UNIVERSITY OF ALGARVE

"EFFECTS OF HIGH CO_2 ON GROWTH, PHOTOSYNTHESIS AND RESPIRATION OF *PHYMATOLITHON CALCAREUM*"

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Dissertation for the degree of Master of Marine Biology

Work performed under the orientation of Doctor João Miguel Silva and Prof. Doctor Rui Orlando Pimenta Santos

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"Effects of high CO_2 on growth, photosynthesis and respiration of *Phymatolithon calcareum*"

Mestrado em Biologia Marinha

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DEDICATION

I would like to dedicate my thesis to my parents, Svetlana and Yuriy Shulika, without whom none of this would have been possible and my younger brother, Alexander Shulika, who in his 24 reached much more in his life than me. I hope now you also have a reason to be proud of me.

Contents

Abstract

Mäerl is a collective term for different species of non-jointed coralline red algae. *Phymatolithon calcareum* is a mäerl species which is widely distributed in Europe, from Norway to the Mediterranean including Portugal. In Portugal it is mostly present in the south along the coast of Algarve. This species forms highly productive marine benthic systems and is considered a priority species, protected by the European Directive 92/443/EEC - Annex V (Habitats directive).

The increase of atmospheric CO_2 concentration is currently leading to ocean acidification. This phenomenon is particularly worrisome for marine calcifying organism that form biogenic calcium carbonate deposits. For the present study *P. calcareum* was chosen as an example of calcifying species. The effect of high CO_2 on the growth, photosynthesis and respiration of *P. calcareum* was conducted as part of a long-term mesocosm experiment.

During a campaign aboard the "Creoula", a search for mäerl habitats along the coast of Algarve was conducted, as a preliminary objective of this study. Three new spots were detected.

The buoyant weight technique was chosen as a main method to measure growth rate. Weight increments decreased with CO_2 and had a negative correlation with time. However, temperature did not had a significant effect on weight increments, neither on its own nor when combined with pCO_2. At the same time, absolute growth rates were continually increasing with time and showed significant positive correlation.

To investigate the effect of elevated CO_2 on the photosynthesis and respiration rates of *P. calcareum*, light – response curves were determined in an oxygen electrode. Photosynthesis-irradiance (P-I) response curves were built up using Smiths' (1936) mathematical model. Photosynthesis was positively affected by elevated pCO_2, while respiration increased with temperature but not with CO_2.

Key words: Calcification, Calcareous algae, Carbon metabolism, CO_2 increase, *Phymatolithon calcareum*, thallus, growth rate, photosynthesis-irradiance (P-I) response curves, maximum photosynthetic rate (P_{max}), saturation irradiance (I_K), respiration.

Institutions and facilities: All experiments were conducted in the Marine Plant Ecology Group (ALGAE) laboratory and mesocosm facility at CCMAR.

1. Introduction

Justification of the theme:

Mäerl is a collective term for different species of non-jointed coralline red algae. These species are able to form wide-ranging beds, usually in coarse clean sediments of gravels, clean sand and muddy mixed sediment. Since mäerl uses light to photosynthesize, the depth of live beds is limited by water turbidity, therefore they can be found up to 40 m (OSPAR commission, 2010). Mäerl usually occurs in a wide range of temperature regimes, from the north of Norway to the tropical region, nevertheless the species composition of the mäerl beds depends very much on the temperature (Wilson *et al.*, 2004).

Mäerl beds are highly productive marine benthic system and one of the main benthic calcium carbonate sinks (Bensoussan & Gattuso, 2007). Fragments of coralline algae generally are the main source of carbonate sediment for beaches all around the world (Potin *et al.*, 1990, Russell & Johnson, 2000). During millions of years mäerl beds have created carbonate-reach gravel deposit where high benthic biodiversity and productivity can be found (Hall-Spencer *et al.*, 2008). These communities form heterogeneous habitats that are also known as biodiversity hotspots (Nelson, 2009).

Coralline red algae are widely distributed across the world's oceans (Fig. 1.1, a). *Phymatolithon calcareum* is a species of mäerl which is distributed in Europe, from Norway to the Mediterranean (Irvine & Chamberlain, 1994) including Portugal (Fig. 1.1, b), forming extremely important habitats, but still remain somewhat overlooked as benthic marine calcifiers (Foster, 2001). Despite their importance to the carbonate precipitation in the temperate zones, there is still not enough data on mäerl community carbon metabolism, net production, respiration and calcification (Martin *et al.*, 2006). Many aspects of their responses to environmental changes like increasing acidification also remain poorly studied (Wilson *et al.*, 2004).

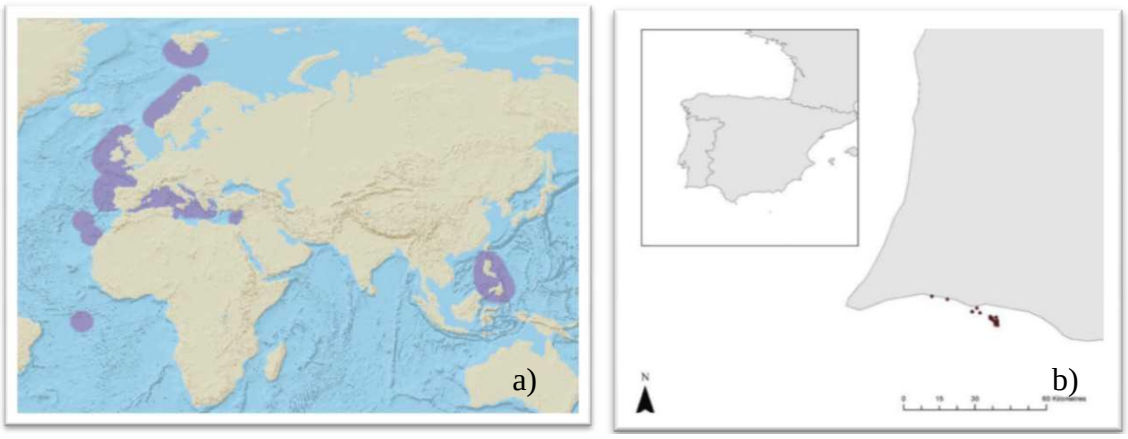

Figure 1.1. a) Mäerl distribution in the world's ocean (Marine animal encyclopedia http://oceana.org); b) distribution of mäerl habitats on southern (Algarve) coast of Portugal from data supplied by Estibaliz Berecibar and Rui Santos (inset, map of the Iberian Peninsula) (OSPAR commission, 2010).

The thalli of these calcified algae are made of a thin crust that consists of inter-connected cell filaments. They grow by division of the filaments and by creating a new cell at the end of each filament (Fig. 1.2, A-C) (Johansen, 1981; Cabioch, 1988; Woelkerling, 1988 for general reference to morphogenesis and anatomy).

The cell walls of the coralline thalli are completely calcified. There are only two exceptions, the reproductive cells and the superficial wall of the outermost epithelial cells in contact with seawater (Fig. 1.2, B) (Basso, 2012).

The precipitation of calcite in living cells is possible because of two aspects. The first is the deposition of the "primary layer" of elongated crystals of magnesium-calcite oriented parallel to the wall surface, following the cell wall in a polysaccharide matrix. The second aspect is the deposition of the "secondary layer" of elongated crystals with an orientation perpendicular to the cell surface (Borowitzka *et al.,* 1974; Flajs, 1977; Cabioch & Giraud, 1986) (Fig. 1.2, B).

The cell walls of the mäerl algae are made of Mg-calcite. The amount of Mg in the calcite depends directly on the temperature and increases with it (Chave, 1954; Basso, 1992; Halfar *et al.*, 2000; Stanley *et al.*, 2002; Basso *et al.,* 2006; Kamenos *et al.,* 2008, 2009). While the Mg of the thallus increases with temperature and calcite saturation (Agegian, 1985), the temperature is negatively correlated to the calcite density of thin-walled cells, with annual or subannual banding patterns (Cabioch, 1966; Adey & McKibbin, 1970; Giraud & Cabioch, 1979; Freiwald

& Henrich, 1994; Basso, 1994, 1995; Halfar *et al.*, 2000; Blake & Maggs, 2003; Rivera *et al.*, 2004; Basso *et al.*, 2006; Kamenos & Law, 2010).

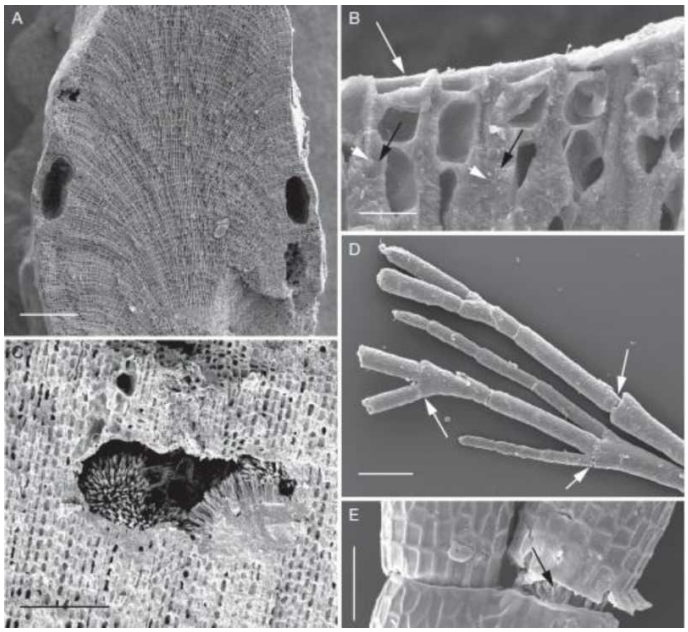

Figure 1.2. SEM photographs of the calcified thallus of coralline algae: **A**, calcified cell filaments in a protuberance of a non-geniculate plant. The empty conceptacle chambers on the border contained the non-calcified spores; **B**, a coralline growing margin showing the outermost non-calcified epithallial cell walls with the primary (white arrowhead) and secondary (black arrow) Mg-calcite layers; **C**, chemical precipitation of carbonate druse inside an empty conceptacle chamber; **D**, a geniculate coralline alga with the articles (intergenicula) connected by flexible joints (genicula) made of non-calcified filaments (arrows); **E**, a close-up of a geniculum showing the non-calcified filaments (arrow). Scale bars: A, D, 250 µm; B, 10 µm; C, 100 µm; E, 50µm (Basso, 2012).

The most common mäerl species are very sensitive to substrate loss, smothering, increase in suspended sediment, abrasion and physical disturbances that can reduce the light reaching the living mäerl and therefore preventing photosynthesis (Jones *et al.*, 2000). There is a wide variety of marine organisms that live amongst or attached to mäerl, or even live inside the coarse gravel or fossil maërl beneath the top living layer, therefore making mäerl beds very important for the biodiversity in the marine environment (Grall & Glemarec, 1997). Even dead mäerl play an important role in marine ecology, although they are not as rich as the living mäerl, they are still able to support diverse communities (Keegan, 1974). Both (dead and live) mäerl deposits are also an important source of subtidal and beach-forming calcareous sediment (Farrow *et al.*, 1978).

Mäerl habitats can be very important nurseries for molluscs and crustaceans. This fact is known at least for a few species such as the black sea urchin *Paracentrotus lividus* and some species of scallops in mäerl deposits in Ireland, France and the western part of Scotland. (Thouzeau, 1991; Keegan, 1974; Birkett *et al.,* 1998).

Besides the direct effect of the physical removal of the mäerl by extraction, there are more direct and indirect impacts that cause extinction of the mäerl species, such as: muddy plumes, exorbitant sediment load, heavy demersal fishing gear, from pollution by finfish and shellfish aquaculture operations in inshore waters (OSPAR commission, 2010).

Human activities such as coastal constructions, active agriculture and sewage discharges may also have an effect on mäerl beds if they increase sediment loads or if ephemeral species of macroalgae will have an excessive growth around mäerl beds (Birkett *et al.,* 1998; De Grave *et al.,* 2000). As mäerl beds have high level of productivity they have been commercially explicated as agricultural fertilizer and organic soil conditioner, especially in Europe (Nelson, 2009). For example, in France more than 450.000 tons of mäerl are dredged every year for commercial use (Potin *et al,* 1990). By reason of slow growth rate, mäerl is considered to be a non-renewable resourse (Hall-Spencer, 1998; Barbera *et al*., 2003).

