature. This study presents the different physiological responses of the Arctic kelps *Saccharina latissima* and *Laminaria solidungula* from Kongsfjorden (Svalbard) cultured at 4 and 9°C in combination with current (390 ppm) and increased (1200 ppm) levels of atmospheric CO₂. Both species were largely unaffected by increased CO₂ conditions. Carbon fixation was not influenced by CO₂, indicating that photosynthesis was C-saturated at present levels, and ¹³C isotopic discrimination values from algal tissue suggested no deactivation of carbon concentrating mechanisms at increased CO₂ levels. Inhibition of photosynthesis by carbonic anhydrases (CAs) inhibitors highlighted the important role of external CAs in inorganic carbon acquisition in Arctic kelps. *Saccharina latissima* showed a significantly higher growth rate at 9°C than at 4°C, probably due to the decrease in the dark respiration rate observed. Growth rate of *L. solidungula* was not affected by temperature or CO₂, and increases in photosynthesis at 9°C could be partially related to a higher dissolved organic carbon release rate. The photochemical performance of both species was not altered by any of the treatments. These results suggest that *S. latissima* might be more benefited than *L. solidungula* in a future warmer Arctic, while both populations seem to be resilient to higher CO₂ concentrations.

Chapter

03

Differences in the response of Arctic versus cold-temperate populations of *Saccharina latissima* to increased CO₂ and temperature



Chapter

3.1

Biochemical composition of temperate and Arctic populations of *Saccharina latissima* after exposure to increased CO₂ and temperature reveals ecotypic variation

Mark Olischläger, Concepción Íñiguez, Francisco J. L. Gordillo, Christian Wiencke

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Biochemical composition of temperate and Arctic populations of *Saccharina latissima* after exposure to increased pCO₂ and temperature reveals ecotypic variation

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Abstract

Previous research suggested that the polar and temperate populations of the kelp *Saccharina latissima* represent different ecotypes. The ecotypic differentiation might also be reflected in their biochemical composition (BC) under changing temperatures and pCO₂. Accordingly, it was tested if the BC of Arctic (Spitsbergen) and temperate *S. latissima* (Helgoland) is different and if they are differently affected by changes in temperature and pCO₂. Thalli from Helgoland grown at 17°C and 10°C and from Spitsbergen at 10°C and 4°C, were all tested at either 380, 800 or 1500 µatm pCO₂, and total C-, total N-, protein soluble carbohydrate and lipid contents, as well as C:N ratio were measured. At 10°C, the Arctic population had a higher content of total C, soluble carbohydrates and lipids, whereas the N- and protein content was lower. At the lower tested temperature, the Arctic ecotype had particularly higher contents of lipids, while content of soluble carbohydrates increased in the Helgoland population only. In Helgoland-thalli, elevated pCO₂ caused an increase in the content of soluble carbohydrates at 17°C, but at 10°C, it lowered the content of N and lipids and increased the C:N ratio. Elevated pCO₂ alone did not affect the BC of the Spitsbergen population. Conclusively, the Arctic ecotype was more resilient to increased pCO₂ than the temperate one, and both ecotypes differed in their response pattern to temperature. This differential pattern is discussed in the context of the adaptation of the Arctic ecotype to low temperature and the polar night.

Chapter

3.2

Increased CO₂ and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*

Mark Olischläger, Concepción Iñiguez, Kristina Koch, Christian Wiencke, Francisco J. L. Gordillo

Planta, accepted September 2016



Increased pCO₂ and temperature reveal ecotypic differences in growth and photosynthetic performance of temperate and Arctic populations of *Saccharina latissima*

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Abstract

Previous research demonstrated that warming and ocean acidification (OA) affect the biochemical composition of Arctic (Spitsbergen; SP) and cold-temperate (Helgoland; HL) *Saccharina latissima* differently, suggesting ecotypic differentiation. The present study analyses the responses to different pCO₂ (380, 800, 1500 μatm pCO₂) and temperature levels (SP population: 4°C, 10°C; HL population: 10°C, 17°C) on the photophysiology (O₂ production, pigment composition, D1-protein content) and carbon assimilation (Rubisco content, carbon concentrating mechanisms (CCMs), growth rate) of both ecotypes. Elevated temperatures stimulated photosynthetic O₂ production in both populations, and also led to an increase in pigment content and a deactivation of CCMs, as indicated by ¹³C isotopic discrimination of algal biomass (ε_p) in the HL population, which was not observed in SP thalli. Generally, pCO₂ effects were less pronounced than temperature effects. High pCO₂ deactivated CCMs in both populations and produced a decrease in the Rubisco content of HL thalli while it was unaltered in the SP population. As a result, the growth rate of the Arctic ecotype increased at elevated pCO₂ and higher temperatures and it remained unchanged in the HL population. Ecotypic differentiation was revealed by a significantly higher O₂ production rate and an increase in Chl *a*, Rubisco and D1 protein content in SP thalli, but a lower growth rate, in comparison to the HL population. We conclude that both populations differ in their sensitivity to changing temperatures and OA and that the Arctic population is more likely to benefit from the upcoming environmental scenario than its Atlantic counterpart.

Chapter

04

Increased temperature and CO₂ alleviate photoinhibition in the Antarctic endemic macroalga *Desmarestia anceps*: insights from physiological performance and transcriptomic analysis in relation to inorganic carbon utilization

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Abstract

Ocean acidification and warming are affecting with special intensity polar regions. In the Southern Hemisphere, the strongest changes are occurring in the western and northern parts of the Antarctic Peninsula. The surrounding rocky sublittoral areas are mainly dominated by highly productive canopy-forming Desmarestiales. This study presents the physiological and transcriptomic responses of the endemic macroalga *Desmarestia anceps* to a combination of different levels of temperature, 2 and 7°C; dissolved CO₂, 380 (A) and 1000 ppmV (C); and irradiance, 65 (LL) and 145 μmol photons m⁻² s⁻¹ (HL). The growth and photosynthetic rates generally increased at high CO₂ conditions, and strongly decreased at 2°C-HL in comparison to 2°C-LL, although this decrease was not observed at 7°C-HL relative to 7°C-LL. This photoinhibition at 2°C-HL was reflected in an elevated dissolved organic carbon release of up to 50% of the total assimilated carbon, a reduction in the pigment contents and a significantly lower maximum electron transport rate (ETR_{max}), photosynthetic efficiency (α) and optimum quantum yield (F_v/F_m). The general increased productivity at elevated CO₂ correlated well with the up-regulation of the *rbcl* gene, suggesting an increase in Rubisco content. The photochemical performance at 7°C-HLC indicated a photo-protective role of CO₂. ¹³C isotopic discrimination values from algal tissue pointed to an absence of CCM down-regulation, despite the 20% decreased dependence of photosynthesis on external carbonic anhydrase (CA) activity. The highest number of differentially regulated transcripts with respect to the control (2°C-LLA) was obtained for 2°C-HL treatments, indicating that irradiance was the abiotic factor that drove the strongest changes in the transcriptome. Instead, high CO₂ induced very few transcriptomic changes in *D. anceps*. Moreover, there was a high and constitutive gene expression of many photochemical and inorganic carbon utilization components, whereas some others were only down-regulated at 2°C-HL. These results are discussed in the context of the strong adaptation of *D. anceps* to the Antarctic environment, and suggest that its productivity would be maintained in near-future scenarios of increased temperature and CO₂, tolerating higher irradiances than at present conditions.

Chapter

05

Rubisco carboxylation kinetics and its thermal dependencies in relation to CCMs operation in seaweeds: a comparative study between polar and cold-temperate populations

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Manuscript for submission



Abstract

Hardbottom substrates of polar coastal regions are mainly dominated by highly productive macroalgal communities which might possess enzymatic adaptations to compensate for the reduction in catalysed reaction rates at low temperature, including the key carboxylating enzyme Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). It has been suggested that polar seaweeds should have a lower requirement of carbon concentrating mechanisms (CCMs) than populations from temperate latitudes due to the higher levels of dissolved CO_2 and the suspected increase in Rubisco affinity for CO_2 at lower temperatures. In order to test this hypothesis, the ^{13}C isotopic discrimination values from algal tissue, the pH compensation point and Rubisco carboxylation kinetics (the Michaelis-Menten affinity constant for CO_2 , K_c , and the catalytic turnover rate, k_{cat}^c , assayed at 4 and 25°C) were analysed in twelve species representative of Arctic and Antarctic ecosystems. When possible, latitudinal comparisons were made for the same species/genus. The rodophyte *Phycodrys rubens*, lacking an active CCM, showed no differences in Rubisco carboxylation kinetics and content in the Arctic relative to the North-Atlantic population. In the rodophyte genus *Palmaria* and the kelp *Saccharina latissima*, the k_{cat}^c and catalytic efficiency (k_{cat}^c/K_c) at 4°C were significantly higher in the polar populations compared to its cold-temperate counterparts, evidencing an enzymatic cold-adaptation. The chlorophyte *Acrosiphonia arcta* did not show significant differences among provenances in Rubisco kinetics at 4°C but a more than two-fold increase in Rubisco content per total soluble protein in the Arctic and Antarctic populations relative to the North-Atlantic counterpart. There was no significant correlation between either K_c or Rubisco content and CCM operation of red and brown seaweeds. Even though Rubiscos from red and brown seaweeds were CO_2 -saturated at 4°C and dissolved- CO_2 concentrations of cold seawater, most analysed polar populations possess the same or even a stronger ability for bicarbonate uptake than their cold-temperate counterparts. These results will be discussed in terms of evolutionary adaptation to cold environments.

