THE ECOPHYSIOLOGY OF *GELIDIUM PRISTOIDES* (TURNER) KUETZING: TOWARDS COMMERCIAL CULTIVATION

By

PAUL-PIERRE STEYN

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Abstract

The ecophysiology of the red alga *Gelidium pristoides* (Turner) Kuetzing was investigated in an effort to establish a technique for commercial cultivation. The seaweed is of commercial importance in South Africa where it is harvested from the intertidal zone rocky shores along the coast. It is dried and exported abroad for the extraction of agar. Yields and quality could be improved by cultivation in commercial systems. However, attempts at growing the seaweed in experimental systems have all ended in failure. This study aimed to describe the conditions in which the seaweed grows naturally; and investigate its physiological response to selected physical conditions in the laboratory in order to determine suitable conditions for mariculture.

Ecological studies showed that *G. pristoides* grew above the spring low tide water level. The upper limit of the seaweed's vertical distribution range, as well as its abundance, was largely dependent on wave exposure. The zone normally inhabited by *G. pristoides* was dominated by coralline turf in sheltered areas, while the abundance of *G. pristoides* increased towards more exposed rocky shore sites. The seaweed occurred among species such as Pattelid limpets and barnacles, but was usually the dominant macroalga in this zone, with coralline turf and encrusting algae being the only others.

Physical conditions in the part of the intertidal zone inhabited by *G. pristoides* were highly variable. During low tide temperatures could vary by as much as 10℃ within the three hours between tidal inundation of the seaweed population, while salinity varied by up to 9 ppt, and light intensity by as much as 800 µmol m^{-2} s^{-1}. During these exposure periods the seaweed suffered up to 20% moisture loss. Laboratory experiments on the seaweed's response to these conditions indicated that it was well adapted to such fluctuations. It had a broad salinity (20 and 40 ppt), and temperature tolerance range (18 to 24℃), with an optimum of temperature of 21℃ for photosynthesis, while there was no difference in the photosynthetic rate of the alga within the 20 to 40 ppt salinity range. The alga had a low saturating irradiance (*ca*. 45 – 80 µmol m^{-2} s^{-1}) equipping it well for photosynthesis in turbulent environments, with high light attenuation, but poorly to un-attenuated light conditions.

Exposure resulted in an initial increase in photosynthetic rate followed by a gradual decrease thereafter. pH drift experiments showed that low seawater pH, and associated increased carbon dioxide availability, resulted in an increase in photosynthetic rate. This response suggests that the seaweed has a high affinity for carbon dioxide, while the reduction in photosynthetic rate in response to bicarbonate use inhibition indicates that it also has the capacity for bicarbonate use.

The high affinity of *Gelidium pristoides* for carbon dioxide as an inorganic carbon source appears to be the primary reason for the low abundance of the alga on sheltered rocky shore areas, and

also explains the failure of the alga to grow in tank or open-water mariculture systems. Exposed rocky shores have experience heavy wave action, and the resultant aeration and mixing of near-shore waters increases the availability of carbon dioxide, which is considered a limiting resource. The absence of such mixing and aeration at sheltered site makes this less suitable habitat for *G. pristoides*. Periodic exposure also makes high levels of atmospheric carbon dioxide available from which the seaweed benefits. The traditional mariculture systems in which attempts have been made to cultivate the seaweed failed to satisfy either of the above conditions.

Key Words:
Ecophysiology, *Gelidium pristoides*, Rhodophyte, Intertidal, Rocky shores, inorganic carbon

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Chapter 1

Introduction

Rationale & Approach to Study

Introduction

Gelidium pristoides (Turner) Kützing is a common Rhodophyte on the south coast of South Africa. Currently the seaweed is harvested from the wild by labourers hand-picking the algae off the rocks in the intertidal zone (Plate 1). The seaweed is dried in the sun after which it is packed into bales and exported to the Far East for the extraction of agar. Seaweed harvesting rights in South Africa are granted for a particular species per section of coastline. These sections of coastline (there are 23 in total along the coast) are referred to as Seaweed Concession Areas. Approximately 60 to 100 tonnes (dry mass) of *G. pristoides* is harvested in Concession area 1 (from the former Transkei region to Plettenberg Bay) per year (Anderson *et al*. 1991). So far attempts at cultivating the species have failed. The research described here investigated the ecophysiology of this important commercial species in order to determine optimum cultivation conditions for the seaweed.

Plate 1.1 (a) Harvesting *Gelidium pristoides* from the rocky shore near Port Elizabeth. (b) Bag of harvested *G. pristoides* ready for transport to a drying area. (c) *G. pristoides* drying in the sun at Schoenmakerskop.

Research methodology

The methodology used in this study towards cultivating *Gelidium pristoides* has been based on the physiological approach outlined by Hansen (1983) for the mariculture of red algae. The pathway suggested requires initial laboratory and field studies, which can then be followed by large-scale intensive mariculture design. The protocol includes studying the ecology of the candidate species, selecting an appropriate strain, completing physiological studies on the selected species or strain, and finally conducting cultivation trials.

Ecological studies

Seasonal biomass, reproductive state, standing crop, growth rate, agar properties, life-history stage composition and the effects of harvesting are generally included in this component of the

pathway. This data makes it possible to predict the feasibility of harvesting from natural populations, elucidate the mode of propagation by the species, and provides a starting point for physiological ranges to be tested. In the case of *Gelidium pristoides*, much of this data has already been collected in earlier studies, and is outlined in the literature review (Chapter 2). Some additional ecological data (i.e. community analysis, vertical and horizontal distribution ranges, physical conditions on the intertidal rocky shore) was gathered in this study as it was felt that data on the physical conditions in the habitat and interactions with other species was lacking.

Strain selection

In the strain selection stage, the candidate species would normally be collected from a natural population and cultivated to produce enough biomass for product assays. In the case of *Gelidium pristoides* this was not considered necessary or practical. Agar assays for *G. pristoides* had been done on a number of occasions in previous studies for material collected from natural populations (Carter & Anderson 1986, Onraet & Robertson 1987). Growing *G. pristoides* to obtain biomass for assays was not possible in the initial stages of the study since the species has proven to be very difficult to maintain in culture. One of the aims of the research was to develop a culture method towards the end of the study. Specimens of *G. pristoides* from local populations on the rocky shores of Algoa Bay were used to study the physiology of the seaweed.

Physiological studies

In the physiology component of the approach used by Hansen (1983) it is suggested that short-term variation in photosynthesis and respiration rates be determined in relation to a variety of physical conditions. These are light intensity, air and water temperature, submerged and immersed conditions and inorganic carbon concentration. These data define the conditions that should be met by the culture method or system, and gives an indication of the alga's basic requirements for growth.

The physiological tolerance ranges of the alga for physical variables tested in the laboratory can be compared with the physical conditions occurring in the natural environment in which the alga is found. Differences between the tolerance range for a particular environmental variable determined in the laboratory and the range of that variable in the alga's natural distribution range could indicate additional mechanisms at work, or specific requirements that may have been overlooked, i.e. some parameter that may not have been measured or considered important, or a species interaction affecting the alga's success in the natural habitat. E.g. *Gelidium pristoides* transplanted to lower

elevations on the intertidal zone are prone to overgrowth by epiphytic bryozoans (Carter & Anderson 1991).

Cultivation trials

Data gathered in the physiological experiments should provide the necessary information to predict the type of mariculture system in which to cultivate *Gelidium pristoides*, and can be used to determine production estimates. This would influence water quality considerations and the culture system design requirements. It is only in cultivation trials that problems like epiphyte control and stocking density can be assessed and addressed.

The main aim of the research undertaken in this study was to determine why *Gelidium pristoides* degrades and perishes when maintained in submerged culture in seawater tanks. Initial culture trials and experimentation have shown that this seaweed can not be maintained in vegetative culture for longer than a week (Hampson 1996 pers com.). After two or three days in culture tanks the alga starts to degrade. Once this degradation process and the factors responsible are understood it should be possible to develop a culture method for *G. pristoides* that will include the factors that promote the alga's growth and exclude factors responsible for the alga's degradation. This may make cultivation possible, facilitating long-term physiological research and possibly commercial mariculture.

From initial observations and existing information on the seaweed a number of hypotheses could be generated. Testing these hypotheses could give information as to why the seaweed is so difficult to cultivate and methods proposed to overcome these problems. Figure 1.1 shows the flow of information and the methodology followed during these investigations.

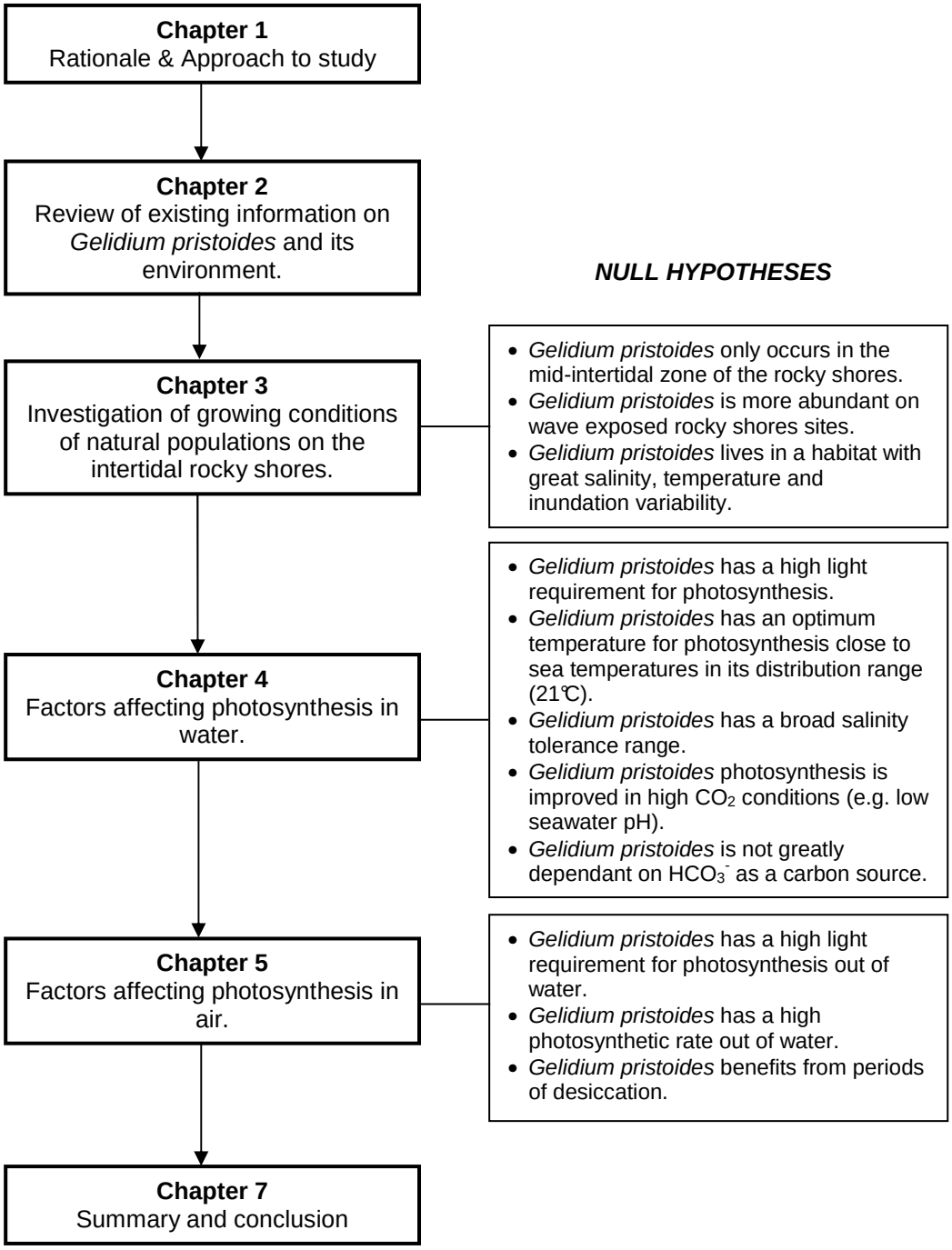


Figure 1.1 Flow of information and hypotheses tested during the investigation of G*elidium pristoides* eco-physiology and potential factors affecting its growth in culture.

Chapter 2

Literature review

2.1 Research on *Gelidium pristoides* (Turner) Kützing

The South African agarophyte *Gelidium pristoides* has been the subject of a number of studies in the past twenty years. Most of these were ecological studies that presented data on the growth and reproduction of the species (Beckley 1981, Carter 1985, McQuaid 1985, Carter & Anderson 1985, Carter & Simons 1987, Gibbons 1988, Carter & Anderson 1991, Carter 1993, Toefy *et al.* 2003), while some also investigated it's agar quality and possible cultivation (Carter & Anderson 1986, Onraet & Robertson 1987, Aken *et al*. 1993). Anderson *et al.* (1991) have reviewed the biology of *Gelidium pristoides,* while Tronchin *et al.* (2003) reviewed the economic use of the seaweed in Southern Africa. The phylogenetic relationships and taxonomic status of the seaweed was assessed by Tronchin *et al*. (2002) and McQuaid's (1985) work considered some aspects of the alga's ecophysiology, no other work has been done to describe the physiological response of *G. pristoides* to changes in environmental conditions.

Other species of *Gelidium*, from different parts of the world, have received considerable attention in this respect. The effects of different physical parameters on the growth of Chilean *Gelidium pusillum, Gelidium filicinum, Gelidium lingulatum* and *Gelidium spinulosum* have been documented (Oliger & Santelices 1981), as well as some physiological work on *Gelidium robustum*, *Gelidium vagum*, and *Gelidium chilense* (D'Antonio & Gibor 1985, Patwary *et al*. 1993, Santelices & Varela 1994).

The most studied species of this genus is *Gelidium sesquipedale* from Europe. Its physiological response to environmental conditions has been the subject of exhaustive investigation (Gorostiaga 1994, Santos 1994, Vignon *et al*. 1994a, Vignon *et al*. 1994b, Torres *et al*. 1995, Duarte & Ferreira 1995, Hernandez *et al*. 1995, Carmona *et al*. 1996, Gomez & Figueroa 1998). While it would be useful if *G. pristoides* and *G. sesquipedale* had comparable physiological characteristics, *G. pristoides* occurs exclusively in the littoral zone of South African rocky shores, while the *G. sesquipedale* latter species is found in turbulent sub-tidal environments on the eastern Atlantic coast, indicating that the two probably differ significantly in this respect.

Many *Gelidium* species, although good sources of agar, are small macroalgae occurring in low biomass volumes (Mairh & Sreenivasa 1978, Aken *et al*. 1993, Ramiro Rojas *et al*. 1996) making them prime candidates for cultivation in mariculture systems. However cultivating *Gelidium* species has proved to be difficult (Santelices 1988, Aken *et al*. 1993) and a number of investigations into certain aspects of developing cultivation methods have been completed. *Gelidium rex* is a Chilean species of commercial importance. Preliminary studies of its potential for cultivation have dealt with seeding or re-attachment of the alga for artificial propagation. Cultivation from spores showed successful settlement of propagules onto limpet shells. These were grown for two months in

laboratory tanks with Provasoli Enriched Seawater at 14℃ and 32 ppt salinity. They later successfully transplanted to the sea (Ramiro Rojas *et al*. 1996). Salinas (1991) also investigated the potential of *G. sesquipedale* for mariculture by attempting re-attachment in spray cultivation systems. Carter (1985) attempted to induce sporeling settlement on glass slides. The study showed germination percentages of *ca*. 10% for bispores and 30% for carpospores. The germlings were cultivated in the laboratory in Provasoli Enriched Seawater at a light intensity of 50 µmol m^{-2} s^{-1} and best growth was obtained at a temperature of 23℃. The seaweed that developed from these spores never developed thalli similar to those in nature and mature individuals could not be obtained for transplantation. However seeding of glass petri dishes and subsequent growth in a study done on *Gelidium robustum*, showed normal morphology developing (D'Antonio & Gibor 1985).

Using fragments of mature seaweed from natural populations for cultivation seems to be another viable option for seeding substrate for *Gelidium* cultivation. Santelices and Varela (1994) have shown that *Gelidium chilense* can successfully re-attach to calcareous substrates once broken off from the parent thallus or from whole thalli being torn from the substrate. Creeping axes develop from upright fragments, and from these, bundles of re-attachment cells arise to penetrate the substratum and anchor the thallus. This characteristic of some *Gelidium* species was tested as a seeding mode for the cultivation of *Gelidium rex*. Sections of fronds were placed in close association with shell substrates by holding them in place with nylon mesh. The treatments were placed in tanks with Provasoli Enriched Seawater at 14℃ and 32 ppt. After only ten days the thalli had completely attached to the shells, with rhizoids penetrating the substrate (Ramiro Rojas *et al*. 1996). The use of *Gelidium* thallus fragments in intensive culture has been suggested in work by Titlyanov & Titlyanova 2006, Titlyanov *et al.* 2006. Whole thalli have been used in attempts to cultivate *Gelidium* species. In *Gelidium pusillum*, translocation of whole individuals to laboratory conditions showed good growth in Von Stosch medium (Mairh & Sreenivasa Rao 1978). Transplanting whole individuals of *Gelidium pristoides* to open water rope and net cultures at varying depth were less successful. This was mostly due sediment accumulation on the thallus surface and probably inappropriate site selection. After two weeks submerged most of the cultures had started deteriorating, while the rope cultures grown at the water surface were the most successful (Aken *et al*. 1993).

2.2. Stress in intertidal seaweeds

Intertidal seaweeds inhabit a highly unpredictable environment. The duration of atmospheric exposure that intertidal seaweeds experience varies with the tidal cycle. For example the lower portion of the intertidal zone is exposed for longer periods during spring tidal cycles than in neap tidal cycles. During immersion the algae experience wave action, currents and lower light

intensities. When the algae are exposed they are subject to evaporative water loss, temperature extremes, high light intensities, and sometimes to fresh water. Such variable surroundings would be considered stressful to most organisms. The relative thermal and osmotic stability of seawater offers the least stressful condition for these algae, while desiccation due to evaporative water loss is probably the most important stressor while the algae are exposed (Abe *et al*. 2001). Desiccation tolerance in intertidal seaweeds varies between species and it has been suggested that these differences are related to their zonation in the littoral zone (Abe *et al*. 2001). Information of the mechanisms employed to tolerate desiccation stress in intertidal algae seems lacking. Work by Sampath-Wiley *et al.* (2008) suggests that antioxidants play an important role in reactive oxygen metabolism and stress tolerance in the intertidal seaweed *Porphyra umbilicalis*.

Davison and Pearson (1996) distinguish between two types of stress in intertidal seaweeds. Limitation stress is stress due an inadequate supply of resources to the organism, causing a reduction in growth rate. The other type of stress is called disruptive stress, which is the result of adverse physical conditions that results in damage or a loss in production (due to resource allocation to prevent or repair damage). These concepts outline the potential negative effects of stress on algae, and assume that all stress results in a negative response.

Lichtenthaler (1996), in a review on vegetation stress, considers stress to potentially have a positive effect on plants, and not only adverse effects. In some cases, mild stress could trigger beneficial metabolic responses or increase physiological activity. This is type of stress is called *eu-stress*. Stress resulting in damage to the plant, reducing its overall success or productivity is called *dis-stress*. There is a gradual transition between *eu-stress* and *dis-stress*. The stress dose and the organism's tolerance limits would determine the type of stress experienced.

Stress can be measured in a number of ways. The most obvious signs of stress would be a lowering of productivity, which is evident from a drop in the growth rate of the organism. Shorter-term stress responses in algae have generally been determined by measuring photosynthetic rate as oxygen evolved or CO_2 uptake (Johnson *et al*. 1974, Beer & Eshel 1983, Surif & Raven 1990, Dawes & Kovach 1992, Abe *et al*. 2001). Lichtenthaler (1996) in review of the stress concept in plants cites the measurement of respiration, transpiration, stomatal conductance, water potential, photosynthetic pigment content, and concentration of stress metabolites as traditional methods for detecting stress, while a more recent non-invasive approach includes the measurements of chlorophyll-*a* fluorescence induction kinetics.

2.3. Chlorophyll-*a* fluorescence as a measure of photosynthetic efficiency in algae

Kautsky (1931) described an inverse relationship between photosynthetic CO_2 assimilation and chlorophyll-a fluorescence. It has since been shown that there is a close, immediate relationship between chlorophyll-*a* fluorescence and carbon assimilation during photosynthesis (Sivak & Walker 1985). This has made chlorophyll-a fluorescence an important tool in photosynthesis research and investigations into plant stress (Sivak & Walker 1985, Lichtenthaler 1992, Gomez *et al*. 2001).

Photosynthetically active radiation incident on a plant excites the chlorophyll molecules. Most of this excitation energy is used in photosynthetic quantum conversion, while about 5 to 15% is dissipated as heat, and about 2% is re-emitted as red and far-red chlorophyll-a fluorescence. When the plant is stressed photosynthetic quantum conversion is lowered and more energy is dissipated as fluorescence (Lichtenthaler 1996). These fluorescence changes can be measured to detect stress in the plant before they become visibly evident. Recent technological advances in the measurement of chlorophyll-*a* fluorescence, namely the development of accurate pulse amplitude modulation fluorimeters have provided a very effective tool for measuring short-term photosynthetic responses in marine algae (Herrmann *et al*. 1995, Magnusson 1997, Cordi *et al*. 1997, Gomez & Figueroa 1998, Silva *et al*. 1998, Hader *et al*. 1998, Casper-Lindley & Bjorkman 1998, Gomez *et al.* 2004).

Chlorophyll-a fluorescence induction kinetics follow a recognised pattern, and present a number of measurable parameters that can be used to determine photosynthetic performance. The terminology used by Lavorel & Etienne (1977) is commonly used to describe these characteristics (Figure 2.1). According to Sivak & Walker (1985) the early events in the fluorescence induction do not relate directly to carbon fixation.

Figure 2.1 below indicates the recognised stages in chlorophyll –a fluorescence induction (as per Lavorel & Ettiene 1977), where O is the moment when illumination is started and variable fluorescence increases above background fluorescence, rises through I, makes a slight dip, D, and rises further to the peak, P. From P there is a steady drop to the steady state S and sometimes a secondary peak, M, and after which a steady state value T is reached.

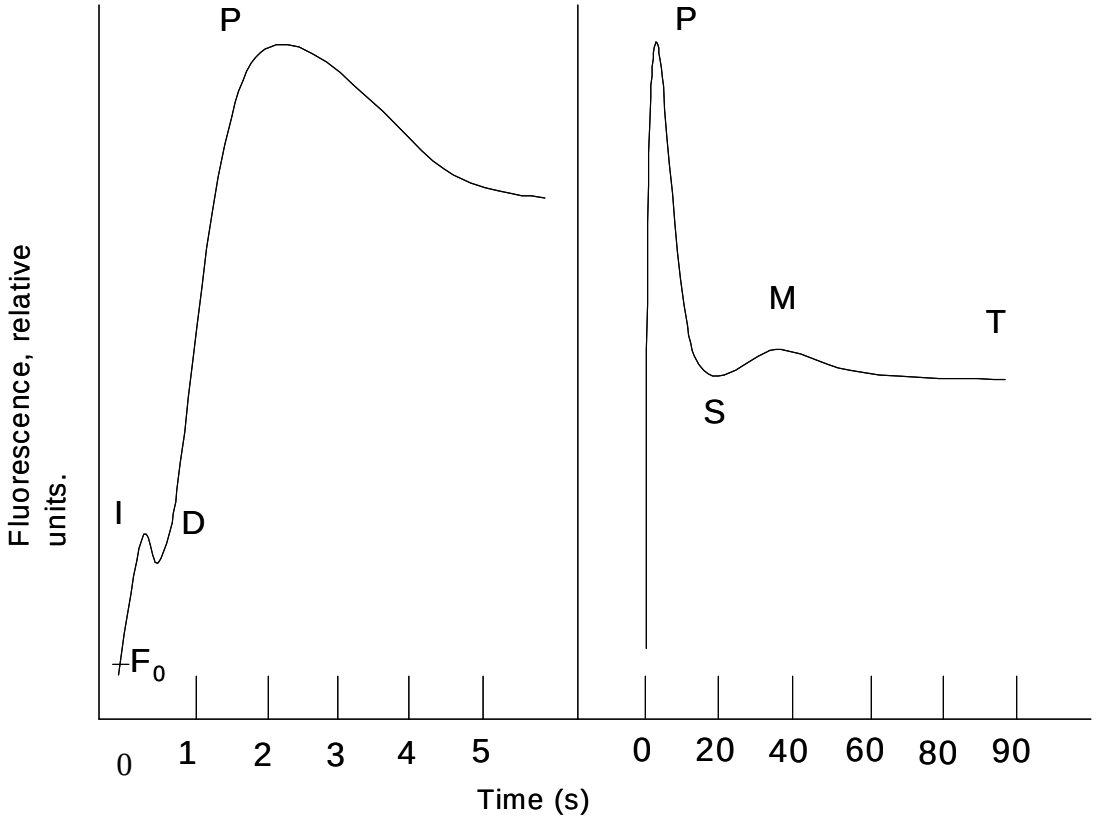


Figure 2.1. The terminology used by Lavorel & Etienne (1977) from Sivak and Walker (1985).

The initial changes (phases O to P) occur very rapidly with the onset of illumination and are a reflection of the condition of the electron transport chain, and changes from the dark to light state, rather than carbon metabolism effects. About ten seconds after the onset of illumination fluorescence characteristics can be related to carbon assimilation (stages P to T) (Sivak & Walker 1985).

Parameters commonly measured with the aid of a PAM fluorimeter have a slightly different terminology but are closely related to that used by Lavorel & Etienne (1977).

F_0 , the minimal fluorescence yield, is defined as the fluorescence from the sample when all the reaction centres are open, i.e. Q_A oxidised (Q_A – primary quinone acceptor of PSII). This is measured by a non-actinic measuring beam, and reflects steady state fluorescence when the sample is dark-adapted. Upon the onset of illumination the fluorescence usually increases to a maximum, F_M. This peak corresponds to "P", and decreases to steady state as the proton gradient across the thylakoid membrane is restored (energy dependant quenching - q_e) and photochemical quenching (q_Q) (delay in re-oxidation of Q_A) is established. The steady state fluorescence, achieved after all these processes have been completed while the plant is being illuminated, is known as F_S and corresponds to the "T" in Lavorel and Etienne's terminology (Sivak & Walker 1985, Cunningham *et al*. 1996). F_V is the difference between F_M and F_0, and is known as variable

fluorescence. By using these fluorescence parameters, calculations can be made providing further information, relating not only to activity on the level of the chloroplast, but to the overall photosynthetic and production performance of algae.

The relationship of the fluorescence parameters F_v (variable fluorescence) and F_m (maximal fluorescence) (F_v/F_m ratio, also called optimal quantum yield) gives an indication of the photosynthetic efficiency (quantum efficiency) of an alga, which can be related to stress and productivity (Herrmann *et al*. 1995, Cordi *et al*. 1997, Magnusson 1997, Gomez & Figueroa 1998). A decrease in the F_v/F_m ratio generally indicates a lowering of photosynthetic efficiency. The F_v/F_m ratio in higher plants and the green algae when photosynthesising efficiently is normally *ca*. 0.8. In cyanobacteria and red algae this ratio tends to be lower, around 0.4 - 0.7 (Cunningham *et al*. 1996). These differences in F_v/F_m among algal classes can be attributed to a number of factors including: [1]a high proportion of uncoupled antennae molecules (phycobillisomes) lowering F_0, [2]highly efficient spillover from PSII to PSI due to their smaller spatial separation in the chl-*c* containing algae (decreasing F_m) and [3]a decrease in F_m due to 'energy dependant quenching' (Cunningham *et al*. 1996). It may be worth keeping in mind that Davison & Pearson (1996) caution against the use of F_v/F_m to quantify photoinhibition, as a decrease in the ratio could indicate either damage to the photosynthetic apparatus, or other energy dissipation mechanisms via the xanthophyll cycle or PSII reaction centres closing down.

The photosynthetic quantum yield for algae can also be calculated from fluorescence yield data:

$$\textit{Effective Quantum Yield (Y)} = \frac{(F_m' - F_s)}{F_m'}$$ (Genty *et al.*$_{(b)}$ 1989, Hader *et al*. 1998).

Where *Y* is the Effective Quantum Yield, F_m' is the maximal fluorescence[1] for light adapted samples and F_s is the current steady state fluorescence. There is a linear relationship between this fluorescence parameter and the quantum yield of photosynthesis. Theoretically this makes it possible to use fluorescence to determine photosynthesis-irradiance curves and measurement of photosynthetic carbon assimilation without having to measure CO_2 uptake or O_2 evolution in the traditional fashion (Genty *et al*. 1989). Further it is possible to determine relative electron transport rate (*rETR*) in seaweed from the effective quantum yield when the irradiance and frond absorbtance of the alga is known.

Relative electron transport rate (*rETR*) is calculated as: $rETR = Y \times Irradiance \times K$, calculated for each irradiance used, where *Y* is the *Effective Quantum Yield*. K can be calculated from $K = 0.5(1 - T - R)$, where T is transmittance through the sample; R is the fraction of light reflected

[1] F_m is usually used to refer to maximal fluorescence in dark adapted samples. In this instance the term F_m' is used to indicate maximal fluorescence in light adapted samples.

by the sample. 0.5 is a correction factor added due to the fact that the transport of one electron requires the absorption of two quanta of light (Gomez & Figueroa 1998, Silva *et al*. 1998).

A novel way of estimating daily productivity in algae by means of fluorescence parameters has been applied to algae by Magnusson (1997); using α_{net} (maximal productivity that can be obtained for the alga), F_v/F_m $_{max}$ and daily weighted F_v/F_m:

$$Fv/Fm_{weighted} = \left[\frac{Fv/Fm(1) \times PI(1)}{PI(1)+PI(2)+...PI(n)} + \frac{Fv/Fm(2) \times PI(2)}{PI(1)+PI(2)+...PI(n)} + ... \right].$$

and

$$\alpha net_{corrected} = \frac{\alpha net \times (Fv/Fm_{weighted})}{Fv/Fm_{max}},$$

α_{net} can be obtained from literature or measurement, and F_v/F_m $_{max}$ can be determined in a laboratory; e.g. F_v/F_m has been shown to be about 0.83 for green algae (Magnusson 1997).

2.4. Photosynthesis in intertidal macroalgae

Algae like *Gelidium pristoides*, which inhabit the mid- to lower-eulittoral zone, are periodically exposed and immersed. During periods of exposure the algae are often subject to temperature increases and desiccation. Different species persisting in this environment respond to these periods of exposure in different ways. Much of the work done on the responses of intertidal algal to exposure and desiccation has been based on measurements of oxygen-evolution or inorganic carbon uptake as measures of photosynthetic rate (Johnson *et al.* 1974, Dromgoole 1980, Dring & Brown 1982, Beer & Eshel 1983, Surif & Raven 1990). Oxygen evolution is used to measure photosynthetic rate in water, whereas CO_2 uptake measured by infrared gas analysis is commonly used as a measure of photosynthetic rate in air. These values are only comparable if assumptions about, or measurement of the photosynthetic quotient of the algae are made.

Work done on *Ulva* sp. investigated the effects of desiccation on the alga when exposed to air. CO_2 uptake was measured and results indicated a constant rate of photosynthetic CO_2 uptake down to 80% relative water content. Thereafter rates decreased but maintained a positive net photosynthesis down to *ca*. 35% relative water content. Exposure periods that this *Ulva* species would experience in its natural habitat would still allow a positive net rate of photosynthesis for a whole day in all but the highest tidal elevations in which it is found (Beer & Eshel 1983).

Similar work done on *Pelvetia canaliculata*, *Fucus spiralis*, *Fucus vesiculosus*, *Fucus serratus* and *Laminaria digitata* showed an increase in photosynthetic rate with water loss down to 25%. These phaeophytes showed a correlation between their height on the intertidal gradient and

photosynthetic recovery after desiccation (Dring & Brown 1982). In *Fucus spiralis*, desiccation down to tissue water contents of 92 to 96% seemed to stimulate photosynthetic rates in air to 110 to 148% of rates in fronds with no water loss. Photosynthetic rate decreased in a linear fashion with further water loss (Madsen & Maberly 1990). Using oxygen evolution and CO_2 uptake, Surif and Raven (1990) showed that, even taking into account a photosynthetic quotient of 1.1 - 1.2, *Fucus spiralis*, *Fucus vesciculosus*, *Fucus serratus* and *Ascophyllum nodosum* had higher photosynthetic rates when exposed than when immersed. The most notable difference in immersed vs. emersed photosynthetic rate in this study was that for *Pelvetia canaliculata*, which had a rate of 13.2 µmol O_2 g^{-1} fresh weight h^{-1} when submerged and a CO_2 uptake rate of 42 µmol g^{-1} fresh weight h^{-1} when emersed (Surif & Raven 1990). This research was done in seawater and air, with near ambient CO_2 levels at a temperature of 10℃ and saturating lig ht intensities.