Because of the increasing CO_2 concentration in the atmosphere, red calcifying algae became under severe threat (Kleypas *et al.,* 2006). With further increasing of atmospheric CO_2 concentration and as a result of increasing ocean acidification it will be more and more complicated for marine calcifying organism to form biogenic calcium carbonate (Kleypas *et al.,* 2006). This effect has been scarcely studied for mäerl species yet. Previous studies related with impacts of ocean acidification were carried out mainly on calcifying marine organisms such as corals, foraminifera and coccolithophores (Kleypas *et al.,* 2006). One of the studies that was done in Hawaii showed that calcification and recruitment rates are declining at lower carbonate saturation state (Kuffer *et al.,* 2008), but still not many studies were done connected to red calcifying algae.

In terms of high CO_2 concentrations in the ocean, some studies were carried out to investigate the effect on marine organisms composition. For example, dramatic shifts in the nearshore benthic community were quantified in the area nearby the natural subsurface volcanic CO_2 vents. In the region near these vents with a high CO_2 concentration and low-pH, scleretinian corals were absent and sea urchins, coralline algae and gastropods were found to be very rare. This vent area was dominated by seagrasses and mostly non-native, invasive species (Hall-

Spencer *et al.*, 2008).

Decreasing pH in the ocean will have a major effect on calcifying algae in terms of calcium carbonate deposition and in the photosynthetic process. Unfortunately, the relationship between photosynthesis and calcification is poorly understood, but this information needs to be found out for us to understand the present threatening situation and to be able to predict future changes. Coralline algae can show different effects from decreases calcification, for instance a) decreasing the ability to compete with non-calcifying organisms (Kuffer *et al*., 2008); b) a decrease in the age of sexual maturity; c) changes in buoyancy; and d) changes to light behavior in the water column (Tyrrell *et al*., 1999).

Ocean acidification is thought to decrease calcium carbonate ($CaCO_3$) saturation states and increase dissolution rates. There is a possibility that the ocean capacity to take up CO_2 from the atmosphere will increase (Doney *et al.,* 2009) as calcium carbonate dissolution consumes CO_2. Acidification does affect a wide range of marine organisms, particularly those who incorporate calcium carbonate in their tissues such as planktonic coccolithophores, pteropods, mollusks, echinoderms, corals and coralline algae (Doney *et al.,* 2009).

P. calcareum is considered a priority species, protected by the European Directive 92/443/EEC - Annex V (Habitats directive). There are some other types of mäerl habitats that are under the protection of the European Nature Information System (EUNIS) at the European Environment Agency (EEA). It is hard to say how ecologically sensitive the mäerl species are, since there has not been many studies done due to the difficulties of working with slow-growing calcified algae (Birkett *et al*., 1998).

Some studies about mäerl population have already started in southern Portugal as part of the research project "Conservation status of the mäerl communities in the Atlantic coast of Iberian Peninsula". CGL2006-03576/BOS, "Ministerio de Educación y Ciencia", Spain. Mäerl communities are highly distributed along the Algarve's open coast, at depths between 15 and 20 meters. In previous research strong seasonal patterns in the photosynthetic activity of rhodoliths was found, so it was expected that such seasonality might be reflected at the same time by the community carbon metabolism and calcification rates. Such hypothesis is one of the aims of "Mäerl calcification, photosynthesis and metabolism in an acidified ocean" project of CCMAR (Center of Marine Sciences) at University of Algarve.

It has been predicted that by the year 2100 the atmospheric partial pressure of CO_2 (pCO_2) will have increased to twice the amount registered nowadays, and as a result, the pH of the seawater

will decrease significantly (Iglesias-Rodriguez *et al.,* 2008). This drop in pH will strongly affect the carbonate system of the oceans and evidently will have major consequences for all marine organisms that have a calcium carbonate skeleton (Riebesell *et al.,* 2000).

In this context, it is highly pertinent to investigate the effects of ocean acidification on the growth and calcification of these calcareous red algae.

Objectives:

This thesis has the following main objectives:

1. To assess the distribution of mäerl along the Algarve coast.
2. To investigate the effect of CO_2 on the growth rates of the calcareous red algae *Phymatolithon calcareum*.
3. To assess the effects of CO_2 on the photosynthetic light response curves of *Phymatolithon calcareum.*
4. To evaluate the combined effects of CO_2 and temperature on the respiration rate of *Phymatolithon calcareum*.

2. Ocean acidification, calcification and photosynthesis

2.1. Ocean acidification

Ocean acidification is a result of chemical reactions that appear in the ocean while establishing a balance of components such as CO_2 between the atmosphere and oceans.

CO_2 can be produced and released to the atmosphere from burning fossil fuels, cement manufacture, agriculture and deforestation. Similarly to other gases, CO_2 obeys Henry's Law, which means that increasing CO_2 concentration in the atmosphere will also increase it in the ocean (Royal Society, 2005).

To maintain chemical equilibrium, CO_2 reacts with the water to form carbonic acid. Some of these extra carbonic acid molecules react with a water molecule to give a bicarbonate ion and a hydronium ion, thus increasing the ocean's "acidity" (H^+ ion concentration) (Jacobson, 2005).

CO_2 exchange is a two-way process that includes absorbing and releasing CO_2 between atmosphere and oceans (Fig. 2.1). Therefore, decreasing the amount of CO_2 that has been absorbed by the oceans will mean that relatively more CO_2 will be in the atmosphere (Royal Society, 2005).

The ability of the oceans to absorb CO_2 from the atmosphere is a reason for the decrease in the pH of the oceans and making them more acidic (Royal Society, 2005).

Dissolving CO_2 in seawater increases the hydrogen ion (H^+) concentration in the ocean, and thus decreases ocean pH, as follows:

$$CO_{2\,(aq)} + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2\,H^+$$

The more acid a solution, the more hydrogen ions are present and the lower the pH. Therefore, the amount of CO_2 that dissolves in seawater has a strong effect on the resultant acidity (alkalinity) and pH of the oceans.

At the same time there are three main inorganic forms of CO_2 dissolved in the oceans, collectively named as dissolved inorganic carbon (DIC). These are: 1 – aqueous CO_2 (around 1 %, including H_2CO_3, of the total); 2 – biocarbonate (HCO_3^-, about 91 %); 3 – carbonate ions (CO_3^-, about 8%) (Royal Society, 2005; Dickson, 2010).

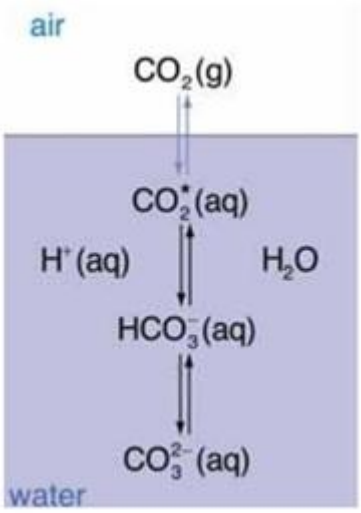

Figure 2.1. The chemical equilibria of the carbon dioxide system in seawater (Riebesell *et al.*, 2010).

2.2. Calcification

Calcification is one of the main oceanic processes through which one carbon is stored all over the ocean; in the open water, in shallow water and in sediments.

This chemical process can be described by the following equation:

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$$

This process shows that, for each mole of carbonate precipitated, one mole of dissolved CO_2 is released to the surrounding water (Frankignoulle *et al*, 1995).

This released CO_2 will next react with bases that are already present in the system to reach the equilibrium conditions (Wollast *et al*, 1980, Frankignoulle & Gattuso, 1993). The final situation is determined by the following physicochemical conditions which specify the aquatic system:

- if the system is closed (i.e. no exchange of CO_2 with the atmosphere), the partial pressure of CO_2 will increase and the final value of it will depend on the buffering capacity of the water (Riley & Skirrow, 1975).
- if the system is open (there is a connection with the atmosphere), the partial pressure of CO_2 needs to be in equilibrium between the water and atmosphere, so it has to be constant and equal (Frankignoulle *et al*, 1995).

Calcification is the process that includes the precipitation of dissolved ions into solid $CaCO_3$ structures, such as coccoliths. The product of the concentrations of calcium and carbonate ion ($[Ca_2^+]\times[CO_3^{2-}]$) must have exceeded the solubility product of calcite or aragonite, otherwise the $CaCO_3$ would have dissolved (Zeebe & Westbroek, 2003).

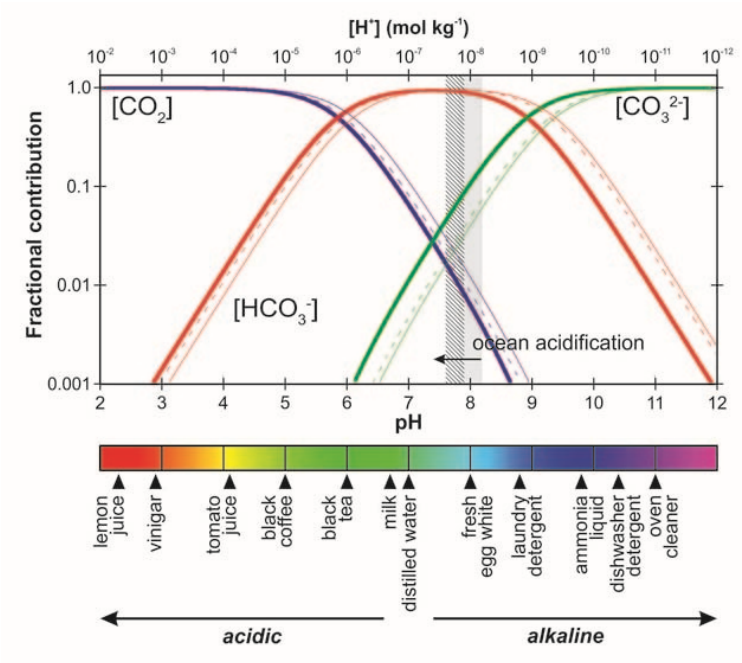

Figure 2.2. Bjerrum plot: Change in carbonate system of seawater from ocean acidification. Concentrations of CO_2, HCO_3^-, CO_3^{2-}, H^+, and OH^- as functions of pH (Barker & Ridgwell, 2012).

We can see in the Bjerrum plot (Fig. 2.2) that as the pH changes and the CO_2 increases in the ocean the oceans' concentrations of the different forms of dissolved inorganic carbon (DIC) also changes. When there is a decrease of CO_3^{2-} concentration, it decreases saturation state (Ω) of seawater and therefore makes $CaCO_3$ dissolution more likely (Royal Society, 2005).

Calcium carbonate is present in the oceans in two polymorphs: aragonite and calcite. Aragonite is one of the most soluble forms of calcium carbonate but it is widely used by marine calcifiers (http://www.sos.noaa.gov). Thus, the calcite saturation horizon is always nearer to the surface than the calcite saturation horizon (Raven *et al.*, 2005). Therefore, those organisms that produce aragonite may be more sensitive to changes in ocean acidity, than other marine organisms that produce calcite (Orr *et al.*, 2005). Increasing CO_2 concentration entails decreasing pH level. Consequently, decreases in the saturation state of $CaCO_3$ and increases in the level of the saturation horizons of both forms places them closer to the surface (Royal Society, 2005). This decrease in the saturation state is thought to be one of the major factors that lead to the decreasing of calcification in marine organisms, as it is known that the inorganic precipitation of $CaCO_3$ is directly proportional to its saturation state (Marubini *et al.*, 2008).

The saturation state of seawater is a measure of the thermodynamic potential for the mineral to form or to dissolve, and can be described by following equation:

$$\Omega = \frac{\left[Ca^{2+}\right]\left[CO_3^{2-}\right]}{K_{sp}}$$

Where Ω is the calculated saturation state; dissolved calcium $[Ca^{2+}]$ is the seawater concentration of dissolved calcium ions, $[CO_3^{2-}]$ is the seawater concentration of carbonate ions and K_{sp} is the solubility of aragonite in seawater (Atkinson & Cuet, 2008).

There is a natural horizontal boundary in seawater that is formed as a result of different parameters such as: temperature, pressure and depth; and called as the saturation horizon (lysocline) (Raven *et al.*, 2005).

When $\Omega = 1$, the seawater is exactly in equilibrium or saturation with respect to aragonite. In other words, the aragonite does not dissolve or precipitate.

When $\Omega > 1$, the seawater is said to be supersaturated with respect to aragonite and $CaCO_3$ does not readily dissolve. Most calcifying organisms live in such waters (Raven *et al.*, 2005).