Chapter

06

General discussion and conclusions



General discussion

The results presented in this thesis have shown that the effects of elevated CO₂ on the growth rate of polar macroalgae are species-specific, and that usually do not parallel the effects (or lack of it) on photosynthesis. Instead, changes in growth rate were due to a reorganization of the energetic and carbon budget of the cell. It has been previously shown that diatoms respond to increasing CO₂ by differentially expressing gene clusters that regulate transcription and chromosome folding, altering the transcription of gene clusters involved in different physiological processes under steady-state elevated CO₂ via cAMP signalling (Matsuda et al. 2001; Hennon et al. 2015), and presumably this sensory system would be present in macroalgae as well. These results suggest that exposure to elevated CO₂ first causes a shift in regulation, and then a metabolic rearrangement, indicating that changes in environmental CO₂ could potentially affect physiological processes other than photosynthesis. However, most of studies based on the effects of OA in seaweeds up to date have been mainly focused on inorganic carbon acquisition, photosynthetic performance or growth, but less attention has been given to the effects on carbon losses due to respiration and DOC release, or organic carbon accumulation in the form of storage biomolecules as another carbon sink, and merely none of those studies have reported measurements of all these processes together (except Gordillo et al. 2001a, 2003a for *U. rigida*), in order to calculate a closed cellular carbon balance. The results of Chapter 2.1 revealed that the growth rate of *D. aculeata* was negatively affected at high CO₂ conditions due to an increase in respiration, in accumulation of storage biomolecules and in the rate of DOC release, whereas the growth rate of *A. esculenta* was enhanced due to a decrease in respiration rate and in the accumulation of storage biomolecules, despite the unchanged carbon fixation rates of both species at high CO₂ conditions. These results highlight the necessity of measuring all carbon gain and loss processes of the cell in order to have a whole picture of the physiological effects of increased CO₂.

Furthermore, almost no previous study on macroalgae has measured all steps involved in the photosynthetic performance in the same experiment, at the level

of chlorophyll *a* measurements, oxygen evolution and carbon fixation. The results from this thesis demonstrate that there is an uncoupling between those processes and that the extrapolation of chlorophyll *a* measurements and oxygen evolution to productivity has to be taken with much care. Changes in different abiotic factors, such as CO₂ and temperature, can modulate the ratio between photons drained to the electron transport rate and O₂ production, and between O₂ production and CO₂ fixation (photosynthetic quotient), as reported in Chapter 2 (2.1 and 2.2), complicating the calculation of productivity from O₂ evolution measurements.

Carbon fixation rates of all analysed Arctic species were not altered by increased CO₂ levels (Chapter 2), indicating that their photosynthesis must be C-saturated at current CO₂ conditions. The results from Chapter 5 revealed that all analysed red and brown seaweeds, including the Arctic species of Chapter 2, possess Rubiscos with a K_c for CO₂ at 4°C of about 2–4 μM. Thus, their Rubiscos are saturated at 22 μM CO₂, which is the concentration that corresponds to air-equilibrated seawater at 4°C. This significant increase in the affinity for CO₂ at lower temperature observed in all analysed species suggest that polar seaweeds are more likely to possess C-saturated photosynthetic rates than temperate species at their usual environmental temperature, independently of their ability to use HCO₃⁻ for photosynthesis.

A review of > 100 marine macro-autotroph species from warm to cold-temperate latitudes revealed that most of them were capable of utilizing HCO₃⁻ but the majority were not saturated at current CO₂ levels (Koch et al. 2013). These results lead the authors to conclude that photosynthetic and growth rates of warm to cold-temperate marine macro-autotrophs must increase under elevated CO₂, in a similar way to terrestrial C₃ species, although some other studies revealed that photosynthesis was not altered by increased CO₂ conditions in some temperate seaweeds (Israel and Hophy 2002; Sarker et al. 2013; Fernández et al. 2015). Nevertheless, in most cases, inorganic C undersaturation was deduced from measurements of photosynthetic rates as a function of external inorganic C concentration (P-C curves), which are usually done in the presence of buffers that could dissipate the contribution of proton extrusion to the internal accumulation of CO₂, leading to overestimated semi-saturation constants for DIC (K_{DIC}).

Despite the fact that polar seaweeds are more likely to possess C-saturated photosynthetic rates, all analysed polar seaweeds, except the lower sublittoral rhodophytes *P. rubens* and *P. gunneri*, were able to actively use HCO_3^- in a similar or even higher extent than cold-temperate populations, as indicated by their $\delta^{13}\text{C}_{\text{alga}}$ and pH compensation points (Chapter 5).

The observed active HCO_3^- usage for photosynthesis at low temperature could be related to the fact that equilibrium conditions for CO_2 are not frequently met in cold oceans, due to the intensive biological activity in macroalgal beds in spring and summer, and the slow equilibration of CO_2 between the surface of the oceans and the atmosphere relative to that between CO_2 and the other dissolved inorganic species (Raven and Falkowski 1999). Moreover, there is a considerable decrease in the uncatalysed rate of CO_2 supply from bicarbonate (Eggleston et al. 2010) and in the diffusion rate of CO_2 (Boudreau 1997) in cold waters relative to warmer latitudes. Regarding photosynthetic carbon fixation, if the diffusive supply rate of CO_2 to Rubisco is slower than the maximum carbon fixation rate at CO_2 -saturated conditions, then the CO_2 concentration around Rubisco will notably decrease with respect to the external CO_2 concentration, becoming not saturating for Rubisco. In addition, exposure of seaweeds to almost continuous high irradiances in combination with low temperatures after the sea-ice break-up in late spring and summer might increase the demand for CCMs, as photochemical reactions are independent of temperature (Raven and Geider 1988), whereas the enzymatic reactions involved in the photosynthesis and carbon acquisition are significantly decreased at low temperature ($k_{\text{cat}}^{\text{C}}$ of Rubisco decreased almost 10 times from 25 to 4°C, see Chapter 5). Hence, these environmental conditions may result in a surplus of photochemical energy that could lead to chronic photoinhibition if excess of photons are not dissipated, so, in this situation, CCM operation could help dissipating excess of energy and ensuring a continuous CO_2 -saturation of Rubisco during steady-state photosynthesis. However, the ability of polar macroalgae for using HCO_3^- for photosynthesis has been shown to be related to depth zonation (according to benthic light availability) as reflected in Fig. 5.1, with higher $\delta^{13}\text{C}_{\text{alga}}$ and pH compensation points values for species inhabiting

the upper sublittoral/lower eulittoral relative to those from the mid/lower sublittoral.

Contrastingly to the results obtained for Arctic seaweeds, the photosynthetic rates of the Antarctic endemic *D. anceps* increased at elevated CO₂ (Chapter 4) despite its CCM operation and the suspected high affinity for CO₂ of its Rubisco, which might lead to C-saturated photosynthetic rates under current CO₂ conditions. The increase in photosynthetic rates at elevated CO₂ observed in *D. anceps* might be related to an increase in Rubisco content, as suggested by a general significant enhancement of the *rbcL* gene expression at high CO₂. Nevertheless, this is only a hypothesis, since Rubisco content and kinetics were not measured in this species due to difficulties in the protein extraction procedure, and an increase in the *rbcL* gene expression does not necessarily mean an increase in the quantity of active Rubisco. In this respect, the activation and regulation processes of form ID Rubisco is supposed to be significantly different from those of Form IB Rubisco (green-type) but it is largely unknown and would require further research in order to be able to fully understand the carbon acquisition and assimilation mechanisms in these seaweeds.

Three out of the five polar macroalgae analysed in this thesis, the Arctic *S. latissima* and *L. solidungula* and the Antarctic *D. anceps*, did not show down-regulation of its CCMs at increased CO₂ concentrations in the range of 1000-1200 ppm CO₂. In this regard, Trimborn et al. (2013) revealed the operation of very efficient CCMs in four different Antarctic phytoplankton species that were not down-regulated after acclimation to elevated CO₂ levels. However, down-regulation of CCMs is a frequent response in temperate seaweeds when cultured at increased CO₂ conditions (Johnston and Raven 1991; Magnusson et al. 1996; Cornwall et al. 2012), since these mechanisms are energetically expensive, although there are other temperate species that did not show this response (Fernandez et al 2015). The partial down-regulation of CCMs saves energy that could be invested in other physiological processes, resulting in some cases in an increase in the growth rate (Gordillo et al. 2001a). The latter response was also observed for the Arctic *A. esculenta* in Chapter 2.1.