Pena *et al*. (1999), investigated the rates of photosynthesis of *Caloglossa leprieurii* and *Bostrychia calliptera* on mangrove prop roots. They found that emersed photosynthesis contributed 17% of the daily carbon gain in *B. calliptera* and 12% in *C. leprieurii*. *Caloglossa leprieurii* occurs higher up in the intertidal range and is exposed for longer (29% vs. 21% of photoperiod), resulting in both gaining a similar amount of carbon from emersed photosynthesis (Pena *et al*. 1999). This indicates that the ability to tolerate exposure is not the only factor determining intertidal algal zonation, but that the ability to take advantage of the exposed conditions is also important.

Emersed conditions present intertidal macroalgae with a number of potential benefits and constraints. Emersed algae will experience a higher incident photon flux density than submerged algae, although it is uncertain how well these algae are able to photosynthesise at high light intensities in the absence of other necessary resources (Lüning 1990). These seaweeds suffer reduced nutrient supply during emersion, however they have access to inorganic carbon in the form of CO_2. Carbon dioxide acquisition from air is less constrained by diffusion boundary layers since CO_2 has a 10000 times greater diffusion coefficient in air than in water (Raven 1999). The time that the algae have to take advantage of increased inorganic carbon availability depends on the desiccation rate. As water is lost there is often an initial increase in photosynthesis, with a decline with further desiccation (Surif & Raven 1990). It seems that the longer the algae takes to reach this threshold moisture content, or the lower the threshold moisture content is, the better it will be able to exploit the greater availability of inorganic carbon in the form of CO_2 (Johnson *et al*. 1974). Further investigation of carbon acquisition mechanisms and their implication for the amount of carbon fixed per unit water lost will show how some algae are more successful in the intertidal zone than others (Raven 1999).

2.5. Irradiance response

While exposed, seaweeds inhabiting the intertidal zone are exposed to high light intensities. However the change in environment presents these algae with a peculiar condition. Are they high light or low light adapted, or do they have rapid light acclimation rates so that they can be successful both when exposed and submerged? The implications of being low light adapted and exposed to high light intensities include photoinhibition and damage to the photosynthetic apparatus (Hader *et al*. 1998, Hader *et al*. 2001, Hanelt 1992). While Silva *et al* (1998) showed that high light adapted individuals of *Gelidium sequipedale* from shallow water environments, exhibit lower photosynthetic rates than low light adapted individuals when exposed to similar low light intensities (< 100 µmol m^{-2} s^{-1}).

Work by Dawes and Kovach (1992) compared light response in subtidal and intertidal populations of a number of tropical macroalgal species. I_k (saturation irradiance) and I_c (light compensation irradiance) values for subtidal samples were generally slightly higher (*ca*. 5 µmol m^{-2} s^{-1}) than those of intertidal samples at 35 ppt and 27℃ (I_k = *ca*. 30 to 90 µmol m^{-2} s^{-1}; I_c = *ca*. 10 to 20 µmol m^{-2} s^{-1}). Some members of the Gelidiales from the lower intertidal of the Canary Islands show similar low I_k values. (*Gelidium canariensis* – *ca*. 20 – 65 µmol m^{-2} s^{-1}, *Gelidium arbuscula*, *ca*. 30 – 90 µmol m^{-2} s^{-1}, *Pterocladia capillacea*, *ca*. 77 – 122 µmol m^{-2} s^{-1}). These algae also showed an increase in the I_k value at increased inorganic carbon concentrations (Mercado *et al*. 2001). Intertidal algae from the rocky shores of Greenland exhibited I_c of 40 – 100 µmol m^{-2} s^{-1}, and I_k of approximately 140 to 400 µmol m^{-2} s^{-1} (Prahl 1979). These low values may be a reflection of lower rates of photosynthesis at the low temperatures used in the experiments (*ca*. 5℃).

When light intensity increases, the rate of photosynthetic carbon fixation increases concomitantly (Kremer 1981). When light intensity increases to above the rate at which photosynthetic carbon fixation in the Calvin cycle can occur, excess energy is dissipated by energy dependent quenching (q_e), which adapts the rate of energy supply by the light reactions of photosynthesis to the demands of the Calvin cycle (Hanelt 1996). A further increase in irradiance results in a decrease in the efficiency of PSII (e.g. decreasing number of active PSII centres, or aggregation of LHCs), increasing non-radiative energy dissipation (Hanelt 1996). This decrease in efficiency is known as dynamic photoinhibition. The D_1-protein of the reaction centres of PSII is continually degraded and repaired during photosynthesis. An increase in irradiance above the dissipation capacity of dynamic photoinhibition results in the D_1-protein of PSII being degraded faster than it can be repaired (chronic photoinhibition) (Ohad *et al.* 1984).

Both the effective quantum yield and the optimal quantum yield for fluorescence, and actual photosynthetic rate measured as oxygen production have been used to quantify photoinhibition in marine macrophytes (Hanelt 1992, Hanelt *et al*. 1992, Jiminez *et al*. 1998, Hader *et al*. 2001). In

marine macroalgae from the intertidal and subtidal zones of Spain, it was found that both photosynthetic oxygen production and electron flow rates were lowest when the irradiance was at a maximum, around noon. This effect was more pronounced in shade-type species, normally occurring in crevices, under overhangs or subtidally. Photosynthetic recovery from photoinhibition was also faster in the sun-type algae than in the shade-type algae, based on faster increase in optimal quantum yield after exposure to high light intensities (Jiminez *et al*. 1998, Hader *et al*. 2001). Hanelt (1992) working in the littoral zone in the South China Sea found similar results. It was also found that photoinhibition was reduced in water with increased turbidity during heavier wave action, and that the mid-day reduction in photosynthetic capacity depended solely on the irradiance levels and was not related to any circadian rhythms (Hanelt 1992). The duration of exposure to high irradiances affects the extent and rate of photosynthetic recovery in seaweeds, with a larger reduction in photosynthetic quantum yield with increased exposure time. It was interesting that the reduction in photosynthetic quantum yield was apparent even in algae at their natural growth sites at midday when the sun was at its highest angle (Hader *et al*. 2001). Photoinhibition and photosynthetic recovery from photoinhibition also seems to be temperature dependant (Hanelt *et al*. 1992, Hanelt 1996).

2.6. Temperature tolerance and adaptation

A typical short-term response of light saturated algal photosynthesis to an increase in temperature is an increase in rate, up to an optimum temperature, followed by a decrease with further rise in temperature. At low temperatures, photosynthetic electron transfer is limited, lowering photosynthetic rates and light saturation at low intensities. The initial increase can be described by the term Q_{10} , which is related by the equation $Q_{10} = \frac{P_t + 10}{P_t}$, where P_t is the photosynthetic rate at temperature *t*, and P_t+10 is the photosynthetic rate a temperature *t*+10. The Q_{10} value for photosynthesis in algae is generally close to 2.0, but may vary from 2.0 to 5.4 (Kremer 1981, Davison 1991).

Beyond the optimum temperature, the decrease in photosynthetic rate is usually rapid. According to Davison (1991) increasing temperature may reduce photosynthetic rate in a number of possible ways: [1]Temperature effects on rate limiting enzymes (both K_m and V_{max} are temperature dependant). [2]Temperature sensitive steps in the Inorganic Carbon (IOC) uptake/concentrating mechanism (temperature affects membrane permeability, carbonic anhydrase activity and diffusion). [3]Disruption of energy transfer between phycobillisomes and PSII in red algae. [4]The K_m of Rubisco for O_2 increases more slowly with increasing temperature than for CO_2, thus the potential for photorespiration increases with increasing temperature.

Simon and co-workers (1999) have shown that both photosynthetic and dark respiration rates increased as seawater temperatures were elevated. In *Gelidium sesquipedale* the highest quantum efficiency was measured in algae acclimated to summer conditions (18.5℃) and then grown at a higher temperature (26℃) (Duarte & Ferreira 1995). Hansen (1983) measured photosynthetic rates of *Gelidium coulteri* in both air and water at different temperatures. The submerged temperature optimum was *ca*. 25℃, and net photosynthesis was about 5 times higher when submerged than in air, however it is not clear from this work, to what temperature the algae were adapted. Work by Oliger and Santelices (1981) showed that there were surprising differences in the temperature optima for growth of four species of *Gelidium* from the same site grown at different temperatures. *Gelidium filicinum* had a temperature optimum of 20℃, while *Gelidium lingulatum* and *Gelidium spinulosum* grew best at 15℃. *Gelidium pusillum* showed good growth performance at both 15 and 20℃. The algae were all from different tidal elevations but there seemed to be no relationship between elevation on the shore and temperature optimum. *Gelidium pusillum* is an upper intertidal species and had the widest temperature tolerance range of the species investigated (Oliger and Santelices 1981). Temperature was shown to be the key factor affecting

growth rate in *Gelidium* species investigated by Boulus *et al*. (2007), where it improved growth rates as well as agar gel strength.

2.7. Inorganic carbon use by marine macroalgae

Carbon dioxide occurs in the atmosphere at a concentration of *ca*. 300 ppm (*ca*. 13 µM) and diffuses into plant cells along a concentration gradient. Plants assimilate inorganic carbon directly from the atmosphere by combination of ribulose-1,5-bisphosphate (RuBP) with CO_2 to form two molecules of 3-phosphoglyceric acid (3-PGA), a reaction catalysed by the enzyme Ribulose-bisphosphate carboxylase / oxygenase (Rubisco). The 3-PGA is used to form tetrose, pentose, hexose and heptose sugar phosphates while some is converted back to RuBP in the photosynthetic carbon reduction cycle (Calvin cycle / C3 photosynthetic pathway) in the stroma of chloroplasts (Salisbury & Ross 1991).

Marine algae use CO_2 as an inorganic carbon source in the same way that plants do, after diffusion of CO_2 across the cell membrane along a concentration gradient (Raven 1974 in Lobban *et al* 1985). Dissolved CO_2 in seawater in equilibrium with the atmosphere is found at a concentration of *ca*. 10 µM (Lobban et al.1985).

The limiting effect of the low CO_2 concentration on algal photosynthesis is exacerbated by the low diffusion rate of dissolved CO_2 in water, which can be several thousand times slower than in air (Holbrook, *et al.* 1988). The concentration of CO_2 in seawater is further affected by water temperature, salinity and pH (Kremer 1981). In the intertidal seaweed *Porphyra haitanensis* it was found that even at atmospheric CO_2 concentrations emersed photosynthesis was not saturated (Zou & Gao 2002).

Dissolved CO_2 occurs in equilibrium with a number of other inorganic carbon species related by the equation:

$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$ - Equation 1.1

At the pH of seawater (*ca*. 8.0), the dominant form of IOC is bicarbonate (HCO_3^-) (Fig. 1.2).

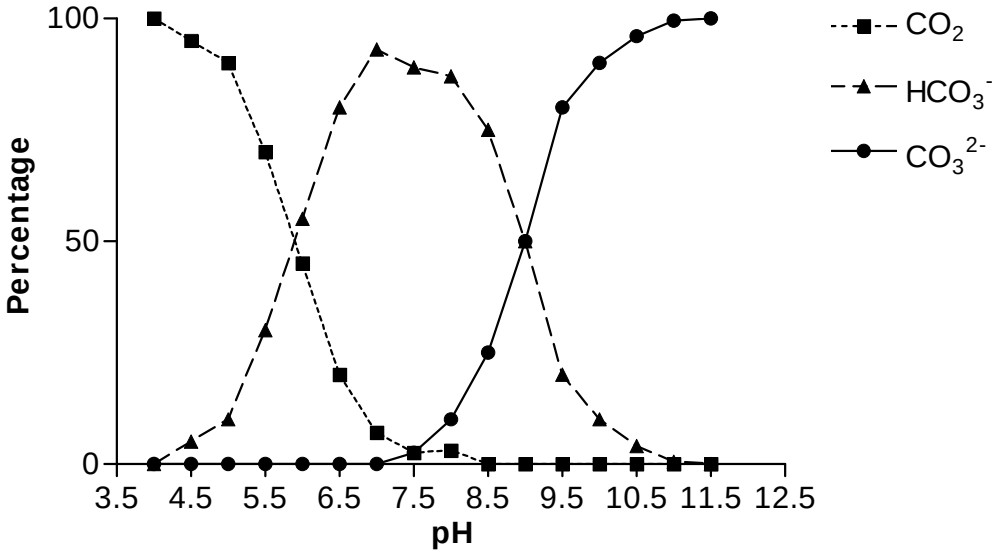


Figure 2.2. Relative amounts of different forms of inorganic carbon in seawater over a pH range *(from Marine Chemistry, Horne).*

2.7.1. Use of bicarbonate in seawater by marine macrophytes

Some algae are also able to use bicarbonate as an IOC source to a certain degree (Maberly 1990, Mercado *et al*. 1998). Murru and Sandgren (2004) investigated carbon use in 38 species of red macroalgae using pH drift experiments. These results suggested that while subtidal algae are able to use dissolved carbon dioxide, intertidal algae showed the ability to use both bicarbonate and dissolved carbon dioxide. Because Rubisco uses CO_2 as a substrate, bicarbonate needs to be converted to CO_2 before entering the Calvin cycle (C_3 metabolism). This reaction is catalysed by the enzyme carbonic anhydrase (Salisbury & Ross 1991).

It has been shown that certain algae assimilate IOC in the dark and are able to use bicarbonate directly by combination with phoshoenolpyruvate (so called 'C_4' metabolism) (Morris 1980). It has been found that some macroalgal species produce malate and aspartate directly from bicarbonate using this pathway (Patil & Joshi 1970). In a study by Holbrook *et al*. (1988) it was shown that Rubisco was the main carboxylation enzyme in *Cladophoropsis membranacea*, *Dilophus guineensis*, *Turbinaria turbinata*, *Lobophora variegata*, and *Laurencia papilllosa*.

The 'C_4'-like photosynthesis shown in *Udotea flabellum* exhibited a 50% decrease when the activity of phosphoenolpyruvate carboxykinase (PEPCK) was inhibited, suggesting that this pathway may be important in some marine macroalgal species (Reiskind & Bowes 1991). However the weak

activity of phosphoenolpyruvate carboxylase (PEPC) in many macroalgae investigated indicates that the 'C_4'-like pathway generally makes only a minor contribution to the total inorganic carbon assimilated by marine algae (Ting 1976, Kremer & Kuppers 1977).

How does the inorganic carbon contributed by bicarbonate enter the cells? Figure 2.3 indicates some potential pathways for IOC entry into the cells, which includes the following:

1. Active extrusion of protons creating an acidic zone where the non-catalysed extra-cellular conversion of HCO_3^- to CO_2 takes place and subsequent passive diffusion (Hellblom *et al*. 2001).
2. Passive diffusion of CO_2 from the boundary layer outside the cell membrane after dehydration catalysed by external CA (Mercado *et al*. 1997).
3. Passive diffusion into the cells and subsequent internal carbonic anhydrase (CA) aided dehydration to CO_2 (Mercado *et al*. 1997).
4. Co-transport of HCO_3^- and H^+ into the cells, and subsequent dehydration or directly to 'C_4' path (Hellblom *et al*. 2001).
5. Energy dependent transport into the cells against a concentration gradient (Hellblom *et al*. 2001).

In a study by Maberly (1990), representatives of the Chlorophyta showed the greatest ability to use bicarbonate. Some exhibited HCO_3^--uptake so effective that these species were able to raise the pH of seawater to more than 10.5, depleting all the CO_2 and half of the total inorganic carbon in the water (Maberly 1990).

At high pH, the concentration of CO_2 in equilibrium with other forms of inorganic carbon is very low. Using HCO_3^- by external conversion to CO_2 will become less effective as the conversion becomes increasingly difficult at higher pH (Shiraiwa *et al*. 1993, Axelsson *et al.* 1995).

Under emersed conditions, intertidal algae can take up CO_2 from the thin film of water around them. As CO_2 is removed the equilibrium shifts and the pH increases to create a HCO_3^- dominated microenvironment. This would also lead to a decrease in photosynthetic rate in macroalgae that don't have the ability to use HCO_3^- (Mercado & Niell 2000).

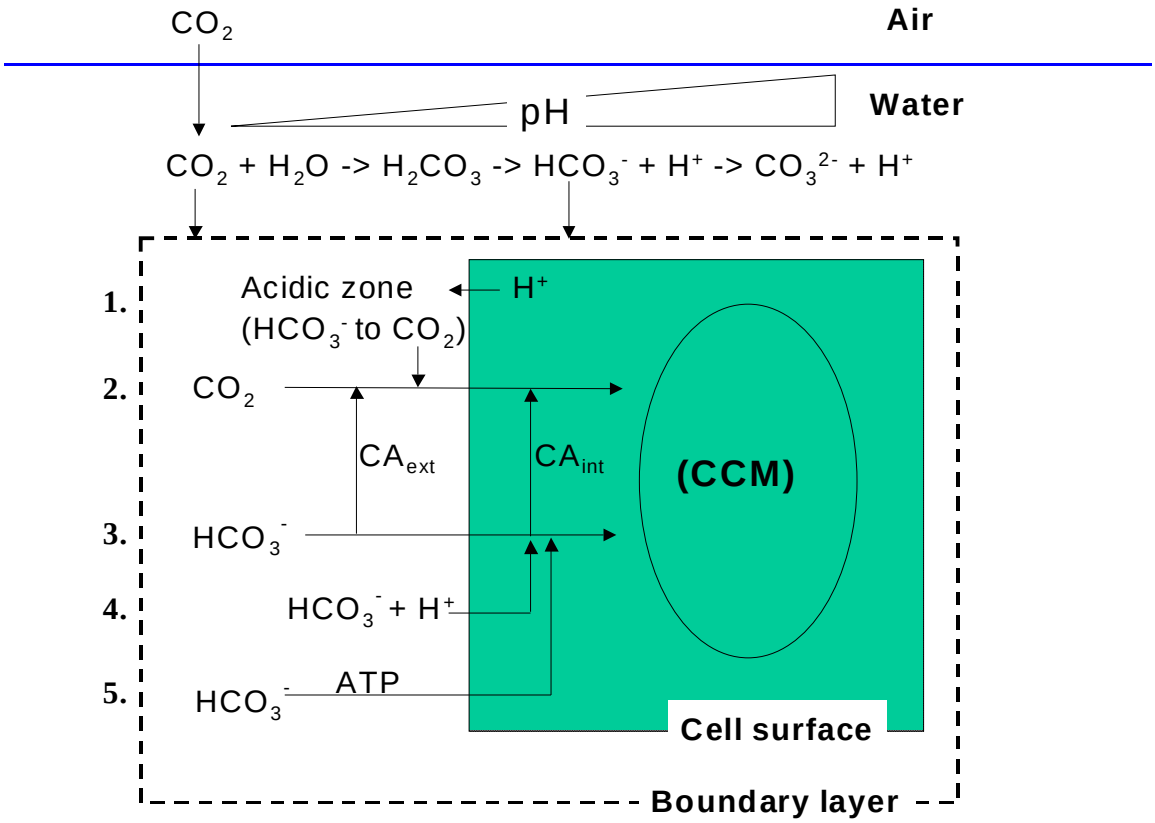


Figure 2.3. Possible mechanisms for the entry of inorganic carbon into the cells of marine macroalgae. ***1. Active extrusion of protons creating an acidic zone where the non-catalysed extra-cellular conversion of HCO_3^- to CO_2 occurs and subsequent passive diffusion (Hellblom et al. 2001). 2. Passive diffusion of CO_2 from the boundary layer outside the cell membrane after dehydration catalysed by external CA (Mercado et al. 1997). 3. Passive diffusion into the cells and subsequent internal CA aided dehydration to CO_2. 4. Co-transport of HCO_3^- and H^+ into the cells, and subsequent dehydration or directly to 'C_4' path. 5. Energy dependent transport into the cells against a concentration gradient.*** *ATP – Adenosine triphoshate, CA_{ext} – External carbonic anhydrase, CA_{int} – Internal carbonic anhydrase, CCM – Inorganic Carbon concentrating mechanism.*

The conversion of bicarbonate to CO_2 is a relatively slow process, so that at high photosynthetic rates CO_2 at the algal surface becomes depleted very quickly (Horne,1969). The subsequent low CO_2 concentration results in the oxygenase activity of Rubisco becoming higher than the carboxylating activity and as a result some of the carbon fixed is released through photorespiration (Kremer 1981a). An inorganic carbon concentrating mechanism (CCM) has been found in some algal species that increases CO_2 concentrations at the site of Rubisco thereby increasing the carboxylating activity of the enzyme and suppressing its oxygenase function (Raven 1996). Shiraiwa *et al*. (1993) showed that the external conversion of HCO_3^- to CO_2 in the medium directly surrounding algal cells can raise the pH of the medium. This alkalinization of the medium ceased at

a pH of between 9 and 10, but continued once the medium had been acidified to a pH of between 6 and 8. The alkalinization was linked to oxygen evolution and the availability of light, i.e. occurred only while the algae were photosynthesizing. This alkalinization phenomenon happened more rapidly in algae that had been adapted to low CO_2 concentrations and had developed an inorganic carbon concentrating mechanism (essentially a HCO_3^- to CO_2 conversion system).

Two commonly accepted mechanisms for bicarbonate assimilation are the direct uptake of HCO_3^- via anion exchange proteins; and the external dehydration of HCO_3^- followed by uptake of CO_2 (Beer & Eshel 1983, Larsson & Axelsson 1999). In *Laminaria saccharina*, HCO_3^- is assimilated by external CA catalysed dehydration and uptake as CO_2, which is augmented by proton extrusion, while *Ulva lactuca* has an inducible mechanism for direct HCO_3^- uptake (Axelsson *et al*. 2000). The induction of HCO_3^- use seems common amongst a number of green macroalgal species (Larsson & Axelsson 1999). C*haetomorpha linum* and *Chaetomorpha melagonium* showed a steady increase in photosynthetic rate with increasing time submerged in seawater at a high pH (9.8), reaching a maximum after about 30 hours (Larsson & Axelsson 1999). *Ulva lactuca* seems to have the ability to utilize HCO_3^-, however it is unknown whether it takes up HCO_3^- directly via an inorganic carbon concentrating mechanism (CCM) at the plasmalemma (Beer & Eshel 1983). It has been suggested that anion exchangers at the level of the cell membrane are employed to facilitate direct uptake up HCO_3^-, as the first step in an inorganic carbon concentrating mechanism in this alga (Drechsler *et al*. 1993). Evidence also exists to indicate that external CA mediated HCO_3^- dehydration and CO_2 uptake is the main mode of IOC uptake at high pH, with direct CO_2 uptake at lower pH (Bjork *et al*. 1992). It is likely that a number of pathways of IOC entry into the cells operate simultaneously and the individual activity of each of these mechanisms is dependent on habitat and physical conditions (light availability, exposure, IOC concentration, pH) (Maberly 1990, Beer 1996b).

While a number of red algal species have been shown to have the ability to use HCO_3^- (Israel & Friedlander 1998, Beardal & Roberts 1999, Mercado *et al*. 2000), the absence of an inorganic carbon concentrating mechanism and HCO_3^- use has been reported for other red algae (Raven & Beardall 1981, Maberly 1990). Species that are incapable of utilizing HCO_3^- show markedly reduced photosynthetic rates at pH values above 8.2. At pH above that normally found in the sea the concentration of CO_2 decreases while HCO_3^- concentration increases. However little indisputable data showing an inability to use HCO_3^- exist, and it is more likely that most species of marine macroalgae are able to take up HCO_3^- to some degree. Those algae performing very poorly at high pH may simply be inefficient at using HCO_3^-. It has been shown that the ability to assimilate HCO_3^- is generally highest in the Chlorophyta and lowest in the Rhodophyta, with the Phaeophyta showing intermediate ability to use HCO_3^- (Maberly 1990).

Carbonic anhydrase speeds up the conversion of HCO_3^- to CO_2 and plays a very important role in CO_2 concentration. Most of the models of inorganic carbon concentrating mechanisms proposed have been based on work done on microalgae (Marcus *et al*. 1984, Moroney & Tolbert 1985, Shiraiwa *et al*. 1993, Moroney & Chen 1998, Matsuda *et al*. 1998, Sultemeyer 1998, Moroney & Somanchi 1999). The existence of an inorganic carbon concentrating mechanism in macroalgae has been suggested by a number of authors (Johnston 1991, Axelsson *et al*. 1995, Raven 1995, Raven 1996). The inorganic carbon concentrating mechanism for macroalgae proposed by Sultemeyer (1998) depends on different types of carbonic anhydrase and their distribution between cell compartments (Figure 1.4).

The fact that membrane impermeable CA inhibitors, lowers the photosynthetic rate in many algae; and that under low CO_2 conditions carbonic anhydrase activity increases; strongly suggests that these algae use bicarbonate by extracellular conversion (e.g. periplasmic carbonic anhydrase outside the cell membrane) to CO_2 and subsequent uptake. Many macroalgae have intracellular carbonic anhydrase, which seems to play an important role in macroalgal photosynthesis (Kremer 1981). The suppression of intracellular CA results in a suppression of photosynthesis (Johnston 1991, Jiminez del Rio *et al*. 1995). The presence of cytoplasmic CA and its possible role depends on how the inorganic carbon is transported into the chloroplast. Where PEPC is involved cpCA (cytoplasmic carbonic anhydrase) is necessary for the conversion of CO_2 to HCO_3^-, which is the substrate for PEPCase. Where PEPCK is involved cpCA would not be required as PEPCKase uses CO_2 as substrate. However the data supporting these assumptions are lacking and there is a need for more work on this aspect of carbon fixation in macroalgae (Sultemeyer 1998).

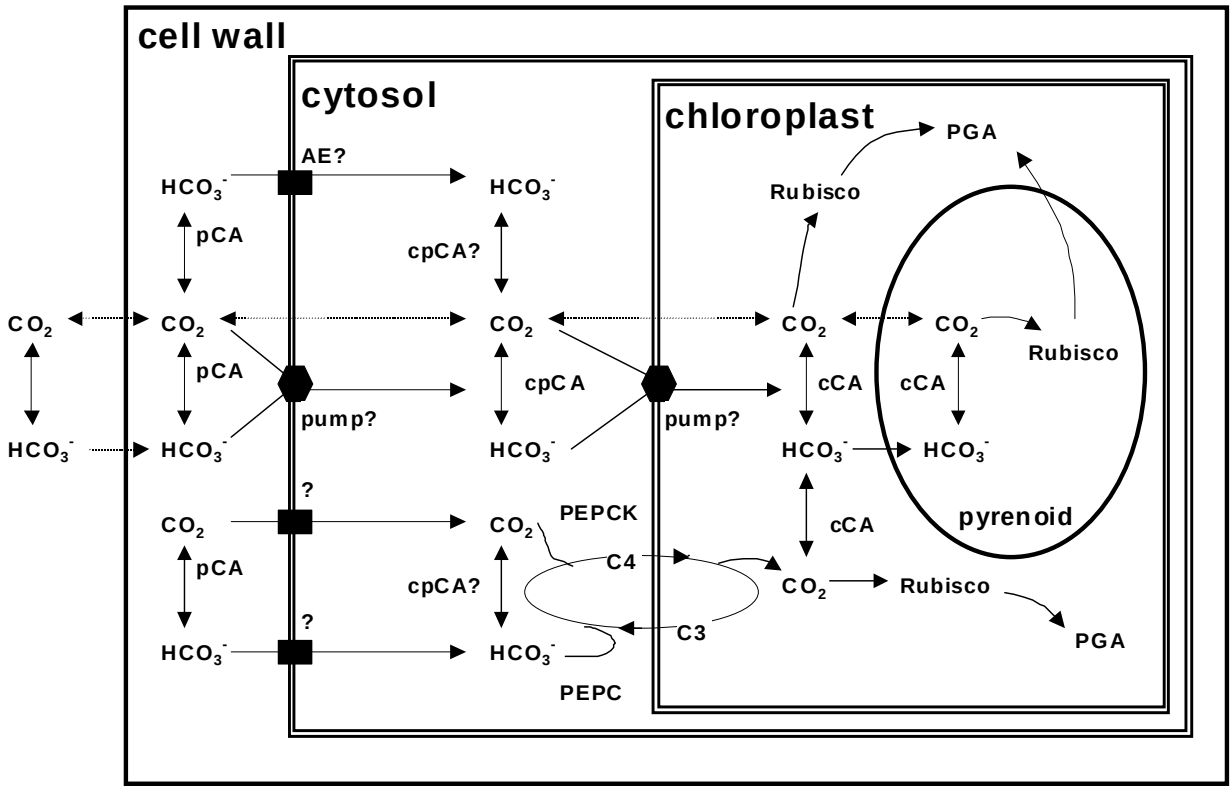


Figure 2.4. Generalised diagram of the CO_2 concentrating mechanism in macrolgae showing the locations of the different types of carbonic anhydrase as described by Sultemeyer (1998). *Reference is made to the C_4 – like metabolism shown in some algae where phosphoenolpyruvate carboxylase (PEPC) or phosphoenolpyruvate carboxykinase (PEPCK) forms C_4 intermediates from inorganic carbon. AE – anion exchanger (protein?), pCA – periplasmic carbonic anhydrase, cpCA – cytoplasmic carbonic anhydrase, cCA – chloroplastic carbonic anhydrase.*

2.8. Salinity tolerance and adaptation

Salinity varies in the ocean over vertical and horizontal gradients. Waters of tropical oceans have a higher salinity than those in polar seas (Nybakken 1993). Salinity gradients exist in intertidal areas, where the salinity at any point is related to tidal exposure and inundation, and associated with duration of evaporation (Branch & Branch 1981).

In the intertidal zone salinity increase and desiccation often occur simultaneously, and the two stresses are analogous since both result in tissue water potential being lowered. The main difference between the two is that desiccation usually leaves ionic ratios unchanged, while algae can undergo changes in ion ratios during salinity stress due to selective permeability to certain ions (Kirst 1989).

Changes in the salinity of water surrounding seaweed can affect them in three basic ways.

1. It may affect cellular water potential causing osmotic stress.

2. It may cause stress due to the differential uptake or loss of ions during osmotic acclimation.
3. Because of the selective permeability of membranes, it may cause stress due to ionic imbalances, or changes in ionic ratios (Kirst 1989).

Salinity stress in macroalgae (hypo- or hyperosmotic stress) results in a decrease in photosynthesis and respiration. The increased permeability of thylakoid membranes to ions (e.g. Na^+ and Cl^-) during osmotic stress negatively affects electron transport between photosystem I and II, inhibiting photosynthesis (Gilmour *et al*. 1985). This suppression of photosynthetic activity, and the longer-term energetic costs of osmotic acclimation, generally results in a decrease in growth rate of algae under osmotic stress. On the other hand, species of *Fucus*, *Ulva* and *Porphyra* from the Adriatic showed an increase in photosynthetic rate with increasing salinity from 0 to 37‰ (Zavodnik 1975). Experiments done on typical estuarine species to investigate the effects of long term salinity changes showed broad salinity tolerance ranges. This salinity tolerance was associated with full osmotic acclimation, but the optimum salinity for growth was still close to that of natural seawater (Young *et al*. 1987, Guo & Mathieson 1992, Fong *et al*. 1996, Raikar *et al*. 2001, Taylor *et al*. 2001). In addition it has been shown that salinity tolerance is reduced when an alga is exposed to temperatures near its tolerance limits (especially low salinity tolerance at high temperatures) (Fralick & Mathieson 1975, Thomas *et al*. 1988).

The initial step in osmotic acclimation involves rapid changes in turgor pressure, or volume changes, as a result of water moving into or out of the cells. Water fluxes are usually fast processes (minutes to hours in the case of macroalgae). This is a passive process, not under metabolic control but rather dependent on membrane permeability and elasticity, which lessens the osmotic stress in the short term (Kirst 1989).

According to Kirst (1989) the fast initial response to salinity stress is followed by osmotic adjustment by means of differential ion transport, or changing the concentration of organic osmolytes. These processes are metabolically controlled and involve energy investment. This phase is a great deal slower and may take two to three days in macroalgae. Changes in membrane turgor alter the physical and electrical properties of the membrane, which in turn affects the activity of ion transport channels. The principle ions involved in osmotic acclimation are K^+, Na^+ and Cl^-. Cytoplasm is usually high in K^+, but low in Na^+ and Cl^-. Ion-specific carriers, driven by membrane potential appear to regulate ion concentrations. Diffusion via ion-selective channels in the membrane is also important during rapid changes in ionic composition (Beilby 1985).

Vacuoles are often formed in macroalgal cells in response to osmotic stress. It is thought that these may serve as compartments for the storage of Na^+ and Cl^- ions. In many types of seaweed

the vacuole dominates osmotic relations since it occupies a large proportion of the cell volume (Kirst 1989). The accumulation of ionic solutes occurs predominantly in algae with large central vacuoles, while compatible solutes are usually accumulated in algae without such structures (Raven 1976). Compatible solutes are organic osmolytes that may be accumulated in the cytoplasm during time of salinity stress. These organic compounds can accumulate in the cytoplasm at a high concentration (100 mM), raising the osmotic potential without inhibiting enzyme activity, as ion accumulation at the same concentration would. These organic osmolytes are often simply the main photosynthetic product, e.g. mannitol in Phaeophyta, sucrose in Chlorophyta, and floridoside in Rhodophyta, although more exist (Dawes 1998). Not all organic osmolytes are “compatible solutes”, for example sucrose accumulation would interfere with general cell metabolism at high concentrations, and is therefore should not really be classified as a compatible solute (Kirst 1989).