When $\Omega < 1$, the seawater is said to be undersaturated with respect to aragonite and $CaCO_3$ will dissolve. However, if its production rate is high enough to offset dissolution, $CaCO_3$ can still occur where Ω is less than 1.

The carbonate compensation depth occurs at the depth in the ocean where production is exceeded by dissolution (Thurman & Trujillo, 2004).

When CO_2 is absorbed from the atmosphere by surface seawater, the acidity of surface seawater is increased. Therefore there are relatively less carbonate ions (CO_3^{2-}) in seawater, and thus the value of Ω decreases and so does the saturation state of seawater with respect to aragonite (Zeebe & Westbroek, 2003).

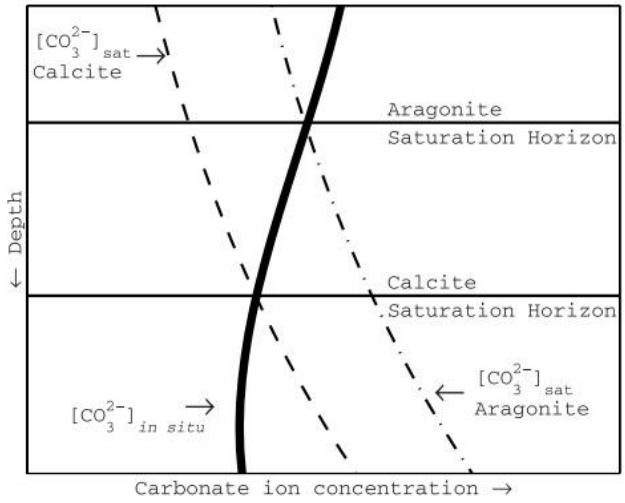

Figure 2.3. Calcite and aragonite saturation horizon in the ocean [after Broecker and Peng, 1987]. As pressure increases with depth, so does the solubility of calcite and aragonite ($[CO_3^{2-}]_{sat}$). The crossover between the in situ carbonate ion concentration (solid curve) and the saturation concentration for calcite (dashed curve) and aragonite (dot-dashed curve) determines the saturation horizon (Zeebe & Westbroek, 2003).

The depth of calcite saturation horizon (lysocline) (Fig. 2.3) is mainly controlled by the saturation state of the bottom water. Essentially, it is controlled by $[CO_3^{2-}]$ because the Ca^{2+} can be considered as constant and much larger than that of CO_3^{2-}. It can be written as the following equation:

$$\Omega = \frac{[CO_3^{2-}]_{sw}}{[CO_3^{2-}]_{sat}}.$$

The crossover of the in situ carbonate ion concentration in seawater ($[CO_3^{2-}]_{sw}$) and the carbonate ion concentration at calcite saturation ($[CO_3^{2-}]_{sat}$) is called the calcite saturation horizon (SH), see Figure 2.3. (Zeebe & Westbroek, 2003).

Since calcite solubility increases with pressure and depth correspondently, there is supersaturated water above saturation depth and undersaturated water below.

2.3. Methods to measure calcification rates

There are various methods that can be used; some of them are used to measure calcification in a small scale such as for individuals and others at the community level.

1. Organisms scale:

1.1 ^{45}Ca incorporation method (assumes that calcification is equal to the rate of incorporation of ^{45}Ca into the skeleton);

1.2 Coral density banding (assumes that calcification rate can be obtained by using long-lived coral colony, by taking a corn from the colony and measuring the distance between the annual bands and the density of the skeletal material);

1.3 Buoyant weighting method (assumes that increasing of the mass caused by calcification. Advantages – it is a very easy technique to use, fast, non-destructive and can be applied for a big community);

1.4 Alkalinity depletion or anomaly method (assumes that there is a direct relationship between calcification rates and total alkalinity changes (TA) such as Calcif = $-0.5\Delta TA$ and NCP = $\Delta DIC - 0.5\Delta TA$. Thus it measures net calcification);

1.5 Ca^{2+} change method (makes a direct measurement of the calcification process);

Coralline algae, just like corals, are slow growers, which makes them really hard to evaluate (Frantz *et al*., 2005). Usually, growth rates in coralline algae are defined through linear increases, and for that, micrometers, microscopes and image analysis are used (Adey, 1970; Garrabou & Ballesteros, 2000; Rivera *et al*., 2004). To determine the biomass increase of coralline algae, the buoyant weight technique has been commonly used, as it is used also for corals (Potin *et al*.,

1990). This method assumes that changes in biomass are the results of $CaCO_3$ inclusion and so can be used to evaluate long-term calcification rates in marine coralline algae (Steller *et al*., 2007). Steller *et al*. (2007) determined the organic matter and $CaCO_3$ composition in the coralline alga by eliminating $CaCO_3$ through acidification, and found that coralline algae for 92% are composed of carbonates.

2. Community scale:

2.1 Slack water method (assumes that there is equation $Calcif = -0.5\Delta TA\rho h/\Delta t$, where ρ is the density of seawater, h – average water depth and Δt – duration of the observation period);

2.2 Lagrangian method (assumes that changes in TA are related to the calcification by the following formula $Calcif=-0.5(TA_2-TA_1)\rho h/(t_2-t_1)$, where TA_1 and TA_2 are the total alkalinity of the water parcel at time t_1 and t_2 accordingly, ρ is seawater density and h – the average water depth);

2.3 Eulerian method (TA is measured simultaneously in the two different locations, upstream and downstream from the site, so calcification can be calculated accordingly the following formula $Calcif=-0.5(TA_d-TA_u)\rho uh/L$, where TA_u and TA_d is the total alkalinity of the water at the up and down of the stream, *u* is the current speed and L - the transect length);

2.4 Total alkalinity depletion/water mass residence method (assumes that temporally and spatially the averaged calcification rate is proportional to the salinity-normalized difference in TA between a source region and region of interest and inversely proportional to the residence time of the water in the study site. This method is based on following formula : $Calcif=-0.5(NTA_r-NTA_o)\rho h/\tau$, where NTA_r and NTA_o are the salinity –normalized total alkalinity of the study and source areas, ρ – seawater density, h – average water depth and τ is the water residence time. Residence time can be estimated by knowing such parameters as circulation, salt or water budget or geochemical tracer);

2.5 Standing crop and turnover or Calcimass method (makes statistics of the all important $CaCO_3$ that has been produce by species in a study area and multiplying by the average turn over time characteristic of each species).

Beside that there are some more new techniques such as Control volume method and Boundary layer-gradient flux measurements:

- Control volume method. Where the flux of water and material of control volume of water is monitored continuously using current meters and chemical sensors.
  J_{bio}=local accumulation – advection – $J_{gas\ exch}$

This method has some advantages such as instantaneous rates measured under natural conditions and disadvantages, where is just one suitable sensor which is O_2, therefore only net community production (NCP) can be measured. There are also some limitations such as depth (measurements can be done just in shallow waters), money (high expenses in terms of equipment) and biofouling.

- Boundary layer-gradient flux measurements.

As an advantages – quite simple; non-invasive; rates measured under natural conditions; gives instantaneous rates and can be employed at any depth; as an disadvantages – requires stable and fast response chemical sensors.

2.4. Relationship between alkalinity and calcification

"Alkalinity is the quantitative capacity of water to neutralize an acid" (The WQA Glossary of Terms, 2000).

Alkalinity (A_t) measures the capacity of a solution to neutralize hydrogen ions to equivalent point of carbonate or biocarbonate.

The total alkalinity of a sample of seawater is a type of mass-conservation expression for hydrogen ions relative to a chosen zero value. It is expressed in moles per kilogram of solution and can be estimated using some form of acidimetric titration.

Changes in CO_2 concentration in solution do not change the alkalinity. The reason of that is that net reaction produces the same number of equivalent contributing species of positively contributing species (H^+) as negative contributing species (HCO_3^- and/or CO_3^{2-}).

At neutral pH values:

$$CO_2 + H_2O \rightarrow HCO_3^- + H^+$$

At high pH values:

$$CO_2 + H_2O \rightarrow CO_3^{2-} + 2H^+$$

Nevertheless there are some processes that might increase or decrease alkalinity.

Processes that increase alkalinity

Alkalinity can be formed in the ocean as a result of many different processes. The dissolution of $CaCO_3$ to form Ca^{2+} and CO_3^{2-} is the most well known way. The carbonate ion has the potential to absorb two hydrogen ions. As a result, it affects a net increase in ocean alkalinity. At the same time dissolution of the calcium carbonate is an indirect result of ocean acidification. It might have great negative effect on coral reef ecosystems, but seems to have a relatively low effect on the total alkalinity (Thomas *et al*., 2008).

Other processes that have much bigger effects on ocean alkalinity are anaerobic degradation processes (denitrification and sulfate reduction). These processes occur in the deep water, where there is a lack of oxygen. Denitrification and sulfate reduction during their chemical reactions consume hydrogen ions and release quasi-inert gases (N_2 or H_2S), which eventually get into the atmosphere. Therefore, consumption of H^+ increases the alkalinity (Kim & Lee, 2009).

Processes that decrease alkalinity

In general, anaerobic processes increase alkalinity. Aerobic degradation on the contrary can decrease A_T. This process takes place in the part of the ocean where oxygen is present. As a result of it there is production of hydrogen ions and dissolved organic matter (Chen & Cai, 2010). Thus, with increasing of H^+ alkalinity is decreasing. However, these hydrogen ions could be consumed by base functional groups that dissolved organic matter may have, and in the result disprove their effect on alkalinity. Therefore, aerobic degradation might have a relatively low impact on the overall oceanic alkalinity (Bates *et al.*, 1996).

All the processes that were mentioned before are chemical processes, but in addition there are some physical processes might also have an effect on A_T. According to global warming, the melting of polar ice caps is an increasing concern as they can contribute to the decrease in oceanic alkalinity. Melting of the ice will increase the overall volume of the ocean. As alkalinity is a concentration value (mol/L), changes in the volume would change the concentration of the chemical elements and thus decrease A_T (Brewer & Goldman, 1976).

In the open ocean the distribution of alkalinity is mainly a conservative function of salinity and those physical factors that have been regulating it (e.g. mixing, evaporation, precipitation and water mass movement; Broecker & Peng, 1982). Some non-conservative processes, such as the

precipitation or dissolution of calcium carbonate, and mineralization or nitrate uptake (Brewer & Goldman, 1976), might be also facilitate the open-ocean variability of TA.

In typical seawater alkalinity is set equal to:

$$A_T = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] - [H^+]$$

There is a clear and simple stoichiometric relationship between TA and calcification: TA is lowered by two equivalent moles for each mole of $CaCO_3$ precipitated (Dickson, 2010). Rationalization has been presented by Bates *et al.* (1996), in that the depletion of TA and changes in TCO_2, and pCO_2 were as a result of calcification.

Accordingly to calcification and the uptake of HCO_3^-, TA and TCO_2 should decrease in ratio between 1:1 and 2:1, depending on the amount of organic carbon production concomitant carbonate production (Bates *et al.*, 1996).

2.5. Relationship between calcification and photosynthesis in calcareous algae

The most popular calcareous algae in the world are coralline (i.e. marine red macroalgae of the family Corallinaceae (Borowitzka, 1981). There is a special growth form of crust-forming coralline algae that are called rhodoliths. Rhodoliths are free-living nodular forms that are able to build up extensive beds, which can provide marine organisms of economical importance, such as crustaceans, fish and molluscs with food, shelter and nursing grounds (Foster, 2001; Kamenos *et al.*, 2004; Wilson *et al*., 2004).

The ability to calcify is present amongst all algal groups. In calcareous macroalgae, calcification refers to the participation of precipitation $CaCO_3$ within or on the cell walls (Kangwe *et al.*,2006). Usually calcification is stimulated by light and as laboratory experiments show it is proportional to the rate of photosynthesis. The main chemical processes can be expressed by following formulas:

$$6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2 - \text{photosynthesis}$$

$$\left.\begin{array}{l} Ca_2^+ + CO_3^{2-} \leftrightarrow CaCO_3 \\ Ca^{2+} + 2HCO_3^- + H_2O \leftrightarrow CaCO_3 + CO_2 + H_2O \end{array}\right\} \text{ calcification}$$

Photosynthesis can affect on internal pH by two ways:

1) increasing the concentration of CO_3^{2-} through CO_2 utilization (Borowitzka, 1977);
2) increasing the pH to favor $CaCO_3$ precipitation by the conversion of HCO_3 into CO_2 (Borowitzka, 1977; Raven, 1970).

