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**Growth and physiology of the green microalga, Coccomyxa subellipsoidea**

*a comparison of an Antarctic and temperate strain and the influence of temperature and irradiance*

# Growth and physiology of the green microalga, *Coccomyxa subellipsoidea*: a comparison of an Antarctic and temperate strain and the influence of temperature and irradiance

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# Abstract

*Coccomyxa* is a genus of unicellular green algae with a cosmopolitan distribution. Species of this genus have been suggested to be physiologically plastic due to their diverse range of lifestyles. The genome of an Antarctic isolate of *Coccomyxa subellipsoidea* was the first polar green alga to be sequenced and it became a model organism for cold tolerance because it was revealed to possess a range of genes associated with survival at low temperature. It has been proposed that *C. subellipsoidea* may be a potential candidate for biofuel generation due to the large quantity and diversity of lipids synthesised. However, knowledge of the growth, physiology and biochemistry of this species is scarce. The aim of this thesis was to investigate these aspects of *C. subellipsoidea* and to determine if latitudinally-separated strains are physiologically distinct. The growth, photosynthetic activity, pigment composition and rate of respiration were measured for two strains: a strain isolated from algal peat in the Antarctic and a strain isolated from a lichen in a temperate region. Both were grown across a range of temperatures and irradiances. The Antarctic strain was tolerant of low temperature and demonstrated eurythermal behaviour with remarkably consistent levels of growth, photosynthetic activity and respiration between 6 and 18 °C. However, the temperate strain was much more sensitive to temperature, with a significant impairment of growth and photosynthetic activity at 6 °C. The Antarctic strain may be relatively resilient to climate change, whereas the temperate strain could be more sensitive to climate change. These findings suggest that *C. subellipsoidea* is a plastic species which may be segregated into physiologically distinct populations, each uniquely adapted to their latitude of origin. The cold tolerance genes previously identified in the genome of the Antarctic strain may not be present or expressed to same extent in temperate strain. Future studies could employ molecular techniques to compare gene expression levels in these strains of *C. subellipsoidea*.

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# Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED: Amy Thorpe DATE: 16/12/2019

# Contents

# List of figures

# List of tables

# List of equations

# List of abbreviations

**3N-BBM+V:** Bold Basal medium with 3-fold nitrogen and vitamins

**Chl a:** chlorophyll a

**Chl b:** chlorophyll b

**DMF:** dimethylformamide

**$E_k$:** light saturation coefficient

**ETR:** electron transport rate

**$ETR_{mPot}$:** maximum potential electron transport rate

***F*:** fluorescence yield before saturation pulse

**$F_m$:** maximal fluorescence in the dark adapted state

**$F_m$':** maximal fluorescence in the light adapted state

**$F_o$:** minimal fluorescence in the dark adapted state

**$F_v/F_m$:** maximum quantum yield of photosystem II

**$F_v$:** variable fluorescence

**NPQ:** non-photochemical quenching

**OD:** optical density

**PAM:** pulse amplitude modulated fluorometry

**PAR:** photosynthetically active radiation

**PSII:** photosystem II

**rETR:** relative electron transport rate

**$rETR_m$:** relative maximum electron transport rate

**RLC:** rapid light curve

**ROS:** reactive oxygen species

**Y(II):** quantum yield of photochemical energy conversion

**Y(NO):** quantum yield of non-regulated non-photochemical quenching

**Y(NPQ):** quantum yield of regulated non-photochemical quenching

***α*:** light utilisation coefficient

# Chapter 1: General introduction

## 1.1. Overview

Many species of microalgae can survive in cold environments in the polar and alpine regions (Russell, 1990; Elster, 2002; Davey *et al*., 2019). In these environments, algae may be exposed to low and fluctuating temperatures and freeze-thaw cycles, desiccation and a high irradiance (Callaghan *et al*., 2004; Leya *et al*., 2009; Yallop *et al*., 2012). Microorganisms capable of surviving in cold conditions are collectively considered to be cryophiles (Morgan-Kiss *et al*., 2006). Cryophiles may be further classified as psychrophiles, which Morita (1975) defined as microorgansisms which have an optimal growth rate below 15 °C. Others may be psychrotolerant, which Morita (1975) defined as having an optimal growth rate at 20-25 °C, but also have the capacity to grow at lower temperatures. Mesophiles have an optimal growth temperature above 15 °C and below 45 °C but are unable to grow at lower temperatures (Castenholz, 1969; De Maayer *et al*., 2014). Psychrophilic and psychrotolerant algae have various adaptations to allow them to survive in their stressful environment which may be unique among polar and alpine populations (Hoham and Duval, 2001). Long-term isolation in the Antarctic may have allowed distinct strains to develop with unique adaptations to the extreme environment (Vincent, 2000). Ecophysiological comparisons between polar and temperate populations of algae are required to fully understand how processes of adaptation or acclimation to the climate have acted upon different populations (Lawley *et al*., 2004).

The green soil alga, *Coccomyxa subellipsoidea* (Schmidle, 1901), has a cosmopolitan distribution (Gustavs *et al*., 2016) with populations being found in temperate (Jaag, 1933) and polar (Pfaff *et al*., 2016) regions. *Coccomyxa* have diverse lifestyles, with free-living (Holm-Hansen 1964), lichen symbiont (Jaag, 1933) and parasitic life stages (Trémouillax-Guiller and Huss, 2007). A strain of *C. subellipsoidea* isolated from the Antarctic was previously identified

as *Chlorella vulgaris* due to the simple morphology of this genus. The Antarctic strain of *C. subellipsoidea* was the first polar green alga to be fully sequenced, revealing it to be a distinct species from *C. vulgaris*. Analysis of the genome also revealed that it possessed various adaptations associated with cold tolerance (Blanc *et al*., 2012). It has been proposed that *C. subellipsoidea*, along with other species in the genus *Coccomyxa*, may have a high degree of physiological plasticity with the potential to adapt to environmental change (Barcytė and Nedbalova, 2017). It had also been suggested that due to the high quantities of lipids produced by *Coccomyxa*, they may be ideal candidates to generate biofuels (Peng *et al*., 2016). However, research into the growth, physiology and environmental tolerance of this species is limited (Gustavs *et al*., 2017). Further research into the physiology and ecology of microalgae is becoming increasingly necessary with climate change because their distribution and community structure may be changing (Convey, 2011). Data on the physiology of cryophilic algae and how it is influenced by the climate can inform predictions on how they respond to climate change (Elster, 2002; Brown *et al*., 2016; Cvetkovska *et al*., 2017).

Chapter 1 of this thesis will introduce the terms commonly used to define the temperature preferences of algae. The diversity and physiology of algae that can be found in cold environments and their potential responses to climate change will be discussed. Finally, the study organism for this research, *C. subellipsoidea*, will be introduced and an overview of the immense diversity and biotechnological applications of the genus *Coccomyxa* will be provided.

## 1.2. Defining temperature preferences of microorganisms

The tolerances of species to a specific range of temperatures can serve as a useful tool to characterise the behaviour of different groups of algae (Butterwick *et al*., 2005). Cryophilic algae can be categorised into psychrophilic or psychrotolerant algae, depending on the temperature at which they achieve an optimal growth rate (Cvetkovska *et al*., 2017). Many

variations in the definition of these two terms exist, which differ in the precise temperature separating psychrophilic behaviour from psychrotolerant behaviour (Cavicchioli, 2016). Terms frequently used to describe the temperature preferences of microorganisms are defined in Table 1.1. For each term, there are variations in the temperatures at which growth can occur and assigning microorganisms into any one of these groups is difficult. For example, Morita (1975) and Gounot (1986) defined a psychrophile as being unable to grow above 20 °C, whereas Komárek and Nedbalová (2007) suggested psychrophiles do not grow above 10 °C. Some confusion arises as a number of definitions are based on growth rather than optimal growth. For example, Komárek and Nedbalová (2007) suggested that a microorganism that can grow between 10 and 25 °C should be described as psychrotolerant, but according to Morita (1975), microorganisms with an optimum growth temperature of 15 °C should be defined as psychrophilic. Terms based on the size of the temperature range microorganisms can grow at have also been introduced. Eurythermal species are capable of growth over a wide temperature range whereas growth of stenothermal species is restricted to a narrower temperature range (Lowe, 1974; Seaburg *et al*., 1981). Many of these definitions were introduced for bacteria (Morita, 1975; Jay, 1987; Zeikus, 1979; Moyer and Morita, 2001) but have since been applied to algae (Teoh *et al*., 2013; Cao *et al*., 2016; Lee *et al*., 2018) with the assumption that bacteria and algae would respond to temperature in the same way. Among these definitions, the bacterial-specific definition developed by Morita (1975) appears to be the most widely accepted and is commonly applied to all microorgansisms (Russell, 1998). However, irradiance is more important for growth for phototrophic organisms compared to heterotrophic bacteria as irradiance can be critical for growth of phototrophic organisms who lack alternative means of obtaining their energy resource. Irradiance has not been considered in these definitions and this may limit the suitability of these bacterial-specific terms for algae as irradiance levels may influence their optimum growth temperature.

Table 1.1. Definitions of terms associated with the growth temperature preferences of microorganisms.

| Term | Definition | Group of organisms | Reference |
|---|---|---|---|
| Cryophilic | Thrive at low temperatures | Microorganisms | Morita (1975) |
| Psychrophilic | Optimum growth temperature ≤ 15 °C, cannot grow ≥ 20 °C | Bacteria | Morita (1975) |
| | Can grow at 0 °C, but not ≥ 20 °C | Microorganisms | Gounot (1986) |
| | Live in permanently cold environments | Microorganisms | Russell (1990) |
| | Grow only at 0-10 °C | Algae | Komárek and Nedbalová (2007) |
| Psychrotolerant | Optimum growth temperature 20-25 °C, but can grow at lower temperatures | Bacteria | Morita (1975) |
| | Can grow at 0 °C and ≥ 20 °C | Microorganisms | Gounot (1986) |
| | Live in temporarily cold environments | Microorganisms | Russell (1990) |
| | Grow only at 10-25 °C | Algae | Komárek and Nedbalová (2007) |
| Psychrotrophic | Optimum growth at a moderate temperature (20 °C), but cannot grow ≥ 35 °C | Bacteria | Jay (1987) |
| | Optimum growth temperature ≥ 15 °C, but cannot grow ≥ 20 °C | Bacteria | Moyer and Morita (2001) |
| Mesophilic | Optimum growth temperature at 30-40 °C | Bacteria | Jay (1987) |
| | Optimum growth temperature ≥ 45 °C | Cyanobacteria | Castenholz (1969) |
| | Cannot grow at low temperatures and optimum growth temperature ≥ 15 °C | Microorganisms | De Maayer *et al*. (2014) |
| Thermophilic | Grow at extreme high temperatures and optimum growth temperature ≥ 60 °C | Bacteria | Zeikus (1979) |
| Eurythermal | Grow over a wide temperature range of ≥ 15 °C units | Diatoms | Lowe (1974) |
| | Grow over a broad temperature range of 20-30 °C units | Microorganisms | Seaburg *et al*. (1981) |
| Stenothermal | Grow over a temperature range of ≤ 5 °C | Diatoms | Lowe (1974) |
| | Grow over a narrow temperature range of ≤ 20 °C units | Microorganisms | Seaburg *et al*. (1981) |

## 1.3. Diversity and distribution of cryophilic algae

Cryophilic algae have a worldwide distribution, with species being described from North America (Wharton and Vinyard, 1983; Takeuchi, 2001), South America (Takeuchi *et al*., 2001b; Takeuchi and Kohshima, 2004), Asia (Segawa *et al*., 2005; Takeuchi *et al*., 2009), Africa (Duval *et al*., 1999), Australia (Marchant, 1982), Europe (Light and Belcher, 1968; Kvíderová, 2010), the Arctic (Kim *et al*., 2008; Harding *et al*., 2011) and Antarctica (Broady, 1996; Vinocur and Izaguirre, 1994). Algae have colonised multiple terrestrial habitats in these locations (Elster, 2002). Ice algae inhabit freshwater ice (Yallop *et al*., 2012), snow algae inhabit permanent and semi-permanent snowfields and cryophilic soil algae colonise soil and rock surfaces in cold climates such as those frequently covered by snow (Gounot, 1999; Remias *et al*., 2005; Frenette *et al*., 2008; Leya, 2013). Some species are specialists to each of these habitats whilst others may be generalists and can occupy multiple habitats (Elster, 2002). The community of algae in each of these habitats is influenced by the tolerances of each species to environmental conditions such as temperature and irradiance (Newton, 1982; Elster, 1999).

### 1.3.1. Ice algae

Ice algae can be found growing on glaciers such as those in Greenland (Williamson *et al*., 2018), Antarctica (Wharton *et al*., 1985) and the Himalayas (Takeuchi *et al*., 2001a). The ice environment is associated with a high irradiance, low temperature and limited nutrients (Yoshimura *et al*., 1997; Hoham and Duval, 2001). The ice algal community has a low diversity with a small number of specialist species (Remias *et al*., 2009; Remias *et al*., 2012; Yallop *et al*., 2012). *Cylindrocystis brebissonii*, *Ancylonema nordenskiöldii* and *Mesotaenium berggrenii* are green algae in Zygnematophyceae which are frequently found on ice and are considered to be glacial ice specialists (Kol, 1968; Williamson *et al*., 2019). Ice algae can tolerate relatively low temperatures (Stamenković and Hanelt, 2017). For example, *C. brebissonii* can survive

freeze-thaw cycles and has been defined as a psychrophile with an optimum growth temperature of 10 °C (Hoham, 1975; Morris *et al*., 1986).

### 1.3.2. Snow algae

Snow algae grow on the surface and upper layers of snow in the Arctic, Antarctic and alpine regions such as the Austrian Alps (Remias *et al*., 2005; Cook *et al*., 2017). The snow may cover glaciers, frozen lakes, rocks or soil (Elster, 2002). Similar to the ice environment, the snow environment is characterised by high irradiance, low temperature and limited nutrients (Yoshimura *et al*., 1997). However, in comparison to ice, the snow environment typically has a more changeable volume of meltwater and is less stable as the snow may not last the entire year (Holzinger *et al*., 2016; Cvetkovska *et al*., 2017). The majority of snow algae are freshwater species of green algae in the phylum Chlorophyta (Round, 1981; Hoham and Duval, 2001). Species commonly found in the snow include those in the classes Chlorophyceae, such as *Chlamydomonas nivalis* (Cd) and *Chloromonas nivalis* (Cr), and Trebouxiophyceae, such as *Raphidonema nivale* and *Raphidonema brevirostre* (Kol, 1942; Yoshimura *et al*., 1997; Takeuchi *et al*., 2006). Hoham (1975) defined snow algae as true snow algae if they complete their life cycle entirely within snow meltwater. True snow algae are psychrophiles with an optimum growth temperature below 10 °C (Hoham, 1975; Stibal and Elster, 2005). For example, *Cd. nivalis* and *Chlainomonas rubra*, both collected from snow in Washington, USA have some of the lowest optimum growth temperatures of the snow algae at 0-2 °C and 0-4 °C, respectively (Hoham, 1975). Some algae isolated from snow have a low optimum growth temperature but are able to survive over a wide temperature range (Hoham, 1989). For example, *R. nivale* has an optimum of 5 °C but has been found to grow between 0 and 20 °C (Hoham, 1975). Rather than being a true snow alga, *R. nivale*, and other species with wide temperature ranges, may be generalists which grow in polar soils but can grow in the snow environment if transported onto the surface by wind (Stibal and Elster, 2005).

### 1.3.3. Soil algae

Some species of cryophilic algae colonise polar and alpine soils where temperatures may be higher than in snow or ice, but they are still subjected to temperatures lower than that in temperate or low altitude soils (Campbell and Claridge, 1987). There is a high degree of variability in the environmental conditions of polar and alpine soils (Yergeau and Kowalchuk, 2008). They can experience significant temperature fluctuations diurnally and seasonally, be subject to frequent freeze-thaw cycles, be covered by snow which reduces light for prolonged periods of time or be exposed to direct sunlight (Johansen and Shubert, 2001; Karsten *et al*., 2010). Cryophilic soil algae can survive in soil crusts on the surface of soil and rocks which develop in habitats where moisture availability is low (Omelon *et al*., 2006; Warren *et al*., 2019). Soil crusts are aggregations of green algae, namely Chlorophyceae and Trebouxiophyceae, bound with soil particles by a mucilaginous film and a mixture of proteins and polysaccharides excreted as extracellular polymeric substances by some species of algae (Elster, 2002; Lawley *et al*., 2004; Borchhardt *et al*., 2017). These soil crusts may also associate with cyanobacteria, bacteria, fungi, mosses and lichens (Elster, 2002). The mucilage can accumulate nutrients and protect the microorganisms within it by reducing the risk of desiccation and freezing (Durrell and Shields, 1961; Elster, 2002). Shading by cells in the outer layers of soil crusts can protect cells within deeper layers from damaging levels of radiation and more photo-sensitive species may be arranged in these deeper layers (Karsten and Holzinger, 2014). Fragments of soil crusts are easily distributed by the wind and the species composition of Antarctic soil crusts share many similarities with those in the Arctic, suggesting bipolar dispersal of soil algae (Jungblut *et al*., 2012; Warren *et al*., 2019).

Cryophilic soil algae contribute significantly to the ecological functioning of polar and alpine regions (Chong *et al*., 2010). They are primary colonisers in succession of soils recently exposed by glacier retreat and snow melt (Elster and Svoboda, 1996). The soil algae break

down and bind soil particles which stabilises and helps to retain moisture in the poorly developed polar soils (Bailey *et al*., 1973; Davey and Rothery, 1993). Algal soil crusts and their mucilage protect the soil against wind and water erosion, and accumulate nutrients (Karsten and Holzinger, 2014; Borchhardt *et al*., 2017; Warren *et al*., 2019). Over time, the nutrient and moisture content of the soil increases, and plants and invertebrates can establish as the otherwise barren soil becomes more suitable for plant growth (Convey *et al*., 2008). Cryophilic soil algae can also grow on the surface and into the cracks of rocks. They accelerate the breakdown of these rocks and release inorganic mineral nutrients such as iron, manganese and phosphorous into the environment where they can be utilised by other organisms (Havig and Hamilton, 2019). As a result of their versatility to tolerate extreme and variable conditions, cryophilic soil algae are able to initiate succession and contribute to making polar and alpine environments more habitable, allowing other trophic levels to develop (Teoh *et al*., 2004; Kaštovská *et al*., 2005).

Chlorophytes have been reported to be the most diverse group of algae colonising alpine, Antarctic and Arctic soils with species such as *Prasiola crispa*, *Chlorella vulgaris* and *Stichococcus bacillaris* commonly being found (Broady, 1996; Cavacini, 2001; Kaštovská *et al*., 2005). Isolates of *P. crispa* has been shown to continue to photosynthesise following exposure to -15 °C, freeze-thaw cycles and periods of desiccation (Becker, 1982; Jacob *et al*., 1992; Jackson and Seppelt, 1995). Antarctic *C. vulgaris* has been shown to grow between 4 and 30 °C (Hu *et al*., 2008). Antarctic *S. bacillaris* also has a wide temperature range, growing between 4 and 25 °C (Chen *et al*., 2012). In addition to being psychrophiles and psychrotrophs due to their ability to grow at low temperatures, algae which can grow over a wide range of temperatures may also be defined as eurythermal (Teoh *et al*., 2004). Eurythermal algae such as *C. vulgaris* and *S. bacillaris* are not restricted to the polar regions but have also been isolated from low altitude temperate soils (Kvíderová and Lukavsky, 2005; Hu *et al*., 2008). Instead of

being a soil specialist, *C. vulgaris* may be a generalist as in addition to polar soils, it has been found growing on the snow surface suggesting its eurythermal behaviour enables it to colonise multiple habitats (Chong *et al*., 2011; Wong *et al*., 2015). The ability to tolerate a wide range of conditions and having a high degree of physiological plasticity may be key to successfully colonising the highly variable microclimate of polar and alpine soils (Chong *et al*., 2011; Hodač *et al*., 2016). Eurythermal behaviour enables the soil algae to become widespread across different habitats and climatic zones and could provide the soil algae with a competitive advantage over more specialist ice and snow algae under environmental change (Elster, 1999; Karsten *et al*., 2010).

## 1.4. Physiological responses to the environment

Rates of photosynthesis, respiration and growth of phototrophs are influenced by the climate (Ensminger e*t al*., 2006; Munir *et al*., 2015). Enzyme activity rates typically decline, and proteins can become denatured at low temperature (Collins and Margesin, 2019). Many species of cryophilic algae have evolved mechanisms which enable their cellular processes to continue under stressful levels of temperature and irradiance (Komarek and Nedbalova, 2007). For example, some species such as *Koliella* and *Stichococcus* isolated from the Antarctic possess cold active enzymes which differ in structure compared to the enzymes of mesophilic species, and these structural differences allow them to function at low temperatures when the activity of enzymes of mesophiles would slow down (Chen *et al*., 2012; Di Martino Rigano *et al*., 2006; Collins *et al*., 2008). Plasma membranes of cryophilic algal cells may also undergo structural changes to maintain integrity at low temperature, allowing the electron transport chain in photosynthesis and respiration to continue (Morgan-Kiss *et al*., 2006; Dolhi *et al*., 2013). Cryophilic algae may also synthesise cryoprotective proteins, such as ice nucleating agents and antifreeze proteins, in response to cold to protect against cellular freezing damage (Lyon and Mock, 2014; Collins and Margesin, 2019).

### 1.4.1. Photosynthesis and photoinhibition

Photoinhibition, which is a down-regulation of photosynthetic capacity as a result of excessive light energy absorption, can be induced when low temperatures are combined with high irradiance (Ensminger *et al*., 2006). Algae inhabiting polar and alpine habitats are vulnerable to photoinhibition (Bidigare *et al*., 1993). Light energy may be in excess when low temperatures reduce the rate of enzyme activity and electron transport, resulting in an imbalance between light energy absorption and use (Ensminger *et al*., 2006; Hüner *et al*., 2012). Absorbed light energy which is not used in photosynthesis can lead to the generation of reactive oxygen species (ROS) which may inactivate photosystems and proteins and increase mutation rates by attacking DNA (Halliwell, 1987; Neidhardt *et al*., 1998; Mallick and Mohn, 2000).

Many species of algae have various mechanisms which enable them to survive in typically stressful environments by avoiding or reducing photoinhibitory damage (Hoham and Duval, 2001). For example, antioxidant enzymes such as superoxide dismutase can deactivate ROS to reduce the risk of photooxidative damage (Mallick and Mohn, 2000). Algae may employ a process known as regulated non-photochemical quenching (NPQ) where excess light energy is diverted away from chloroplasts to reduce the risk of photoinhibition and the associated damage (Hüner *et al*., 2012). Overexcitation of the photosystems by this excess energy can be prevented and generation of ROS can be reduced (Ensminger *et al*., 2006). The process of NPQ does not require *de novo* protein synthesis so is a rapid and reversible response to a fluctuating light environment (Hüner *et al*., 2012). Having the ability to rapidly balance light absorption with energy utilisation is essential for survival in the polar regions where there can be significant fluctuations in light and temperature on a diurnal scale (Pérez-Torres *et al*., 2004). Flexibility in photosynthesis is also important depending on the microhabitat. Algae may temporarily be covered by snow or suddenly exposed to a higher irradiance as the snow melts

(Rivas *et al*., 2016). Shading by other cells in biofilms and soil crusts can also cause variations in the light environment (Karsten and Holzinger, 2014).

There is a more gradual change in irradiance in the polar regions as the photoperiod changes from 24 hours of light in the summer to 24 hours of darkness in the winter (Larose *et al*., 2013). Algae must be able to respond to this change to protect their photosynthetic apparatus from high irradiance in the summer and optimise the use of limited irradiance towards the winter (La Rocca *et al*., 2015). Algae can do this by adjusting concentrations of primary pigments in the light harvesting complexes of the photosystems (Neidhardt *et al*., 1998). In response to high irradiance, the concentration of light harvesting pigments can be reduced to limit light absorption and therefore reduce the risk of photoinhibition. When light availability is limited, an increase in light harvesting pigments can increase light absorption to maintain photosynthesis (Wilhelm and Wild, 1984; Smith *et al*., 1990; MacIntyre *et al*., 2002). Modifying pigment concentrations requires regulation of gene expression and protein synthesis so is a slower process compared to NPQ (Hüner *et al*., 2012).

Some species of algae may use secondary pigments as a form of photoprotection (Rivas *et al*., 2016). Unlike primary pigments, secondary pigments are not used directly in the light harvesting stage of photosynthesis but instead function in photoprotection (Bidigare *et al*., 1993). Astaxanthin is a secondary carotenoid produced by many species of snow algae, such as *Cd. nivalis* (Anesio *et al*., 2017). The pigment surrounds and shelters chloroplasts from high irradiances by absorbing a proportion of the incident light and dissipating it as heat. By diverting this energy away from chloroplasts, the risk of photoinhibition and the associated photodamage can be reduced (Remias *et al*., 2016). Ice algae lack astaxanthin but instead achieve photoprotection from a secondary phenol, purpurogallin (Remias *et al*., 2012; Yallop *et al*., 2012; Remias *et al*., 2016). Production of these pigments can be up-regulated in response to exposure to high irradiance (Leya, 2013). Snow algae may develop into non-flagellated

spores, known as hypnoblasts (Holzinger *et al*., 2016) during the summer when irradiance is high. During this stage of their lifecycle, secondary carotenoids are produced to maximise shading of the chloroplasts (Remias *et al*., 2005). The secondary carotenoids may act as a carbon source to allow metabolism to continue when photosynthesis cannot be maintained (Remias *et al*., 2016). Some species modify their cell wall composition in response to stress (Elster and Benson, 2004). Synthesis of the biopolymer, sporopollenin, and the group of secondary metabolites known as mycosporine-like amino acids may be up-regulated to shade cells from high irradiance and ultraviolet radiation (Xiong *et al*., 1997; Holzinger and Pichrtova, 2016). These compounds are produced by several members of *Chlamydomonas* and *Prasiola* in response to high radiation (Weiss, 1983; Blokker *et al*., 1999; Gorton and Vogelmann, 2003; Karsten *et al*., 2005) and sporopollenin is thought to exist in the membrane of *Coccomyxa* (Honegger and Brunner, 1981).