Whether an organism down-regulates CCMs at increased CO₂ or not might be determined by the ability of the species to increase the inorganic carbon concentration around Rubisco, as the replacement of CCMs by diffusive CO₂ entry would require an external CO₂ concentration in excess of what is usually achieved internally by CCMs (Hepburn et al. 2011; Raven et al. 2012). However, it can also be determined by the cellular energy balance, that is frequently altered at increased CO₂ conditions.

The partial down-regulation of CCMs in the Arctic *D. aculeata*, as suggested by $\delta^{13}\text{C}_{\text{alga}}$ values of Chapter 2.1, could be related to an increased energy demand for counteracting the external pH reduction (Wu et al. 2010). There are also reports of similar negative effects of increased CO₂ on growth rate of other non-calcifying species (Mercado et al. 1999; Israel and Hophy 2002; Gutow et al. 2014), as those observed in the present study for *D. aculeata*, and it has been suggested that changes in extracellular pH could alter the membrane electrochemical potential and proton or ion channels by altering the structure of periplasmic proteins (Sültemeyer 1998; Kramer et al. 2003; Beardall et al. 2009; Lü et al. 2011). The biochemical explanation underlying this negative effect observed in some non-calcifying species, presumably due to the concomitant lower pH associated to increased CO₂ levels, is still largely unknown and must be a potential field for future research.

In either case, it is clear that the majority of the analysed polar seaweeds would need more than 1000 ppm CO₂ to down-regulate their CCMs, despite the observed high affinity for CO₂ of their Rubiscos at low temperature. This highlights the importance of CCM operation in polar environments as part of a mechanism that ensures high photosynthetic rates in cold waters, of the same order of magnitude as those from temperate latitudes at their respective environmental temperature (Wiencke et al. 1993; Weykam et al. 1996; Eggert and Wiencke 2000).

This thesis also reports clear evidences of an important role of external CA activity in the inorganic carbon acquisition of all tested species (the Arctic *S. latissima* and *L. solidungula*, and the Antarctic *D. anceps*), as reflected in a strong inhibition of about 50-80% of net photosynthesis by DBS (inhibitor of external CA activity).

These results are in agreement with the elevated external CA activities reported by Gordillo et al. (2006) for Arctic seaweeds, suggesting that this might be part of a general adaptation to cold waters. Moreover, the dependence of photosynthesis on external CA activity was not reduced at increased CO₂ conditions in neither *S. latissima* nor *L. solidungula*, in agreement with the absence of CCM down-regulation suggested by the unaltered $\delta^{13}\text{C}_{\text{alga}}$ values. In this regard, Gordillo et al. (2016) reported no decrease in external CA activity at increased CO₂ levels for six Arctic seaweed species, again supporting the idea of a higher requirement for facilitated carbon acquisition in polar environments, most likely related to a photoprotection mechanism under a continuous solar radiation during the spring-summer period. Conversely, a decreased activity of external or internal CAs has been often reported at increased CO₂ conditions in temperate seaweeds (Björk et al. 1993; Mercado et al. 1997; Klenell et al. 2004) which might be related to a down-regulation of its CCMs overall. However, a decrease of about 20% in the photosynthetic dependence on external CA activity at high CO₂ was found in the Antarctic *D. anceps* (Chapter 4), even though unaltered $\delta^{13}\text{C}_{\text{alga}}$ values and the constitutive expression of genes encoding for CCM components (including those of CAs) indicated that there was no significant down-regulation of CCM operation.

The observed physiological effects of the projected increase in temperature on the analysed polar seaweeds was in accordance with the previous knowledge, with the Arctic population of a cold-temperate distributed species being benefited, the Arctic endemic species being not affected and the Antarctic endemic species being negatively affected, as reflected in their growth rates. This response will depend on their temperature requirements for growth, reproduction and survival of the species (Lüning 1990; Müller et al. 2009) and it has been shown that optimum temperature for growth and UST of the Antarctic endemic *D. anceps* are considerably lower than the Arctic endemic *L. solidungula*, and the latter showed lower optimum temperature for growth and UST than *S. latissima* (tom Dieck 1992; Bischoff-Bäsmann and Wiencke 1996), which reflect their degree of adaptation to cold environments. A poleward shift in the geographic range of major subtidal kelp species has been suggested for future-projected climate scenarios in marine coastal ecosystems (Merzouk and Johnson 2011) and a northward distribution of

Arctic species has also been predicted due to the change in their summer isotherm boundary (Müller et al. 2009). These predictions are in agreement with the results from Chapter 2.2, which suggest that the Arctic population of the cold-temperate distributed *S. latissima* might be more benefited than the Arctic endemic *L. solidungula* in a near-future warmer Arctic, while both populations seem to be highly resilient to higher CO₂ concentrations. Thus, temperature, rather than CO₂, could drive major changes in Arctic kelp-dominated communities.

Interactions between temperature and CO₂ are particularly relevant to photosynthesis. The C assimilation rate at a given temperature depends on the activity of different limiting processes at different CO₂ levels, namely the inorganic P regeneration of ATP synthesis, the Rubisco capacity and the ETR. Moreover, photosynthesis saturation at a given CO₂ changes at different temperatures. Nevertheless, fewer interactions between CO₂ and temperature have been observed in the analysed polar seaweeds than generally reported for other ecosystems (Darling and Coté 2008). The same response was found in six Arctic seaweeds after acclimation to increased CO₂ and temperature (Gordillo et al. 2016). This might indicate that polar seaweed communities are more resilient than expected as compared to their cold-temperate counterparts (Connell et al. 2013). Accordingly, significant interactions were only found in 14% of the analysed variables in relation to the biochemical composition in *S. latissima* from Kongsfjorden compared to 29% in *S. latissima* from Helgoland (Chapter 3.1). Even more, there were no significant interactions in the physiological performance of any of those populations (Chapter 3.2).

The significant differences in the acclimation responses to increased CO₂ and temperature observed in the Arctic and the Atlantic populations of *S. latissima* (Chapter 3) indicate that conclusions obtained from previous studies on seaweeds have to be restricted to the population analysed and not the species, since populations from different latitudes might show different biochemical and physiological patterns of acclimation, even though they belong to the same species (ecotypic differentiation). As observed in Chapter 2.2, changes in temperature seem to exert stronger effects than changes in CO₂ on the physiology of both populations. A similar ability for HCO₃⁻ use of both populations was

deduced from $\delta^{13}\text{C}$ measurements. However, CCM down-regulation was only suspected at 1500 $\mu\text{atm CO}_2$ in the Arctic ecotype, whereas a down-regulation already at 800 $\mu\text{atm CO}_2$ in the Atlantic one, but only at the higher temperature, further supports the hypothesis that lower temperature promotes a higher requirement for CCM operation.

The ecotypic differentiation between both populations was also reflected in significantly different Rubisco carboxylation kinetics (Chapter 5), suggesting a genetically fixed cold-adaptation of the Arctic population of *S. latissima*. This cold-adaptation was reflected in a significant increase in the $k_{\text{cat}}^{\text{c}}$ and the catalytic efficiency ($k_{\text{cat}}^{\text{c}}/K_{\text{c}}$) at 4°C relative to its cold-temperate counterpart due to a reduced thermal dependence of the Arctic Rubisco. A similar cold-adaptation in Rubisco kinetics was also found in the Arctic relative to the Atlantic population of *P. palmata*, although no increase in Rubisco content was observed in any of those species. Conversely, the chlorophyte *A. arcta* did not show significant differences in its Rubisco kinetics at 4°C but a more than two-fold increase in the Rubisco content per TSP in the polar populations in comparison to their North-Atlantic counterpart, revealing various alternative ways of adaptation to cold waters involving Rubisco. However, the rhodophyte *P. rubens*, lacking CCMs, showed no differences in Rubisco carboxylation kinetics and content in the Arctic relative to the North-Atlantic population, presumably due to its lower subtidal habitat, where selection pressure for photoprotection via CCMs and a more efficient/abundant Rubisco is absent.

Until now, there has been almost no information about the Rubisco kinetics for carboxylation in seaweeds and its thermal dependencies, even though this is an essential information for understanding the functioning of carbon acquisition and assimilation mechanisms in these organisms.

Despite this modest improvement in the Rubisco carboxylation kinetics of the Arctic *S. latissima* relative to the Atlantic population, an increased Rubisco activation state must also be required in order to explain the two-fold higher photosynthetic rates observed in the Arctic *versus* the Atlantic population of *S.*

latissima when both species were cultured at 10°C, as the content of Rubisco was not significantly different (see Chapter 3.2).

