



# PHYSIOLOGICAL RESPONSES OF SWEDISH MAERL TO OCEAN ACIDIFICATION AND WARMING

Master's Thesis



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## ABSTRACT

Maerl, (Corallinales, Rhodophyta), are free-living calcareous algae found in coastal ecosystems. They form biogenic beds with complex structures in which other species can find refuge or on which other species can settle, which highlights their importance as an ecosystem. While many species have been investigated worldwide, maerl from the Swedish west coast are poorly studied. This report investigated both acidification and warming impacts on different physiological functions of Swedish maerl, including photosynthesis, respiration and calcification. The maerl were exposed to different pH levels and temperatures in both light and dark conditions to determine their physiological thresholds, where photosynthesis and respiration were measured via oxygen fluctuations, photosynthetic efficiency via PAM fluorometry and calcification via alkalinity titrations. It was found that neither photosynthetic nor respiratory oxygen exchange showed positive or negative trends when exposed to changes in pH. On the contrary, photosynthesis peaked at the natural ambient temperature of 16°C and respiration increased with increasing temperature. Photosynthetic efficiency also did not show any trends to pH changes. However, calcification showed a significant ( $p < 0.05$ ) negative response to pH in both light and dark conditions, with the response more severe in dark conditions. This suggests that decreasing pH may induce skeletal dissolution, and that photosynthesis could help buffer internal responses to external conditions. Carbonate production at ambient conditions in the light was calculated to be  $556 \pm 54 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$ , showing that Swedish maerl are just as, if not more, productive than maerl found elsewhere. Overall, this report showed that photosynthetic and respiratory thresholds may not be reached with acidification and that temperature increases could instead have much more severe consequences. It also showed that calcification thresholds will be met sooner rather than later, depending on acidification rates, in darker conditions for maerl found in temperate and possibly polar regions.

*Keywords:* Maerl, mitochondrial respiration, photosynthesis, calcification, thresholds

# 1. INTRODUCTION

## 1.1 *What is maerl and where is it found?*

Calcareous red algae (Corallinales, Rhodophyta) are part of a relatively understudied group of macroalgae that come in many shapes, sizes, morphotypes and growth forms (Costa et al., 2023). They can be geniculated or non-geniculated, where the non-geniculated morphotype has two different growth forms: encrustations that are attached to rocks or shells (Birkett et al., 1998) or free-living 3D structures that can be more spherical (Bosellini & Ginsburg, 1971; Teichert et al., 2014) or branched (Birkett et al., 1998; Foster, 2001) also known as rhodoliths or maerl. Nomenclature regarding these growth forms has been inconsistent throughout the literature (Bulleri et al., 2024; Costa et al., 2023; Foster, 2001; OSPAR Commission, 2008), with the terms rhodolith and maerl being used synonymously. Jardim et al. (2025) has recently described two different ways that both maerl and rhodolith terminology can be used as a way to unify the scientific community and decrease their previously interchangeable nature. Both terms can either include *sensu stricto* or *sensu lato*, or the type of growth form. By definition, rhodoliths can either include all ‘nucleated nodules of non-geniculate coralline algae and other non-coralline algae’ or specifically include only those of ‘free-living non-geniculate coralline algae’ (*sensu lato* and *sensu stricto* respectively). Maerl, on the other hand, can either be all ‘free-living non-geniculate coralline algae’ or ‘non-nucleated nodules of free-living non-geniculate algae and fragments of branching non-geniculate coralline algae’ (*sensu lato* and *sensu stricto* respectively). Based on these new suggestions and for the sake of this report, the term maerl will be used to describe the organisms used in this experiment while the term rhodolith will be used only when regarding other experiments and their organisms.

Maerl can accumulate to form large and structurally complex beds (Hall-Spencer et al., 2003) with a mix of living and dead pieces (Littler et al., 1991) that could potentially be classified as one of the four major benthic communities alongside kelp forests, seagrass meadows and non-geniculate coral reefs (Foster, 2001). These beds can provide refuge and stable microhabitats for some species (Bulleri et al., 2024), enhance recruitment for others (Foster, 2001; McCoy & Kamenos, 2015), both sessile and mobile, and also lay the foundation (as the deemed foundation species) for local and diverse facilitation cascades (Bulleri et al., 2024). These ecosystems are influenced by the physiology and morphology of the algal species laying the foundation, which can vary based on many factors and the relationship between those factors, with hydrodynamics, temperature and depth at the forefront (Bulleri et al., 2024; Illa-López et al., 2023).

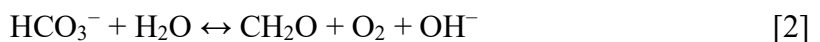
Maerl and maerl beds have a very large geographical range, from the tropics to the arctic circle (Bulleri et al., 2024), where temperature is one of the main driving factors for the location of certain species (Teichert et al., 2014). On a more local scale, temperature combines with other factors to have an influence on diversity, abundance and distribution, with those factors including depth, currents, substrata, salinity, irradiance and oceanic chemistry (Beer et al., 2014; Bosellini & Ginsburg, 1971; Canals & Ballesteros, 1997; Hall-Spencer et al., 2008a; OSPAR Commission,

2008). Maerl have been found at some of the deepest places known thus far to be inhabited by macroalgae (Voerman et al., 2022), between 3 and 200 meters (Littler et al., 1991; Roberts et al., 2002) with densities and percent coverage typically higher at lower depths (Teichert & Freiwald, 2014). Any spatial gaps between the different depths could be caused by the physical environmental (Illa-López et al., 2023; Littler et al., 1991), type of substrata (Steller & Moss, 1995; Teichert et al., 2014), and/or varying degrees of protection from wind and water currents (Bosellini & Ginsburg, 1971; Bosence & Wilson, 2003; Steller & Moss, 1995).

### 1.2 Physiology – Photosynthesis, respiration, and calcification

The influence that irradiance has is very important, as they require a sufficient amount of light for photosynthesis (Beer et al., 2014), which leads to calcification and growth (Schubert et al., 2024). Maerl have an ability to acclimate to low light intensities while simultaneously maintaining photosynthetic efficiency, largely due to their phycobilisome pigments (Voerman et al., 2022). Experimentally, the efficiency of the algae’s ability to utilize this light to trigger electron transport through from photosystem II to I can be measured via fluorescence (Beer et al., 2014). When a modulated beam of light flashes at the algae in either dark or light conditions, the light hits the pigment molecules in the antenna complex which transfers energy to their reaction centers and will either send the photons to the photosystems or be emitted as heat or fluorescence. This fluorescence is measurable and usable for quantifying the photosynthetic yield and efficiency. The measurements can be performed with a specific piece of equipment that utilizes a principle called pulse-amplitude modulated (PAM) fluorometry. With this equipment, one can obtain i. the maximum quantum yield from dark-exposed algae to show the degree of photoinhibition and any signs of stress and ii. the effective quantum yield from light-exposed algae (see more detailed explanations in section 2.3; Beer et al., 2000, 2014).

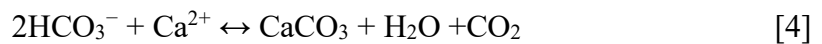
As photosynthesis (left to right) and mitochondrial respiration (right to left) have an inverse relationship, their chemical processes can be visualized via these reactions:



where the arrows represent the light (Beer et al., 2014). Reaction [1] shows the simplistic way of photosynthesis consuming  $\text{CO}_2$  and producing  $\text{O}_2$  (with respiration consuming  $\text{O}_2$  and producing  $\text{CO}_2$ ). As the preferred form of inorganic carbon ( $\text{C}_i$ ), uptake of  $\text{CO}_2$  can be done via two different mechanisms (Hurd et al., 2009): diffusion, which tends to be seen in algae found in environments with less light, or active transport with the help of carbonic anhydrase (CA) which is seen in environments with more light (Beer et al., 2014). Reaction [2] shows that  $\text{HCO}_3^-$  is converted into  $\text{CO}_2$  which can then be used in the Calvin-Benson cycle during photosynthesis (Beer et al., 2014). This reaction also supports the idea, which has experimentally been shown by Cook et al. (1988), that some macroalgae use  $\text{HCO}_3^-$  as a source of carbon for their photosynthesis instead of  $\text{CO}_2$ .

However, this usage varies along the latitudinal gradient, with species in more temperate or polar regions utilizing  $\text{HCO}_3^-$  less than those found in more tropical regions (Hofmann & Heesch, 2018).

The sum of the bases ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ) found in the water is defined as the total alkalinity (Beer et al., 2014). This presents one way in which calcification can be measured, because any changes in total alkalinity can be attributed to changes in speciation within the  $\text{C}_i$  system (Hurd et al., 2009). Alkalinity decreases when both bases are taken out of the water. The  $\text{C}_i$  form of  $\text{HCO}_3^-$  can either be decomposed into  $\text{CO}_3^{2-}$  and then used (Zhao et al., 2019) or used directly (Martin & Hall-Spencer, 2017) in combination with  $\text{Ca}^{2+}$  (sometimes replaced with  $\text{Mg}^{2+}$ ) to precipitate as  $\text{CaCO}_3$  in the form of either calcite or aragonite (McCoy et al., 2023). The difference between calcite and aragonite is mainly the structure, where calcite is more stable, and aragonite tends to be more soluble (Beer et al., 2014; Hill et al., 2015). However, another form of calcite called Mg-calcite is even more soluble than aragonite (Basso, 2012) and is the form that maerl deposit at their cell walls (Koch et al., 2013), with nucleation of these depositions being influenced heavily by photosynthesis (Frankignoulle et al., 1994; McCoy et al., 2023; Schubert et al., 2024) and regulated by intercellular pH (McCoy et al., 2023). Rates of maerl calcification vary based on geographical and temporal location, with a range of 8- to 75-  $\text{g CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$  (Van Der Heijden & Kamenos, 2015). The calcification process can be visualized via these reactions:



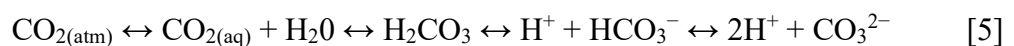
where reaction [3] shows the use of  $\text{CO}_3^{2-}$  and reaction [4] shows the use of  $\text{HCO}_3^-$  to form  $\text{CaCO}_3$  (calcium carbonate or sometimes in the form of calcite; Martin & Hall-Spencer, 2017). This deposition of calcium carbonate has been suggested to occur simultaneously as the growth of new cell walls (McCoy et al., 2023). Growth rates for maerl have shown much variability between reports and location, with Amado-Filho et al. (2012) reporting growth rates between 1- and 1.5-  $\text{mm y}^{-1}$  depending on depth, Littler et al. (1991) finding rates of less than 0.05  $\text{mm y}^{-1}$  in deep water rhodoliths, and Bosence & Wilson (2003) showing rates of between 0.1- and 1-  $\text{mm y}^{-1}$  in temperate species.

Regardless of whether growth and calcification do occur at the same time at and around the cell walls, both require the mineral saturation states of  $\text{Ca}^{2+}$ ,  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  to be higher than 1, allowing for easier precipitation of calcium carbonate (McCoy et al., 2023; Teichert et al., 2014). The saturation states are another factor that determines their location and ability to grow (Basso, 2012; Teichert et al., 2014). This has important implications for the concept of global carbonate production and their involvement and/or contribution to blue carbon (carbon storage from coastal marine and freshwater environments; Alongi, 2023). Carbon storage, also called carbon sinking, can be accomplished via carbon sequestration, which is defined as the “long-term storage of carbon dioxide from the atmosphere through biological, chemical or physical processes” (Hill et al., 2015). Algae are able to do this via the calcium carbonate they produce during calcification, however, the process of precipitating this produces  $\text{CO}_2$  which returns to the water (Koch et al., 2013) and is

therefore called carbon release. The impact of this output of CO<sub>2</sub> is still a bit uncertain, because the amount depends on many factors including water movement (Frankignoulle et al., 1994) as well as the environment and if other inhabitants in the area include photosynthesizers (Kalokora et al., 2020). This leads to much conversation and controversy throughout the literature around whether calcareous algae in general are able to sequester more carbon than they release, leading to either a net contribution to blue carbon (carbon sink), or a net release (carbon source). Teed et al. (2020) suggested that the weight loss of dead rhodoliths could account for up to 75% of carbonate gross production, and that the underestimations could be due to differences in the ways that growth is quantified. In addition, Cornwall et al. (2023) studied crustose coralline algae and found trade-offs in the carbonate production in conjunction with coral reefs after they had experienced major degradation, where the algae produced as much carbonate as the coral for a period of time. Both of these studies support the idea that they are carbon sources rather than sinks, though to varying degrees.

### 1.3 Anthropogenic effects

Unfortunately, the environment and atmosphere are changing at such a rapid pace that the capacity for maerl physiology to function properly and allow for long-term survival and growth is greatly hindered. Though there are many anthropogenic effects that negatively impact maerl, one of the most impactful is climate change and the altered oceanic chemistry and increasing temperatures that come along with it. Atmospheric CO<sub>2</sub> is increasing at a rate never seen before, where the increase over the past 17 years has exceeded rates previously recorded over a normal 1,000-year time scale. The annual means of global atmospheric CO<sub>2</sub> levels have already increased by over 40% compared to the pre-industrial levels and about 50% of that increase occurred after the 1980's (European Environment Agency, 2024). In order to reach equilibrium with the atmosphere, the oceanic uptake rates of CO<sub>2</sub> increase which increases the concentrations of dissolved C<sub>i</sub> (Koch et al., 2013) as well as H<sup>+</sup> concentrations. This then directly translates to more acidic conditions or lower pH levels (Orr et al., 2005). The entire process of carbonate chemistry can be visualized via this series of reactions that Doney et al. (2009) described:



It shows the process of atmospheric CO<sub>2</sub> dissolving, combining with water to create carbonic acid that easily loses H<sup>+</sup> ions to form HCO<sub>3</sub><sup>-</sup> and ultimately creating CO<sub>3</sub><sup>2-</sup>, meaning that the CO<sub>2</sub> increase affects both HCO<sub>3</sub><sup>-</sup> CO<sub>3</sub><sup>2-</sup> and thus calcification processes (as described in section 1.2). However, the increase in extra H<sup>+</sup> ions also means a decrease in pH. Over the same period as the change in atmospheric CO<sub>2</sub>, pH decreased from 8.11 to under 8.05 (this was in 2021; Copernicus Marine Service, 2021) which is equivalent to a 15% decrease since just 1985 and an even higher decrease of 40% since pre-industrial levels (European Environment Agency, 2024). Under different future scenarios, including best case and worse case, at the end of the century, the change in pH is expected to be -0.16 ± 0.002 and -0.44 ± 0.005 respectively while temperature changes are expected to be +1.42 ± 0.32 and +3.47 ± 0.78 respectively (Kwiatkowski et al., 2020). The degree

of change is not only variable with scenario, but it is also variable depending on the region and location (Feely et al., 2004; Orr et al., 2005), where higher changes are seen at higher latitudes (IPCC, 2007; Kwiatkowski et al., 2020) due, possibly, to the idea that gasses dissolve quicker and are held in higher concentrations in cold water compared to warm water (Weiss, 1974). It is important to note, however, that these estimates are focused on open ocean systems, so estimates for coastal areas could be different as pH fluctuates more as a natural result of biotic and abiotic processes (Beer et al., 2014).