### 1.4.2. Adaptation vs acclimation

Cryophilic algae may acquire tolerance to environmental conditions by adaptation or acclimation (Elster, 1999). Adaptation is a genetically determined response to the environment which occurs across multiple generations. Acclimation is a phenotypic change induced by environmental fluctuations which is often shorter term and arises during the lifetime of an individual (Eggert and Wiencke, 2000; Morgan-Kiss *et al*., 2006). Cold active enzymes and the ability to synthesise certain cryoprotectants are usually adaptive, with changes being made to gene sequences on an evolutionary time scale. Activation of NPQ, changes in pigment composition and the production rates of cryoprotectants are more likely to be brought about by acclimation (Russell, 1990). Acclimation is often regulated by changes in gene expression (Valledor *et al*., 2013). Upon detection of environmental change, certain genes may be up or down regulated to improve tolerance to the new conditions (Guy, 1990). With declining temperatures, cryophilic algae may upregulate gene expression of cryoprotectant proteins

whilst downregulating processes that generate ROS (Chong *et al*., 2011; Liu *et al*., 2012; Collins and Margesin, 2019). However, the distinction between adaptation and acclimation is often unclear (Raven and Geider, 2003). In more specialist psychrophilic species, genes for cold tolerance may be constitutively expressed, whereas in psychrotolerant species, expression may be transient (Raven and Geider, 2003; Casanueva *et al*., 2010). For example, it has been documented that an Antarctic strain of *Chlorella* showed a high degree of cold tolerance without prior exposure to low temperatures, whereas temperate strains required a pre-acclimatory period of low temperature to show a cold tolerant phenotype and the accompanying changes in gene expression (Hu *et al*., 2008). The Antarctic strain may therefore have adapted to the extreme environment of Antarctica by achieving constitutive cold tolerance through changes in gene sequences rather than inducible changes in gene expression (Hwang *et al*., 2008; Hu *et al*., 2008; Whitehead, 2011).

## 1.5. Climate change and cryophilic algae

### 1.5.1. Climate change

The average global temperature has increased by 0.8 °C since 1880 (Hansen *et al*., 2010). Some of the most significant consequences of climate change are being seen in the Arctic and Antarctic, an effect known as polar amplification. Temperatures have increased in the polar regions by at least 1.5 °C since the 1950s (Vaughan *et al*., 2003). The Antarctic Peninsula has shown the greatest temperature change, with an increase of 3.7 °C per century. With higher rates of melt, snow and ice cover are declining, melting earlier in the spring and many snowfields that once persisted throughout the year are now seasonal and only appear during the winter or have disappeared completely (Vaughan *et al*., 2003). Models suggest that by the end of the 21$^{st}$ century there will be three times as much exposed ground in Antarctica and sub-Antarctic islands could become completely ice-free in the summer (Lee *et al*., 2017).

### 1.5.2. Ecological implications for cryophilic algae

Climate change is likely to have varied consequences for Antarctic ecosystems (Parmesan, 2006). Temperatures in Antarctica are close to the lower threshold for biological activity. A small change in temperature can mean the difference between inactivity and activity so can have significant ecological implications compared to lower latitudes (Convey, 2001). Higher temperatures, earlier and faster spring melt and more frequent rainfall can all extend the growing season and alleviate the extreme climate (Frenot *et al*., 2005; Hennion *et al*., 2006). These changes could have a positive impact on algae, enabling higher rates of productivity, faster growth rates and net population growth (Teoh *et al*., 2004; Convey, 2011). However, climate change may also have negative impacts on algae because higher temperatures and earlier spring melt may result in the supply of liquid water being exhausted sooner and increase the risk of desiccation later in the season (Convey, 2001). Snow and ice habitats may be reduced or lost completely in some areas, and temperatures could exceed the thermal limits for some species (Convey, 2011). Soil algae may benefit from glacial retreat and faster melt rates which expose more bare ground for colonisation whereas snow and ice algae may suffer from habitat loss (Hall and Walton, 1992; Marshall and Chalmers, 1997; Davey *et al*., 2019). Psychrophiles may be unable to tolerate a rise in temperature, but eurythermal algae, which are more likely to cope with temperature change, could grow faster (Teoh *et al*., 2004; Larose *et al*., 2013). They may out compete and cause local extinction of the psychrophilic snow and ice algae (Convey, 2011; Yergeau *et al*., 2012).

Distributional ranges of algae could shift in under climate change (Wall, 2005). Alleviation of the extreme climate may enable many species of algae to expand their ranges as more habitats become suitable for colonisation (Convey, 2011). An expansion in distribution towards the poles has already been observed in several macroalgae species and has been associated with a warming climate (Lima *et al*., 2007). Higher temperatures could enable mesophilic algae,

which may disperse in the wind or be carried by birds and humans from temperate latitudes, to establish an Antarctic population (Pearce *et al*., 2010, Duffy and Lee, 2019). Climate change may favour the growth of these invasive mesophiles which could out-compete indigenous psychrophiles and psychrotrophs if the latter groups are unable to adapt their physiology to tolerate an increase in temperature (Cowan *et al*., 2011). Populations of algae with lower temperature requirements may disappear along the Antarctic Peninsula where temperatures could increase beyond their physiological limits. These populations may experience a southward shift in their distribution as they may survive at higher latitudes where snow and ice habitats are more likely to persist (Walther *et al*., 2002; Rindi, 2011). Many habitats at these higher latitudes may have been uninhabited before the onset of climate change but colonisation and initiation of succession by algae may increase the biodiversity (Lewis-Smith, 1990; Wynn-Williams, 1996). The probability of successful dispersal to these new latitudes may be improved with more extensive snow and ice melt because distances between ice-free patches are reduced, increasing the connectivity between populations. However, increased gene flow between these once isolated populations could lead to a loss of genetic diversity (Lee *et al*., 2017). Homogenisation of sub-populations of algae has already been observed in *P. crispa* which dispersed south from Signy Island to Marguerite Bay, resulting in a loss of the genetically distinct Marguerite Bay population (Hughes and Convey, 2010). The ecological implications of climate change for algae are poorly understood and further research into their ecology, physiological limits and biogeography is necessary to identify which species may be vulnerable to climate change and what impact distributional changes may impose on Antarctic ecosystems (Litchman, 2010; Duffy and Lee, 2019).

## 1.6. *Coccomyxa*

### 1.6.1. Characteristics of the genus *Coccomyxa*

*Coccomyxa* (Schmidle, 1901) is a genus of Chlorophyte in the class Trebouxiophyceae (Honegger and Brunner, 1981). Species within this genus are small (3-9 μm long), unicellular and non-motile with a single parietal chloroplast (Honegger and Brunner, 1981; Peng *et al*., 2016). The genus was originally defined by morphology, such as an ellipsoidal or spherical shape and the production of mucilage (Komarek and Fott, 1983; Gustavs *et al*., 2017). However, the small and simple morphology of *Coccomyxa* with no clear defining characteristics and features which are highly variable depending on culture conditions, has led to many misidentifications (Darienko *et al*., 2010; Malavasi *et al*., 2016; Maltsev *et al*., 2019). For example, *C. subellipsoidea* was previously classified as *Chlorella* until analysis of the genome revealed it to be a distinct species (Blanc *et al*., 2012). Molecular data has since been used to divide the genus into 7 separate species (Darienko *et al*., 2010). Building upon this phylogeny, ecological and morphological data were combined with molecular data and collectively, this expanded the genus from 7 to 27 species segregated based on their ecology and behaviour (Malavasi *et al*., 2016).

Species in the genus *Coccomyxa* have a worldwide distribution. They colonise freshwater, marine and terrestrial habitats and can be found free-living, in symbiotic associations with lichens or as parasites (Zoller and Lutzoni, 2003; Belzile and Gosselin, 2015; Cao *et al*., 2018). Many species of *Coccomyxa* are extremophiles and are capable of tolerating conditions usually considered to be unfavourable for the growth of most Chlorophytes (Malavasi *et al*., 2016). Table 1.2. summarises a range of *Coccomyxa* species to illustrate their diversity of lifestyles.

Table 1.2. Selection of *Coccomyxa* species and summary of their locality and characteristics. The strain number is given if the isolate has been deposited in a culture collection. Strains used for the current research are in bold.

| **Species of *Coccomyxa*** | **Culture collection number** | **Habitat and Location** | **Characteristic** | **Reference** |
|---|---|---|---|---|
| *C. actinabiotis* | CCAP 216/25 | Nuclear reactor water pool, Grenoble, France | Tolerance of high levels of ionising radiation | Rivasseau *et al*. (2013) |
| | | | Heavy metal tolerance | Leonardo *et al*. (2015) |
| *C. astericola* | N/A | Starfish tissue, Rongesundet, Norway | Parasite of marine invertebrate | Mortensen and Rosenvinge (1933) |
| *C. elongata* | N/A | Hromnické Lake, Hromnice, Czech Republic | Acidophile | Barcytė and Nedbalova (2017) |
| *C. fottii* | CCALA 428 | Thermal spring, Piešťany, Slovakia | Thermophile | Barcytė and Nedbalova (2017) |
| *C. greatwallensis* | FACHB-2139 | Lichen (*Psoroma hypnorum*), King George Island, Antarctica | Lichen symbiont | Cao *et al*. (2018) |
| *C. melkonianii* | SCCA048 | Rio Irvi river, Sardinia, Italy | Heavy metal tolerance | Malavasi *et al*. (2016) |
| *C. onubensis* | SAG 2510 | Tinto river, Huelva, Spain | Heavy metal tolerance | Vaquero *et al*. (2012) |
| | | | Acidophile, halophile | Fuentes *et al*. (2016) |
| *C. parasitica* | CCAP 216/18 | Scallops, Newfoundland, Canada | Parasite of marine invertebrate | Stevenson and South (1974) |
| *C. peltigerae* | SAG 216-5 | Lichen (*Peltigera aphtosa*), Switzerland | Lichen symbiont | Jaag (1933) |
| ***C. subellipsoidea*** | **SAG 216-13** | **Lichen (*Lichenomphalia umbelifera*), Innsbruck, Austria** | **Lichen symbiont** | **Jaag (1933)** |
| | **C-169** | **Dried algal peat, Victoria Land, Antarctica** | **Cold tolerance** | **Blanc *et al*. (2012)** |
| *Coccomyxa* sp. | N/A | *Ginkgo biloba* root, leaf and embryo, Asia, North America and Europe | Plant parasite | Trémouillax-Guiller and Huss (2007) |

### 1.6.2. *Coccomyxa subellipsoidea*

Blanc *et al*. (2012) sequenced the genome of *C. subellipsoidea* (C-169). It is the only polar green alga with a sequenced genome and has been described as a model organism for cold adaptation (Blanc *et al*., 2012; Pfaff *et al*., 2016). Previously, the only fully sequenced genome belonging to a species closely related to many cryophilic algae was the mesophilic Chlorophyte, *Chlamydomonas reinhardii* (Lutz *et al*., 2015). Analysis of the genome of the Antarctic isolate of *C. subellipsoidea* by Blanc *et al*. (2012) revealed several features associated with survival at low temperatures, suggesting that it has adapted to the cold climate of Antarctica (Hopes *et al*., 2017). For example, this strain was found to possess three fatty acid desaturase genes which are not found in other Chlorophytes. Fatty acid desaturases help to maintain structural integrity of membranes at low temperature (Collins and Margesin, 2019). Blanc *et al*. (2012) also identified genes encoding proteins which help to prevent deleterious protein aggregation and stabilise enzymes at low temperature (Hopes *et al*., 2007). It is not known whether other strains of *C. subellipsoidea* share the same traits of cold tolerance identified in the genome of C-169, or if this strain has adapted to the extreme environment of Antarctica in isolation from other geographically distinct populations (Pfaff *et al*., 2016). A comparison of the physiology of *C. subellipsoidea* strains isolated from different climatic regions may help to better understand how the physiology of cryophilic algae is influenced by their climate and when combined with molecular data, could reveal if an Antarctic subclade of *C. subellipsoidea* exists (Pfaff *et al*., 2016). Gustavs *et al*. (2017) identified a need for further research into *Coccomyxa* metabolism to improve our understanding of its physiological plasticity which could help to explain the mechanisms underpinning their worldwide distribution. Furthermore, a comprehensive knowledge of the physiological plasticity of cryophilic algae can also help to predict the extent to which their population and distribution may be influenced by climate change in the future (Davey *et al*., 2019).

### 1.6.3. *Coccomyxa* in biotechnology

Exploiting the lipids synthesised by algae for biofuel production has been proposed as a potential alternative to non-renewable, polluting fossil fuels (Nicolo *et al*., 2010; Allen *et al*., 2017). Mesophilic algae have previously been used to produce biofuels but their high optimum temperature for lipid production increases the energy cost of incubation (Barnard *et al*., 2010). For example, strains of *C. reinhardtii* which have commonly been used have an optimum of 32 °C (James *et al*., 2012). The ability of cryophilic algae to grow at low temperatures whilst still producing large quantities of lipids make them attractive candidates for biofuel production (Varshney *et al*., 2015).

Species in the genus *Coccomyxa* have been proposed as ideal candidates for biofuel production (Peng *et al*., 2016; Liu *et al*., 2018). Several studies have suggested that members such as *C. subellipsoidea* have fragile cell walls, allowing for easy extraction of lipids for biofuels (Peng *et al*., 2016; Yu *et al*., 2018; Ajayan *et al*., 2019; Wang *et al*., 2019; Yang *et al*., 2019). Several species, particularly *C. subellipsoidea*, have been reported to produce large quantities of lipids under a wide range of culture conditions (Ruiz-Domínguez *et al*., 2015; Řezanka *et al*., 2019; Wang *et al*., 2019). *Coccomyxa* produce omega-3 polyunsaturated fatty acids in a higher abundance than many other microalgae (Lang *et al*., 2011; Maltsev *et al*., 2019). These fatty acids could serve as a useful source for pharmacology and food supplements (Ryckebosch *et al*., 2012). Furthermore, *C. subellipsoidea* has been shown to grow heterotrophically on an organic carbon source so cultivation costs could be reduced by using organic waste as a nutrient source (Zajic and Chiu, 1970; Perez-Garcia *et al*., 2011; Wang *et al*., 2019). The cold tolerance of Antarctic *C. subellipsoidea* may enable biofuel production to take place in colder climates where the land is less suitable for agriculture, heating costs of incubators can be reduced and the low temperatures can reduce the risk of contamination by other microbes (Msanne *et al*., 2012; Allen *et al*, 2017; Soru *et al*., 2018). However, some findings challenge the applicability

of *C. subellipsoidea* in biotechnology. Microscopic analysis of the structure of the cell wall of *Coccomyxa* suggests it is composed of three layers, with an inner layer formed of an amorphous material of variable thickness, an intermediate layer of a matrix of cellulous microfibrils and an outer layer consisting of a trilaminar sheath, potentially associated with resistant polymers such as sporopollenin (Honegger and Brunner, 1981; Baudelet *et al*., 2017; Muggia *et al*., 2018). The cell wall may therefore be more resistant to degradation than previously assumed and the energy input required to extract the lipids may be higher compared to species with weaker cell walls which lack these resistant polymers (Dunker and Wilhelm, 2018). Further research into the physiology of biofuel candidates such as *Coccomyxa* is necessary to allow the most suitable species and the most productive strain to be selected and to optimise the culturing conditions for growth (Maltsev *et al*., 2019).

## 1.7. Aim and thesis outline

The aim of this thesis is to explore the physiological responses of *C. subellipsoidea* to temperature and irradiance to determine whether latitudinally-separated populations show distinct responses to these factors. To achieve this, the growth rates of these strains of *C. subellipsoidea* will be compared in Chapter 2. In Chapter 3, the physiology and biochemistry of the Antarctic and temperate strain will be investigated by assessing the photosynthetic activity, pigment composition and rate of respiration in response to temperature and irradiance. Finally, Chapter 4 brings together the conclusions from each chapter and places the findings in the wider context of climate change and potential for use of *C. subellipsoidea* in biotechnology.

# Chapter 2: A comparison of the growth rates of an Antarctic and temperate strain of *Coccomyxa subellipsoidea* at low temperatures

## 2.1. Introduction

### 2.1.1. Biogeographic differences in optimum growth conditions

Some researchers have suggested that many species of algae have adapted or can acclimate to optimise their physiology to the conditions typically encountered in their habitat (Kvíderová and Lukavský, 2005). Their capacity to photosynthesise and grow may be limited to the range of temperatures and irradiance levels associated with their habitat, and deviations from these conditions may result in an impairment of photosynthesis and growth (Stamenković and Hanelt, 2017). It has been argued that the geographical distribution of algae may therefore be restricted by their tolerance range, with psychrophilic species potentially being limited to permanently cold environments (Russell, 1990; Teoh *et al*., 2013). However, other researchers argue that populations of algae may be more phenotypically plastic compared to others. Those with a greater capacity for phenotypic plasticity may acclimate to environmental conditions, enabling them to cope with temperature and irradiance fluctuations, and this could provide them with the ability to tolerate a wider range of conditions (Elster, 1999). In comparison to adaptation, this acclimation response is a shorter term reaction to fluctuating environmental conditions which involves phenotypic changes as opposed to genetic changes (Morgan-Kiss *et al*., 2006). Species with a capacity for acclimation may be able to respond rapidly to environmental change and optimise their physiology to the present conditions to which they are exposed, not only to the conditions experienced throughout their evolutionary history (Elster, 1999; Eggert and Wiencke, 2000).

Latitudinal-specific differences in optimum growth temperature have been observed in different species of the green algae, *Chlamydomonas* (Teoh *et al*., 2013) and *Chlorella* (Lee *et al*., 2018) isolated from polar, temperate and tropical regions. The polar species exhibited lower optimum growth temperatures when compared to the temperate and tropical species within the same genus (Teoh *et al*., 2013; Lee *et al*., 2018). Evidence suggests that these patterns in optimal growth conditions depending on the latitude of origin of each species may also arise between strains within the same species (Claquin *et al*., 2008; Barati *et al*., 2018), such as between polar and temperate strains of *Chlorella pyrenoidosa* (Cao *et al*., 2016) and *Stichococcus bacillaris* (Kvíderová and Lukavský, 2005; Chen *et al*., 2012). These studies have been based on strains sourced from culture collections, so they may have been exposed to conditions differing from their origin for several years (Kvíderová and Lukavský, 2005; Teoh *et al*., 2013; Cao *et al*., 2016; Lee *et al*., 2018). Despite this, preferences for conditions reflecting those typical for their original latitude have been maintained over time in culture collections or through cryopreservation, suggesting adaptations to their original habitat have persisted over time (Stamenković and Hanelt, 2017).

Polar strains of the green algae *Cosmarium crenatum* var. *boldtianum* and *Cosmarium punctulatum* var. *subpunctulatum* appear to display an optimisation of photosynthesis and growth at a higher irradiance compared to their temperate counterparts, a pattern which correlates with the higher irradiance experienced by the polar strains at high latitudes and in habitats surrounded by reflective snow and ice (Stamenković and Hanelt, 2013). However, unlike these polar *Cosmarium* strains, a polar strain of *S. bacillaris* did not show an optimisation of growth at a higher irradiance compared to a temperate strain (Kvíderová and Lukavský, 2005). Stamenković and Hanelt (2017) suggested that rather than responding to the typical irradiance experienced at the surface at a given latitude, strains may show preference for the irradiance experienced in their microhabitat, such as a low irradiance within soil crusts

and a higher irradiance in the upper layers of a water body. However, research investigating the relationship between irradiance and growth for strains originating from different latitudes is limited and has been less intensively studied compared to the influence of temperature on growth (Kvíderová and Lukavský, 2005).

### 2.1.2. Growth optima of *Coccomyxa subellipsoidea*

Blanc *et al*. (2012) published the first complete genome of an alga isolated from a polar environment, the green alga *Coccomyxa subellipsoidea* (C-169), identifying the presence of a range of cold tolerance mechanisms in the genome. Since 2012, a few researchers have adopted this strain as a model organism to better understand the metabolic and gene regulation pathways involved in lipid synthesis (Allen *et al*., 2015; Allen *et al*., 2017; Yu *et al*., 2018). Others have manipulated the growth conditions, such as the nutrient source (Msanne *et al*., 2012; Tevatia *et al*., 2014; Liu *et al*., 2018) and the concentration of $CO_2$ (Peng *et al*., 2016) to optimise lipid production for use in biotechnology. A review of recent literature demonstrates that this polar strain is capable of growing at relatively high temperatures, between 25 and 30 °C (Msanne *et al*., 2012; Tevatia *et al*., 2014; Allen *et al*., 2015; Pfaff *et al*., 2016; Allen *et al*., 2017; Liu *et al*., 2018; Yu *et al*., 2018). Growth at these temperatures would not be expected for an organism that was described as cryophilic (Blanc *et al*., 2012; Pfaff *et al*., 2016; and Table 1.1. in this thesis). As a model species for cold tolerance, *C. subellipsoidea* offers an excellent opportunity to address questions relating to temperature tolerance. Only a limited number of studies have been performed to understand the physiological responses of *C. subellipsoidea* to temperature. Pfaff *et al*. (2016) identified 12.9 °C as the optimum growth temperature for this strain, suggesting it may be psychrophilic according to Morita (1975) because the optimum growth temperature was below 15 °C. However, Blanc *et al*. (2012) reported the optimum growth temperature of this strain was 20 °C but provided no detail about the growth rate or growth conditions.

The irradiance levels that have been used for culturing Antarctic *C. subellipsoidea* in previous studies are varied. Although many studies have used an irradiance of 60 μmol photons $m^{-2}$ $s^{-1}$ (Peng *et al*., 2016; Allen *et al*., 2017), some have grown this strain of *C. subellipsoidea* at 30 μmol photons $m^{-2}$ $s^{-1}$ (Kováčik *et al*., 2015; Pfaff *et al*., 2016) whilst other studies have grown cultures at 200 μmol photons $m^{-2}$ $s^{-1}$ (Tevatia *et al*., 2014). The ability of the Antarctic strain of *C. subellipsoidea* to grow over this range suggests this strain can tolerate a wide range of irradiance levels as well as temperatures. However, no study appears to have been dedicated to characterising the growth rate in response to both irradiance and temperature for any strain of *C. subellipsoidea*. Physiological data is particularly scarce for the lichen symbiont strain of *C. subellipsoidea* (SAG 216-13) isolated from a temperate region (Pfaff *et al*., 2016; Gustavs *et al*., 2017), and a comparison of the responses of the non-lichen symbiont Antarctic strain with that of the lichen symbiont temperate strain could enable questions to be addressed regarding phenotypic plasticity within this species.

Knowledge of the growth responses of algae to environmental conditions in relation to the climatic region strains originated from is required to understand the extent to which they have adapted or acclimated to their habitat, whether their tolerance range may restrict their distribution, and how they may be influenced by climate change, both in their abundance and distribution (Teoh *et al*., 2013). A comparison of the growth responses between isolates from different latitudes can help to determine whether geographical isolation of populations has segregated a cosmopolitan species into locally adapted populations (Stamenković and Hanelt, 2017). Information on growth rates under different temperatures is particularly relevant in the context of climate warming to understand which groups may currently be surviving close to the limits of their thermal tolerances, and are therefore vulnerable to climate change, compared to those which have a wide tolerance range, and therefore a greater capacity to cope with temperature change (Somero, 2010).

### 2.1.3. Aim and hypotheses

The aim of this chapter is to characterise the growth behaviour of an Antarctic strain of *C. subellipsoidea* and confirm if the two strains can be separated according to their growth responses to temperature. Based on previous research on strains of different species of green algae spread across a range of latitudes (Kvíderová and Lukavský, 2005; Stamenković and Hanelt, 2013), it was hypothesised that:

(i) the Antarctic strain of *C. subellipsoidea* would have an optimum growth temperature at 10 °C, making it a psychrophile, and would maintain a higher growth rate at a lower temperature than the temperate strain, due to the relatively lower long-term temperatures experienced in the Antarctic,

(ii) the temperate strain of *C. subellipsoidea* would show a growth response more closely matching the definitions for a psychrotolerant organism with an optimum growth temperature at 18 °C, and would have a higher growth rate at a higher temperature than the Antarctic isolate, due to the relatively higher temperatures typically experienced in temperate regions,

(iii) the Antarctic strain of *C. subellipsoidea* would show a greater rate of growth at a higher irradiance at each temperature compared to the temperate strain due to the higher irradiance typically experienced in the polar regions (Stamenković and Hanelt, 2013).

## 2.2. Methods

### 2.2.1. Strains and culture conditions

Growth responses of an Antarctic strain of *C. subellipsoidea* were compared against those of a temperate strain of *C. subellipsoidea*. The Antarctic strain of *C. subellipsoidea* was originally isolated from dried algal peat in Marble Point in Victoria Land, Antarctica (Holm-Hansen, 1964) and was supplied by the National Institute for Environmental Studies Microbial Culture

Collection, Japan, under strain number C-169. The temperate strain of *C. subellipsoidea* was originally isolated from a lichen in Innsbruck, Austria (Jaag, 1933) and was supplied by the Culture Collection of Algae and Protozoa, United Kingdom, under strain number SAG 216-13.

Stock cultures of each strain were maintained in 25 ml Bold Basal medium with 3-fold nitrogen and vitamins (3N-BBM+V) (Culture Collection of Algae and Protozoa, Scotland). The concentration of each element in 3N-BBM+V is given in Table 2.2. The 3N-BBM+V had a pH of 6.3-6.5. Cultures were kept in autoclaved 50 ml Pyrex Erlenmeyer flasks with a cotton wool plug. Preliminary experiments were undertaken to determine if each isolate could grow over the range of temperatures selected. The cultures were initially acclimated for a minimum of 30 days (Chen *et al*., 2012) at the experimental temperature of 6, 10 or 18 °C in temperature controlled cabinets (LMS, Kent, England). The range of temperatures were chosen to potentially allow separation of psychrotolerant growth, which may be greater at 18 °C, and psychrophilic growth, which may be higher at a lower temperature, such as 6 °C. The irradiance during this acclimation period was 40 μmol photons $m^{-2}$ $s^{-1}$ on a light: dark cycle of 12:12 hours. The irradiance level was measured using a spherical light probe (US-SQS/L, Spherical Micro Quantum Sensor, Walz, Germany) connected to a light meter (ULM-500, Universal Light Meter and Data Logger, Walz, Germany). Every 7-14 days throughout the acclimation period, cultures were centrifuged (5810 R, Eppendorf, Germany) at 1500 rpm for 10 minutes at the growth temperature to remove exudates and old media. A subset of cells was resuspended in fresh 3N-BBM+V in autoclaved 50 ml flasks. The procedure maintained the algae in a semi-continuous state so that cells could be harvested in exponential phase for experiments.