With tidal water level fluctuation and concomitant exposure and inundation, intertidal algae are exposed to rapid changes in salinity. Seaweed found high up in the intertidal zone generally have a broad salinity tolerance range and can tolerate short periods of salinity stress without osmotic adjustment (Russel 1987, Reed 1990, Kirst 1995). In seaweeds inhabiting this environment the time lag involved in reaching osmotic equilibrium by adjusting osmolytes, especially organic osmolytes, (two to three days) would be too long to be effective in the tidal regime. The energetic costs of regular biosynthesis of organic osmolytes, and active ion-transport would also be very high. These algae often opt for incomplete turgor pressure regulation (Dickson *et al*. 1980). Ionic concentrations do change with fluctuating salinity, but significant changes in turgor pressure also occur. These algae usually have the ability to tolerate variations in turgor pressure. Cell compartments such as cell walls and vacuoles are important in buffering the immediate effects of salinity stress (Kirst 1989).

In intertidal algae exact turgor pressure control is sacrificed in favour of a more flexible and energetically favourable mechanism of short-term passive tolerance (Kirst 1989). The extent to which seaweeds can employ osmotic stress tolerance has been shown to be associated with its vertical position on the intertidal gradient (Smith & Berry 1986). However in work done on *Gelidium* species (*G. filicinum, G. lingulatum, G. spinulosum, G. pussilum*), that tested growth rate response to salinity, all four species showed salinity optima of 35‰ for growth. The results showed no relationship with the particular species’ vertical distribution range (higher salinity tolerance in upper intertidal species vs. lower salinity tolerance in lower intertidal species) (Oliger & Santelices 1981).

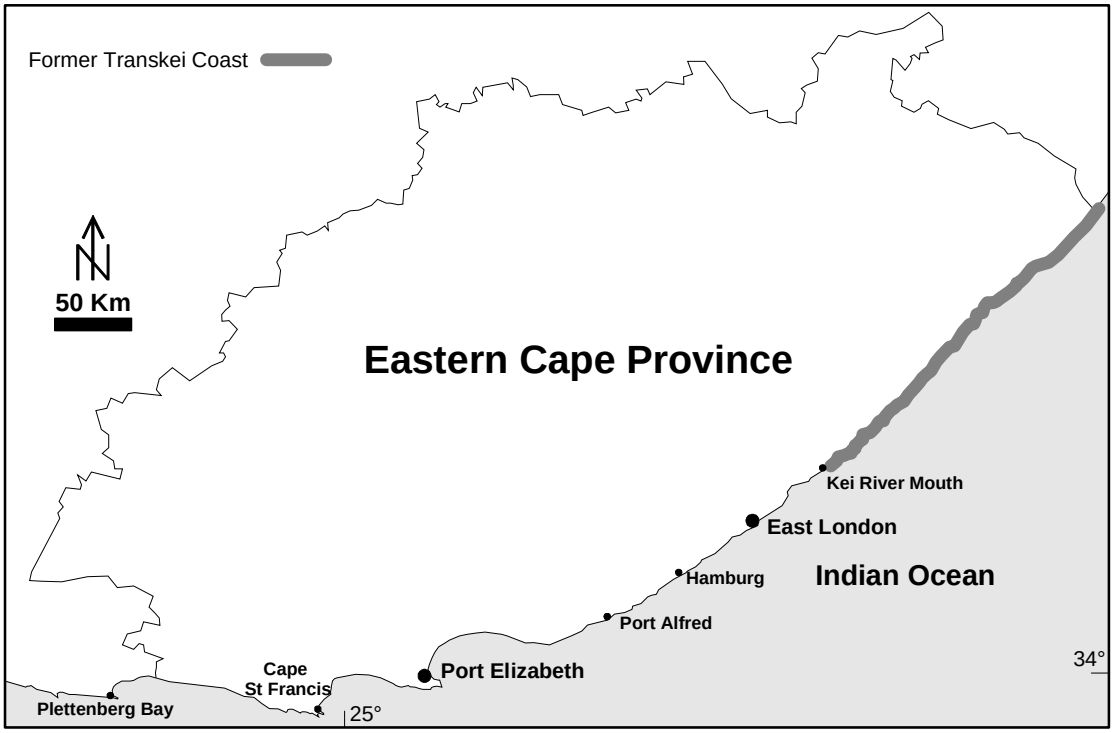
Chapter 3

Habitat and environment of *Gelidium pristoides*

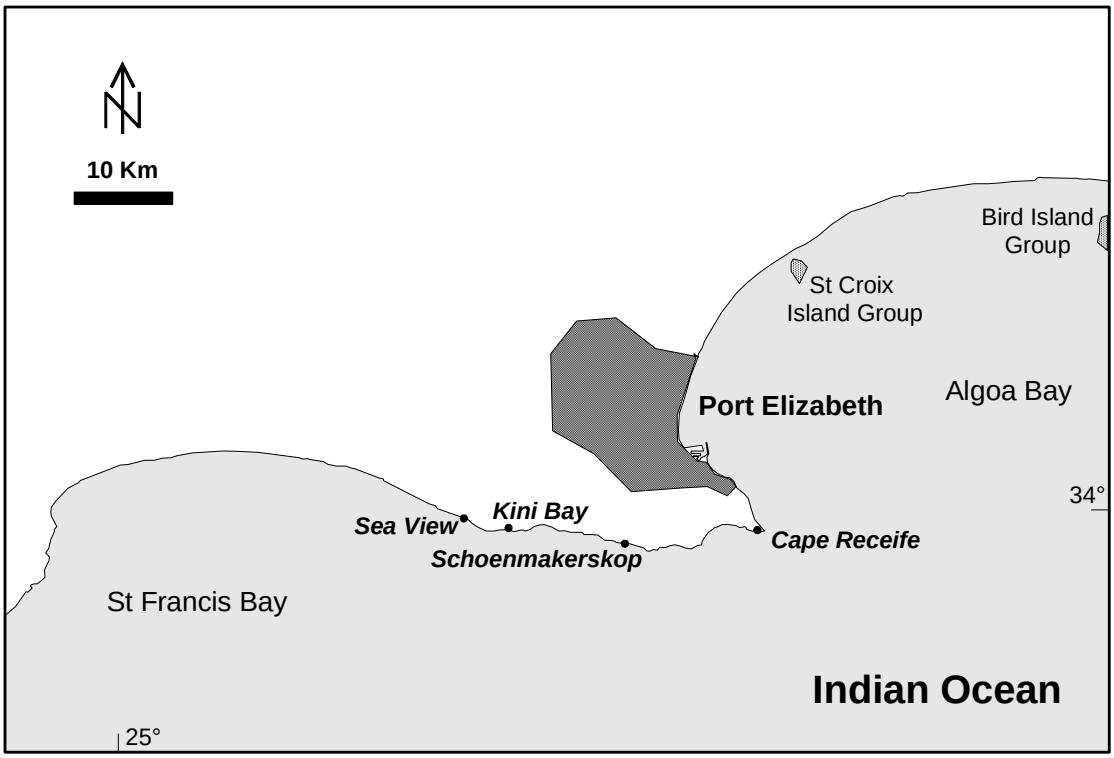
3.1. Introduction

Gelidium pristoides (Turner) Kuetzing occurs in the mid- to lower intertidal zone (lower balanoid) on rocky shores and extends from the Eastern Cape through to Sea Point (in Cape Town) in the Western Cape of South Africa (Day 1974, Onraet & Robertson 1987, Stegenga *et al*. 1997) (Map 3.1). *Gelidium pristoides* has been investigated on a number of different parts of the South African coast. Populations from Port Alfred (Carter 1986, Carter & Anderson 1991), St Croix Island (Algoa Bay) (Beckley & McLachlan 1979, Beckley & McLachlan *et al.* 1981, Beckley 1982), and False Bay (Cape Town) (Gibbons 1988) have been studied, and some of these studies include useful data on the physical and biological environment in these areas. Information on the conditions in the *Gelidium pristoides* habitat in Algoa Bay is limited to the work done on the St Croix Island populations. These data, however useful, are considered to be specific to the island and cannot be extrapolated to the rocky shores of the mainland because of differences in, for example, the presence of guano, runoff, wave conditions, and exposure. Some work focusing on other aspects of the biota of the Port Elizabeth intertidal areas also contains limited information on conditions in the intertidal zone of the rocky shores of Algoa Bay (McLachlan *et al*. 1981).

Map 3.1. Southern Africa showing the distribution of *Gelidium pristoides* along the SA coast. The work in this study was conducted in the Eastern Cape Province (EC) near the city of Port Elizabeth.



Map 3.2. The Eastern Cape Province (EC) showing Port Elizabeth and Port Alfred where earlier work on *Gelidium pristoides* had been done. The northern portion of the EC coast corresponds to the former Transkei Coast.



Map 3.3. Algoa and St Francis Bay showing the city of Port Elizabeth and the location of the sampling sites referred to in this study.

Gelidium pristoides populations were investigated on the coast at Port Alfred by Carter and Anderson (1991). The rocky shore in this area consists of quartzitic sandstone with many gullies,

and steep, vertical faces. In this area *Gelidium pristoides* is distributed from about 0.2 – 0.8 m above MLWS (mean low water spring). It occurs in association with *Cymbula oculus* and higher up on the intertidal with the barnacle *Tetraclita serrata* in the lower balanoid zone. Size distribution along the intertidal zone at their study sites did not show a normal distribution. At the lower intertidal end of its vertical distribution range the frond length was a maximum of about 6 to 12cm. Frond length decreased with increasing elevation to about 2 to 4 cm at the upper end of its distribution in the intertidal zone. *Gelidium pristoides* also showed a preference for shells as substrate, with the highest proportion of individuals growing attached to limpet and barnacle shells. Most of those individuals growing on the rock surface occurred in crevices. Limpet and barnacle shells probably provide refuges from grazers for newly settled propagules. Coralline algae dominated the sublittoral fringe areas, and algae transplanted into this zone were soon overgrown by epiphytes (Carter & Anderson 1991). The climate in the area is temperate, with seawater temperatures of 14 to 22℃ (Carter & Anderson 1986) .

On the Port Elizabeth coast the annual inshore temperatures in the shallows, where *Gelidium pristoides* occurs, are between 11 to 25℃, with means of 21℃ in summer and 15℃ in winter (Lubke & De Moor 1998). Cooler conditions have been recorded to the west of Cape Receife (Map 3.33) where occasional upwelling can decrease seawater temperatures to below 11℃, while temperatures in Algoa Bay on the eastern side of Cape Receife are relatively stable (Beckley 1983). The rocky shores in Algoa Bay (east of Cape Receife) are by and large gently sloping Table Mountain sandstone and a matrix of calcareous sandstones and pebbles. The water has a higher turbidity than on to the west of Cape Receife. The shore to the west of Cape Receife consists of rugged, folded and weathered sandstones, with many rock pools and gullies. Work done here mentions the dominant algal species to be; *Laurencia natalensis*, *Pterosiphonia cloiophylla*, *Lithothamnion* sp. and *Plocamium corallorhiza* east of Cape Receife; and *Gelidium pristoides*, *Lithothamnion* sp., *Ralfsia verrucosa*, *Plocamium corallorhiza*, and *Arthrocardia* sp. to the west of Cape Receife (McLachlan *et al*. 1981).

Studies of *Gelidium pristoides* populations on St Croix Island in Algoa Bay (Map 3), describe the intertidal rocky shore at this site as Table Mountain sandstone with many gullies (Beckley & McLachlan 1979). Wave action is considered to be the most important physical factor on these intertidal rocky shores. Mean annual air temperature can be considered to be the same as for Port Elizabeth, and is around 17℃. Annual rainfall in the area is about 600 mm with peaks in spring and autumn. Water temperatures range from 11℃ in winter to 25℃ in summer. McLachlan *et al*. (1981) described the area as having a semidiurnal tidal regime with a mean spring range of 1.61 m, and a mean neap range of 0.51 m. The *G. pristoides* belt in exposed areas on the island was widest where wave action was moderate. Maxima in *G. pristoides* biomass were apparent in spring

and autumn. The alga occurred in the same zone as the barnacles *Tetraclita serrata*, *Chthamalus dentatus*, *Octomeris angulosa*, and the mussel *Perna perna*. Algae found in the same zone as *G. pristoides* on the island included; *Ulva rigida*, *Lyngbya* sp., algal turf, *Chaetangium erinaceum*, *Ceramium* sp., *Jania* sp. and *Lithothamnion* sp. (Beckley & McLachlan 1979, Beckley 1982).

The geographic distribution of the alga between the Kei River and Cape St Francis (Map 3.2) is patchy, but it appears that the highest biomass is reached on the coast between Hamburg and Kini Bay (Map 3.2 & 3.3), west of Port Elizabeth (Jarman *et al*. 1988). The vertical distribution of *Gelidium pristoides* in the intertidal zone extends from 0.2 to 0.8 m above mean low water spring (MLWS), a part of the rocky shore referred to as the lower- to mid-eulittoral zone (Carter & Anderson 1985). The width of the intertidal zone, and similarly the width of the zones into which the littoral is subdivided, is determined by wave action, tidal range, and the slope of the rocky shore (Lewis 1964). This has been illustrated on St Croix Island where it has been shown that the vertical distribution of *G. pristoides* within the intertidal zone, and the mean width of the zone occupied by *G. pristoides* were determined by wave exposure. At sheltered and exposed sites the zone occupied by *G. pristoides* was narrower than at sites with moderate exposure, while the vertical position of the *G. pristoides* zone shifted higher up on the intertidal gradient with an increase in exposure (Beckley 1982). *Gelidium pristoides* tufts exhibited a decrease in abundance and frond length from the lower to the upper reaches of the zone occupied by the alga.

The substrates colonized by *Gelidium pristoides* are relatively diverse. Rock, limpets, tubeworms, encrusting coralline algae and barnacles have all been shown to be suitable substrates for attachment of the alga (Carter 1985, Jarman *et al*. 1988). At Port Alfred the greatest numbers of tufts were found attached to rock, especially in crevices. However as a percentage of the total substrate available, limpets are also a very important substrate, especially lower down in the intertidal zone where limpet abundance is higher. Higher up in the intertidal zone (mid-eulittoral), barnacles provide important attachment sites. The security of basal attachment to limpets and barnacles is much higher than on rock, suggesting that removal by wave action is less likely for individuals colonizing these substrates. It also appeared that recruitment favoured limpet shells as a substrate in the absence of grazers (Carter & Anderson 1991).

Some information is available on the biological interactions involved in structuring the lower distribution limits of *Gelidium pristoides* (Carter & Anderson 1991). *Patella oculus* is a common herbivore in the lower eulittoral and may be responsible for low recruitment of all the algae within this zone. Transplant experiments have shown that the lower distribution limit of *G. pristoides* may be set by epiphytic encrusting corallines overgrowing the seaweed. Grazing by sea urchins and periwinkles has also been suggested as a contributor to the limitation of *G. pristoides* distribution in

the subtidal fringe and lower down (Carter 1985). There is a lack of microclimate data on the intertidal environment in which *G. pristoides* is abundant. Important physical variables such as light intensity, day-length, tidal exposure, inundation duration, temperature on the rock surface and salinity changes have until now been omitted in studies on *G. pristoides*. These data will give an indication of the habitat requirements of the species, limit the range of conditions to be tested when its physiological responses to these are investigated, and aid in determining the ideal conditions under which the alga could be cultivated.

No work relating the vertical distribution and abundance of *Gelidium pristoides* to changes in the environment along the intertidal gradient has been published. However some research on the effects of physical conditions along such a gradient on the morphology and anatomy of *G. pristoides* has been done (Ballantyne 2003).

The aims of this investigation were to determine the physical conditions on the rocky shores on which *Gelidium pristoides* populations around Port Elizabeth occur. In doing this it is expected that an indication of the alga's physical tolerance limits will be obtained.

3.2. Materials and methods

3.2.1. Study area

The physical conditions that *Gelidium pristoides* populations are exposed to in the intertidal zone were measured at Schoenmakerskop (Map 3.3) near Port Elizabeth. Map 3.3 shows the position of Schoenmakerskop on the shore of St. Francis Bay (34°02'S, 25°34'E), South Africa. Laboratory experiments were also carried out on material collected from *G. pristoides* populations in this area. The sites were chosen because of their close proximity to the laboratory and culture facilities. This minimized the stress induced by transporting the algae from the shore. The study site is on a stretch of coastline that is regularly exposed to large south westerly swell generated by south-westerly winds. South-westerly winds dominate throughout the year, with easterly winds playing a role in summer months (Beckley 1983). The coastline just east of Cape Receife, for example, is somewhat protected from this direct swell by refraction of these south-westerly wave fronts around the Cape Receife headland. The rocky shores at Schoenmakerskop consist mainly of Table Mountain sandstone with many steep sloping gullies, most of which open to the sea in a south-easterly direction. Much of the sampling was done along transects from the high tide to the low tide mark along the intertidal gradient, with specific attention given to the zone populated by *Gelidium pristoides*. These transects were chosen to be similar in geology, slope and aspect, but sometimes varied in the amount of wave action induced water movement they experienced. This was usually due to the presence of a shallow reef or rock outcrop just seaward of these sheltered sites. Sites considered exposed were not protected from direct surf action by such structures and were

exposed directly to south-westerly swell and surf coming on-shore. A few sites were considered intermediate between these two extremes. Sites were chosen in this fashion to make it possible to investigate the effect of water movement on the distribution of *Gelidium pristoides* along the shore.

Plate 3.1. The study area at Schoenmakerskop.

About 40 to 50 km off-shore of Port Elizabeth, the warm (20 - 28℃) Agulhas current moves in a south-westerly direction southward at a rate of *ca*. 2 m s^{-1}, and even though it flows a considerable distance offshore it has an important influence on inshore seawater temperatures in the region (Lutjeharms 1983). Its influence on seawater temperature is evident down the entire eastern length of South African coast, which has high inshore water temperatures in the East Coast where the Agulhas current flows close inshore. As the current moves south along the coast, the width of the continental shelf increases, forcing the current further off-shore and resulting in a gradual decrease of inshore seawater temperatures towards the south coast (Branch & Branch 1981). Closer inshore in the Algoa Bay region, the dominant current is a northward flowing long-shore counter current, which has the greatest influence on the sediment and water movement in Algoa Bay and St Francis Bay, and is in part responsible for the formation of these half-heart (log-spiral) bays (Branch & Branch 1981). Schumann *et al.* 1995 reported mean monthly seawater temperatures of 21.01℃ for summer and 15.53℃ for winter for Port Elizabeth. These values were calculated from twenty years of temperature data measured in Algoa Bay (1972 to 1992). There is occasional localized upwelling of cold, deeper water causing temperatures to drop to as low as 8℃ (Beckley

1983). Light intensity above water on the rocky shores has not been measured on this particular coastline and is assumed to be related to local weather conditions and time of year. Winds in the area are predominantly south-westerly and westerly winds, with some easterly winds during summer months (Lubke & De Moor 1998). This is also reflected in the predominantly south-westerly swell direction. These swells refract around the headland at Cape Receife and break on the shores of Algoa Bay at an angle, producing the alongshore drift northwards in the bay (Branch & Branch 1981). The study site falls in the Agulhas marine province (Hommersand 1986) and within a warm-temperate marine region (Stephenson 1948).

3.2.2. Long-term climatic data

Long-term data represent the temperatures, day-lengths and light intensities to which natural *Gelidium pristoides* populations are regularly exposed. Long-term air temperature, seawater temperature and sunrise and sunset data for Algoa Bay were obtained from the South African Weather Service. Daylengths were calculated from data on the time of sunrise and sunset at Port Elizabeth. Approximations of tidal water level fluctuations on the coast around Port Elizabeth were generated using the tide simulation software WXtide32 (© Mike Hopper 1999). This data was adjusted and verified using information from Ocean Rhythm (www.adfi.co.za/OceanRhythm, © ADF Interlink 2002). The data presented by Ocean Rhythm is based on information supplied by the Hydrographer of the South African Navy for 2002.

3.2.3. Spatial change of conditions along the intertidal zone in the *Gelidium pristoides* habitat

The intertidal zone was considered as the area of rock extending from the lowest water level during a spring low tide, inland, up to the zone where *Nodilittorina africana* and *Porphyra capensis* occurred. The area above this zone is not regularly covered by seawater during normal spring high tides. It includes the area described as the intertidal zone by Stephenson & Stephenson (1972), and Branch & Branch (1981).

3.2.3.1. Topography

Along the Port Elizabeth coast, spring low tide was usually between 9h00 and 11h00 in the morning (and again between 21:00 and 23h00 at night). Sampling was done during the day at spring low tides, which in Port Elizabeth, were always between 9h00 and 11h00 on the day of spring tide. The topography of the study / sampling site was measured during a spring low tide. Permanent transects were set up perpendicular to the shoreline, extending from the lowest water level at spring low, to above the upper distribution limit of *Gelidium pristoides*. The lowest water level at spring low tide on the day of sampling was assumed to be very close to mean spring low water level, and the boundary between the intertidal and subtidal environments. The transect line

traversed the entire *G. pristoides* vertical distribution range, and included intertidal rocky shore areas both above and below the species' vertical distribution limits.

A tape measure was laid along the transect line to determine approximate horizontal distances. A Seikosha theodolite was used to determine shore elevation along the transect line. The theodolite was set up at the shoreward end of the transect line. Elevation and horizontal distance (as opposed to sloping distance) were measured every meter, starting at the low tide water level and along the transect line to where the theodolite was positioned.

Elevation data were adjusted to metres above spring low tide water level using the following equation:

$E_{adjusted} = -(h-h_{theod}) + (h_{low}-h_{theod})$ ..... Equation 3.1

Where $E_{adjusted}$ = the elevation data adjusted to metres above low tide level (m),

h = elevation measured with theodolite (m),

h_{theod} = the height of the theodolite above the rock surface (m), and

h_{low} = elevation measured at spring low tide water level (m).

3.2.3.2. Distribution of Biota

The abundance of dominant intertidal species at the study site was determined by estimating percentage cover of species occurring in a 0.16 m^2 (0.4 x 0.4 m) quadrat placed at 0.5 m intervals along transect lines. Abundance of biota along the intertidal gradient was plotted as kite diagrams to show which species may potentially be interacting with *Gelidium pristoides*.

Mean *Gelidium pristoides* frond length was determined at three points along a transect within the *G. pristoides* population. This was done by randomly selecting three tufts of *G. pristoides* within the *G. pristoides* population at these points along the transect line (a quadrat was placed at the sampling point and three tufts nearest the corners of the quadrat taken). These were removed with a paint scraper to ensure that entire thalli, including holdfasts, were removed. Samples were placed in plastic bags and transported to the laboratory for measurement. The tufts were carefully separated into individuals and the longest frond for each individual was recorded.

3.2.3.3 Exposed vs. Sheltered Intertidal Communities

There appeared to be a difference in the composition of dominant biota at sites directly exposed to incoming waves, and sites where little wave action was experienced. The exposed sites were often near the seaward edge of gullies, while higher up in these gullies less wave action was experienced. This difference in communities from exposed and sheltered habitats has been documented by Stephenson & Stephenson (1972) for the Schoenmakerskop area. Their data

showed that foliose algae common at exposed rocky intertidal habitats were replaced by coralline turf in sheltered habitats investigated.

Gelidium pristoides also appeared to be most abundant at wave exposed turbulent rocky shore sites. The seaweed seemed to become less abundant in more sheltered areas, and seemed to be absent or only present in very low abundances at the very sheltered intertidal rocky shore sites. The reasons for its absence in sheltered areas may provide information on the reasons *Gelidium pristoides* cannot be successfully cultivated in tank systems. It is for this reason that this phenomenon was investigated. This was done by measuring abundance of biota along six transects at “exposed” rocky intertidal habitats, and at six “sheltered” habitats, at Schoenmakerskop and at Sea View further to the west along the coast. The sites chosen were similar with respect to slope, aspect and rock type, but differed in the amount of wave action they received, based on visual estimates. Species abundance data was analyses using DCA (Canoco Ver. 4.52.). These data were also compared in kite diagrams and summarized in a figure showing changes in biota along the intertidal gradient between the two shore types.
The following physical data were gathered along the transect line at spring low tide:

3.2.3.4. Temperature

Substrate surface temperature was measured with a Bailey thermocouple (Sensortek). During high tide the entire intertidal zone was covered with seawater, and as such inaccessible. During this time the intertidal rock surface was assumed to have a uniform temperature very similar to that of the seawater temperature on the day. During the low tide, five replicate temperature measurements were made at each of four regularly spaced points along the transect line. The first set of measurements was taken at the spring low tide water level, and the last at the landward extent of the transect line (among the *Nodilittorina africana* and *Porphyra capensis*). Where *Gelidium pristoides* was encountered along the transect line temperature was measured inside the seaweed tuft.

3.2.3.5. Salinity

Salinity was measured using a hand-held optical refractometer (Atago). The refractometer was calibrated at the laboratory each day before measurements were taken during a site visit. This was achieved by placing a couple of drops of distilled water on the measuring stage of the refratometer, and adjusting the salinity value given by the instrument to zero using the calibration screw. For salinity measurement in the field a couple of drops of water were drawn from the relevant water source using a Pasteur pipette, and placed on a hand-held optical refractometer.

During high tides the intertidal rock surface was inaccessible. During this time the zone was covered with seawater and was once again assumed to have uniform salinity, that of the seawater on the day. When the tide was out, salinity along the intertidal gradient was measured only where surface water was available. Most of the rock surface under the transect-lines could be classified as emergent surface from which the seawater drains as the tide recedes. Many of the small indentations, cracks and pools in the rock surface do not drain completely during low tide and are called non-emergent surfaces. These retain water during low tides. During low tide the salinity of water from such reservoirs was measured. Three regularly spaced points along the transect line were chosen, with the first near the low tide water level and last near the upper extent of the transect line amongst the *Nodilittorina africana* and *Porphyra capensis*. The five pockets of water nearest to each of these points were sampled to serve as replicate salinity measurements at each tidal level along the intertidal transect. Only small water reservoirs (*ca*. < 0.5 dm^3) of similar size were sampled to avoid variability in salinity due to different rates of salinity change as a result of volume differences. However a great deal of variation was still expected even with this additional precaution.

3.2.3.6. Irradiance

The transects did not include any large rocks or overhangs, and generally had a gentle slope from the upper to lower intertidal, so that there was very noticeable little shading of intertidal habitat along the transect lines. During high tides the intertidal zone was covered by water and inaccessible so no light measurements were made during this time. Measurements were taken during spring low tides and the few hours before and after low tide. South Africa has a semi-diurnal tidal regime with two low and two high tides per day. Along the Port Elizabeth coast, spring low tide was usually between 9h00 and 11h00 in the morning (and again between 21:00 & 23h00 at night). Spring low tides on this part of the South African coast always fall during this time. The even gradient and regular topography resulted in no noteworthy shading of the rock surface during low tide (See Fig. 3.8 for examples of the topography). During the time of the spring low tide exposure of the intertidal rocky shore at the sampling site the sun was also quite high in the sky, and the large angle of the sun's rays with the rock reduced shading further. Because none of the rock surface was affected by shading during the time that the measurements were taken, light intensity could be assumed to be similar along the entire transect line. Five replicate representative light intensity measurements were made in the centre of the transect (midway between the top and bottom of the transect lines). Irradiance was measured as µmol photons m^{-2} s^{-1} with a handheld quantum sensor (LI-Cor Model LI-189). The sensor was placed on a horizontal surface in the middle of the transect line, at the level of the rock surface.

3.2.4. Temporal change of conditions in the *Gelidium pristoides* habitat

Seawater in the oceans is thermally, physically and chemically relatively stable over periods of days or weeks. Therefore conditions in the intertidal zone are rather stable when the area is inundated by seawater. However when the intertidal zone is not covered by water conditions may change considerably with increasing duration of exposure. The temporal changes in physical conditions during exposed periods in the intertidal zone, and more specifically within the *Gelidium pristoides* population, were investigated. Measurements were made at three sites at different elevations in the *G. pristoides* population. These were taken at the lower distribution limit, at the upper distribution limit and at a point half way between the two extremes. The measurement series was started at low tide. Physical variables were measured every half-hour for the entire time the *G. pristoides* population was exposed. Environmental conditions in the submerged *Gelidium pristoides* population during high tide were considered to be the same as the seawater in the area, and the algae assumed to be fully hydrated during these periods of inundation. There was no difference in the moisture content of seaweed collected just after exposure during low tide, and seaweed from fully submerged conditions (T_0 and T_3 in Fig. 3.18.).

3.2.4.1. Temperature

Temperature was measured using a Bailey thermocouple temperature probe. The probe was placed inside the tufts of *Gelidium pristoides* in the centre of the zone inhabited by the algae. Five replicate measurements were made every 30 minutes after low tide. Measurements were stopped when the incoming tide had increased seawater levels to the point where the zone of the intertidal inhabited by *G. pristoides* had been covered with water once again, and accurate measurements could not be made.

3.2.4.2. Irradiance

Light intensity was measured by placing a LI-Cor Handheld Quantum sensor on a horizontal rock surface exactly halfway between the upper and lower distribution limits of *Gelidium pristoides*. Irradiance measurements were made at half-hourly intervals after low tide, until the tide had covered the study area. Irradiance was recorded as µmol photons m^{-2} s^{-1}.

3.2.4.3. Desiccation rate

Gelidium pristoides samples were gathered in the middle of the *G. pristoides* population's vertical distribution range along a transect line. Five replicate samples were collected every 30 minutes after the seaweed was exposed at low tide, until the tide had come in and started covering the seaweed with seawater once again. These samples were sealed in plastic bags and transported to the laboratory in the dark for determination of moisture content. This ensured that the seaweed

would remain cool and not loose any further moisture during the collection and transportation process. Once in the lab, the samples were blotted dry, and weighed to determine wet mass of the seaweed only, without any residual surface water adhering to the thallus. Dry mass was determined by weighing the samples after drying at 60℃ in an air-circulating oven for 48h. Moisture content was calculated as [(Wet mass – Dry mass)/Wet mass] X 100. Moisture content was plotted against time to show changes in hydration during exposure.

3.3. Results

3.3.1. Long-term climate data

Port Elizabeth has a tidal range of approximately two metres (Fig 3.1). The six hours and one hour indicated on the graph represent the maximum amount of time that *Gelidium pristoides* beds on the intertidal rocky shore could spend exposed during low tides on a spring and neap cycle respectively.

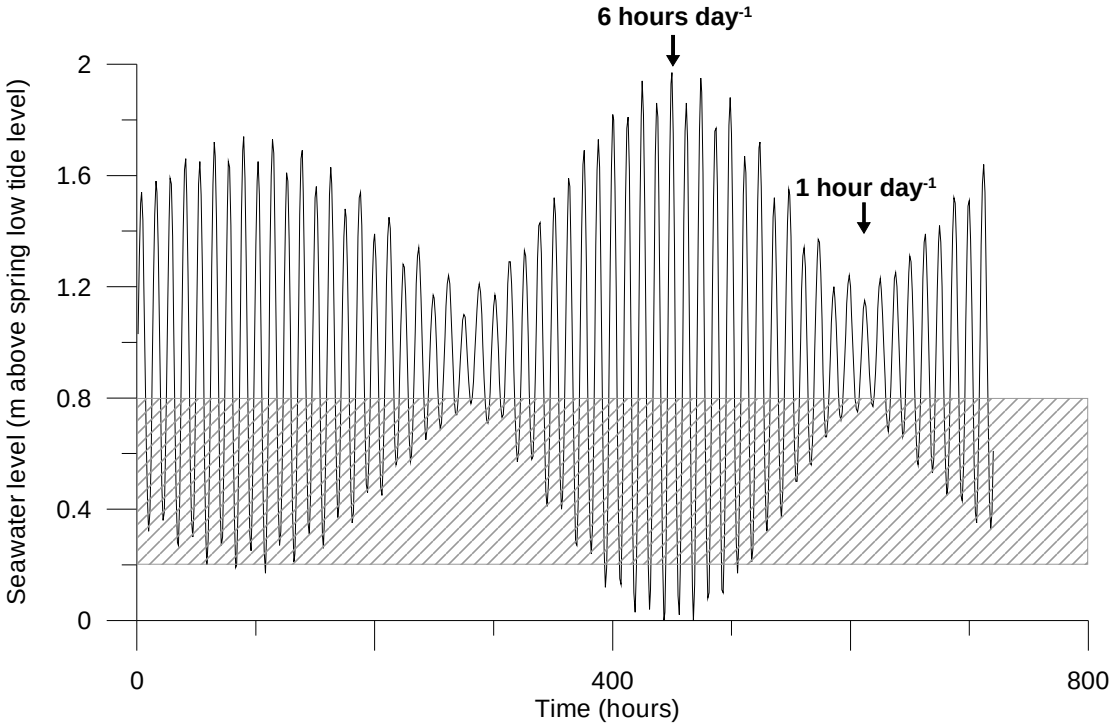


Figure 3.1. Tidal seawater fluctuations in Algoa Bay over a month showing spring and neap tidal ranges. The maximum potential exposure duration for Gelidium pristoides occurring within a vertical distribution range, (cross hatched region) from 0.2 to 0.8 m above spring low tide water level, is indicated.