Consequently, maerl photosynthesis, respiration and calcification processes exhibiting heightened sensitivities to these changes are variable and species-specific (Costa et al., 2023) and can be either reduced (Martin & Hall-Spencer, 2017) or accentuated (Noisette et al., 2013b) as a result of their location in higher latitudes because of the higher degree of change in acidification and temperature predicted for these regions (European Environment Agency, 2024; IPCC, 2007). This will put higher levels of stress at an increased pace on the algae, which will have ramifications on their enzymatic process and abilities to photosynthesize, respire and grow (Martin & Hall-Spencer, 2017). In addition, reaction [5] shows the way in which increased CO<sub>2</sub> uptake and H<sup>+</sup> concentrations potentially control the carbon species that are readily available for these processes (Koch et al., 2013), and this in turn influences the saturation states of vitally important minerals like calcite, which is used by maerl in the precipitation of their calcareous structures (section 1.2). If the saturation states get below 1, their structures will have a higher chance of dissolving (Feely et al., 2004; Orr et al., 2005) and this is also amplified in the polar and temperate regions (Andersson et al., 2008; Basso, 2012).

Both increases in temperature and acidification can be simulated via isolated experimental adjustments of pH levels and temperature, or these two variables can be combined to try and understand the added influence of lowered pH on thermal stress. When studied in isolation, pH has been shown to impact growth both indirectly – by causing abnormal development in young algal thalli compromising their ability and future potential to grow (Bradassi et al., 2013) – and directly, through reduced structural integrity (Ragazzola et al., 2013). Increased pCO<sub>2</sub> can cause reduced calcification and growth while eliciting highly variable responses in photosynthetic rates (Koch et al., 2013), where different components of photosynthesis are affected disparately. The components correlated to oxygen consumption and usage could decrease (Semese et al., 2009) while electron transport rates could remain largely unaffected (Schermer et al., 2016). Temperature, independently, also affects photosynthetic components, where the rates may increase (Sordo et al., 2019) until a threshold is met and a crash is induced (Dorey et al., 2013). Maximum photosynthetic yield (Fv/Fm) has been shown to be impacted by large decreases in pH but were unimpacted by temperature increases (Schermer et al., 2016), despite changes in photosynthetic-, respiratory- or calcification rates (Kroeker et al., 2010; Martin & Hall-Spencer, 2017). Respiration has also been shown to not be affected by changes in pH (Semese et al., 2009) but instead increased with increasing temperature (Noisette et al., 2013a). When both pH and temperature were combined,

the responses seen under thermal stress were accentuated when exposed to decreased pH (Martin et al., 2013; Martin & Hall-Spencer, 2017; Mccoy & Kamenos, 2015).

#### 1.4 Global and local relevance and importance

In a global context, maerl are invaluable in terms of the biodiversity they support and the microenvironments they are able to create (Bulleri et al., 2024). These algae may have the ability to acclimate or even adapt to the changing environmental conditions (Martin & Hall-Spencer, 2017), however, as climate change is occurring at such a fast rate, they might not have the time due to the fact that they are extremely slow growers (Birkett et al., 1998; Mccoy & Kamenos, 2015) and are sensitive to such changes. Once they have disappeared from a certain location, it is unlikely they will return (HELCOM, 2013; OSPAR Commission, 2008). This is especially concerning in higher latitudes where it is feared they could be significantly reduced by 2100 (Brodie et al., 2014) and taken over by other types of more fleshy algae that are more resilient (Martin & Hall-Spencer, 2017; Mccoy & Kamenos, 2015; OSPAR Commission, 2008; Peña et al., 2021). Some reports do not include maerl in their biodiversity as functional algae and instead report them as only substrate for other algae (Hily et al., 1992; Johansson et al., 1998). Those who do study maerl specifically have reported them around Europe in the Northeast Atlantic, near Ireland, Norway, the UK (Birkett et al., 1998; Hall-Spencer et al., 2008a) and around the Mediterranean (Peña et al., 2021), with highlighted species including *Boreolithothamnion glaciale*, *Lithothamnion corallioides*, *Lithothamnion tophiforme* and *Phymatolithon calcareum* (Costa et al., 2023).

Given the importance of maerl and the need to understand their functioning on a higher level, this report will concentrate on the highly understudied maerl found in Swedish waters (HELCOM, 2013; Johansson et al., 1998; King & Schramm, 1982; Rosenberg & Nilsson, 2005), with a focus on the species *Phymatolithon calcareum* and its physiological responses and calcification to acidification and warming specific to this location. Most of the studies focusing on this area of Europe are from over 20 years ago, hardly mention calcareous algae, and are only aimed at biodiversity and distribution. Though it is understudied, this species and *Lithothamnion corallioides* are both included in Annex V (b) of the EC Habitats Directive, occur in other habitats that are part of Annex I, and are also included in the UK Biodiversity Action Plan long list (Birkett et al., 1998). The 1992 Oslo Paris convention (OSPAR), with the aim of guiding international cooperation towards the protection of marine environments in this region, Northeast Atlantic, has also established maerl beds as threatened and in dire need of higher priority in terms of protection (Hall-Spencer et al., 2008a). Due to the fact that maerl is on the EU regulation list of marine habitats, Sweden is obligated to protect and restore these ecosystems. This led to the start-up of a project in 2022 at the University of Gothenburg, conducted by Lina Rasmusson, called “Fantastic maerl and where to find it”. This project is focused specifically on maerl beds off of Sweden’s west coast, including the Kattegat area, and has three main work packages aimed at providing insights into their distribution, biodiversity of organisms living in and among them, and their physiology and physiological responses to climate change and acidification (Rasmusson, 2021). This report has been designed based off of the third package.

### *1.5 Aims and hypotheses*

The aims of this report were to 1. gain a deeper understanding of maerl physiology, including calcification, photosynthesis and respiration, 2. to gain knowledge regarding physiological responses when exposed to different pH levels and temperatures (with a note that calcification and photosynthetic efficiency/maximum capacity were only assessed with regards to acidification), 3. to assess maerl production and contribution to local and global blue carbon and 4. to begin the development of methods suitable for gathering trustworthy data relating to their physiology and responses to acidification. In order to study these aims, oxygen exchange techniques was used to determine photosynthesis and respiration rates, alkalinity titrations was used to establish calcification rates and carbonate production, and PAM fluorometry was used to address photosynthetic efficiency and maximum capacity, all occurring in a laboratory setting. In order to address these aims, two main research questions were answered and tested via a number of hypotheses.

Research Question 1: At what pH and temperature will the species-specific tipping points for calcification, photosynthesis and respiration occur?

Hypotheses: i. Calcification rates will decrease with decreasing pH. ii. Photosynthetic efficiency and maximum capacity will be negatively impacted by decreasing pH. iii. Photosynthesis and respiration will increase with both decreasing pH and increasing temperature.

Research Question 2: What is the contribution of Swedish maerl to local and global carbonate production?

Hypothesis: i. Carbonate production contributions will vary depending on changes in pH on a local scale. ii. Carbonate production in Swedish waters at ambient conditions will be comparable to other global production rates found in the literature worldwide.

## **2. METHODS AND MATERIALS**

### *2.1 Site of interest*

The area within Kattegat, specifically Fladen, off of the Swedish west coast is unique to this region due to diverse offshore banks and this is where maerl beds can be found. This area is located in the northern part of the transitional zone between the North Sea with its marine water and the Baltic Sea with its brackish water. The brackish water coming up from the Baltic Sea floats at the surface and the distance it travels north is highly dependent on wind direction and strength. This forms a halocline at about 15m depth (SMHI et al., 2016), which when combined with tidal fluctuations and atmospheric pressure (Pedersén & Snoeijs, 2001), creates specific oceanic conditions that influence the distribution, structure and composition of the algae throughout the area (Pedersén & Snoeijs, 2001; Rinne & Kostamo, 2022). Species under this halocline have also been shown to be more diverse due to the increased stability in temperature and salinity (Rosenberg & Nilsson, 2005).

## 2.2 Specimen Collection

Hundreds of individual specimens of the maerl species *Phymatolithon calcareum* used for these experiments were collected at the offshore bank of Fladen, located within Kattegat (57.41333°N, 11.865278°W), by scuba diving at a depth of 23 meters. The maerl was contained in aerated water during the time of transport between this location and the Kristineberg Marine Research Center, which is located on the west coast in Kristineberg, just south of Lysekil (Figure 1).

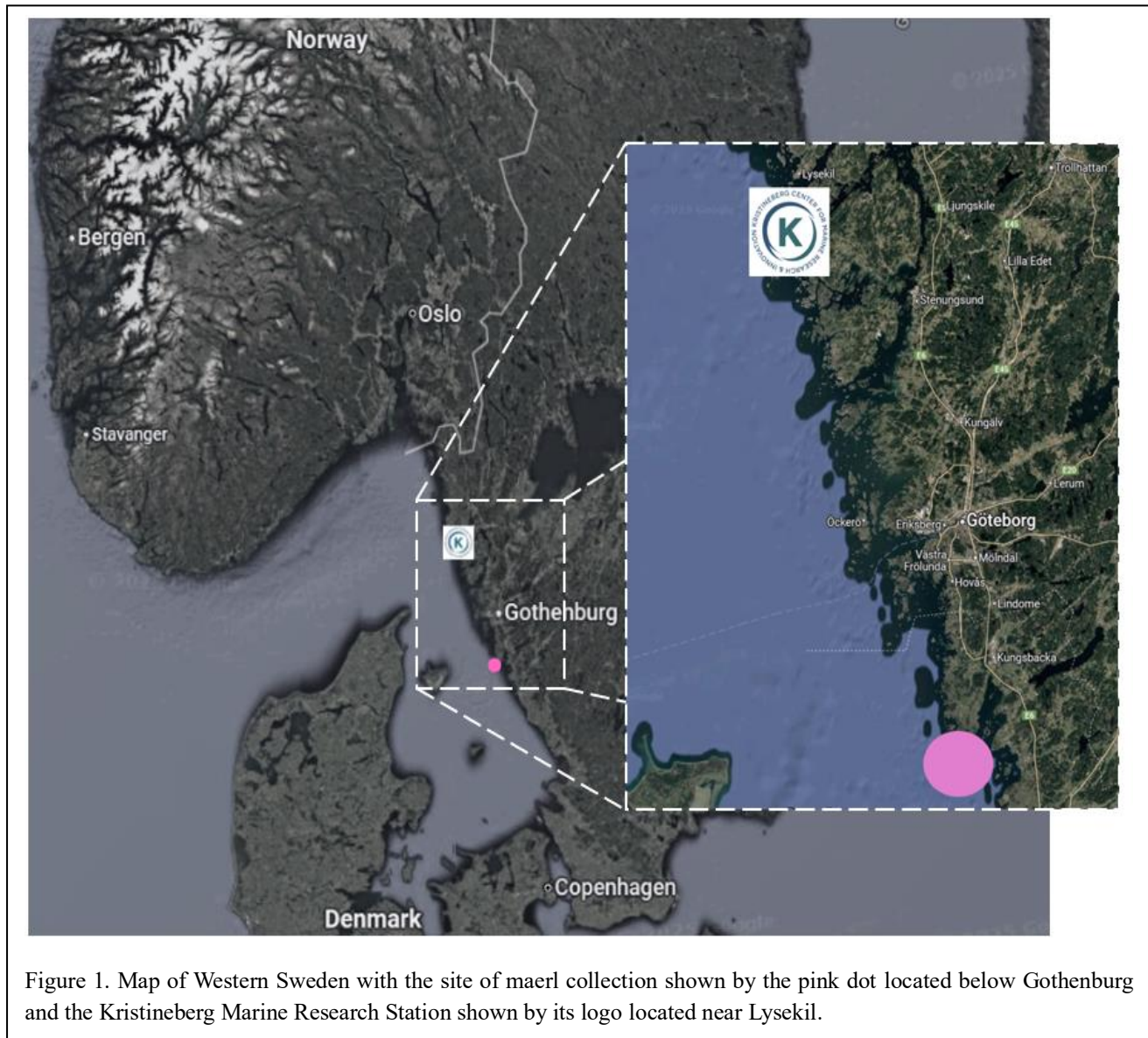


Figure 1. Map of Western Sweden with the site of maerl collection shown by the pink dot located below Gothenburg and the Kristineberg Marine Research Station shown by its logo located near Lysekil.

## 2.3 Acidification experimental procedures

### 2.3.1 Location

The experiments for varying pH levels were conducted at the Kristineberg Marine Research Center. All of the maerl specimens were kept in three separate acclimation tanks with constant running water that was pumped and filtered directly from the Gullmarsfjord at a depth of 35 meters with an average measured salinity of 30 ppt, exposing the algae to natural fluctuations in salinity, pH and dissolved ions and minerals. Prior to the start of the experiments, the maerl were flushed with seawater to remove any debris and visible biota. They were also exposed to an irradiance of about  $10 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR (photosynthetically active radiation) via illuminating tubes that were programmed on a 12:12 daily timer to mimic natural day and night oscillations.