Table 2.2. Elemental composition of 3N-BBM+V medium.

| Element | Concentration (mg $l^{-1}$) |
|---|---|
| Oxygen | 626.62 |
| Sodium | 212.84 |
| Nitrogen | 124.49 |
| Potassium | 116.50 |
| Chlorine | 27.36 |
| Phosphorous | 10.52 |
| Sulfur | 9.89 |
| Hydrogen | 9.67 |
| Magnesium | 7.50 |
| Calcium | 6.75 |
| Carbon | 2.78 |
| Iron | 0.12 |
| Manganese | 0.07 |
| Cobalt | 0.04 |
| Zinc | 0.01 |
| Molybdenum | 0.01 |

### 2.2.2. Experimental conditions

For each strain, cells were harvested and spun at 1500 rpm for 10 minutes at the growth temperature at which they had been acclimated (6, 10 or 18 °C) in an Eppendorf centrifuge. Exudates and old 3N-BBM+V were removed, and a subset of the cells were used to inoculate 600 ml of fresh 3N-BBM+V with an initial cell concentration of $1.5 \times 10^5$ cells $ml^{-1}$. Cell counts with a haemocytometer (Mod-Fuchs Rosenthal, Hawksley, England) were performed to calculate the inoculation volume required to achieve this initial concentration. The 600 ml culture was divided between twelve 150 ml Pyrex Erlenmeyer flasks each containing 50 ml of the culture. The flasks were then placed in temperature controlled growth chambers (Micro Clima Series Economic Lux Chambers, Snijders Labs, Netherlands) at either 6 ± 0.3, 10 ± 0.3

or 18 ± 0.3 °C. At the end of the 10 °C experimental period, the temperature in this Micro Clima cabinet was adjusted to 18 °C. The experiment at 6 °C was performed in a separate Micro Clima cabinet.

The experimental irradiance differed from the initial culturing irradiance. The experimental irradiance levels were constant over a 24-hour period and were supplied by Cool White fluorescent linear lights (F36 W/T8, BriteGro, Sylvania, Massachusetts). The lights were arranged vertically across the left and right sides of the growth chambers behind thermal glass. Four culture flasks of each set were positioned on three different shelves at an irradiance of 30, 60 or 100 μmol photons $m^{-2}$ $s^{-1}$. The irradiance range was chosen to reflect the higher irradiance that may be received on the surface, and the lower irradiance that may be experienced in a shaded habitat, such as within lichen or algal peat (Nash, 2008; Gustavs *et al*., 2017). Neutral density filters were used to vary the irradiance levels (0.6 ND 210, LEE filters, Andover, England). The highest average irradiance of 100 ± 2.6 μmol photons $m^{-2}$ $s^{-1}$ was achieved without any neutral density filters. To achieve an average irradiance of 60 ± 2.1 μmol photons $m^{-2}$ $s^{-1}$, one layer of the filter was positioned over the glass covering the lights on both sides of the shelf. Two layers of the filter were positioned over the glass to reduce the irradiance to 30 ± 0.9 μmol photons $m^{-2}$ $s^{-1}$. The average irradiance for each shelf was calculated from measurements taken in five positions from front to back across the centre of each shelf using the spherical light probe and light meter.

Four culture flasks were positioned in a line from front to back across the centre of each shelf and adjacent to four flasks of the other strain. Flasks containing cultures of the Antarctic strain were positioned to the left of those containing cultures of the temperate strain. Each flask was shaken gently every 2-4 days to resuspend any cells that may have settled and then returned to the same position in the cabinet. For each strain, the four flasks were sampled as independent

replicates to measure the growth rate. Measurements of the cell concentration for the growth rate started at the time of inoculation.

### 2.2.3. Quantification of the optical density-cell concentration relationship for use as an indirect measure of biomass

To determine whether optical density (OD) could be used as a reliable predictor of the cell concentration, the relationship between these variables was measured and a standard curve was constructed. Using stock cultures of exponentially growing Antarctic and temperate strains of *C. subellipsoidea*, a series of dilutions were made with an OD ranging from 0.05 to 2.5 Abs. The stock cultures were acclimated to 10 °C and an irradiance of 60 μmol photons $m^{-2}$ $s^{-1}$. Prior experimentation revealed the relationship between cell concentration and OD did not vary with growth temperature or irradiance. A spectrophotometer (WPA Biowave II, Biochrom, England) was used to measure the OD. Each dilution was transferred into a 1 cm diameter Fisherbrand polystyrene cuvette and the OD was measured at 750 nm, as recommended by Griffiths *et al*. (2011). The spectrophotometer was zeroed at this wavelength with 1 ml of 3N-BBM+V. Following measurement of the OD, each dilution was fixed in an Eppendorf tube with 10 μl of Lugol's iodine reagent ($I_2$, $KI^-$ and glacial acetic acid). The fixed sample was pipetted on to the haemocytometer grid and after the cells had settled, the number of cells on the grid was counted under a light microscope (CH B, Olympus, Japan) at 400x magnification. The cell concentration was calculated for each sample using the average number of cells counted per $mm^2$ of the haemocytometer grid and accounting for the depth of the grid (0.2 mm). To reduce errors in the cell counts, samples with an OD greater than 0.2 were diluted so that no more than 30 cells were counted per $mm^2$. The cell count was then multiplied by the dilution factor to give the actual number of cells. The cell concentration was plotted against OD for each strain. The relationship was linear (Fig. 2.1), and OD was considered to be a reliable predictor of the cell concentration for both strains.

Following inoculation, 1 ml was sub-sampled from each culture every 2-7 days over the 37-day period. Each culture was gently shaken to resuspend cells that may have settled and returned to the growth conditions. The sample was transferred into the 1 cm diameter cuvette and the OD was measured immediately at a wavelength of 750 nm, using the Biochrom spectrophotometer. Using linear regression of the relationship between OD and cell concentration, the cell concentration of each sample was predicted from the measured OD. The initial cell concentration was subtracted from that achieved on the final day of the experiment to calculate the day-37 cell yield.

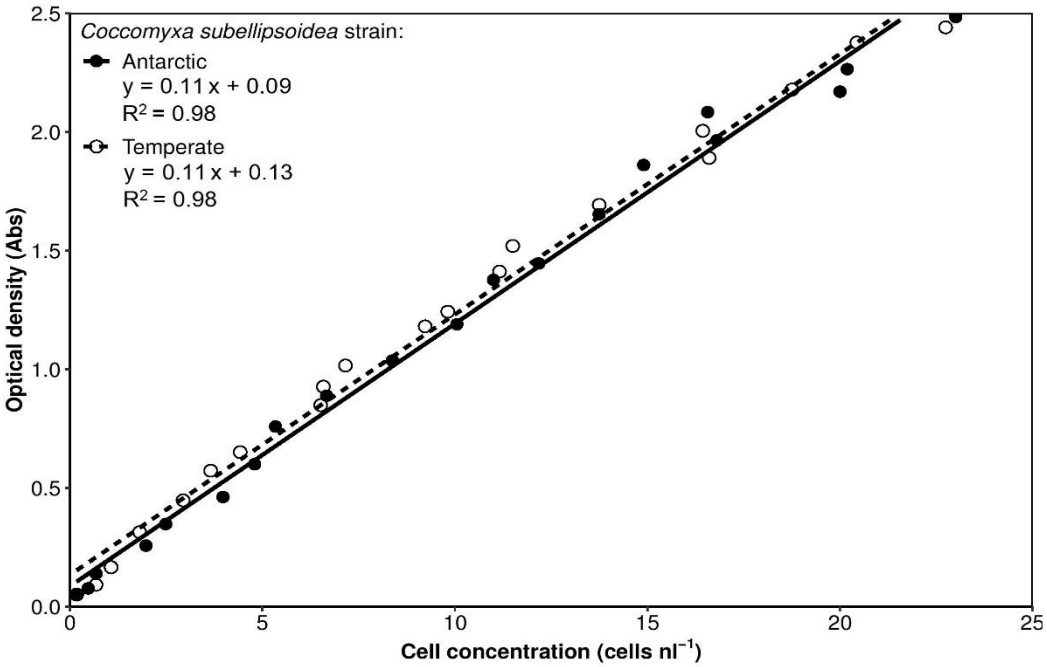


Figure 2.1. Standard curve of the cell concentration and optical density measured at 750 nm of an Antarctic and temperate strain of *C. subellipsoidea*. Based on cultures grown at 10 °C and constant irradiance of 60 μmol photons $m^{-2}$ $s^{-1}$.

### 2.2.4. Calculation of growth rates

The predicted cell concentration was logarithmically transformed and plotted against time in days. The exponential growth phase was then identified from the curve for each sample. Using the predicted cell concentration at the start and end of this exponential phase, the growth rate for each sample was calculated according to equation 2.1.

$$\mu = \frac{(\ln N2 - \ln N1)}{t2 - t1} \quad (2.1)$$

Where:

$\mu$ = specific growth rate ($day^{-1}$)

t1 and t2 = beginning and end of the exponential growth phase, respectively

N1 and N2 = cell concentration at t1 and t2, respectively

ln = natural log

### 2.2.5. Statistical analysis

Statistical analyses were performed in R V3.6.0 (R Core Team, 2019) and plots were constructed using the ggplot2 package (Wickham, 2016). Linear regression was used to predict the cell concentration from the OD. The means and standard errors were calculated from four independent replicates. A Shapiro-Wilk test was performed to test for normal distribution of residuals and a Levene's test was used to test for homogeneity of variances. To determine whether temperature and irradiance have a significant effect on the day-37 cell yield and growth rate, and if there is a significant interaction effect between temperature and irradiance, a two-way ANOVA was performed. A one-way ANOVA was also performed to identify if there was a significant difference between the growth rate of the Antarctic and temperate strain within each set of temperature and irradiance conditions. A significance level of $P < 0.05$ was considered significant, and where significant differences were identified, a Tukey's pairwise comparison *post-hoc* test was used.

## 2.3. Results

### 2.3.1. Growth curves of an Antarctic strain of *Coccomyxa subellipsoidea*

The Antarctic strain of *C. subellipsoidea* grew across the selected range of temperatures (6-18 °C) and irradiance levels (30-100 µmol photons $m^{-2}$ $s^{-1}$) for the duration of the 37-day experiment (Fig. 2.2 A-C). There was some variation in the shape of each growth curve depending on the temperature. However, the shape of the growth curve at each irradiance level within a temperature showed little variation. The most notable lag phase, when the rate of increase in the cell concentration over time was relatively low, occurred at 6 °C where the lag phase lasted until day 9 (Fig. 2.2 A). Each growth curve showed an exponential phase of growth, which was a period of accelerated growth following the lag phase. At 10 °C, the exponential phase lasted for 17 days (Fig. 2.2 B), 14 days at 18 °C (Fig 2.2 C) and 13 days at 6 °C (Fig. 2.2 A), after which there was a slight decline in the rate of increase in the cell concentration as the cells started to enter early stationary phase.

For the Antarctic strain of *C. subellipsoidea*, the day-37 cell yield was highest at 10 °C where the yield was between $1.8 \times 10^7$ and $2.0 \times 10^7$ cells $ml^{-1}$ and lowest at 6 and 18 °C where the yield was between $8.0 \times 10^6$ and $9.3 \times 10^6$ cells $ml^{-1}$ (Fig. 2.2 A-C). Within each temperature, the day-37 cell yield increased with irradiance. A two-way ANOVA identified a significant difference in the cell yields for the Antarctic strain depending on the temperature ($F_{[2,27]} = 194.885$, $P < 0.001$), but not irradiance ($F_{[2,27]} = 0.286$, $P > 0.1$), and there was no significant interaction effect ($F_{[4,27]} = 1.210$, $P > 0.1$). *Post-hoc* Tukey tests identified that the cell yield at 10 °C was significantly higher compared to that at 6 and 18 °C ($P < 0.001$), but there was no significant difference in the day-37 cell yield between 6 and 18 °C ($P > 0.1$).

### 2.3.2. Growth curves of a temperate strain of *Coccomyxa subellipsoidea*

The temperate strain of *C. subellipsoidea* grew across the selected range of temperatures and irradiance levels (Fig. 2.2 D-F). The lag phase at 6 °C was most notable and lasted for 9 days (Fig. 2.2 D). The duration of the exponential phase was 17 days at 10 °C (Fig 2.2 E) and 15 days at 18 °C (Fig 2.2 F). The temperate strain grown at 6 °C maintained a relatively constant rate of increase following the lag phase and did not enter stationary phase before the end of the experimental period (Fig 2.2 D). There was little variation in the shape of each curve depending on irradiance at 18 °C (Fig 2.2 F), but at 6 and 10 °C there was some separation in the curves following day 15, where the rate of increase in the cell concentration was slightly greater at a higher irradiance (Fig 2.2 D and E).

For the temperate strain, the cell yield achieved on day 37 increased with temperature and irradiance (Fig. 2.2 D-F). The highest occurred at 18 °C where the yield was between 2.5 $\times 10^7$ and 2.9 $\times 10^7$ cells $ml^{-1}$ whilst the lowest yield was between 2.5 $\times 10^6$ and 4 $\times 10^6$ cells $ml^{-1}$ at 6 °C. A two-way ANOVA indicated that there was a significant difference in the yield depending on the temperature ($F_{[2,27]} = 812.289$, $P < 0.001$), but not irradiance ($F_{[2,27]} = 0.500$, $P > 0.1$) and there was no significant interaction effect ($F_{[4,27]} = 1.352$, $P > 0.1$). *Post-hoc* Tukey tests revealed that the yield at 18 °C was significantly higher compared to that at 6 and 10 °C ($P < 0.001$), and the cell yield at 10 °C was also significantly higher than at 6 °C ($P < 0.001$). The day-37 cell yields are explored further in Chapter 3.

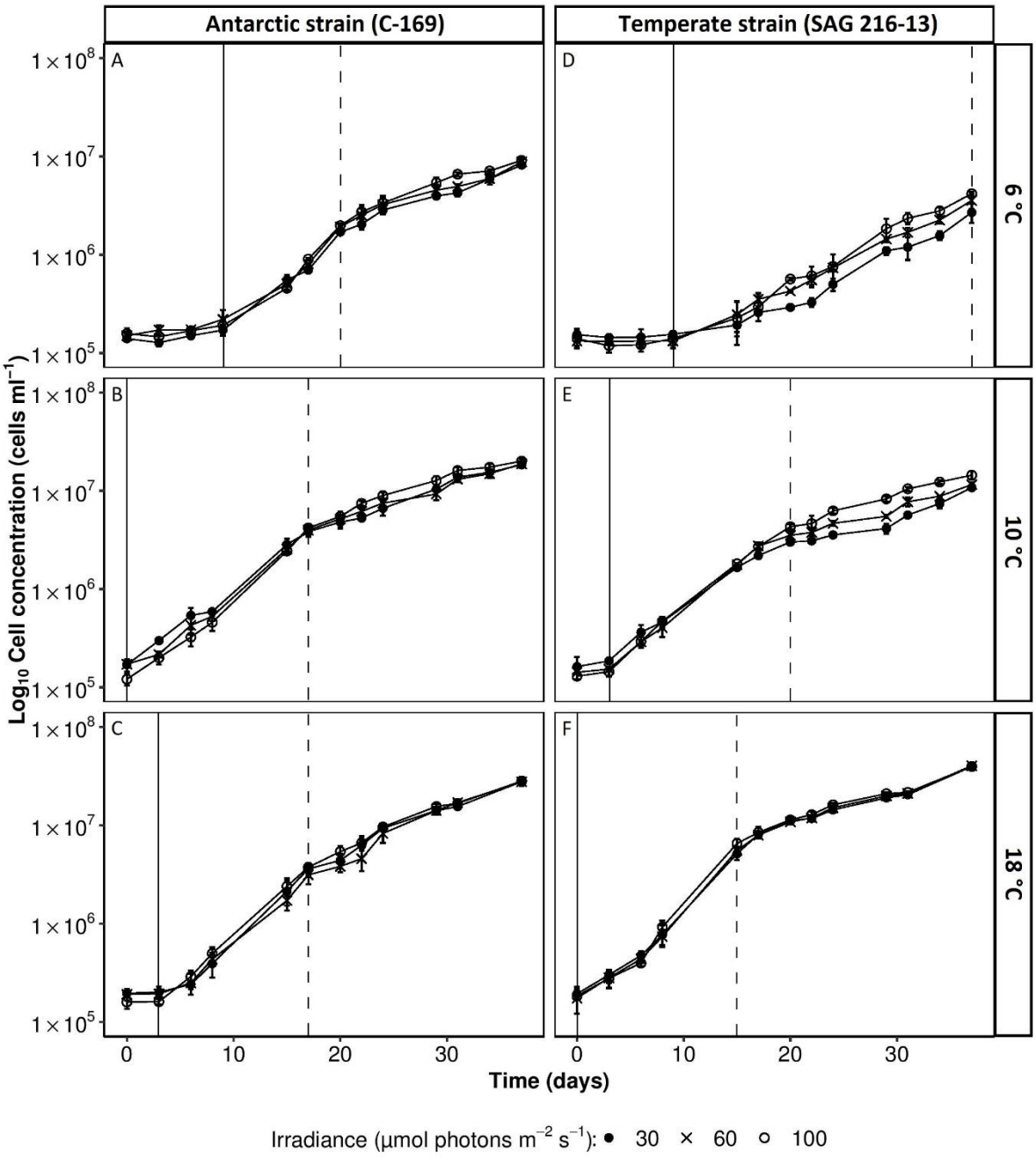


Figure 2.2. Semi-logarithmic growth curves of an Antarctic strain (A-C) and temperate strain (D-F) of *C. subellipsoidea* over a 37 day period at 6 (A, D), 10 (B, E) or 18 °C (C, F) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Solid vertical lines mark the end of the lag phase and dashed vertical lines mark the beginning of the stationary phase. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 2.3.3. The influence of temperature and irradiance on the growth rate of an Antarctic strain of *Coccomyxa subellipsoidea*

The growth rate for each strain at each set of conditions was calculated for the exponential growth phase (Fig. 2.3 A-C). The highest growth rate for the Antarctic strain was 0.25 ± 0.01 $day^{-1}$ which was recorded at 10 °C and an irradiance of 100 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 2.3 B). The lowest growth rate was 0.20 ± 0.01 $day^{-1}$ which was recorded at 6 °C and 30 µmol photons $m^{-2}$ $s^{-1}$ and was 1.2-fold lower compared to the maximum growth rate (Fig. 2.3 A). A two-way ANOVA did not indicate a significant interaction effect between temperature and irradiance ($F_{[4,27]} = 0.314$, $P > 0.1$), but did indicate a significant difference depending on temperature ($F_{[2,27]} = 10.682$, $P < 0.001$). A Tukey's *post-hoc* test indicated that the growth rate was significantly higher at 10 °C compared to 6 °C ($P < 0.001$) and 18 °C ($P < 0.05$), but there was no significant difference between 6 and 18 °C ($P > 0.1$). Significant differences were also identified depending on irradiance ($F_{[2,27]} = 5.715$, $P < 0.01$). Within each temperature, the growth rate at 100 µmol photons $m^{-2}$ $s^{-1}$ was significantly higher compared to that at 30 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.01$), but not 60 µmol photons $m^{-2}$ $s^{-1}$ ($P > 0.1$).

### 2.3.4. The influence of temperature and irradiance on the growth rate of a temperate strain of *Coccomyxa subellipsoidea*

The growth rate of the temperate strain of *C. subellipsoidea* increased with increasing temperature and irradiance (Fig. 2.3 A-C). A two-way ANOVA for the temperate strain identified significant differences in the growth rate between temperatures ($F_{[2,27]} = 546.71$, $P < 0.001$) and irradiances ($F_{[2,27]} = 31.14$, $P < 0.001$), but no significant interaction effect ($F_{[4,27]} = 1.98$, $P > 0.1$). The highest growth rate experienced by the temperate strain was 0.31 ± 0.01 $day^{-1}$ at 18 °C and an irradiance of 100 µmol photons $m^{-2}$ $s^{-1}$ (Fig 2.3 C), and this was significantly higher ($P < 0.001$) compared to the lowest rate which was 5.4-fold lower at 6 °C

and 30 µmol photons $m^{-2}$ $s^{-1}$ where the growth rate was 0.06 ± 0.0 $day^{-1}$ (Fig. 2.3 A). The growth rate at 10 °C was also significantly higher compared to that at 6 °C ($P < 0.001$) and significantly lower compared to that at 18 °C ($P < 0.001$). At each temperature, the irradiance which gave rise to the highest growth rate was 100 µmol photons $m^{-2}$ $s^{-1}$, where the growth rate was significantly higher compared to that at 30 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.001$) and 60 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.05$).

### 2.3.5. Comparison of the growth rate between an Antarctic and temperate strain of *Coccomyxa subellipsoidea*

The Antarctic and temperate strains each experienced highest growth rates at the highest irradiance tested of 100 µmol photons $m^{-2}$ $s^{-1}$ at each temperature. At each temperature, the growth rate at 100 µmol photons $m^{-2}$ $s^{-1}$ was therefore considered as optimal for this study and these growth rates were compared between the Antarctic and temperate strains (Fig. 2.3 A-C). A one-way ANOVA detected a significant difference between the growth rates of the Antarctic and temperate strain of *C. subellipsoidea* where the Antarctic strain displayed a significantly higher growth rate at 6 °C ($F_{[1,6]} = 157$, $P < 0.001$) and 10 °C ($F_{[1,6]} = 10.91$, $P < 0.01$) compared to the temperate strain. At 18 °C, the Antarctic strain had a significantly lower growth rate compared to the temperate strain ($F_{[1,6]} = 45.03$, $P < 0.001$). The difference in the growth rate between the strains was greatest at 6 °C, where the growth rate for the Antarctic strain was 2-fold higher (Fig. 2.3 A). The temperate strain displayed more pronounced differences in the growth rate between each temperature, with a difference of 0.25 $day^{-1}$ between the highest and lowest growth rates. Whereas the Antarctic strain, which grew at a relatively similar rate at each temperature, varied only by 0.05 $day^{-1}$ between the maximum and minimum growth rates.

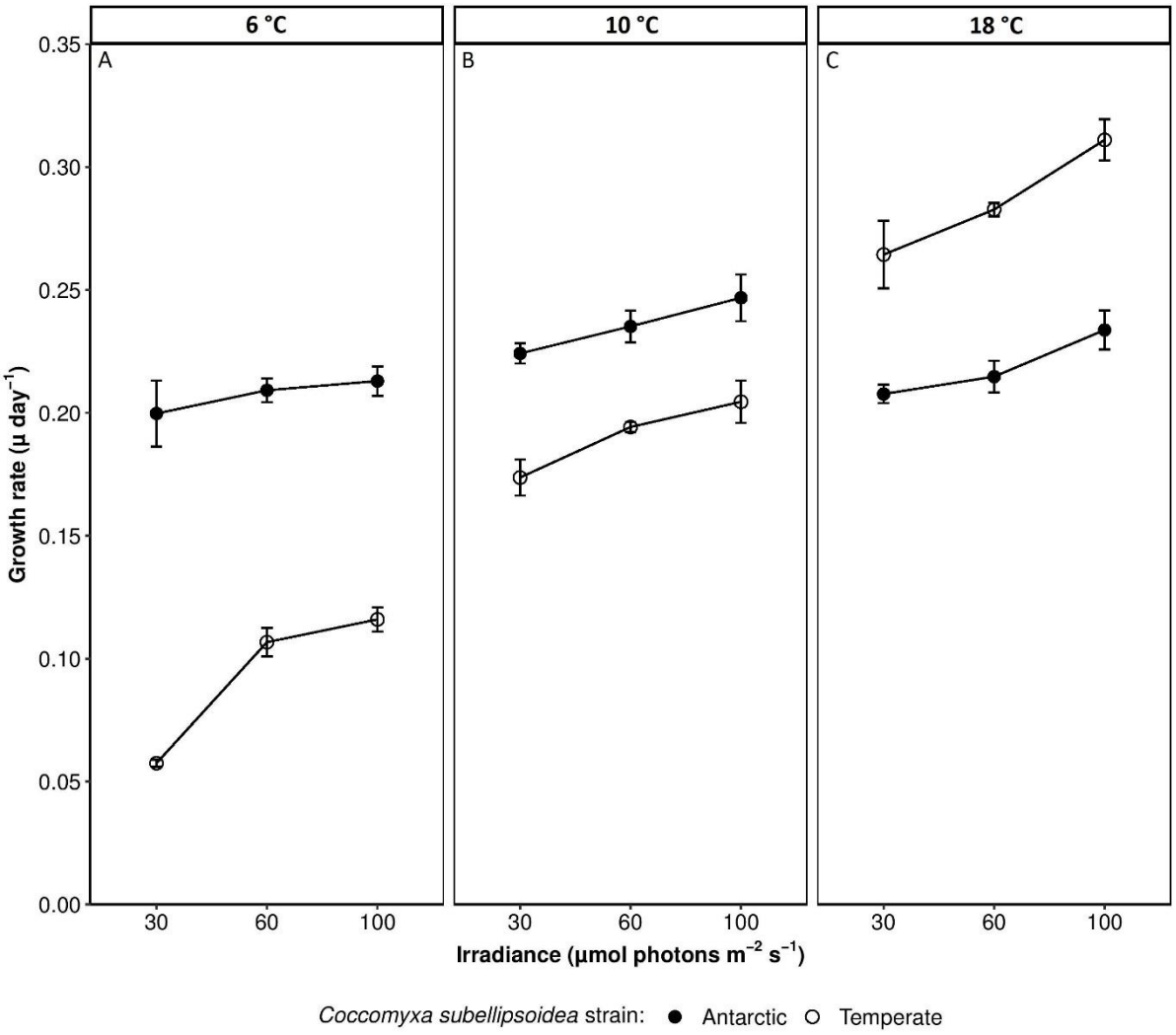


Figure 2.3. Growth rates of an Antarctic strain and temperate strain of *C. subellipsoidea* grown at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 2.3.6. Summary of results

The Antarctic strain of *C. subellipsoidea* displayed a distinct response to temperature compared to the temperate strain. The highest growth rate of the Antarctic strain occurred at 10 °C, whereas the highest growth rate of the temperate strain occurred at 18 °C. The differences in the growth rate between the strains were most notable at 6 °C, where the growth rate of the Antarctic strain was at least 60% higher compared to that of the temperate strain. Antarctic *C. subellipsoidea* had the ability to maintain growth rates within a narrow band varying by only

0.05 $day^{-1}$ across the 12 °C temperature range studied, whereas the growth rate of the temperate strain varied by 0.25 $day^{-1}$.

## 2.4. Discussion

### 2.4.1. Can the two algal strains be classified as psychrophilic or psychrotolerant?

#### 2.4.1.1. Antarctic *Coccomyxa subellipsoidea*

The aim of this chapter was to characterise the growth responses of Antarctic and temperate isolates of *C. subellipsoidea*. The Antarctic strain was hypothesised to be psychrophilic and have an optimum growth temperature at 10 °C. The highest growth rate of the Antarctic strain did occur at 10 °C, indicating that the optimum growth temperature of this strain likely lies close to 10 °C, suggesting it may be psychrophilic according to Morita (1975). Based on the environment-specific definition introduced by Russell (1990), the Antarctic strain could also be classified as a psychrophile considering it was isolated from continental Antarctica, a permanently cold environment.