The area experiences almost two high and two low tides in a 24-hour cycle. These semidiurnal tides are of unequal amplitude (Fig 3.2). The lowest low tide experienced in the area is 0.01 m above chart datum, while the highest high tide is 2.09 m above chart datum.

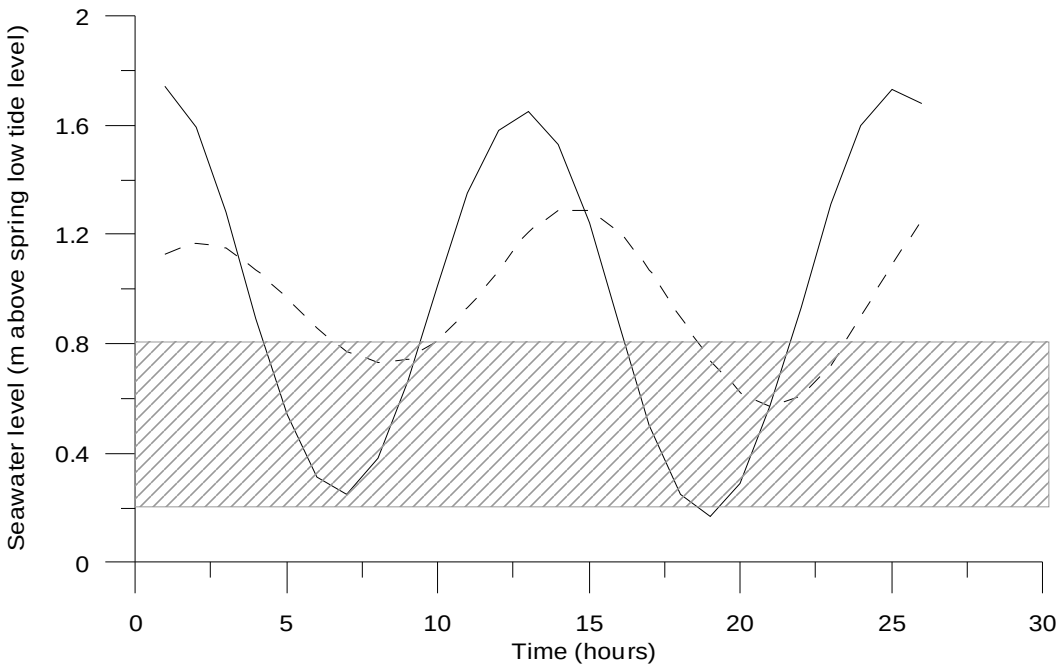


Figure 3.2. Tidal seawater fluctuation in Algoa Bay over a 24-hour period during a spring (solid line) and a neap tide (broken line). Crosshatched area indicates the vertical distribution range of *Gelidium pristoides*.

Data from intertidal transects presented in this thesis are relative to the elevation above the water level at a spring low tide (lowest water level on the day of sampling), which generally varied between 0.1 and 0.3 m above chart datum.

Air temperatures in Algoa Bay (Fig 3.3) vary seasonally and may influence *Gelidium pristoides* productivity when the algae are exposed to the atmosphere during low tides. Mean monthly summer temperatures are around 21℃, while winter t emperatures are *ca*. 14℃. However it should be noted that these data do not reflect the considerable diurnal variability in air temperatures, which have a daily range of approximately 8.6 ± 0.3℃ in summer and 13.1 ± 0.8 ℃ in winter (SA Weather Service 2000 - 2002).

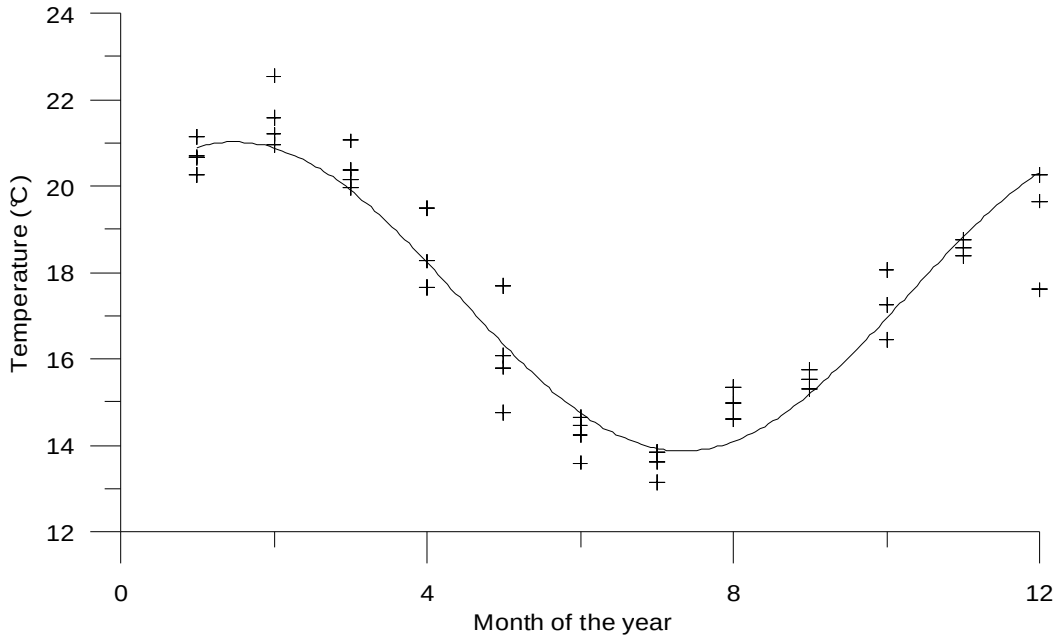


Figure 3.3. Mean monthly air temperatures for Port Elizabeth for 2001 (r^2 = 0.91).

Mean monthly seawater temperatures in Port Elizabeth (Fig 3.4) also follow a seasonal pattern, with mean monthly seawater temperatures ranging from 16℃ in winter to 21℃ in summer. While there is some diurnal variability in coastal seawater temperatures (Schumann *et al*. 1995), this variability is generally expected to be smaller than the diurnal variability in the air temperatures on the shore.

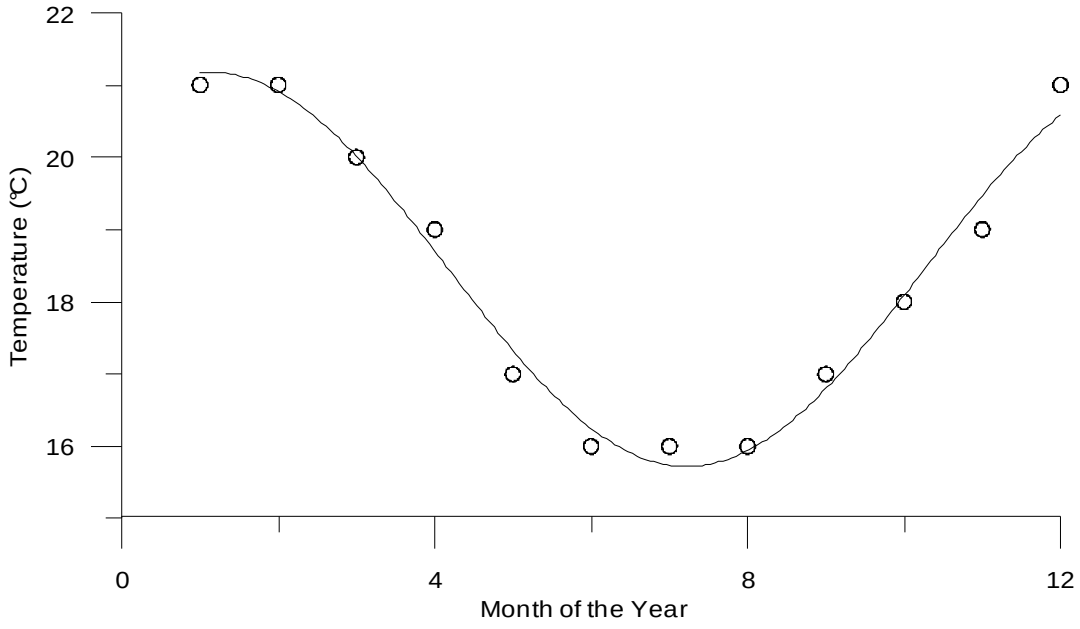


Figure 3.4. Mean monthly seawater temperatures for Port Elizabeth for 2001 (r^2 = 0.98).

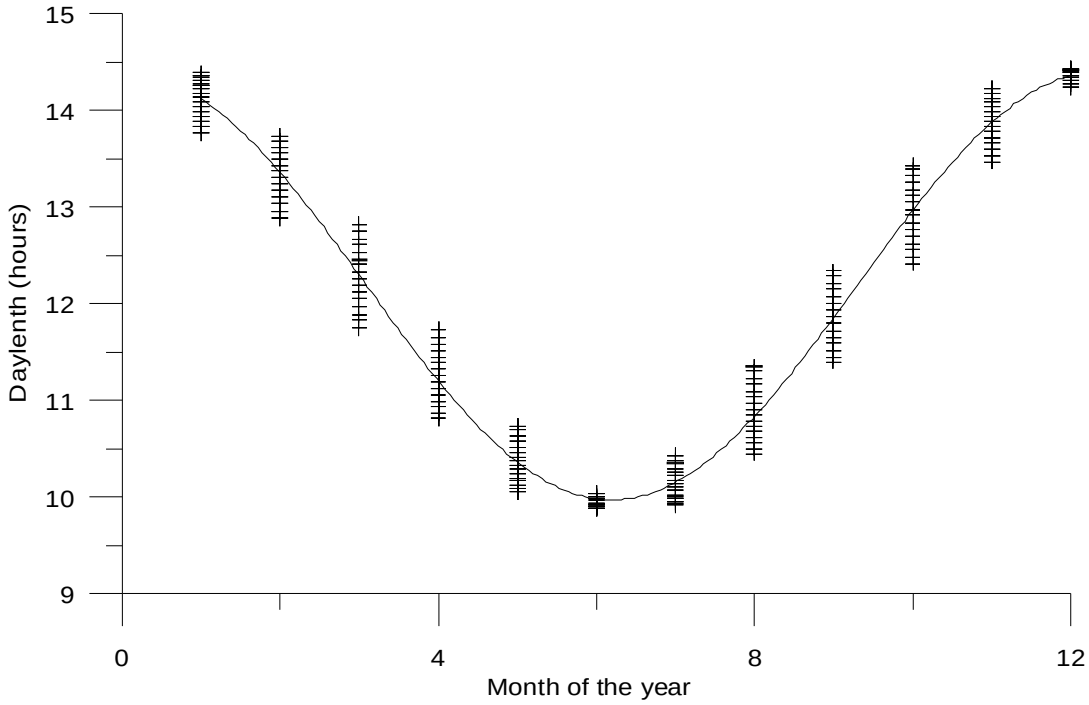


Figure 3.5. Mean monthly day lengths for Port Elizabeth for 2001 (r^2 = 0.97).

Monthly means of day lengths (time from sunrise to sunset) for Port Elizabeth vary seasonally (Fig 3.5.). The longest day occurred in summer (14 hours 24 min) and the shortest day, in winter, was 9 hours 54 min long.

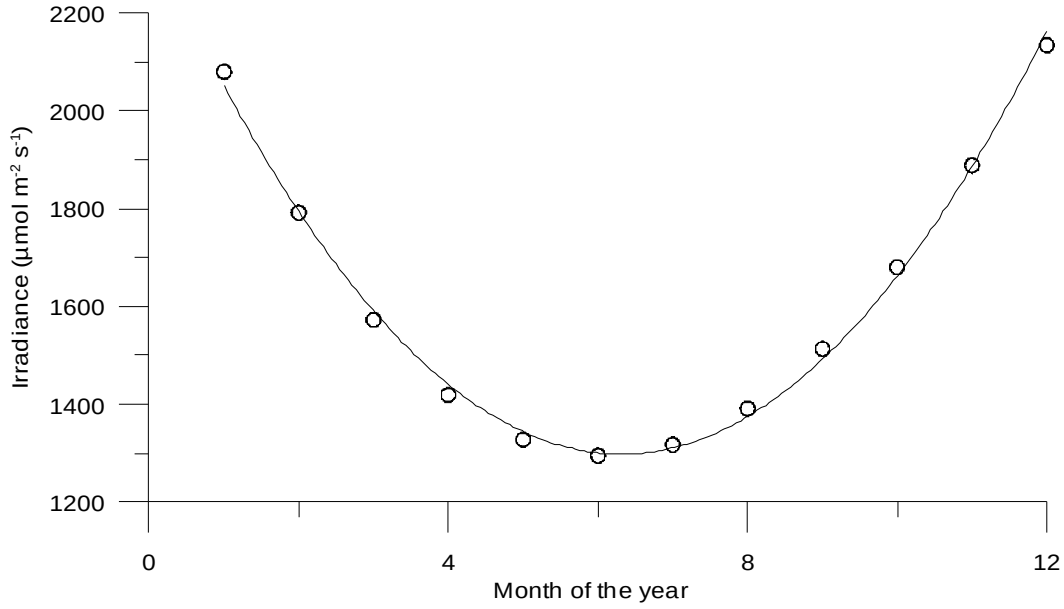


Figure 3.6. Change in surface irradiance levels during a year (Calculated from Sloff, 1984) (Y = 2430.8 Sin (0.1X +3.7) + 3723.5; r^2 = 0.99).

The mean daily sunlight intensity when averaged on a monthly basis (Fig 3.6.) shows similar seasonal variation, from *ca*. 2100 µmol m^{-2} s^{-1} in summer to *ca*. 1300 µmol m^{-2} s^{-1} in winter. The

data presented in Figure 3.6 were calculated from Sloff (1984) and do not account for light attenuation due to cloud cover, fog or other atmospheric phenomena, which will also have a marked effect on sunlight reaching the surface of the intertidal zone during low tides; since they are theoretical values.

3.3.2. Spatial change in the intertidal zone

3.3.2.1. Topography

The rock at Schoenmakerskop is folded, jointed Table Mountain sandstone, which has been weathered to produce a highly variable topography (Plate 3.1). The coast is characterized by a number of gullies both perpendicular and parallel to the coastline. The site chosen for the study presented a relatively even slope from the supralittoral to the sublittoral. The highest point on the transect lines was *ca*. 2.0 metres above the spring low tide water level. The transect was gently sloping rock with a relatively flat area from 5 to 8 meters from the water's edge. This flat area was fairly pitted and had a number of small (5 – 10 cm diameter) puddles of trapped water (Plate 3.2). These small pools (non-emergent surfaces) are reflected in the apparent patchy distribution of coralline turf, limpets, *Siphonaria* sp. and *Ulva* sp. along the intertidal gradient (See Plate 3.3).

Plate 3.2. The rocky slopes along which the transect data was gathered showing the pitted surface and numerous small puddles of trapped water.

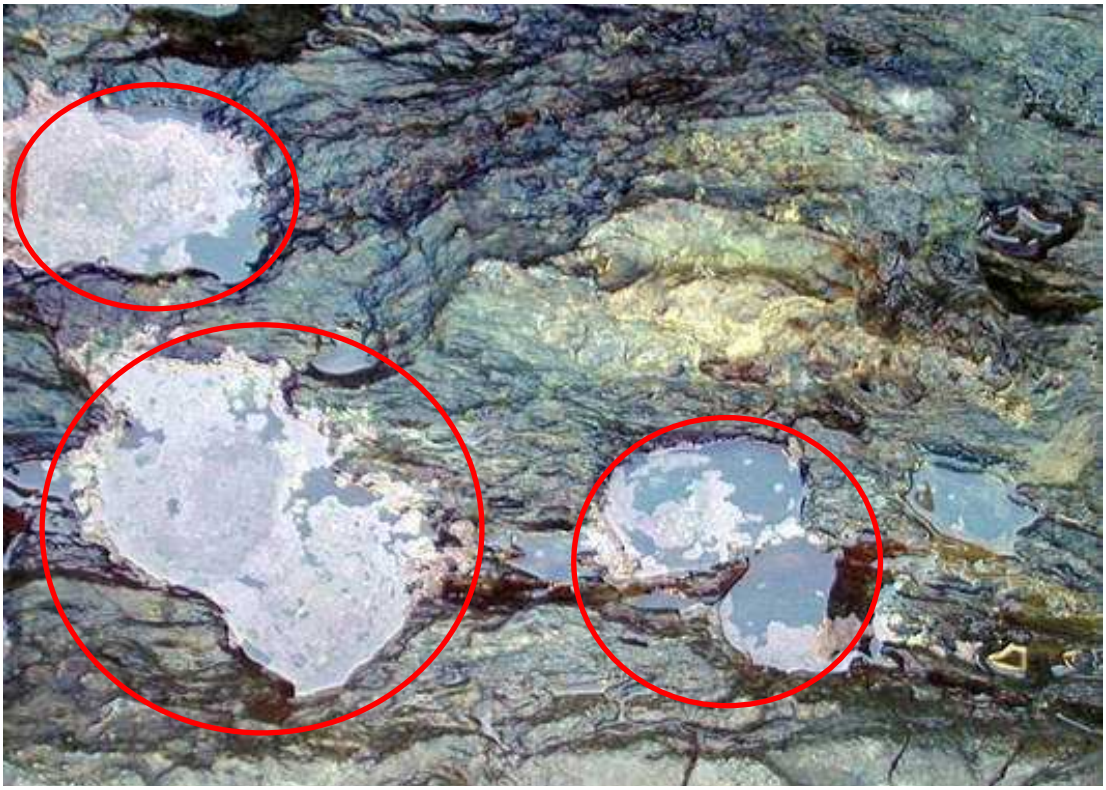


Plate 3.3. Pitted and pock-marked rock surface showing the patchy distribution of encrusting coralline algae which are confined to the small puddles formed in the rock.

3.3.2.3. Distribution of Biota

The distribution of biota on the intertidal gradient along the rocky shores at various sites at Schoenmakerskop is shown in Figure 3.7 below.

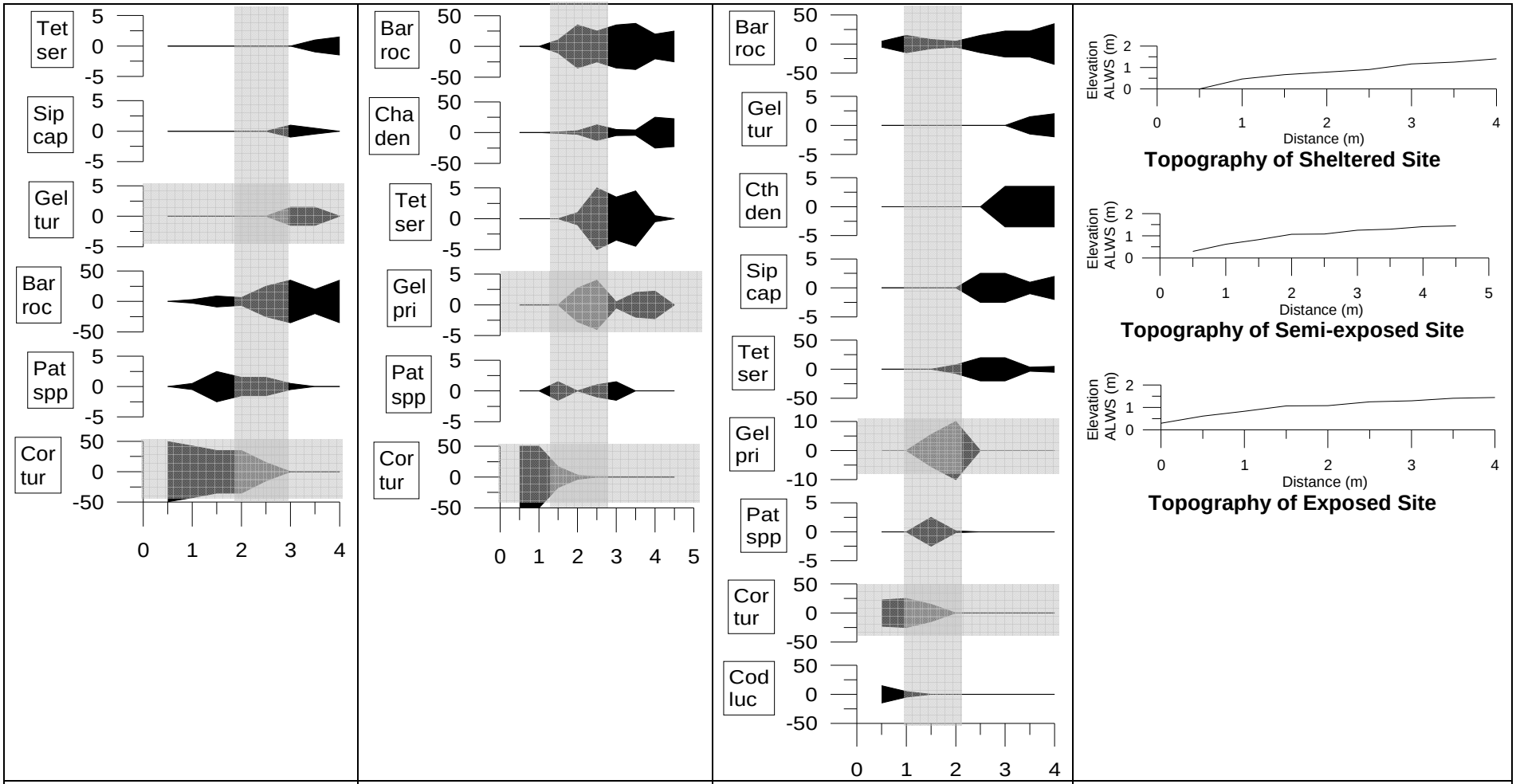


Figure 3.7. Kite diagrams and profiles of three of the rocky shore sites sampled showing the distribution of biota along the intertidal gradient, and changes in the communities between sites with varying degrees of wave exposure. Tet ser – *Tetraclita serrata***, Sip cap –** *Siphonaria capensis***, Gel tur -** *Gelidium pristoides* **truf, Bar Roc –** Bare Rock**, Pat spp. –** *Patella* species**, Cor tur –** Coralline Turf**, Cha den –** *Chthamalus dentatus***, Gel pri –** *Gelidium pristoides* foliose**, Cod luc –** *Codium lucasii*

G. pristoides was found to occur from about 0.2 metres above spring low tide level (ASL) to 0.8 m ASL (Fig. 3.7). Densities varied from 5% near its lower distribution limit; to ca. 40% at 0.6 – 0.8 m ASL, and decreased to a minimum of <5% at 1.2 metres ASL. The lower boundary of the distribution range appeared to coincide with the increased abundance of coralline turf. There is an inverse relationship between the percentage cover of *G. pristoides* and coralline turf, as shown in the kite diagrams of Figure 3.8. Correlation analysis yielded correlation coefficients of -0.98, -0.99, and -0.82 for the exposed, semi-sheltered and sheltered sites respectively.

Coralline turf algae covered almost 80% of the rock surface from 0.2 m ASL down to the subtidal; while it diminished in abundance up the intertidal to *ca*. 5% at 0.8 m ASL. Most of the Patellid limpets were confined to the lower intertidal, and in many instances were found in gaps among the coralline turf. Where these limpets occurred amongst the coralline turf *G. pristoides* was often found attached to their shells.

Gelidium pristoides turf distribution extended up the intertidal zone to a maximum elevation of 1.2 meters above low tide level. In this part of the intertidal it occurred amongst the barnacles *Octomeris angulosa* and *Tetraclita serrata*. *G. pristoides* and the barnacles showed similar vertical distribution ranges, and in many cases *G.pristoides* was found attached to the shells of these barnacles.

At the upper extreme of its vertical distribution range *Gelidium pristoides* was found together with *Ulva* sp., and some coralline turf, while the encrusting corallines occurred in varying abundances along the entire transect. No other macroalgal species were apparent in the zone inhabited by *G. pristoides*. *Siphonaria* sp., *Patriella* sp., and *Porphyra capensis* inhabited the zone above *G. pristoides*, hence interaction between with these species is considered unlikely.

Coralline turf consisted of the following species:

1. *Arthrocardia sp.*
2. *Ceramium arenarium*
3. *Cheilosporum sp.*
4. *Gelidium applanatum*
5. *Haliptilon subulata*
6. *Hydrichia woelkerlingii*
7. *Jania sp.*

3.3.2.4. Intertidal community structure at exposed vs. sheltered habitats

DCA plots of sheltered and exposed sites are shown in Fig. 3.8 below, with the corresponding species plots for dominant taxa found at the different sites shown in figure 3.9.

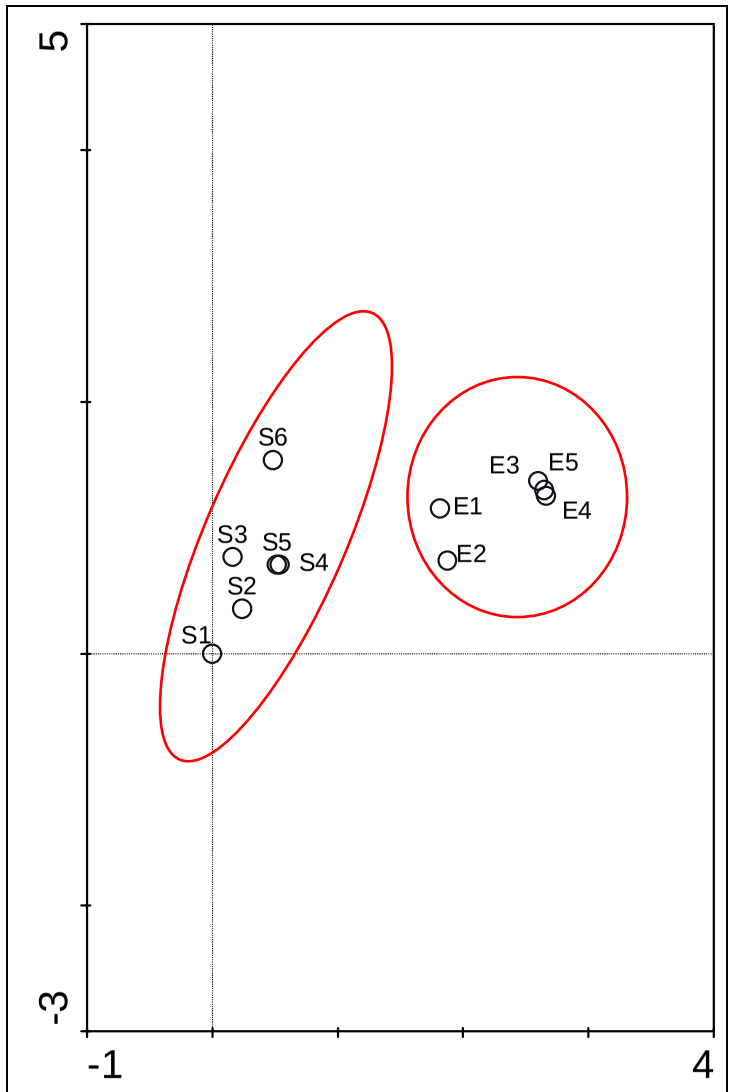


Figure 3.8. DCA of exposed and sheltered intertidal rocky shore sites (S – Sheltered Site, E – Exposed Site).

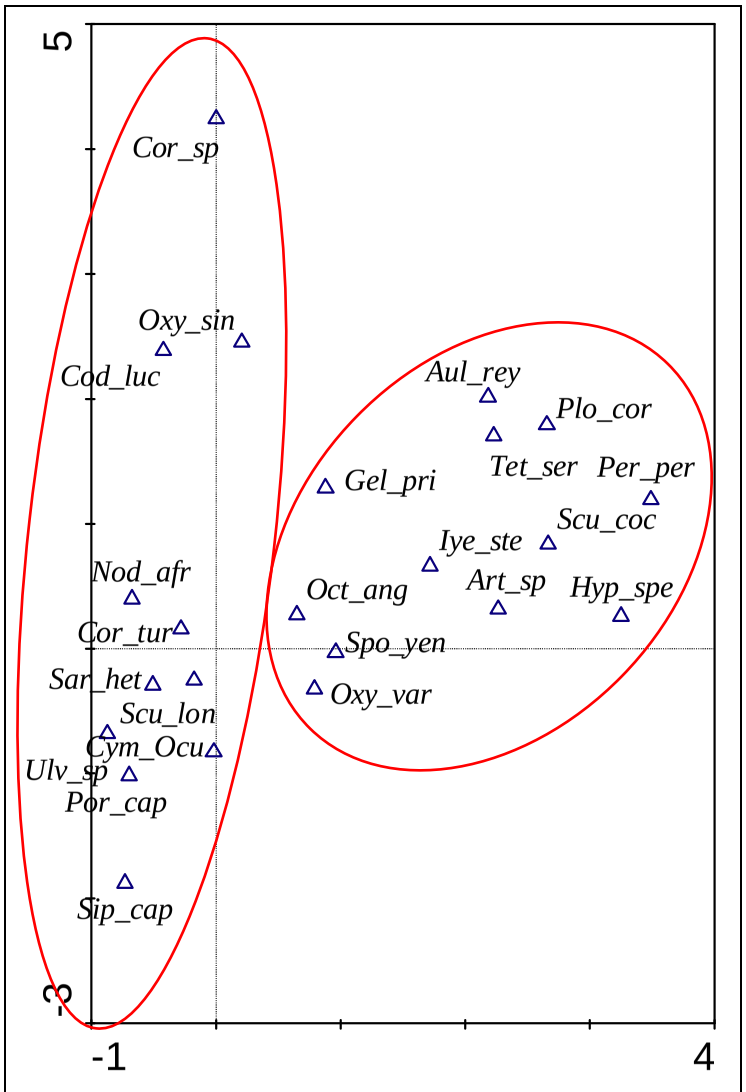


Figure 3.9. DCA of dominant taxa at sheltered and exposed intertidal rocky shore communities.

The groupings encircled show the separation of the two community types. The further separation within the indicated groupings is the result of samples from two different localities. Sites S1, S2 and S3 were sampled at Schoenmakerskop, and sites S4, S5, and S6 were sampled at Sea View. Similarly sites E1 and E2 were from Sea View, and sites E3, E4 and E5 were from Schoenmakerskop.

The separation between species from exposed and sheltered habitats is less distinct. The major difference between the two communities is the dominance of *Gelidium pristoides*, *Plocamium corallorhiza* and *Hypnea spicifera* in exposed sites, and the dominance of coralline turf, and *Codium lucasii* at sheltered sites. *G. pristoides* lies approximately between the two groupings as it is found at both sheltered and exposed sites, however it occurs at much higher densities at eh exposed sites (in gaps in the turf or on limpet shells in gaps within the turf).

3.3.2.5. Temperature

There was very little change in the temperature of the substrate along the intertidal transects in winter where the temperature during the study period (over low tide when the intertidal was exposed) remained approximately 18 – 20ºC (Fig. 3.10). In summer there was clear increase in temperature up the intertidal gradient from approximately 20 to 25ºC at the top of the intertidal (2m ALWS). These values showed considerable variability with temperatures high on the intertidal ranging from 19 to 33 ºC resulting in the large standard error (± 4.7 ºC).

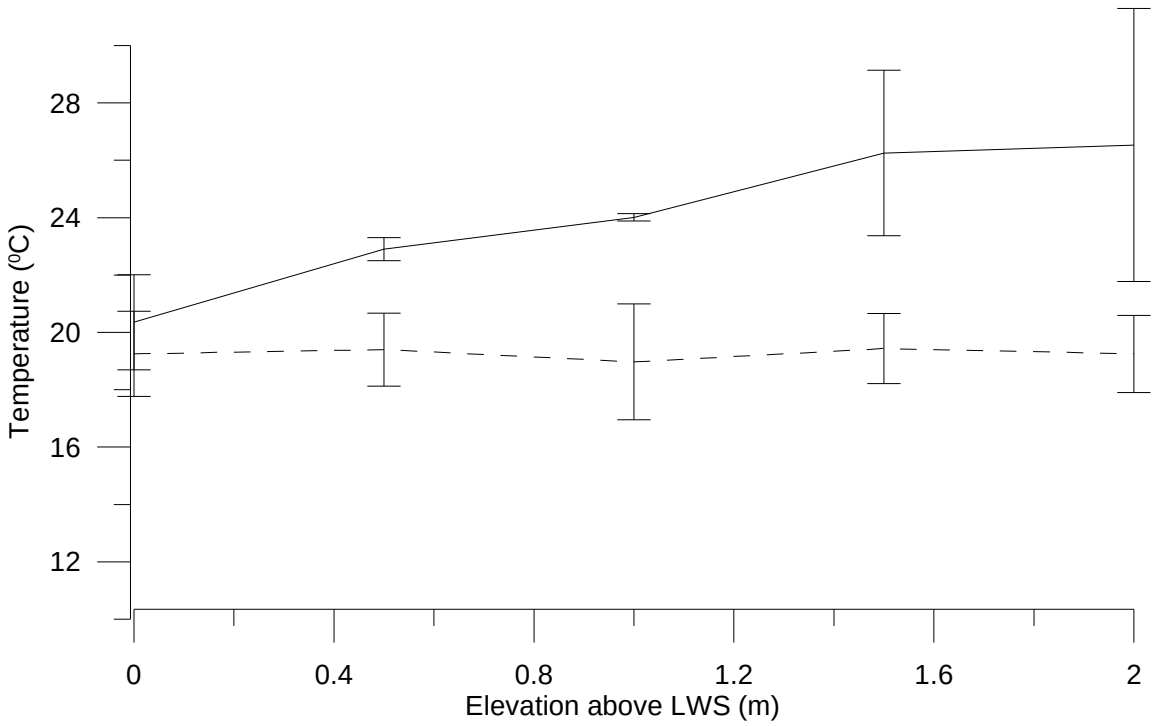


Figure 3.10. Substrate temperature along an intertidal transect at the study site near Schoenmakerskop, Bar = ±SE, n = 7. (Solid Line - Summer, Dashed Line – Winter)

3.3.2.6. Salinity

The salinity of free surface water along the transect measured on a warm summer day exhibited an increase with increased elevation above spring low tide water level (Fig. 3.11). The salinity of surface water on algae and rock near the low tide mark, and up to 0.4 meters, was ca. 35 ‰ (‰ – parts per thousand). Near the top of the transect the salinity of water was close to 43‰.