There were very few interactions between temperature and CO₂ in the endemic Antarctic *D. anceps* (Chapter 4), in agreement with the results from Arctic species of Chapter 2.2. Nevertheless, a strong interaction in almost all analysed variables was obtained between irradiance and temperature. It has been previously shown that photosynthetic DIC saturation is generally highly modulated by light energy availability (Gao et al. 2012; Hepburn et al. 2011) and temperature. This was reflected in a strong inhibition of growth rate at 2°C when thalli were acclimated to excessive irradiance, which was not observed at 7°C, suggesting that increased temperature alleviated chronic photoinhibition in *D. anceps*. Transcriptomic analysis of the same experiment revealed that gene expression of photosynthetic and carbon utilization components in *D. anceps* are less regulated than in the few marine photoautotrophic organisms analysed up to date, such as *Emiliania huxleyi* (Rokitta et al. 2012) and *Phaeodactylum tricornutum* (Li et al. 2015), in response to abiotic changes in temperature and CO₂, that might be due to a strong adaptation to continuous low temperature. This lack of genetic regulation might suppose a disadvantage in comparison to cosmopolitan eurithermic species in near-future scenarios, as a constitutive high gene expression involves an extra energy that may be saved by species with a stronger gene expression regulation in response to abiotic changes.

All experiments of this thesis were performed in summer conditions (nearly continuous light) but it is unknown neither how the analysed polar seaweeds will respond to the increase in CO₂ and temperature during the polar night, nor how it will affect other life-cycle stages different from the sporophytes.

It needs also to be taken into account that most of the predictions derived from OA simulation experiments on macroalgae are made assuming an inorganic carbon system in the natural waters around the algae being close to or at air equilibrium. However, it is clear that the concentration of CO₂ in the light in macroalgal beds can be well below air equilibrium due to an elevated photosynthetic activity (Raven and Hurd 2012), the contrary occurring during the

long and dark respiration-prominent winter. Nevertheless, increasing atmospheric CO₂ will produce an increase in CO₂ and a decrease in pH superimposed on these biologically induced effects. It is still to be determined how the presence/absence of ice cover is modifying the level of CO₂ present in the coastal system at a given time of the year, although ongoing continuous monitoring in Kongsfjorden might elucidate this issue in the upcoming future.

Future perspectives

A deeper understanding on how CCMs (including external CA) might contribute to photoprotection under photoinhibitory conditions could be a focus of further research. The use of inhibitors of different CCM components along with measurements on photoinhibition-related variables such as ROS accumulation might shed light into these mechanisms. The knowledge about the physiological and biochemical response to the increase in CO₂ and temperature during the polar night is also crucial to a complete understanding of potential changes derived from the up-coming scenarios in polar seaweeds, as both factors could alter respiration rates, compromising the survival of the sporophytes until the next spring.

Measurements of Rubisco oxygenation kinetics and the specific factor for carboxylation over oxygenation, along with sequencing of the large and small subunits will also shed light into the understanding of carbon fixation from polar and temperate seaweeds, which might have important implications in productivity and ecosystem functioning in near-future scenarios.

Moreover, experiments of longer-term acclimation to increased CO₂ and temperature are also needed to extrapolate their responses to the future environment, as there could be complex effects different from short-term physiological responses. However, these experiments are more difficult to carry out in seaweeds relative to phytoplankton due to the long and complex life-cycle of the formers.

Conclusions

1. The effect of the projected increase in CO₂ levels on the growth rate of Arctic macroalgae has been shown to be species-specific, with some positive (*Alaria esculenta*), negative (*Desmarestia aculeata*) and neutral responses (*Saccharina latissima* and *Laminaria solidungula*), whereas the Antarctic endemic *Desmarestia anceps* has been shown to be benefitted by increased CO₂ levels.
2. Increased CO₂ concentrations produced a reorganization of the cellular carbon budget of the alga and did not reflect a direct effect on photosynthesis in most of the analysed species, but rather a combination of effects on respiration, organic carbon accumulation and/or DOC release, along with a readjustment of the cellular energetic requirements, which ultimately determine growth rate.
3. Photosynthesis was not altered by increased CO₂ in any of the analysed polar species, except for the Antarctic *D. anceps*, suggesting that most polar seaweeds must be C-saturated at current CO₂ conditions.
4. There were no significant down-regulation of CCMs at the projected increase in CO₂ levels in the Arctic endemic *L. solidungula* and the Antarctic endemic *D. anceps*, while a significant down-regulation was observed in the Arctic *A. esculenta* and *D. aculeata*, evidencing species-specific differences. Regulation of CCM operation in the Arctic *S. latissima* was dependent on the external dissolved CO₂ concentration, showing a down-regulation at 1500 ppm of CO₂ but not at lower concentrations.
5. External CA activity plays a major role in the carbon acquisition mechanisms of all analysed polar seaweeds, *S. latissima*, *L. solidungula* and *D. anceps*, showing a 60 to 80% inhibition of photosynthesis by a specific inhibitor of external CAs, while internal CA activity seems to be marginally relevant. The dependence of photosynthesis on external CA activity was unchanged at increased CO₂ levels in *S. latissima* and *L. solidungula*, although it was decreased by 20% in *D. anceps*.

6. The projected increase in temperature, when analysed alone, positively affected the growth rate of the Arctic population of *S. latissima*, did not affect the growth rate of the Arctic endemic *L. solidungula* and negatively affected the growth rate of the Antarctic endemic *D. anceps*.
7. There were very few interactions between CO₂ and temperature among the analysed physiological variables for *S. latissima*, *L. solidungula* and *D. anceps*, and none of them were observed on growth rate.
8. *Saccharina latissima* would be benefited by the upcoming environmental scenario, mainly due to the projected increase in temperature, while *L. solidungula* may retreat to northernmore latitudes in West Spitsbergen as it would not be benefited by the increase in temperature.
9. The projected warming would significantly increase the tolerance of *D. anceps* to excessive irradiance (increasing the irradiance at which the growth rate of the alga becomes photoinhibited), and so, this species would maintain its productivity in near-future scenarios of increased temperature and CO₂ in a wider range of irradiances than at present conditions, since the increase in biomass due to elevated CO₂ conditions would compensate for the reduction produced by the increase in temperature.
10. Transcriptomic analysis revealed that there was a high and constitutive gene expression of many photochemical and inorganic carbon utilization components in *D. anceps* that were not altered by increased CO₂ and temperature, whereas excessive irradiance produced the strongest changes in the transcriptome, but only at low temperature.
11. Arctic and Atlantic populations of *S. latissima* differ in their physiological and biochemical responses to increased CO₂ and temperature. However, the physiological and biochemical variables analysed in the present study were more affected by temperature than by CO₂ in both populations. The Arctic population is more likely to benefit from the upcoming environmental scenario than its Atlantic counterpart, mainly due to the beneficial effect of the increase in temperature on its growth rate.

12. Most polar macroalgae possess similar or even more effective CCMs when compared with their cold-temperate counterparts, despite the observed higher affinity for CO₂ of their Rubiscos at low temperature and the higher ambient level of dissolved CO₂ in air-equilibrated cold waters, suggesting that Rubisco from polar seaweeds are more likely to be saturated at current CO₂ conditions than species from other latitudes.
13. There are evidences of cold-adaptation in Rubisco carboxylation kinetics and Rubisco content for some polar macroalgae possessing CCMs when compared to its cold-temperate counterparts, showing species-specific patterns. Despite of the improvement in the catalytic properties of polar Rubiscos at low temperature, photosynthesis of polar red and brown seaweeds must be constrained by carbon fixation rates, requiring for high Rubisco activation states in comparison to other latitudes.
14. Different alternative ways of adaptation to cold environments involving Rubisco have been identified: an increase in $k_{\text{cat}}^{\text{C}}$ and $k_{\text{cat}}^{\text{C}}/K_{\text{C}}$ at 4°C due to a decrease in E_{a} for $k_{\text{cat}}^{\text{C}}$ in the Arctic *S. latissima*, a general increase in $k_{\text{cat}}^{\text{C}}$ and $k_{\text{cat}}^{\text{C}}/K_{\text{C}}$ without a change in E_{a} for $k_{\text{cat}}^{\text{C}}$ in the Arctic *P. palmata*, and a significant increase in the percentage of total soluble protein being Rubisco ([Rubisco]/[TSP]) in the Arctic and Antarctic *A. arcta*, relative to their cold-temperate counterparts.

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Resumen



Las regiones polares se caracterizan por ser ambientes extremos de temperaturas constantemente bajas y una fuerte estacionalidad en las condiciones de luz, lo que las diferencia de todas las demás regiones del mundo. A pesar de estas duras condiciones, sus zonas costeras rocosas están dominadas por comunidades de macroalgas que forman densos bosques subacuáticos y representan una contribución trófica importante a estos sistemas, desempeñando un papel dominante en los flujos de carbono a escala regional. Además, proporcionan alimento, refugio y sustrato para un número elevado de animales y otras algas.