In their study on the Antarctic strain of *C. subellipsoidea*, Pfaff *et al*. (2016) reported that the optimum growth temperature was 12.9 °C, which places the strain within the range expected for a psychrophile (Morita, 1975). The highest growth rate of this strain measured in the present investigation occurred at 10 °C, which is close to the optimum identified by Pfaff *et al*. (2016). There was a reduction in the growth rate between 10 and 18 °C, indicating that the optimum growth temperature for this strain is unlikely to be at 20 °C, which was reported by Blanc *et al*. (2012) as the optimum growth temperature for the same strain of *C. subellipsoidea*. Blanc *et al*. (2012) did not provide any detail on the growth conditions or growth rate to justify this optimum growth temperature. Although there was a slight reduction in the growth rate, the Antarctic strain was able grow at 18 °C and previous research has shown growth in this strain up to 30 °C (Msanne *et al*., 2012; Tevatia *et al*., 2014; Allen *et al*., 2015; Pfaff *et al*., 2016;

Allen *et al*., 2017; Liu *et al*., 2018; Yu *et al*., 2018). The ability of this strain to grow at 30 °C (Pfaff *et al*., 2016) implies that it cannot be classified at psychrophilic because Morita (1975) and Gounot (1986) suggested that psychrophiles cannot grow above 20 °C. The temperature typically experienced in continental Antarctica is much lower than 18 °C, so the ability of the Antarctic strain to grow at and above this temperature suggests that it may have mesophilic ancestors and the capacity to grow at higher temperatures could be evidence of having ancestors which originated from a warmer climate and lower latitude and could have been transported to the Antarctic by the wind (Cao *et al*., 2016).

Pfaff *et al*. (2016) reported that the growth rate of the Antarctic strain of *C. subellipsoidea* was 0.46 $day^{-1}$ at 12.9 °C and 0.35 $day^{-1}$ at 9.6 °C. Each of these growth rates are higher than the highest growth rates identified for the same strain in the present investigation of 0.23-0.25 $day^{-1}$ at 10 °C and an irradiance of 30-100 µmol photons $m^{-2}$ $s^{-1}$. The difference in the growth rates may be a consequence of the different culturing conditions. Although the irradiance level of 40 µmol photons $m^{-2}$ $s^{-1}$ used by Pfaff *et al*. (2016) was within the same range of irradiances, Pfaff *et al*. (2016) grew the cultures under a light: dark cycle of 16:8 hours, compared to the constant photoperiod used in the present study. It could be suggested that a constant photoperiod was suboptimal for the growth of *C. subellipsoidea* and may explain the lower growth rates, however, previous studies have shown that a constant photoperiod compared to a shorter photoperiod enhanced the growth rate of the green algae *Coccomyxa melkonianii* (Pasqualetti *et al*., 2015), *Cosmarium* (Bouterfas *et al*., 2006), *Scenedesmus* and *Botryococcus* (Krzemińska *et al*., 2014). A longer photoperiod may be beneficial for growth because the period over which photosynthesis can occur may be extended (Krzemińska *et al*., 2014). Alternatively, the method used to estimate growth could account for the difference in growth rate. Pfaff *et al*. (2016) monitored Chlorophyll a (Chl a) fluorescence over the growth period as a proxy for the cell concentration. Chl a fluorescence is indicative of the Chl a concentration, and with information

on the Chl a content per cell, this allows an estimate to be made on the cell concentration (Gustavs *et al*., 2009). However, an increase in Chl a per cell over time may increase the Chl a fluorescence reading, and this could lead to an over estimation of the cell concentration (Kruskopf and Flynn, 2005; Baulch *et al*., 2009; van de Poll *et al*., 2013). Using OD as a biomass indicator can overcome this problem by measuring the absorbance at 750 nm which avoids interference by changeable pigment concentrations (Griffiths *et al*., 2011). Changes in pigment composition are discussed further in Chapter 3.

#### 2.4.1.2. Temperate *Coccomyxa subellipsoidea*

The temperate strain of *C. subellipsoidea* was hypothesised to by psychrotolerant and have an optimal growth temperature at a higher temperature. The temperate strain was able to grow at the low temperature of 6 °C, but the greatest rate of growth achieved at 18 °C suggests it may reach optimal growth at a temperature exceeding the 15 °C requirement to be classified as psychrophilic and may instead be classified as psychrotolerant (Morita, 1975). However, psychrotolerant growth of the temperate strain cannot be confirmed because growth at 20-25 °C was not determined, and psychrotolerant organisms show optimum growth within this temperature range according to Morita (1975). The temperate strain may also be classified as psychrotolerant according to Russell (1990) as it was isolated from Austria, where it may experience periodic exposure to relatively low temperatures.

The growth responses of this strain, to my knowledge, have not been reported, but the growth temperature at which temperate isolates of *Coccomyxa melkonianii* and *Coccomyxa onubensis* achieved optimal growth rates at have been reported to be 25 °C (Pasqualetti *et al*., 2015; Bermejo *et al*., 2018). The growth rate of the temperate strain of *C. subellipsoidea* may increase further above 18 °C to achieve optimal growth within the same range as *C. melkonianii* and *C. onubensis*. However, having an optimum growth temperature close to 18 °C would place the

temperate strain of *C. subellipsoidea* within the range of optimum growth temperatures experienced by other temperate Trebouxiophyte photobionts which range from 15 to 23 °C (Ahmadjian, 1965). The temperate strain also displayed positive, sub-optimal growth rates at the lower temperatures of 6 °C and 10 °C, suggesting it may be capable of survival in colder climates, although the reduced growth rate at these lower temperatures may give it a competitive disadvantage against more specialised psychrophilic algae which have relatively higher growth rates at low temperature (Teoh *et al*., 2013).

The definition proposed by Gounot (1986) is not appropriate for these results as this definition relies on obtaining information on the upper and lower lethal temperatures, which were not measured in this study. The algal-specific definition introduced by Komárek and Nedbalová (2007) is also not appropriate as growth was recorded within in the range suggested for psychrophilic algae (0-10 °C) and the range suggested for psychrotolerant algae (10-25 °C), so a clear distinction cannot be made. Although the definitions for psychrophily and psychrotolerance introduced by Morita (1975) potentially allow separation of the growth responses of the Antarctic and temperate strains of *C. subellipsoidea*, these definitions were introduced for bacteria and not phototrophic organisms, so the influence of irradiance was not considered (Morita, 1975). The results of the present study demonstrated that irradiance may also influence the growth rate of *C. subellipsoidea*. Alternative definitions that do not rely on the optimum growth temperature, such as eurythermal and stenotherm, may be more appropriate to describe the growth responses seen in these results.

### 2.4.2. Are there alternative descriptors to better define temperature responses for these strains of green algae?

The Antarctic strain was able to maintain a much more constant growth rate across the range of temperatures studied compared to the temperate strain. The growth patterns observed

between the Antarctic and temperate strains of *C. subellipsoidea* examined during this research are similar to those reported in previous studies on other species of green algae across different latitudes (Teoh *et al*., 2013; Cao *et al*., 2016; Lee *et al*., 2018). The distinct growth responses of each strain suggest that the effects of temperature on growth in *C. subellipsoidea* is influenced by the latitude of origin, despite more than 50 years of exposure to culture collection or laboratory conditions, and these patterns may be common among green algae. The growth responses to temperature of *C. subellipsoidea* recorded during this study are similar to those of *Chlorella pyrenoidosa* observed by Cao *et al*. (2016). In their study, a temperate strain of *C. pyrenoidosa* achieved a maximum growth rate of 0.26 $day^{-1}$ at 27 °C at a constant irradiance of 45 µmol photons $m^{-2}$ $s^{-1}$ and the growth rate declined significantly by 80% as temperature decreased from 27 to 3 °C, the temperate at which slowest growth was recorded. An Arctic strain used in their study experienced a maximum growth rate of 0.32 $day^{-1}$ at 15 °C and 45 µmol photons $m^{-2}$ $s^{-1}$, and the growth rate varied only by 16% from 27 to 3 °C. Cao *et al*. (2016) concluded that the Arctic strain exhibited eurythermal behaviour because it was able to maintain a relatively constant growth rate across a wide range of temperatures. In the present study, growth of the Antarctic strain of *C. subellipsoidea* was also less sensitive to temperature compared to the temperate strain as it showed only a 5-10% change in the growth rate between 6 and 18 °C, whereas the temperate strain varied by 60-70% between these temperatures. The Antarctic strain of *C. subellipsoidea* may therefore demonstrate much more eurythermal behaviour compared to the temperate strain which could be considered stenothermal. The ability to tolerate a wide range of temperatures appears to be widespread among Antarctic green algae, particularly in soil algae (Seaburg *et al*., 1981; Elster, 1999). Eurythermal behaviour could be an adaptation to an environment associated with frequent temperature fluctuations (Teoh *et al*., 2013; Lee *et al*., 2018). The polar soils and algal peat in which populations of this Antarctic strain of *C. subellipsoidea* may grow can be a particularly unstable environment

where temperature may fluctuate on a diurnal and seasonal scale, freeze-thaw cycles are common and snow cover, which impacts irradiance levels, can be variable (Elster, 1999; Karsten *et al*., 2010). Daily soil temperature fluctuations can be up to 20 °C in Marble Point, Antarctica, where the Antarctic strain was isolated from (Aislabie *et al*., 2006; Cary *et al*., 2010). Eurythermal behaviour may also provide an advantage under climate change as it could allow the strain to be more resilient to temperature change (Teoh *et al*., 2013). In comparison, the temperate strain may not experience as frequent or extreme temperature fluctuations at the lower latitude where it normally grows and when growing symbiotically, the lichen may provide a more stable climate within the thallus (Honegger, 1998). Therefore, the temperate strain may be more sensitive to temperature change.

At 6 °C, the Antarctic strain of *C. subellipsoidea* was able to maintain a 3-4-fold higher growth rate compared to the temperate strain, which experienced a significant reduction in the growth rate at this lower temperature. Similarly, Cao *et al*. (2016) found Arctic *C. pyrenoidosa* was able to maintain a 6-fold higher growth rate compared to the temperate strain at 9 °C. The ability of the Antarctic strain of *C. subellipsoidea* to maintain a relatively high growth rate at 6 °C, suggests that it may continue to grow below this temperature. However, the much lower growth rate of the temperate strain at this temperature suggests 6 °C may be close to the lower thermal limit of the temperate strain.

### 2.4.3. Growth responses to irradiance of an Antarctic and temperate strain of *Coccomyxa subellipsoidea*

It was hypothesised that the Antarctic strain of *C. subellipsoidea* would show a higher growth rate at a higher irradiance compared to the temperate strain as irradiance is often more intense at polar latitudes. The results did not support this hypothesis as both strains experienced a significant increase in the growth rate at the higher irradiance of 100 µmol photons $m^{-2}$ $s^{-1}$

compared to at the lower irradiance of 30 µmol photons $m^{-2}$ $s^{-1}$ within each temperature. The increase in the growth rate with irradiance indicates that the growth rate at 30 µmol photons $m^{-2}$ $s^{-1}$ was limited by irradiance. Kvíderová and Lukavský (2005) also found that the growth responses to irradiance of temperate and polar *S. bacillaris* were each optimised at a relatively high growth irradiance above 500 µmol photons $m^{-2}$ $s^{-1}$. The Antarctic strain of *C. subellipsoidea* appeared to be less sensitive to irradiance as there was no significant difference in the growth rate between 60 and 100 µmol photons $m^{-2}$ $s^{-1}$ at each temperature, indicating growth saturation at this level, whereas the growth rate of the temperate strain increased significantly between these irradiance levels at each temperature. The growth rate of the temperate strain may therefore have increased further if exposed to an irradiance above 100 µmol photons $m^{-2}$ $s^{-1}$.

It could be argued that the growth responses to irradiance may be influenced by the microhabitat of the strains (Stamenković and Hanelt, 2017). The temperate strain, as well as markedly changing the growth rate at difference temperatures, was relatively more responsive to irradiance with an increase from 30 to 100 µmol photons $m^{-2}$ $s^{-1}$ of up to 90%, whereas the increase in the growth rate of the Antarctic strain between these irradiance levels was only up to 10%. These results suggest that the temperate strain may have a greater capacity to acclimate to variable irradiance and grow faster at a higher irradiance. The temperate strain may experience fluctuations in irradiance as it transitions between a free-living and lichenised life stage (Gustavs *et al*., 2017). The capacity to rapidly acclimate to irradiance may therefore benefit lichen symbiont strains of *C. subellipsoidea* by allowing them to optimise growth under these fluctuating irradiances and this strain of *C. subellipsoidea* may even tolerate higher irradiances compared to the Antarctic strain. However, the Antarctic strain may also be exposed to irradiance fluctuations due to varying levels of snow cover (Pérez-Torres *et al*., 2004; Larose *et al*., 2013). The light environment of the microhabitat of each strain is yet to be described and

knowledge of the environmental conditions in the field is required to better understand their responses to irradiance.

### 2.4.4. Conclusions

The two strains of *C. subellipsoidea* differed significantly in their growth responses to temperature. The Antarctic strain maintained a relatively constant growth rate between 6 and 18 °C, whereas the growth rate of the temperate strain was much more sensitive to temperature. Classifying these strains as psychrophilic and psychrotolerant may not be the most appropriate terms and instead, the Antarctic strain could be described as being more eurythermal compared to the temperate strain, which could be stenothermal. The eurythermal behaviour of the Antarctic strain may allow it to tolerate the highly variable temperature of polar soils and make it relatively resilient to climate warming, whereas the temperate strain may be more sensitive to temperature change.

The temperate and Antarctic strains of *C. subellipsoidea* displayed an increase in the growth rate with a higher irradiance, irrespective of temperature. The temperate strain appeared to be better able to deal with a higher irradiance, with a significant increase in the growth rate between 30, 60 and 100 µmol photons $m^{-2}$ $s^{-1}$. The microclimate from which each strain was isolated may influence the response to both of these variables. More information on the respective microhabitats is therefore required to investigate this. To better understand the growth responses of each strain of *C. subellipsoidea*, further knowledge on how the pigment composition and rates of photosynthesis and respiration are be influenced by temperature and irradiance are necessary, a topic which is addressed in Chapter 3.

# Chapter 3: Photophysiology, pigment composition and respiration of an Antarctic and temperate strain of *Coccomyxa subellipsoidea* in response to temperature and irradiance

## 3.1. Introduction

The physiology and biochemistry of algae is highly sensitive to the climate (Ensminger *et al*., 2006). A suboptimal temperature can have deleterious effects on the photosynthetic and respiratory capacity of algae. The effects on photosynthesis in particular are exacerbated when stressful temperatures and high irradiances are experienced together as photoinhibition and photodamage can be induced (Powles, 1984). Many species of algae are able to rapidly regulate their physiology in response to the climate to minimise the risk of damage to proteins and avoid reductions in rates of photosynthesis and growth (Teoh *et al*., 2004; Lutz *et al*., 2017). Some cryophilic algae, such as an Antarctic isolate of *C. subellipsoidea*, may have cold tolerant and cryoprotective proteins which could enable metabolism to continue at low temperatures (Blanc *et al*., 2012). Algae may also adjust their pigment compositions to tolerate different irradiance levels (La Rocca *et al*., 2014). By having a high degree of plasticity in their physiology, some species of algae are able to survive in a wide range of different environments (Fujita *et al*., 2001). Cryophilic algae may have adapted or have the capacity to acclimate to increase the efficiency of photoprotective and repair mechanisms or even to optimise their physiology to low temperatures (Kosugi *et al*., 2010; Stamenković and Hanelt, 2017).

### 3.1.1. Photosynthesis and photoinhibition

If absorption of light energy exceeds the rate the energy can be utilised in metabolism, the excess light energy can impair photosystems, photoinhibition can occur and the rate of photosynthesis declines as a result of photodamage (Neidhardt *et al*., 1998). The risk of

photoinhibition is high when low temperatures and high irradiances are experienced together, making polar algae particularly vulnerable to photoinhibition (Bidigare *et al*., 1993). The rate photons are absorbed by photosystems is high under a high irradiance, but a low temperature can decrease the activity rates of enzymes involved in electron transport and catabolism and growth rates can decline, reducing use of photosynthate (Ensminger *et al*., 2006; Hüner *et al*., 2012). The imbalance between the light dependent rate of light absorption and temperature dependent rate of energy utilisation can result in photoinhibition, which may have damaging consequences for algae (Davison, 1991). Photoinhibition can lead to the generation of reactive oxygen species (ROS) when the electron transport chain slows, and electrons are lost from the chain and instead taken up by singlet oxygen (Sharma *et al*., 2012). These ROS are highly reactive and can induce oxidative damage of DNA and inhibit photosynthetic proteins and enzymes involved in repair processes (Mallick and Mohn, 2000).

### 3.1.2. Non-photochemical quenching

Light energy can take different pathways when received by a photosynthetic organism. The photons can be absorbed by light harvesting pigments and converted into photochemical energy in photosynthesis, but if irradiance is high, photosystems can become inactivated and further absorbed light energy cannot be converted into photochemical energy. The excess light energy then takes an alternative pathway known as non-photochemical quenching (NPQ) (Hüner *et al*., 2012). There are two forms of NPQ, regulated NPQ and non-regulated NPQ (NO) (Klughammer and Schreiber, 2008). Regulated NPQ is a photoprotective process which involves the xanthophyll cycle in plants and green algae. Alternative NPQ mechanisms exist in other photosynthetic organisms. In the xanthophyll cycle, an uncoupling of light capture and electron transport due to high irradiances triggers acidification of the thylakoid lumen. Violaxanthin de-epoxidase is activated in response to a decrease in pH and this enzyme converts the light harvesting pigment, violaxanthin, into the non-light harvesting pigments,

antheraxanthin and then zeaxanthin. Instead of transferring photons to chlorophyll where the energy can be utilised in photosynthesis, zeaxanthin dissipates the energy as heat (Müller *et al*., 2001). In comparison, NO is a constitutive and passive energy dissipation process where excessive energy is transferred to chlorophyll, but rather than being converted into photochemical energy, the energy is lost as fluorescence due to inactive photosystems (Figueroa *et al*., 2019). The conversion processes within chlorophyll molecules leading to the emission of this fluorescence generates singlet oxygen, resulting in the formation of ROS when it reacts with unpaired electrons (Moustaka *et al*., 2015). Organisms with a large capacity for regulated NPQ are able to safely dissipate excess energy away from chlorophyll molecules and maintain a balance between excitation by photons and the rate of temperature dependent electron transport, preventing photoinhibition. The loss of electrons from the electron transport chain can therefore be minimised and the generation of ROS can be reduced (Kitao *et al*., 2019). However, if regulated NPQ is not initiated, the excess light energy is forced to take the NO route where the energy is not safely dissipated away from chlorophyll and it can cause photodamage by generation of ROS (Klughammer and Schreiber, 2008).

### 3.1.3. Alterations in pigment composition

The primary light harvesting pigment in green algae is chlorophyll a (Chl a). Green algae also contain the accessory pigments, chlorophyll b (Chl b) and carotenoids, which transfer absorbed light energy to Chl a in the core light harvesting complexes of the photosystems (Lichtenthaler, 1987). The core light harvesting complexes of the photosystems contain Chl a whilst Chl b can be found in the auxiliary complexes. The composition of the pigments within the auxiliary complexes can vary depending on the light environment (Lichtenthaler, 1987). In response to a relatively high irradiance, algae typically respond by decreasing the concentration of Chl b to avoid absorption of excessive light energy, resulting in less Chl b relative to the Chl a in the core complexes and therefore a high Chl a to b ratio (Chl a/b). In response to low irradiance,

the concentration of light harvesting pigments may be increased to maintain light absorption, resulting in a higher concentration of Chl b relative to Chl a, and therefore a lower Chl a/b ratio (Smith *et al*., 1990). Carotenoids include beta-carotene, lutein, violaxanthin and neoxanthin and in addition to functioning as accessory pigments, this group can act as photoprotective pigments, shielding chloroplasts from a high irradiance (Ibelings *et al*., 1994; Marizcurrena *et al*., 2019). Some carotenoids demonstrate this photoprotective function as their content in a number of freshwater cyanobacteria species has been reported to be higher at a higher growth irradiance of 120 μmol photons $m^{-2}$ $s^{-1}$ compared to at 15 μmol photons $m^{-2}$ $s^{-1}$ (Schager and Müller, 2006).

### 3.1.4. Biogeographic differences in physiology

The sensitivity of the photosynthetic apparatus to irradiance and temperature, the ability of algae to initiate NPQ and the capacity to acclimate by modulation of their pigment composition may depend on the climate to which algae have adapted or acclimated (van de Poll, 2006). Stamenković and Hanelt (2017) proposed that strains of microalgae isolated from different latitudes display dissimilar photosynthetic behaviour depending on the climate of their original habitat. Previous research by Cao *et al*. (2016) on Arctic and temperate strains of *Chlorella pyrenoidosa* suggested the photosynthetic apparatus of the Arctic strain is more suited to low temperature compared to the temperate strain, as shown by higher photosynthetic activity of the Arctic strain relative to the temperate strain at low temperatures. Green algae such as *Koliella antarctica* isolated from the Antarctic have been shown to demonstrate a rapid and strong regulated NPQ response to a relatively high irradiance of 150 μmol photons $m^{-2}$ $s^{-1}$ to protect against photoinhibition and associated photodamage, in addition to a reduction in chlorophyll a and b (La Rocca *et al*., 2014). A reduction in light harvesting pigments appears to be a conserved response to high irradiance among algae (Maxwell *et al*., 1994; Tanaka and Melis, 1997; Guo *et al*., 2015; Stamenković and Hanelt, 2017). Pigment synthesis may be

influenced by temperature so this response could depend on the sensitivity of enzymes to temperature (Cao *et al*., 2016).

Previous research has suggested that the rate of respiration increases with temperature due to an increase in the rate of activity of respiratory enzymes (Eggert and Wiencke, 2000; Vona *et al*., 2004; Karsten *et al*., 2014). Many species of cryophilic algae appear to have cold active enzymes, suggesting they may be able to maintain high respiration rates at lower temperatures, allowing them to continue to generate energy for growth and cellular processes from organic carbon at a low temperature (Vona *et al*., 2004; Di Martino Rigano *et al*., 2006; Chen *et al*., 2012). However, research on how the influence of temperature and irradiance on respiration may vary between latitudinally separated populations is limited. Physiological data for *C. subellipsoidea* is scarce, and no research group appears to have attempted to uncover the photosynthetic and respiratory activity of any strain of *C. subellipsoidea*. Information on the physiology and biochemistry of this species would be valuable in understanding physiological plasticity in green algae and how their physiology is influenced by the climate (Gustavs *et al*., 2017).

### 3.1.5. Aim and hypotheses

It was previously reported that the Antarctic and temperate strains of *C. subellipsoidea* had significantly different growth rates at 6, 10 and 18 °C (Chapter 2). The aim of this chapter is to investigate the differences in their physiology and biochemistry when grown across the range of temperature and irradiance levels that may account for the variation in their growth rates. It was hypothesised that:

(i) the Antarctic strain would show a higher level of photosynthetic activity at the lower temperature of 6 °C compared to the temperate strain due to exposure to lower temperatures for longer periods of time in the Antarctic,

(ii) the Antarctic strain of *C. subellipsoidea* was hypothesised to be better able to avoid photodamage at the low temperature of 6 °C when combined with a high irradiance compared to the temperate strain, as shown by a higher level of regulated NPQ measured at a high irradiance during a rapid light curve (RLC),

(iii) the Chl a/b ratio was hypothesised to increase with irradiance for both strains, but at the low temperature of 6 °C, the Antarctic strain may be better able to adjust the ratio depending on growth irradiance as the enzymes involved in pigment synthesis may be more cold-tolerant compared to those of the temperate strain,

(iv) the carotenoid content per cell of the Antarctic strain was hypothesised to show a greater increase at a low temperature and high irradiance compared to the temperate strain as carotenoids may have photoprotective properties, allowing cells to reduce the risk of photodamage, and the Antarctic strain may have more cold-tolerant enzymes to synthesise carotenoids at low temperature,

(v) the rate of respiration was hypothesised to increase with temperature for both strains as rates of enzyme activity may increase, but the Antarctic strain may be better able to maintain the rate of respiration at a lower temperature compared to the temperate strain as it could have more cold-tolerant respiratory enzymes.

## 3.2. Methods

### 3.2.1. Culturing and experimental conditions

Stock cultures of an Antarctic (C-169) and temperate (SAG 216/13) strain of *C. subellipsoidea* were maintained in 3N-BBM+V at the experimental temperature of 6, 10 or 18 °C in an LMS fridge for a minimum of 30 days. Irradiance over this period was 40 μmol photons $m^{-2}$ $s^{-1}$ on a light: dark cycle of 12:12 hours. The stock cultures of each strain at each temperature were centrifuged, and the pelleted cells were resuspended in fresh 3N-BBM+V with a cell

concentration of 1.5 x$10^5$ cells $ml^{-1}$ which was divided between twelve flasks per strain, each containing 50 ml of the culture. The twelve flasks of each set were kept in Snijders Labs Micro Clima growth chambers at 6, 10 or 18 °C. Four flasks were grown under an irradiance of 30 μmol photons $m^{-2}$ $s^{-1}$, another four at 60 μmol photons $m^{-2}$ $s^{-1}$, and the final four at 100 μmol photons $m^{-2}$ $s^{-1}$. Irradiance during the experimental period was constant over 24 hours. Each set of four flasks were sampled as independent replicates over a 37 day period to measure photosynthetic activity, pigment composition and the rate of respiration. Photosynthetic activity was measured on day 16, pigment composition on day 15 and respiration rates on day 37. The delay between setting up the cultures and placing them under experimental conditions (day 0) and taking these measurements was to allow time for the cells to acclimate to the new irradiance levels and constant photoperiod.

Culturing, maintenance of strains and experimental conditions are described in detail in Chapter 2 sections 2.2.1. and 2.2.2.

### 3.2.2. Pulse amplitude modulated (PAM) fluorescence to measure photosynthetic activity

Pulse amplitude modulated (PAM) fluorometry was used to assess photosynthetic activity after 16 days of growth at each set of temperature and irradiance conditions. The PAM fluorometer measures chlorophyll fluorescence which acts as a useful measure of the performance of PSII by distinguishing between the different pathways taken by absorbed light energy (Consalvey *et al*., 2005). Electrons within chlorophyll molecules are excited by a light pulse emitted by the light-emitting diodes (LEDs) of the Water-PAM system. The chlorophyll molecules may transfer this energy to an acceptor molecule in the electron transport chain by photochemical quenching where this energy can be used in photosynthesis. Alternatively, the electrons may re-emit the energy as heat or fluorescence (Maxwell and Johnson, 2000). These dissipation pathways include regulated NPQ, where energy is dissipated by activation of photoprotective

mechanisms such as the xanthophyll cycle, or NO which is induced by closure of reaction centres (Klughammer and Schreiber, 2008). The energy re-emitted as fluorescence is measured by the PAM system and gives an indication of the proportion of the absorbed energy which is lost in NPQ compared to what is utilised in photochemistry. Determining the fate of absorbed energy based on these fluorescence measurements can therefore allow estimates to be made on the efficiency of photosynthesis (Maxwell and Johnson, 2000).