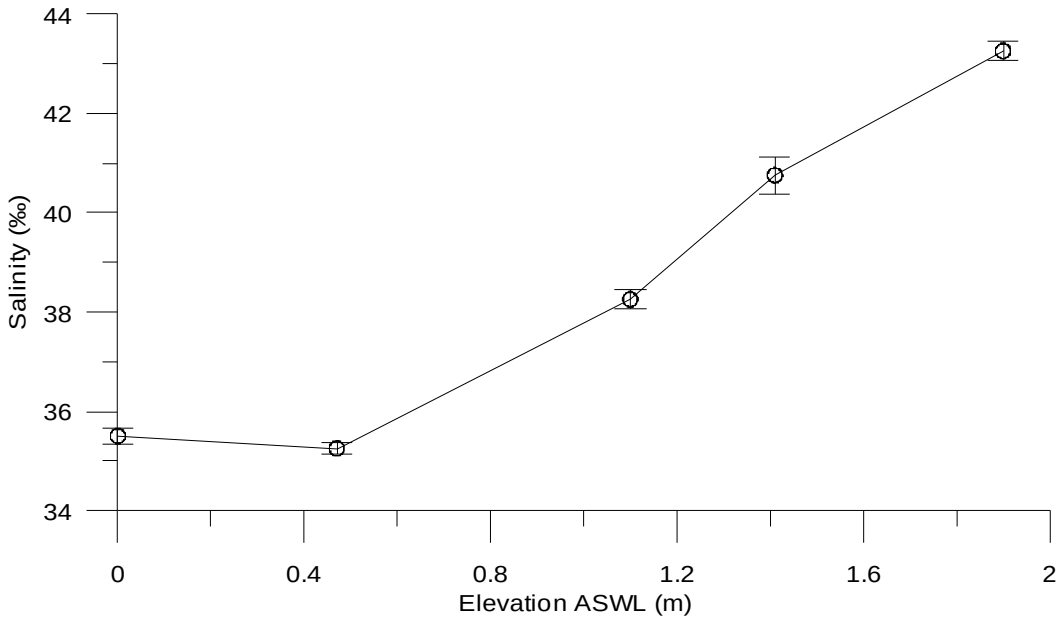


Figure 3.11. Salinity of surface water along the intertidal transect at the study site near Schoenmakerskop, Bar = ±SE, n = 5 (‰ - parts per thousand).

3.3.2.7. Light intensity

Figure 3.12 shows the averaged frond length of *Gelidium pristoides* at the bottom, middle and upper portions of the population in the intertidal. The frond length, and correspondingly, the tuft thickness of *G. pristoides* decreased up the intertidal zone.

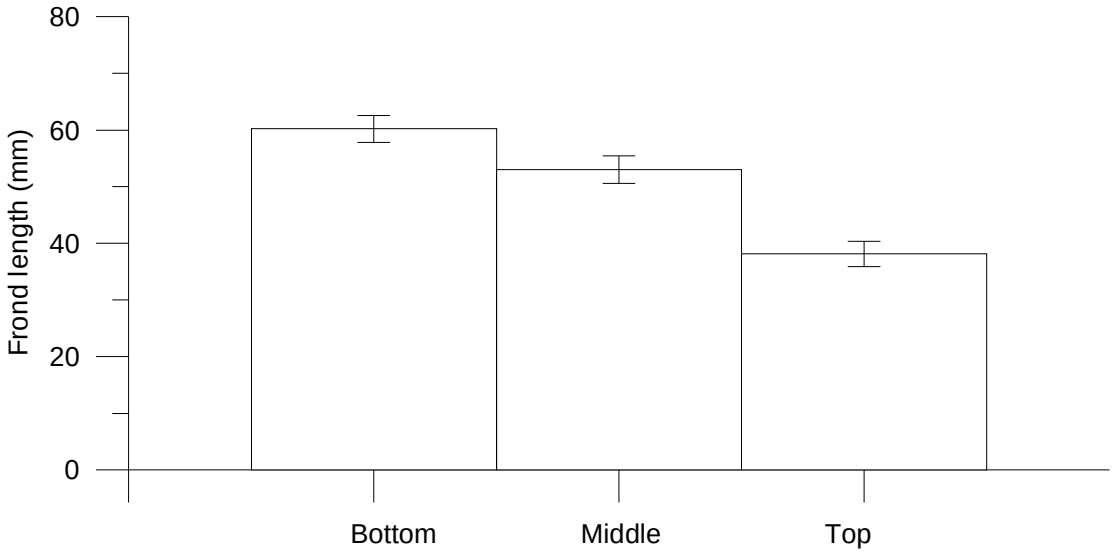


Figure 3.12. Frond length of *Gelidium pristoides* at three elevations along the intertidal transect at Schoenmakerskop, Bar = SE, n = 6.

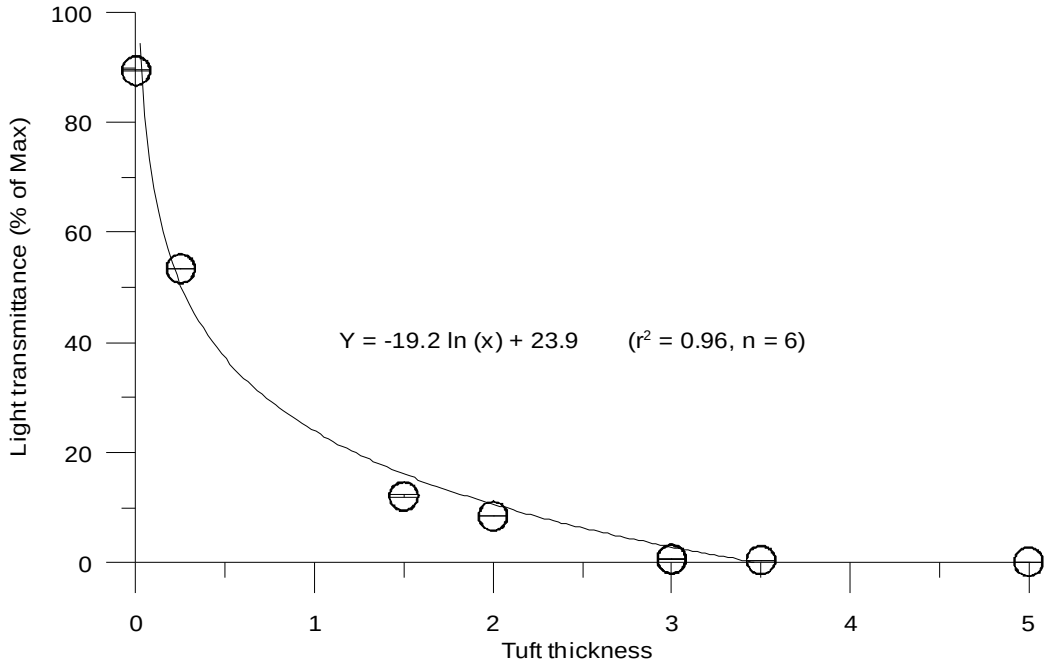


Figure 3.13. Light transmittance (percentage of the maximum incident radiation) through *Gelidium pristoides* tufts of different thickness, Bar = ±SE, n = 3

Figure 3.13 shows the attenuation of light penetrating the seaweed tufts for tufts of varying thickness. Tests on the amount of light penetrating tufts of different thickness indicated that there is a negative relationship between the two variables (Fig. 3.13). The light attenuation was described by: I = -19.2 ln (Tt) + 23.9, r^2 = 0.96, where I – light transmittance (% of maximum intensity), and Tt – tuft thickness (cm).

3.3.3. Temporal change in the intertidal zone

3.3.3.1. Temperature

Figure 3.14 shows surface temperature measured in the centre of the *Gelidium pristoides* population on the intertidal zone. Surface temperature did not show a significant increase with time after low tide. Within the *Gelidium pristoides* tufts there was a jump in the temperature from 16℃ to 18℃ between 30 min after low tide and 1 ho ur after low tide. Thereafter the temperature remained around 18℃ until the incoming tide had covered the algae again after 2.5 hours. Seawater temperature would have had no direct influence on the *Gelidium pristoides* population during the time of measurement, as it was not in contact with the seaweed until 2 hours after low tide. The seawater temperature decreased from 14 to 11℃ as the tide came in, replacing the water confined in the shallow gully with water from the open sea.

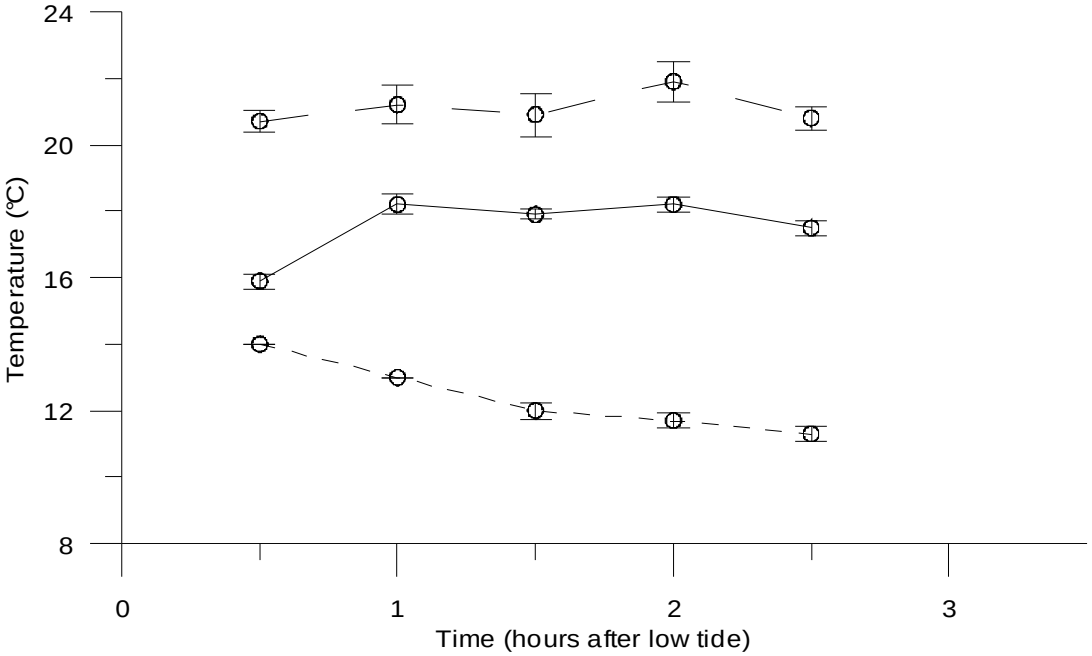


Figure 3.14. Temperature change in the *Gelidium* population on the intertidal zone at different times after a spring low tide (Solid line – Algal tufts, Broken line – Rock surface, dashed line – Seawater), Bar = ±SE, n = 5.

3.3.3.2. Light intensity

The light intensity in the intertidal zone showed an increase after low tide, from1400 µmol m^{-2} s^{-1} to 1800 µmol m^{-2} s^{-1} after 2.5 hours (Fig 3.15). The light measurements were made between 10h30 and 12h00 so that the final light intensity measured (at midday) represents the maximum for the day. (Measurements terminated when intertidal zone was covered by the incoming tide.)

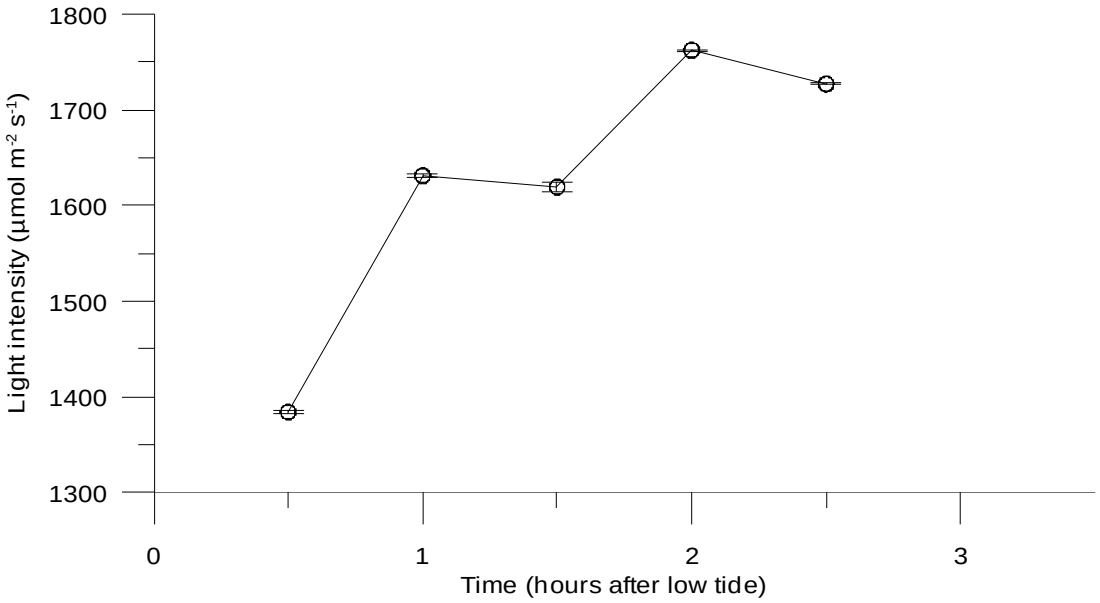


Figure 3.15. Change in the surface light intensity on the rocky intertidal zone at Schoenmakerskop at different times after a spring low tide, Bar = ±SE, n = 8.

3.3.3.3. Desiccation

Gelidium pristoides showed a drop in moisture content from 70% at low tide to 50% 2.5 hours after low tide (Fig.3.16). Three hours after low tide the incoming tide had started covering the seaweed again, resulting in rapid re-hydration to a moisture content of 75%.

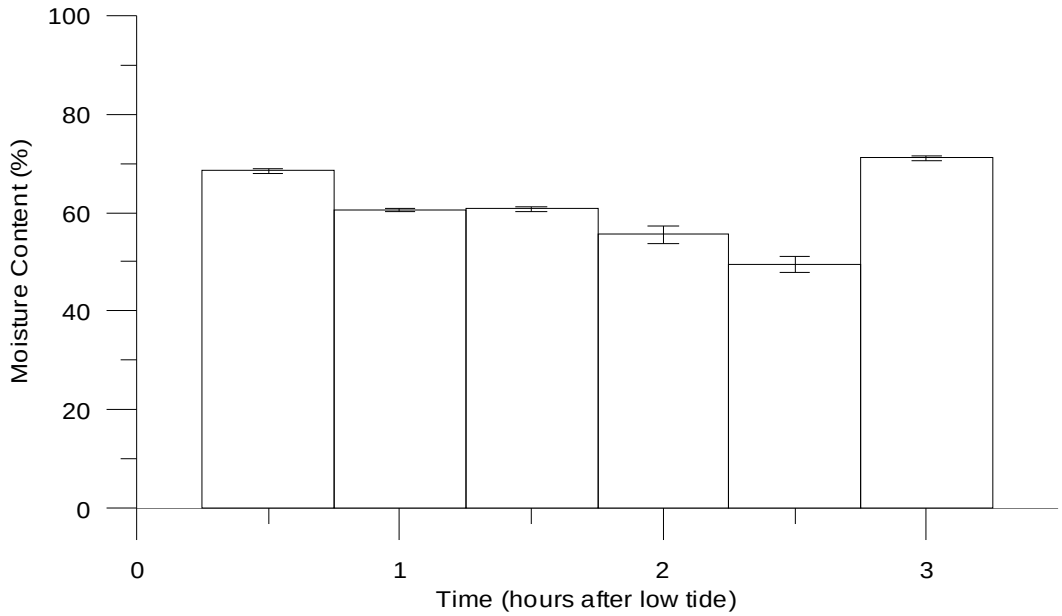


Figure 3.16. Moisture content of *Gelidium pristoides* thalli on the rocky intertidal zone at Schoenmakerskop at different times after a spring low tide, Bar = ±SE, n = 3.

3.4. Discussion

Gelidium pristoides has been documented to occur between 0.2 and 0.8 meters above tidal chart datum on rocky shores measured at Port Alfred, 140 km east of Port Elizabeth (Carter & Anderson 1991). Data from sites around Port Elizabeth have indicated that *G. pristoides* is found from 0.2 to 1.2 meters above spring low tide level. Local variability in the vertical distribution range of the algae has been documented above and had been documented by Stephenson (1948), and most likely explains the differences in distribution data. (Tidal chart datum is close to spring low tide water level and has only been adjusted by seven centimetres since 1991).

Using the vertical distribution data for local populations of *Gelidium pristoides* around Port Elizabeth, the elevation of the middle of the zone inhabited by *G. pristoides* would be 0.8 m above tidal chart datum. Using this as the average elevation of *G. pristoides* at the study site, it can be calculated that the algae would be exposed for up to 6 hours at a time during a spring tide, and only for an hour or less during a neap tide. The alga on average, therefore, has to endure variations of exposure durations between 1 to 6 hours in a two weekly cycle. Of course those at higher elevations will be exposed for even longer, while those lower down in the intertidal for less.

Long-term climatic data indicates that during the exposed times the alga may be exposed to air temperatures ranging from 15℃ in winter to 22℃ in summer. This does not accurately represent the thermal environment the alga experiences as the substrate temperature with which the alga is closely associated may reach temperatures in excess of these means due to solar insolation. Data collected on the exposed rocky shore of Port Elizabeth sites (during spring low tides) shows summer temperatures ranging from 19 to 33℃. Winter data recorded on the rocky shores in a similar area during spring low tide exposure on rocky shores show more stable lower temperatures between 18 and 20℃. The long term winter data also does not account for wind chill, and actual minimum temperatures the alga may experience when exposed may be substantially lower than the seasonal mean minimum temperature of 15℃.

Seawater temperatures showed the same seasonal pattern as the air temperatures. The mean monthly summer temperature of 21℃ may be close to the optimum for growth of *G. pristoides* as the highest growth rate measured for natural populations in the area have been shown to occur during spring and summer (Carter & Anderson 1985). The increase in growth rate may be attributed to increased seawater temperature, increased day lengths and increased light intensity during this time of the year.

Observations in the intertidal at the Schoenmakerskop study site have shown that *Gelidium pristoides* distribution and abundance is variable on a small spatial scale, apparently related to the level of wave exposure. Such variability in intertidal flora of the coast at Schoenmakerskop has been previously documented by Stephenson (1948). The lower distribution limit for *Gelidium pristoides* at Schoenmakerskop is similar to that found by Carter & Anderson (1991) at Port Alfred, and McQuaid (1985) in False Bay. In these studies the lower limit also coincided with the increased abundance of coralline turf in the intertidal fringe. The epiphytic encrusting coralline alga, *Synarthrophyton patena* (J.D. Hooker & Harvey) R.A. Townsend, which has been shown to negatively affect *G. pristoides* growth in the subtidal fringe (Carter & Anderson 1991), was not found amongst the algae in the coralline turf at Schoenmakerskop. In coralline turf dominated areas it appears that competition for space and secure substrate limits colonization by *Gelidium pristoides*. Attaching to the coralline algae in the littoral fringe, the seaweed would soon be torn from the substrate as the turf algae are not very securely attached to the rock. These corallines avoid detachment by wave action by having a dense short mat-like growth form. As soon as the upright portions of *G. pristoides* develop, the algae would most likely be torn from the substrate by wave shear, along with its host coralline alga. Toefy *et al.* (2003) also reported differences in *G. pristoides* thallus size from sheltered and exposed sites, with those from exposed sites being larger. Potential reasons for the low abundance of *G. pristoides* at turf dominated sheltered rocky shores are discussed in Chapter 6, and appears to be related to inorganic carbon availability.

Gelidium pristoides favours the calcareous substrates provided by barnacle and limpet shells for attachment as these provide security of attachment, while probably providing refuge from herbivores as well (Jarman *et al* 1988). It has been shown that some *Gelidium* species attach to calcareous substrates more readily than other potential settlement surfaces (Ramiro-Rojas *et al*. 1996, Santelices & Varela 1994). This could explain the similar distribution ranges observed for the alga and these animals (Fig 3.7).

Limpets and barnacles, as well as *Gelidium pristoides* show diminishing abundances higher up the intertidal zone, most likely due to increased physical stress. This stress is associated with the time the algae are exposed. After low tides the algae can show a reduction in moisture content from 70% to 50% at a substrate temperature of 20℃, with likely higher rates of water loss at temperatures above this. There is also good evidence of increasing salinities higher up the intertidal zone after a low tide (Fig. 3.11). This is related to the time the surface has been exposed to evaporative water loss. The reduction in *G. pristoides* frond length up the intertidal gradient has been investigated by Ballantyne (2003) and indicated that this change in morphology is related to desiccation stress tolerance. Similar findings have been made in work

done in subtropical and tropical shores (Tittley & Neto, 2000, Taylor & Hay 1984). The alga's response to change in salinity has not been well investigated. According to the marked changes in salinity up the intertidal gradient, *G. pristoides* would have to be well adapted to this added stress.

The very short, turf like form of *Gelidium pristoides* is often found at the highest point of its intertidal distribution. Ballantyne (2003) showed the turf like form of *G. pristoides* to be an adaptation to desiccation. Prathep *et al.* (2009) showed shorter frond lengths for *Gelidium pusillum* from wave exposed sites and also suggested reduced water loss as a reason for the persistence of the turf like growth form. The longer *Gelidium pristoides* frond length and resultant larger tuft size lower on the intertidal may also be a reflection of lower incidence of nutrient limitation, related to the longer mean monthly immersion time experienced by these individuals (Carter & Anderson 1991). The larger tuft size has been shown to increase self-shading (Fig. 3.13). This may enable algae to better cope with high light intensities and the associated photoinhibition; slow down water loss in the tufts; and maintain lower temperatures inside the tuft. Those fronds inside the tuft could maintain a net positive photosynthetic rate at tolerable temperatures and light intensities, while the fronds on the outside of the tuft may show net respiration due to desiccation stress and photoinhibition. This type of response has been shown for algal communities which reached light saturation at much higher irradiances than individual fronds (Binzer & Middelboe 2005). This is thought to be related to more diffuse light distribution within a canopy of seaweed communities, which reduces overall suppression of photosynthesis by photoinhibition.

Chapter 4

Gelidium pristoides photosynthesis in water.

4.1. Introduction

In terrestrial plants photosynthetic rate is generally determined by measuring the amount of carbon dioxide taken up per unit biomass over time. This method is commonly used since the medium in which plants photosynthesize best is air, and changes in CO_2 concentration can be measured relatively easily by Infra-red Gas Analyzers. Traditionally photosynthetic rate in marine macroalgae is measured in submerged samples. This is because the most favorable condition for photosynthesis in seaweed is typically when the material is immersed in seawater. In seawater it is easier to measure changes in photosynthetic oxygen concentration than it is to measure carbon dioxide. Carbon dioxide occurs in equilibrium with various other forms of inorganic carbon in seawater and the atmosphere, the relative forms of which is dependant on, and affect the seawater pH. Theoretically in an enclosed volume of water, in which diffusion of atmospheric CO_2 into the medium is prevented, pH can be used as a measure change in carbon dioxide concentration. However the total inorganic carbon concentration, chlorinity, and other seawater chemical variables need to be determined before these calculations can be accurately done. The development of the Clarke-type oxygen electrode makes measurement of oxygen concentrations in seawater relatively quick and simple. This is probably on of the reasons photosynthetic rate in seaweed has usually been measured by monitoring the change in oxygen concentration in an enclosed volume of water, containing a sample of the seaweed under investigation. In these types of experiments photosynthetic rate is expressed as grams, milliliters or moles of oxygen produced per unit biomass over time. A relatively new concept in determining relative photosynthetic rates in plants, and only recently, algae is by using Chlorophyll fluorescence. The measurement of certain fluorescence parameters can give an indication of the relative electron flow rate along the electron transport chain in the chloroplasts (Genty *et al*. 1989a), which is closely linked to photosynthetic carbon fixation and oxygen liberation (Hanelt *et al*. 1992).

The aims of these experiments were:

- To attempt to determine whether fluorescence measurement, in particular relative electron flow rate, could replace traditional measurement of photosynthesis by monitoring O_2 evolution.
- Determining the optimum light conditions, temperature, salinity, and pH for *Gelidium pristoides* photosynthesis.

4.2. Materials and Methods

4.2.1. Photosynthetic response to different irradiance levels

To ensure that the maximum rates were measured during the photosynthetic measurements conducted on *Gelidium pristoides*, and in an attempt to avoid measuring diurnal patterns in photosynthetic rate, all photosynthetic measurements were taken over the period from 11 am to 2 pm daily. Photosynthesis irradiance response curves were generated by placing samples of freshly collected seaweed in a sealed cuvette. The production or use of oxygen at varying light intensities was measured using a Clarke-type oxygen electrode. *Gelidium pristoides* was collected from the rocky shores in the morning and transported to the lab in a closed, darkened insulated container. At the laboratory the material was stored in a controlled environment chamber at a temperature of 18 ℃ in a container wi th fresh seawater, at a light intensity of *ca*. 180 µmol m^{-2} s^{-1} between experiments. The water in the container was continually aerated to prevent boundary layers effects, and to ensure sufficient gas exchange. Samples free of macroscopic epiphytes were selected to minimize the effect of epiphytic photosynthesis on the photosynthetic rate measurement in the samples. Sub-samples for experimentation were removed from the container and placed in a water jacketed cuvette with a volume of 3.1 milliliters. The oxygen electrode was inserted at the base of this cuvette. The glass cuvette was surrounded by a stainless steel water jacket. Light could reach the glass cuvette only through glass ports in the side of the stainless steel water jacket (see Fig. 4.1.).

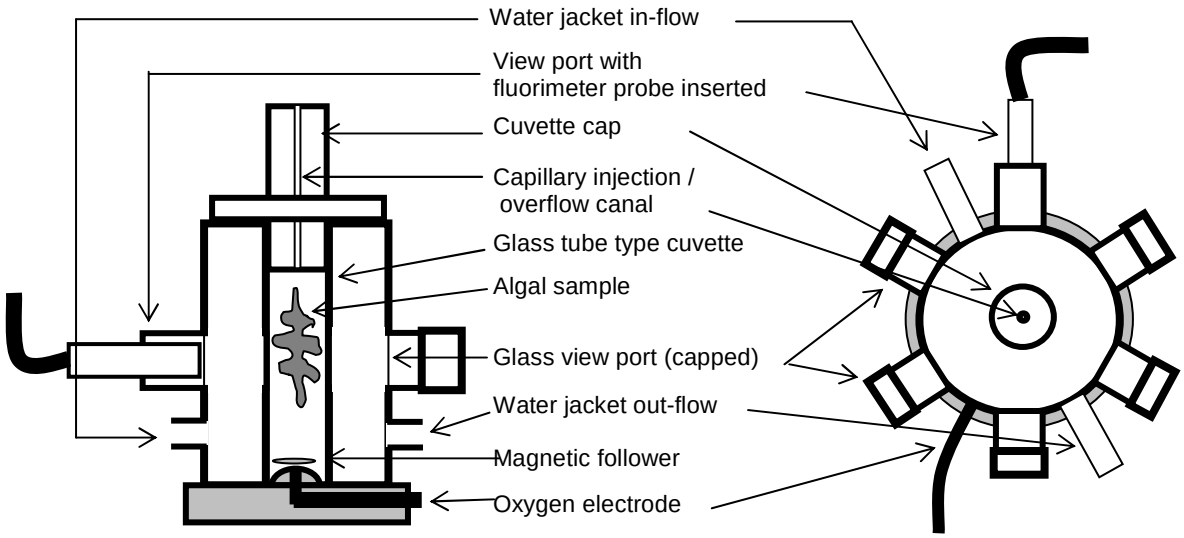


Figure 4.1. Oxygen electrode / fluorimeter cuvette assembly (in section – left, and in plan view – right).

The temperature in the cuvette was kept constant by circulating water from a thermally controlled water bath, set to the desired temperature, through the water jacket. Attached to one of the glass ports on the cuvette water jacket was the optical fibre probe of a Hansatech PAM fluorimeter. The probe could also be used to deliver pulses of actinic light from a light emitting

diode. The fluorimeter probe was used as a light source in the photosynthesis irradiance response curve experiments. The light intensity delivered could be set at a stable light intensity dependant on a remote controlled setting. The settings used and the corresponding light intensities are illustrated in figure 4.2.

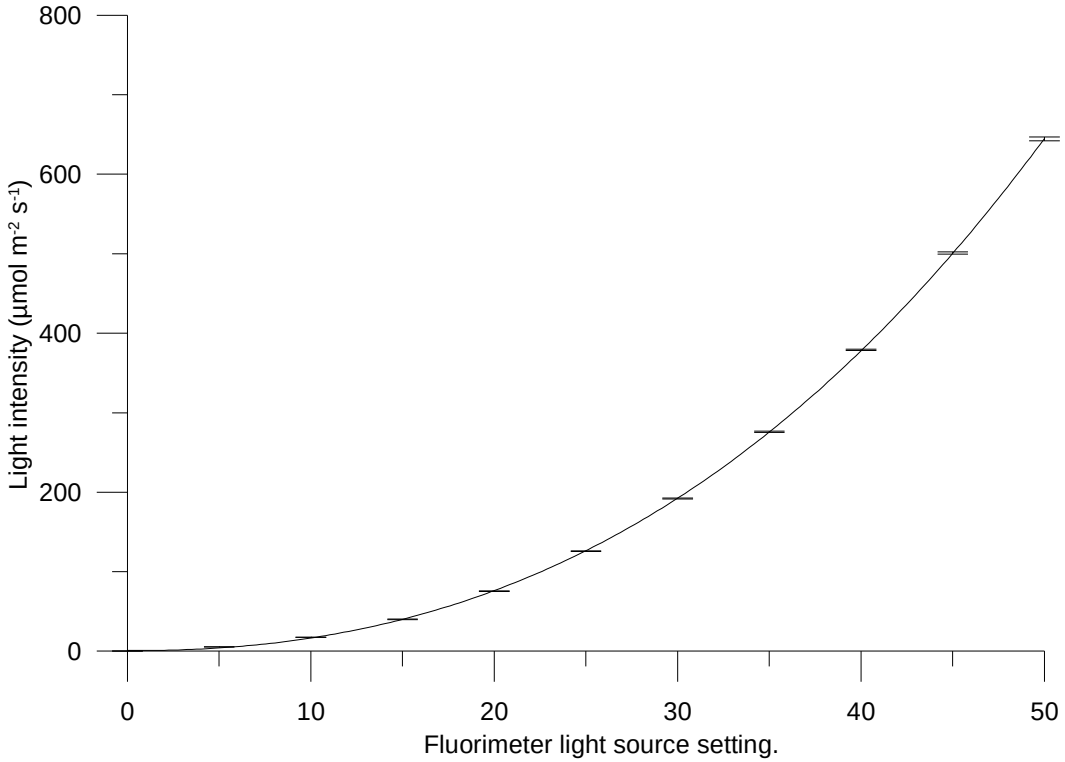


Figure 4.2. Different light intensities produced by the PAM fluorimeter light source, used in photosynthesis experiments, at different fluorimeter settings, Bar = SE.

With the equipment configuration used it was possible to determine fluorescence parameters while measurement of photosynthetic oxygen production was taking place. With the aim of evaluating the usefulness of Relative Electron Flow rate as a measure of photosynthesis, the following fluorescence parameters were measured.

F_m, the maximal fluorescence yield for light adapted sample, and F_s, the current or steady state fluorescence for the sample. Genty *et al*. (1989) suggest that the term (1-T-R) is assumed to be one in the absence of frond transmittance (T) and the reflectance (R) values. In *Gelidium pristoides* Relative Electron Flow Rate (rEFR) could be calculated as follows:

rEFR = [(Fm-Fs) / Fm] x Irradiance x 1. ..... Equation 4.1

The measurement of changes in the oxygen concentration in the cuvette were recorded as voltage output from the oxygen electrode. The signal from the electrode was amplified by a

Hansatech electrode control box and transmitted, via a digital analogue converter, to a personal computer. Voltage change over time was recorded by the computer fitted with a digital-analogue converter with the aid of DASYLab Ver 3.5 and WaveView for Windows Ver 2.0.6.10. This software was also used to calibrate and manipulate the voltage signal from the electrode. Before and during the experiment, the seawater used in the experiment was stored in a measuring cylinder that was placed in a thermally controlled water bath. Water from the water bath was also circulated trough the cuvette water jacket. This ensured that the seawater in the cuvette, as well as seawater destined for the cuvette, was always at the same temperature. The ambient air was bubbled through the water in the measuring cylinder. This ensured that the oxygen concentration in the seawater to be used in the cuvette was in equilibrium with atmospheric levels. This characteristic also made it possible to relate voltage output to actual oxygen concentration.