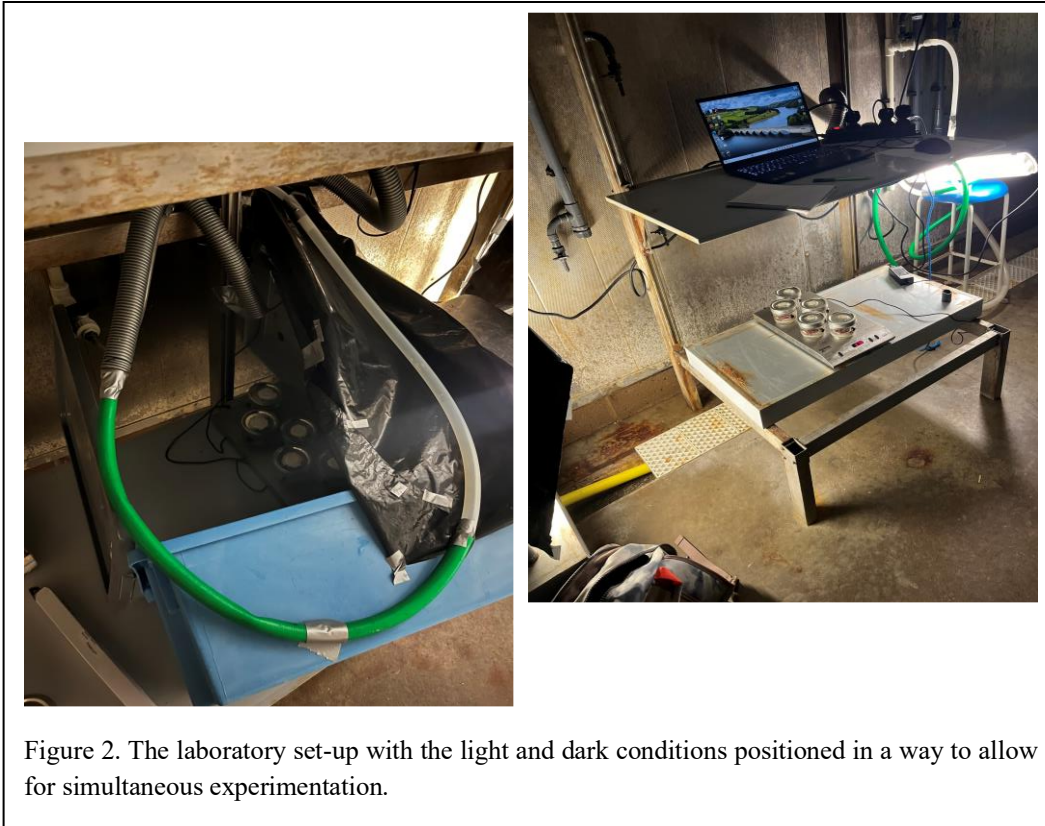
### 2.3.2 Randomization

Before the start of the experiments, maerl specimens were randomly selected from the acclimation tanks and divided into two groups within a thermo-constant ‘wet’ laboratory (including air and water). These two groups were kept in two containers with the same water, which was supplied to them within those acclimation tanks, at about  $17^{\circ}\text{C}$ . This was done in order to allow the maerl to rest between experiments, as the two groups were used in rotation. From within each group, the maerl were randomly selected again for each replicate of each experiment. They were also more thoroughly cleaned to remove any growth that had not previously been cleaned off.

### 2.3.3 Set-up

The laboratory was set up so that it was separated into light and dark conditions in order to trigger photosynthesis in the light and respiration in the dark simultaneously. Figure 2 shows how this was possible, with light conditions on one side and dark conditions on the other. Both set-ups had constant air flow from the pressurized air valves in the room to regulate temperature. Pressurized air was used because the Center did not have fans that could be used inside of a “wet” laboratory. Other materials that were used to set up the room were found in the general storage or in the laboratory being used, including various containers and black plastic bags that could not be penetrated by the light, tubing for the air, tape and two large (around one meter each) LED light strips with waterproof casings.

The containers and bags were used to create a wall-like barrier to prevent light from contaminating the dark area. The LED light strips were placed in such a way that the irradiance measured for each replicate was that of natural light conditions:  $10 + 0.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  PAR. This was measured using a light meter (Li-1000 Data Logger LI-COR Environmental; Nebraska, USA), where the sensor was placed facing the light source at  $180^{\circ}$  directly through the 250 ml glass jars used for each replicate (10 per experiment) and through the filtered water (see section 2.3.4) within each jar. This was done to account for the two factors (glass and water) in these experiments that would affect the amount of light reaching the maerl specimens, to make it



so that a more accurate measurement would be obtained. The set-up for each condition also included a 12-position magnetic stirrer, to ensure constant stirring within each jar for the entire duration of each experiment so that no diffusive boundary layers around the maerl would form and prevent any exchange of ions (Hurd et al., 2009). Both stirrers were set at 370 RPM. Each jar contained a plastic mesh platform on which the algae were placed to prevent any disturbance from the mixer.

#### 2.3.4 Experiment

Two controls were completed during the trial phase (Appendix 2 Figure 1) before the experiments started, one in the light and one in the dark, using the same filtered water as all other replicates. This was done to see if there was any extra growth in the water that would create background oxygen changes and would subsequently affect oxygen measurements and changes over the course of the experiments, and thus affect photosynthetic and respiratory rates. Due to lack of resources, these types of controls were only done for the ambient pH of 8.035.

On the first day of experimentation, two standards were created. First, to establish the photosynthetic light saturation of the maerl, rapid light curves were performed using a Pulse-amplitude modulated (PAM) fluorometer (Mini-PAM; Walz, Germany). The irradiance levels automatically produced were 0, 24, 45, 65, 91, 126, 192, 288, 424 and 627  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , and the output was the effective quantum yield ( $\Delta F/F_m'$ ; see section 2.5.1). This was done six separate times, with two curves being created on three random groups of maerl pieces. However,

only two measurements were recorded at 627 PAR. The measurements taken by the PAM device were converted into photosynthetic rates, as described in section 2.5. Second, a calibration and configuration of the oxygen sensors, which were in the form of spots, (PyroScience Oxygen Spots, V2.05; Aachen, Germany) was performed. The spots were pre-attached to the inside of each jar and a 2-meter-long fiber optic cable was used to detect oxygen molecules through these spots from the outside. The measurements were converted into oxygen partial pressure (hPa) and displayed on the pyro data inspector software on a computer. The configurations were based on the temperature of the water used, around 16°C, with an LED intensity of 40% to increase the sensitivity, and the detector amplification set at 10 M (400x), to reduce background noise and enhance any weak signals the sensors might experience (Pyro Developer Tool, n.d.).

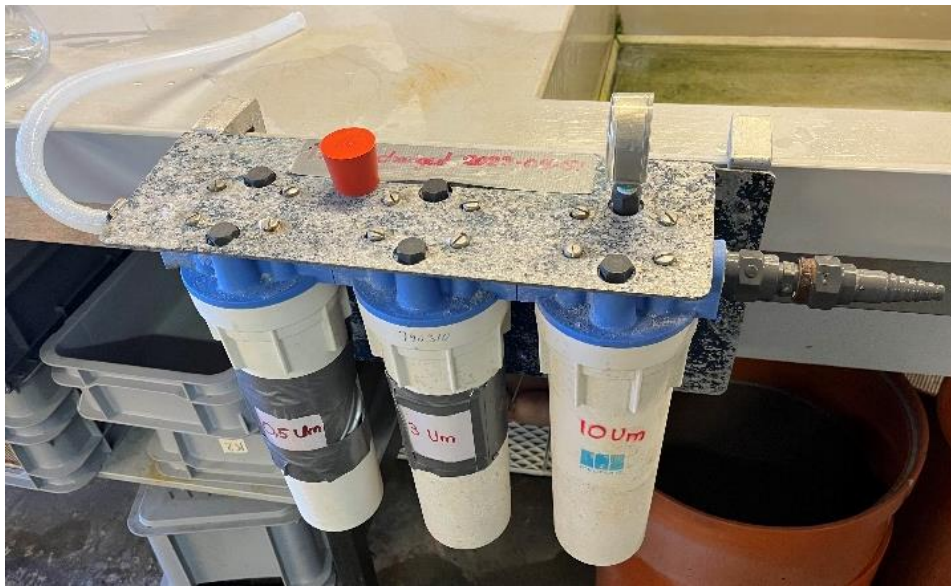


Figure 3. The deep water was cleaned via this 3-step filter before being used in the experiments, going from left to right, through the 10, then 3 and finally the 0.5  $\mu\text{m}$ .

A few hours before the commencement of each experiment, 500 ml of water was taken from the main deep-water source and filtered using the filter shown in Figure 3. The water was then transferred into the laboratory and bubbled with oxygen. The amount of water stored in the jar was large enough that the temperature did not have time to acclimatize, and the opening of the jar was small enough to reduce the gas exchange between the water and air, keeping the oxygen

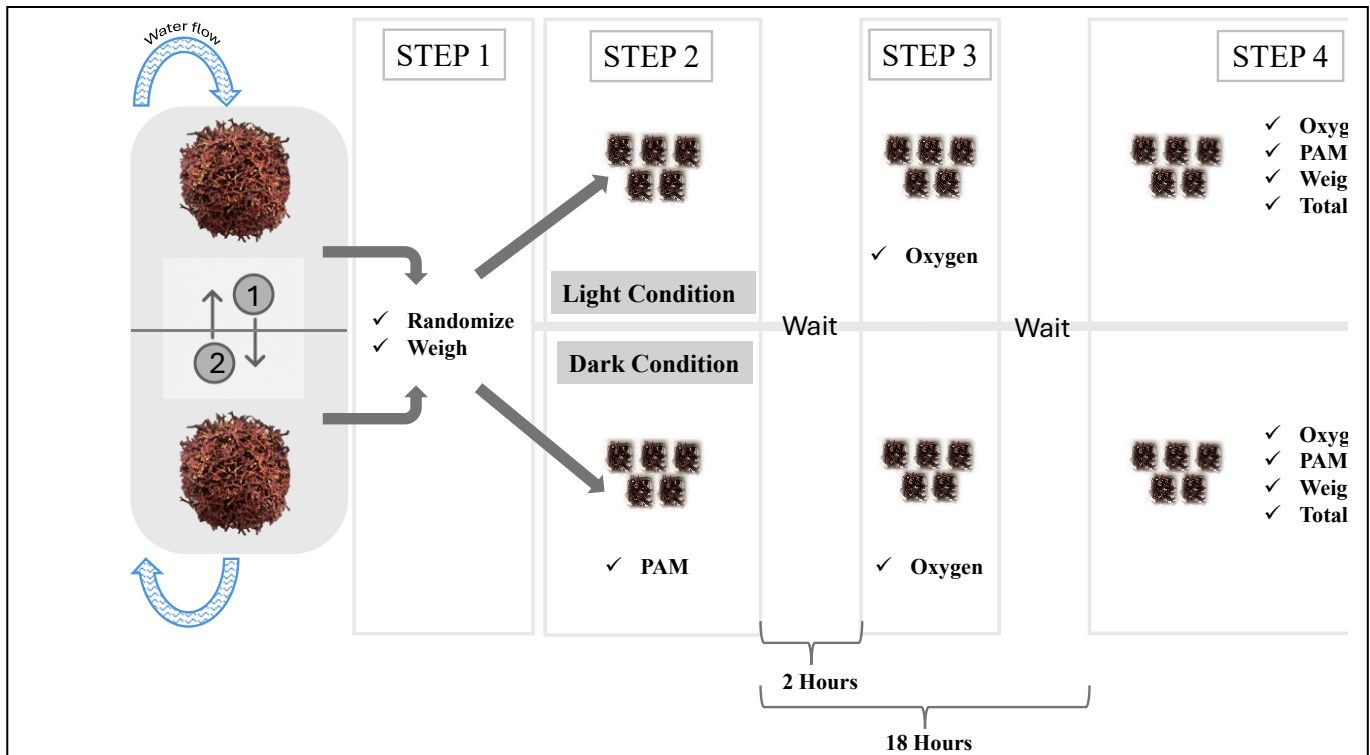


Figure 4. The experimental process (right to left) once the various pH levels had been created for each replicate of algae. The algae were first in two different groups (1 and 2) in separate containers with constant flowing water. The groups alternated for the experiments, giving time for the algae to rest. The algae from that experiment's designated group were then picked at random and weighed, using a 'wet' lab scale that measured to the nearest full number, into 10 different replicates of  $8 \pm 0.5g$  [step 1]. They were then further randomized into either light or dark conditions [step 2], where the photosynthetic maximum capacity ( $Y = F_v/F_m$ ) was measured (averaged from 5 replicate measurements) with a device using Pulse-amplitude modulated (PAM) fluorometry for only the replicates going into the dark. After two hours, the oxygen measurements were taken for both conditions on all replicates for 10 minutes with one-second intervals. The oxygen (hPa) measurements were then used to calculate photosynthetic and respiratory rates. This 2-hour delay in oxygen measurements was determined during the trial period, as this was the duration of time the oxygen levels took to regulate properly and to give a more accurate and consistent reading. Another 16 hours lapsed before the conclusion of the experiment. The oxygen and PAM measurements were taken again for both conditions, where the photosynthetically effective quantum yield ( $Y' = \Delta F/F_m'$ ) was measured (averaged from 5 replicate measurements) using the PAM device for the replicates in the light and  $Y'$  was measured again in the dark. The algae were also weighed again using the same scale, and two 25-ml samples were taken from each replicate to be brought to an alkalinity titration machine (SI-Analytics TitroLine alpha plus connected to automated TW alpha plus; Mainz, Germany), alongside two controls with 25ml each, to measure total alkalinity ( $T_A$ ) following the recommendations put forth by Dickson et al. (2007) with a precision of  $mmol\ kg^{-1}\ SW$ . These measurements were used for calculating the physical and chemical parameters of the water samples using the software  $CO_2\ sys.xls\ v.\ 10$  (Lewis & Wallace, 1998) as well as the calcification rates.

saturation steady at around 92-95%. It was found during the trial stages that oxygen saturation could not reach 100% consistently.