On day 16 of the experiment, 2 ml was taken from each culture and transferred to a Falcon tube. Samples were dark adapted for 30 minutes before measuring to ensure all reaction centres were open and ready to respond to a light pulse. To standardise the time of dark adaptation, samples were taken in batches of four. Fluorescence measurements were taken using the Cuvette Water-PAM and PAM-Control units which were controlled by the accompanying software, WinControl V3.29 (Walz, Germany). Adjustments of the sample volume were made to obtain a minimal fluorescence ($F_o$) value between 300 and 1000 to prevent maximal fluorescence ($F_m$) exceeding the measurement range.

#### 3.2.2.1. Maximum quantum yield of photosystem II ($F_v/F_m$)

Among the key measurements that can be made with PAM fluorometry is the maximum quantum yield of PSII, or $F_v/F_m$, which provides information on the proportion of reaction centres available to engage in photosynthesis and is often used as an indicator of stress (Maxwell and Johnson, 2000). To induce a fluorescence response, the PAM system exposed the dark adapted sample in the cuvette to a series of light pulses emitted from the LEDs. The first of these light pulses is a low irradiance measuring light which is too weak to induce photosynthesis but allows measurement of $F_o$, the minimal fluorescence when all reaction centres are open. A saturating light pulse followed which induced closure of the reaction centres and allowed measurement of $F_m$, the maximal fluorescence when reaction centres are

closed. The difference between $F_m$ and $F_o$ gives the variable fluorescence ($F_v$) which is representative of the number of active reaction centres. The ratio, $F_v/F_m$, which is equivalent to the maximum quantum yield of PSII, is calculated according to equation 3.1. (Genty *et al*., 1989; Brooks and Niyogi, 2011).

$$\frac{F_v}{F_m} = \frac{F_m - F_o}{F_m} \tag{3.1}$$

Where:

$F_v/F_m$ = maximum quantum yield of PSII

$F_v$ = variable fluorescence

$F_o$ = minimal fluorescence in the dark adapted state

$F_m$ = maximal fluorescence in the dark adapted state

**3.2.2.2. Rapid light curves (RLCs)**

Rapid light curves (RLCs) of the relative electron transport rate (rETR) measured against photosynthetically active radiation (PAR), applied in short steps increasing in irradiance, can also be generated using PAM fluorometry. The rETR is used as a measure of photosynthetic activity (Consalvey *et al*., 2005). These RLCs are frequently used to evaluate the relationship between photosynthetic activity and light and to provide information about the photoadaptive state of samples (Stibal *et al*., 2007). They can show short-term responses to light over the course of the RLC, and these responses can be influenced by the long-term history of their light environment (Ralph and Gademann, 2005). Immediately following measurement of $F_v/F_m$, RLCs were run for each sample by exposing the sample to a sequence of increasing PAR levels of 27, 137, 211, 312, 477, 712, 1011, 1403 and 2294 μmol photons $m^{-2}$ $s^{-1}$. Each step lasted 20 seconds and at each irradiance, the fluorescence yield ($F$) was measured. A saturating light

pulse was then applied for 0.6 seconds to close reaction centres and measure maximal fluorescence in the light ($F_m$') (Brooks and Niyogi, 2011). The rETR was plotted against PAR in the RLC. To allow for quantitative comparison of each RLC, the curves were fitted to a function introduced by Platt *et al*. (1980) by the accompanying PAM software, WinControl V3.29. The function, described in equation 3.2, allows for a decline in rETR at a high PAR, which is typical of a sample which has become photoinhibited and activity in PSII has been down regulated (White and Critchley, 1999). Each RLC displayed photoinhibition so this function was chosen over alternative functions suggested for RLCs which do not allow for photoinhibition (Ralph and Gademann, 2005). From each fitted curve, the WinControl V3.29 software generated a series of parameters describing the photosynthetic performance. These included the light utilisation coefficient (α), which is proportional to the efficiency of light utilisation and was estimated from the initial slope of the curve, the maximum rETR ($rETR_m$), which was calculated from equation 3.3. and is a measure of the rETR when photosynthesis becomes light saturated and the curve plateaus, and the light saturation coefficient ($E_k$), which was calculated from equation 3.4. and is a measure of the minimum irradiance which saturates photosynthesis, when the curve begins to plateau (Ralph and Gademann, 2005). Derivation of these parameters from a fitted curve are illustrated in Fig. 3.1.

$$\mathrm{rETR} = \mathrm{ETR_{mPot}} \times \left(1 - e^{-\frac{(\alpha \times \mathrm{PAR})}{\mathrm{ETR_{mPot}}}}\right) \times e^{-\frac{(\beta \times \mathrm{PAR})}{\mathrm{ETR_{mPot}}}} \qquad (3.2)$$

Where:

rETR = relative electron transport rate (µmol electrons $m^{-2}$ $s^{-1}$)

$ETR_{mPot}$ = maximum potential (without photoinhibition) electron transport rate (µmol electrons $m^{-2}$ $s^{-1}$)

*e* = exponential function

$\alpha$ = light utilisation coefficient

$\beta$ = photoinhibition parameter

PAR = photosynthetically active radiation (µmol photons $m^{-2}$ $s^{-1}$)

$$rETR_m = ETR_{mPot} \times \left(\frac{\alpha}{\alpha + \beta}\right) \times \left(\frac{\beta}{\alpha + \beta}\right)^{\frac{\beta}{\alpha}} \quad (3.3)$$

Where:

$rETR_m$ = relative maximum electron transport rate (µmol electrons $m^{-2}$ $s^{-1}$)

$$E_k = \frac{rETR_m}{\alpha} \quad (3.4)$$

Where:

$E_k$ = light saturation coefficient (µmol photons $m^{-2}$ $s^{-1}$)

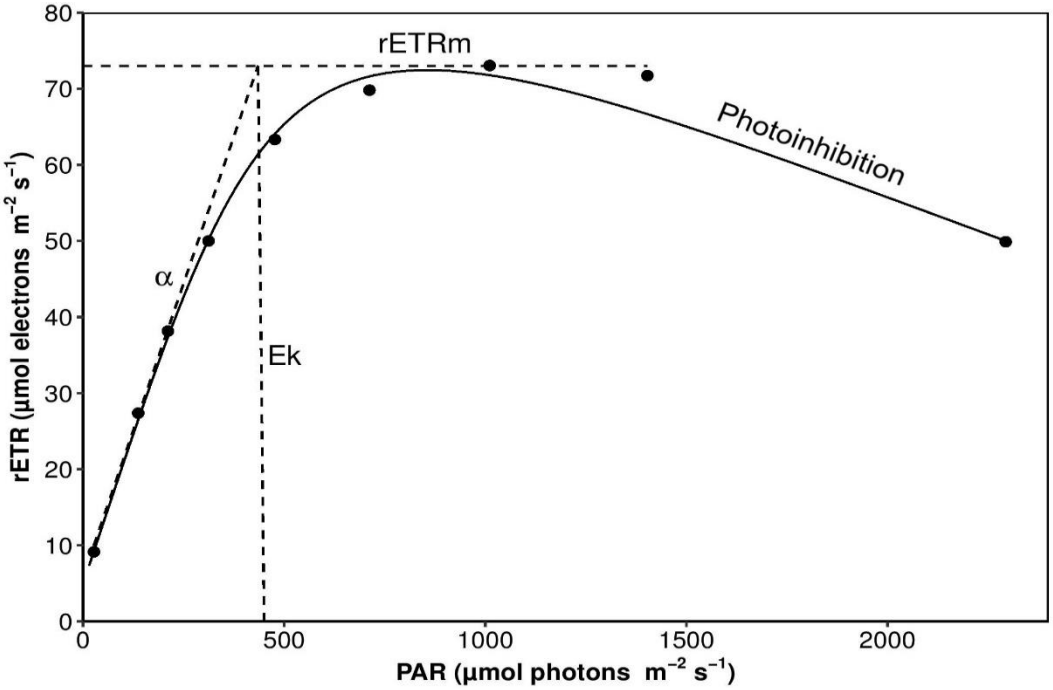


Figure 3.1. Typical rapid light curve fitted to the function introduced by Platt *et al*. (1980) illustrating estimation of the light utilisation coefficient (α), the relative maximum electron transport rate ($rETR_m$) and the light saturation coefficient ($E_k$). Photoinhibition is shown as the decline in rETR after reaching the $rETR_m$ at a relatively high level of photosynthetically active radiation (PAR). The curve was generated using Antarctic *C. subellipsoidea* grown at 10 °C and a constant growth light of 60 µmol photons $m^{-2}$ $s^{-1}$ for 16 days.

### 3.2.2.3. Non-photochemical quenching (NPQ)

Energy conversion routes in PSII were calculated from the fluorescence measurements at each light step during the RLC as the quantum yields (Y) of photochemical energy conversion (Y(II)), regulated NPQ (Y(NPQ)), and non-regulated NPQ (Y(NO)), as shown in equations 3.5-3.7. (Klughammer and Schreiber, 2008). These values are expressed as fractions and Y(II), Y(NPQ) and Y(NO) give a sum of 1 (Kramer *et al*., 2004). To compare the energy conversion routes taken by each sample in response to a high irradiance, the ratio of Y(NPQ) to Y(NO) when exposed to the highest PAR level of 2294 µmol photons $m^{-2}$ $s^{-1}$ was calculated (Figueroa *et al*., 2019). The process was repeated for each sample and the cuvette was washed out with distilled water between replicates and with ethanol followed by distilled water between different sample groups.

$$\mathrm{Y(II)} = \frac{(F_m{}' - F)}{F_m{}'} \tag{3.5}$$

Where:

Y(II) = quantum yield of photochemical energy conversion in PSII

$F_m$' = maximal fluorescence in the light adapted state

$F$ = fluorescence yield before saturation pulse

$$\mathrm{Y(NPQ)} = \left(\frac{F}{F_m{}'}\right) - \left(\frac{F}{F_m}\right) \tag{3.6}$$

Where:

Y(NPQ) = quantum yield of regulated non-photochemical energy conversion in PSII

$$\mathrm{Y(NO)} = \frac{F}{F_m} \tag{3.7}$$

Where:

Y(NO) = quantum yield of non-regulated non-photochemical energy conversion in PSII

### 3.2.3. Extraction of pigments

Pigment concentrations were measured on day 15 of the experiment. Aseptic techniques were adopted throughout to avoid contamination of samples. After 15 days of growth, 2 ml of a well-mixed culture was transferred from each flask into a Falcon tube with the exception of the 6 °C samples where 5 ml was taken because of the lower cell concentration. Samples were centrifuged at 4000 rpm for 15 minutes and the supernatant was discarded. The pelleted cells were resuspended in 2.5 ml of pure ethanol and vortexed (Analog Vortex Mixer, VWR, Leicestershire, England). Samples were stored for 24 hours in a 5 °C fridge (LMS, Kent, England) in the dark. After 24 hours, the samples were vortexed and centrifuged again at 4000

rpm for 15 minutes to separate the pigment extract from the cells. Using the Biochrom WPA spectrophotometer, 1 ml of extract from each sample was transferred to a cuvette and absorbance was measured at 665 nm for Chl a, 649 nm for Chl b, 470 nm for carotenoids and 750 nm to correct for turbidity after zeroing the spectrophotometer with pure ethanol.

Using the equations taken from Lichtenthaler and Welburn (1983) below, the concentration of Chl a (3.8), Chl b (3.9) and carotenoids (3.10) were calculated for each sample where A is the absorbance at the given wavelength (nm). Absorbance at 750 nm was subtracted from the absorbance for each pigment to account for turbidity in the sample. The absorbance was multiplied by the absorption coefficient for each pigment determined by Lichtenthaler and Welburn (1983). To correct for the sample volumes and solvent volumes used, the concentration was multiplied by the extract volume (ml) and divided by the volume of the sample spun (L). The final pigment concentration (pg $L^{-1}$) was then divided by the cell concentration (cells $L^{-1}$) to determine the concentration of each pigment per cell (pg $cell^{-1}$). The ratio of Chl a to Chl b was then calculated for each sample.

$$\text{Chl a} = \big(13.95 \times (\text{A665} - \text{A750})\big) - \big(6.88 \times (\text{A649} - \text{A750})\big) \tag{3.8}$$

$$\text{Chl b} = \big(24.96 \times (\text{A649} - \text{A750})\big) - \big(7.32 \times (\text{A665} - \text{A750})\big) \tag{3.9}$$

$$\text{Carotenoids} = \frac{\big(1000 \times (\text{A470} - \text{A750})\big) - \big(2.05 \times (\text{A665} - \text{A750})\big) - \big(114.8 \times (\text{A649} - \text{A750})\big)}{245} \tag{3.10}$$

### 3.2.4. Measurement of the rate of respiration

The rate of respiration was measured for the four replicate cultures at each set of temperature and irradiance conditions after 37 days of growth. On day 37, 4 ml was transferred from each culture into a Falcon tube and kept at the growth temperature for a maximum of 15 minutes until measurements were taken. The Hansatech Instruments Oxylab liquid phase oxygen electrode control unit with a DW2 electrode chamber was used to measure oxygen the

concentration which was recorded in $O_2$ View V2.06 software (Hansatech Instruments, England). The temperature in the chamber was maintained at the growth temperature using a circulating water bath (Thermo Haake C10, Germany). At each temperature, the system was calibrated using air saturated deionised water and then sodium hydrosulfite was added to remove oxygen from the water. For each sample, 2 ml was loaded into the empty electrode chamber and the oxygen concentration was measured per second for a minimum of 5 minutes whilst the sample was stirred at a rate of 85 rpm with a magnetic stirrer. The electrode chamber was washed out with distilled water between samples.

The rate of respiration for each sample was calculated from the change in oxygen concentration (μmol $O_2$ $ml^{-1}$ $h^{-1}$) after 1.5 minutes, to allow the cells to adjust to the chamber conditions, until 5 minutes. For 6 and 18 °C, the remaining 2 ml of each sample was used to calculate the concentration of Chl a to normalise the rate of respiration for biomass. The pigment extraction methods are described in section 3.2.3. The concentration was corrected for the sample volume and solvent volume as before. The rate of respiration was divided by the concentration of Chl a (mg $ml^{-1}$) to give the rate in μmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$.

Pigment extractions were not performed for the 10 °C samples on the same day respiration was measured. Therefore, there was no matching pigment data to normalise the 10 °C respiration rates for biomass with. To overcome this, the concentration of Chl a for these samples was predicted using the cell concentration of the respiration samples, which was measured on the same day respiration was measured. To produce a standard curve, a stock culture of each strain grown at 10 °C and 60 μmol photons $m^{-2}$ $s^{-1}$ was diluted to give an OD range of 0.05 to 2. The cell concentration was predicted using the OD according to the relationship described in Chapter 2 section 2.2.3. The pigments were extracted using the methods outlined in section 3.2.3. A standard curve was constructed by plotting the Chl a concentration against the cell concentration (Fig. 3.2) and based on the cell concentration of the day 37 10 °C samples, the

Chl a concentration was predicted using linear regression. The rate of respiration was divided by the predicted Chl a concentration for each 10 °C sample to give the biomass-specific rate in μmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$, enabling comparisons to be made between samples varying in their biomass.

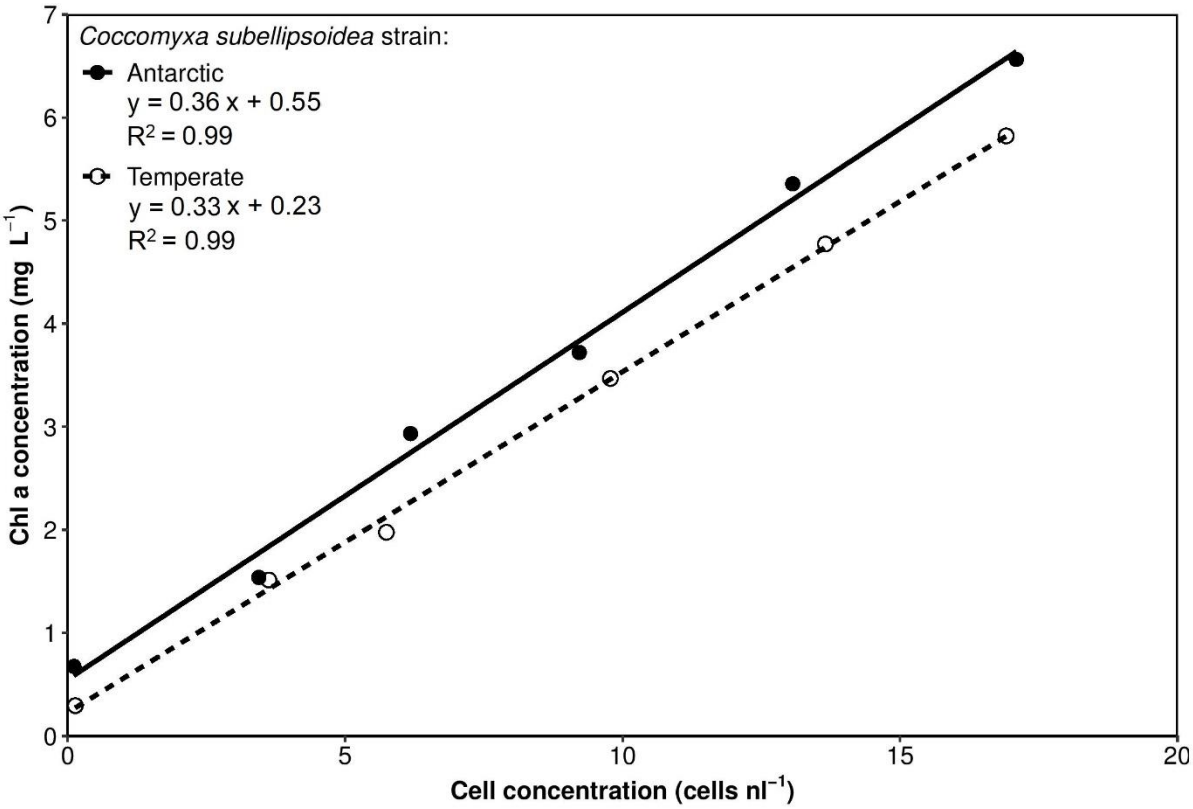


Figure 3.2. Standard curve of the chlorophyll a (Chl a) concentration and cell concentration of an Antarctic and temperate strain of *C. subellipsoidea*. Based on cultures grown at 10 °C and constant irradiance of 60 μmol photons $m^{-2}$ $s^{-1}$.

### 3.2.5. Statistical analysis

Statistical analyses were performed in R V3.6.0 (R Core Team, 2019) and plots were constructed using the ggplot2 package (Wickham, 2016). Linear regression was used to predict the cell concentration from the OD and the Chl a concentration from the cell concentration. The means and standard errors were calculated from four independent replicates. A Shapiro-Wilk test was performed to test for normal distribution of residuals and a Levene's test was used to test for homogeneity of variances. To determine whether temperature and irradiance

had a significant effect on the photosynthetic activity parameters, pigment composition and the rate of respiration, and if there was a significant interaction effect between temperature and irradiance, a two-way ANOVA was performed. A one-way ANOVA was used to identify if there was a significant difference in each of these parameters between the Antarctic and temperate strain. A significance level of $P < 0.05$ was considered significant, and where significant differences were identified, Tukey's pairwise comparison *post-hoc* test was used.

## 3.3. Results

### 3.3.1. Photosynthetic activity in response to temperature and irradiance

#### 3.3.1.1. Maximum quantum yield of PSII ($F_v/F_m$)

The Antarctic strain of *C. subellipsoidea* displayed little variation in the maximum quantum yield of PSII, or $F_v/F_m$, which was maintained between 0.60 and 0.70 at each temperature and irradiance (Fig. 3.3). A two-way ANOVA for temperature and irradiance followed by a Tukey's pairwise comparison showed the $F_v/F_m$ at 10 °C to be significantly higher compared to that at 18 °C ($F_{[2,27]} = 3.371$, $P < 0.05$). There was no significant difference in the $F_v/F_m$ depending on the irradiance ($F_{[2,27]} = 1.649$, $P > 0.1$).

The $F_v/F_m$ of the temperate strain at 6 °C was significantly lower compared to that at 10 and 18 °C ($F_{[2,27]} = 260.217$, $P < 0.001$) (Fig. 3.3). Variation in the $F_v/F_m$ between 10 and 18 °C was small and non-significant ($P > 0.05$). There was also no significant difference depending on irradiance ($F_{[2,27]} = 259.28$, $P < 0.001$).

The Antarctic strain of *C. subellipsoidea* experienced a higher $F_v/F_m$ at each temperature and irradiance compared to the temperate strain (Fig. 3.3). The largest differences between the strains occurred at 6 °C (Fig. 3.3 A). A one-way ANOVA followed by Tukey's pairwise

comparison indicated that the $F_v/F_m$ of the Antarctic strain was only significantly higher compared to that of the temperate strain at 6 °C ($F_{[19,52]} = 38.03$, $P < 0.001$).

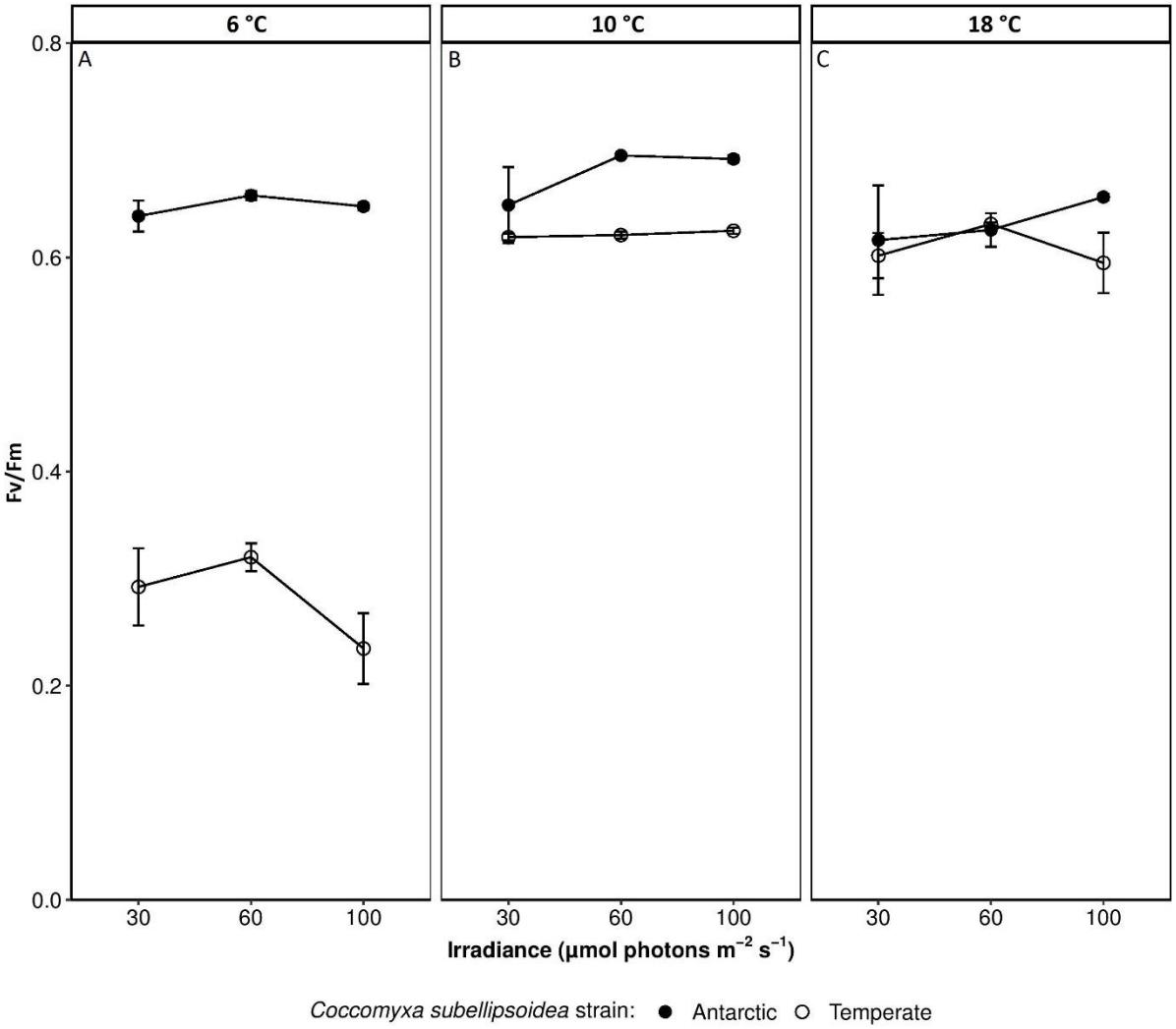


Figure 3.3. Maximum quantum yield of PSII ($F_v/F_m$) of a temperate and Antarctic strain of *C. subellipsoidea* measured after 16 days of growth at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

#### 3.3.1.2. Rapid light curves (RLCs)

The Antarctic strain of *C. subellipsoidea* displayed a higher rETR at each temperature and irradiance compared to the temperate strain (Fig. 3.4). The difference in the profile of the RLCs between the strains was most notable at 6 °C (Fig. 3.4 A-C), whereas at 10 and 18 °C (Fig. 3.4 C-I), the difference between the strains was reduced.