A pesar de que las macroalgas son elementos comunes de las zonas litorales rocosas de ambas regiones polares, proporcionando estructura a la comunidad, hay diferencias sustanciales en la composición de especies de algas marinas del Ártico y la Antártida. La mayoría de las macroalgas del Ártico tiene un origen Atlántico o Pacífico debido a la conexión de las aguas del Ártico con otras zonas climáticas, con pocas especies cosmopolitas o endémicas. Por el contrario, las regiones antárticas poseen un 35% de especies endémicas, muchas de ellas pertenecientes al filo Heterokontophyta. La mayoría de las especies de algas marinas de ambas regiones polares se encuentran en el sublitoral y muy pocas especies habitan el eulitoral.

Una de las áreas del Océano Ártico mejor estudiadas y con una elevada diversidad de especies es el Kongsfjorden en Spitsbergen (Islas Svalbard). Las zonas sublitorales rocosas del Kongsfjorden están dominadas por una elevada biomasa de algas marinas. El sublitoral superior se caracteriza por la presencia de las algas pardas *Fucus distichus*, *Pylaiella littoralis*, *Chordaria flagelliformis* y la laminaria *Saccorhiza dermatodea*, la macroalga endémica roja *Devaleraea ramentacea* y las algas verdes del género *Acrosiphonia*. La zona del sublitoral medio está dominado por las laminarias *Alaria esculenta*, *Laminaria digitata* y *Saccharina latissima*. Las algas pardas *Desmarestia viridis* y *Desmarestia aculeata* también se encuentran en esta zona, aunque son menos abundantes que las anteriormente mencionadas. Las especies características del sublitoral inferior son las algas rojas *Phycodrys rubens* y *Ptilota gunneri*, que crecen hasta 30 m de profundidad. La especie endémica del Ártico *Laminaria solidungula* está retrocediendo hacia el norte de la isla y cada vez es menos abundante en el Kongsfjorden, encontrándose

predominantemente en las zonas interiores del fiordo. Sin embargo, en las costas de Alaska y el Ártico canadiense, *L. solidungula* es la especie dominante junto con *A. esculenta* y *S. latissima*.

Las costas rocosas a lo largo de la parte norte de la Península Antártica occidental y las islas adyacentes están dominadas por extensas comunidades de macroalgas submareales, a menudo cubriendo más del 80% de la superficie. Uno de las zonas costeras mejor estudiadas de la Península Antártica son las pertenecientes a la isla Rey Jorge (Islas Shetland del Sur). El sublitoral a menudo está colonizado por especies como las algas rojas *Palmaria decipiens*, *Porphyra endivifolium* y *Iridaea cordata*, y el alga parda *Adenocystis utricularis*. Por debajo de esta zona hay bosques de grandes algas pardas altamente productivas que dominan el sublitoral. Estas grandes algas marinas son perennes y pertenecen al orden Desmarestiales, sustituyendo ecológicamente a los bosques de laminarias característicos de aguas templadas y árticas. Estas especies son *Desmarestia menziesii*, *Desmarestia Anceps* y *Himantothallus grandifolius*. Tanto las comunidades de laminarias del Ártico como las de Desmarestiales de la Antártida presentan una productividad similar a la de los bosques de algas de latitudes templadas.

Las macroalgas polares están adaptadas a los regímenes locales de temperatura, luz y nutrientes de las regiones polares. Muchos de los esporofitos de macroalgas endémicas de la Antártida pueden crecer hasta a 5°C y algunas de estas especies poseen tasas de crecimiento óptimo a 0°C. En contraste, los esporofitos de las pocas especies endémicas del Ártico que se conocen, como *L. solidungula*, tienen tasas de crecimiento óptimo a 5-10°C, pero pueden crecer hasta a 15°C. Además, los valores máximos de fotosíntesis de macroalgas polares son comparables a los observados para macroalgas de climas templados a sus respectivas temperaturas ambientales. Hasta ahora, la cantidad de datos sobre los procesos bioquímicos que explican las respuestas fisiológicas en el crecimiento y la fotosíntesis de macroalgas del Ártico y Antártida es muy escasa. Como se ha observado en microorganismos fotosintéticos adaptados al frío, las características metabólicas que influyen en el crecimiento y la fotosíntesis a bajas temperaturas podrían incluir el mantenimiento de la fluidez de la membrana mediante la incorporación de ácidos grasos poliinsaturados, la adaptación molecular de enzimas de manera que

compense la reducción en las tasas de reacción, y las adaptaciones en la cadena de transporte de electrones fotosintética que equilibran la transducción de la energía luminosa en energía química a bajas temperaturas.

La adaptación a los ecosistemas polares incluye la supervivencia de las algas marinas sublitorales durante los meses de oscuridad continua. Algunas macroalgas antárticas endémicas requieren dosis anuales de luz de sólo la mitad de lo que comúnmente se necesita para especies de macroalgas templadas, lo que es indicativo de la fuerte adaptación a las bajas condiciones lumínicas que experimentan a lo largo del año. Sin embargo, justo después de que el hielo marino se rompa en primavera, se pueden alcanzar altas irradiancias en profundidad. Esta situación de bajas temperaturas combinadas con altas irradiancias podría conducir a un desequilibrio entre las reacciones fotoquímicas, insensibles a la temperatura, y las reacciones enzimáticas, sensibles a la temperatura, ya que la absorción de luz puede exceder los requerimientos energéticos metabólicos del organismo. Por lo tanto, las algas polares del submareal no sólo tienen una fuerte adaptación a las bajas condiciones lumínicas, sino que también pueden hacer frente a condiciones de luz alta, debido a que poseen diferentes mecanismos fotoprotectores.

En relación con la adaptación a la fuerte estacionalidad del régimen de luz, algunas algas polares empiezan a crecer bajo condiciones de días cortos al final del invierno o principios de primavera, a menudo bajo el hielo marino. Su crecimiento se basa probablemente en ritmos circanuales sincronizados por la duración de la luz del día, en contraste con el resto de las especies que no inician su crecimiento antes de que las condiciones de luz sean suficientemente favorables. En el Ártico no hay sólo una fuerte variación estacional con respecto a la luz, sino también con respecto a los niveles de nitrato y fosfato, que son altos en invierno y bajos en verano. Por ello, algunas algas Árticas muestran una alta acumulación de biomoléculas de reserva durante el verano que permiten el crecimiento durante el invierno, cuando los niveles de nutrientes en el medio son altos.

El sistema Ártico contribuye en un 5-14% al balance global de CO₂ y actúa como sumidero. Como consecuencia del aumento de la emisión de CO₂ a la atmósfera y de su posterior disolución en las capas superficiales del océano, la

concentración de carbono inorgánico disuelto ha aumentado durante las últimas décadas y el pH ha disminuido. Este proceso, conocido como "acidificación oceánica", tendrá unas dimensiones de hasta 0.32 unidades de pH en zonas polares para finales de siglo según el último informe del IPCC de 2013, debido principalmente a la mayor disolución de CO_2 en aguas frías. Esta disminución de pH junto con el descenso en CO_3^{2-} , esencial para organismos con conchas y otras estructuras carbonatadas, y el aumento en el CO_2 disuelto tienen enormes consecuencias para multitud de procesos biológicos. Además, debido al efecto invernadero causado por la emisión de estos gases, se prevé un aumento de la temperatura global de 2.6 a 4.8°C que puede dar lugar a un aumento de más de 2°C de la temperatura de las capas más superficiales del océano para finales de siglo. El Ártico y la Península Antártica ya están experimentando este calentamiento que está ocurriendo a una tasa tres veces más rápida que en el resto del mundo, debido en parte a las corrientes oceánicas, a la circulación atmosférica y al efecto albedo. A parte del aumento en temperatura y de la acidificación oceánica, también se están observando otros cambios en estas regiones polares tal como la reducción en las masas de hielo, el retroceso de los glaciares, el deshielo del permafrost y el acortamiento en la duración del hielo estacional, lo que puede provocar cambios en la turbidez (y por lo tanto en los niveles de irradiancia en el sublitoral), salinidad del agua y en la concentración de nutrientes.

El CO_2 es el sustrato para la reacción de la carboxilación de la ribulosa-1,5-bisfosfato carboxilasa oxigenasa (Rubisco), principal enzima de la producción primaria, la cual suministra más del 99% del C en forma de materia orgánica a las redes tróficas marinas. En principio, el aumento de los niveles de CO_2 disuelto debería incrementar la tasa de fotosíntesis, dado que su concentración actual es menor que las constantes de semi-saturación de la Rubisco de la mayoría de especies. Sin embargo, son minoría las especies que parecen tener su fotosíntesis restringida a la difusión de CO_2 desde el medio, ya que la mayoría de ellas disponen de mecanismos que generan altas concentraciones de CO_2 en el interior celular. Estos mecanismos son conocidos como mecanismos concentradores de carbono (CCMs), y consisten en el transporte facilitado o activo de CO_2 y/o HCO_3^-

al interior celular, y el control de los flujos de carbono inorgánico acumulado intracelularmente. Una de las principales enzimas de los CCMs es la anhidrasa carbónica (CA), tanto externa como interna, que cataliza la conversión (reversible) de HCO_3^- a CO_2 .