Excluding the ambient pH (8.035) experiment, oxygenated water from the jar was taken right before each experiment to be spiked with  $CO_2$  to decrease the pH. The levels of pH that were first used in the experiments were 8.035, 7.8, 7.6, 7.5 and 7.4. In order to create a more complete

picture as well as to find the physiological tipping points, pH levels in between these were chosen, resulting in further experiments with pH 7.9, 7.7, 7.45 and 7.42. All pH levels were measured separately in each replicate jar using a pH and temperature sensor (SI Analytics, Lab 870; Mainz, Germany) set up in a separate ‘dry’ laboratory. The algae were then randomized into these jars according to the experimental procedures shown in Figure 4, where they underwent 18 hours of exposure to each pH.

## *2.4 Temperature experimental procedures*

### 2.4.1 Location and Randomization

The maerl was taken from the Research Center and kept in aerated water during transport to a laboratory at the Department of Ecology, Evolution and Plant Sciences, Stockholm University. Approximately 50 liters of sea water from the station’s deep-water pump was brought as well to ensure that the same water source as the pH experiments would be used for both detaining the algae and temperature experimental procedures. Prior to experimentation, the maerl specimens were kept in aerated aquaria with a measured light PAR of 10 and kept on a light and dark 12:12 daily timer at a temperature of 12°C. The specimens used were randomly chosen and taken to the laboratory for the experiment.

### 2.4.2 Set-Up and Experiment

The experiments were conducted in 3 ml airtight chambers connected to Clark-type electrodes (model DWA1, Hansatech, King’s Lynn, UK). This system allows for between 3-5 chambers to be connected so that multiple replicates for that particular temperature can be exposed simultaneously. In each of the three chambers used for the experiment, sea water of the desired temperature was added. The pH was determined to 8.1 and salinity was ~27 ppm. Small plastic mesh was also placed inside the chambers to prevent any disturbances from the magnetic stirring bars which were used for ensuring constant water movement inside each chamber. During experiments, randomly chosen maerl specimens were put into the chambers for each temperature, with the manipulated temperatures being 6, 10, 14, 16, 20, 24 and 27°C. Each temperature was tested on at least two occasions with 1-3 replicates for each, totaling 4-6 replicates per temperature. Temperatures were controlled via a water bath (model RC20, Lauda, Lauda-Königshofen, Germany) which allowed for constant running water through water ‘jackets’ that surround each chamber to ensure stable temperatures for the entire duration of the experiments (Figure 5). During light exposure, a PAR of 25  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  was provided by a cold light source. In each experimental round, the set-up was first exposed to 30 minutes of dark conditions in order to acquire respiration rates followed by 30 minutes in light conditions immediately afterwards to acquire oxygen measurements for photosynthesis. After finishing this procedure, the algae were left to dry in an oven at 60°C for 24 hours before weighing to obtain dry weight.

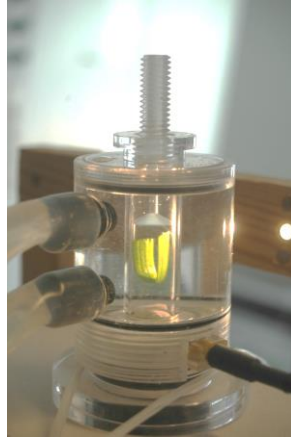


Figure 5. Airtight chamber used to measure the oxygen exchange from the red algae (shown here is green algae). The tubes attaching to it are for the continuous water circulation, and the mesh and oxygen electrodes are attached at the bottom.  
Photo: Lina Rasmusson

## 2.5 Calculations

### 2.5.1 Fluorescence

For the algae exposed to the light condition, the photosynthetically effective quantum yield (YII) was calculated as:

$$Y(II) = \Delta F / F_m' = (F_m' - F) / F_m' \quad [6]$$

where  $F$  is the fluorescence emitted at a certain light irradiance when some of the reaction centers are closed, and  $F_m'$  is the maximum fluorescence at that same light irradiance over a short time period (0.8 s) of saturating light when all of the reaction centers are closed, according to the guidelines from the PAM Walz, Germany. To create the light curves, the efficient quantum yield was used to calculate the relative electron transport rate (rETR;  $\mu\text{mol electrons m}^{-2} \text{s}^{-1}$ , relative):

$$rETR = Y(II) \times PAR \times 0.5 \quad [7]$$

where the PAR ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) is the irradiance measured for the experiment (10 PAR) and 0.5 is based on the assumption that half of the photon capture distribution goes to Photosystem I and the other half goes to Photosystem II, which is the one being targeted specifically. This can only be calculated as relative ETR due to the fact that the algae have a calcareous structure that will not allow for the light to pass through and therefore they do not have an absorption factor (Beer et al., 2014). For the algae exposed to the dark condition, the maximum photosynthetic quantum yield was calculated according to:

$$F_v / F_m = (F_m - F_0) / F_m \quad [8]$$

where  $F_m$  is the maximum fluorescence and  $F_v$  is the variable fluorescence measured as  $F_m$  minus the minimum fluorescence ( $F_0$ ).

### 2.5.2 Photosynthetic and respiratory rates

In order to calculate the net photosynthetic (NP) and respiratory ( $R_d$ ; in dark condition) rates for the pH experiments, the oxygen measurements (hPa) taken from the light and dark conditions respectively were used. The difference between the start and end measurements over the full 10 minutes were determined for each replicate. The differences were then adjusted for any background respiration found via control measurements taken prior to the start of the experiments (section 2.3.4). The rate of change (slope) over the 16 hours (Figure 4) was calculated in excel by finding the average of all measurements and then dividing by time. The rates were then calculated according to Kamermans et al. (2022):

$$\text{NP or } R_d (\mu\text{mol O}_2 \text{ g}^{-1} \text{ s}^{-1}) = ( (b/a) \times V_j / (\Delta t \times \text{FW}_a) ) \quad [9]$$

where  $b$  is the slope previously calculated ( $\text{hPa s}^{-1}$ ),  $a$  is the solubility ( $\text{mgO}_2 \text{ L}^{-1} \text{ hPa}^{-1}$ ) calculated from the Loligo website (<https://www.loligosystems.com/resources/online-oxygen-converter/>) depending on temperature, salinity and atmospheric pressure,  $V_j$  is the volume of water in the jars,  $\Delta t$  is the exposure time (s) and  $\text{FW}_a$  is the fresh weight (g) of algae as an average of the starts and ends of all replicates in light or dark for each pH. Gross photosynthesis was calculated as:

$$\text{GP} = |\text{NP}| + |R_d| \quad [10]$$

For the temperature experiments, NP and  $R_d$  were calculated as:

$$\text{NP or } R_d (\mu\text{mol O}_2 \text{ g}^{-1} \text{ s}^{-1}) = ([\text{O}_2] \times V_c) / (\Delta t \times D_a) \quad [11]$$

where  $[\text{O}_2]$  represents the concentration of  $\text{O}_2$  ( $\mu\text{mol O}_2 \text{ ml}^{-1}$ ),  $V_c$  is the volume (ml) of water in the chamber,  $\Delta t$  is exposure time (seconds). The GP was calculated according to equation [10] and the ratio between photosynthesis and respiration was calculated as  $\text{GP} : |R_d|$  for both pH and temperature.

### 2.5.3 Calcification and carbonate production

From the 25 ml samples of water taken from each replicate, the total alkalinity was measured, as described in Figure 3, and was used to further calculate the calcification via the alkalinity anomaly technique (Smith & Key, 1975) where there was a ratio of 1:2 between the precipitation of 1 Mol of calcite and a reduction in total alkalinity ( $T_A$ ) of two molar equivalents (Chisholm & Gattuso, 1991). Thus, calcification rates for light ( $G_l$ ) and dark ( $G_d$ ) were calculated according to:

$$G (\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ min}^{-1}) = -1 ( \Delta T_A \times V_j ) / ( \text{FW}_a \times 2 \times \Delta t ) \quad [12]$$

where  $\Delta T_A$  ( $\mu\text{mol O}_2 \text{ L}^{-1}$ ) is the change in total alkalinity from the control divided by  $\text{FW}_a$  and  $\Delta t$  (min). The  $T_A$  was also used to estimate the carbonate system parameters (Table 1) through the use of the software  $\text{CO}_2 \text{ sys.xls v. 2.1}$  (Lewis & Wallace, 1998), including the saturation levels of

calcium and aragonite ( $\Omega$ ). To measure the carbonate production (CP), based on the area in which  $8 + 0.5$  g of algae fit, the  $T_A$  was used according to:

$$CP \text{ (g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}) = -1 \text{ ( } \Delta T_A \times V_j \times B \text{ ) / ( FW}_a \times 2 \times \Delta t \text{ )} \quad [13]$$

where  $T_A$  (g CaCO<sub>3</sub> kg<sup>-1</sup>) is multiplied by volume (L) and the biomass of the algae (B; g m<sup>-2</sup>) and is divided by 2 (Chisholm & Gattuso, 1991), the weight (g), and  $\Delta t$  (yr).

## 2.6 Statistical Analysis

Microsoft excel (v. 2504) was used for data calculations and R Studio (v. 4.3.2; RStudio Team, 2023) was used for all statistical analyses. The packages used were: dplyr, ggplot, ggpubr, ggtext, readr, scales and tidy. The  $\alpha$  and  $\beta$  slopes, as well as the  $E_k$ , were calculated directly in R from the light saturation curves data. For testing normality and homogeneity, the Shapiro-Wilks test and Fligner test were used based on their robustness to small data sets for the fluorescence, photosynthetic, respiratory and calcification data sets. For analysis on light and dark conditions separately over all pH levels, an ANOVA test was performed for fluorescence, for the light start and dark start and end data sets, photosynthesis, respiration, calcification and carbonate production. An ANOVA test was also performed on photosynthesis for varying temperatures, though the Kruskal-Wallis test was performed for respiration. This test was also performed for the rETR and gross photosynthesis : respiration ratios. T-tests were performed when comparing start and end values for fluorescence or comparing light and dark between each pH level for calcification. TukeyHSD or dunnTests were performed if any of the ANOVA or Kruskal-Wallis tests showed significance. It must also be noted that a few major outliers were removed prior to analysis due to potential equipment or human error.

## 3. RESULTS

### 3.1 Fluorescence

The light curves measured prior to experimentation can be seen in Appendix 2, with calculated  $\alpha$  and  $\beta$  slopes as 0.019 and -0.006 respectively and an  $E_K$  of 314  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Appendix 2 Figure 2).

The photosynthetically effective quantum yield ( $Y_{II}$ ;  $\Delta F/F_m'$ ) calculated across all pH levels showed no significance (ANOVA,  $p > 0.05$ ), and this was also seen for the rETR (Kruskal-Wallis,  $p > 0.05$ ; Table 2). The values for  $\Delta F/F_m'$  ranged between 0.54 and 0.63 and the values for rETR ranged between 2.71  $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$  and 3.15  $\mu\text{mol electrons m}^{-2} \text{ s}^{-1}$  with the same variation in values as  $\Delta F/F_m'$  due to their relationship.

The maximum photosynthetic yield ( $F_v/F_m$ ) showed significance in the values taken before the experiments across pH levels, with the difference between pH 7.7 and 7.4 driving the significance seen (Appendix 2 Table 1; Figure 4). Significance was also seen between the before and after exposure values within each pH level (Appendix 2 Table 1; Figure 4). The values for 'before' ranged between 0.59 and 0.68 and those taken afterwards ranged between 0.42 and 0.64.

Table 2. The relative electron transport rates for maerl when exposed to different pH levels calculated based off of the  $\Delta F/F_m'$  (photosynthetically effective quantum yield), as explained in section 2.5.1.

pH	$\Delta F/F_m'$	rETR
8.035	0.57	2.86
7.9	0.61	3.03
7.8	0.58	2.89
7.7	0.56	2.79
7.6	0.54	2.71
7.5	0.60	3.02
7.45	0.63	3.15
7.42	0.59	2.97
7.4	0.57	2.86

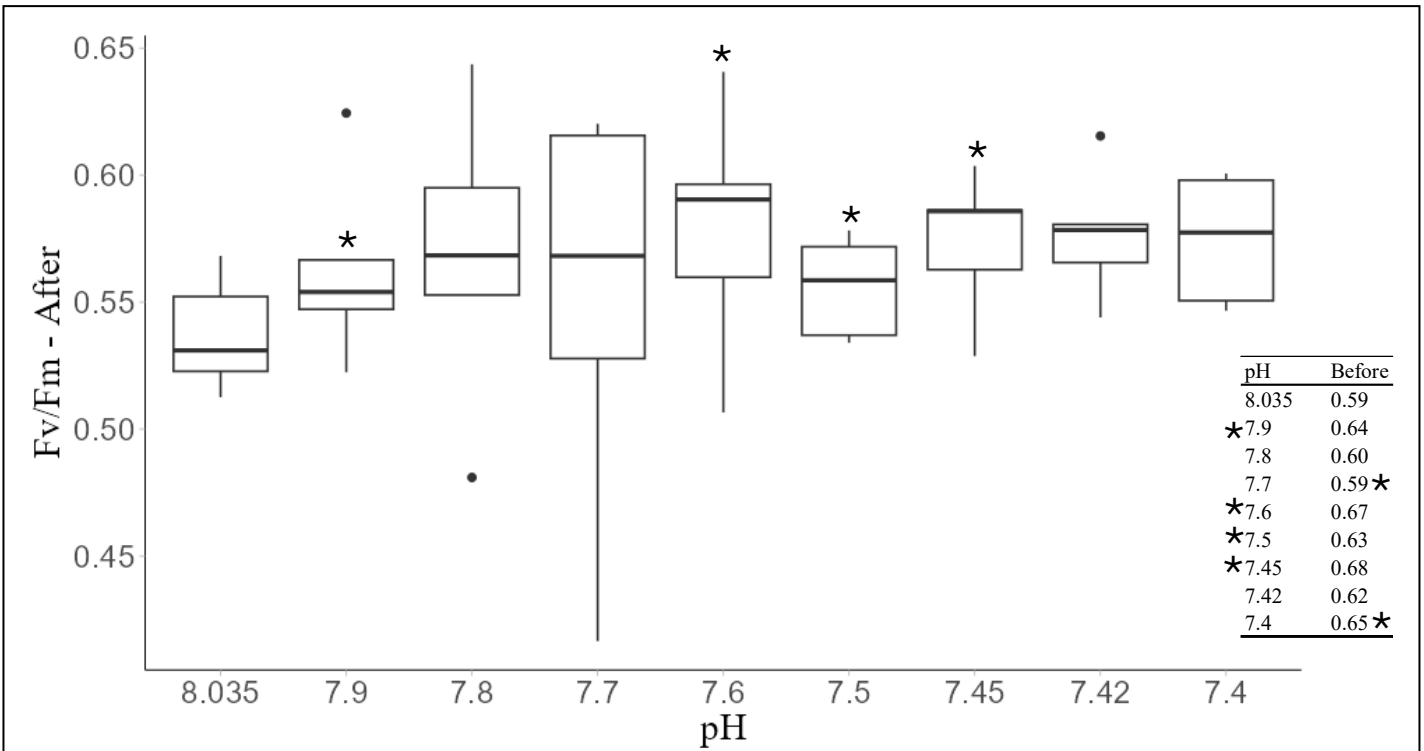


Figure 6. The maximum photosynthetic yield ( $F_v/F_m$ ) of maerl after exposure to different pH levels for 18 hours in dark conditions. The table within shows the  $F_v/F_m$  values before the maerl were exposed across all pH levels. Significance was seen for the before values across pH levels ( $p > 0.05$ ) and between the start and end values across pH levels ( $p > 0.05$ ), with the asterisks on the right side of the table representing the pH levels driving the significance within the before values and the asterisks on the left side of the table representing the pH levels driving the significance between the start and end values as indicated by Tukey HSD tests ( $p > 0.05$ ).