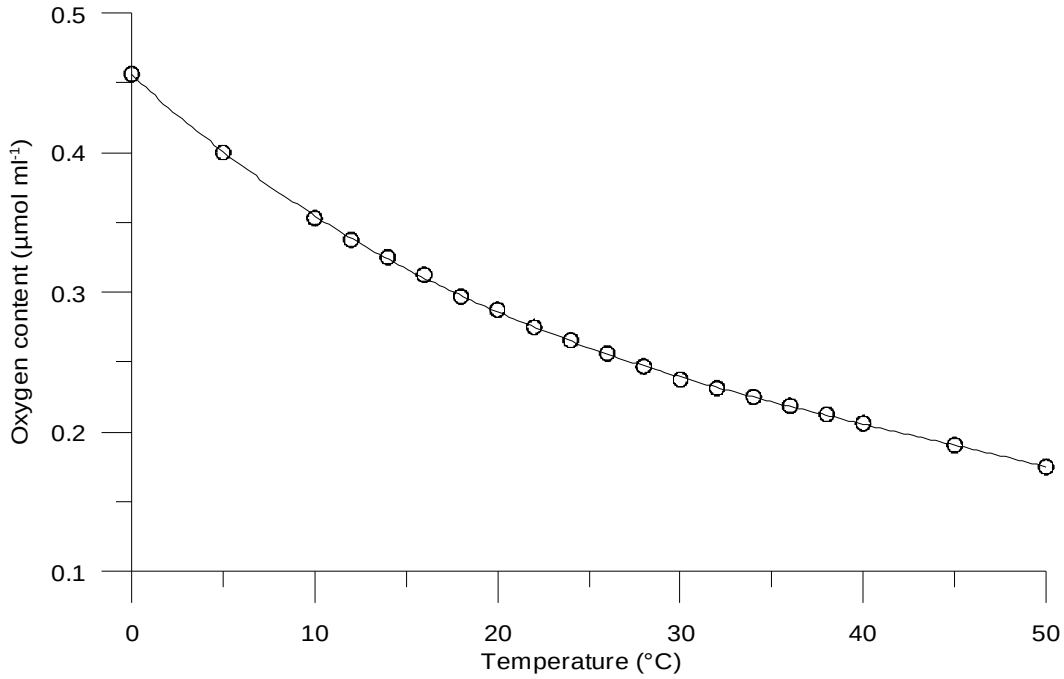


Figure 4.3. Oxygen content of distilled water saturated with air at various temperatures (adapted from Campbell 1986).

Before experiments commenced the electrode voltage was always zeroed by scrubbing all oxygen from the water in the cuvette. This was achieved by the addition of Sodium dithionite, which would drop the oxygen, and correspondingly, the voltage to a minimum value. The voltage output was then corrected, by adding a constant in the arithmetic function of the Dasylab software, so that voltage output recorded registered zero. The cuvette was then washed twice with distilled water to remove all traces of the scrubbing chemical, and subsequently filled with the air-equilibrated seawater. The voltage recorded for this oxygen concentration was representative of the amount of oxygen content of seawater in equilibrium

with atmospheric oxygen levels. These values were obtained from (Campbell 1986, PhD thesis) and are represented in Fig.4.3. The curve is described by the equation $y = 0.455 – 0.0119x + 0.00021x^2 – 1.599 \times 10^{-6}x^3$ ($r^2 = 0.999$). When air saturated seawater was placed in the cuvette, the voltage reading obtained could be related to the corresponding air saturated seawater oxygen content, for the particular temperature of seawater being used. The system was then considered calibrated.

The voltage-signal sampling rate was set to 10 Hz, and every ten measurements were averaged to give one voltage value every second. Linear regression was used to determine the rate of voltage change (d_V/d_t or rate of change in oxygen concentration in the cuvette).

The rate voltage change was converted to photosynthetic rate as µmol O_2 g^{-1} (ww) s^{-1} using the following equation:

$$f = \frac{a \times b \times (0.455 – 0.0119g + 0.00021\,g^2 – 1.1599\times10^{-6}g^3)}{c \times d} \quad \text{.....Equation 4.2}$$

Where

a = Volume of the electrode cuvette (mL).

b = Rate of voltage change at the electrode (V s^{-1}).

c = Electrode voltage for water in equilibrium with atmospheric oxygen at the operating temperature *[calibration voltage]* (V).

d = Weight of sample (g).

f = Photosynthetic rate (µmol O_2 g^{-1} s^{-1}).

g = Temperature of water in cuvette (℃). (*The term describing the O_2 concentration of seawater in equilibrium with atmospheric O_2 levels at a particular temperature ($\underline{0.455 – 0.0119g + 0.00021g^2 – 1.599 \times 10^{-6}g^3}$) was derived from a 3rd order polynomial curve fit of seawater temperature-oxygen data from Campbell 1990, $r^2 = 0.9998$.)*

10 mm portions of *G. pristoides* fronds were cut and placed in the cuvette with fresh seawater. Seaweed samples had a weight of *ca*. 0.05 – 0.1 g (wet weight). The samples were subject to periods of five to ten minutes at each light intensity, separated by periods of darkness. Light intensities between 0 and 700 µmol m^{-2} s^{-1} were tested. The seawater in the cuvette was replaced with fresh seawater when the oxygen concentration approached a value double that of initial concentrations to avoid suppression of photosynthesis.

F_s and F_m was measured at after each five to ten minute period of photosynthetic measurement at a particular light intensity. This was done to avoid the high light pulse used to measure F_m from affecting subsequent measurement of photosynthetic rate. F_s is a measure of steady state

fluorescence at a particular light intensity. Therefore it was always measured before F_m, for the same reasons as stated above.

4.2.2. Effect of short-term temperature change on photosynthetic rate

The photosynthetic response of *G. pristoides* to temperature change was measured in short term experiments only. This type of experimental design was used for two reasons. The reliability of data gathered over a period of more a day was questionable due the alga's tendency to deteriorate when placed in artificial seawater systems. The alga deteriorates markedly in laboratory culture within four to six days of being placed in containers outside its natural environment.

The goal of the experiments was to gather data about the alga's photosynthetic rate when the tide recedes. During these times water surrounding the thallus may be heated up or cooled over a relatively short period as a result of insolation or wind chill respectively. Algal samples were collected and stored between experiments in the same way as for the irradiance response curves. Seawater for the experiments was stored in a flask immersed in temperature controlled water bath, and bubbled continuously. The temperature of the water bath was set to the desired temperature and allowed to adjust. Water from the water bath flowing through the cuvette water jacket ensured that the seawater in the cuvette remained the same as that of the treatment seawater. Samples of *G. pristoides* (0.05 – 0.1 g) were placed in the cuvette and photosynthetic rate determined as described above, with the difference that only one light intensity was used for all the temperatures tested. All photosynthetic rates were determined at a light intensity of *ca*. 300 µmol m^{-2} s^{-1}. Preliminary irradiance response data suggested an I_k value for photosynthesis of *ca*. 154 µmol m^{-2} s^{-1} and an irradiance higher than this value was considered sufficient for accurate measurement of temperature effects on photosynthetic rate. After a number of replicate photosynthetic rates had been determined at a particular temperature. The temperature setting on the water bath was changed to the next temperature to be tested. The water bath was left to adjust to this temperature and measurements of photosynthetic response commenced as described above. A range of temperatures both above and below the annual mean temperature for Algoa Bay (*ca*. 21℃) were tested (12, 15, 18, 21, 24, 27, 30℃).

4.2.3 Effect of short-term salinity change on photosynthetic rate

As exposure duration continues *Gelidium pristoides* will experience evaporative water loss. This water loss will occur first from the small pool of water in which it may be attached, then from the external water adhering to the thallus surface, and finally water will be lost from the thallus itself. With evaporation from the small pool of seawater associated with the seaweed tuft, or more commonly from the external water adhering to the thallus, there will be marked short term

salinity changes. It is expected that *G. pristoides* will be well adapted to cope with this potential salinity stress. Experiments were done to ascertain the photosynthetic response of *G. pristoides* to such short-term salinity changes.

G. pristoides was collected and pre-treated the same as in the experiments discussed above. Seawater of different salinities was prepared by adding either distilled water or sodium chloride to normal seawater. The salinities were measured using an Atago handheld refractometer. Seawater with salinities of 10, 20, 30, 40, 50 and 60 ‰ were prepared in this way. Seaweed samples (0.05 – 0.1 g) were removed from the stock stored in normal seawater (35‰), in the controlled environment chamber, and placed in the cuvette with seawater with adjusted salinities. Photosynthetic rate was recorded for these samples at a light intensity of *ca*. 300 µmol m^{-2} s^{-1}, and a temperature of 18℃. Four replicate measurements were made of the photosynthetic rate at each salinity.

4.2.4. Inorganic carbon use in *G. pristoides*

4.2.4.1. Effect of pH on photosynthetic rate

In an attempt to determine the inorganic carbon form used by *G. pristoides* the effect of pH on the photosynthetic rate was determined. The dominant forms of inorganic carbon in seawater vary according to seawater pH. At low pH carbon dioxide is the dominant form, at a pH of 7 – 9 bicarbonate is the dominant form, while most of the IOC in seawater at a pH above 9 occurs as carbonate (Section 1.6 in literature review). By monitoring photosynthetic rate at different pHs it is possible to gain information on the form of IOC the alga uses (Larsson & Axelsson 1999, Bjork *et al*. 1992, Axelsson *et al*. 2000, Maberly 1990, Menendez *et al*. 2001).

The same oxygen electrode – cuvette system, as was described for the experiments in sections 4.2.1 and 4.2.2 was used for the pH experiments. The cuvette cap had small aperture at the top through which excess seawater in the cuvette could escape. This aperture presented a very small (<0.5 mm) seawater – atmosphere interface which ensured that gas exchange between the treatment water and the atmosphere was minimal. This system was as close to a closed system as was practical.

Seaweed samples were placed in the cuvette and photosynthetic rates measured from changes in the oxygen concentration in the cuvette. All the photosynthesis measurements were taken at a seawater temperature of 18℃ and an irradiance above the I_k value for photosynthesis (300 µmol m^{-2} s^{-1}). Initial measurements were taken in normal seawater with a pH of between 8.2 and 8.5. Thereafter various amounts of 1 N HCl were added to the system to obtain the required seawater pH.

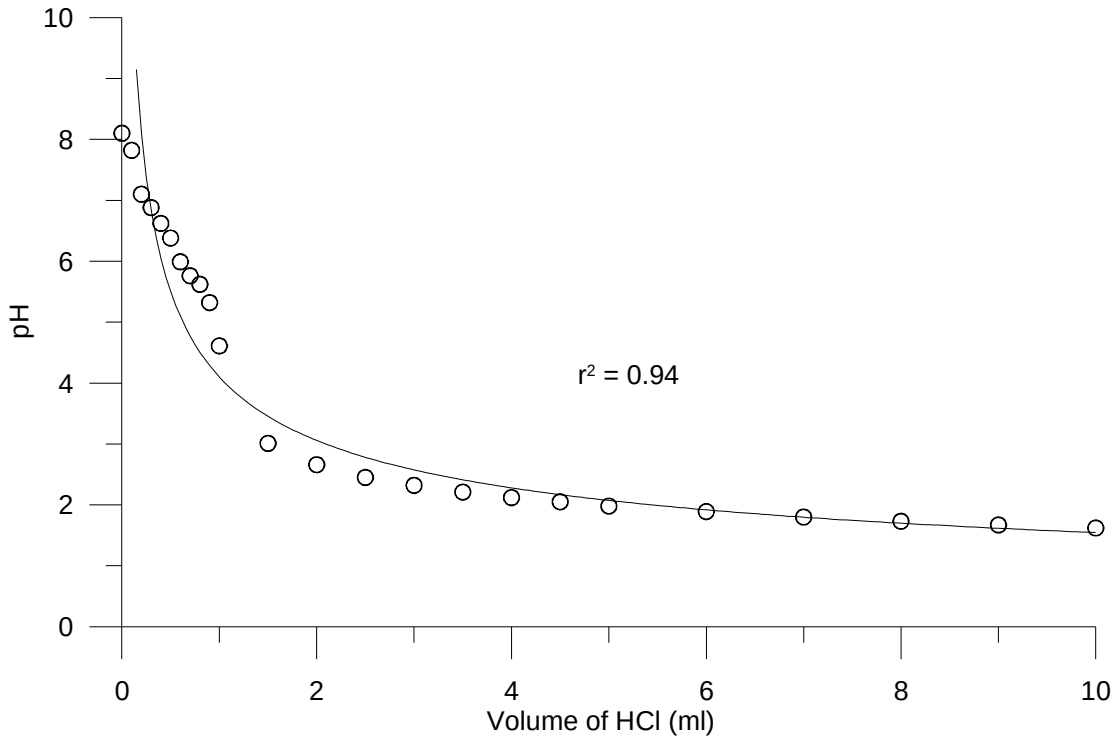


Figure 4.4. Acid-seawater titration curve used to determine the appropriate volumes of HCl to add to seawater to obtain a desired pH for the pH-response experiments.

The amounts of 1 N HCl that had to be added to the 3.1 ml cuvette was established before the experiment could be conducted. HCl was titrated into a known volume of seawater in a closed system (Fig 4.4.). The amounts added were adjusted to the amounts required for the smaller cuvette system. A 50 µL Hamilton syringe was used to add the very small amounts of HCl required to drop the pH in the cuvette to appropriate levels. Photosynthetic rate was measured for seawater pH of 8.2 (normal seawater), 7, 6, 5, 4, and 3.

4.2.4.2. Effect of carbonic anhydrase (CA) inhibitors on photosynthetic efficiency

Results obtained in the pH experiment indicated that *Gelidium pristoides* may be predominantly a carbon dioxide user, with very limited capacity for using the dominant from of inorganic carbon in seawater, bicarbonate. The ability to use bicarbonate is dependant largely on the dehydration of bicarbonate, either inside or outside the cells, by carbonic anhydrase (see Section 2.7.1). It is possible to investigate the role of carbonic anhydrase and the relative importance of bicarbonate as an inorganic carbon source by using specific carbonic anhydrase inhibitors.

Acetozolamide (AZ) and 6-ethoxyzolamide (EZ) are compounds that inhibit the activity of carbonic anhydrase. Acetazolamide is generally accepted to be membrane impermeable, and as such only able to inhibit CA occurring outside the cells. Ethoxyzolamide is membrane permeable and inhibits the activity of all CA, both inside and outside the cell (Mercado *et al*.

1998). This experimental protocol had been successfully used to investigate inorganic carbon use in a number of marine macroalgae (Bjork *et al*. 1992; Axelsson *et al*. 1995; Israel & Friedlander 1998; Larsson & Axelsson 1999; Mercado *et al*. 1998; Axelsson *et al*. 2000).

Fresh, filtered seawater was used in the experiments testing the effect of these two CA inhibitors on photosynthesis in *Gelidium pristoides*. Solutions of acetazolamide and ethoxyzolamide were made up to a final concentration of 40 µM in this seawater. These chemicals have a very low solubility in water, and it was necessary to use a miscibility agent to improve the solubility of AZ and EZ in the seawater. The AZ and EZ were dissolved in Dimethylsuphoxide (DMSO) before being added to the seawater. The amount of DMSO used was such that the final concentration did not exceed 2% on a volume basis. This concentration appeared to have no negative affect on photosynthesis in *G. pristoides*. For each experiment 100 milliliters of treatment water was made up in the following way:

AZ treatment - 40 µmoles acetazolamide was dissolved in 2 mL DMSO. To this solution 98 mL of the filtered seawater was added.
EZ treatment - 40 µmoles of ethoxyzolamide was dissolved in 2 mL DMSO. To this solution 98 mL of filtered seawater was added.
DMSO treatment - 2 mL of DMSO was added to 98 mL of filtered seawater.
Control - 100 ml of filtered seawater.

These solutions were poured into Petri dishes in a sufficient quantity to cover thallus pieces *ca*. 200 mL. The treatments were placed in Petri dishes to ensure a large surface area for gas exchange between the water and the atmosphere. These dishes were agitated regularly (every 5 to 10 minutes) to ensure mixing in the treatment water.
Into each Petri dish, an intact thallus piece of approximately 50 mm length was placed in each dish. The seaweed samples were freshly collected from the rocky shores of the Port Elizabeth coast no more than thee hours before experiments commenced. The samples were kept in a container with fresh seawater, also collected at the sampling site, and provided with vigorous aeration.

Thallus pieces (12 replicates) were incubated in this way at a light intensity of 800 µmol photons m^{-2} s^{-1} in the laboratory at room temperature (*ca*. 18 – 20℃). After 30 minutes the lab was darkened and the algae allowed to dark adapt for 7 minutes, after which the photosynthetic efficiency (F_v/F_m) was determined using a Hansatech PAM fluorimeter. This process was repeated a number of times and the response of the seaweed samples to the different treatments recorded. It may be appropriate to mention that while we have not found the use of

relative electron flow rate a reliable measure of photosynthetic rate in *Gelidium pristoides*, the use of a PAM fluorimeter to determine photosynthetic efficiency (F_v/F_m) is a well documented, and used, tool in the investigation of stress in plants and algae (Herrmann *et al*. 1995, Magnusson 1997, Cordi *et al*. 1997, Gomez & Figueroa 1998, Silva *et al*. 1998, Hader *et al*. 1998, Casper-Lindley and Bjorkman 1998). Photosynthetic efficiency in healthy plants and green algae, under little or no stress, is normally about 0.8 relative units. In red algae the photosynthetic efficiency of healthy fronds is lower, at approximately 0.4 - 0.7 relative units (Cunningham *et al*. 1996). Reasons for this lowering in F_v/F_m ratios in red algae and the cyanobacteria are outlined in chapter two, section 2.3. The above method was considered a reliable measure of stress due to carbon limitation in an experimental system where all other conditions where kept optimal.

4.3. Results

4.3.1. Photosynthetic response vs relative electron flow rate (fluorescence methods)

Preliminary measurements of photosynthesis using both fluorescence and oxygen evolution indicated that Relative Electron Flow Rate (rEFR) cannot be directly related to photosynthetic measurement using the oxygen electrode. Application of a photosynthesis-irradiance model (Jassby & Platt, 1976) to the fluorescence and oxygen data yielded very different curve shapes and I_k values (154.2 vs. 245.1 µmol m^{-2} s^{-1}).

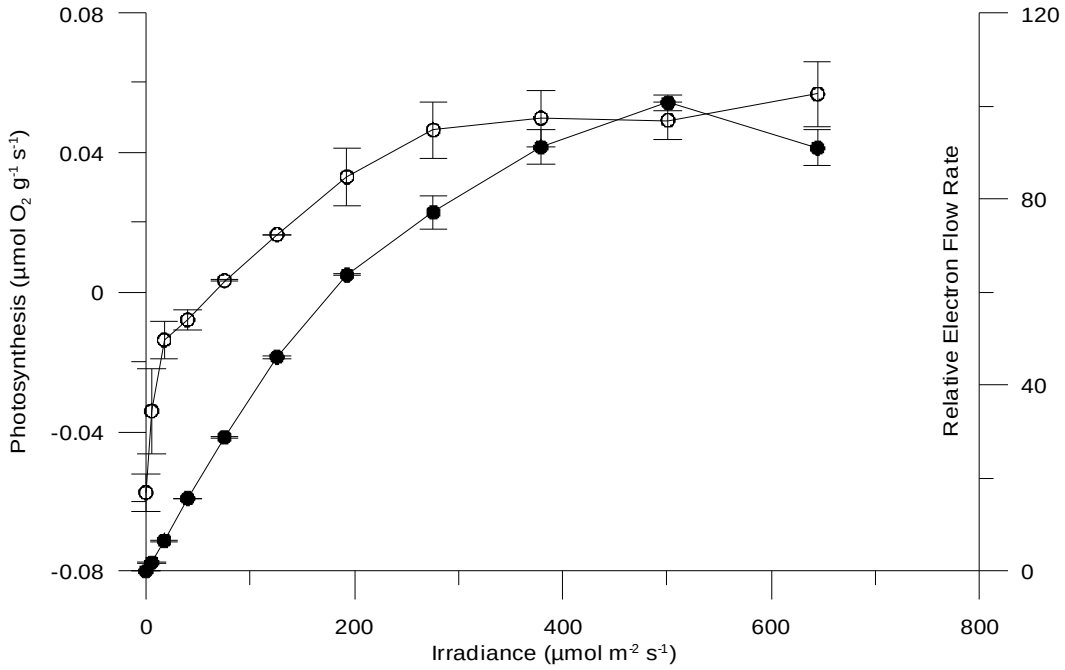


Figure 4.5. Photosynthetic rate (Open circles) and relative electron flow rate (Solid circles) in *Gelidium pristoides* at varying light intensities in seawater at 18℃, Bar = ±SE.

The rEFR method also does not reflect respiration rates as it only measures to light induced electron flow at the chloroplast. The use of fluorescence in PI curves may be of value at high light intensities where a regression analysis had indicated a good correlation between oxygen evolution data and the rEFR method (r^2 = 0.933). However at lower light intensities there is not a significant correlation between rEFR and photosynthesis (r^2 = 0.735). The method was abandoned as it was unclear how this data should be interpreted and the traditional method of measuring photosynthesis using O_2 production seemed widely accepted and more easily comparable to existing data.

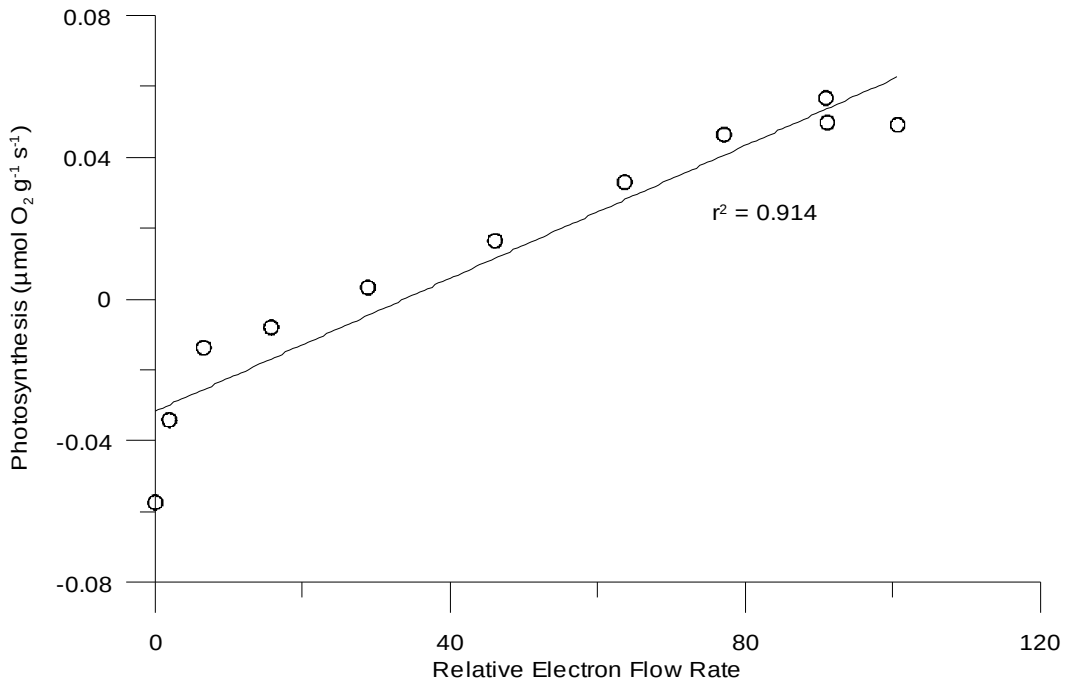


Figure 4.6. Relative Electron Flow Rate vs. Photosynthetic rate measured in *Gelidium pristoides* showing deviation from the linear relationship at low irradiances (Line = Linear regression, r2 = 0.914).

4.3.2. Photosynthetic response to different irradiance levels

The photosynthetic rate of *Gelidium pristoides* increased with an increase in light intensity (Fig. 4.7). A number of photosynthesis-irradiance (PI) models were fitted to the data. The best fit was obtained from the model by Parker (1974), followed by the models of Bannister (1979), Henley (1993) and Jasby & Platt (1976).

The Parker model does not include an "α" term describing the initial slope of the curve with which to calculate I_k , as it uses a "convexity index" (m). The parameters α (initial slope of the curve), P_{max} (Maximum photosynthesis) and R_d (Respiration) are regarded as the most important in describing photosynthetic light response curves, and the parameter I_k can usually be calculated from α and P_{max} (Henley 1993). The Bannister model appeared to underestimate I_k (P_{max} / α = I_k = 5.14 µmol m^{-2} s^{-1}), and as such was considered unsuitable. The Henley model provided a good fit, while providing realistic values for the parameters of the light response curve. It gives an I_k value nearly half of that calculated by the Jasby and Platt model (79.48 vs. 153.93 µmol m^{-2} s^{-1}), while it predicted a P_{max} of 0.1139 µmol O_2 g^{-1} s^{-1} from the data. The Henley model was the one considered as best representing the data, and is the curve fitted to the data presented in Fig.4.7. The I_k value for submerged photosynthesis was 79.48 µmol m^{-2} s^{-1}, while the initial rate of increase in photosynthetic tempo was 0.001 (µmol O_2 g^{-1} s^{-1} / µmol m^{-2} s^{-1}). The maximum photosynthetic rate was 0.114 µmol O_2 g^{-1} s^{-1}, while the alga exhibited a dark respiration rate of 0.046 µmol O_2 g^{-1} s^{-1}.

Table 4.1. PI models fitted against photosynthesis vs. irradiance response data of *Gelidium pristoides* in seawater at 18℃, and the approximate values fo r some of the model parameters (α – Initial slope of PI Curve, P_{max} - Maximum Photosynthetic Rate, R_d – Respiration Rate, I_k, - Light Saturation Parameter).

Author	Equation	α	P_{max}	R_d	I_k	R^2
Parker (1974)	$P = P_{\max}((I/I_s)\exp(1-I/I_s))^m + R_d$	-	0.113	-0.056	-	0.991
Bannister (1979)	$P = P_{\max}((\alpha I)/\sqrt[c]{P^c{}_{\max} + (\alpha I)^c}) + R_d$	0.055	0.282	-0.056	5.14	0.987
Henley (1993)	$P = P_{\max}(\alpha I/(P_{\max} + \alpha I)) + R_d$	0.001	0.114	-0.046	79.48	0.972
Jassby & Platt (1976)	$P = P_{\max}\tanh(\alpha I/P_{\max}) + R_d$	0.001	0.09	-0.039	153.9	0.950

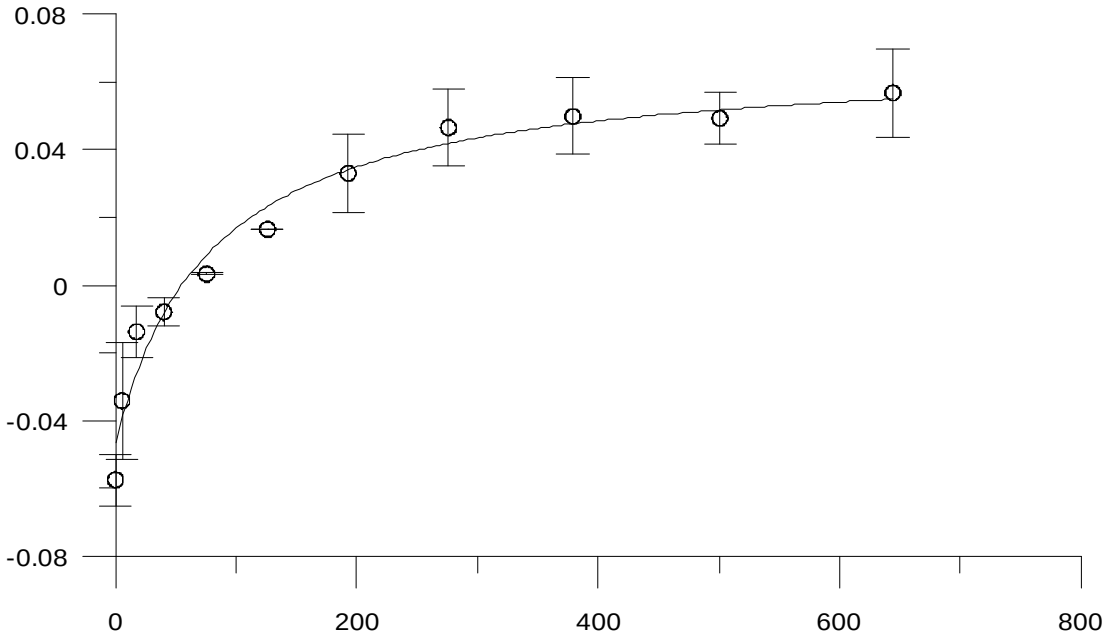


Figure 4.7. Photosynthetic rate of *Gelidium pristoides* at various light intensities measured in seawater at 18℃ (Henley Model (1993) c urve fit r^2 = 0.972, Bar = ±SE, n = 5).

4.3.3. Effect of temperature on photosynthetic rate

Gelidium pristoides showed an initial exponential increase in photosynthetic rate from 0.011 µmol O_2 g^{-1} s^{-1} at 12℃ to 0.02 µmol O_2 g^{-1} s^{-1} at 21℃ (Fig. 4.7) The initial increase can be described rate was described by the following equation:

$P = e^{(0.0643T - 5.379)}$, ... Equation 4.3

where P is photosynthetic rate (µmol O_2 g^{-1} s^{-1}), and T is water temperature (℃); r^2 = 0.909.

The Q_{10} for rate increase was calculated from the following equation:

$$Q_{10} = \frac{P_t + 10}{P_t}$$, ... Equation 4.4

where P_t is the photosynthetic rate at temperature *t*, and P_t+10 is the photosynthetic rate at temperature *t* +10.

The Q_{10} for *Gelidium pristoides* was calculated to be 1.902.

Above 21 ℃ the photosynthetic rate decreased in a linear fashion with a further increase in temperature (P = -0.00123T + 0.046, P is photosynthetic rate (µmol O_2 g^{-1} s^{-1}), and T is water temperature (℃); r^2 = 0.945) to a minimum rate of *ca*. 0.009 µmol O_2 g^{-1} s^{-1}, recorded at 30℃. Unpaired t-test on the data indicated that rates recorded at 21℃ were significantly higher than rates recorded at both higher and lower temperatures (P < 0.05, n = 3), while rates at 12, 15, 18 and 27℃ were similar (p > 0.05, n =3).

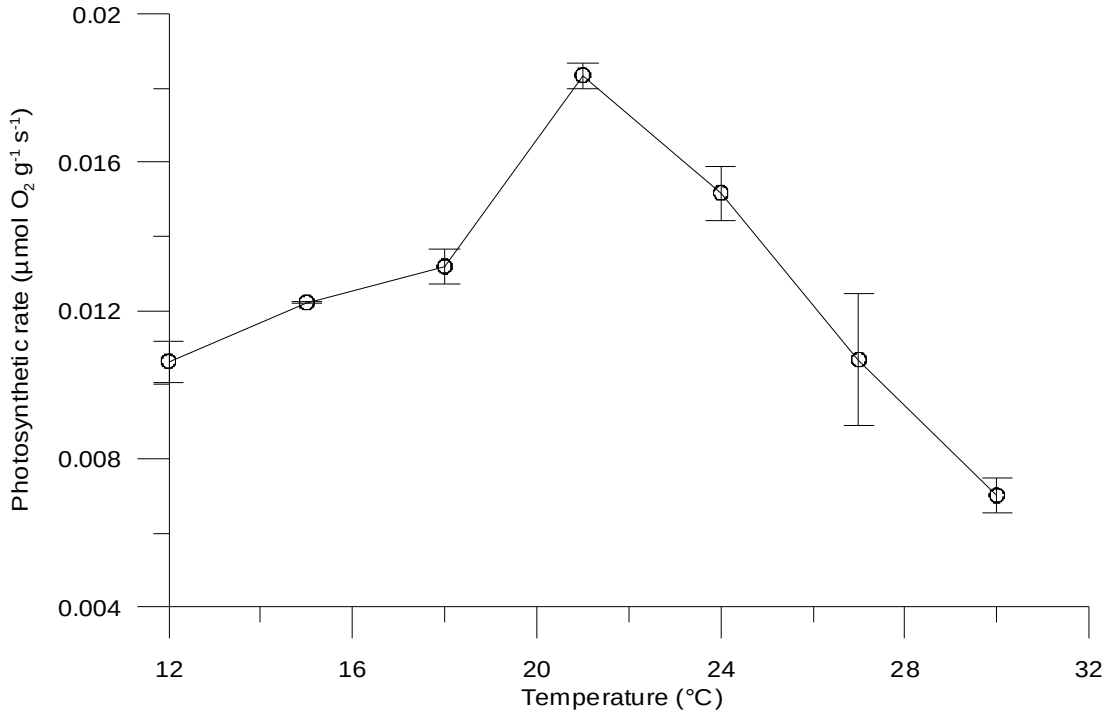


Figure 4.8. Photosynthetic rate of *Gelidium pristoides* at different temperatures measured in seawater, Bar = ±SE.