Respiration, the opposite to photosynthesis, reduces calcification rates as released CO_2 decreases the intra- and intercellular pH (Borowitzka, 1977, 1981, 1982; McConaughey & Whelan, 1997).

HCO_3^- is the main substrate for photosynthesis by intracellular production of CO_2:

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O \rightarrow CH_2O + O_2$$

Using of HCO_3^- is possible due to the simultaneous calcification using a second HCO_3^-, which provides the required proton:

$$HCO_3^- + Ca_2^+ \rightarrow CaCO_3 + H^+$$

Therefore, HCO_3^- is the only substrate for calcification (Buitenhuis *et al.*, 1999)

Indirect effects of pCO_2 on calcification can be happen through effects on photosynthesis (Borowitzka, 1977, 1981; Borowitzka & Larkum, 1976), contrary to the direct effects of increasing pCO_2 on the physical kinetics of carbonate chemical reactions.

It is known that calcification in coralline algae is strongly coupled with photosynthesis (Chisholm, 2000). It can be a direct effect of photosynthesis on calcification through the supplying of ATP for the energetic expenses of calcification. Moreover, photosynthesis can affect calcification indirectly by increasing the internal pH at the site of calcification (Gattuso *et al.*, 1999; Goreau, 1963).

During the high CO_2 concentration, CO_2 dissolution may have exceed photosynthetic "CO_2" removal in the light time, on the result pH decreased to some constant point where the two processes (photosynthetic "CO_2" removal and CO_2 dissolution) will be balanced. Nevertheless, in the dark time, during the respiration, the addition of CO_2 reduces the pH. During the light time, concentration of Ca_2^+ decreases according to the low CO_2 level where pH increases, while the Ca_2^+ concentration is stable as pH decreased in the high CO_2 level. Therefore, calcification in *Coralline pilulifera* considered to be a process that is dependent on pH (Gao *et al.*, 1993). Borowitzka (1981) saw that the calcification rates in the other species of the coralline red algae such as *Arnphiroa anceps* increased proportionally to increased pH due to photosynthesis in the

pH 7.0 to 9.0 range, possibly because of increasing CO_3^{2-} under the increased pH (Gao *et al*., 1993).

Addition of free CO_2, by lowering the pH, does not enhance calcification but that the addition of carbonate and bicarbonate do enhance this process (Gao *et al*., 1993).

2.6. Effect of increasing CO_2 on calcification and productivity of calcareous algae

Every year the rate of anthropogenic CO_2 has been increasing. The partial pressure of CO_2 in the atmosphere has increased from 280 μatm to 388 μatm since pre-Industrial times (IPCC, 2007). At the same time, global temperature has been increasing annually by 0.2-0.3 °C (IPCC, 2007).

More than 30% of anthropogenic CO_2 is absorbed by surface waters of the ocean, simultaneously increasing CO_2 concentration in seawater that causes changes to the carbonate chemistry of seawater, associated with ocean acidification (Caldeira & Wickett, 2003; Feely *et al*., 2004; Sabine *et al*., 2004). These changes include reduction in pH, available CO_3^{2-}, and the saturation state of $CaCO_3$ (Ω) (Orr *et al*., 2005) and increases in pCO_2 and HCO_3^- (Caldeira & Wickett, 2003; Gattuso & Buddemeier, 2000).

Ocean acidification can reduce biogenic calcification by decreasing the availability of CO_3^{2-} , increasing HCO_3^-, and decreasing the saturation state of $CaCO_3$ mineral forms (Ω) (Borowitzka, 1981; Steneck, 1983, 1986), and represents a balance between calcification and dissolution.

A meta-analysis of biological responses to ocean acidification demonstrated overall negative effects of increased pCO_2 on survival, calcification, growth and reproduction of marine biota (Kroeker *et al*., 2010). Increased pCO_2 and decreases in Ω have been correlated directly with decreased calcification and increased dissolution (Gattuso *et al*., 1998; Langdon *et al*., 2000; Marubini *et al*., 2001, 2003).

Crustose coralline algae (CCA) play an important ecologic role in reef ecosystems, with such ecological functions as cementation and maintenance of reef stability (Adey, 1998; Camoin & Montaggioni, 1994; Littler, 1973; Payri *et al*., 2001), and primary production (Adey & Macintyre, 1973; Borowitzka, 1981; Chisholm, 2003; Littler, 1971).

Biogenic calcification of CCA takes place within the cell walls of the algal thallus and consists of the $CaCO_3$ polymorph high magnesium calcite (Borowitzka, 1977). High-Mg calcite is the most soluble mineral form of $CaCO_3$, because of incorporation of magnesium into the carbone

crystal lattice. Therefore, they are very sensitive to chemical changes associated with ocean acidification (Andersson *et al*., 2008; Morse *et al*., 2006).

Studies that have been done with controlled laboratory mesocosms have documented negative effects of ocean acidification on recruitment (Kuffner *et al.,* 2008), growth (Jokiel *et al*., 2008), and calcification (Gao *et al*., 1993; Semesi *et al*., 2009) of crustose coralline algae by manipulating only pCO_2.

The combined effects of pCO_2 and temperature might have even bigger impacts than either one considered alone (Anthony *et al*., 2008; Diaz-Pulido *et al*., 2012; Reynaud *et al*., 2003), therefore it is important to investigate the joint effects of temperature and pCO_2 at the same time. A reason of the variable biogenic calcification response to warming and increasing of pCO_2 might be related to effects of pCO_2 on photosynthesis (Borowitzka, 1977, 1981; Borowitzka & Larkum, 1976), as contrary to the direct effects of increasing pCO_2 on the physical kinetics of carbonate chemical reactions.

As an example *Hydrolithon onkodes* net calcification rates were affected significantly by the both these treatments pCO_2 and temperature and there was a significant interaction between them on the net calcification rate of *H. onkodes*. There is also significant interactive effect of increasing CO_2 concentration and warming on calcification rate of this species (Johnson & Carpenter, 2012).

Reis *et al.* (2009) found a parabolic response of the crustose coralline *Neogoniolithon* to ocean acidification, and the highest calcification rate was found at intermediate CO_2 level. Therefore, drastic increases in CO_2 concentration have overall negative effect for biogenic calcification. Increases in pCO_2 within the realm of natural diel pCO_2 variability may stimulate calcification, at least for a short time (Pandolfi *et al*., 2011). Anthony *et al.* (2008) and Diaz-Pulido *et al.* (2012) found that warming increased the sensitivity of coralline algae to ocean acidification. Johnson and Carpenter (2012) found that at high concentrations of CO_2, temperature could alleviate the drastic effects of increased pCO_2 on *H. onkodes* net calcification. In response to ocean acidification there was a 24% decrease in *H. onkodes* calcification rates. Ragazzola *et al*. (2012) found that *Lithothamnion glaciale* had lower growth rates and some loss of structural integrity after exposure to high pCO_2.

Martin and Gattuso (2009) showed 200-400% increase in net dissolution of the *Lithophyllum cabiochae* in response to increasing of pCO_2 and warming.

Johnson and Carpenter (2012) were the first to find the direct evidence linking decreased calcification rates of *H. onkodes* to increased susceptibility of this coralline algae to sea urchin grazing. Crustose coralline algae play important ecological roles and decreased calcification rates with increased receptivity to herbivory could have negative effects on reef primary productivity, coral larval settlement and reef cementation stabilization (Adey *et al*., 1982; Chisholm, 2003; Harrington *et al*., 2004; Littler, 1971).

Therefore, the physiological effects of ocean acidification and increased seawater temperature may have community effects that could damage the biodiversity and coral reef stability.

2.7. The potential mutualistic effects between calcification and photosynthesis

Considering that pH may affect calcification and photosynthesis rates, Semesi *et al*. (2009) studied the impact of pH on these rates of the calcareous red and green algae in their natural environment, taking in account diel variations in pH caused by photosynthesis.

According to ocean acidification it was just recently admitted that high pH values could affect the photosynthesis of the calcareous algae (Björk *et al*., 2004; Middelboe & Hansen, 2007). Moreover, seagrasses can elevate the pH of the surrounding seawater. Invers *et al*. (1997) documented a pH increase of up to 0.5 units in seagrass meadows, which has a negative effect on photosynthesis rates of three seagrasses such as: *Posidonia oceanica, Cymodocea nodosa* and *Zostera noltii*. Also, Beer *et al*. (2006) showed that dominant species could increase the pH to 8.5–9.2, but some species, in that case it was *Halophila ovalis*, could not grow with certain species such as *Thalassia hemprichii* at the same site because they could not photosynthesise at the high pH values generated.

Semesi *et al*. (2009) found that calcification rate of *Hydrolithon* sp. and *Mesophyllum* sp. increased with incubation time in the presence of seagrasses, while photosynthetic rates dropped at the end of incubation period. The same as coralline algae, *Halimeda renschii* showed significantly higher rates of calcification with the presence of seagrasses (Fig. 2.4).

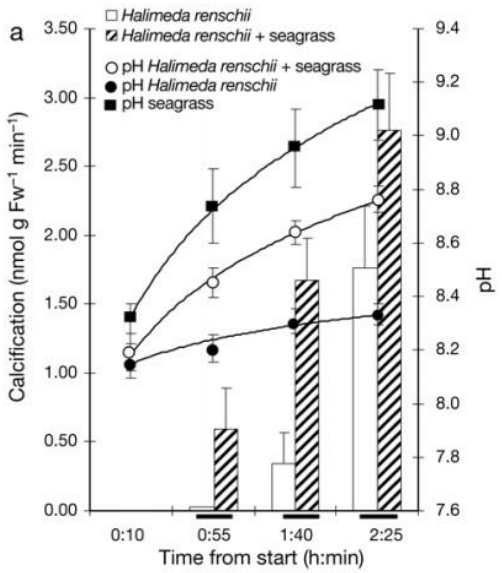

Figure 2.4. *Halimeda renschii*. Irradiance, pH, rates of calcification. Calcification rates with algae only (open bar) and algae together with sea-grasses (hatched bar), and the pH generated in each treat-ment: seagrasses only (■) algae only (•) and algae together with seagrasses (o); lines below bars indicate a significant difference between treatments ($p \leq 0.05$); ww: wet weight (Semesi *et al*., 2009).

Semesi *et al*., (2009) conclude that algal calcification within seagrass meadows is appreciably increased by the photosynthetic activity of the seagrass, which in turn increases the seawater pH.

Therefore, the main conclusion from this mutualistic relationship between seagrasses and calcareous algae is that calcification is higher when seagrasses are present and the pH does not increase so much, which means that there is more CO_2 available for seagrasses photosynthesis.

3. Material and methods

3.1. Distribution of *Phymatolithon calcareum* along the Algarve coast

The distribution of *P. calcareum* along the Southern coast of Portugal was investigated during the expedition for studying marine biodiversity along the Algarve coast, organized by M@rbis (Information System for Marine Biodiversity) and EMEPC (Task Group for the Extension of the Continental Shelf) and conducted aboard the UAM Creoula (Naval Auxiliary Units) from 21st June to 11th July 2013. During this campaign samples of *P. calcareum* were taken at different sites by SCUBA and coordinates were taken using GPS (Global Positioning System).

In the dives where *P. calcareum* was found samples were collected and taken to the boat, where they were dried and conserved for later analysis in the laboratory at the University of Algarve.

Those sites where *P. calcareum* was found were marked on the map using program Google Earth with the GPS coordinates of the dive spots.

3.2. Effects of CO_2 on growth, photosynthesis and respiration of *P. calcareum*

3.2.1. Sampling sites and algal material

To investigate the effect of high pCO_2 on the calcification rate of the calcareous red algae *P. calcareum*, a long term experiment was done in the Marine Plant Ecology Group (ALGAE) laboratory and mesocosm facility at CCMAR.

For that using SCUBA diving in the bay of Armaçao de Pera, in the Algarve, Portugal (Fig. 3.1), on the 8th December 2011 samples of *P. calcareum* were collected. Sampling was done at the depth of approximately 20 m, where the mäerl occupies an area around three km^2 between 13 and 25 m depth, with the thickness of the layer up to five cm. After that, samples were stored in the seawater and transported to the university field station Ramalhete.

Figure 3.1. Location of the mäerl sampling, Armaçao de Pera, Portugal (earth.google.com).