Anteriormente se pensó que el impacto de la acidificación oceánica en la producción de algas con CCMs sería limitada, sin embargo, estos mecanismos son costosos energéticamente y se inhiben en condiciones de alta concentración de CO_2 , por lo que una atmósfera enriquecida en CO_2 libera a la célula de la demanda energética de los CCMs, pudiendo ésta ser empleada en otros procesos como la asimilación de otros nutrientes, teniendo como consecuencia el aumento en la tasa de crecimiento de estas especies. Por otra parte, un aumento en los niveles de CO_2 no siempre trae consecuencias positivas a las macroalgas. Hay evidencias tanto en especies de climas templados como polares de un efecto negativo de este aumento de CO_2 , presumiblemente por una incapacidad de equilibrar los flujos de C y otros nutrientes, o una falta de control del pH intracelular.

Se ha planteado que la presencia o ausencia de CCMs podría relacionarse con el hábitat y la taxonomía, proponiéndose algunos modelos. Estudios previos han hipotetizado que en las regiones frías, debido a una mayor solubilidad de los gases, entre ellos el CO_2 , las algas sin CCM tendrían ventaja competitiva sobre aquellas con CCM. Por tanto, la proporción de especies con CCM sería menor en regiones polares respecto a las regiones templadas. A pesar de esta hipótesis, otros estudios corroboraron que la mayoría de especies polares eran capaces de usar HCO_3^- para la fotosíntesis.

La falta de correlación entre la actividad de CCMs, los niveles de CO_2 en el medio y la capacidad de asimilación de C por parte de la célula se complica aún más cuando se tienen en cuenta las propiedades cinéticas de la Rubisco. La cantidad de Rubisco activa es generalmente mayor a bajas temperaturas, pero menor a concentraciones elevadas de CO_2 , por lo que es difícil predecir cuál sería la consecuencia de aumentar el CO_2 en algas polares. Tanto la cantidad como la cinética de la Rubisco en macroalgas del Ártico es desconocida, e inferir las

consecuencias del aumento de CO₂ sobre éstas a partir de estudios realizados con algas de otras latitudes podría llevar a conclusiones erróneas.

A pesar de la alta productividad y relevancia ecológica de las comunidades de macroalgas en estos ecosistemas polares, y de que dichas regiones estén experimentando los efectos más fuertes y más rápidos del cambio climático en comparación con cualquier otra región de la Tierra, los estudios sobre la respuesta de aclimatación fisiológica de macroalgas polares a los escenarios ambientales proyectados de aumento de CO₂ y de temperatura son muy escasos. Además, los estudios sobre los mecanismos moleculares que subyacen a estas respuestas fisiológicas son casi inexistentes en macroalgas polares.

Por lo tanto, el objetivo de esta tesis ha sido caracterizar la respuesta de aclimatación fisiológica de algas polares ecológicamente relevantes, a un aumento de temperatura y CO₂ disuelto correspondiente a los niveles esperados para finales de este siglo.

El presente estudio ha abordado las siguientes cuestiones específicas: ¿De qué manera puede verse alterado el balance de carbono celular de macroalgas polares por el aumento de CO₂?, ¿Refleja la tasa de crecimiento el efecto del aumento de CO₂ en la fotosíntesis?, ¿Se desactivan los CCMs de estas especies en respuesta al aumento de CO₂?, ¿En qué proporción depende la fotosíntesis de estas macroalgas de la actividad de CA externa e interna y cómo se ve modificada esta proporción por el aumento de CO₂?, ¿Cómo pueden verse afectadas las macroalgas polares por un aumento de la temperatura?, ¿Existen interacciones entre los efectos del aumento de CO₂ y de temperatura?, ¿Es esta interacción dependiente de los niveles de irradiancia?, ¿Existe una respuesta fisiológica y bioquímica diferente en relación a un aumento de CO₂ y de temperatura entre las poblaciones polares y las poblaciones de zonas templadas?, ¿Van a verse más beneficiadas por el aumento de CO₂ y temperatura las poblaciones Árticas de especies ampliamente distribuidas por zonas templadas en comparación con las especies endémicas del Ártico?, ¿Cómo se ve alterada la respuesta en la expresión génica que subyace a la respuesta fisiológica de estas especies por estos factores del cambio global?, ¿Muestran las macroalgas polares CCMs menos eficaces que sus especies homólogas de zonas templadas?, ¿Cómo son sus cinéticas de

carboxilación de la Rubisco a bajas temperaturas?, ¿Está la Rubisco de estas especies saturada a los niveles actuales de CO₂?

Para contestar a estas preguntas se realizaron diferentes experimentos:

En el capítulo 2.1 se analizaron los cambios en el balance celular de carbono (fijación de carbono, crecimiento, respiración, excreción de carbono orgánico y acumulación de biomoléculas de reserva) en respuesta a un aumento de CO₂ (1300 ppm) en las poblaciones árticas de las especies *Desmarestia aculeata* y *Alaria esculenta* del Kongsfjorden (Svalbard).

En el capítulo 2.2 se analizó la respuesta fisiológica (crecimiento, fotosíntesis, respiración, excreción de carbono orgánico disuelto, actividad de los CCM y relevancia de las CAs en la adquisición de carbono) de las laminarias árticas *Saccharina latissima* y *Laminaria solidungula* del Kongsfjorden después de haber sido incubadas a 4 y 9°C en combinación con concentraciones presentes (390 ppm) y futuras (1200 ppm) de CO₂.

En el capítulo 3 se analizó la variación en la composición bioquímica (proteínas, lípidos, carbohidratos, composición pigmentaria y contenido en las enzimas Rubisco y D1) y la respuesta fisiológica (crecimiento, fotosíntesis y actividad de los CCMs) a un aumento de CO₂ (380, 800 y 1500 ppm) y temperatura (población ártica: 4 y 10°C, población atlántica: 10 y 17°C) de una población atlántica de *S. latissima* en comparación con una población ártica de la misma especie.

En el capítulo 4 se analizó la respuesta fisiológica (crecimiento, fotosíntesis, respiración, excreción de carbono orgánico disuelto, actividad de los CCMs y relevancia de las CAs en la adquisición de carbono) y de la expresión génica de la especie endémica de la Antártida *Desmarestia anceps* a un aumento en temperatura (2 y 7°C), CO₂ (380 y 1000 ppm) e irradiancia (65 y 145 μmol fotones m⁻² s⁻¹).

Por último, en el capítulo 5 se analizó la relevancia del funcionamiento de los CCMs en doce especies de macroalgas representativas de los ecosistemas Árticos y Antárticos mediante medidas de discriminación isotópica del ¹³C del alga ($\delta^{13}\text{C}_{\text{alga}}$) y experimentos de deriva del pH que permiten determinar el pH de compensación. También se determinó la cinética de carboxilación a 4 y 25°C de la Rubisco de estas especies (constante Michaelis-Menten de afinidad por el CO₂,

K_c , y la tasa catalítica de recambio de la carboxylación, k_{cat}^c) y se hicieron comparaciones latitudinales de poblaciones de la misma especie, con el fin de detectar posibles adaptaciones al frío en las poblaciones polares en comparación con las poblaciones templadas.

Los resultados presentados en esta tesis han demostrado que los efectos de niveles elevados de CO_2 en la tasa de crecimiento de las macroalgas polares son específicos de cada especie, y que por lo general no van paralelos a los efectos en la fotosíntesis. En su lugar, los cambios en la tasa de crecimiento se deben a una reorganización en el balance de energía y de carbono de la célula. La mayor parte de los estudios basados en los efectos de la acidificación oceánica en macroalgas realizados hasta la fecha se han centrado principalmente en analizar la adquisición de carbono inorgánico, el rendimiento fotosintético o el crecimiento, pero se ha prestado menos atención a los efectos sobre las pérdidas de carbono debido a la respiración y la liberación de carbono orgánico disuelto, o la acumulación de carbono orgánico en forma de biomoléculas de almacenamiento como otro sumidero de carbono, y no existe casi ningún estudio en el que se hayan medido todos estos procesos en el mismo experimento con el fin de calcular un balance de carbono. Los resultados del capítulo 2.1 revelaron que la tasa de crecimiento de *D. aculeata* se vio afectada negativamente en condiciones de elevado CO_2 debido a un aumento en la respiración, en la acumulación de biomoléculas de almacenamiento y en la tasa de liberación de carbono orgánico disuelto, mientras que la tasa de crecimiento de *A. esculenta* aumentó debido a una disminución en la tasa de respiración y en la acumulación de biomoléculas de almacenamiento, a pesar de que no se apreciaron diferencias en las tasas de fijación de carbono a los diferentes niveles de CO_2 ensayados. Estos resultados ponen de manifiesto la necesidad de medir todos los procesos de ganancia y pérdida de carbono de la célula con el fin de tener una visión completa de los efectos fisiológicos del aumento de CO_2 .