### 3.2 Photosynthetic and respiratory rates

The photosynthetic rates, despite showing significance (ANOVA,  $F = 2,51$ ,  $p < 0.05$ ), did not show any specific post hoc significance or trend after exposure to the varying pH levels. However, the highest rates on average were found at the ambient pH level of 8.035 ( $2.8 \times 10^{-9} \mu\text{mol O}_2 \text{ L}^{-1} \text{ s}^{-1}$ ) and the lowest on average ( $1.6 \times 10^{-9} \mu\text{mol O}_2 \text{ L}^{-1} \text{ s}^{-1}$ ) were measured pH 7.4 (Figure 5a). In contrast, the photosynthetic rates after temperature exposure showed the highest rates at the ambient temperature of 16°C ( $2.1 \times 10^{-4} \mu\text{mol O}_2 \text{ L}^{-1} \text{ s}^{-1}$ ) and decreased with both decreasing and increasing temperatures. Significance was seen via a post-hoc test specifically between the rates for 16°C and to both 27°C ( $5.9 \times 10^{-5} \mu\text{mol O}_2 \text{ L}^{-1} \text{ s}^{-1}$ ) and 10°C ( $9.2 \times 10^{-5} \mu\text{mol O}_2 \text{ L}^{-1} \text{ s}^{-1}$ ; Appendix 2 Table 2; Figure 5b). Overall, photosynthetic rates were lower in pH experiments compared to those in the temperature experiments (Figure 5).

The respiration rates also did not show any positive or negative trend after exposure to decreasing pH levels, with the highest rates measured at the ambient pH 8.035 ( $-3.3 \times 10^{-9} \mu\text{mol O}_2 \text{ L}^{-1} \text{ s}^{-1}$ ; Figure 5c). There was significance seen with a post hoc test between pH 8.035 and the

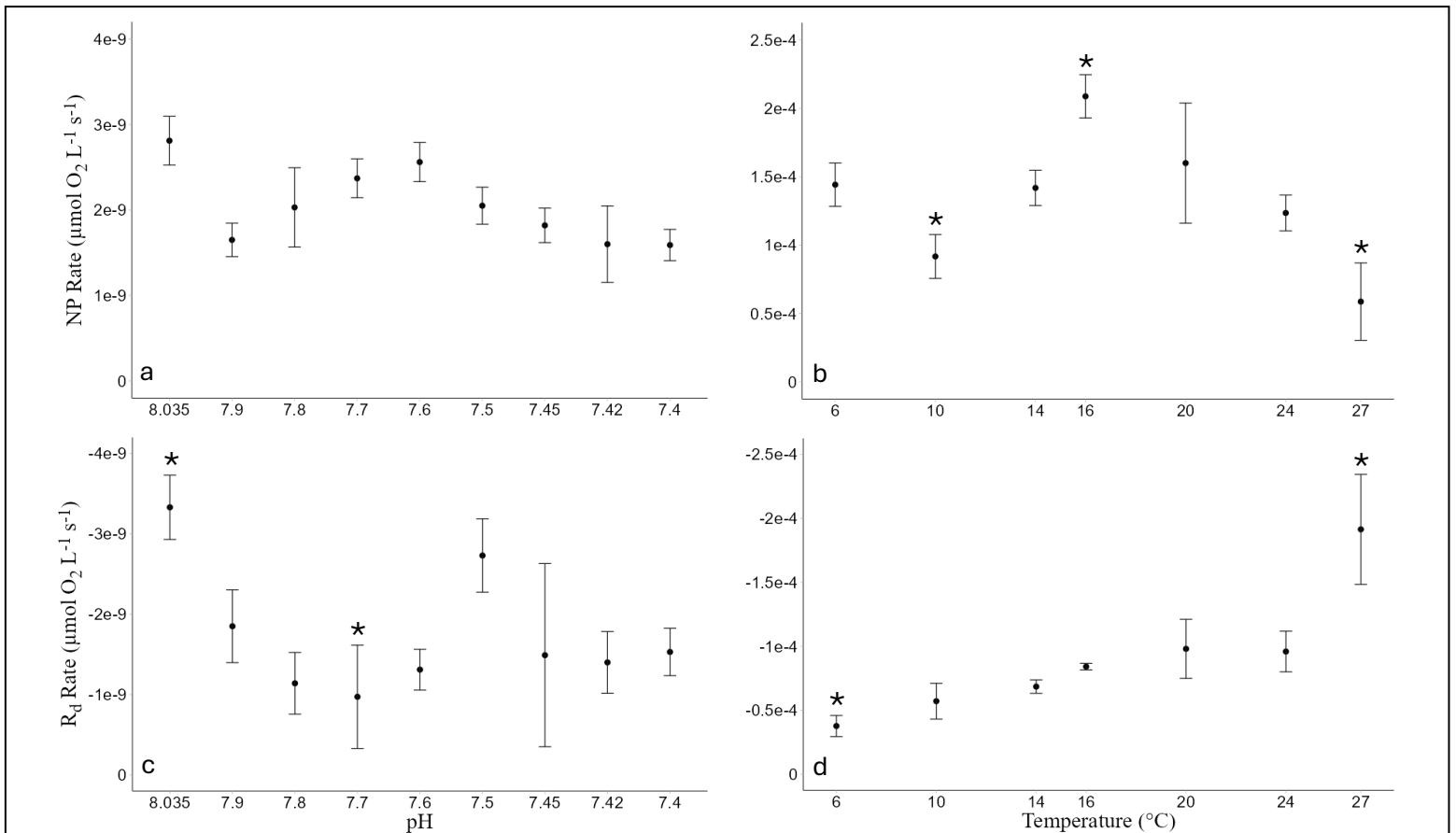


Figure 5. Net photosynthetic (NP; a and b) and dark respiratory (R<sub>d</sub>; c and d) rate responses of maerl when exposed to a range of pH levels and temperature as shown by averages of all replicates with SE lines. Significance is seen in all four experiments ( $p < 0.05$ ) and the asterisks show the ANOVA Tukey HSD adjusted significance ( $p < 0.05$ ) for b. and c. and the Kruskal-Wallis dunnTest adjusted significance ( $p < 0.05$ ) for d. No ANOVA Tukey HSD adjusted significance was found in a.

lowest rates for respiration found at pH of 7.7 ( $-9.7e-10 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ; Appendix 2 Table 3) and not at pH 7.4 like with photosynthesis (Figure 5c). There was also a spike in rates at pH 7.5 ( $-2.7e-9 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ; Figure 5c). On the other hand, the respiratory rates when exposed to changes in temperature showed a significant increase with increasing temperature, with a post-hoc test revealing the lowest temperature of  $6^\circ\text{C}$  ( $-3.8e-5 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ) and the highest of  $27^\circ\text{C}$  ( $-1.9e-4 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ; Figure 5d) driving the significant result (Appendix 2 Table 4). When looking at the standard error for each measurement, though, there was less variation at  $16^\circ\text{C}$  and  $14^\circ\text{C}$  compared to the rest of the temperatures. Respiratory rates overall were also much lower in the pH experiments compared to the temperature experiments.

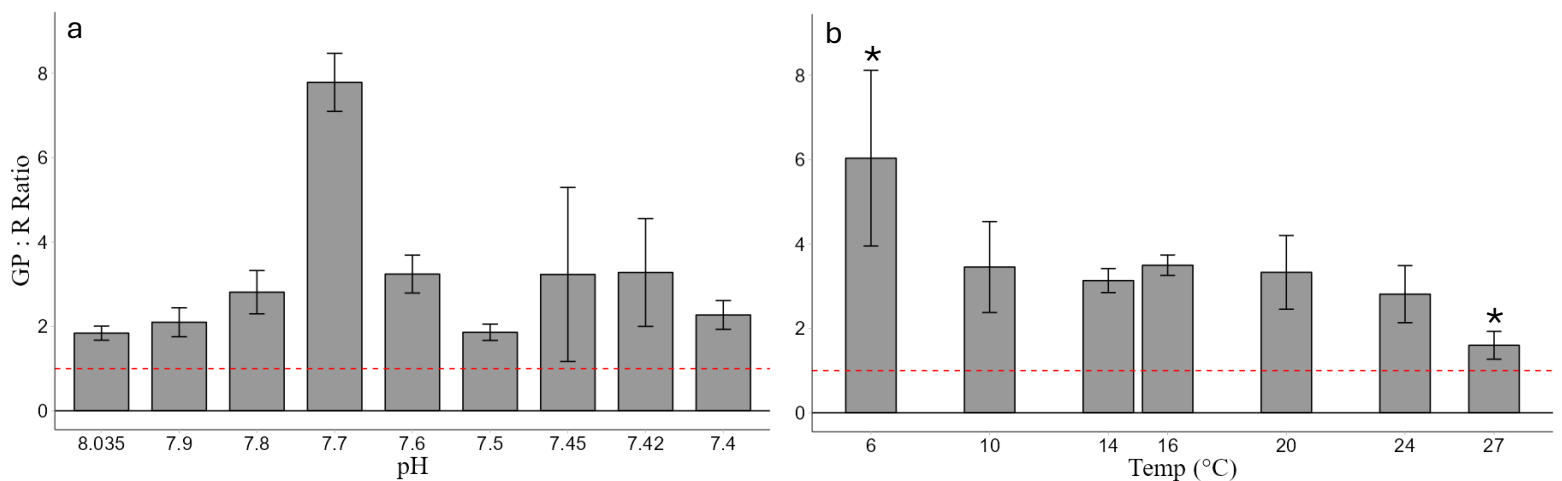


Figure 6. The gross photosynthesis : respiration (GP : R) ratio for the maerl over varying pH levels (a) and temperature (b), with a dotted red line indicating a ratio value of 1. The values are shown as averages of all replicates with SE lines. The asterisks shows that the significance seen across temperatures ( $p < 0.05$ ) is driven by the  $6^\circ\text{C}$  and  $27^\circ\text{C}$  based on the dunn Test performed ( $p < 0.05$ ). No significant effects of pH were found.

Gross photosynthesis (GP) showed an increasing trend twice over the various pH levels. The first was from the ambient pH 8.035 ( $-3.5e-10 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ) to 7.7 ( $1.4e-9 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ) and the second was from pH 7.5 ( $-6.7e-10 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ) to 7.4 ( $5.6e-11 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ; Appendix 2 Figure 3). On the contrary, gross photosynthesis had significant differences across temperatures, with a general decrease with increasing temperature (Appendix 2 Figure 3). The significant differences were driven, via a post-hoc test, by the differences from the highest temperature of  $27^\circ\text{C}$  ( $-1.3e-4 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ) to  $20^\circ\text{C}$  ( $6.2e-5 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ),  $16^\circ\text{C}$  ( $1.3e-4 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ),  $14^\circ\text{C}$  ( $7.3e-5 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ) and  $6^\circ\text{C}$  ( $1.1e-4 \mu\text{mol O}_2 \text{L}^{-1} \text{s}^{-1}$ ; Appendix 2 Table 2; Appendix 2 Figure 3). When comparing gross photosynthesis to respiration as a ratio (GP : R), there was no trend across pH levels, with the exception of an increase at pH 7.7 up to a value of 7.8 (Figure 6a). On the contrary, GP : R showed a decreasing trend with increasing temperature, with significance specifically seen, after a post-hoc test, between the highest value found at  $6^\circ\text{C}$  and the lowest at  $27^\circ\text{C}$  with values of 6.0 and 1.6 respectively (Figure 6b; Appendix 2 Table 2). All values across all pH levels, as well as temperatures, had values above 1.

### 3.3 Calcification and carbonate production

Calcification rates showed a significant difference in tipping point (threshold) between light and dark conditions in relation to decreased pH levels (Appendix 2 Table 5). Calcification rates transitioned from positive calcification to dissolution between pH 7.9 and 7.8 with averages of  $0.27 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ m}^{-1}$  and  $-0.44 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ m}^{-1}$  respectively in the dark and between pH 7.42 and 7.4 with averages of  $0.0082 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ m}^{-1}$  and  $-0.24 \mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ m}^{-1}$  respectively in the light (Figure 7). Significant differences in the rates across the pH levels, as shown via a post-hoc test, were seen specifically between ambient pH 8.035 and pH 7.4 in the dark and between the two lowest pH levels (7.42 and 7.4) to the rest of the pH levels (Appendix 2 Table 6; Figure 7).