3.2.2. Experimental setup (mesocosm)

Since coralline algae are slow-growing organisms, the changes in the growth rates can only be determined after a long period of time (Kleypas *et al*., 2006). For a long term experiment, a permanent mesocosm system was built in CCMAR field station, where *P. calcareum* is being cultivated in the CO_2 enrichment conditions. The mesocosm helps to keep and control the experimental conditions and to have a greater control of species community.

The mesocosm functioned as an open system with low flow-rates. For this experiment, natural seawater from the station was used, to make sure that the algae had all nutrients that they are supposed to have. Water ran through a preliminary reservoir before it reached the aquaria. In that reservoir, the water was enriched with CO_2 at the specified concentration by adding commercial CO_2 from a dedicated tank (Fig. 3.2). The flushing rate of CO_2 was controlled by a solenoid valve connected to a pH controller, a constant pCO_2 was maintained at the same time in the reservoir. A control level and two enrichment levels were maintained based on the International Panel on Climate Change (IPCC IS92) CO_2 emission perspectives (Houghton *et al*., 2001). The two enrichments relate to the predicted situation for the year 2060 with pCO_2 = 595 ppm, and for the year 2100 with pCO_2 = 750 ppm. The control was ran without any enrichment at pCO_2 = 380 ppm.

In each of nine aquariums per treatment there were fifteen rhodoliths that have been tagged and weighted, among other individuals. Salinity, temperature, pH and oxygen were measured regularly. Every month the marked samples were taken from the aquarium and measured the weight using the buoyant weight technique.

Figure 3.2. Aquariums and mesocosm in the Ramalhete field station; a) six aquariums in each system with controlled level of CO_2, b) two mesocosm systems with blue tanks were seawater

became enriched with fixed level of CO_2, c) system where CO_2 concentration has been maintained and controlled.

In the experiment for measuring the response curves of different light intensities according to the different CO_2 concentrations the Clark-type oxygen electrode was used with eight rhodoliths. These samples were taken from each level of CO_2 concentration to the Campus of Gambelas of the University of Algarve. For the next experiment the response curves to the light and respiration rates in terms of different temperatures and levels of CO_2 were measured, again using the Clark-type oxygen electrode, six rhodoliths from each system of CO_2 level were taken to the Campus of Gambelas and stored in the plant chamber "walk in" with temperature and light level controlled. In this chamber samples were stored for two days for acclimatization to the specified temperature: 12, 14, 16, 18, 20, 22, 24 and 26 ºC.

3.2.3. Buoyant Weight Technique

The weight of *P. calcareum* was determined in the laboratory using the buoyant weight technique (Fig. 3.3) that was firstly used for corals by Jokiel *et al*. (1978). The buoyant weight method is a very simple technique used to estimate the increase of the amount of $CaCO_3$ in living coralline algae. This technique is based on measuring the initial and final weights of the organisms in the water. In this case marked samples of the *P. calcareum* were placed in the water and weighted. The weight of $CaCO_3$ was calculated based on Archimedes' Principle using the following equation:

$$W_{cc} = W_b + (V_{cc} \times D_w)$$

where, W_{cc} is the dry weight of the $CaCO_3$ of the coralline alga, W_b is the buoyant weight of the sample, V_{cc} is the volume displaced by the $CaCO_3$ of the coralline alga, and D_w is the density of the seawater used to suspend the samples during weighing (approx. 1.03 g cm^{-3}). The product of $V_{cc} \times D_w$ is equal to the weight of the seawater displaced by the sample's $CaCO_3$ (Steller *et al*., 2007). At present study calculations were done based on following equation, where density of seawater and $CaCO_3$ were substituted.

$$W_{cc} = 1.61 * W_b$$

This technique was shown to be effective for determining calcification in *L. margaritae* even during a relatively short-term experiment of less than 60 h (Potin *et al*., 1990). It was also used to estimate growth rate in other coralline algae like *L. corallioides*, in an experiment conducted in France, with an incubation period of over 250 days (Potin *et al*., 1990).

The method assumes that: the whole calcareous skeleton of the algae is made of carbonates; that there are no air spaces within the skeleton, because otherwise it might cause weighing errors. The density of organic tissue is the same to that of seawater (Steller *et al*., 2007). Growth is calculated from the difference between initial and final weights of the organism.

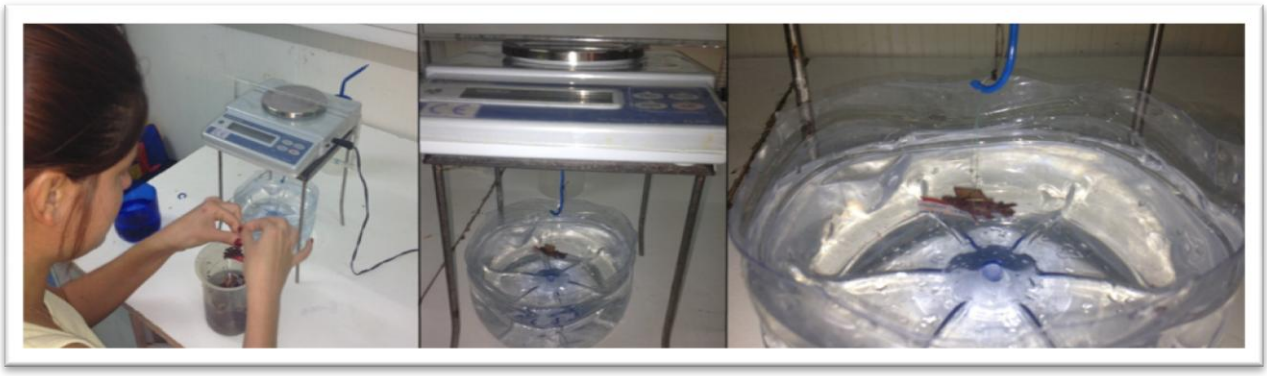

Figure 3.3. Buoyant Weight Technique in the field station Ramalhete, where tagged rodolith were measured in the seawater using the balance.

To represent results "Sigmaplot" (Copyright © 2008 Systat Software, Inc. Germany, Sigmaplot for Windows Version 11.0) program was used to build the graphs.

3.2.4. Clark-type oxygen electrode

Hansatech version of the Clark-type electrode comprises platinum cathode and a silver anode immersed in, and connected by, an electrode. Electrodes are set in a plastic disc; where the cathode is at the center of a dome and the anode is in a circular groove. The electrodes are protected by polythene membrane which is pervious to oxygen. The idea of the dome is to stretch the membrane smoothly over the surface of the platinum cathode and to allow it to be secured in position by an O-ring. The membrane also traps a thin layer of electrolyte, as a potassium chloride (KCl) solution, over the surface of the electrodes (Walker, 1987). To provide a uniform layer of electrolyte between anode and cathode a special paper is placed beneath the membrane.

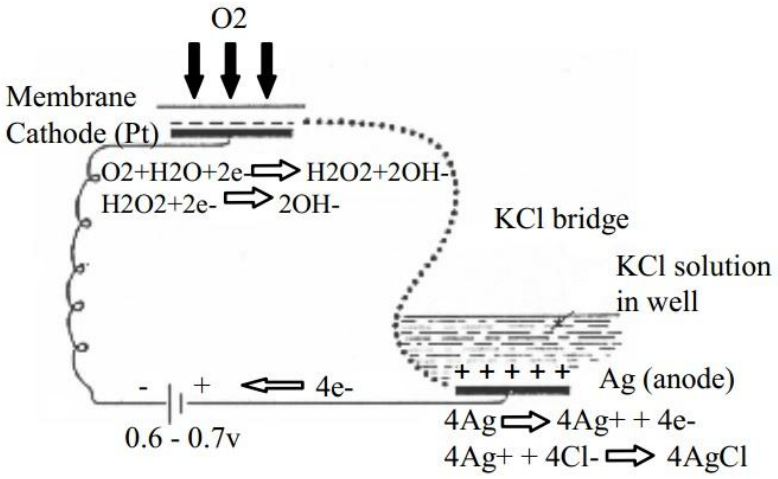

Figure 3.4. Oxygen electrode reactions in the Clark-type oxygen electrode. The platinum (Pt) and the silver (Ag) electrodes are changing charge according to the potentiating voltage that was applied across them. Oxygen diffuses through the membrane and is reduced at the cathode surface so that a: current flows through the circuit. The current is recorded as a voltage, on a pen-recorder (Walker, 1987).

The electrical current that has been generated by the reduction of oxygen at the cathode converts to a voltage output signal by the Hansatech CB1 or CBI-D control box.

The difference between zero oxygen and the electrical zero is used as calibration and can be done by passing nitrogen over the electrode. To calibrate the electrode, a 2-point calibration was done using 100% air-saturated seawater and oxygen-free seawater; the 100% air-saturated seawater was created by bubbling ambient air through 14°C seawater and anoxic seawater was created by adding N to seawater in the electrode chambers. Total μmoles of dissolved O_2 could then be determined from known standardized tables (Dickson *et al*., 2007).

During photosynthesis light energy has been captured by the antenna pigments, where centers of the reaction photosystems I and II are been converted into chemical energy and reducing in power afterwards this chemical energy is used in the process of reduction of CO_2 to carbohydrates in the Calvin cycle (Lambers *et al*. 1998). During this process the water molecule is oxidized, and trough the electron transport due to photosynthesis process oxygen is released.

The photosynthetic process can be represented by the following chemical equation (Lambers *et al*. 1998)

$$6CO_2 + 6H_2O \xrightarrow{\text{light}} C_6H_{12}O_6 + O_2$$

Therefore, if a photosynthetic organism is contained in a chamber where it is possible to focus photosynthetically active light, and in this chamber there is a source of CO_2 for the Calvin cycle, the rate of the released oxygen will be proportional to the rate of CO_2 fixation.

The oxygen released by the photosynthetic organism is detected by the oxygen electrode which is connected to a recorder (Fig. 3.4) (Lessler & Brierly, 1969).

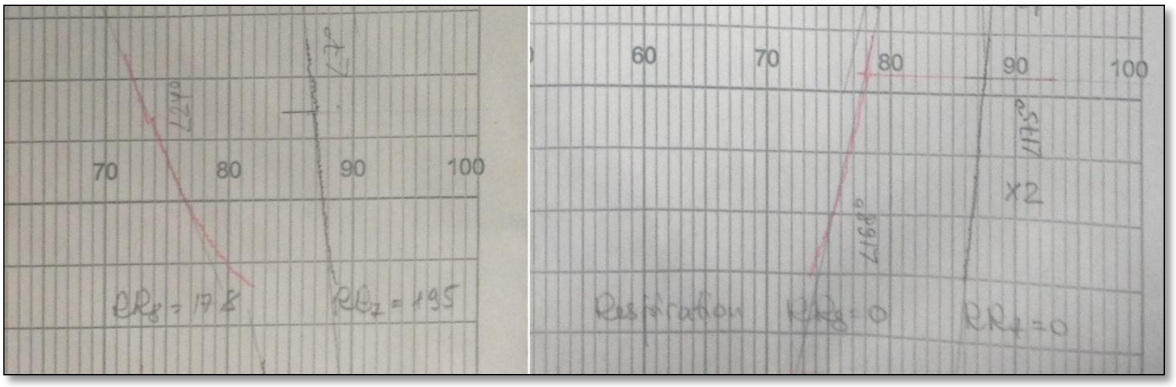

Figure 3.5. Graphic recording of the evolution of the amount of oxygen of the two samples of the rodolith (red and black line) in contact with the electrode.

It is also possible to measure the dark respiration for the same organism by measuring the consumption of oxygen without the light. The records of the evolution of oxygen in the incubation chambers were then interpreted: with a ruler and protractor light. A line was drawn up on the same slope that represents the photosynthetic response, after that the angle of the slope was measured (Fig. 3.5). The value of this angle is used in the equation as the data needed to assess the rate of release consumption of oxygen.

3.2.5. Light – response curves

To characterize impacts of elevated pCO_2 on the photosynthetic performance of each *P. calcareum* photosynthesis-irradiance (P-I) response curves were constructed under three pCO_2 levels; 380 ppm, 550 ppm and 750 ppm. It helped to compare photosynthetic efficiency (α) under non-saturating irradiances, maximum rates of photosynthesis (P_{max}) under saturating irradiances, and the saturation irradiance itself (I_K) under the three pCO_2 levels.