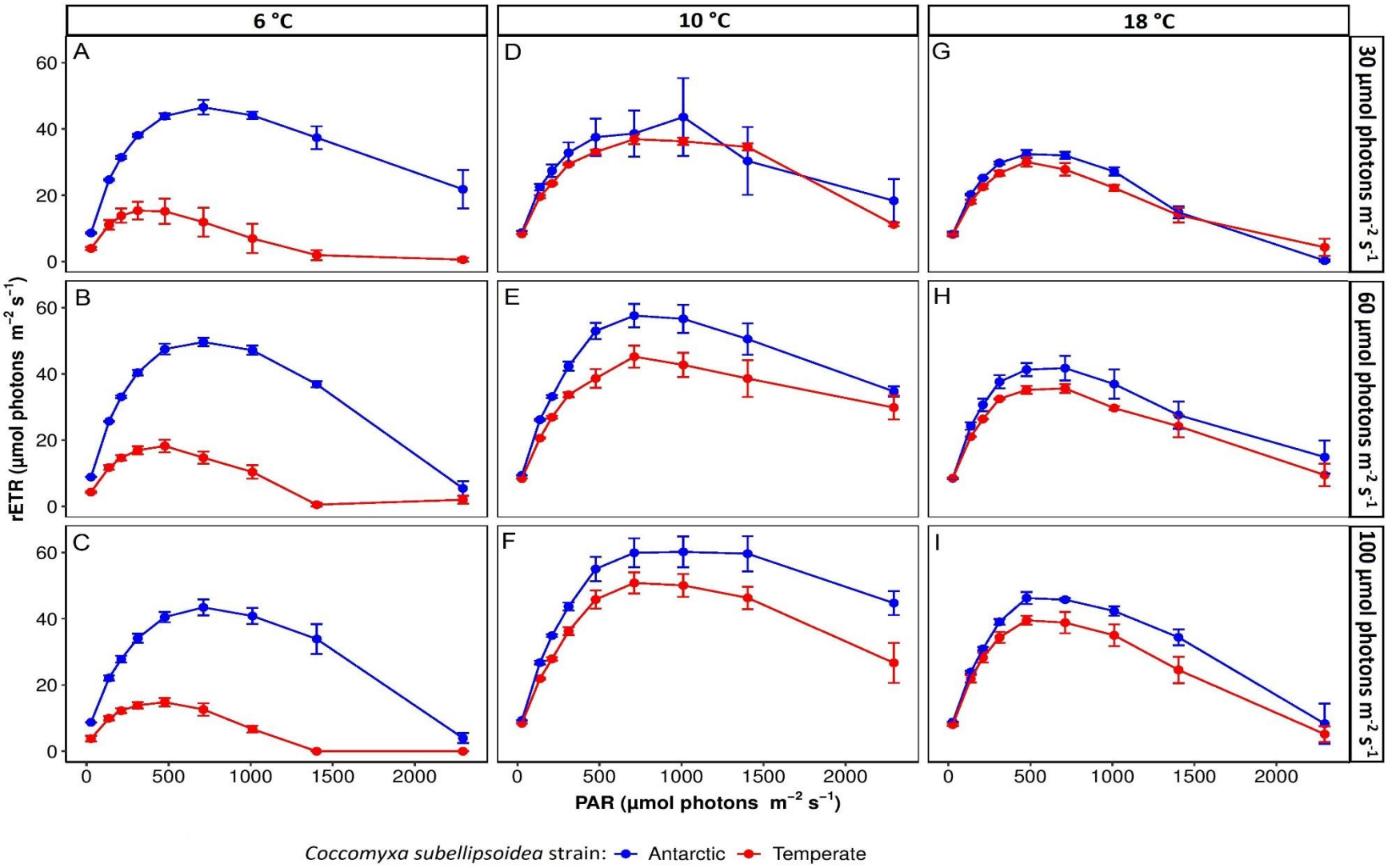


Figure 3.4. Rapid light curves of the relative electron transport rate (rETR) at increasing steps of photosynthetically active radiation (PAR) for an Antarctic and temperate strain of *C. subellipsoidea* grown for 16 days at 6 (A-C), 10 (D-F) or 18 °C (G-I) and constant irradiance of 30 (A, D, G), 60 (B, E, H) or 100 µmol photons $m^{-2}$ $s^{-1}$ (C, F, I). Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 3.3.1.3. Light utilisation coefficient (α)

The α of the Antarctic strain was between 0.24 ± 0.0, achieved at 18 °C and 100 µmol photons $m^{-2}$ $s^{-1}$, and 0.18 ± 0.0, achieved at 6 °C and 100 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 3.5 A and C). A two-way ANOVA indicated a significant interaction effect between temperature and irradiance ($F_{[4,27]}$ = 15.568, $P < 0.001$). A Tukey's *post-hoc* test revealed that for the Antarctic strain grown at 10 and 18 °C, there was a significant increase in α with increasing irradiance ($P < 0.05$), but at 6 °C, the α was significantly higher α at 30 and 60 µmol photons $m^{-2}$ $s^{-1}$ compared to at 100 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.01$).

The highest α of the temperate strain was 0.213 ± 0.00 which occurred at 18 °C and 100 µmol photons $m^{-2}$ $s^{-1}$, whilst the lowest was 0.112 ± 0.01 also at 100 µmol photons $m^{-2}$ $s^{-1}$, but at 6 °C (Fig. 3.5 A and C). The α was significantly lower at 6 °C than at 10 and 18 °C ($F_{[4,27]}$ = 14.211, $P < 0.001$). The relationship with irradiance within each temperature varied. At 6 °C, there was a significant decrease in α with increasing irradiance, but the relationship was reversed at 18 °C with a significant increase with irradiance ($P < 0.05$).

The α of the Antarctic strain was greater compared to that of the temperate strain at all temperatures and irradiance levels (Fig. 3.5), but was only significantly higher, compared to the temperate strain, at 6 and 10 °C ($F_{[19,52]}$ = 37.97, $P < 0.001$). The difference was most notable at 6 °C, where the α of the Antarctic strain was 0.18-0.22 but that of the temperate strain was 0.11-0.14 (Fig. 3.5 A).

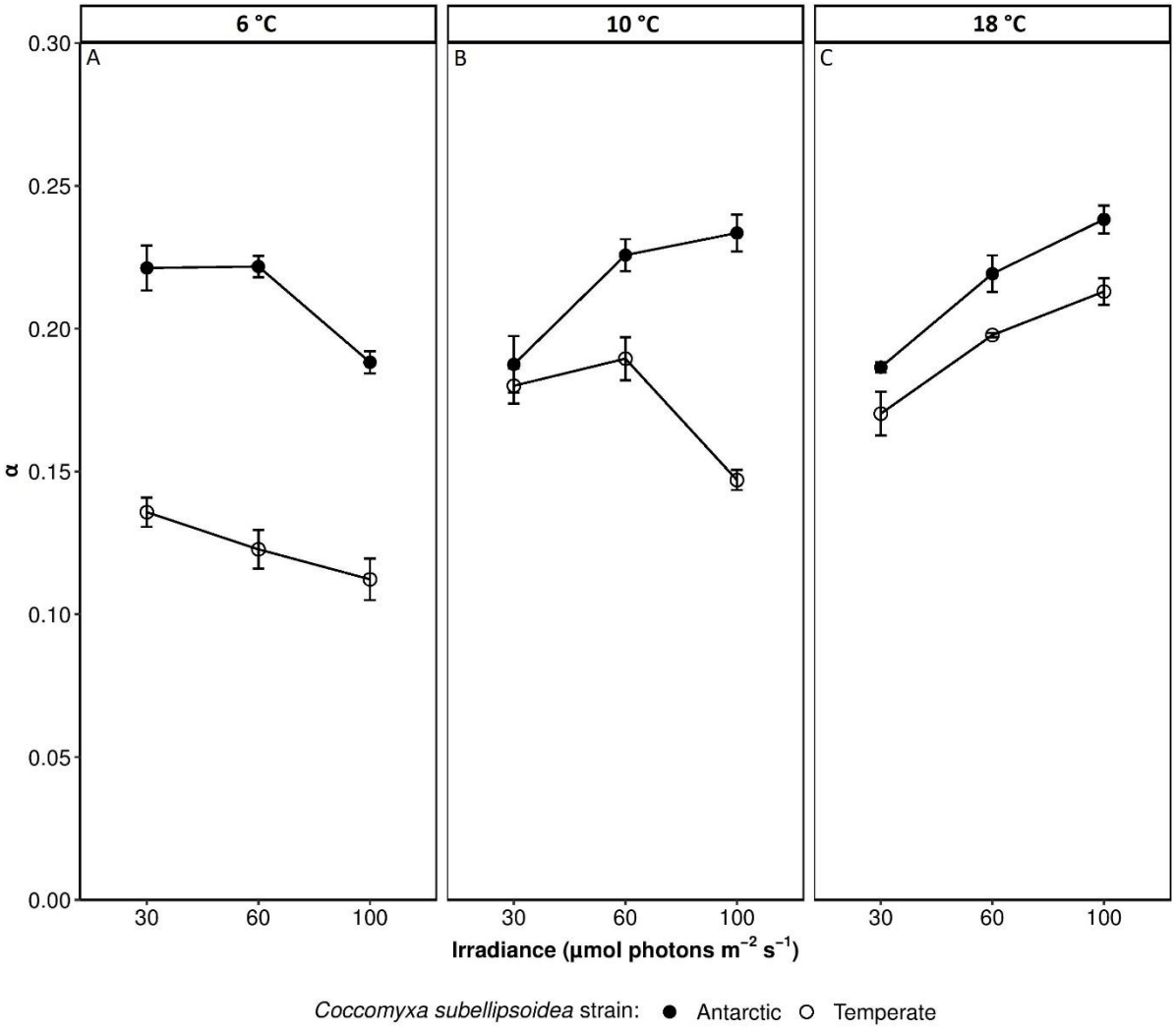


Figure 3.5. Light utilisation coefficient (α) of a temperate and Antarctic strain of *C. subellipsoidea* measured 16 days of growth at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 3.3.1.4. Relative maximum electron transport rate ($rETR_m$)

The lowest $rETR_m$ of the Antarctic strain was 33.92 ± 0.8 µmol electrons $m^{-2}$ $s^{-1}$ which was seen at 18 °C and 30 µmol photons $m^{-2}$ $s^{-1}$. The highest was almost double this, at 61.73 ± 4.8 µmol electrons $m^{-2}$ $s^{-1}$ which occurred at 10 °C and 100 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 3.6 B and C). The $rETR_m$ at 10 °C was significantly higher compared to that at 18 °C at all irradiance levels and compared to that at 6 °C at 60 and 100 µmol photons $m^{-2}$ $s^{-1}$ ($F_{[4,27]} = 3.203$, $P < 0.05$), as revealed by a two-way ANOVA followed by a Tukey's *post-hoc* test. Within each temperature, the $rETR_m$ also differed depending on the irradiance. At 10 and 18 °C, there was a significant increase with irradiance ($P < 0.01$).

The $rETR_m$ of the temperate strain of *C. subellipsoidea* was significantly lower at 6 °C, where it was 15-18 µmol electrons $m^{-2}$ $s^{-1}$, compared to at 10 and 18 °C, where it was 30-50 µmol electrons $m^{-2}$ $s^{-1}$ ($F_{[4,27]} = 5.558$, $P < 0.05$) (Fig. 3.6). The only significant difference between irradiance levels within a temperature occurred at 10 and 18 °C where the $rETR_m$ at 100 µmol photons $m^{-2}$ $s^{-1}$ was significantly higher compared to that at 30 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.01$).

The Antarctic strain had a 3-fold higher $rETR_m$ at 6 °C compared to the temperate strain, and this difference was significant ($F_{[19,52]} = 26.25$, $P < 0.001$) (Fig. 3.6 A). At 10 and 18 °C, there were no significant differences in $rETR_m$ between the two strains ($P > 0.05$) (Fig. 3.6 B and C).

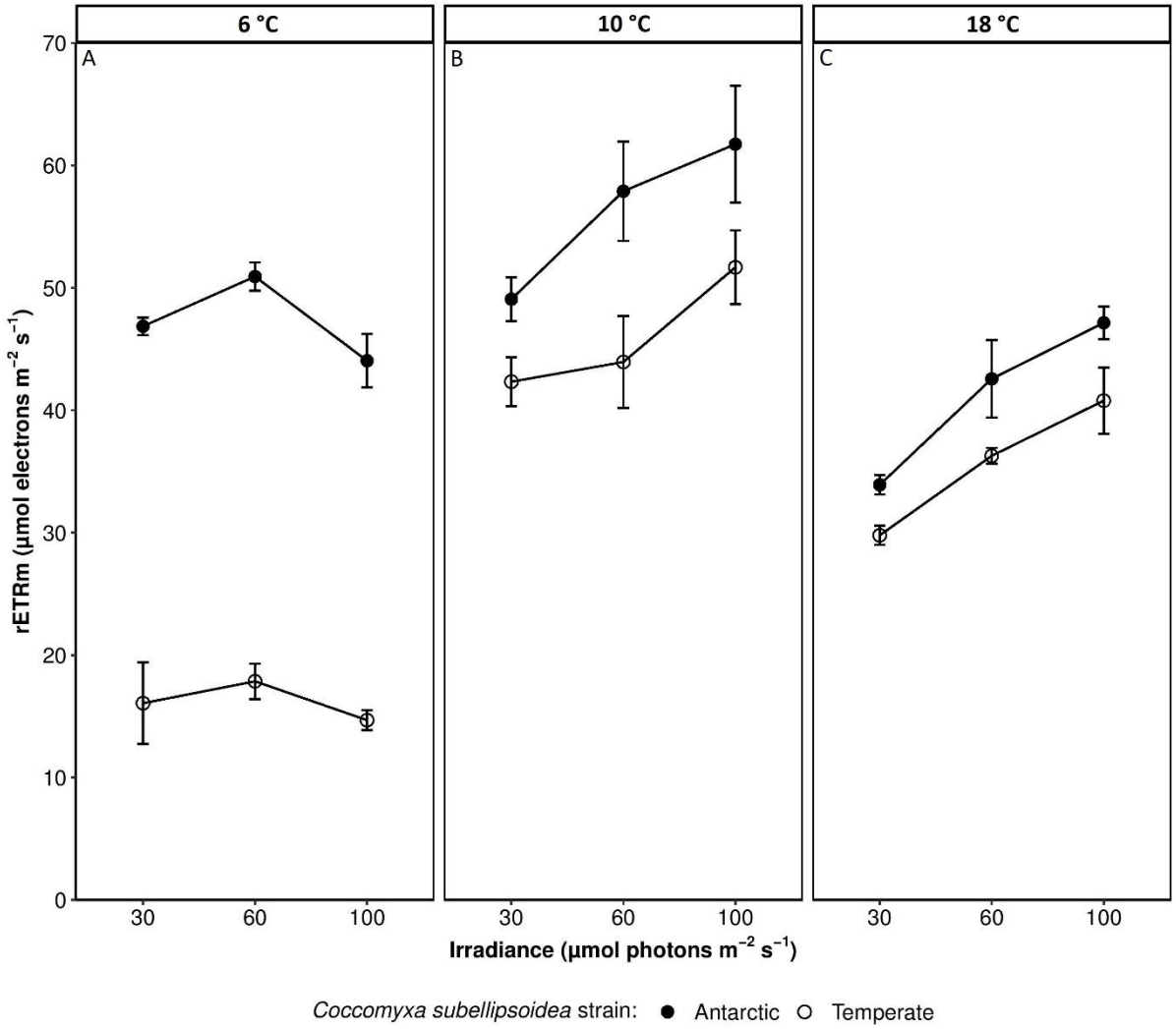


Figure 3.6. Relative maximum electron transport rate ($rETR_m$) of a temperate and Antarctic strain of *C. subellipsoidea* measured after 16 days of growth at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 3.3.1.5. Light saturation coefficient ($E_k$)

For the Antarctic strain, the highest $E_k$ of 266.06 ± 26.2 µmol photons $m^{-2}$ $s^{-1}$ occurred at 10 °C and 100 µmol photons $m^{-2}$ $s^{-1}$ and the lowest of 181.96 ± 5.2 µmol photons $m^{-2}$ $s^{-1}$ occurred at 18 °C and 30 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 3.7 B and C). A two-way ANOVA followed by a Tukey's *post-hoc* test indicated that the $E_k$ at 10 °C was significantly higher compared to that at 6 °C and 18 °C ($F_{[2,27]} = 14.696$, $P < 0.001$). There was no significant difference in $E_k$ depending on irradiance at any temperature ($F_{[2,27]} = 0.488$, $P > 0.1$).

The temperate strain experienced a greater $E_k$ at 10 °C, where it was 235-350 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 3.7 B) and a lower $E_k$ at 6 °C, where it was 117-145 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 3.7 A). The $E_k$ at 6 °C was significantly lower compared to that at 10 and 18 °C ($F_{[4,27]} = 4.923$, $P < 0.01$). There was no significant difference between irradiance levels within a temperature at 6 or 18 °C ($P > 0.05$), but at 10 °C the $E_k$ at 100 µmol photons $m^{-2}$ $s^{-1}$ was significantly higher compared to that at 30 and 60 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.01$).

There was a 2-fold higher $E_k$ for the Antarctic strain compared to the temperate strain at 6 °C and the difference was significant ($F_{[19,52]} = 10.07$, $P < 0.001$) (Fig. 3.7 A). The Antarctic strain also had a higher $E_k$ at 18 °C at all irradiances but only at 30 and 60 µmol photons $m^{-2}$ $s^{-1}$ at 10 °C (Fig. 3.7 B and C). At 10 °C and 100 µmol photons $m^{-2}$ $s^{-1}$, the temperate strain had a 1.3-fold higher $E_k$ compared to the Antarctic strain, but this was the only instance where the temperate strain had a higher $E_k$. There was no significant difference between the strains at 10 or 18 °C ($P > 0.05$).

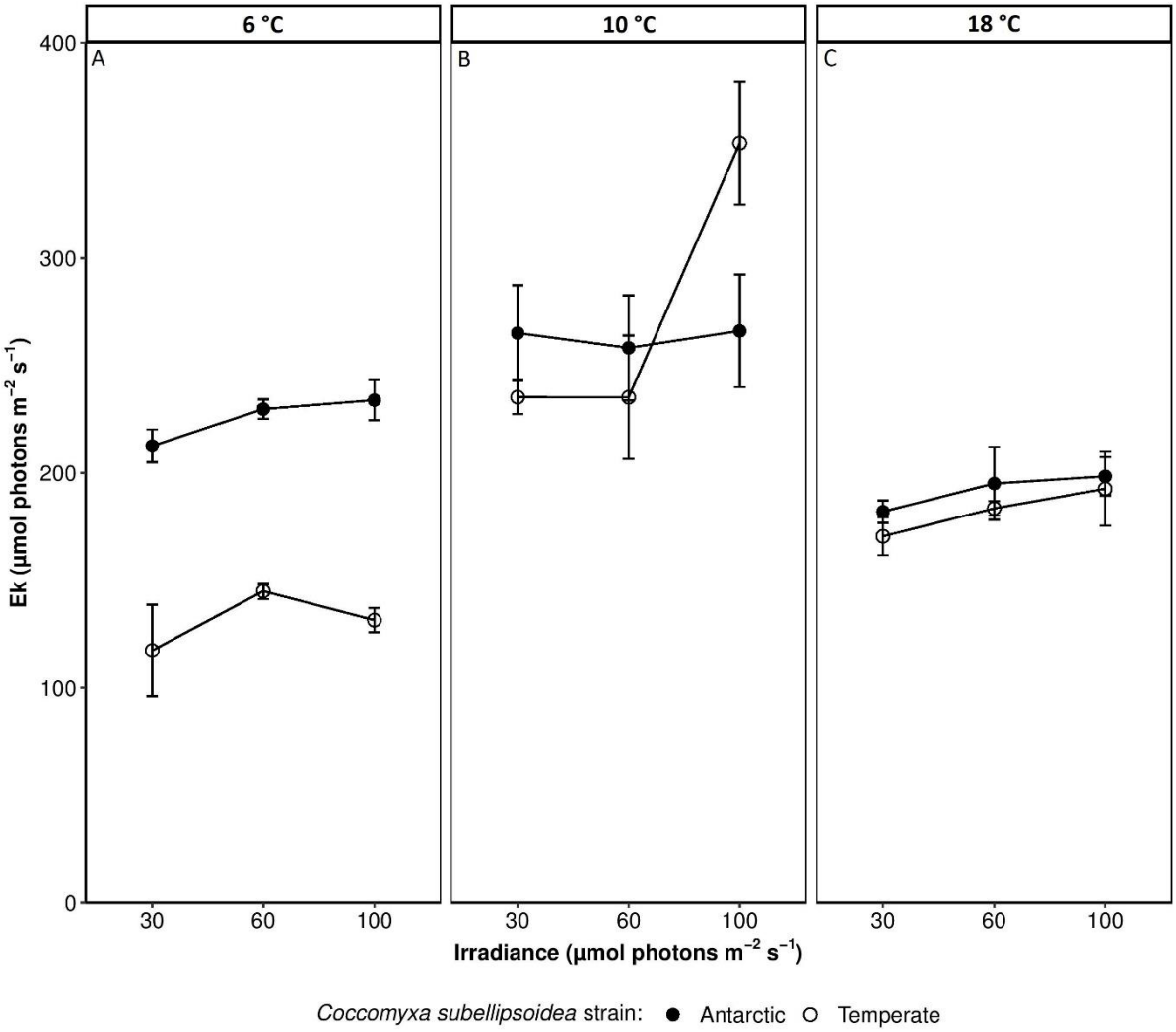


Figure 3.7. Light saturation coefficient ($E_k$) of a temperate and Antarctic strain of *C. subellipsoidea* measured after 16 days of growth at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 3.3.1.6. Ratio of the quantum yields of regulated non-photochemical quenching (Y(NPQ)) to non-regulated non-photochemical quenching (Y(NO))

The Y(NPQ)/Y(NO) of the Antarctic strain decreased with temperature and increased with irradiance (Fig. 3.8). A two-way ANOVA followed by a Tukey's *post-hoc* test revealed that the Y(NPQ)/Y(NO) at 6 °C was significantly higher compared to that at 10 and 18 °C ($F_{[4,27]} = 22.83$, $P < 0.001$). There was no significance in the Y(NPQ)/Y(NO) between 10 and 18 °C ($P > 0.05$). Within each temperature, the increase with irradiance was significant between each irradiance level ($P < 0.001$).

There were small variations in the Y(NPQ)/Y(NO) of the temperate strain depending on temperature (Fig. 3.8). The Y(NPQ)/Y(NO) was significantly higher at 10 °C compared to at 6 and 18 °C, but only at 60 µmol photons $m^{-2}$ $s^{-1}$ ($F_{[4,27]} = 7.194$, $P < 0.001$). The Y(NPQ)/Y(NO) at 18 °C was significantly lower compared to that at 6 and 10 °C, but only at 100 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.001$). There was a significant increase with irradiance within each temperature, with a larger difference between irradiances at 6 °C ($P < 0.05$).

The Y(NPQ/Y(NO) was 2-3-fold higher for the Antarctic strain of *C. subellipsoidea* at 6 °C, ranging between 2 and 3.5, but that of the temperate strain was between 0.5 and 1.7 (Fig. 3.8 A). The only significant differences between the strains occurred at 6 °C where the Y(NPQ)/Y(NO) of the Antarctic strain was significantly higher ($F_{[19,52]} = 53.56$, $P < 0.001$).

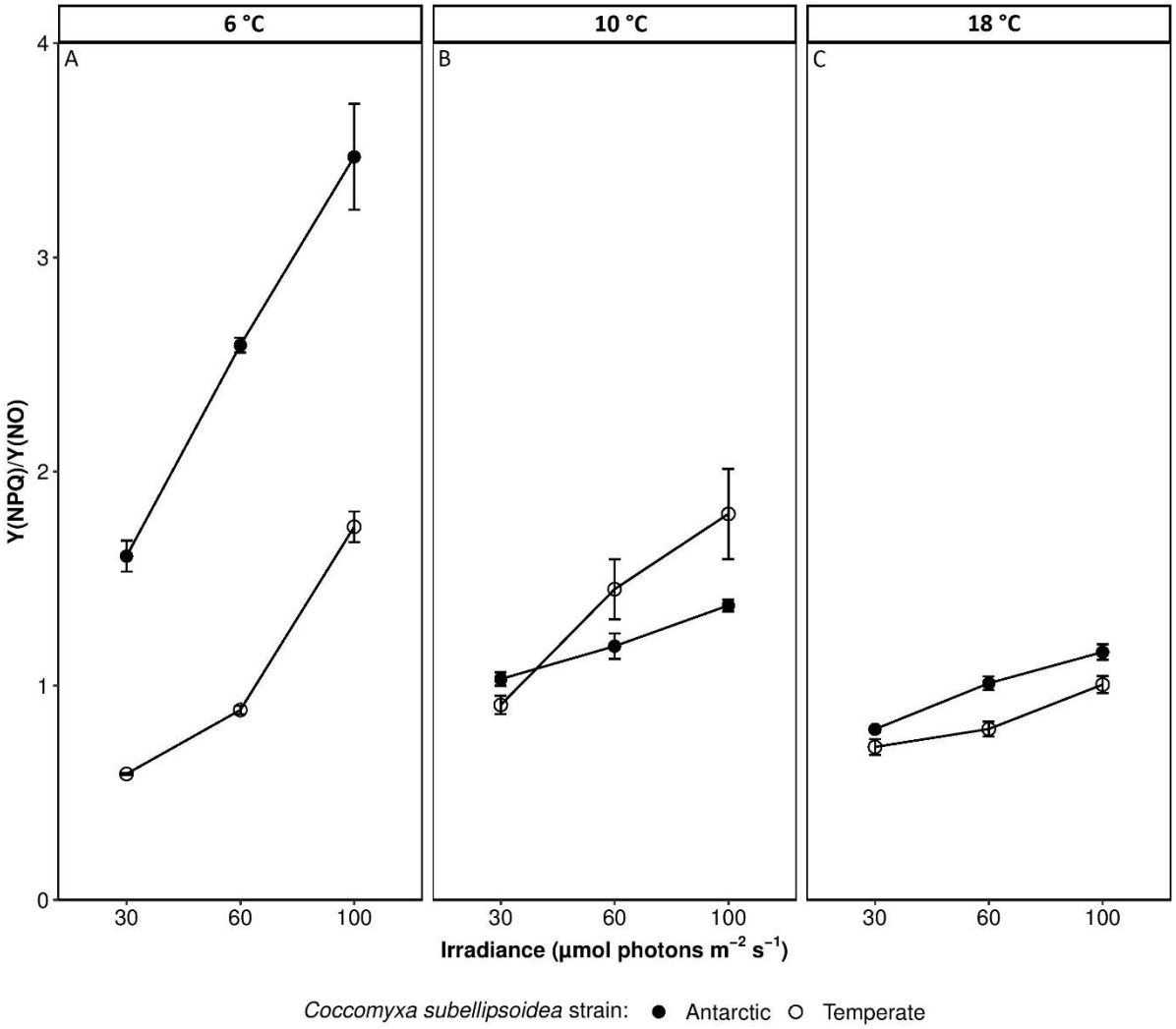


Figure 3.8. Ratio of the quantum yield of regulated (Y(NPQ)) to non-regulated (Y(NO)) non-photochemical quenching at an irradiance of 2294 µmol photons $m^{-2}$ $s^{-1}$ measured during a rapid light curve for a temperate and Antarctic strain of *C. subellipsoidea* after 16 days of growth at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 3.3.1.7. Summary of photosynthetic activity

The $F_v/F_m$, $rETR_m$ and $E_k$ of the Antarctic strain were significantly higher at 10 °C and lower at 18 °C. The relationship between α and temperature depended on the irradiance, where the α was greater at a higher irradiance at 10 and 18 °C, but lower at a higher irradiance at 6 °C. The $rETR_m$ was also higher at a higher irradiance at 10 and 18 °C, but was reduced at a higher

irradiance at 6 °C. The $F_v/F_m$ and $E_k$ displayed little variation depending on the irradiance. The Y(NPQ)/Y(NO) decreased with temperature and increased with irradiance.

The $F_v/F_m$, α, $rETR_m$ and $E_k$ values for the temperate strain were significantly lower at 6 °C compared to at 10 and 18 °C. There was little variation in $F_v/F_m$ depending on irradiance. The α, $rETR_m$ and $E_k$ were each higher at a higher irradiance at 18 °C, but lower at a higher irradiance at 6 °C. The Y(NPQ)/Y(NO) was lowest at 18 °C and increased with irradiance.