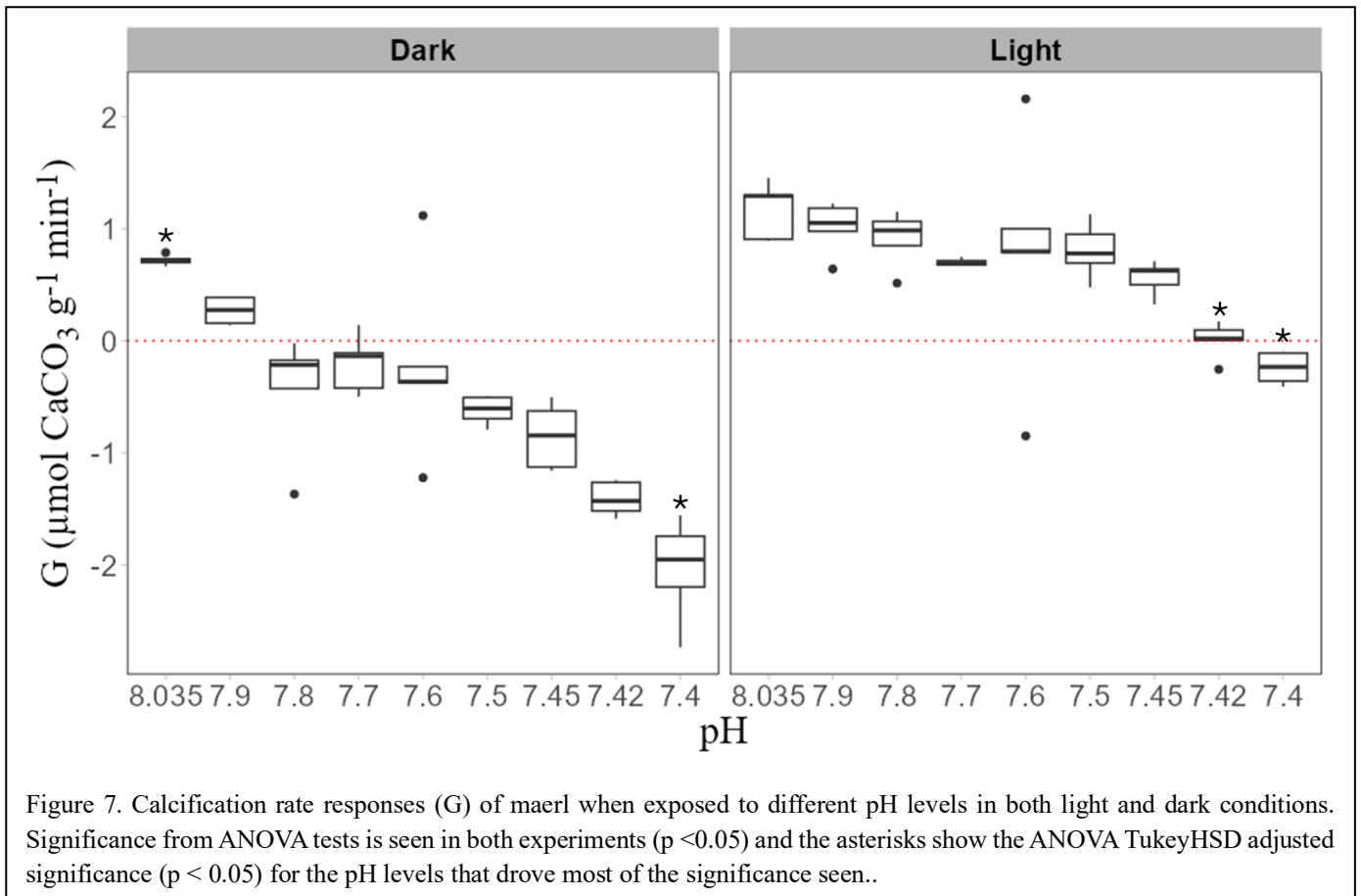


Figure 7. Calcification rate responses (G) of *maerl* when exposed to different pH levels in both light and dark conditions. Significance from ANOVA tests is seen in both experiments ( $p < 0.05$ ) and the asterisks show the ANOVA TukeyHSD adjusted significance ( $p < 0.05$ ) for the pH levels that drove most of the significance seen..

The carbonate production in the light at ambient pH 8.035 was calculated to be  $556 \pm 54 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  and is shown in Table 3 with comparisons to other production rates found in other reports. Table 3 also shows that this study's production rate is within the range of rates found for temperate and polar regions. Carbonate production, across all pH levels, showed a significant decrease between (Appendix 2 Table 7) and within both the light and dark conditions (Appendix 2 Table 8), with shifts from positive to negative production values seen at the same pH levels as

Table 3. Synopsis of carbonate production as  $\text{g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  ordered from south to north as reference for the production from this present study.

Location	Depth (m)	Latitude	Condition	Species	Region	Carbonate Production ( $\text{g CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ )	Reference
Abrohols Shelf, Brazil	20-100	19.75°S	<i>In situ</i> ; Depth variation	<i>Lithothamnion glaciale</i> as majority	Temperate	1000 ± 700	Amado-Filho et al. 2012
Algarve, Portugal	?	37.18°N	Temp; $\text{pCO}_2$	<i>Phymatolithon lusitanicum</i>	Temperate	237 - 1743	Sordo et al. 2019+
Balearic Platform, Mediterranean	40-85	40.50°N	<i>In situ</i> ; Depth variation	Various Maerl Species	Temperate	210	Canals and Ballesteros, 1997
Conception Bay, Canada	16	47.59°N	Percent DW change	<i>Lithothamnion glaciale</i>	Subpolar	196 ± 7	Teed et al. 2020
Conception Bay, Canada	16	47.59°N	Extension rates	<i>Lithothamnion glaciale</i>	Subpolar	326 ± 17	Teed et al. 2020
Bay of Brest, France	<10	48.35°N	<i>In situ</i> ; Ambient, Seasonality	<i>Lithothamnion corallioides</i> as majority	Temperate	487	Martin et al. 2007
Bay of Brest, France	0-10	48.35°N	<i>In situ</i> ; Ambient	<i>Lithothamnion corallioides</i>	Temperate	876	Potin et al. 1990
Mannin Bay, Ireland	<10	53.46°N	Ambient	<i>Lithothamnion corallioides</i>	Temperate	212 - 1197	Bosense and Wilson, 2003
Mannin Bay, Ireland	<10	53.46°N	<i>In situ</i> ; Ambient	<i>Phymatolithon calcareum</i>	Temperate	79 - 422	Bosense 1980
Mannin Bay, Ireland	<10	53.46°N	<i>In situ</i> ; Ambient	<i>Lithothamnion corallioides</i>	Temperate	29 - 164	Bosense 1980
<b>Kattegat, Sweden</b>	<b>23</b>	<b>57.41°N</b>	<b>pH 8.035; Light</b>	<b><i>Phymatolithon calcareum</i></b>	<b>Temperate</b>	<b>556 ± 54</b>	<b>Present Study</b>
Cape Rubin, Svalbard	40-50	80.32°N	Seasonality, pH 8.07-7.52	<i>Lithothamnion glaciale</i>	Polar	314 ± 78	Büdenbender et al. 2011

+The production rates were converted from  $\mu\text{mol CaCO}_3 \text{ g}^{-1} \text{ hr}^{-1}$  where the assumed biomass (g) per  $\text{m}^2$  was the same as that found in this study, and the conversion from  $\mu\text{mol}$  to g came from the molarity (M) of  $\text{CaCO}_3$ .

with the calcification rates (Appendix 2 Table 9). The change from pH 7.9 to 7.8 showed a change in average production values from  $135 \pm 34 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  to  $-218 \pm 119 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  respectively. Values at pH 7.8 and 7.6 also had much higher SE deviations from the average compared to the other values in the dark, as well as in the light. In the light conditions, the change from positive to negative average values between pH levels 7.42 and 7.4 were found to be  $4.2 \pm 37 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  and  $-124 \pm 32 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  respectively (Appendix 2 Table 9).

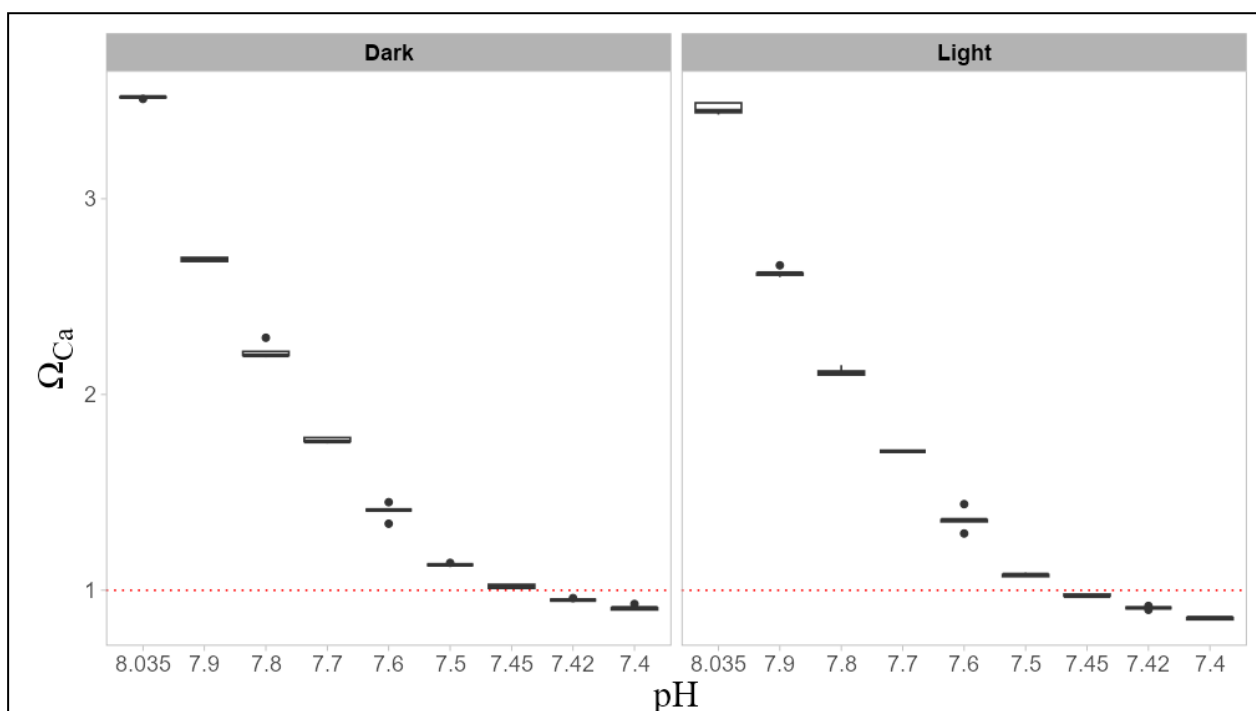


Figure 8. The saturation state ( $\Omega$ ) of calcite in dark and light conditions when exposed to increasing pH levels, with a red horizontal line at 1 showing the tipping point between saturated and unsaturated.

The calcite used for this carbonate production shows a similar tipping point in both light and dark conditions, changing from a saturated to an unsaturated state at around pH 7.45, though the exact tipping point for the dark is between 7.45 and 7.42 and for the light is between 7.5 and 7.45 (Figure 8 and Table 1).

## 4. DISCUSSION

The results of this report show that Swedish maerl photosynthetic efficiency and maximum capacity are not largely impacted by changing pH levels. It also shows that photosynthetic and respiratory rates do not show any specific reaction to changing acidification levels but do in fact react to increasing temperatures, with photosynthesis having the highest rates in ambient temperatures found in Kattegat and respiration increasing gradual with increasing temperatures. This report also suggests that, due to the difference in thresholds for the transition from calcification to dissolution in dark and light conditions, calcification could be coupled to photosynthesis.

### 4.1 Fluorescence

The light saturation curve (or Rapid Light Curve – RLC) generated in this report showed that the point of saturation for *Phymatolithon calcareum* was higher than that of *Phymatolithon lusitanicum* measured by Sordo et al. (2020) in Portugal at a depth of 13 to 25 m. This indicates that Swedish maerl require higher irradiance levels to become fully saturated and are more acclimated to an environment with overall higher light levels available for photosynthetic activities (Appendix 2 Figure 2; Beer et al., 2014). The saturation point of 314  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  is also not one that would likely naturally be found in depths of 23 m. Other Rhodophyta species found in Kattegat at around 20 m had saturation points between 40-100  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  while others found at depths of  $\leq 5\text{m}$  had saturation points between 100-280  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Johansson & Snoeijs, 2002) suggesting that the saturation point Swedish maerl are reaching is one typically found in depths of less than 5 m. It also shows that there could be potential crashes in the system due to photoinhibition ( $\beta$  slope) once this saturation point is reached (Beer et al., 2014), and this was not necessarily evident in the study done by Sordo et al. (2020) which had a more horizontal curve once the saturation point had been reached. Both the point at which Swedish maerl reach their saturation and the crashes afterwards indicate that further research should be done on irradiance levels and how possible mixing of the Baltic and North Seas as well as run-off from the Gothenburg area could influence these important factors.

The effective and maximum photosynthetic yield of *P. calcareum* ( $\Delta F/F_m'$  directly related to the relative electron transport rate – rETR and the  $F_v/F_m$  respectively) remained relatively unchanged despite the exposure to different pH levels. However, there was a slight decline in  $F_v/F_m$  at pH 7.9, 7.6, 7.5 and 7.4 before and after the experiments (Table 2; Figure 4) indicating a certain stress on the plant that is not related to acidification and therefore leads to a rejection of hypothesis ii – photosynthetic efficiency and maximum capacity will be negatively impacted by

decreasing pH – related to research question 1 – At what pH and temperature will the species-specific tipping points for calcification, photosynthesis and respiration occur? For a different species of maerl, *Sporolithon australe*, pH levels of around 7.7 and < 7.4 (according to the pCO<sub>2</sub> values from Table 1) also had no impact on the photosynthetic efficiency (Cavalcanti et al., 2018). In other species of macroalgae, the maximum quantum yields were significantly different across varying pH levels, though these differences were most likely enhanced by the added exposure to increased nutrients as well as low irradiance levels (Celis-Plá et al., 2015). For the rhodolith *Neogoniolithon* sp., there was a lower maximum quantum yield under higher temperatures compared to pH levels (Vásquez-Elizondo & Enríquez, 2016). Additionally, two seagrass species found off of the coast of Sweden had similar trends. The maximum quantum yields for *Zostera marina* decreased with increasing temperature (Rasmusson et al., 2021a) and for *Ruppia cirrhosa* they decreased with increasing exposure times to warmer conditions, despite the effective quantum yield have variable results to those same conditions (Rasmusson et al., 2021b).