P-I curves were constructed for dissolved O_2 production (in μmol O_2 produced per gram of dry weight algae per hour) under each pCO_2 level. P_{max}, α and I_K were calculated for each curve. Specifically, dissolved O_2 was measured using a Clark-type oxygen electrode.

Response curves to the light and respiration rates were measured with the Clark-type oxygen electrode (Hansatech Instruments, Norfolk, UK) coupled with incubation chambers (DW3, Hansatech Instruments, Norfolk, UK). In the incubation chambers parameters such as temperature and light intensity can be manipulated (Walker, 1987). During the measurements, each incubation chamber remained bound to a magnetic stirrer to ensure homogeneity of water. The water temperature was set at the same level 14°C by means of a thermostatic bath with outer recirculation system (Julabo HC, Julabo Labortechnik, Seelbach, Germany). As a source of actinic light a slide projector was used (Pradovit 150, Leica, Germany) equipped with a halogen lamp (Osram XENOPHOT 150W). At the conclusion of the measurements the algae were removed from the chambers and dried for 48 hours at 64°C, and after weighed with balance.

Net Photosynthesis was studied in each of the CO_2 levels. To build P-I curves mathematical models were used that were developed by: Smith (1936), Jassby and Platt (1976), Platt *et al* (1980) and Bannister (1979). The model that Bannister (1979) designed has been found as the most reliable for light-response curves for *P. calcareum*. The study setting of Photosynthetic light and the remaining statistical treatment of the data was done with the program "Sigmaplot." (Copyright © 2008 Systat Software, Inc. Germany, Sigmaplot for Windows Version 11.0).Two Way ANOVA was used to compare photosynthesis rates between three different levels of pCO_2.

3.2.6. Effects of CO_2 and temperature on respiration

To measure the respiration rates were used Clark-type oxygen electrode. For that six replicates were taken from each of the system with three different CO_2 levels (380 ppm, 550 ppm and 750 ppm). All replicates were acclimatized for two days to the eight different levels of temperature (12°C, 14°C, 16°C, 18°C, 20°C, 22°C, 24°C and 26°C). For each temperature level new mäerl samples were used. After respiration measurements all samples were dried for 48 hours in the oven at a temperature of 64°C. To calculate Net Photosynthesis per area a photo was made (Fig. 3.6) of all the samples and using “ImageJ” (http://rsbweb.nih.gov) software the area of each replicates was measured. The determination of the fresh weight, dry weight and area of the analyzed rhodoliths were used to calculate dark respiration.

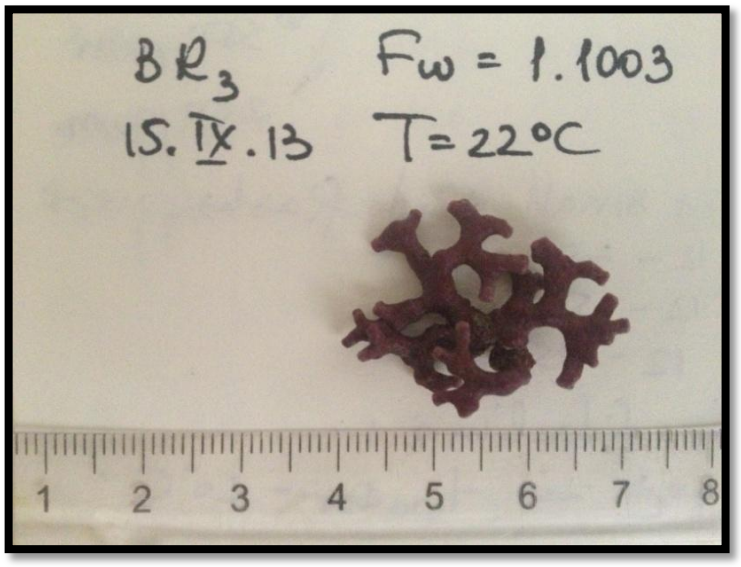

Figure 3.6. Photographic record and information about rhodoliths *P. calcareum* to calculate the area with the "ImageJ" software.

To analyze the data the "SigmaPlot" program was used. To evaluate the differences of respiration between different pCO_2 and temperature a Two Way ANOVA statistical analysis was done.

4. Results

4.1. Distribution of *Phymatolithon calcareum* along the Algarve coast

During the campaign on the "Creoula", 18 dives were conducted at different sites and *P. calcareum* was found in four of these sites. Mäerl was found on sandy sediments, in areas with high marine biodiversity. Diving spots marked as dives # 7 and #10 were characterized as an area with high density of mäerl and at site #7 a mäerl bed with a layer of around five cm was found. All geographical coordinates and presence/absence of the mäerl can be found on the Table 4.1 and are also represented in the Fig. 4.1.

All dive spots had sandy bottoms with different levels of water turbulence and current strengths.

Figure 4.1. Diving sites along the Algarvean coast, red spots represent the places where mäerl was found, numbers from 1 to 18 are the numbers of the dives. Numbers 4, 7, 8 and 10 are sites where *P. calcareum* was found.

Table 4.1. Geographical coordinates of the diving spots with present (+) or absent(-) of the mäerl

Dive №	Latitude	Longitute	Mäerl	Dive №	Latitude	Longitute	Mäerl
1	37°3'38,9"	7°19'55,16"	-	10	37°3'49,536"	8°22'3,0354"	+
2	37°4'59,95"	7°19'18,47"	-	11	37°3'19,728"	8°20'56,7594"	-
3	37°2'7,836"	7°22'38,6394"	-	12	37°3'29,7"	8°21'4,0674"	-
4	37°3'25,619"	7°35'49,2354"	+	13	37°2'46,1394"	8°26'44,0874"	-
5	37°1'7,4994"	8°2'53,232"	-	14	0'49,968"	8°35'35,124"	-
6	37°1'44,5074"	8°6'16,632"	-	15	37°0'47,088"	8°34'39,648"	-
7	37°1'20,4954"	8°11'33,1074"	+	16	37°0'42,1919"	8°34'35,0754"	-
8	37°2'18,0954"	8°13'54,264"	+	17	37°0'2,6274"	8°33'33,5154"	-
9	37°3'51,624"	8°19'55,8474"	-	18	37°3'46,2474"	8°34'13,1154"	-

4.2.Effects of CO_2 on growth, photosynthesis and respiration of *P. calcareum*

4.2.1. Growth

Weight increments of *P.calcareum* were calculated per each CO_2 level and have been represented in the Figure 4.2.

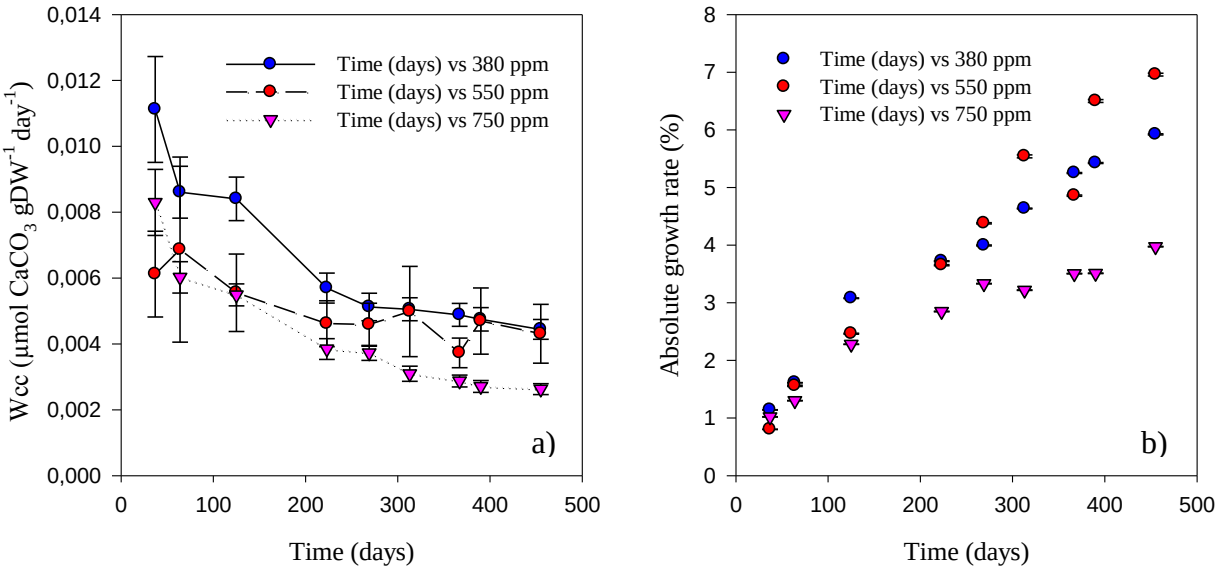

Figure 4.2. a) Weight increments of *P. calcareum*. On the graph mean values of weight increments and bars of standard errors have been displayed; b) absolute growth rate (%) of *P. calcareum*, represented by mean values and bars of standard errors.

During the entire period of the mesocosm culturing experiment, increases in mäerl buoyant weight were recorded. Growth rates were calculated separately for each system of CO_2 level as means of the all values for tagged samples. Weight increments rate of *P. calcareum* decreased regularly during all time of the experiment for all systems (Fig. 4.2, a). Two way ANOVA shows a significant difference in the mean values of weight increments among the time ($P<0.001$) and CO_2 ($P<0.001$) (Table 4.2). At the same time there is no significant difference between the two systems with CO_2 level of 550 ppm and 750 ppm. The higher weight increments have been observed on the system with CO_2 level of 380 ppm.

Table 4.2. Two-Way ANOVA Table of results for weight increments with fixed factors time and pCO_2.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Time	8	0,0000691	0,00000864	11,477	<0,001
pCO_2	2	0,0000215	0,0000108	14,290	<0,001
Residual	16	0,0000120	0,000000753		
Total	26	0,000103	0,00000395		

Absolute growth rate (%) was measured as mean values of the tagged samples by each system and has been represented on the Figure 4.2. (b), and calculated as a percentage from the initial weight of the organisms. Growth rate has been increased during the experiment for all of the

systems. Two Way ANOVA shows a significant correlation with time (P<0.001) and pCO_2 (P<0.001) (Table 4.3).

Table 4.3. Two-Way ANOVA Table of results for absolute growth rates (%) with fixed factors time and pCO_2.

Source of Variation	DF	SS	MS	F	P
Time	8	61,384	7,673	19,460	<0,001
pCO_2	2	8,791	4,396	11,148	<0,001
Residual	16	6,309	0,394		
Total	26	76,484	2,942		

Average growth rate of the *P.calcareum* was found to be 0,202 (g $CaCO_3$/day) for system with pCO_2 = 380; 0.196 (g $CaCO_3$/day) for a system with pCO_2 =550 and 0,120 (g $CaCO_3$/day) at the system with the higher level of CO_2 – 750 ppm.

Three Way ANOVA was calculated to perform the relationship between weight increments and temperature (that was measured during weight buoyant technique) and pCO_2. It was not found a significant difference in the mean values of weight increments among the temperature (P=0.796) and CO_2 (P=0.408) (Table 4.4). Therefore, simultaneous effect of temperature and CO_2 was not ascertained (P=1.000) in the present study.

Table 4.4. Three-Way ANOVA Table of results for weight increments with fixed factors temperature and pCO_2.

Source of Variation	DF	SS	MS	F	P
pCO_2	2	0,0000178	0,00000892	1,044	0,408
T	6	0,0000251	0,00000419	0,490	0,796
pCO_2 x T	12	0,00000472	0,000000394	0,0461	1,000
Residual	6	0,0000513	0,00000855		
Total	26	0,000103	0,00000395		

4.2.2. Photosynthesis

Among the mathematical models tested, the one that globally had the best fit to the photosynthetic light response curves obtained for each CO_2 level was Smith's (1936). All the models based on the positive values, and the best adjustment for the data was chosen according to such parameters as R^2 and P_{max}. The model of Platt *et al*. (1980) shows higher R^2 values (Table 4.5) nevertheless it is not able to calculate P_{max} for the data set of the samples for the control system. Therefore, Bannister's model was chosen for further calculations. Photosynthetic

light response curves have been measured under different level of elevated pCO_2 and have been significantly different ($p<0.001$).