Marked differences in photosynthetic activity were recorded between the Antarctic and temperate strain at 6 °C, where the $F_v/F_m$, α, $rETR_m$, $E_k$ and Y(NPQ)Y(NO) were significantly lower for the temperate strain compared to the Antarctic strain. Smaller differences were recorded between the strains at 10 and 18 °C.

### 3.3.2. The influence of temperature and irradiance on the pigment composition

#### 3.3.2.1. Ratio of chlorophyll a to b (Chl a/b)

The Chl a/b ratio (Table 3.1) was significantly higher at 10 °C compared to the ratio 18 °C for the Antarctic ($F_{[2,27]} = 4.083$, $P < 0.05$) and temperate strain ($F_{[2,27]} = 34.420$, $P < 0.001$), as indicated by the two-way ANOVA followed by a Tukey's *post-hoc* test. There was a significant increase in the Chl a/b ratio with increasing irradiance for the Antarctic ($F_{[2,27]} = 14.549$, $P < 0.001$) and temperate strain ($F_{[2,27]} = 21.406$, $P < 0.001$).

Variation in the Chl a/b ratio between each strain was relatively small (Table 3.1). The Antarctic strain had a lower Chl a/b ratio compared to that of the temperate strain at 10 °C at all irradiances and at 6 and 18 °C at 30 µmol photons $m^{-2}$ $s^{-1}$. However, the Chl a/b ratio of the Antarctic strain was only significantly lower compared to that of the temperate strain at 10 °C ($F_{[19,52]} = 6.543$, $P < 0.001$).

### 3.3.2.2. Carotenoid content per cell

The carotenoid content per cell decreased significantly with increasing temperature for the Antarctic ($F_{[2,27]} = 76.155$, $P < 0.001$) and temperate strain ($F_{[2,27]} = 142.077$, $P < 0.001$) (Table 3.1). The carotenoid content decreased significantly with increasing irradiance for the Antarctic ($F_{[2,27]} = 152.008$, $P < 0.001$) and temperate strain ($F_{[2,27]} = 145.509$, $P < 0.001$).

The Antarctic strain of *C. subellipsoidea* had a significantly higher carotenoid content per cell at each temperature and irradiance compared to the temperate strain ($F_{[19,52]} = 55.97$, $P < 0.001$) (Table 3.1). The difference in the content between each strain was greatest at 18 °C, where the carotenoid concentration of the Antarctic strain was 1.4-2-fold higher. The difference in the concentration between the strains at 6 and 10 °C was 1.2-fold.

Table 3.1. Ratio of chlorophyll a to b (Chl a/b) and carotenoid content per cell of an Antarctic and temperate strain of *C. subellipsoidea* measured after 15 days of growth at 6, 10 or 18 °C and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$.

| **Temperature (°C)** | **Irradiance (µmol photons $m^{-2}$ $s^{-1}$)** | **Chl a/b** | | **Carotenoid content (pg $cell^{-1}$)** | |
|---|---|---|---|---|---|
| | | **Antarctic** | **Temperate** | **Antarctic** | **Temperate** |
| 6 | 30 | 1.186±0.06 | 1.587±0.08 | 0.314±0.01 | 0.275±0.01 |
| | 60 | 1.750±0.47 | 1.667±0.22 | 0.226±0.01 | 0.188±0.01 |
| | 100 | 2.452±0.10 | 2.275±0.07 | 0.168±0.00 | 0.163±0.00 |
| 10 | 30 | 1.607±0.23 | 1.907±0.07 | 0.234±0.01 | 0.214±0.01 |
| | 60 | 1.787±0.23 | 2.317±0.18 | 0.176±0.01 | 0.142±0.01 |
| | 100 | 2.801±0.17 | 3.186±0.23 | 0.102±0.01 | 0.093±0.00 |
| 18 | 30 | 1.101±0.10 | 1.186±0.06 | 0.218±0.02 | 0.160±0.01 |
| | 60 | 1.573±0.14 | 1.536±0.09 | 0.119±0.02 | 0.061±0.01 |
| | 100 | 1.822±0.36 | 1.621±0.22 | 0.083±0.01 | 0.051±0.01 |

### 3.3.3. The influence of temperature and irradiance on the rate of respiration

#### 3.3.3.1. Rate of respiration of an Antarctic strain of *Coccomyxa subellipsoidea*

For the Antarctic strain, the rate of respiration decreased by 1.4-2-fold from 6 to 18 °C (Fig. 3.9). A two-way ANOVA identified a significant interaction effect between temperature and irradiance ($F_{[4,27]} = 4.559$, $P < 0.01$). A Tukey's *post-hoc* test indicated the rate at 18 °C was significantly lower than the rate at 6 °C at all irradiance levels and the rate at 10 °C at 30 and 60 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.05$). There was no significant difference in the rates between 6 and 10 °C ($P > 0.1$). The highest respiration rate was seen at 100 µmol photons $m^{-2}$ $s^{-1}$ for each temperature which was significantly higher than the lowest rate which occurred at 30 µmol photons $m^{-2}$ $s^{-1}$ at 10 and 18 °C, but at 60 µmol photons $m^{-2}$ $s^{-1}$ at 6 °C ($P < 0.01$).

#### 3.3.3.2. Rate of respiration of a temperate strain of *Coccomyxa subellipsoidea*

The temperate strain experienced a 3-4-fold decrease in the rate of respiration from 6 to 18 °C (Fig. 3.9). The respiration rate at 18 °C was significantly lower compared to that at 6 and 10 °C at each irradiance level ($F_{[4,27]} = 31.151$, $P < 0.001$). The rate at 10 °C was significantly lower compared to that at 6 °C at 60 and 100 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.001$). There were also significant differences in the rate within a temperature depending on irradiance. At 6 °C, the rate at 60 µmol photons $m^{-2}$ $s^{-1}$ was significantly higher compared to that at 30 and 100 µmol photons $m^{-2}$ $s^{-1}$. At 10 °C, the rate at 30 µmol photons $m^{-2}$ $s^{-1}$ was significantly higher compared to that at 60 and 100 µmol photons $m^{-2}$ $s^{-1}$ ($P < 0.001$). The increase with irradiance at 18 °C was not significant between any irradiance level ($P > 0.1$).

#### 3.3.3.3. Comparison of the respiration rate between an Antarctic and temperate strain of *Coccomyxa subellipsoidea*

Between the strains, the rate of respiration was higher for the temperate strain compared to the Antarctic strain of *C. subellipsoidea* at each temperature and irradiance, apart from at 18 °C

and 100 µmol photons $m^{-2}$ $s^{-1}$, where the rate of the Antarctic strain was slightly higher (Fig. 3.9). Both strains experienced their lowest rate of respiration at 18 °C and 30 µmol photons $m^{-2}$ $s^{-1}$ (Fig. 3.9 C). The lowest respiration rates of each strain were similar. The Antarctic strain had a minimum rate of 8.99 ± 0.3 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$ and that of the temperate strain was 10.51 ± 1.2 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$. There was a greater difference in the maximum rates of respiration between the strains. Both strains experienced their maximum rate of respiration at 6 °C, but at a different irradiance (Fig. 3.9 A). The maximum rate of respiration of the Antarctic strain was 23.12 ± 1.0 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$ at 100 µmol photons $m^{-2}$ $s^{-1}$ whereas the maximum rate of the temperate strain was almost double this at 50.88 ± 0.7 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$ and was experienced at 60 µmol photons $m^{-2}$ $s^{-1}$. The temperate strain showed much more variation in the rate of respiration between each temperature, with a difference of 40.37 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$ between the minimum and maximum rates, whereas the Antarctic strain varied only by 14.13 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$. A one-way ANOVA indicated that there was a significant difference in the respiration rate between each strain ($F_{[17,54]} = 137.3$, $P < 0.001$). Tukey's pairwise comparison indicated that the respiration rate of the temperate strain was significantly higher than that of the Antarctic strain at all irradiances at 6 °C ($P < 0.001$) and at 30 and 60 µmol photons $m^{-2}$ $s^{-1}$ at 10 °C ($P < 0.01$). There were no significant differences between the rate of respiration of each strain at 18 °C for any irradiance ($P > 0.1$).

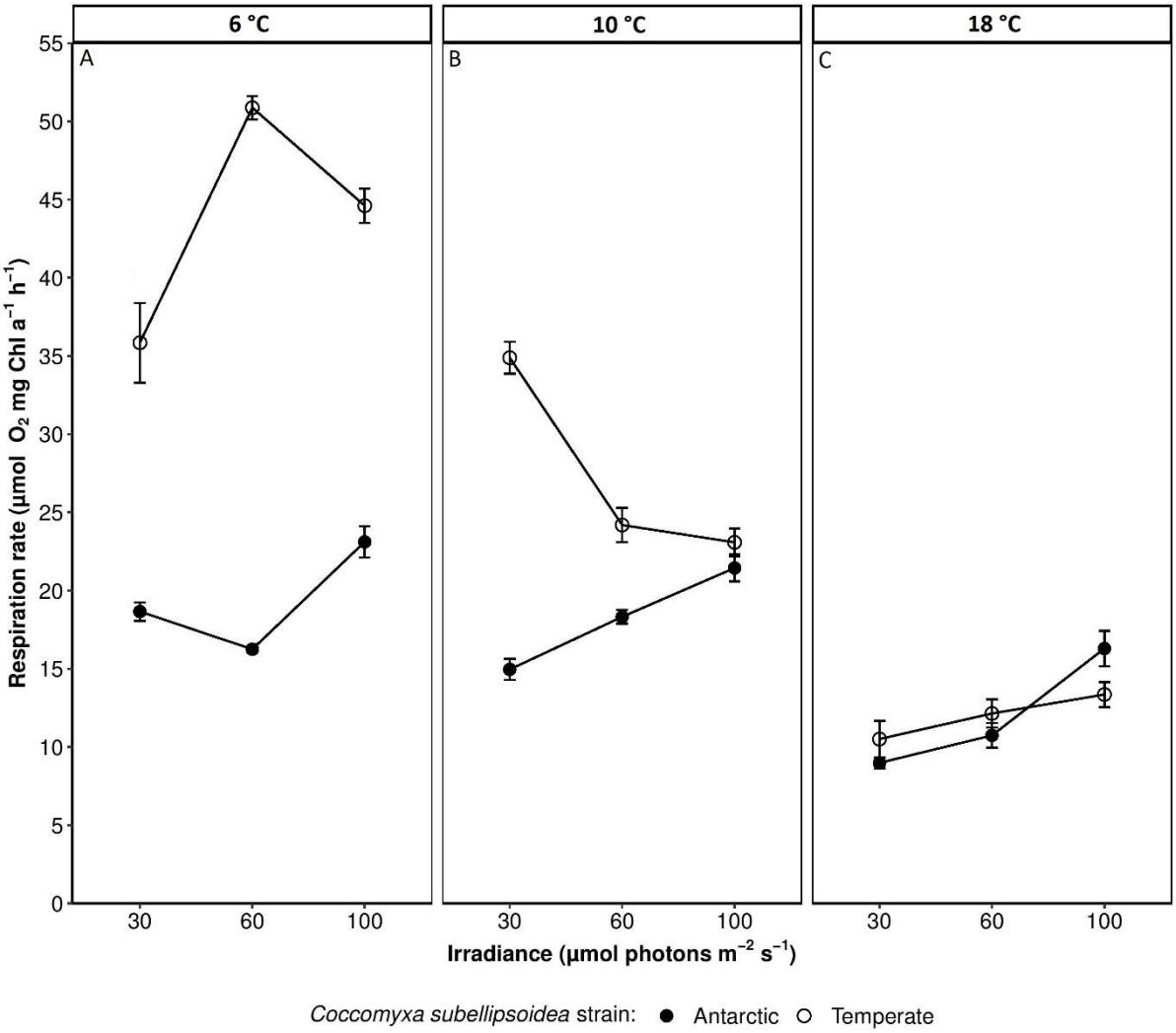


Figure 3.9. Rate of respiration of a temperate and Antarctic strain of *C. subellipsoidea* measured after 37 days of growth at 6 (A), 10 (B) or 18 °C (C) and constant irradiance of 30, 60 or 100 µmol photons $m^{-2}$ $s^{-1}$. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

### 3.3.4. Rate of respiration and cell yield

The rate of respiration was compared against the day-37 cell yield, which was described in Chapter 2 sections 2.3.1 and 2.3.2. The temperate strain grown at 6 °C had the highest respiration rate and the lowest day-37 cell yield (Fig. 3.10).

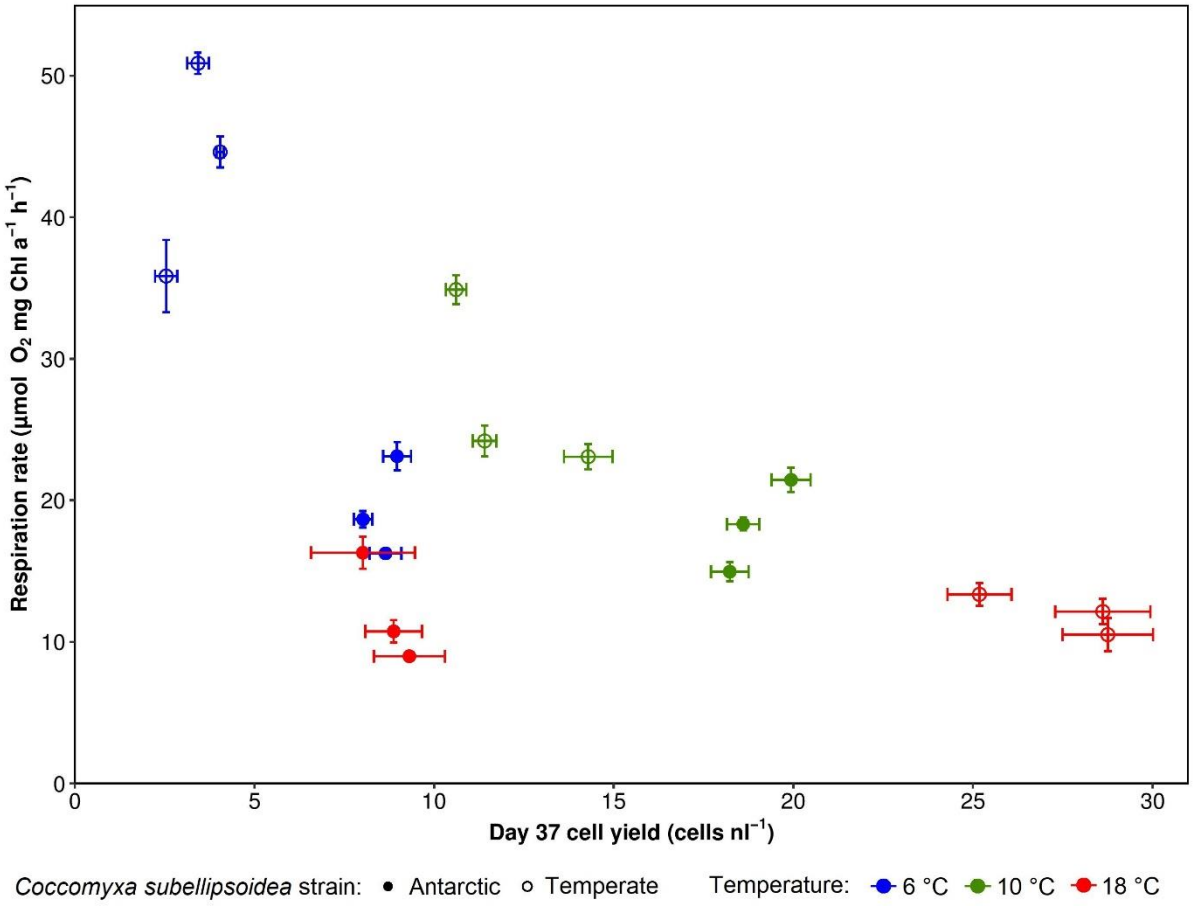


Figure 3.10. Rate of respiration and cell yield achieved after 37 days of growth at 6, 10 or 18 and constant irradiance between 30 and 100 µmol photons $m^{-2}$ $s^{-1}$ for a temperate and Antarctic strain of *C. subellipsoidea*. Each datapoint represents the mean calculated from four independent replicates. Error bars show mean ± standard error.

## 3.4. Discussion

### 3.4.1. Can the Antarctic strain of *Coccomyxa subellipsoidea* maintain a higher level of photosynthetic activity at a low temperature compared to the temperate strain?

It was hypothesised that the Antarctic strain of *C. subellipsoidea* would demonstrate a higher level of photosynthetic activity at the low temperature of 6 °C relative to the temperate strain. The results support this hypothesis because at 6 °C, the $F_v/F_m$, α, $rETR_m$, $E_k$ and Y(NPQ)/Y(NO) were each significantly higher for the Antarctic strain compared to the temperate strain.

The maximum quantum yield of PSII, or $F_v/F_m$, has been used as a sensitive indicator of stress in photosynthetic organisms as it acts as a measure of the fraction of active PSII reaction centres, which can be damaged by stressful temperatures and irradiance levels (Torzillo and Bernardini, 1998; Maxwell and Johnson, 2000; Morgan-Kiss *et al*., 2002). The Antarctic strain of *C. subellipsoidea* maintained an $F_v/F_m$ between 0.60 and 0.70 at each set of conditions studied. The temperate strain also maintained an $F_v/F_m$ between 0.60 and 0.70 at 10 and 18 °C (Fig. 3.3). These ranges are consistent with those recorded for isolates of *Coccomyxa* sp. (Tomaselli *et al*., 2002; Mudimu *et al*., 2015). Healthy, unstressed green algae typically have an $F_v/F_m$ close to 0.60, although the $F_v/F_m$ that is considered as healthy can vary between species (White *et al*., 2011). Maintenance of the $F_v/F_m$ between 0.60 and 0.70 by the Antarctic strain of *C. subellipsoidea* indicates that this strain did not become stressed and the photosynthetic apparatus did not accumulate damage across the temperature and irradiance range studied. The ability of the temperate strain of *C. subellipsoidea* to maintain an $F_v/F_m$ between 0.60 and 0.70 at 10 and 18 °C also suggests that the photosystems did not become impaired at these temperatures. However, the temperate strain of *C. subellipsoidea* grown at 6 °C had an $F_v/F_m$ between 0.23 and 0.32. The significantly lower $F_v/F_m$ of this strain at 6 °C

compared those grown at higher temperatures and the Antarctic strain indicates that the cells may have been stressed and could have experienced greater photodamage to the PSII proteins at a low temperature compared to the Antarctic strain. A lower $F_v/F_m$ for a temperate strain compared to a polar strain at low temperatures has also been recorded by Stamenković and Hanelt (2013) where a temperate strain of *C. punctulatum* var. *subpunctulatum* experienced a $F_v/F_m$ of 0.30 whereas a polar strain of this species had an $F_v/F_m$ of 0.60 when grown at 7 °C and 30 µmol photons $m^{-2}$ $s^{-1}$. Similarly, a temperate strain of *C. pyrenoidosa* had an $F_v/F_m$ of 0.40 after 15 days of growth at 9 °C and 45 µmol photons $m^{-2}$ $s^{-1}$, but an Arctic strain had an $F_v/F_m$ of 0.60 at this temperature (Cao *et al*., 2016). The inability of the temperate strain of *C. subellipsoidea* to maintain a high $F_v/F_m$ at the lowest temperature studied of 6 °C suggests that it may be more susceptible to photodamage, whereas the photosynthetic apparatus of the Antarctic strain may be better equipped to tolerate low temperatures and avoid accumulation of photodamage, similar to the polar strains of *C. punctulatum* var. *subpunctulatum* (Stamenković and Hanelt, 2013) and *C. pyrenoidosa* (Cao *et al*., 2016).

More photodamage was expected to occur at a high irradiance when combined with low temperature. The $F_v/F_m$ of the Antarctic and temperate strains of *C. subellipsoidea* did not appear to be influenced by irradiance, as the $F_v/F_m$ of both strains was relatively consistent between 30 and 100 µmol photons $m^{-2}$ $s^{-1}$ at each temperature. However, Wilson and Hüner (2000) found evidence of greater photodamage at a higher irradiance in *C. vulgaris* which experienced an $F_v/F_m$ of 0.30 at 250 µmol photons $m^{-2}$ $s^{-1}$ compared to 0.70 at 100 µmol photons $m^{-2}$ $s^{-1}$. Similarly, Gordillo *et al*. (2001) reported a decline in the $F_v/F_m$ from 0.70 to 0.35 with an increase in the irradiance from 250 to 1500 µmol photons $m^{-2}$ $s^{-1}$ in *D. viridis*. He *et al*. (2015) also noted a decrease in the $F_v/F_m$ at an irradiance above 200 µmol photons $m^{-2}$ $s^{-1}$ in *Chlorella* sp. Despite the relatively high growth temperature of 25 °C used in the above studies (Wilson and Hüner, 2000; Gorillo *et al*., 2001; He *et al*., 2015), photodamage as a

consequence of a high irradiance was still evident. El-Sabaawi and Harrison (2006) also found that the $F_v/F_m$ of the subarctic marine diatom, *Pseudo-Nitzschia granii* declined when grown at the low temperature of 8 °C and the relatively high irradiance of 150 µmol photons $m^{-2}$ $s^{-1}$. These studies exposed cells to a higher irradiance compared to the maximum irradiance of 100 µmol photons $m^{-2}$ $s^{-1}$ used in the present study. One limitation for this study was that the range of growth irradiances from 30 to 100 µmol photons $m^{-2}$ $s^{-1}$ used may not have provided a steep enough gradient to identify if the $F_v/F_m$ would decline with increasing irradiance in *C. subellipsoidea*.

The Antarctic strain of *C. subellipsoidea* maintained a relatively constant α of 0.18-0.23 across the range of temperature and irradiance conditions, whereas the temperate strain experienced a significantly lower α at 6 °C of 0.11-0.14 (Fig. 3.5). The lower α of the temperate strain indicates that at a low temperature, it had a reduced efficiency of light utilisation compared to the Antarctic strain. These results suggest that the temperate strain may be more susceptible to photodamage at a low temperature. A relatively constant α between 0.20 and 0.25 across a wide temperature range of 3 to 27 °C was also reported for the Arctic strain of *C. pyrenoidosa* whereas a temperate strain of this species suffered a decline in α below 0.15 at 9 °C (Cao *et al*., 2016). Cao *et al*. (2016) concluded that the ability of the Arctic strain to maintain light utilisation irrespective of the temperature enabled it to continue to photosynthesise and grow across a wide temperature range. In comparison, the temperate strain may have been unable to maintain photosynthesis under low temperatures due to the inefficiency of light utilisation and susceptibility to photodamage. Therefore, similar to the temperate strain of *C. pyrenoidosa*, the lower α of the temperate strain of *C. subellipsoidea* may also limit photosynthesis at a low temperature, particularly when combined with a high irradiance as the α at 100 µmol photons $m^{-2}$ $s^{-1}$ was significantly lower compared to that at 30 µmol photons $m^{-2}$ $s^{-1}$ at 6 °C.

The $rETR_m$ acts as a measure of the rate of electron flow through the electron transport chain via PSII in photosynthesis (Ralph and Gademann, 2005). As electron transfer is closely related to oxygen evolution, the $rETR_m$ has been used as an estimate of the photosynthetic performance of algae (Juneau *et al*., 2005; Hancke *et al*., 2008). The rate of electron transport is a temperature dependent process (Davison, 1991) and the effects of temperature can be seen in these results. The $rETR_m$ of the Antarctic and temperate strains of *C. subellipsoidea* were significantly higher at 10 °C compared to at 6 and 18 °C (Fig. 3.6), suggesting that the photosynthetic performance of both strains was maximised at 10 °C. An isolate of *Chlorella* sp. from a snow surface in Antarctica also had a maximal $rETR_m$ at 10 °C (Rivas *et al*., 2016). Similarly, the $rETR_m$ of the temperate diatoms, *Amphora coffeaeformis* and *Cocconeis sublittoralis* were maximised at 10-15 °C (Salleh and McMinn, 2011). The $rETR_m$ has been shown to be sensitive to high temperatures, and a decline in $rETR_m$ at temperatures exceeding 15 °C has been noted in temperate and polar strains of *C. pyrenoidosa* (Cao *et al*., 2016) and *Chlorella* sp. (Rivas *et al*., 2016; Barati *et al*., 2018; Lee *et al*., 2018) and in the marine Chlorophyte *Dunaliella tertiolecta* (MacIntyre *et al*., 1997). It has been suggested that the decline in $rETR_m$ at high temperature is related to deactivation of the temperature sensitive enzymes involved in carbon fixation (MacIntyre *et al*., 1997; Barati *et al*., 2018).

The Antarctic strain maintained a 3-fold higher $rETR_m$ at 6 °C compared to the temperate strain. These results suggest that the rate of electron transport through PSII and therefore the photosynthetic performance of the temperate strain was impaired at the lowest temperature studied. Similar to the Antarctic strain of *C. subellipsoidea*, the polar strains of *C. punctulatum* var. *subpunctulatum* (Stamenković and Hanelt, 2013) and *C. pyrenoidosa* (Cao *et al*., 2016) also maintained a higher $rETR_m$ at temperatures below 9 °C compared to their temperate counterparts (Cao *et al*., 2016). Antarctic *Chlorella* sp. maintained a relatively high $rETR_m$ at 5 °C (Rivas *et al*., 2016). A relatively high $rETR_m$ at low temperatures has been associated with

maintenance of membrane structural integrity, cold tolerant enzymes and higher concentrations of photosynthetic enzymes in cryophilic algae which allow them to continue to photosynthesise at temperatures which could impair photosynthesis in less cold tolerant algae (Eggert *et al*., 2006; Salleh *et al*., 2010).

The $E_k$ values did show some variation depending on the temperature. Similar to the $rETR_m$, the $E_k$ was highest at 10 °C for both strains (Fig. 3.7), suggesting this temperature is most favourable for a higher level of photosynthetic activity. There was a decline in the $E_k$ between 10 and 18 °C for both strains of *C. subellipsoidea*. A decrease in $E_k$ has also been reported for marine phytoplankton (Hancke *et al*., 2008) and temperate, tropical and polar strains of *Cosmarium* (Stamenković and Hanelt, 2013) at temperatures above 16 °C. The $E_k$ of the temperate strain of *C. subellipsoidea* declined by at least 236 µmol photons $m^{-2}$ $s^{-1}$ from 10 to 6 °C and such a large decline was not seen in the Antarctic strain which had a significantly higher $E_k$ at 6 °C compared to the temperate strain. The reduction in $E_k$ of the temperate strain at 6 °C suggests light capture and the rate of electron transport became unbalanced at a lower irradiance compared the Antarctic strain and compared to when grown at higher temperatures. The lower $E_k$ of the temperate strain further supports the evidence seen in the $F_v/F_m$, α and $rETR_m$ that this strain suffers from more photodamage at 6 °C compared to the Antarctic strain.