4.3.4. Effect of salinity on photosynthetic rate

The figure 4.9 shows the results of the salinity experiments and indicates much lower photosynthetic rates than expected across the entire range of salinities tested. The optimum salinity for photosynthesis appears to be between 20 and 40 ‰, with photosynthetic rates of ca. 0.01 µmol O_2 g^{-1} s^{-1}. At salinities above and below this range there is a decrease in photosynthetic rate. At salinities of 0 and 60 ‰ the alga showed net respiration of 0.002 µmol O_2 g^{-1} s^{-1}. Differences rates for salinities between 20 and 50‰ were not statistically significant (P > 0.05, n = 3).

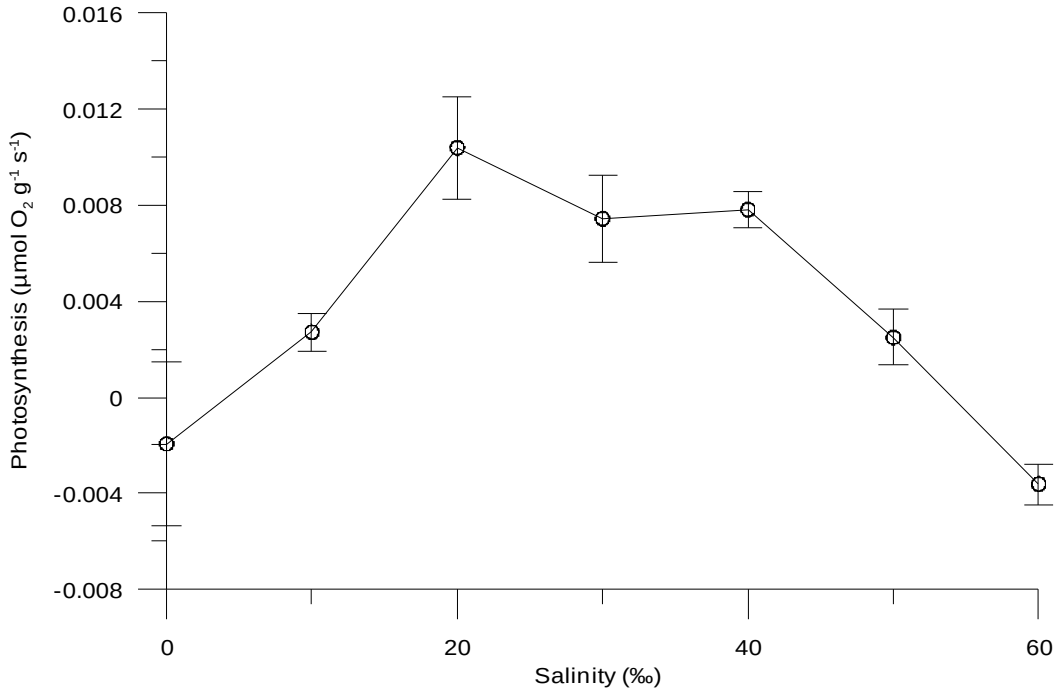


Figure 4.9. The effect of different salinities on the photosynthetic rate of *Gelidium pristoides* at 18℃, Bar = ±SE.

4.3.5. Effect of pH on photosynthetic rate

The lowest photosynthetic rate (0.035 ± 0.004 µmol O_2 g^{-1} s^{-1}) was recorded at the normal pH of seawater (8.4). With a decrease in seawater pH the photosynthetic rate increased to a maximum value at pH 6. With a further decrease in pH there was no change in the photosynthetic rate, which remained at ca. 0.09 µmol O_2 g^{-1} s^{-1} down to a pH of 3. Unpaired t-tests for the data indicate that there was no significant difference between photosynthetic rates at pH 3, 4, 5, and 6 ($P > 0.05$, $n = 6$). Rates at pH 7 and 8 differed significantly from each other and all the other pHs ($P < 0.05$, $n = 6$) (Fig. 4.10).

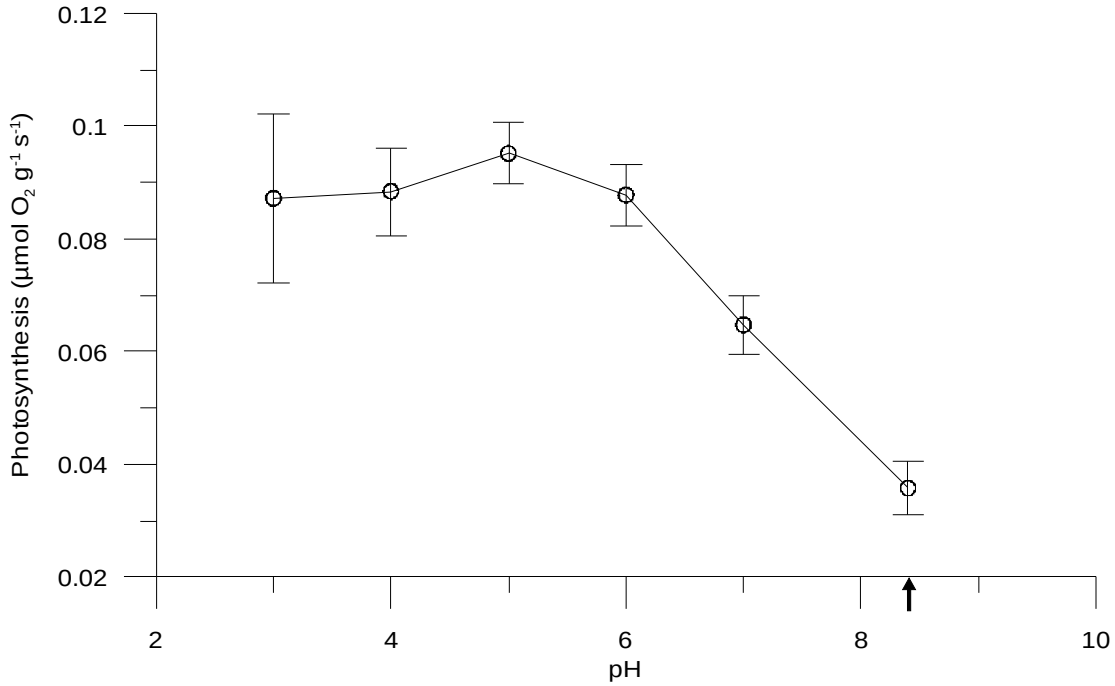


Figure 4.10. The effect of different pH on the photosynthetic rate of *Gelidium pristoides* at 18℃, Bar = SE, Arrow indicates natural seawater pH at 8.4.

4.3.6. Effect of carbonic anhydrase (CA) inhibitors on photosynthetic efficiency

Figures 4.11 and 4.12 show the response of *Gelidium pristoides* to carbonic anhydrase inhibitors.

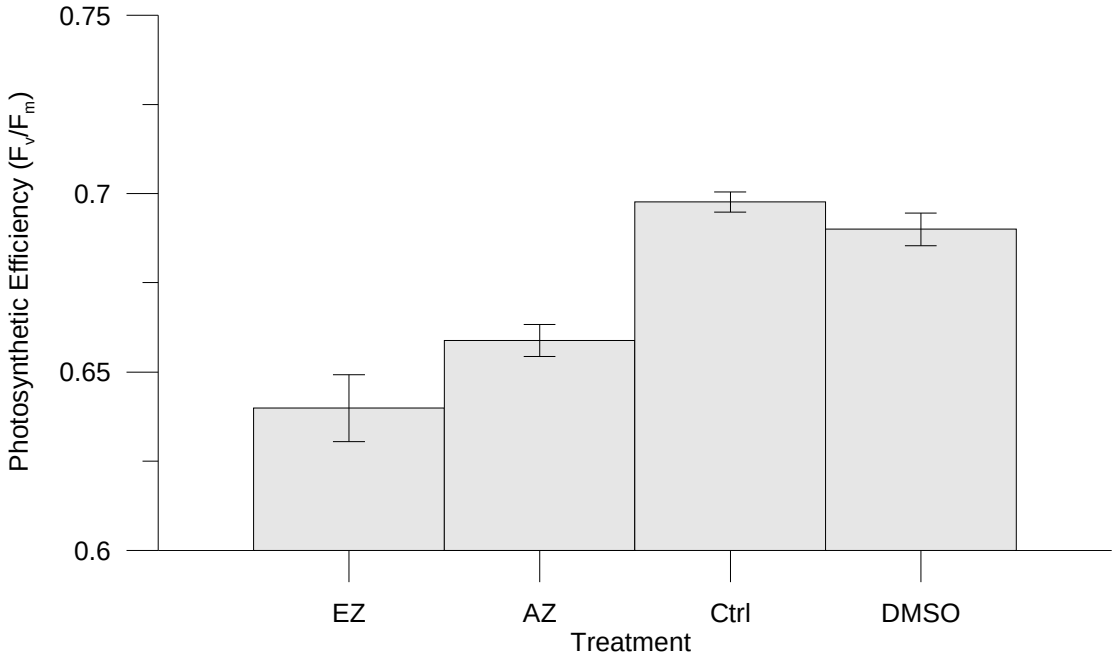


Figure 4.11. The effect of carbonic anhydrase inhibitors on the photosynthetic efficiency of *Gelidium pristoides* (EZ – Ethoxyzolamide, AZ – Acetazolamide, Ctrl – Control, DMSO – Dimethylsulphoxide, Bar = SE).

The DMSO did not have a significant effect on photosynthetic efficiency (n = 12, p = 0.07). The F_v/F_m ratio remained at approximately 0.7 for both the control and the DMSO treatment. Both the CA inhibitors resulted in a reduction in photosynthetic efficiency relative to the control (n = 12, p < 0.001), with F_v/F_m ratios of 0.659 ± 0.004 and 0.640 ± 0.009 for AZ and EZ respectively. The addition of EZ resulted in a significantly lower F_v/F_m in *G. pristoides* (n = 12, p = 0.013).

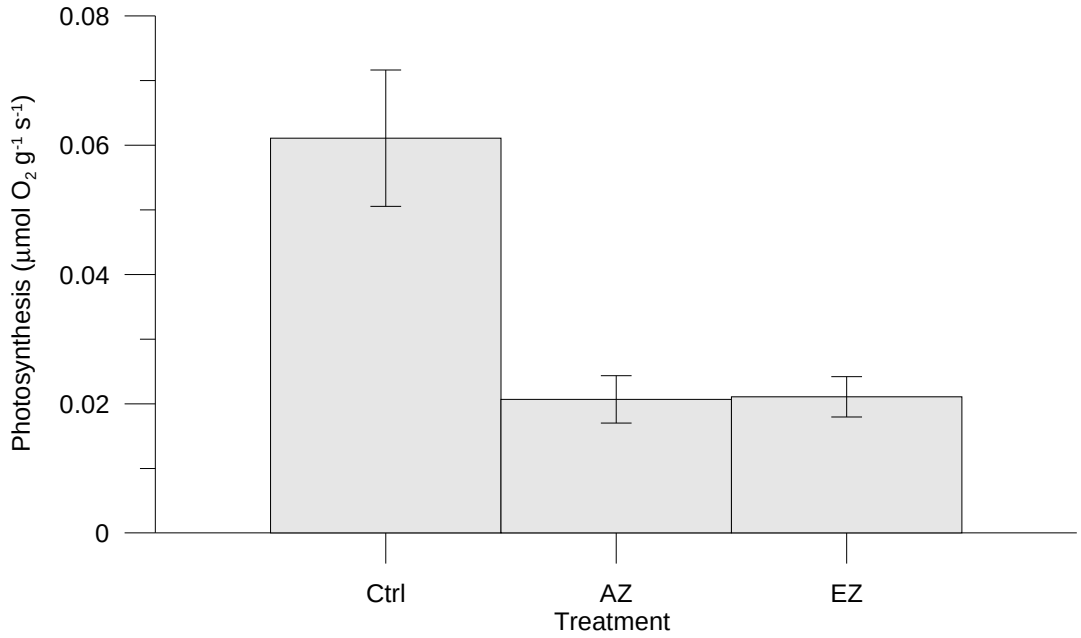


Figure 4.12. The effect of carbonic anhydrase inhibitors on the photosynthetic rate of *Gelidium pristoides* (EZ – Ethoxyzolamide, AZ – Acetazolamide, Ctrl – Control, Bar = SE).

This response was also reflected in the photosynthetic rate of the alga which decreased from 0.06 ± 0.01 µmol O_2 g^{-1} s^{-1} to approximately 0.02 ± 0.003 µmol O_2 g^{-1} s^{-1} with the addition of Acetozolamide as well as Ethoxyzolamide. This represents a decrease of approximately 60% in the photosynthetic rate as a result of inhibiting bicarbonate use in the alga (n = 5, p = 0.01). The photosynthetic rate did not differ for Acetozolamide and Ethoxyzolamide (n = 5, p = 0.9)

4.4. Discussion

Fluorescence methods have been used for the measurement of photosynthetic efficiency in marine macroalgae by a number of authors (Gomez and Figueroa 1998, Silva *et al*. 1998, Magnusson 1997, Cordi *et al*. 1997, Herrmann *et al*. 1995, Hader *et al*. 1998, Casper-Lindley and Bjorkman 1998). The term most often used, photosynthetic efficiency, incorporates variable fluorescence of a dark-adapted sample irradiated by a non-actinic light source, and maximal fluorescence after irradiating the sample with a saturating light source. The term gives an indication of the state of photochemical fluorescence quenching. The use of fluorescence in measuring photosynthetic responses in marine algae with accessory pigments (phycobilins) can be limited and the interpretation of results using these methods confusing, as is outlined by Ting and Owens (1992). While method is useful in examining the photosynthetic physiology of samples, it is based on to photosynthetic electron transport at the chloroplast, and does not account for photorespiration. This appears to be one of the major factors making it inappropriate to directly relate fluorescence data and net photosynthesis data gathered by traditional methods (e.g. oxygen liberation / CO_2 uptake), especially at low irradiances. Genty *et al.* (1989a) used a method whereby fluorescence parameters may be measured and used to describe the relative rate of electron flow (rEFR) during photosynthesis. This method was quick, simple and non-disruptive and appeared to be very closely related to actual photosynthesis.

A comparison of the rEFR, and net photosynthesis measured using an oxygen electrode, indicated that the two methods are not directly comparable. The reason for this is particularly apparent at low light intensities. The rEFR equation takes the quantum yield of photosynthesis into account, and in achieving this incorporates a factor for irradiance. When the photosynthetic response in the dark is calculated, multiplication by an irradiance quantity zero forces the rEFR curve to 0 at this light intensity. While this may be correct in terms of photosynthetic electron transport (which would cease at I = 0), traditional photosynthetic measurements would give an indication of dark respiration. This limitation of rEFR measurement results in potential overestimation of I_k, and gives no indication of the compensation irradiance of the sample. The incomparability of the two methods is most pronounced at low irradiances where the parameters mentioned above are defined; however, at higher irradiances the methods are well correlated. The method may still prove to be very useful in calculating daily productivity estimated in practical applications, e.g. mariculture or agriculture. For physiological work done in this study the method was considered inappropriate, as the experiments were done at irradiances between I_k and I_{Pmax}, which is the irradiance range where rEFR data is not directly related to photosynthesis.

The irradiance response for *G. pristoides* in seawater showed the typical curve, with PI-curve parameters within the range expected for intertidal marine macroalgae of the genus. The curve giving the most acceptable fit was one by Henley (1993). The maximal rate of photosynthesis is considerably higher than most of the other groups, shown in Table 4.2., but is close to the value for *Gelidiella acerosa*. The slope "alpha" for *G. pristoides* is also considerably smaller than the values for other similar species. The slope "alpha" is a measure of light harvesting efficiency and photosynthetic energy conversion efficiency. The variability in the different estimates for "alpha", and the related I_k values, obtained for the same data set (Table 4.1.) using different PI models, make interpretation of this data somewhat difficult. Many PI-curves' data are too variable, or do not include enough data in the light-limiting region making slope estimates unreliable (Henley 1993).

Table 4.2. Photosynthetic parameters for a number of representatives of the Gelidiales.

Species	P_{max} (µmol O_2 g^{-1} s^{-1})	I_k (µmol m^{-2} s^{-1})	Alpha	Source
Gelidium pristoides	**0.114**	**79.48**	**0.001**	**This study**
Gelidium canarienesis	**0.04**	**19.1**	**0.07**	**Mercado *et al*. (2000)**
Gelidium arbuscula	**0.045**	**28.9**	**0.05**	**Mercado *et al*. (2000)**
Pterocladiella capillacea	**0.058**	**77.3**	**0.02**	**Mercado *et al*. (2000)**
***Gelidiella acerosa* (0.6 m)**	**0.031**	**54**	**0.03**	**Dawes & Kovach (1992)**
***Gelidiella acerosa* (5 m)**	**0.06**	**74**	**0.05**	**Dawes & Kovach (1992)**
Gelidiella acerosa	**0.416**	**74**	**0.324**	**Ganzon-Fortez (1997)**
Gelidium sesquipedale	**0.026**	**154.5**	**0.02**	**Carmona *et al*. (1996)**

The I_k for *Gelidium pristoides* (79.48 µmol m^{-2} s^{-1}) was similar to other similar rhodophytes, and is a reflection of many rhodophytes' adaptation to lower light intensities, and growth under water where the red and green wavelengths are unavailable. It was expected that *G. pristoides* might have a higher I_k, since it grows in the intertidal where it is exposed to direct sunlight periodically. However considering that for much of the time it is covered by water in a highly turbulent (foaming) environment, the ability to photosynthesize under lower light conditions may offer a distinct advantage. I_k values ranging from 50 to 400 µmol m^{-2} s^{-1} were found for intertidal algae in work done by Gomez et al. (2004), with the subtidal *Gelidium lingulatum* having an I_k of 335.6 ± 21.2 µmol m^{-2} s^{-1}.

G. pristoides has a temperature optimum for photosynthesis of 21℃. This value agrees well with both environmental and growth data for the seaweed. This has been shown to be the monthly mean seawater temperature for shallow seawater around Port Elizabeth for summer

months (McLachlan *et al*. 1981). *G. pristoides* shows it best growth from spring to summer (October – February), as seawater temperatures approach this optimum (Anderson *et al*. 1991). Further, the maximum abundance of *G. pristoides* in its natural environment is encountered on the Eastern Cape coast between Cape St. Francis and Hamburg west of East London (Jarman *et al*. 1988). Seaweed geographical distribution boundaries are often set by the long-term effect of seasonal extreme temperatures. These temperatures may act by being lethal to the particular species, or may limit growth rates or reproduction (Luning 1990). Mean summer temperatures in this region appear to be close the temperature optimum for *G. pristoides* (Lubke & De Moor 1998).

G. pristoides has a wide salinity tolerance range, and could maintain as similar rate of photosynthesis for salinities from 20 to 40‰. *Gelidium filicinum* and *G. lingulatum* showed similar broad salinity tolerance ranges between 25 and 45‰, while *G. spinulosum* and *G. pusillum* were more stenohaline with salinity optima at 35‰ (Oliger & Santelices 1981). This broad tolerance of short-term salinity change is to be expected in an alga that lives on the intertidal rocky shores. When the alga is exposed, seawater affecting the seaweed may be trapped in a few possible ways. Some water will adhere to the thallus surface; seawater may be retained in spaces between the fronds and the rocky surface; water may be retained in pits or depressions (small pools) on the rock surface on which the alga is growing; or be trapped amongst the fronds in each tuft. During low tides this seawater will be exposed to evaporation and resultant increase in salinity. This effect will become more marked higher up the intertidal zone, as these upper reaches are uncovered for greater periods of time. Measurements in this study did not show this salinity change clearly, since the volumes of this adhesive water are generally very small, and difficult to measure. However it is highly likely that the water associated with the thallus of *G. pristoides* is subject to great fluctuations in salinity on a regular basis, during tidal exposure. *G. pristoides* would have to be able to cope well with salinities, from near fresh (surface runoff and rainfall during low tide exposures) and highly saline (extended exposure may lead to evaporation of all surface water and salt crystallization). The data from this study supports this assumption. Intertidal species often show tolerance of salinities from 0.1 to 3.5 times that of normal seawater (*ca*. 4 – 130 ‰) (Luning 1990). These species often experience increased temperature and salinity stress simultaneously, and both types of stress affect the alga by affecting cellular hydration. Often tolerance of one such "dehydration stressor" also offers the alga good resistance to other similar stressors acting in the same way (i.e. freezing, drying, high salinity) (Davison & Pearson 1996). The salinity tolerance range of *G. pristoides* indicates that the alga may well be cultivated in seawater ranging in salinity from 20 to 40 ‰, but as in most cultivated seaweeds will most probably do best at the salinity of natural seawater (*ca*. 35-37 ‰). If the species were to be cultured the

seaweed grower would not be under pressure to exercise strict salinity control, as the seaweed is flexible in its salinity optimum. However maintaining the alga at a constant salinity would most probably be more economical, since osmotic adjustment required for changing salinities would require an energy investment, which would most likely cause a drop in growth and production (Kirst 1989).

Gelidium pristoides showed a great increase in photosynthetic rate with a drop in pH from 8.2 of natural seawater to a pH of 6. *Ulva* sp. showed exactly the same response in work by Menendez *et al*. (2001), while *Gracilaria* and *Chaetomorpha* from the same study showed the initial increase in rate between pH 9 and 6, with a decrease at lower pH. *Laurencia papillosa*, *Dilophus guineensis*, and *Cladophoropsis membranaceae* showed similar increase in photosynthetic rate with decreasing pH from 8.2 to 6.8. The rate increase was biggest in the *Dilophus* (Phaeophyta), and the lowest in the *Cladophoropsis* (Chlorophyta) (Holbrook *et al*. 1988). *Gracilaria lemaneiformis* also showed a drop in photosynthetic rate with pH increases above 8, but still maintained higher than expected rates indicating some ability to use bicarbonate as an inorganic carbon source (Zou *et al.* 2004).

Between pH 8 and 6, bicarbonate is the dominant form of inorganic carbon (*ca*. 2000 µM), with carbon dioxide representing only *ca*. 10µM. At pH 6 half of the total inorganic carbon in seawater occurs in the form of carbon dioxide (*ca*. 1000 µM) (Lobban *et al*. 1985). With a further decrease in pH the total inorganic carbon decreases but the CO_2 fraction of this becomes even larger (*ca*. 1995 µM) (Zeebe & Wolf-Gladrow 2001). This suggests strongly that *G. pristoides* is predominantly a carbon dioxide user, and is inorganic carbon limited under natural conditions in seawater. Both exclusive and preferential use of carbon dioxide as inorganic carbon source has been documented in marine macroalgae. The carbon source used does not seem related to the particular group to which the alga belongs. Within the genus *Gelidium* it has been found that *Gelidium amansii* is a bicarbonate user while *Gelidium cartilagineum* is a carbon dioxide user. Similarly *Porphyra schizophylla* and *Porphyra endiviifolia* is a carbon dioxide user, while *Porphyra tenera* is a bicarbonate user (Beardall & Roberts 1999, Kremer 1981a). Maberley (1990) found six of the species investigated to be carbon dioxide users and all were rhodophytes. However Beardall (1984) work showed that most of the algae from the group they investigated where chlorophytes. Maberly (1990) argued that the species exhibiting exclusively carbon dioxide use were subtidal and, in such an environment, would seldom experience inorganic carbon limitation. The other algae tested in that study were mostly intertidal rock pool algae, which regularly experience carbon dioxide depletion, and elevated pH, and thus would have to be able to use bicarbonate to survive.

G. pristoides is seldom encountered in rock pools on the intertidal zone, and when they are found in this environment, it is usually only on the fringe of such pools. *Gelidium pristoides* grows in a highly turbulent environment when immersed. Waves washing over, and breaking on the intertidal zone ensures that this water is always well aerated, and diffusion boundary layers do not exist. One of the greatest impediments of depending on carbon dioxide as an inorganic carbon source is its low concentration in seawater, and its low diffusion across the velocity boundary layer. In turbulent environments the high water turnover across the thallus surface, the high aeration rate and the continuous rapid breakdown of the velocity boundary layer means that the usual limitations of using only carbon dioxide do not apply. Further, the alga may be able to benefit greatly during low tidal exposure times, from the increased availability and high concentration (13 µM) of carbon dioxide in the air. The addition of both internal and external CA inhibitors resulted in a drop both in photosynthetic efficiency and rate. This indicates that the seaweed is also reliant on bicarbonate as an inorganic carbon source.

It seems that for *Gelidium pristoides* the optimum conditions for photosynthesis are: i) a light intensity of 81 µmol m^{-2} s^{-1} or above, as no upper photo inhibitory intensity has been determined; and ii) the optimum temperature appears to be in the region of 21℃, while the alga has a broad salinity tolerance range centered on 30 ‰. Maintaining the alga in a medium at a pH of 6 or lower, or increasing the carbon dioxide concentration in the medium to 1000 µM or above would increase photosynthesis by an order of magnitude. These results all reflect short-term physiological responses, and should be applied with caution as long term acclimation responses may also play an important role in the alga's success or productivity.

Chapter 5

Gelidium pristoides photosynthesis in air

5.1. Introduction

Observations in the intertidal zone inhabited by *Gelidium pristoides* (Chapter 3) have indicated that the seaweed may be exposed for prolonged periods during spring low tides, and to a lesser degree during periods between spring tides during the lunar tidal cycle. During neap tides the seaweed is exposed for very short periods. Calculations of the mean duration of exposure have indicated that the alga may be exposed for more than six hours per day on a spring tide, and not at all during a neap tide. However the maximum amount of time algae growing in the zone that *Gelidium pristoides* inhabits may spend continuously inundated, is six days (three days before and three days after neap tide). Earlier work has also indicated that *G. pristoides* is intolerant of extended periods of immersion (Hampson 2000 *pers com*., Aken *et al.* 1993).

It is not well understood how the alga deals with the periods of exposure. Davison & Pearson (1996) indicate that there are a number of negative impact that seaweeds suffer during emersion, however these algae may also benefit from increased availability of inorganic carbon in the form of carbon dioxide, and a stable high light intensity. In exposure tolerant species indirect benefits may also include the inhibition of epiphytic growth (usually thin, delicate filamentous algae which are less tolerant of water loss), and the temporary exclusion of grazers. A number of authors have reported elevated or sustained photosynthetic rates in intertidal seaweeds during periods of exposure (Johnson et al 1974, Beer & Eshel 1983, Dring & Brown 1982, Madsen & Maberly 1990, Surif and Raven 1990).

The abundance of *Gelidium pristoides* on the intertidal zone of Southern African rocky shores suggests that it is well adapted to an intertidal lifestyle and periodic exposure. However no information on the photosynthetic response of *G. pristoides* to exposure periods has been published. The aim of this section was to investigate the effects of exposure on the photosynthetic rate in *G. pristoides*, in order to determine whether the alga benefits from exposure by exhibiting higher photosynthetic rates exposed than those measured in seawater.

5.2. Materials and methods

Measurements of photosynthetic rate were done by monitoring carbon dioxide uptake by using an infrared gas analyzer (IRGA). An open system was used to avoid inhibition of photosynthesis by CO_2 depletion, which was expected to occur in a closed system. Passing air in a closed system through the required H_2O vapor scrubbers would also have resulted in the air passing over the algae being dried out continuously which may lead to increased desiccation rates in the algal samples.

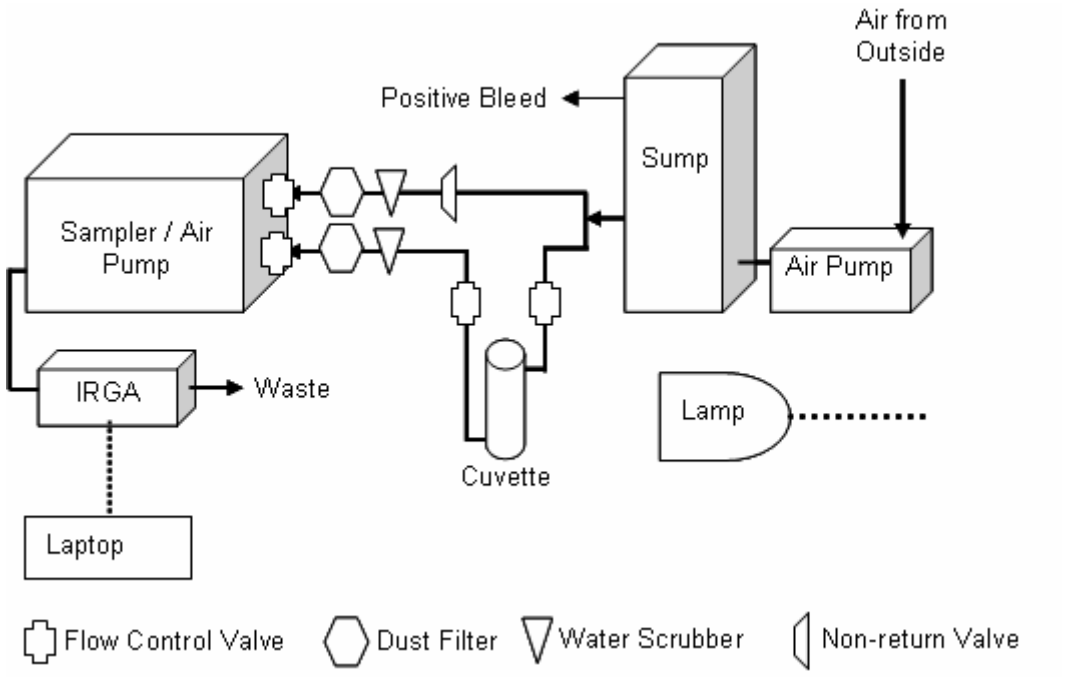


Figure 5.1. Schematic representation of the equipment setup used to measure photosynthetic carbon dioxide uptake in *Gelidium pristoides* in air.

Air from outside was pumped into a mixing sump (*ca*. 25 liters) using an air pump. Some of the air pumped into the sump was allowed to bleed off to release pressure inside the container and avoid pressure build-up down stream. The airflow was then split into two paths, one to the cuvette, and one directly to the IRGA. The air to the cuvette was passed through a flow meter to measure rate of air flow into the cuvette, through the cuvette containing the sample, and then through another flow meter which measured flow coming out of the cuvette. The glass cylinder used as a cuvette was ca. 300 mL in volume. This cuvette size ensured a fast response in the carbon dioxide concentration signal from the sample. Airflow at this point was maintained at just above 0.3 liter per minute. The air from the cuvette was then passed through a H_2O scrubber containing Sodium perchlorate that removed water vapour from the air, and a dust filter before it entered the sampler pump system along its path towards the IRGA. A positive bleed outlet was placed between the cuvette and the H_2O scrubber. This positive pressure in the sample line was maintained to ensure no backflow of air from the cuvette containing the sample would enter the air stream split where ambient CO_2 air was directed off towards the IRGA. Air from the cuvette

was finally passed through the sampler pump system with flow control and to the IRGA where [CO2] was measured. The air stream split off between the mixing sump and the cuvette was aimed at measuring the ambient level of CO_2 of the air from the sump. This air was also directed through a H_2O scrubber and a dust filter before passing into the sampler pump system, and to the IRGA. The IRGA was set up to measure the [CO_2] in one air stream at a time only. The function of the sampler pump system was to sample the different air streams periodically. The sampler switched flow through the IRGA between ambient air and air from the cuvette at regular intervals. The difference in CO_2 concentration between the ambient air and the air flowed through the cuvette was used as a measure of CO_2 uptake by the sample. Airflow through the sampler to the IRGA was set to 0.3 liters per minute. Ambient air was sampled for a minute followed by air from the cuvette for two minutes (i.e. giving a resolution of three minutes per measurement).

Before sampling commenced ambient air was flowed allowed to flow through the cuvette line as well (empty cuvette) the system was considered sealed and accurate when no difference in the CO_2 concentration of air from the ambient line and air from the cuvette line was apparent. Before each experiment the IRGA was allowed to warm up to 26℃ at which point the sample cell temperature stabilized. The IRGA was then zeroed by flowing CO_2 free gas (scrubbed by soda lime) through system, the span was set by flowing calibration gas (352 ppm) through the analyzer. With these parameters set and calibrated experiments could proceed. Carbon dioxide concentration of airflow through the IRGA was logged by a PC connected to the IRGA via a serial port. The IRGA software was set to measure carbon dioxide concentrations every ten seconds. This data was saved directly into a file for later manipulation and analysis. Raw data relating rate of CO_2 uptake were extracted by calculating the difference between the CO_2 concentrations of the ambient and the sample air for each three minute cycle ($\Delta[pCO_2]$). CO_2 concentration data were measured as partial pressure (in Pascal) of CO_2 in the air flowing through the IRGA. This data was converted to µmol CO_2 taken up by the sample per unit time by using the ideal gas law (PV=nRT) modified as follows:

CO_2 Uptake Rate = [(A/101325) x B] / [C x (D+273)]/E x (6×10^7) ..... Equation 5.1.