Table 4.5. The R^2 values obtained in the application of different mathematical models to photosynthetic responses of rhodoliths to the light at each CO_2 level. High values (>0.5) are represent that more than 50% of the data has been adjusted by the model; as higher the numbers as better the model fits to the data.

pCO_2	Smith model (1936)	Jassby and Platt model (1976)	Model of Platt *et al* (1980)	Bannister model (1979)
380 ppm	0,5198	0,5234	0,5275	0,5274
550 ppm	0,8701	0,8661	0,8731	0,8729
750 ppm	0,8375	0,8339	0,8424	0,8386

P-I curves based on Bannister model for each system are represented on the Figure 4.3.

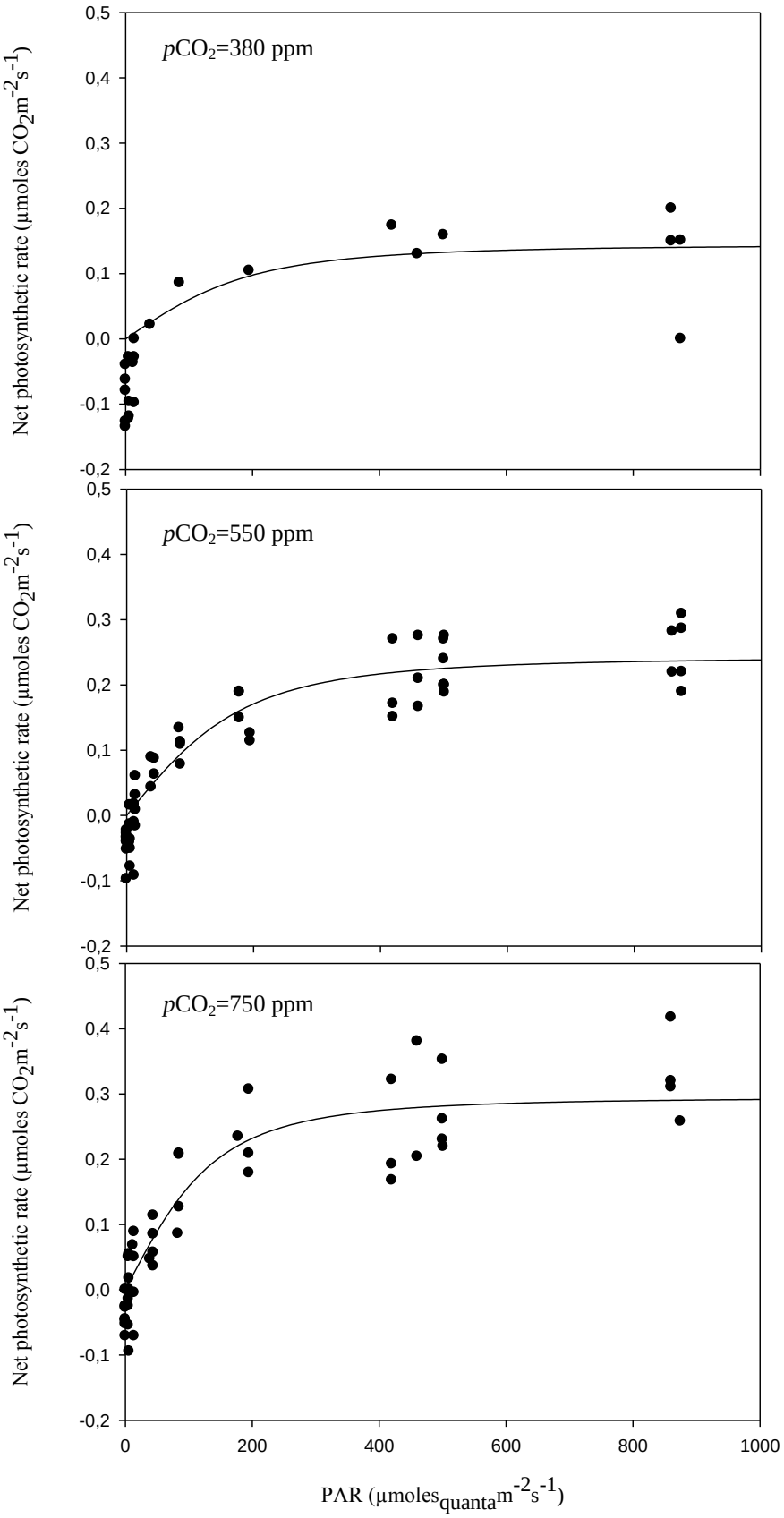

Figure 4.3. Photosynthesis Irradiance response curve of *P. calcareum* ambient at 380 ppm, 550 ppm and 750 ppm using Smiths' (1936) model.

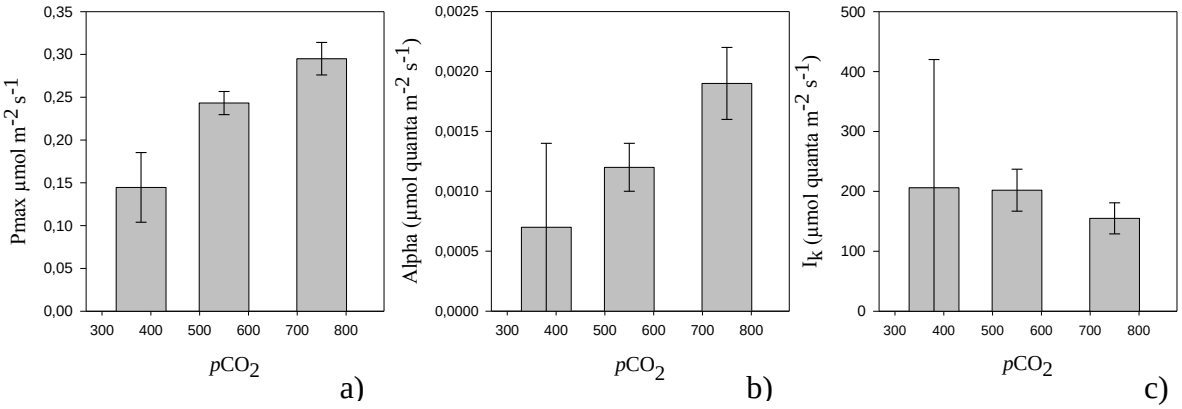

Figure 4.4. (a) Maximum photosynthesis (P_{max}), (b) initial slope of the P-I curves (α), (c) irradiance at saturation (light adaptation index) (I_k) of *P. calcareum* acclimatized to different level of CO_2 (One Way ANOVA: a) P=0.003, b) P=0.165, c) P=0.936); error bars are standard errors.

Maximum photosynthetic rates were observed at system with level of CO_2 750 ppm, while minimum rates were observed at the level of 380 ppm (Fig. 4.4, a). Increased rates of maximum photosynthesis (P_{max}) under elevated pCO_2 indicate higher carbon uptake via photosynthesis according to increase of CO_2 concentration (Fig. 4.4, a). One Way ANOVA shows statistically significant difference of Pmax between the different level of CO_2 with P=0.003 (Table 4.6).

Table 4.6. One-Way ANOVA Table of results of P_{max} with fixed factors pCO_2.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0,0628	0,0314	8,888	0,003
Residual	14	0,0494	0,00353		
Total	16	0,112			

The initial slope of the P-I curve (α) and I_k did not change significantly P=0.165 and P=0.936 accordingly (Fig. 4.4, b, c; Table 4.7-4.8).

Table 4.7. One-Way ANOVA Table of results of initial slope (α) with fixed factors pCO_2.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	0,00000402	0,00000201	2,054	0,165
Residual	14	0,0000137	0,000000979		
Total	16	0,0000177			

Table 4.8. One-Way ANOVA Table of results of I_k with fixed factors pCO_2.

Source of Variation	DF	SS	MS	F	P
Between Groups	2	9296,118	4648,059	0,0669	0,936
Residual	14	972950,000	69496,429		
Total	16	982246,118			

4.2.3. Respiration

Respiration is increasing with increasing of the temperature (Fig. 4.5). There is no significance of CO_2 levels (P=0.924), but there is clear effect temperature on the respiration rate (P<0,001) (Table 4.9). No significantly interaction between CO_2 concentration and temperature (P=0.985) was found.

Table 4.9. Three-Way ANOVA Table of results for respiration rates with fixed factors temperature and pCO_2.

Source of Variation	DF	SS	MS	F	P
Temperature	7	20,615	2,945	19,915	<0,001
pCO_2	2	0,0235	0,0118	0,0796	0,924
Temperature x pCO_2	14	0,732	0,0523	0,354	0,985
Residual	119	17,597	0,148		
Total	142	38,966	0,274		

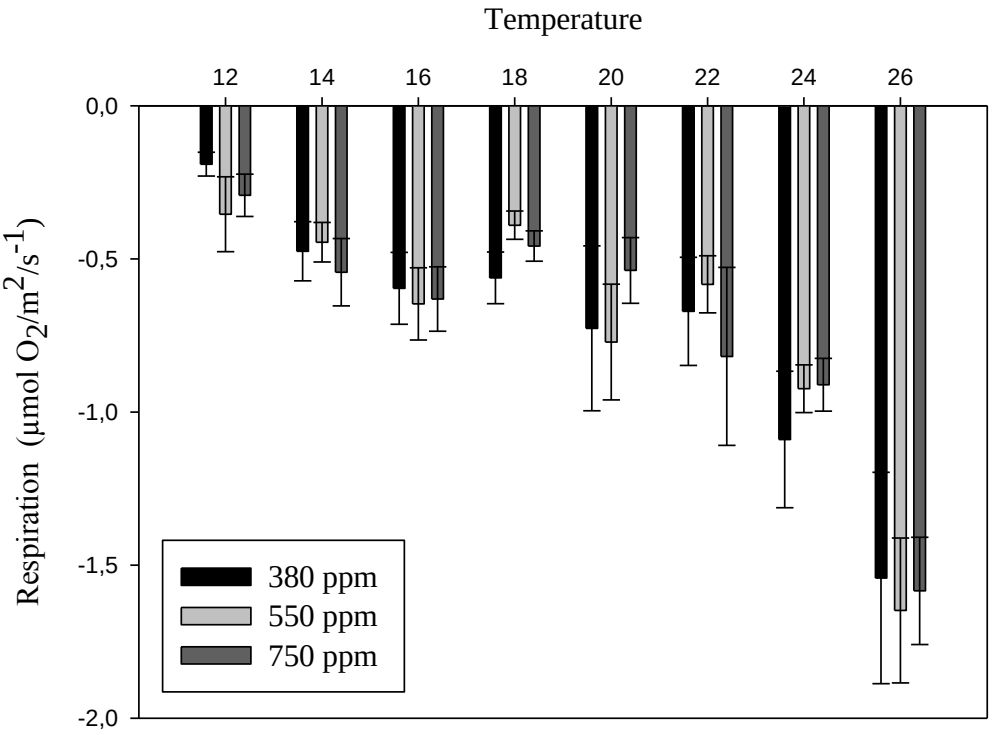

Figure 4.5. Mäerl respiration rates with errors bars according to the different level of CO_2 and exposed to the different temperature.

5. Discussion

5.1. Distribution

Phymatolithon calcareum is widely distributed all over the Atlantic Ocean, particularly in: the British Isles (Woelkerling & Irvine, 1986), Ireland (De Grave *et al*., 2000), France (Mendoza & Cabioch, 1998), Spain (Adey & McKibbin, 1970; Peña & Bárbara, 2008), Italy (Basso, 1998), NW Pacific (Konar *et al*., 2006), Canary & Madeira Islands (Afonso-Carrillo *et al*., 1985), Azores (Rosas-Alquicira *et al*., 2009) and Portugal (OSPAR commission, 2010).

During EMEPC campaign all dives where mäerl was found have been done on the depth between 17 and 20 m. The most abundance species was *P. calcareum*. As a result, tree new spots were found with mäerl species that have not been recorded anywhere else before. Two of them were found in front of Albufeira which has been considered as a new spot in Algarve coast. Therefore, these spots are required for more careful and detailed exploration in the future.

5.2. Growth

Buoyant Weight Technique was chosen for measuring the growth rate of *P. calcareum* because it is a simple and relatively accurate technique for the estimation of calcium carbonate mass (Potin *et al*., 1990). Also, it has such advantages as: relative independence from the amount of water contained in the plants; the amount of organic matter and the procedure of weighing, beside that it is nondestructive technique that keeping alive organisms. The same as at the work of Potin *et al* (1990) in this work BWT gives valuable results only for an aggregate of mäerl and not for individuals. Seasonal variations could not be measured since the only condition that was controlled is pCO_2.