The $E_k$ is a measure of the photoacclimation state of photosynthetic organisms and is indicative of the highest irradiance at which a balance between light absorption and electron transport can be achieved (Salleh and McMinn, 2011). The $E_k$ is typically higher in algae acclimated to a high irradiance, as shown in the diatom, *Nitzschia palea* which had a significantly higher $E_k$ when grown at an irradiance of 400 µmol photons $m^{-2}$ $s^{-1}$ compared to 20 µmol photons $m^{-2}$ $s^{-1}$ (Cruz and Serôdio, 2008). Marine cyanobacteria strains of the genus *Synechococcus* also displayed an increase in $E_k$ with increasing growth irradiance from 10 to 145 µmol photons $m^{-2}$ $s^{-1}$ (Jodłowska and Śliwińska, 2014). Antarctic sea ice algae growing under the ice surface

have been shown to be acclimated to the dark conditions with $E_k$ values below 10 µmol photons $m^{-2}$ $s^{-1}$ (McMinn *et al*., 2010). However, there was no clear evidence of photoacclimation to the growth irradiances in the $E_k$ values of *C. subellipsoidea* as there was little variation in $E_k$ between cultures grown at a different irradiance level from 30 to 100 µmol photons $m^{-2}$ $s^{-1}$. The $E_k$ for both strains of *C. subellipsoidea* was between 130 and 353 µmol photons $m^{-2}$ $s^{-1}$ across the range of temperatures and irradiances studied, indicating that the cells could acclimate to a relatively high irradiance, and this was higher than the highest growth irradiance of 100 µmol photons $m^{-2}$ $s^{-1}$. Temperate, tropical and polar strains of *Cosmarium* also achieved an $E_k$ within the same range and Stamenković and Hanelt (2013) concluded that these strains of *Cosmarium* were high light acclimated. High light acclimation of *C. subellipsoidea* was unexpected because the Antarctic strain was originally isolated from within algal peat and the temperate strain from within a lichen thallus and the irradiance may be lower within these habitats (Gustavs *et al*., 2017). Algae isolated from similar habitats have been shown to be acclimated to a low irradiance. For example, the filamentous green alga *Klebsormidium dissectum* collected from within alpine soil crusts had a low $E_k$ of 18 µmol photons $m^{-2}$ $s^{-1}$ when grown at 20 °C and 30 µmol photons $m^{-2}$ $s^{-1}$ (Karsten and Holzinger, 2012). Green algae isolated from lichens have been proposed to be low light adapted (Deming-Adams *et al*., 1990). However, as the temperate strain of *C. subellipsoidea* is not an obligate photobiont, adaptation to a low irradiance may limit photosynthesis during free-living stages when the ambient irradiance may be higher than within a lichen thallus (Gustavs *et al*., 2017). Similarly, the algal peat the Antarctic strain was isolated from may not have been a permanent habitat and the irradiance experienced within the peat may fluctuate and depend on the depth of the algal layer (Gustavs *et al*., 2017). The photoacclimation state of these strains in their natural habitats may not have been preserved over the multiple years of cultivation in laboratory conditions (Stamenković and Hanelt, 2017).

### 3.4.2. Has the Antarctic strain of *Coccomyxa subellipsoidea* got a greater capacity for regulated non-photochemical quenching (NPQ) at a low temperature compared to the temperate strain?

A higher Y(NPQ)/Y(NO) indicates that a larger proportion of excess light energy takes the regulated NPQ energy conversion route relative to the NO route (Figueroa *et al*., 2019). The Y(NPQ)/Y(NO) of both the Antarctic and temperate strains of *C. subellipsoidea* was significantly higher at 6 or 10 °C compared to at 18 °C and increased with increasing irradiance (Fig. 3.8), indicating that regulated NPQ was stronger under low temperatures and high irradiances, conditions which are likely to induce photoinhibition (Hüner *et al*., 2012). Similar responses have been reported for the brown macroalga, *Fucus serratus* which experienced greater Y(NPQ)/Y(NO) when grown at 8 °C compared to when grown at higher temperatures up to 34 °C (Figueroa *et al*., 2019). Polar isolates of *Chlorella* sp. also experienced an increase in regulated NPQ with a decrease in temperature below 15 °C (Lee *et al*., 2018). An Antarctic isolate of *K. antarctica* demonstrated a stronger regulated NPQ response when grown at 4 °C and an irradiance of 150 µmol photons $m^{-2}$ $s^{-1}$ compared to 15 µmol photons $m^{-2}$ $s^{-1}$ (La Rocca *et al*., 2014). A stronger regulated NPQ response under low temperatures and high irradiances allows the cells to reduce the risk of photoinhibition by initiating the xanthophyll cycle. In this cycle, the excess light energy can be diverted away from chlorophyll molecules, avoiding an imbalance between light capture and electron transport. Passive energy dissipation as fluorescence in the NO route can be minimised and generation of ROS can be reduced which lessens photodamage of the photosystems (Hüner *et al*., 2012).

Although both the Antarctic and temperate strains of *C. subellipsoidea* experienced a significantly lower Y(NPQ)/Y(NO) at 18 °C compared to that at lower temperatures, the Y(NPQ)/Y(NO) at 6 °C was similar, or slightly lower compared to that at 10 °C for the temperate strain, but there was a significant upregulation in Y(NPQ)/Y(NO) from 10 to 6 °C

for the Antarctic strain. The Antarctic strain of *C. subellipsoidea* had a much higher Y(NPQ)/Y(NO) at 6 °C compared to the temperate strain. These results followed the hypothesis that the Antarctic strain would have a greater capacity for regulated NPQ compared to the temperate strain at a low temperature. Similarly, polar *C. punctulatum* var. *subpunctulatum* also showed a stronger regulated NPQ response compared to a temperate strain when grown below 7 °C (Stamenković and Hanelt, 2013). The lower regulated NPQ response at low temperatures of the temperate strain of *C. subellipsoidea* in the present study and temperate *C. punctulatum* var. *subpunctulatum* (Stamenković and Hanelt, 2013) coincided with significant reductions in $F_v/F_m$, α, $rETR_m$ and $E_k$. The decline in photosynthetic capacity may be explained by the inability of these temperate strains to maintain sufficient regulated NPQ at lower temperatures. With a weaker regulated NPQ response, a larger fraction of the excess light energy may have been absorbed by chlorophyll, but due to inactive photosystems at high irradiances, the energy was likely passively dissipated as fluorescence in NO. The emission of this fluorescence generates ROS which can induce photodamage of photosystems and impair photosynthetic activity (Moustaka *et al*., 2015; Figueroa *et al*., 2019). In comparison, these results indicate that the Antarctic strain achieved effective regulated NPQ as a photoprotective mechanism at 6 °C, enabling avoidance of large reductions in $F_v/F_m$, α, $rETR_m$ and $E_k$ and therefore maintenance of a relatively high level of photosynthetic activity at 6 °C. Lee *et al*. (2018) suggested that regulated NPQ responses can be impaired by stressful temperatures. As the xanthophyll cycle is enzyme dependent, the enzymes involved in this process, such as violaxanthin de-epoxidase (Müller *et al*., 2001), may be less efficient below 10 °C in the temperate strain, but continue to function at temperatures as low as 6 °C in the Antarctic strain of *C. subellipsoidea*.

### 3.4.3. Is there evidence of pigment adjustment in the Antarctic and temperate strains of *Coccomyxa subellipsoidea* in response to temperature and irradiance?

As hypothesised, the Chl a/b ratio of both the Antarctic and temperate strains of *C. subellipsoidea* increased significantly with increasing irradiance (Table 3.1) and this response to irradiance appears to be conserved among algae (Falkowski and LaRoche, 1991). An Antarctic isolate of *K. antarctica* had a significantly higher Chl a/b ratio when grown at an irradiance of 150 µmol photons $m^{-2}$ $s^{-1}$ compared to at 15 µmol photons $m^{-2}$ $s^{-1}$ (La Rocca *et al*., 2014). The green alga, *D. tertiolecta* also had a higher Chl a/b ratio when grown at 600 µmol photons $m^{-2}$ $s^{-1}$ compared to 45 µmol photons $m^{-2}$ $s^{-1}$ (Falkowski *et al*., 1981). A higher Chl a/b ratio indicates that there was a reduction in the size of PSII antenna at a higher irradiance and this was likely due to a decrease in the concentration of Chl b, which specifically binds antenna complexes, relative to Chl a bound to reaction centre complexes. A reduction in the size of PSII antenna can reduce light absorption (Falkowski and LaRoche, 1991; La Rocca *et al*., 2014). These photoacclimatory responses may allow cells to minimise the risk of photodamage at a high irradiance by decreasing excessive light absorption (Smith *et al*., 1990). The Chl a/b ratio of both strains was adjusted depending on irradiance at 6 °C, but there was no significant difference in the Chl a/b ratio between each strain at this temperature. Contrary to the hypothesis, this finding suggests that the Antarctic strain is not better able to adjust the chlorophyll content in response to irradiance at a low temperature relative to the temperate strain. Enzymes involved in chlorophyll synthesis may continue to function at low temperature for both strains of *C. subellipsoidea*, unlike a temperate strain of *C. pyrenoidosa* which was unable to maintain chlorophyll synthesis at 9 °C compared to an Arctic strain (Cao *et al*., 2016).

The carotenoid content per cell was hypothesised to be higher at a low temperature and high irradiance as these pigments may have photoprotective properties which allow them to reduce the risk of photodamage. The carotenoid content for the Antarctic and temperate strains of *C.*

*subellipsoidea* did increase as temperature declined from 18 to 6 °C (Table 3.1). Orset and Young (1999) also found that the carotenoid content increased as growth temperature decreased from 30 to 10 °C in the green alga, *Dunaliella salina*. The carotenoid content may increase at a lower temperature as a photoprotective strategy (Orset and Young, 1999). Some carotenoids may shade chloroplasts to minimise the risk of photodamage at lower temperatures (Takaichi, 2011). The Antarctic strain of *C. subellipsoidea* had a significantly higher concentration of carotenoids compared to the temperate strain. Cao *et al*. (2016) also found the Arctic strain of *C. pyrenoidosa* had a significantly higher concentration of carotenoids compared to the temperate strain. The higher carotenoid concentration of the polar strains could be linked to a higher photoprotective capacity at low temperatures, contributing to the ability of these strains to maintain photosynthetic activity and avoid photodamage at low temperatures (Bidigare *et al*., 1993). However, carotenoids also function in light absorption (La Rocca *et al*., 2014) and the carotenoid content decreased with increasing irradiance, suggesting the carotenoids of *C. subellipsoidea* are reduced to prevent excessive light absorption. The extraction method used to measure the carotenoid content of *C. subellipsoidea* did not allow separation of carotenoids which function in light absorption from those with photoprotective properties.

### 3.4.4. Was the Antarctic strain of *Coccomyxa subellipsoidea* better able to maintain the rate of respiration at a low temperature compared to the temperate strain?

Contrary to the hypothesis, the respiration rate of the Antarctic and temperate strains of *C. subellipsoidea* declined significantly with increasing temperature and was lower for the Antarctic strain compared to the temperate strain, with a much larger difference between the strains at a lower temperature. The opposite relationship with temperature has been reported in previous studies. The respiration rate of green algae increased from 5 to 30 °C for Antarctic *K. antarctica*, alpine *Chlorella saccharophila* and temperate *Chlorella sorokiniana* (Vona *et al*.

2004) and for Antarctic and temperate *Stichococcus bacillaris* (Chen *et al*., 2012). The respiration rates of *K. antarctica* and *C. saccharophila* at 20 °C were 13.3 and 19.8 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$, respectively, which was similar to those of Antarctic and temperate *C. subellipsoidea* in the present study at 18 °C, where the respiration rate was between 10 and 16 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$. However, there were large differences between the respiration rate recorded in the present study and in the studies by Vona *et al*. (2004) and Chen *et al*. (2012) at lower temperatures. The respiration rate of *K. antarctica*, *C. saccharophila*, *C. sorokiniana* (Vona *et al*., 2004) and *S. bacillaris* (Chen *et al*., 2012) was 0.4-10.9 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$ between 5 and 10 °C, whereas at 6 °C, Antarctic and temperate *C. subellipsoidea* in the present study had a respiration rate of 18-23 and 36-51 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$, respectively. Vona *et al*. (2004) and Chen *et al*. (2012) concluded that a higher respiration rate was achieved at high temperatures due to increased activity rates of the respiratory enzyme, nitrate reductase. Also in contrast to the results of the present study, the Antarctic and alpine strains in these studies had higher respiration rates compared to the temperate strains at low temperatures, and this was suggested to be a result of the Antarctic and alpine strains having cold tolerant enzymes which can maintain higher reaction rates at lower temperatures (Vona *et al*., 2004; Chen *et al*., 2012). The high rate of respiration of both strains of *C. subellipsoidea* at 6 °C indicates that their respiratory enzymes may also be relatively cold tolerant. The higher respiration rate at a low temperature could be attributed to an increased need to utilise energy from stored organic substances to support cellular processes and synthesis of substances required for survival at low temperature (Geider and Osborne, 1989; Price and Sowers, 2004). The increase in respiration with decreasing temperature was more notable for temperate *C. subellipsoidea*, which ranged from 10 to 51 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$, compared to the Antarctic strain, which maintained a rate between 10 and 23 µmol $O_2$ mg Chl $a^{-1}$ $h^{-1}$ from 18 to 6 °C. An elevated rate of respiration to generate metabolic energy at low temperature may be more important for the

temperate strain of *C. subellipsoidea* because it may have a limited ability to synthesise organic substances for energy in photosynthesis due to the large reduction in photosynthetic activity at 6 °C. In comparison, Antarctic *C. subellipsoidea* maintained a higher level of photosynthetic activity between 6 and 18 °C so was likely able to maintain production of photosynthate across the range of temperatures. Reliance of the Antarctic strain on respiration for metabolic energy at low temperatures may be lower, explaining the much smaller increase in respiration with decreasing temperature compared to the temperate strain.

Interpretation of the respiration rate must be taken be caution because the day-37 cell yield appeared to influence the rate of respiration. Cultures with a lower day-37 cell yield, such as those of the temperate strain grown at 6 °C, had a higher respiration rate which could have been a consequence of the higher availability of nutrients remaining in the 3N-BBM+V on day 37 compared to cultures with a higher day-37 cell yield which likely experienced greater depletion of nutrients. The variation in the day-37 cell yield between each strain and temperature may be responsible for the different relationship seen between respiration and temperature for this study compared to the results of other research groups (Vona *et al*., 2004; Chen *et al*., 2012). Measuring the rate of respiration at an earlier stage during the experiment may avoid interference of the cell yield on the respiration rate.

### 3.4.5. Conclusions

The Antarctic and temperate strains of *C. subellipsoidea* displayed similar photosynthetic and respiratory responses at the highest temperature studied of 18 °C, but there were clear differences in their responses at the lower temperature of 6 °C. The temperate strain of *C. subellipsoidea* appeared to suffer from photodamage at a low temperature, with significant decreases in $F_v/F_m$, α, $rETR_m$ and $E_k$ at 6 °C. The Antarctic strain of *C. subellipsoidea* maintained a higher and remarkably consistent level of photosynthetic activity between 6 and

18 °C. Both the Antarctic and temperate strain demonstrated an ability to acclimate to irradiance levels in an attempt to reduce the risk of photodamage, as shown by an increase in Chl a/b ratio at a higher irradiance, but only the Antarctic strain achieved effective regulated NPQ at 6 °C to dissipate excess light energy and avoid photodamage to the photosynthetic apparatus. Furthermore, the higher respiration rate of temperate *C. subellipsoidea* at 6 °C indicates that may have experienced a greater level of temperature stress relative to the Antarctic strain. These findings suggest that *C. subellipsoidea* is a physiologically and biochemically plastic species with latitudinally separated populations demonstrating distinct physiological responses to temperature and irradiance. The results contribute to the existing evidence revealing that the physiology of polar strains of green microalgae is more suited to lower temperatures compared to their temperate counterparts (Vona *et al*., 2004; Chen *et al*., 2012; Stamenković and Hanelt, 2013; Cao *et al*., 2016; Lee *et al*., 2018).

# Chapter 4: Synthesis and future directions

## 4.1. Linking the growth and metabolic responses of an Antarctic and temperate strain of *Coccomyxa subellipsoidea*

The Antarctic strain of *C. subellipsoidea* had a maximum growth rate at 10 °C and achieved significantly higher levels of photosynthetic activity at this temperature, as shown by a greater $F_v/F_m$, $rETR_m$ and $E_k$. The higher level of photosynthetic activity at 10 °C combined with the relatively low rate of respiration compared to the temperate strain may have enabled the Antarctic strain to achieve the highest rate of growth at this temperature. Utilisation of photosynthate and stored compounds in respiration may have been low, whilst production of photosynthate may have been high, resulting in more energy allocation for growth at 10 °C (Geider and Osbourne, 1989). The Antarctic strain could be described as psychrophilic considering it had a maximum growth rate at 10 °C (Morita, 1975). However, application of terms such as psychrophily and psychrotolerance is complicated as there many variations in the optimum growth temperature required for algae to be defined by these terms. Application of terms which do not rely on optimal growth temperatures were considered more appropriate to describe the growth behaviour of *C. subellipsoidea*. The Antarctic strain of *C. subellipsoidea* demonstrated eurythermal behaviour because it maintained a relatively steady growth rate from 6 to 18 °C, and previous research has recorded growth in this strain up to 30 °C (Msanne *et al*., 2012; Pfaff *et al*., 2016; Yang *et al*., 2019). The Antarctic strain also maintained a relatively steady level of photosynthetic activity and rate of respiration from 6 to 18 °C. Eurythermal behaviour indicates that this strain may have a high level of physiological plasticity and can acclimate to adjust to different conditions, as shown by the NPQ response where regulated NPQ was significantly upregulated at low temperature and high irradiance. The effective dissipation of excess light energy under these conditions could explain the ability of Antarctic

*C. subellipsoidea* to maintain photosynthetic activity and growth at 6 °C by avoiding accumulation of photodamage which could otherwise result in a reduction in photosynthetic activity and growth (La Rocca *et al*., 2004; Cao *et al*., 2016). The cold tolerance mechanisms identified in the genome of this Antarctic strain by Blanc *et al*. (2012) included a large number of fatty acid desaturase proteins which are associated with regulation of membrane integrity, cryoprotective antifreeze proteins and molecular chaperones which are involved in maintenance of enzyme structure and activity at low temperature. These mechanisms may be responsible for the ability of Antarctic *C. subellipsoidea* to photosynthesise and grow well at a low temperature.

In comparison, the temperate strain of *C. subellipsoidea* was unable to maintain a steady level of photosynthetic activity and rate of growth at 6 °C compared to when grown at the higher temperature of 18 °C. The lower $F_v/F_m$, α, $rETR_m$ and $E_k$ combined with the lack of effective regulated NPQ indicated that the efficiency of photosynthesis was low and photodamage may have occurred at 6 °C. The low temperature was likely stressful for the temperate strain, resulting in a high respiration rate in an attempt to allow cellular processes to continue. A high respiration rate relative to photosynthetic activity may have depleted energy stores and as a consequence, there may have been less energy available for growth (Geider and Osbourne, 1989), resulting in a much lower growth rate at 6 °C compared to the Antarctic strain. The temperate strain was able to grow at the low temperature, but the impaired photosynthetic activity and growth and elevated rate of respiration at 6 °C compared to at 18 °C suggests that a higher temperature is more favourable for this strain. The temperate strain may grow over a much narrower temperature range compared to the Antarctic strain, and it could be defined as stenothermal. Based on these results, the evidence of cold tolerance identified in the genome of the Antarctic strain by Blanc *et al*. (2012) may not be present or expressed at the same level in temperate *C. subellipsoidea*.

At 18 °C, the temperate strain had a significantly higher growth rate compared to the Antarctic strain, but photosynthetic activity and the respiration rate were similar for each strain. Therefore, the elevated growth rate of the temperate strain at 18 °C cannot be explained by photosynthetic or respiratory responses to temperature. Alternatively, the lower growth rate of the Antarctic strain could be a consequence of this strain still expressing cold tolerance mechanisms at 18 °C. It has been suggested that unnecessary investment in cold tolerance mechanisms could be energetically costly, resulting in a reduction in energy allocation for growth (Collins *et al*., 2008; Casanueva *et al*., 2010), which could explain the lower growth rate of the Antarctic strain at a higher temperature compared to the temperate strain. However, a limitation of this study was the mis-match in the timing of the photosynthesis and respiratory measurements. Photosynthetic activity was measured much earlier in the experiment compared to respiration, so the effects of a dense culture, such as self-shading and nutrient depletion, could have influenced the rate of respiration. Therefore, photosynthetic activity cannot be directly compared against the respiration rate to explain the differences in growth rates. Future studies should measure photosynthesis and respiration in close succession to fully understand energy generation and allocation for growth. Measurements of respiration should also be taken earlier on in the experiment to minimise the influence of a dense culture.

## 4.2. Climate change and *Coccomyxa subellipsoidea*

Average global temperatures are increasing and a more severe increase in temperature is being seen in the Antarctic (Vaughan *et al*., 2003). Temperatures are predicted to increase in the Antarctic by 0.34 °C per decade (Convey *et al*., 2009). There are likely to be significant habitat changes for algae, and a change in temperature could be stressful for many species (Convey, 2001). The eurythermal behaviour demonstrated by Antarctic *C. subellipsoidea* indicates that it may have a capacity to tolerate temperature change. Higher temperatures early in the year can lead to earlier spring snow and ice melt and expose more areas of bare soil (Lee *et al*.,

2017). These changes may allow Antarctic populations of soil algae such as *C. subellipsoidea* to expand their range and colonise new ground, whereas more specialised snow and ice algae which may be restricted to snow and ice habitats could suffer a reduction in the growth rate at higher temperatures and could therefore be out-competed by eurythermal soil algae (Teoh *et al*., 2004; Convey, 2011; Davey *et al*., 2019). Changes in the community structure of Antarctic algae could have implications for biodiversity, succession and nutrient cycling (Davey *et al*., 2019). As primary colonisers in succession, soil algae such as *C. subellipsoidea* colonising newly exposed ground could initiate development of new communities of plants and invertebrates, and therefore more cycling of nutrients which could influence other communities downstream (Elster and Svoboda, 1996; Convey *et al*., 2008).

Considering *C. subellipsoidea* is a small alga with a length of just 3-9 µm (Honegger and Brunner, 1981), it may easily be dispersed by the wind (Gustavs *et al*., 2017) and populations may disperse to other latitudes. Whilst the eurythermal behaviour of the Antarctic strain suggests it may tolerate higher temperatures if dispersed to temperate latitudes, the sensitivity of the temperate strain to low temperature may prevent survival at lower temperatures if dispersed to the polar regions. Therefore, the risk of homogenisation of Antarctic populations by temperate strains, as previously observed in *Prasiola crispa* (Hughes and Convey, 2010), may be low. However, as *Coccomyxa* appears to be a physiologically plastic genus, strains may rapidly acclimate and even adapt to new conditions (Gustavs *et al*., 2017).

## 4.3. Biotechnological applications of *Coccomyxa subellipsoidea*

Species of the genus *Coccomyxa* have previously been suggested to be ideal candidates for lipid production for use as biofuel (Peng *et al*., 2016; Liu *et al*., 2018). The Antarctic strain of *C. subellipsoidea* was found to possess four large families of proteins involved in the biosynthesis and metabolism of a diverse range of different lipids and these proteins are of high

interest in biotechnology (Blanc *et al*., 2012). Cultivation at a lower temperature could allow the costs and risk of microbial contamination of biofuel generation to be reduced provided the algae can continue to grow and synthesise lipids at low temperatures. Therefore, the ability of Antarctic *C. subellipsoidea* to maintain photosynthesis and growth at 6 °C may make this strain a more suitable candidate for low temperature biofuel generation compared to the more stenothermal temperate strain. Previous studies examining lipid production in the Antarctic strain of *C. subellipsoidea* have grown cultures at 25-28 °C (Msanne *et al*., 2012; Yang *et al*., 2019), but the results of the present study suggest that these higher temperatures may be unnecessary considering the eurythermal behaviour of the strain. Antarctic *C. subellipsoidea* may be able to grow and yield just as much lipid at a lower temperature, reducing costs of cultivation.

## 4.4. Future directions for the study of *Coccomyxa subellipsoidea*

The results of the present study revealed that the Antarctic and temperate strains of *C. subellipsoidea* could both grow between 6 and 18 °C. Previous research has also demonstrated that the Antarctic strain is able to grow at temperatures up to 30 °C (Msanne *et al*., 2012; Pfaff *et al*., 2016; Yang *et al*., 2019). In this study, the Antarctic strain maintained a relatively high growth rate at 6 °C so could be expected to continue to grow below this temperature. However, no measurements were made of their relative capacity to grow below 6 °C, and future studies could aim to investigate physiological responses of these strains of *C. subellipsoidea* in response to more extreme temperatures to identify the temperatures which may restrict their distribution.

The responses to irradiance were not pronounced and the $E_k$ values indicated that for both strains, photosynthesis would begin to saturate above the highest growth irradiance used in the present study of 100 µmol photons $m^{-2}$ $s^{-1}$ at each temperature. Therefore, to fully understand

how each strain of *C. subellipsoidea* tolerates extremes of irradiance, coupled with low temperature, exposure to a wider range of irradiances may be necessary, similar to the studies on other species of algae which have exposed samples to irradiances between 15 and 1500 µmol photons $m^{-2}$ $s^{-1}$ (La Rocca *et al*., 2014; He *et al*., 2015). Considering photoacclimation responses can be rapid and could be lost in the laboratory, analysis of the photosynthetic activity of isolates of *C. subellipsoidea* measured in the field may allow differences to be identified between isolates with different lifestyles and microhabitats.

Future studies could expand the analysis of the physiology of this diverse genus to other strains, such as those isolated from a tropical region, or those with different lifestyles such as parasitic strains (*Coccomyxa parasitica* and *Coccomyxa astericola*) or isolates which demonstrate tolerance to high temperatures (*Coccomyxa fottii*) to examine how this genus has diversified. Molecular analysis could also be employed to investigate the genetic diversity of the genus, and whether those with similar lifestyles, such as tolerance to extremes of temperature, or from similar environments, such as the Antarctic and Arctic, may have similar phylogeny (Darienko *et al*., 2015).

The major findings of this thesis are the distinct physiological responses of the Antarctic and temperate strains of *C. subellipsoidea* to low temperature. Further, the Antarctic strain demonstrated eurythermal behaviour, indicating that it may continue to photosynthesise, respire and grow well across a wide range of temperatures, and could be relatively resilient to climate change. However, growth and photosynthetic activity of the temperate strain, which to my knowledge have not previously been elucidated, were significantly impaired at a low temperature, indicating it may be much more sensitive to climate change. These strains have adapted their physiology to their latitude of origin, and due to their distinct responses to temperature, they may therefore respond differently to climate change.

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