These studies support the idea that both global warming and other factors like turbidity (a direct relationship to irradiance and nutrient availability; Hall-Spencer et al., 2008a) have and will have a more negative influence on their ability to absorb light especially after being in darkness for a certain period of time. In addition, the rETR remaining unaffected shows that the photosystem, particularly photosystem II, continued to function adequately, meaning none of the important parts of this system, including pigments and proteins, had been affected or degraded from the acidification (Beer et al., 2014). A study done by Noisette et al. (2013a) found that while CO<sub>2</sub> changes had no impact on the pigments associated with photosynthetic activities, temperature did. This then supports the findings from the previous studies that temperature has more of an impact on more aspects of the process of photosynthesis compared to acidification. This was not investigated in the current report due to lack of time and equipment when performing experiments on temperature, so it is suggested that more studies should be done with Swedish maerl to investigate this further.

#### 4.2 Photosynthetic and respiratory rates

With regards to net photosynthesis (NP), the results from this study only showed a slight decrease with relation to decreasing pH, despite performed statistics revealing significant differences across pH levels. The highest rates of NP were found at ambient pH 8.035, however, a second smaller peak in rates was found at pH 7.6 (Figure 5a) which could suggest that the increased levels of CO<sub>2</sub> in the water created a broader range of optimal conditions, even at lower pH levels. This is not the reaction that was anticipated, as hypothesis iii – Photosynthesis and respiration will increase with both decreasing pH (1<sup>st</sup> and 3<sup>rd</sup> ¼ respectively) and increasing temperature (2<sup>nd</sup> and 4<sup>th</sup> ¼ respectively – in relation to research question 1 was formulated due to the idea that higher CO<sub>2</sub> levels and thus lower pH would increase CO<sub>2</sub> uptake for photosynthesis (as well as the idea that the increased productivity and energy from photosynthesis would result in increased respiratory activity). However, the lack of response across lowered pH levels means that the 1<sup>st</sup> ¼ of the hypothesis must be rejected. Despite the rejection of both this hypothesis and the one related

to the effective and maximum photosynthetic yields, the trends were slightly different, and this could be explained by the different processes being used. Photosynthetic rates were not based off of the irradiance and light saturation levels but were instead based off of oxygen exchange measurements. Oxygen exchange is a process much more directly related to seawater chemistry which has been shown to be highly impacted by acidification (section 1.3). On a local scale off of Sweden's coast, pH can fluctuate seasonally between 8.1 and 7.8, with the average being about 8 (Havenhand et al., 2019). Due to climate change, that average is expected to decrease to 7.7 by the end of the century (Gustafsson et al., 2021). Therefore, the pH levels tested in this report were not only to investigate the responses to this prediction, but to also find maerl's threshold, and if there was a crash in any part of the processes of photosynthesis, as well as in respiration and calcification, at even lower pH levels. For photosynthesis, this threshold was not found, so the predicted pH levels in Sweden might not have much of an impact. There could also have been an issue in the methodology with creating the various pH levels, as the temperature in the room with the pH sensor was much higher than that of the experimental laboratory, though any increases in water temperature was mitigated as much as possible by using refrigerators.

The NP results in this report are in agreement with Kamenos et al. (2013), who found no significant differences in the NP rates between control groups and lowered pH in *Boreolithothamnion glaciale*. This is also a species found in temperate regions and could potentially be found off of Sweden's coast, so this suggests that NP rates may not be as species-specific as they are location specific. To expand upon this idea further, Noisette et al. (2013b) did a study in more southern temperate regions with the species *L. corallioides* and found enhanced NP rates with lowered pH. A general compilation done by Martin & Hall-Spencer (2017) on various maerl species found in different regions when exposed to pH showed variation within both region as well as among the species within each region. NP rates were enhanced and reduced in tropical regions and showed no response in temperate regions. On the contrary, to refute this idea, Semesi et al. (2009) studied a tropical species *Hydrolithon sp.* and found that lowered pH levels also showed no significant effects on NP rates in terms of oxygen exchange. They, in turn, suggested that NP rates were enhanced in terms of their uptake of inorganic carbon ( $C_i$ ) instead, which is a factor that the present study did not have the capacity to consider under the given circumstances.

With regards to respiration ( $R_d$ ), the results from this study showed an even less decreasing trend across pH levels compared to NP rates, with another smaller peak at pH 7.5 instead (Figure 5c). This also is not what was expected according to hypothesis iii from research question 1. This means that the 3<sup>rd</sup> ¼ of the hypothesis is rejected. The  $R_d$  results were also supported by Kamenos et al. (2013) who found no response between control groups and lowered pH for respiration either in *B. glaciale* which could also support the idea that responses are more location specific. This lack of response was seen for *L. corallioides* as well by Noisette et al. (2013a) but, just like with photosynthesis, this was later opposed by Noisette et al. (2013b) who found that  $R_d$  decreased with decreasing pH. On the same compilation of studies done by Martin & Hall-Spencer (2017), there were less variations among species within as well as across regions, with no response shown in

either cold/warm temperate- or tropical- regions. This both supports and refutes the idea that reactions are more location specific, however it does help support the results found in the present study that reactions in photosynthesis and respiration are slightly different from each other.

The results from the temperature experiments in this study showed a peak NP rate at 16°C which is the temperature in which maerl inhabit naturally in Sweden. This shows that they might not respond well to any changing temperatures in the future in this region and contradicts a study done on seagrass found in the same area (Kattegat), where the NP rates increased until reaching a certain temperature, denoting a threshold, and immediately decreased afterwards (Rasmusson et al., 2021a), resulting in the hypothesis formed for the present study. As with pH, bottom water temperatures in Kattegat fluctuate annually and seasonally between 4 and 14, especially in the years closer to 2012 (Skjevik et al., 2022), but the predicted changes in surface temperature for this area vary due to the combination of the Baltic and North Seas. The Baltic Sea is predicted to change from 1.3°C to 3.5°C while the North Sea is predicted to change between 1°C and 2.4°C, with more frequent heat waves expected to occur (European Environment Agency, 2025). On average, it can be predicted that Kattegat surface temperatures will change between 1.75°C and 2°C (Climate Change Scenario, 2024). Therefore, the temperatures measured in this report reflect seasonal changes and fluctuations down to about 6°C as well as increased temperatures past the predictions in an attempt to find the maerl's threshold, which was also not found. This means that the 2<sup>nd</sup> ¼ of the hypothesis is rejected.

The results showing that the NP rates are highest at 16°C are emphasized by the fact that the significant differences seen across pH levels are between the ambient 16°C and temperature changes to a lower 10°C and a higher 27°C (Figure 5b). In a study done on rhodolith beds in the mediterranean sea, the optimal temperature for the various beds found at 40-65 m was between 14.5°C and 15°C (Illa-López et al., 2023) which is slightly deeper and colder than what appears to be the optimal temperature for *P. calcareum* on the west coast of Sweden. This was also seen in Mexico with the rhodolith *Neogoniolithon* sp., where under the optimal temperature of 30°C in summer, the overall metabolic rates, including both photosynthesis and respiration, were highest when irradiance was saturating (Vásquez-Elizondo & Enríquez, 2016). This shows that optimal temperatures vary among both species and location and any changes within those areas could cause crashes in photosynthesis to varying degrees.

On the other hand,  $R_d$  in the present study showed a slight positive linear relationship between the lowest temperature tested, 6°C, and the highest, 27°C (Figure 5d). Similar trends were seen with *L. corallioides*, where respiration rates were significantly lower at 10°C than at either 16°C or 19°C (Noisette et al., 2013a) and also with *Neogoniolithon* sp. which showed a linear relationship between 24°C and 32°C (Vásquez-Elizondo & Enríquez, 2016). This was also seen in correlation with seasonality, where respiration rates were much higher in the summer than in the winter, though this was also connected to irradiance and not solely temperature (Qui-Minet et al., 2021). This indicates that respiration rates are partially less species and location specific and could show a general increase with temperature globally in the future. More specifically with Swedish

maerl, the 4<sup>th</sup> ¼ of hypothesis iii relating to research question 1 can be accepted (the other ¾ has previously been rejected), as respiration did increase with temperature. Overall, it is important to locally consider the temperature changes expected in this area, and that with rising temperatures comes rising respiration and possibly inhibited photosynthesis. However, a big consideration needs to be taken with respect to the fact that NP and R<sub>d</sub> rates in pH were taken by different groups of maerl and the rates for temperature were taken by the same groups of maerl for the replicates. This could have implications on fully understanding how well different specimens of maerl switch between the two mechanisms, and that is something that could be improved upon with the methodology.

Another way of looking at photosynthetic efficiency in response to changing conditions is to look at the ratio between gross photosynthesis (GP) and R<sub>d</sub> (GP : R) which shows if a system is producing or consuming more O<sub>2</sub>. For this ratio, values above 1 will indicate a net autotrophic system where more oxygen is being produced than consumed, and those below 1 will indicate a net heterotrophic system where more oxygen is being consumed rather than produced (Alongi, 2023). The values from the present report showed that pH had a variable albeit insignificant influence on this ratio with no visible trend except for a spike in oxygen production at pH 7.7 up to a value of 7.8 (Figure 6a). The increase at pH 7.7 could potentially be due to equipment or human error, or some other uncontrollable factor like the temperature in the room, which had some issues in remaining constant during the duration of the experiments.

Temperature, on the other hand, had a significant influence on the ratio, with a decreasing trend seen from the lowest to the highest temperature (Figure 6b), with the same trend being shown the rhodolith *Neogoniolithon* sp, which also had a decreasing ration with increasing temperatures (Vásquez-Elizondo & Enríquez, 2016). This could translate to seasonal effects during *in situ* experiments, where the ratio is much higher in the winter compared to the summer. Winter would mean lower temperatures and therefore lower respiration rates whereas the summer would bring warmer temperatures and thus increased respiration rates. The higher the respiration rate, the lower the GP : R value will be. This was seen for both *L. corallioides* and *P. calcareum*, where their GP:R ratios were around a mean value of 10 and 6 respectively in the winter and were reduced to about 4 each in the summer (Qui-Minet et al., 2021). All values of the ratio, for both pH and temperature exposure in the present study, were above a value of 1 as well, meaning that regardless of the condition, and regardless of any significance seen, each ‘system’ was autotrophic. It is important to note here that the same maerl specimens were used for both dark and light conditions in the temperature experiments, and different specimens were used for the dark and light conditions in the pH experiments, so this could influence the trends being seen as the temperature results could present a more complete picture.

Temperature and pH have also been shown to have impacts on NP and R<sub>d</sub> when combined. For the species *P. lusitanicum*, temperature seemed to heighten the effects from increased pCO<sub>2</sub>. NP increased with pCO<sub>2</sub> at all temperatures except 16°C and the highest NP rate was seen at 22°C which also had the largest difference between the control group and the level of pCO<sub>2</sub> compared

to the other temperatures. With regards to  $R_d$ , it showed the opposite effect, where  $pCO_2$  was the factor that amplified the effects of temperature. The rates only increased with temperature in the higher  $pCO_2$  exposure treatments (Sordo et al., 2019). It was also seen that for *P. calcareum*, the  $R_d$  rates in the summertime were negatively affected by the future temperature and pH scenarios that were tested, though this was not the case for *L. corallioides* (Qui-Minet et al., 2019), showing species-specific responses to future climate change predictions. This also highlights the importance of studying these two factors of climate change in combination with one another, and not just in separate experiments. Due to time and temperature control issues, the combination of both of these factors was not possible in the current report, so this further emphasizes the need to re-work the methods chosen and performed in this study, improve upon temperature and pH control and investigate the responses of Swedish maerl to these conditions.

#### 4.3 Calcification and carbonate production

The calcification rates in the present report showed a negative relationship to decreasing pH levels, with different thresholds found for dark and light conditions leading to the acceptance of hypothesis i – calcification rates will decrease with decreasing pH – in research question 1. The threshold in the dark conditions was found to be between pH levels 7.9 and 7.8 (Figure 7), showing a rather quick transition from calcification to dissolution within the range of future predictions for acidification, especially within the context of Sweden, as the decrease to pH 7.7 is expected by the end of the century (Gustafsson et al., 2021). This also suggests that dissolution might not be directly related to the saturation state of calcite, due to the fact that the tipping point between saturation and undersaturation for calcite was around pH 7.45 (Figure 8). Instead, the skeleton might be dissolving once it comes in direct contact with the water. Ocean acidification and the increase of atmospheric  $CO_2$  cause a shift in the concentrations of various inorganic carbon species and thus their saturation states (Equation 5; Doney et al., 2009). This could mean that either the saturation state of carbonate or other factors might have a much larger influence than calcite in particular. This idea emphasizes that there is a large web of interconnectedness of various factors which could influence the reactions in calcification processes.

The threshold in the light was not found until between pH 7.72 and 7.4, where the rates were generally higher than those in the dark, and the rate of change from calcification to dissolution was not as steep as that seen in the dark (Figure 7). It is important to note that these pH levels will most likely not be found in natural conditions, so the levels of pH below 7.7 were purely to understand the threshold of Swedish maerl and where the systems might crash, or in this case, transition to dissolution. This trend was also seen in other studies. For the rhodolith-forming *Hydrolithon* sp., Semesi et al. (2009) found a 20% decrease in calcification at lower pH levels, as well as overall lower rates in the dark than in the light. For *L. corallioides*, despite having significance only seen in the light, the rates were generally lower in the dark as well with a decreasing trend and transition to dissolution at the lowest pH levels (Noisette et al., 2013b). *B. glaciale* also showed dissolution at night in low pH levels, however the calcification during the day was significantly higher than the amount of dissolution at night, showing a net calcification

for the system across pH levels (Kamenos et al., 2013). Net calcification or dissolution is not something that was analyzed in the present study as different algae specimens were used simultaneously during the light and dark treatments, which calls for a change in methodology to a system or set-up that can allow for this to be implemented. Any net measurements derived from the results would assume similar calcification rates across all replicates and across all pH levels, which is too large of an assumption to make.