Where:

CO_2 Uptake Rate - Rate of CO_2 uptake as µmol CO_2 g^{-1} DW h^{-1},

A - $\Delta[CO_2]$ Partial pressure CO_2 (Pa),

B - Flow rate ($L.min^{-1}$),

C - Ideal gas constant (0.0821 L.atm/mol.K),

D - Temperature (℃),
E - Dry weight of sample,
(6×10^7) - to change units from mol to µmol (x 1 000 000) and from min^{-1} to h^{-1} (x 60).

CO_2 uptake calculated from changes in the partial pressure of CO_2 in the air flowing through the cuvette, as explained above, was converted to O_2 evolution data for easier comparison to the photosynthesis data obtained by measuring O_2 evolution in seawater. The conversion calculations were done using a photosynthetic quotient of 1.2 ± 0.1, determined as described in section 5.2.4 below.

5.2.1. Temperature change in the experimental system

Measurements of temperature in the cuvette during measurement of photosynthetic rate indicated that the temperature of air flowing through the cuvette remained stable. The thallus, however, exhibited an increase in temperature of *ca*. 1℃ every 20 minutes at the highest light intensity used (*ca*. 1200 µmol m^{-2} s^{-1}). To avoid temperature artifacts on the photosynthesis data for irradiance and desiccation treatments, rates for individual samples (thallus fragment) were never measured for more than 10 minutes.

5.2.2. Irradiance response

Gelidium pristoides material was collected from the study site on the morning of the experiment and transported to the laboratory in a cool, dark container. At the laboratory the material was sorted and all visible epiphytes and fauna removed from the thallus surface. Photosynthetic rate was measured in small (5 cm / 1 g wet weight) seaweed thallus portions that were removed from tufts of seaweed. The sample size was chosen because five-centimeter portions of seaweed frond could be removed from tufts with minimum damage to the thalli. This sample size was also the most appropriate for the cuvette size used and minimized self-shading in the sample. Samples were stored in seawater, which was aerated well by bubbling air through the water. One thallus fragment was used for each replicate measurement (n = 5) at each of the light intensities tested. Carbon dioxide uptake was measured for no longer than 10 minutes for each sample to avoid temperature increase effects. The temperature of the air flowing through the system was *ca*. 20℃.

5.2.3. Desiccation Response

The photosynthetic response of *G. pristoides* to air-drying was also tested in fresh algal material collected from the rocky shores on the day that the experiments were conducted. Material was sorted; cleaned and stored in a similar fashion as described above, and the same sample size was used. The algal material was removed from the seawater and spread out on sheets of

paper, where it was exposed to air at a temperature of *ca*. 20℃ and a light intensity of *ca*. 100 µmol m^{-2} s^{-1}. A number of samples were weighed and dried immediately to determine the initial moisture content of the material. A sample was removed from the batch of exposed seaweed every 3 – 4 minutes and the CO_2 uptake rate measured. After measurement each sample was weighed and dried in a desiccating oven for determination of the moisture content. Three replicate series of measurements were made. The rates measured as change in partial pressure of CO_2 in the air passing through the cuvette was converted to moles of CO_2 taken up per second, and finally converted to O_2-evolution rates using the photosynthetic quotient of 1.2 ± 0.1 as described in 5.2.4 below.

5.2.4. Photosynthetic Quotient

The photosynthetic quotient of *Gelidium pristoides* was determined in order to compare the photosynthetic rates of the alga under exposed and submerged conditions. Photosynthetic quotient was calculated as follows (Knoop 1988):

PQ = Moles of O_2 evolved / Moles of CO_2 taken up ..... Equation 5.2

The ^{14}C method was used to determine photosynthetic carbon assimilation, with simultaneous measurement of photosynthetic oxygen evolution as described in section 4.2.1.
$NaH^{14}CO_3$ seawater stock solution was prepared by adding a 1 mL ampoule of $NaH^{14}CO_3$ standard solution (9.25 MBq.mL^{-1} or 250 µCi.mL^{-1} & 2.15 MBq.μL^{-1} or 58 µCi.uL^{-1}) to 50 mL of fresh seawater. The $NaH^{14}CO_3$ seawater stock (181.3 kBq.mL^{-1} or 4.9 µCi.mL^{-1}) had a specific activity of 77.7 kBq.µmol^{-1} (2.1 µCi.µmol^{-1}). (MBq.mL-1 = Megabecquerel per milliliter)

During experimentation the stock solution was placed in a measuring cylinder in a water bath at the experimental temperature (21ºC) and bubbled with air in order for the dissolved gas concentration in the $NaH^{14}CO_3$ seawater stock solution to equilibrate with atmospheric CO_2 levels.

A 1 mL aliquot of the $NaH^{14}CO_3$ stock solution was removed before experimentation for the determination of initial radioactivity. This sub-sample was transferred directly from the measuring cylinder to a glass scintillation vial, and 10mL of Filtercount added. The vial sealed immediately for later liquid scintillation counting.

The oxygen electrode was then calibrated as described in section 4.2.1, using the bubbled $NaH^{14}CO_3$ seawater stock solution. For the incubations 3.1 mL of the stock solution was added to the cuvette. The cuvette was then sealed and a stable voltage signal, representing the

oxygen concentration of the seawater stock solution, was awaited (10 – 30 seconds). Once a stable signal had been obtained the cuvette was opened, a seaweed frond inserted and the cuvette sealed for incubation. Similar sized frond portions were used in each experiment (approximately 0.1 g wet weight). The cuvette was provided with illumination (*ca*. 300 µmol m^{-2} s^{-1}) and photosynthetic oxygen production recorded as previously described (section 4.2.1). Once the oxygen concentration in the cuvette had reached double the staring concentration, the incubation was stopped (*ca*. 10 – 15 minutes).

The inorganic carbon uptake during the sample incubation was determined by measuring the liquid scintillation counting.

- A 1 mL sample of the incubation water was transferred to a scintillation vial for the determination of exuded organic carbon. Two drops of 0.1N HCl was added and the vial bubbled in a fume hood for 1 hour to drive off any remaining inorganic carbon. After venting 10 mL of Filtercount was added and the vial sealed for liquid scintillation counting.
- The seaweed frond was removed from the cuvette, rinsed in fresh water, transferred to a covered glass Petri dish and placed in a drying oven at 50ºC.

5.2.4.1 Photosynthetic oxygen production

Oxygen produced during the incubation was determined from the oxygen electrode data. The initial oxygen concentration of the incubation water was determined before the algal sample was introduced to the cuvette. The oxygen concentration of the incubation water was determined just after the seaweed sample had been removed from the cuvette. Oxygen uptake was determined as follows:

O_2 uptake = (Final O_2 Concentration – Initial O_2 Concentration) x 3.1..... Equation 5.3

Were:

Final O_2 Concentration (µmol O_2. mL^{-1}) – Oxygen concentration of the incubation water in the cuvette measured at the end of the incubation

Initial O_2 Concentration (µmol O_2. mL^{-1}) - Oxygen concentration of the incubation water in the cuvette measured at the start of the incubation

3.1 (mL) – volume of water in the cuvette to determine total photosynthetic O_2 production

5.2.4.2 Photosynthetic Carbon Uptake

The dried seaweed samples were wrapped in a “Combusto cone” and placed in a test tube. The test tube was sealed with a rubber bung with two glass tubes inserted which allowed the delivery of venting air to the combusting sample in the test tube, and the collection of exhaust

air from combustion. The test tube was heated with a Bunsen burner until the sample combusted under continual venting. The exhaust air from the test tube was bubbled through 15 mL of Carbosorb, which collected all the carbon dioxide released during combustion. Heating was stopped once the entire sample had been combusted with no more smoke being vented from the test tube and only ash remaining of the sample (ca. 20 minutes). The Carbosorb containing the trapped radiocarbon was divided into three 5 mL aliquots which were placed into glass scintillation vials, 10 mL of Permafluor liquid scintillator added to each and the vials sealed.

The samples were counted in a Packard Liquid Scintillation Counter to 2σ%. Counts per minute (CPM) were converted to DPM (disintegrations per minute) by using the sample channels ratio (CPM_B / CPM_A) and a quench correction curve obtained by counting a quenched standard series with known DPM but varying quantities of quenching agent (Figure 5.2). The following equations were used to determine the counting efficiency for the different channels ratios:

Counting Efficiency (%) = CPM_A / DPM ..... Equation 5.4

Channels Ratio = CPM_B / CPM_A..... Equation 5.5

Where:

CPM_A – the counts per minute obtained in channel A

CPM_B – the counts per minute obtained in channel B

DPM – the disintegrations per minute of the quenched standard as indicated on the vial

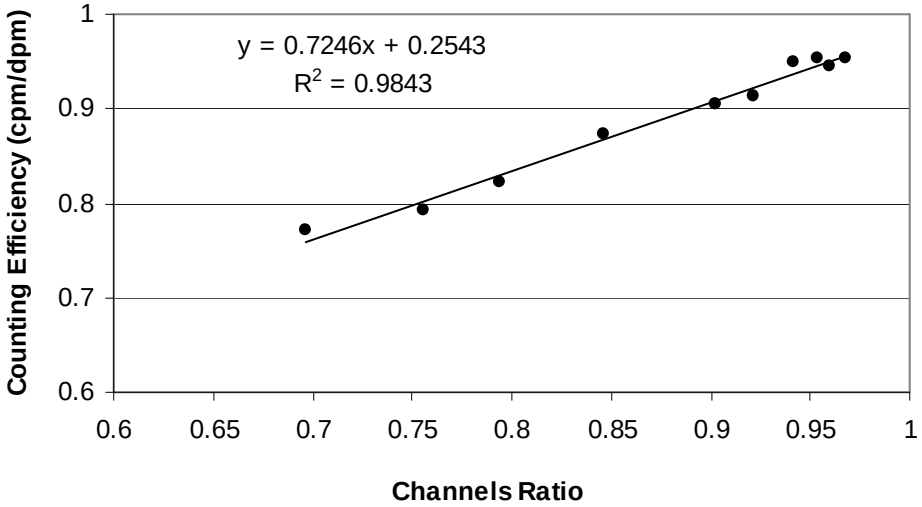


Figure 5.2 Quench correction curve from quenched standard series which was used to determine counting efficiency.

The Inorganic Carbon uptake during the incubation was calculated from the radioactivity (DPM) in the samples as measured by liquid scintillation counting as follows:

Carbon uptake = [($DPM_{seaweed}$ / 2.22 x 10^6) / 58 x 27.1 x 1.06] + [((DPM_{vented} / 2.22 x 10^6)) / 58 x 27.1 x 1.06] ..... Equation 5.6

Where:

$DPM_{seaweed}$ - radioactivity in the seaweed sample

DPM_{vented} - radioactivity in the acidified and vented exudation sample

2.22 x 10^6 DPM. µCi $^{-1}$ – to convert from µCi to DPM

58 *µCi.umol^{-1}* – specific activity of the $NaH^{14}CO_3$ standard

27.1 – to correct for specific activity of $NaH^{14}CO_3$ seawater stock

1.06 – to correct for slower uptake of heavier ^{14}C (Vollenweider *et al.* 1974)

5.2.4.3 DIC and $NaH^{14}CO_3$ Seawater Stock Specific Activity Calculation

The specific activity of the $NaH^{14}CO_3$ Seawater Stock was calculated as follows (Knoop 1988):

Sp Act = Dissolved Inorganic Carbon (DIC) / Labeled DIC ..... Equation 5.7

The DIC in the seawater carrier was determined by acid titration (Skirrow 1965). A 100 mL sample of seawater used to prepare the $NaH^{14}CO_3$ seawater stock was placed in a glass beaker with a pH meter inserted in the solution. The seawater was gently stirred by using a magnetic stirrer while the seawater was titrated with 0.1 N HCl until the inflection point was reached (Figure 5.3).

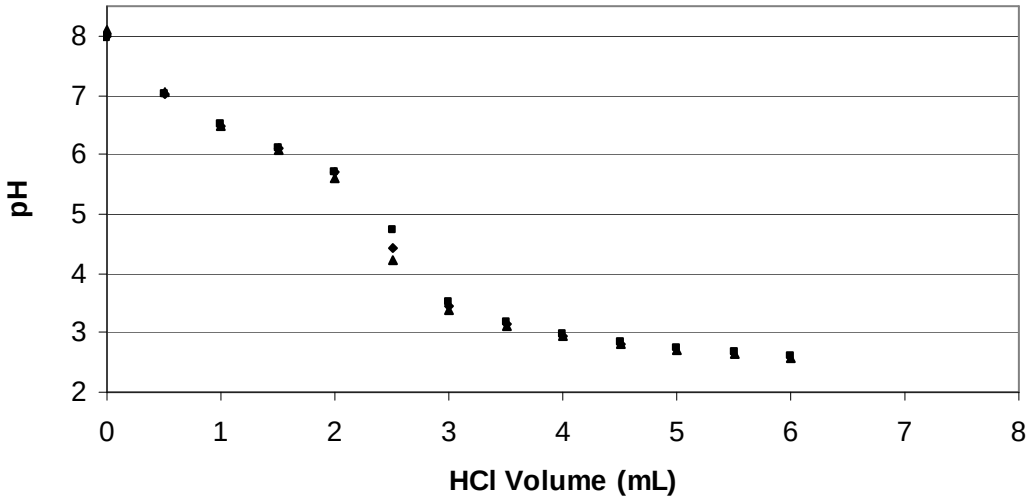


Figure 5.3 Acid titration curve indicating the inflection point at approximately pH4.5 after the addition of 2.25 mL of 0.1N HCl.

The inflection point was reached at approximately pH 4.5 with the addition of 2.25 mL of 0.1 N HCl. The inflection point represents the point at which all the bicarbonate in the seawater has been converted to CO_2 and the amount of HCl added up to the point of inflection is directly proportional to the bicarbonate in the seawater. DIC in the seawater was calculated as follows:

$DIC = V_{acid} \times N / V_{seawater}$ ..... Equation 5.8

Where:

DIC – Dissolved Inorganic Carbon ($\mu mol.mL^{-1}$)

V_{acid} – volume of acid (mL)

N – normality of the acid

$V_{seawater}$ - volume of seawater (L)

5.3. Results

5.3.1. Photosynthetic quotient

The photosynthetic quotient of 1.2 ± 0.1 (n = 5) was found for *Gelidium pristoides*. This number was used to convert photosynthetic rate measurements in air (CO_2 uptake) to O_2 evolution rates in order to allow comparison of exposed and submerged photosynthetic rates for the seaweed. This photosynthetic quotient is similar to the value used by Duarte & Ferreira (1995) in work on *Gelidium sesquipedale*.

5.3.2. Photosynthesis-irradiance response

Photosynthetic rate of *Gelidium pristoides* increased with increasing irradiance under exposed conditions (Fig 5.2). The alga did not show any signs of photoinhibition, even at the highest light intensity tested. The highest light intensity used (1300 µmol m^{-2} s^{-1}) was determined by the capacity of the projector lamp used to illuminate the samples. The same procedure of curve fitting applied to the photosynthesis-irradiance (PI) response data for immersed samples (Chapter 3), was followed for the experiment done on *G. pristoides* photosynthesis in air. Eight PI response models were fitted to the data. The model best fitting the data was that of Bannister (1979), followed by the models of Parker (1974), Henley (1993) and Jassby & Platt (1976).

Table 5.1. PI models fitted against photosynthesis vs. irradiance response data of *Gelidium pristoides* in air at 20℃, and the approximate values for some of the model parameters (α – Initial slope of PI Curve, P_{max} - Maximum Photosynthetic Rate, R_d – Respiration Rate, I_k, - Light Saturation Parameter).

Author	Equation	α	P_{max}	R_d	I_k	r^2
Parker (1974)	$P = P_{\max}((I/I_s)\exp(1-I/I_s))^m + R_d$	-	0.019	-0.005	-	0.987
Bannister (1979)	$P = P_{\max}((\alpha I)/\sqrt[c]{P^c{}_{\max} + (\alpha I)^c}) + R_d$	0.007	0.026	-0.005	3.54	0.988
Henley (1993)	$P = P_{\max}(\alpha I/(P_{\max} + \alpha I)) + R_d$	0.0004	0.018	-0.004	46.85	0.975
Jassby & Platt (1976)	$P = P_{\max}\tanh(\alpha I/P_{\max}) + R_d$	0.0002	0.016	-0.004	76.13	0.914

The Bannister model again appeared to underestimate I_k (P_{max} / α = I_k = 3.54 µmol m^{-2} s^{-1}) and as such was disregarded as a realistic representation of the PI curve. As discussed in Chapter 3, the Parker model was not as useful as the other models, since it does not include the parameters used to calculate I_k. The I_k value is a very useful PI-curve parameter in the context of this research, as it is independent of the photosynthetic units, and can be used to describe the light environment to which the alga is best acclimated (Henley 1993). The Henley model and

the model by Jassby & Platt gave comparable values for P_{max} and I_k, and R_d (Table 4.1). Because the Henley model fit the data better than the Jasby & Platt model, it was considered to best represent the data. This is the curve fitted to the data presented in Fig.5.4. The maximum photosynthetic rate was 0.0179 µmol O_2 g^{-1} s^{-1}, while the respiration rate in the dark was 0.0044 µmol O_2 g^{-1} s^{-1}. The initial rate of increase in photosynthetic tempo was 0.00382 (µmol O_2 g^{-1} s^{-1} / µmol m^{-2} s^{-1}) and the alga had an I_k value of 46.85 µmol m^{-2} s^{-1}).

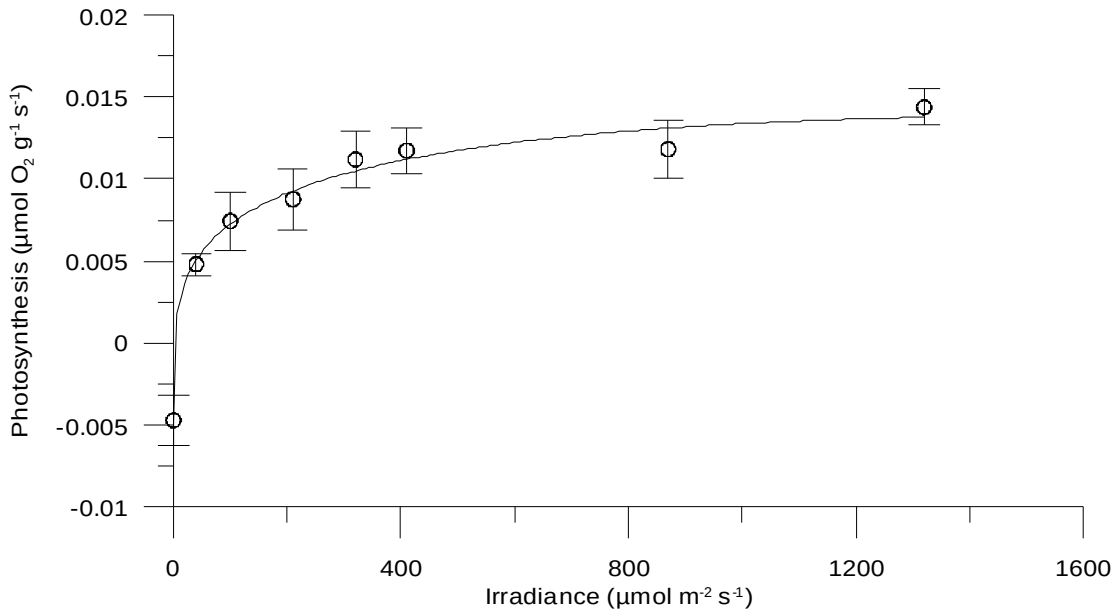


Figure 5.4 Photosynthetic response to different light intensities in *Gelidium pristoides* under exposed conditions measured in the laboratory (Henley Model (1993) curve fit r^2 = 0.975, Bar = SE, n = 4).

5.3.3. Photosynthesis – desiccation response

Moisture content was expressed relative to the initial moisture content of the seaweed (Fig. 5.5.). The initial relative moisture content (100%) corresponds to *ca*. 71.5 ± 0.47 % absolute water content. The moisture content of the seaweed decreased with exposure time in a linear fashion ($Y = -0.527X + 99.302$, r^2 = 0.995). The rate of water loss in this type of experiment was significantly faster (0.5 % min^{-1}) than when the algae are exposed as tufts. Measurements done in the laboratory at 25° (0.13 % min^{-1}, r^2 = 0.977) and in the field at 20℃ (0.2 % min^{-1}, r^2 = 0.932) showed that the rate of water loss was lower than the experimental treatment, in which the tufts were separated into individual fronds.

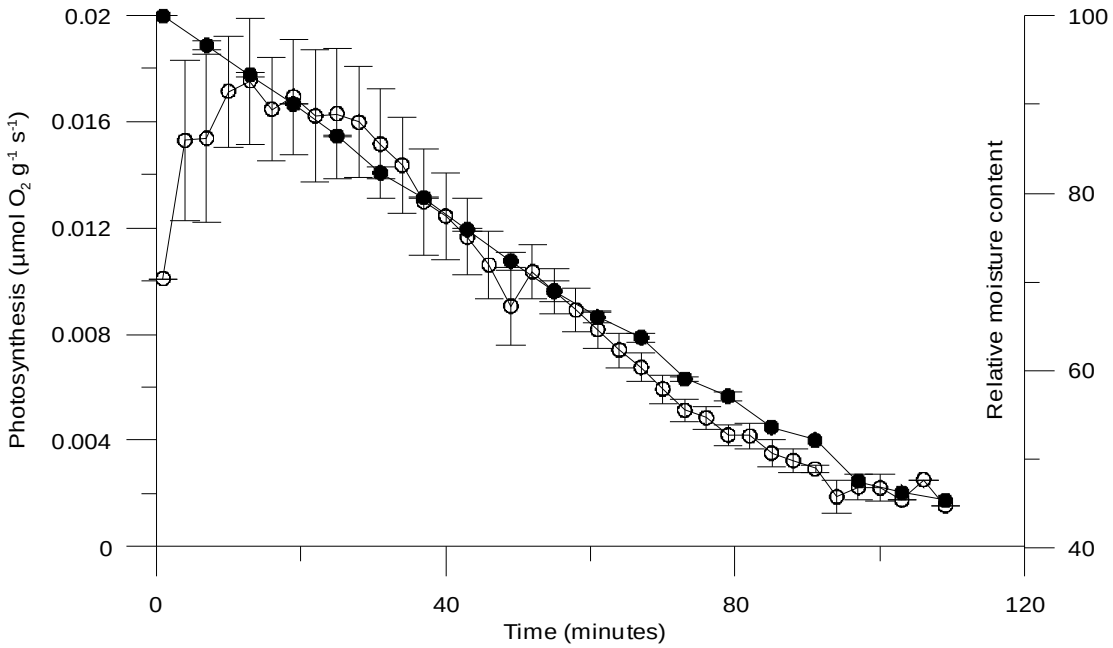


Figure 5.5. Photosynthetic response (Open Circle) and relative moisture (Solid Circle) content of *Gelidium pristoides* exposed for different periods in the laboratory, Bar = ±SE.

Photosynthetic rate did not show the same linear response to exposure time, but rather increased in the first 19 minutes of exposure, reaching a maximum of 0.0169 ± 0.002 µmol O_2 g^{-1} s^{-1} .Thereafter there was an almost linear decrease in photosynthetic rate to a minimum of 0.0015 ± 0.001 µmol O_2 g^{-1} s^{-1}. One-way ANOVA indicated that the means of photosynthetic rate for the different exposure times were significantly different ($F = 9.218$, $P < 0.0001$).

5.4. Discussion

The photosynthesis-irradiance (PI) response curve for *Gelidium pristoides* in air (Fig. 5.2) is similar to the data for submerged samples. P_{max} is considerably lower, but this is most likely due to variability between sampling times. The I_k value is comparable to the light saturation parameter for submerged samples. The value falls within the range for other members of the Gelidiales (Table 3.5), however no data on emersed photosynthetic rates for the group were available. Investigation on the submerged and immersed PI response for *Fucus spiralis* also showed very similar curve shapes, with a slightly higher P_{max} and I_k for photosynthesis in water than in air (Kawamitsu & Boyer 1999). This is similar to the findings in the experiments with *G. pristoides*.

These two intertidal species both grow as tufts in which individual fronds cover each other wholly or partially when the tide is out. This causes a degree of self-shading which may serve to lower desiccation rates, however dense growth forms do serve to limit water loss (Norton et al. 1982). During exposure periods, fronds inside the tuft may still be able to photosynthesize at the lower light intensities available inside the tuft, while the outside fronds had already shut down photosynthesis due to desiccation stress. This may be the reason an alga growing in full sunlight often appears to have the I_k value indicating efficient use of low light intensities (Henley 1993). Elevated photosynthetic rates in emersed samples, photosynthesizing in air vs. immersed samples, where photosynthesis was measured in seawater, have been documented in a number of species. *Pelvetia canaliculata*, *Fucus spiralis*, *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Fucus serratus* have all been shown to have greater photosynthetic rates when exposed than when immersed. These were all intertidal species, and the difference between emersed and submerged rates appeared to be related to the position on the intertidal zone. *Pelvetia*, which grew highest up on the intertidal, showed a large increase in photosynthetic rate when exposed, while *Fucus serratus*, which occurs on the lower intertidal only showed a small increase in rate. Subtidal, or rock pool species showed a reduction in rate when exposed (Surif & Raven 1990). Similar earlier work by Johnson *et al*. (1974) showed similar trends in photosynthesis between exposed and submerged samples. The intertidal species *Endocladia muricatra*, *Fucus distichus*, *Porphyra perforata* and *Iridaea flaccida* had varying increases in photosynthetic rate when exposed (Johnson *et al*. 1974).

During exposure, seaweeds are subject to water loss as a result of evaporation. In a number of seaweed species it has been shown that short periods of partial water loss actually stimulate photosynthetic rate, or at least leave rates unaffected for up to an hour. *Ulva* sp. has been shown to maintain a steady rate of photosynthesis while exposed and desiccating, down to a relative moisture content of about 0.6 (60%) of the initial water content (Beer & Eshel 1983).

In *Gelidium pristoides* there is an initial increase in photosynthetic rate when exposed. This trend is similar to the findings of Ji & Tanaka (2002), who tested the effect of desiccation on the net photosynthetic rate of twelve macroalgal species. *Porphyra yezoensis*, *Gloiopeltis furcata*, *Ishige okamurae*, *Myelophycus simplex*, *Chondracantus intermedius*, *Pterocladia capillaceae*, *Sargassum thunbergii*, *Chondrus occelatus* and *Hizikia fusiformis* all showed varying degrees of increase in photosynthetic rate with desiccation. Most of these species showed an increase in net photosynthesis 110 – 180% of the initial rate for the initial fully hydrated algae. This increase was generally maintained up to relative moisture content of *ca*. 60%. With further dehydration the photosynthetic rates dropped to below the initial rate (Ji & Tanaka 2002). *Gelidium pristoides* exhibited a net photosynthetic rate of 0.01 at 100 % relative moisture content. With a loss of water there was an increase in photosynthesis to ca. 0.018 after 20 minutes exposure time and a relative moisture content of 90%. The rate dropped to the initial rate of 0.01 again after 1 hour, at a relative moisture content of ca. 65%. A similar response was obtained by Ji & Tanaka (2002) working on intertidal algal species. With a moisture content of 65% in the fronds inside a tuft of *G. pristoides* two hours after exposure, these inside frond could continue photosynthesizing. This indicates that the algae would still be able to maintain a net positive rate of photosynthesis in the fronds on the inside of the tuft, while those on the outside are too dry to photosynthesize (moisture content *ca*. 45%). Zou and Gao (2002) showed that in the intertidal seaweed *Porphyra haitanensis* emersed photosynthesis was not saturated even under atmospheric carbon dioxide concentrations.

The tuft like growth form which shades and reduces water loss, coupled with a low I_k value and a high affinity for carbon dioxide as an inorganic carbon source, could be a good strategy for *G. pristoides* to grow on the intertidal zone. While the algae are partially dehydrated, and subject to shaded, lower light intensities, they would also be able to take advantage of the elevated carbon dioxide concentrations in the air.

Chapter 6
General Discussion and Conclusions

The distribution of *Gelidium pristoides* at the Algoa Bay sites investigated in this study was similar to findings from earlier studies on the Southern African Rocky shores (Carter & Anderson 1991, Anderson et al. 1991, Beckley 1981). The seaweed occurred between 0.2 and 1.2 m above spring low tide water level. Data presented in this study has shown that *G. pristoides* was more common at turbulent wave exposed sites sampled than at sheltered sites, where it was found at low abundance as a turf form. Toefy *et al*. (2003) also reported larger thallus sizes for *G. pristoides* from exposed sites than at sheltered sites. A preference for turbulent rocky shore habitat is also reflected in the unsuccessful earlier attempts at cultivating the alga in tank based and open water mariculture systems (Hampson *pers com*. 2000, Aken et al. 1993). The mariculture systems employed in these studies did not simulate the high energy and periodic exposure of rocky shores.

One of the key questions with regards to *Gelidium pristoides* was why it should persist under these apparently stressful conditions of high energy wave action and periodic exposure, but perish under the presumably favorable conditions presented by sheltered rocky shore sites and mariculture systems. The answer appears to be related to the availability of inorganic carbon.

Data from this study shows that when immersed in seawater, *Gelidium pristoides* uses mainly bicarbonate (HCO_3^-) as inorganic carbon source, and when bicarbonate use is suppressed there is a marked reduction (ca. 60%) in its photosynthetic rate. The capacity to use bicarbonate as an inorganic carbon source is important under immersed conditions where the dominant inorganic carbon form is bicarbonate, at a concentration of approximately 2000 µM, while CO_2 is only available at approximately 10 µM (Kremer 1981a). pH drift experiments indicate that *Gelidium pristoides* has a high affinity for CO_2 which it also uses as an inorganic carbon source. The alga shows a doubling in the photosynthetic rate when CO_2 availability in incubation water in increased by lowering the pH from 8.2 to pH 6.

However CO_2 is considered limiting in normal seawater where it represents less than 10% (10 µM) of the total inorganic carbon. In addition to the low concentrations of CO_2 in seawater, a factor further limiting CO_2 use is the slow diffusion of CO_2 in seawater (2.0×10^{-5} cm^2 s^{-1}), which exacerbates the problem of diffusion boundary layers. While calm sheltered conditions may result in the formation a diffusion boundary layer impeding CO_2 uptake during immersed conditions, turbulence and wave action are known to break down boundary layers, facilitating diffusion of CO_2 across the thallus surface (Norton *et al.* 1982, Dring 1982, Hurd 2000). Increased turbulence and mixing would also improve gas exchange (CO_2) at the water surface and further increase CO_2 availability in the seawater (Boettcher *et al.* 2000). This suggests that CO_2 limitation could be more severe at sheltered sites than at wave exposed or turbulent sites.

The affinity of *G. pristoides* for CO_2 and potential CO_2 limitation at sheltered sites explains, at least in part, the lower abundance of *Gelidium pristoides* at sheltered sites on the rocky shores, and the poor performance in the calm conditions in mariculture systems.

The high CO_2 affinity in *Gelidium pristoides* also allows the alga to take advantage of the increased availability of CO_2 during exposure periods. CO_2 occurs at higher concentrations (ca. 15 µM) and has a higher diffusion rate in air (0.16 cm^2 s^{-1}). The seaweed has been shown to maintain a net positive photosynthetic rate for up to 1.5 hours after exposure, with an increased rate during the initial 40 minutes after exposure. Such sustained or elevated photosynthetic rates during periods of exposure have been shown for a variety of seaweeds (Ji & Tanaka 2002, Surif & Raven 1990, Johnson *et al*. 1974, Beer & Eshel 1983).

Gelidium pristoides has been shown to have broad temperature and salinity tolerance range, and a low I_k value. This should make the seaweed suitable for cultivation in a range of systems including sub-tidal areas. However Carter & Anderson (1991) have shown that the *G. pristoides* transplanted to the infra-tidal zone, below its natural distribution range, is prone to overgrowth by epiphytic corallines. This is supported by the inverse relationship between the abundance of *G. pristoides* and the abundance of coralline algae founding the present study. Furthermore, Aken *et al.* (1993) reported a general deterioration in the algae including biomass loss, discoloration and drop in agar content for *G. pristoides* transplanted to a subtidal open-water mariculture system.

The findings of this study indicate that the seaweed derives specific advantage from existing in highly turbulent and periodically exposed habitat. This environment provides the alga with elevated levels of CO_2 for which the alga has a high affinity and is a major inorganic carbon source for the seaweed. Periodic exposure also provides periods of drying which may limit the potential for colonization by epiphytes, including the coralline algae reported by Carter & Anderson (1991). This is supported by the prevalence of encrusting coralline algae to infra- and subtidal habitats, and rock pools of varying size, rather than emergent surfaces.

In the absence of these conditions the alga will not persist or grow, which is the main reason for the failure of previous attempts at culturing the seaweed. A mariculture system which can simulate the turbulence of wave action, as well as periodic tidal exposure may prove to be suitable for the cultivation of the seaweed; however the practicality and cost effectiveness of such a system would need to be considered. Once an appropriate culture system for the seaweed is available and the seaweed can be maintained in culture for extended periods,

further work on nutrient requirements, growth rates and stocking densities can be conducted and a cultivation protocol for the alga refined.

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