Además de esto, casi ningún estudio sobre macroalgas ha medido todos los pasos implicados en el rendimiento fotosintético en el mismo experimento, a nivel de fluorescencia de la clorofila *a*, evolución de oxígeno y fijación de carbono. Los resultados de esta tesis demuestran que hay desacoplamientos entre estos

procesos y que la extrapolación de las mediciones de clorofila *a* y evolución de oxígeno a valores de productividad tiene que realizarse con cuidado. Los cambios en los diferentes factores abióticos, como el CO₂ y la temperatura, pueden modular la relación entre los fotones drenados a la tasa de transporte de electrones y la producción de O₂, y entre la producción de O₂ y la fijación de CO₂, tal y como se indica en el Capítulo 2, lo que complica el cálculo de productividad a partir de mediciones de evolución de O₂.

Las tasas de fijación de carbono de todas las especies del Ártico analizadas no se vieron alteradas por el aumento en los niveles de CO₂ (Capítulo 2), lo que indica que la tasa de fotosíntesis debe estar saturada en carbono a los niveles actuales de CO₂. Los resultados del Capítulo 5 revelaron que todas las algas rojas y pardas analizadas, incluyendo las especies árticas usadas en el Capítulo 2, poseen Rubiscos con una constante de semi-saturación para el CO₂ a 4°C de aproximadamente 2-4 μM. Por lo tanto, sus Rubiscos están saturadas a concentraciones en torno a 22 μM de CO₂, que es la concentración que corresponde al agua de mar en equilibrio con el aire a 4°C. Este incremento significativo en la afinidad por el CO₂ a menor temperatura observado en todas las Rubiscos analizadas sugiere que las macroalgas polares tendrían más facilidad para presentar tasas de fotosíntesis saturadas en carbono que las especies templadas a su correspondiente temperatura ambiental, independientemente de su capacidad de utilizar HCO₃⁻ para la fotosíntesis.

A pesar de que las macroalgas polares tendrían más facilidad para tener tasas de fotosíntesis saturadas en carbono debido a la mayor afinidad por el CO₂ de la Rubisco a bajas temperaturas, todas las macroalgas polares analizadas, excepto las rojas pertenecientes al sublitoral inferior *P. Rubens* y *P. gunneri*, fueron capaces de utilizar activamente HCO₃⁻ en un grado similar o incluso superior a las poblaciones templadas, como indican las medidas de δ¹³C_{alga} y de punto de compensación del pH (capítulo 5).

El uso activo de HCO₃⁻ a bajas temperaturas, a pesar de la mayor afinidad por el CO₂ de la Rubisco y de la mayor concentración CO₂ disuelto en equilibrio con la atmósfera a bajas temperaturas, podría estar relacionado con el hecho de que las condiciones de equilibrio de CO₂ no se llegan a alcanzar en los océanos fríos,

debido a la intensa actividad biológica en los bosques de macroalgas en primavera y verano, y al lento equilibrio de CO_2 entre la superficie de los océanos y la atmósfera en relación con el que hay entre el CO_2 y las otras especies de carbono inorgánico disuelto. Por otra parte, hay una disminución considerable de la tasa no catalizada de suministro de CO_2 por el bicarbonato y en la velocidad de difusión de CO_2 en aguas frías en relación con latitudes más cálidas. En cuanto a la fijación fotosintética de carbono, si la tasa de suministro difusivo de CO_2 a la Rubisco es más lenta que la tasa de fijación de carbono máximo en condiciones de saturación de CO_2 , la concentración de CO_2 alrededor de la Rubisco disminuirá notablemente con respecto a la concentración de CO_2 externa, convirtiéndose en una concentración no saturante para la Rubisco. Además, la exposición de las algas a altas irradiancias casi continuas en combinación con la baja temperatura después de que el hielo marino se rompa a finales de la primavera y el verano podría aumentar la demanda de los mecanismos concentradores de carbono (CCM), ya que las reacciones fotoquímicas son independientes de la temperatura mientras que las reacciones enzimáticas implicadas en la adquisición de la fotosíntesis y de carbono se reducen significativamente a baja temperatura. Por lo tanto, estas condiciones ambientales pueden dar lugar a un excedente de energía fotoquímica que podría conducir a la fotoinhibición crónica si el exceso de fotones no se disipa, por lo que, en esta situación, el funcionamiento de los CCMs podría ayudar a disipar el exceso de energía y asegurar una saturación de CO_2 continua de la Rubisco. La capacidad de las macroalgas polares de usar HCO_3^- para la fotosíntesis ha demostrado estar relacionada con la zonación en profundidad (de acuerdo con la disponibilidad de luz bentónica) tal como se refleja en la Fig. 5.1, con mayores $\delta^{13}\text{C}_{\text{alga}}$ y puntos de compensación del pH para especies que habitan en el sublitoral superior y eulitoral inferior respecto a los que habitan el sublitoral bajo y medio.

En contraste con los resultados obtenidos para las macroalgas del Ártico, las tasas de fotosíntesis de la especie endémica antártica *D. anceps* aumentó a niveles elevados de CO_2 (Capítulo 4), a pesar del funcionamiento de los CCMs y de la alta afinidad por el CO_2 de la Rubisco de algas pardas a bajas temperaturas, lo que podría conducir a tasas de fotosíntesis saturada de carbono en las condiciones

actuales de CO₂. El aumento de las tasas de fotosíntesis a niveles elevados de CO₂ observado en *D. anceps* podría estar relacionado con un aumento en el contenido de la Rubisco, que se sospecha a partir de un aumento significativo en la expresión del gen *rbcL* a alto CO₂. Sin embargo, esto es sólo una hipótesis, ya que el contenido en Rubisco y su cinética no se midieron en esta especie debido a dificultades en el procedimiento de extracción de proteínas, y un aumento en la expresión del gen *rbcL* no significa necesariamente un aumento en la cantidad de Rubisco activa.

Tres de las cinco especies de macroalgas polares analizadas en esta tesis, las árticas *S. latissima* y *L. solidungula* y la antártica *D. anceps*, no mostraron una desactivación de los CCMs al aumento de CO₂ en el rango de 1000 a 1200 ppm. Sin embargo, la desactivación de los CCMs es una respuesta frecuente en las macroalgas de climas templados cuando se cultivan en condiciones de aumento de CO₂ disuelto, ya que estos mecanismos son energéticamente costosos. La desactivación parcial de los CCMs ahorra energía que puede ser invertida en otros procesos fisiológicos, lo que resulta en algunos casos en un aumento de la tasa de crecimiento. Esta respuesta se observó en *A. esculenta* en el Capítulo 2.1.

La capacidad de un organismo para desactivar o no los CCMs va a depender en su mayor parte de su capacidad para aumentar el carbono inorgánico alrededor de la Rubisco, ya que la sustitución de los CCMs por la entrada de CO₂ por difusión requeriría una concentración de CO₂ externa en exceso de la que se logra por lo general internamente por los CCMs, pero también podría estar determinada por una disminución en los requerimientos energéticos de la célula, algo que se ve frecuentemente alterado en condiciones de aumento de CO₂.

La desactivación parcial de los CCMs observada en *D. aculeata* (Capítulo 2.1) podría estar relacionada con un aumento de la demanda de energía para contrarrestar el estrés fisiológico generado por la reducción del pH externo. En otras especies también se han descrito efectos negativos similares a los observados en *D. aculeata*, y se ha sugerido que los cambios en el pH extracelular podrían alterar el potencial electroquímico de la membrana y los canales de protones o iones mediante la alteración de la estructura de las proteínas periplásmicas. La explicación bioquímica subyacente a este efecto negativo

observado en algunas especies sigue siendo en gran medida desconocida y es un campo potencial para la investigación futura.

En cualquier caso, es evidente que una parte representativa de las algas polares analizadas necesitaría más de 1000 ppm de CO₂ para desactivar los CCMs, a pesar de la alta afinidad observada para el CO₂ de la Rubisco de estas algas a bajas temperaturas, poniendo de relieve la importancia del funcionamiento de los CCMs en entornos polares como parte de un mecanismo que asegura altas tasas de fotosíntesis en aguas frías, similares a las de latitudes templadas a su respectiva temperatura ambiental.