From the studies that were done for *P. calcareum* it is known that this species is able to survive down to 2°C and dying at 0.4°C, and an optimal temperature for growth will be temperatures around 12-13°C (Adey & McKibbin, 1970). At the same time Blake and Maggs (2003) did not find an effect of temperature on the growth rate of *P. calcareum* comparing 10, 14 and 18°C. Absence of detectable stress at 25°C indicates that *P. calcareum* is more tolerant to higher temperatures than most of the subtidal red algae (Luning, 1990). In terms of salinity King and Schramm (1982) found that growth rate would be affected by salinities below 24 (practical more tolerant in low salinity conditions than other species (Birkett *et al.,* 1998), but in turn, further studies found that *P. calcareum* is significantly more tolerant than *L. glaciale* at 3 practical salinity units (psu) and 15 psu conditions (Wilson *et al.*, 2004).

In our studies, we can observe that weight increments decreasing with a time (Fig. 4.2, a) with significant difference in time $p<0.001$ (Two Way ANOVA test) that might be a reason of acclimatization to the continuously low light intensity (8 $\mu mol_{quanta} m^{-2} sec^{-1}$). Nevertheless, mäerl keep growing as we can see on the graph with absolute growth rate values (Fig. 4.2, b). As it was shown by Halfar *et al* (2011), temperature and light conditions influence growth rate. Besides that, local factors can influence the amount of light at a given site that explains differences in growth rates between individuals (Halfar *et al*., 2011). At the same time, in the studies made by Kamenos and Law (2010) with *Lithothamnion glaciale* was not found significant correlations between growth-increments width and water temperature. Steneck (1986) was also studing the growth of coralline corresponds with water temperature. In his studies he compared lateral growth rate of the four to five most abundant corallines living in shallow water zones in the different places such as Caribbean, Great Barrier Reef, Gulf of marine, and Pacific Northwest, and find out that thinner crusts generally grow faster than thicker ones . As a result, it was found that they grow faster in warmer water (18, 24ºC).

Blake and Maggs (2003) have established the relation between growth rate and temperature for *L. corallioides,* and they found that the optimal temperature for growth rate is 10.8 ºC. However, comparisons of growth rate at 10, 14 and 18ºC did not show any effect on *P. calcareum* (Blake & Maggs, 2003). Nevertheless, it is necessary to have an acclimation period of at least 5 months after temperature shifts (Adey & McKibbin, 1970).

In the field conditions, growth and calcification rate of the coralline algae used to increase with increasing temperature (King & Schramm, 1982; Blake & Maggs, 2003; Martin *et al*., 2006), but with the temperature above the thermal optimum have a detrimental effect (King & Schramm, 1982). For such species as *Porolithon gardineri* has been reported a 50% decrease in the growth rate and mortality events with increasing temperature for 2.5 – 4.5 ºC (Agegian, 1985). *L. cabiochae* has been even more susceptible to disease in the result of a thermal physiological stress (Martin & Gattuso, 2009).

In the present study for *P. calcareum* there was no significant correlation between weight increments and water temperature ($p=0.796$) but there is a significant correlation with time, which means that in this case mäerl grew with time without dependence of the temperature. Another factor that had influence on the growth rates was CO_2 level with significant correlation $p<0.001$.

5.3. Response of photosynthesis and respiration to elevated pCO_2 and temperature

Several studies have investigated the influence of elevated temperature and pCO_2 on the photosynthesis, calcification, respiration and growth rate of mäerl species (Table 5.1). These algae show a highly diverse set of responses to ocean acidification (Hurd *et al*., 2009) and these responses vary considerably even between species from the same family, depending on the different environment conditions (Harley *et al*., 2012).

With increasing pH values, calcification rates of the algae generally increased as well. But it was also found that for some species such as *Mesophyllum* sp. when incubated together with seagrasses there were increasing photosynthetic rates, thus, higher pH values. At the same time it was found that there were lower pH values obtained when the seagrasses were together with the algae during the photosynthesis than when there were just seagrasses. A reason for that could be a contribution of CO_2 from the algae's calcification process that moderated the pH increase due to seagrass photosynthesis (Semesi *et al*., 2009).

Most of the studies on mäerl reported an increase in photosynthesis under elevated temperature (Martin *et al*., 2006; Semesi *et al*., 2007; Cornwall *et al*., 2011; Borowitzka, 1981). The same trend in mäerl photosynthesis was observed under elevated pCO_2 (Semesi *et al*., 2009; Cornwall *et al*., 2011). In contrast, the opposite trend was reported for three species *Bossiella californica, Calliarthron tuberculosum* and *Corallina officinalis* in the long term of Bulach (2012). Martin *et al.,* (2013) as well found a decrease in photosynthesis under elevated CO_2 for crustose coralline alga *L. cabiochae.* Some of the studies showed weak response in photosynthesis to elevated pCO_2 and had been previously reported by several authors (Hofmann *et al.*, 2012; Semesi *et al*., 2009).

Table 5.1. Responses of mäerl photosynthesis, calcification, respiration and growth rate to reduced salinity and to elevated temperature, pCO_2 alone or in combination. Where "–" is indicated as decrease; +, increase; 0, no effect, *, the mark of the reference. Respiration and photosynthesis rates presented here were determined from measurements of oxygen and C_T exchanges or fluorescence. Calcification rates were determined from alkalinity anomaly or buoyant weight techniques. Growth was determined from variations in fresh weight, red alizarin staining or buoyant weight technique. § - Rhodoliths used in this experiment consisted of a mixed CCA community including *Lithophyllum* cf. *pallescens, Hydrolithon* sp. and *Porolithon* sp. (Martin *et al.*, 2013).

Effects of	↓Salinity	↑TºC	↑CO_2	T + CO_2	Reference
On:					
Growth rate					
Phymatolithon calcareum		0			Blake and Maggs (2003)
		0	-		Present study
Lithothamnion glaciale		0			Blake and Maggs (2003)
Lithothamnion corallioides		+			Adey and McKibbin, (1970); Potin *et al*. (1990); Blake and Maggs (2003)
Lithophyllum margaritae		+			Steller *et al*. (2007)
Hydrolithon sp.		+			Steller *et al*. (2007b)
Photosynthesis					
Phymatolithon calcareum		0			Wilson *et al*. (2004) in Martin *et al*. (2013)
			+		Present study
Bossiella californica			-		Bulach, 2012
Calliarthron tuberculosum			-		Bulach, 2012
Corallina officinalis			-		Bulach, 2012
Lithothamnion corallioides		+			Martin *et al*. (2006) in Martin *et al*. (2013)
			+		Semesi *et al*. (2009),Cornwall *et al*.(2011)
		0	0	0	Noisette *et al*. (2013)
Lithophyllum margaritae		+			Steller *et al*. (2007) in Martin *et al*. (2013)
Hydrolithon sp.			+		Semesi *et al*. (2009) in Martin *et al*. (2013)
Calcification					
Phymatolithon calcareum	-	+			King and Schramm (1982)
Bossiella californica			-		Bulach, 2012
Calliarthron tuberculosum			+		Bulach, 2012
Corallina officinalis			-		Bulach, 2012
Lithothamnion glaciale		+	-	0	Budenbender *et al.* (2011) in Martin *et a*l. (2013)
Lithothamnion corallioides		+	+*		Martin et al. (2006) in Martin *et al*. (2013), Noisette* *et al*. (2013)
Lithophyllum margaritae		+			Steller *et al*. (2007) in Martin *et al*. (2013)
Lithophyllum cf. *pallescens*, *Hydrolithon* sp. and, *Porolithon* sp. §			-		Jokiel *et al*. (2008) in Martin *et al*. (2013)
Hydrolithon sp.			-		Semesi *et al*. (2009) in Martin *et al*. (2013)
Respiration					
Phymatolithon calcareum		+	0		Present study
		+			Freitas (2012)
Lithothamnion corallioides		+	0*	0*	Martin *et al*. (2006) in Martin *et al*. (2013), Noisette* *et al*. (2013)
Lithophyllum margaritae		+			Steller *et al*. (2007) in Martin *et al*. (2013)
Hydrolithon sp			0		Semesi *et al*. (2009) in Martin *et al*. (2013)

However, there are not many studies done focused on the interactive effect of temperature and pCO_2 on the photosynthesis beside the study of Noisette *et al.* (2013), where *L.corallinoides* photosynthesis was not affected by temperature and pCO_2.

Koch *et al.*, (2013) found that in 82% of the experiments reviewed, elevated pCO_2 and temperature had a negative effect such as decline in calcification and growth of Chlorophyta and

Rhodophyta macroalgae. However, the effect on photosynthesis stays unclear, because the results are different for each of the species. A reason for it could be the use of different experimental designs or and/or physiological differences among species thalli.

In the present study *P. calcareum* was affected by elevated CO_2. It has been shown by the main parameters of P-I curves such as P_{max} that increased with higher CO_2 concentration. Such parameters as α and I_k did not show significant difference between the ambient and elevated pCO_2. Absence of the effect of CO_2 on the photosynthetic parameters such as α and I_k could be a feature of a potential acclimatization by shallower mäerl beds to high light intensities (Martin *et al.*, 2006).

One of the objectives of the present study was to test the effects of temperature and CO_2 separately and simultaneously on the respiration rate of *P.calcareum.* According to our results there is a significant effect of temperature on the respiration rate. The temperature effect on respiration rate has been reported for several species of mäerl with a trend of increasing respiration under elevated temperature (Martin *et al*., 2006, 2013; Semesi *et al*., 2009; Steller *et al*., 2007). Similar results were found in a previous study made for the bachelor project of Freitas (2012), where respiration continuously increased with temperature. Low level of respiration activity could be observed in the deep living algae as a strategy to prevent excessive carbon losses that occur through the release of CO_2 (Littler *et al*., 1986; Lüning, 1990). High respiration rates or low photosynthesis rates – both are indicators of low carbon efficiency (Touchette and Burkholder, 2000). Photosynthesis would be expected to decrease with increasing of temperature because respiration increases with temperature faster than does photosynthesis (Sharkey, 2005).

No significant effect of CO_2 was detected on *P.calcareum* respiration rates. This confirms the results from other studies in some species of soft macroalgae (Zou *et al*., 2011) and in crustose coralline algae (Martin *et al*., 2013; Noisette *et al*., 2013; Semesi *et al*., 2009). Zou *et al*. (2011) proposed two coexisting responses of respiration under elevated pCO_2: i) the stimulation of respiration by an increase in respiratory substrates such as soluble carbohydrates due to enhanced photosynthesis and ii) a reduction in maintenance respiration due to a decrease in tissue nitrogen content.

Overall effect of elevated pCO_2 and temperature on respiration rates was not significant, which agrees with the results obtained by Noisette *et al*. (2013).

Therefore, it could be concluded that there were significant effects of elevated pCO_2 on photosynthesis and no effect on respiration rates of *P.calcareum.* The main parameter that had a

significant effect on respiration was temperature. Such a high tolerance to the changes of temperature and level of CO_2 also could explain such a wide distribution of *P.calcareum.*

We can conclude that there is a strong need of further research on this topic. For example, to measure growth rate it would be useful to include such a parameter as temperature, and for respiration rates it would be nice to compare results from the long and short term experiments.

Furthermore, since *P.calcareum* has been shown to be sensitive to CO_2 increase associated with global warming the survival rates of mäerl may be in jeopardy as a result of ocean acidification.

Final synthesis

1. *Phymatolithon calcareum* is one of the most abundant mäerl species in the Algarve coast and was found to be present in the three new spots in Algarve.
2. Weight increments of *Phymatolithon calcareum* decreased with time and CO_2 concentration.
3. There were no correlation between weight increments and water temperature ($p=0.796$). In addition, the simultaneous effect of temperature and CO_2 concentration was found as a non-significant with $p=1.000$.
4. Absolute growth rate (%) of *Phymatolithon calcareum* has a tendency to decrease with time ($p=0.001$). Average absolute growth rate for system with CO_2 concentration at 380 ppm was 0.202 (g $CaCO_3$/day), for system at 550 ppm average value was 0.196 (g $CaCO_3$/day) and under pCO_2 of 750 ppm the mean value of absolute growth rate was 0.120 (g $CaCO_3$/day).
5. Photosynthesis increased under elevated pCO_2.
6. Respiration rate of *P. calcareum* increased with the temperature but not with CO_2 level.
7. Simultaneous high pCO_2 and temperature did not show an effect on respiration rate.

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