Esta tesis también aporta claras evidencias de un papel importante de la actividad de la CA externa en la adquisición de carbono inorgánico de todas las especies analizadas (las árticas *S. latissima* y *L. solidungula*, y la antártica *D. anceps*), tal como se refleja en una fuerte inhibición de alrededor de 50-80% de la fotosíntesis neta por DBS (inhibidor de la actividad de CA externa). Estos resultados concuerdan con las elevadas actividades de CA externa reportadas previamente en macroalgas del Ártico, lo que sugiere que este mecanismo podría ser parte de una adaptación general a las aguas frías. Por otra parte, la dependencia de la fotosíntesis de la actividad de CA externa no se redujo tras la incubación a un incremento de CO₂ ni en *S. latissima* ni en *L. solidungula*, lo que apoya de nuevo la idea de una mayor necesidad de mecanismos activos de adquisición de carbono en ambientes polares, probablemente relacionada con la fotoprotección en condiciones de radiación solar continua durante el período de verano.

Los efectos fisiológicos observados tras un aumento de la temperatura en las algas polares son similares a los obtenidos en estudios previos. El aumento de temperatura fue positivo para *S. latissima*, neutral para la endémica del Ártico *L. solidungula* y negativo para la endémica de la Antártida *D. anceps*, como se refleja en sus tasas de crecimiento. Esta respuesta fisiológica depende de los requisitos de temperatura para el crecimiento, la reproducción y la supervivencia de cada especie, reflejando su grado de adaptación a ambientes fríos. Se prevé un desplazamiento hacia los polos en la distribución geográfica de las principales especies submareales de macroalgas en los futuros escenarios climáticos y una distribución hacia el norte de las especies del Ártico debido a un cambio en la

isoterma límite de verano. Estas predicciones concuerdan con los resultados del capítulo 2.2, lo que sugiere que *S. latissima* podría beneficiarse en mayor medida que la endémica *L. solidungula* en un futuro cercano más cálido, mientras que ambas poblaciones parecen ser altamente resilientes al aumento de CO₂. Por lo tanto, el aumento de temperatura, en mayor grado que el aumento de CO₂, podría conducir a cambios importantes en las comunidades de macroalgas del Ártico.

Las interacciones entre la temperatura y el CO₂ son particularmente relevantes para la fotosíntesis. La tasa de asimilación de C a una temperatura dada depende de la actividad de los diferentes procesos a diferentes concentraciones de CO₂, como son la regeneración de fosfato inorgánico para la síntesis de ATP, la capacidad de la Rubisco y de la tasa de transporte de electrones (ETR). Por otra parte, la saturación de la fotosíntesis cambia a diferentes temperaturas para un determinado nivel de CO₂. Sin embargo, se ha observado un menor número de interacciones entre CO₂ y temperatura en las algas polares analizadas en comparación con otros ecosistemas. Esto podría indicar que las comunidades de algas polares son más resilientes de lo esperado a los futuros cambios ambientales en comparación con sus homólogos de zonas templadas.

Las diferencias significativas en las respuestas de aclimatación al aumento de CO₂ y la temperatura observadas entre poblaciones del Ártico y del Atlántico de *S. latissima* (Capítulo 3) indican que las conclusiones obtenidas a partir de estudios anteriores de macroalgas deben ser restringidas a la población estudiada y no la especie, ya que las poblaciones de diferentes latitudes pueden mostrar diferentes patrones bioquímicos y fisiológicos de aclimatación, a pesar de que pertenecen a la misma especie (diferenciación ecotípica). Como se observa en el capítulo 2.2, los cambios de temperatura ejercen efectos más fuertes que el CO₂ en la fisiología de ambas poblaciones. Una capacidad similar para el uso de HCO₃⁻ de ambas poblaciones se dedujo a partir de los valores de $\delta^{13}\text{C}_{\text{alga}}$. Sin embargo, los CCMs solamente se desactivaron a 1500 ppm de CO₂ en el ecotipo Ártico, mientras que se observó una desactivación a 800 ppm de CO₂ en la población Atlántica, pero sólo a la temperatura más alta, lo que apoyaría la hipótesis de que las bajas temperaturas promueven una mayor demanda de CCMs.

La diferenciación ecotípica entre ambas poblaciones se reflejó también en una cinética de carboxilación de la Rubisco significativamente diferente (Capítulo 5), lo que sugiere una adaptación al frío de la población ártica de la especie *S. latissima*. Esta adaptación al frío se reflejó en un aumento significativo en la k_{cat}^c y en la eficiencia catalítica (k_{cat}^c/K_c) a 4°C con respecto a la población Atlántica, debido a una menor dependencia térmica de la Rubisco polar. También se encontró una adaptación al frío similar en la cinética de la Rubisco de la población ártica de la especie *P. palmata* en relación a la población atlántica de la misma especie. Por el contrario, la macroalga verde *A. arcta* no mostró diferencias significativas en la cinética de carboxilación de la Rubisco a 4°C, pero sí se observó un aumento de más de dos veces en la proporción de proteínas totales solubles correspondiente a la Rubisco en las poblaciones polares respecto a la población atlántica de la misma especie.

Se observaron muy pocas interacciones entre la temperatura y el CO₂ en el alga endémica de la Antártida *D. anceps* (Capítulo 4), en concordancia con los resultados obtenidos para las especies árticas analizadas en el Capítulo 2.2. Sin embargo, se obtuvo una fuerte interacción entre irradiancia y temperatura para casi todas las variables analizadas en el capítulo 4. Se ha demostrado previamente que la saturación de carbono de la fotosíntesis está generalmente modulada por la disponibilidad de energía lumínica y por la temperatura. Esto se reflejó en una fuerte inhibición de la tasa de crecimiento a 2°C cuando los talos se aclimataron a una irradiancia excesiva, que no se observó en 7°C, lo que sugiere que el aumento de temperatura aliviaría la fotoinhibición crónica en *D. anceps*. Los análisis transcriptómicos de este mismo experimento revelaron que la expresión génica de los componentes de la fotosíntesis y la adquisición y asimilación de carbono en *D. Anceps* están menos regulados que en los pocos microorganismos fotosintéticos marinos analizados hasta la fecha, en respuesta a los cambios abióticos en la temperatura y el CO₂. Esta falta de regulación genética podría suponer una desventaja en comparación con especies euritermas cosmopolitas en los escenarios del futuro próximo, ya que una alta expresión génica constitutiva implica un mayor gasto de energía.

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- ϵ_p : ^{13}C isotopic fractionation of organic carbon production
 $\delta^{13}\text{C}_{\text{alga}}$: Stable isotope measurements of the $^{13}\text{C}/^{12}\text{C}$ ratio of algal biomass
 Φ_{PSII} : Effective quantum yield of PS II
 α : Photosynthetic light-harvesting efficiency
 AP: Sum of accessory pigments
 AZ: Acetazolamide
 BC: Biochemical composition
 CA: Carbonic anhydrase
 CCM: Carbon concentrating mechanism
 Chl a: Chlorophyll a
 Chl c: Chlorophyll c
 C: CO_2 concentration around Rubisco during steady-state photosynthesis
 DBS: dextran-bound sulfonamide
 DIC: Dissolved inorganic carbon
 DIDS: 4,4'-diisothiocyanatostilbene-2,2'-disulphonate
 DOC: Dissolved organic carbon
 DPS: De-epoxidation state
 DW: Dry weight
 E_{opt} : Irradiance at which chronic photoinhibition begins
 ETR: Electron transport rate
 E_k : Irradiance required for saturating the photosynthetic rate
 EZ: 6-ethoxazolamide
 FSW: Filtered natural seawater
 F_v/F_m : Optimal quantum yield for PSII fluorescence
 FW: Fresh weight
 HL: Helgoland
 K_c : Michaelis-Menten affinity constant for CO_2
 k_{cat}^c : Carboxylation turnover rate
 k_{cat}^c/K_c : Carboxylation catalytic efficiency
 NPS: Net photosynthesis
 NR: Nitrate reductase
 OA: Ocean acidification
 PAR: Photosynthetic active radiation
 $p\text{CO}_2$: Partial pressure of CO_2
 PFR: Photon fluence rate
 POC: Particulate organic carbon
 PQ: Photosynthetic quotient
 PS I: Photosystem I
 PS II: Photosystem II
 RGR: Relative growth rate
 ROS: reactive oxygen species
 Rubisco: Ribulose 1,5-bisphosphate carboxylase oxygenase
 RuBP: Ribulose 1,5-bisphosphate
 $S_{c/o}$: Specificity factor of CO_2 over O_2
 SP: Spitsbergen
 SWCS: Seawater carbonate system
 TA: Total alkalinity
 TSP: Total soluble proteins
 UST: Upper survival temperature
 UVR: Ultraviolet radiation
 VAZ: Xanthophyll cycle pigment pool (sum of viola-, anthera- and zeaxanthin)



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Effects of the Increase in CO₂ and Temperature on the Carbon Acquisition
and Assimilation Mechanisms in Polar Macroalgae

TESIS DOCTORAL

Concepción Íñiguez Moreno