This is something that could be taken into consideration in future research projects when quantifying calcification for Swedish maerl or any maerl globally. It would contribute to the understanding of the calcification system in a more complete picture for each bed, location or region. This would be due to the fact that the reaction to acidification and the tipping points between calcification and dissolution might be at different pH levels for individual specimens that had been both exposed to dark conditions first and then light, or vice versa. These results would be critical when looking at the reaction in overall biogenic bed systems as well, because each individual specimen of maerl in the entire bed is exposed to light and dark on a continuous scale daily, seasonally and annually, and this would help determine if the system shows net calcification or net dissolution on those various scales.

One of the most likely explanations for the slower transition from calcification to dissolution found in light conditions – and the more rapid transition found in the dark – may be found within the coupling between this process and the metabolic activity of the maerl, including NP and  $R_d$ . It is possible that the activity from photosynthesis could provide a buffer between internal and external chemistry, control the extracellular chemistry at the site of calcification and organically mediate the precipitation of  $\text{CaCO}_3$  as the maerl form their skeletons. This is plausible because an influx of  $\text{H}^+$  ions into the extracellular fluid can cause an increase in pH which favors the conversion of  $\text{CO}_2$  and water into  $\text{HCO}_3^-$  and the formation of calcite. The reason for dissolution in the dark is because respiration reverses this process and decreases the extracellular pH (McCoy et al., 2023).

This coupling could also be accentuated under high  $\text{pCO}_2$  levels (Sordo et al., 2019), where any extra energy being produced from photosynthesis would be directed to the precipitation of  $\text{CaCO}_3$  and be taken away from other processes like growth (Cavalcanti et al., 2018) which could reduce photosynthetic efficiency in the long term (Kamenos et al., 2013). This was seen in a study done by Vásquez-Elizondo & Enríquez (2016) where they found that the rhodoliths needed up to a 3-fold increase in photosynthetic rate to balance the dissolution recorded in *Neogoniolithon* sp. For future research looking into the connection between these processes, it could be insightful to not only look at  $\text{O}_2$  exchange for the photosynthetic and respiratory rates, but also the  $\text{C}_i$  exchange and uptake (Kalokora et al., 2020; Semesi et al., 2009). This could show how much  $\text{C}_i$  is being used for calcification, growth and other processes. It is important to see if this provides another link between these and the external seawater chemistry and to better understand the effects of temperature and pH on this system.

As with NP and  $R_d$ , the combination of both temperature and pH might also enhance the effects seen in calcification. The present study was not able to look at either temperature effects or the combination between temperature and pH due to time and equipment constraints. However, for similar species of maerl, like *P. lusitanicum*, this combination had a significantly positive effect on calcification seen in light conditions, where the rates increased with decreasing pH at higher temperatures. For dark calcification, dissolution was higher than calcification for all treatments in high pCO<sub>2</sub> levels, where the rates also decreased with temperature. Because of this, it was found that the replicates in this study dissolved more than they calcified and it was suggested that this could have a long-term effect on their ability to grow (Sordo et al., 2019). These two studies suggest that if the maerl from the present study had also been exposed to temperature simultaneously, the dissolution found in the dark could have an increased rate, whereas the light calcification could have actually increased, aided by both temperature and photosynthesis.

*L. corallioides*, on the other hand, showed that dark calcification increased significantly with increasing temperatures between 10°C and 16°C, but strongly decreased with increasing pCO<sub>2</sub> levels, which was also seen with the light calcification, though there was no enhancement from temperature (Noisette et al., 2013a). This could suggest something entirely different, where temperature might not have affected the rates found over decreasing pH, and would instead have more of an impact in combination with another external factor. The combination of temperature and pH can also be seen alongside natural seasonal changes, where in the winter with colder temperatures, all calcification rates of *B. glaciale* decreased at the different pCO<sub>2</sub> conditions, with a transition from calcification to dissolution at the higher levels (Büdenbender et al., 2011). Seasonality could have a big influence in Sweden, especially due to the increased darkness and decreased temperatures that occur during winter months, and this is another aspect that could be included in future research to understand how natural fluctuations independently and in conjunction with climate change will affect the system.

The results found in the present study could also be insightful when looking at various life stages of maerl, especially with regards to their ability to precipitate calcium carbonate and to grow. For example, when *Phymatolithon lenormandii* was exposed to small changes in pH in the early life stages, the effect of pH became indirect as the algae grew older, due to the fact that the pH increased the rate of abnormalities in their growth patterns, which lead to higher rates of mortality later and made it more energetically costly for the algae to calcify (Bradassi et al., 2013). The cost of keeping the carbonate skeleton was also seen in *S. australe*, when under high pCO<sub>2</sub> levels of 500 and 1500 (7.7 and <7.4 according to Table 1), there was only tissue loss and not skeletal loss (Cavalcanti et al., 2018), indicating a shift in priorities under stressful conditions. When comparing the difference between a 3 month and a 10 month study, *B. glaciale* showed higher energy demands after 3 months in order to continue calcifying under increased pCO<sub>2</sub> ultimately leading to reduced calcite being deposited into the walls. After 10 months, the cell wall structure recovered and maintained its form, but the growth rate was reduced (Ragazzola et al., 2012, 2013).

This could support the results found in light calcification in the present study as well, due to the likely coupling of calcification and photosynthesis seen as the rates remained relatively steady until the system could no longer handle the amount of CO<sub>2</sub> in the water (Figure 7). It is possible that an increase of energy from this photosynthesis was being put towards calcification in exchange for another process, highlighting the importance of implementing growth alongside both calcification and photosynthesis under various scenarios. Despite there being a reaction in one system, there could be another simultaneous reaction occurring in an additional system. This concept can also be seen in relation to the saturation state of calcite as well, where a decrease in calcite resulted in a loss of structural integrity (Ragazzola et al., 2013) and this could cause even more complications to the maerl's ability to grow and survive in such conditions. With this in mind, it could be beneficial to implement a combination study of laboratory-manipulated settings, *in situ* measurements, and natural analogs from various naturally low pH environments in order to get a more in depth picture of what these and other factors might be doing and changing in these systems over maerl's various life stages.

#### 4.4 Are Swedish maerl carbonate sinks or sources?

The production of CaCO<sub>3</sub> followed the same trend seen with the calcification rates because they are directly related to one another (Appendix 2 Table 8), meaning that the ability for maerl to precipitate calcium carbonate and build their structures is also coupled to photosynthesis and any changes in photosynthetic rates could potentially cause a decrease in skeletal production. Additionally, the decrease in calcium carbonate production leads to the acceptance of hypothesis i – carbonate production contributions will vary depending on changes in pH on a local scale – in research question 2 – What is the contribution of Swedish maerl to local and global carbonate production? On a global scale, the production rate found as an average of the replicates in the ambient light conditions was  $556 \pm 54 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  which is within the range found across different locations in temperate, subpolar and polar regions (Van Der Heijden & Kamenos, 2015) and was at the high end of the production rates found by Bosence (1980) for *P. calcareum* (Table 3). This means that hypothesis ii – carbonate production in Swedish waters at ambient conditions will be comparable to other global production rates found in the literature worldwide – of research question 2 can be accepted as well. However, it is also important to account for dark conditions because calcification rates vary on a daily basis (Figure 7). Thus, the average production between the light and dark in ambient conditions decreases to  $449 \pm 33 \text{ g CaCO}_3 \text{ m}^{-2} \text{ yr}^{-1}$  (Appendix 2 Table 8).

Even so, this could be an overestimation due to the fact that Sweden experiences many dark months over the winter because of its location, which means the rates found in the present study could be more representative of spring and summer production and not annual production. It should also be noted that these production rates in light and dark were also calculated from different replicates and do not show the difference within individuals but instead between them for each condition, just as was the case for calcification. This has implications for understanding the concept of carbonate sinks and sources from the results of the present study alone. These concepts require

understanding the rates of both calcification and dissolution in each specimen in order to calculate if the system has net calcification or net dissolution, as mentioned previously. In addition, not only is it the production of calcium carbonate that needs to be considered but also the amount of carbon that is being stored/accumulated from maerl beds (Van Der Heijden & Kamenos, 2015). This could be found in the form of organic carbon in the tissues (Arina et al., 2020) or fixed carbon in the sediments which could come directly from the maerl (Hill et al., 2015).

The amount of organic carbon stocks in the tissues ( $\text{CaCO}_3$ ) of *Jania adhaerens* has been found to vary annually between May 2018 (highest) and August 2018 (lowest) with amounts between 147 and 73 g  $\text{CaCO}_3 \text{ m}^{-2}$  respectively while the amount of organic carbon stocks in the sediment between August 2018 (lowest) and May 2019 (highest) was 344 and 545 g  $\text{CaCO}_3 \text{ m}^{-2}$  (Arina et al., 2020). It seems that calcium carbonate store into the sediment occurred the most over the winter months, which might not be the case for Swedish maerl *P. calcaerum*, which could be due to the different regions in which the two species inhabit. Nevertheless, that is also something that could be investigated further in conjunction with the calcium carbonate storage within the tissues during calcification and growth.

A major consideration in the discussion of carbon source or sink potential is the release of  $\text{CO}_2$  back into the water, which occurs during the process of calcification, specifically when the calcium carbonate is being precipitated (Frankignoulle et al., 1994; Hill et al., 2015) and is therefore seemingly offsetting the carbon taken out of the water to build the structure in the first place. However, what could offset the  $\text{CO}_2$  being sent back into the carbon system is the dissolution occurring during respiration at night and under lower pH levels (Schubert et al., 2024), or the concept that  $\text{CO}_2$  could be taken back up via photosynthesis (Frankignoulle et al., 1994; Hill et al., 2015), which is counterintuitive and indicates a system that is highly complex and is constantly in a balance between simultaneously being a carbon source and sink. The  $\text{CO}_2$  released can also be taken up by other photosynthesizers in the area, which contributes to the idea that maerl are instead carbon donors (Arina et al., 2020; Hill et al., 2015). Though the present study did not investigate overall carbonate storage in the replicate systems, it does show that Swedish maerl could be productive comparative to other maerl beds globally. However, further studies of this storage independently and in combination with other habitat facilitators and photosynthesizers might provide even more important insights into their contribution to global blue carbon stores.

#### 4.5 Future directions

Aside from the future research topics previously mentioned, more factors should be included in future studies regarding climate change impacts, especially irradiance and salinity, as there has been some evidence that this negatively impacts photosynthesis and growth in particular (King & Schramm, 1982; Roberts et al., 2002; Schubert et al., 2024; Teichert & Freiwald, 2014). On a more local scale (in Sweden), more studies like this one and others that focus on different aspects of physiology and climate change should be conducted on different maerl beds along the coast to deepen our knowledge on whether their reactions are species-specific, location-specific or

both. The more studies conducted on the maerl found here, the better the chance of raising awareness of their ecological importance as well as providing more insights on how they function and grow, translating into ways we can help mitigate climate change affects to better protect them. They are in danger of physical damage which could cause permanent disappearances due to slow growth (OSPAR Commission, 2008), being overtaken by more warm-acclimated (Costa et al., 2023), fleshy (Koch et al., 2013), or lower pH acclimated (Hall-Spencer et al., 2008b) species. This calls for the need to introduce more marine protected areas for them as stand-alone habitats or in accordance with other economically important species (Belgrano et al., 2021; HELCOM, 2013), and to incorporate better monitoring systems (Hiscock, 1998) to ensure that the current maerl beds found globally remain.

#### 4.6 Conclusions

This report has shown the variability of different aspects of maerl physiology and calcification when exposed to climate change factors. Overall, photosynthetic and respiratory rates, albeit with slightly different trends, did not show much of a reaction after exposure to decreased pH. On the other hand, they had different reactions after exposure to changes in temperature, with photosynthetic rates being the highest at the maerl's natural temperature and with respiratory rates increasing with increasing temperature. Calcification thresholds were found in very acidic scenarios for light conditions and within predicted scenarios for dark conditions, meaning dissolution will be much more prevalent within the next century. With both acidification and global warming getting worse in the future, understanding how Swedish maerl react to these scenarios will help the Swedish government better understand the ways in which these maerl can and need to be protected.

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## 6. APPENDIX 1

Maerl are free-living red algae with hard skeletons made out of calcium carbonate that can be found in many different coastal ecosystems worldwide. Gathered together by wind and water currents, they form large and complex beds that are rich in biodiversity – similar to that of coral reefs, though consisting of much smaller organisms. These beds provide important habitats for local fishery species and other various forms of marine life. They may also have high potential for storing carbon – specifically in the seafloor sediments or in their tissues in the form of their calcium carbonate skeletons – which is also similar to coral reefs. While many species of maerl have been studied across the globe, those off of Sweden’s west coast have remained largely unexplored. This makes the present study especially important, as it investigated the effects of climate change (acidification and increasing temperature) on the maerl’s ability to produce and consume oxygen, absorb light and build their calcium carbonate skeletons. In order to do this, the maerl were exposed to different pH levels and temperatures in both light and dark conditions. These tests helped identify their environmental thresholds beyond which the maerl can no longer function properly, where their systems can shut down and their skeletons can begin to disappear.

In order to understand how the maerl responded to these environmental changes, oxygen levels were measured to look further at photosynthesis and respiration, their efficiency and maximum capacity in absorbing light were measured using a technique called Pulse-amplitude modulated fluorometry, and calcification was measured based on the changes in water chemistry. It was found that changes in pH (acidification) did not affect the oxygen levels produced via photosynthesis and consumed via respiration. Their efficiency and maximum capacity also did not show any trends or changes after exposure to decreasing pH. Calcification, on the other hand, decreased with pH due to the impact of acidification on their ability to build and keep their skeletons intact. This was particularly found in the dark conditions where it did not take long for their skeletons to start disappearing. The light conditions, however, showed maintenance of the skeletons until they reached a certain pH level where the system stopped functioning properly and they started to lose their skeletons, just as with the dark conditions. However, the temperature changes did have an influence, though only photosynthesis and respiration were measured after temperature exposure. Photosynthesis peaked at the natural temperature found in their habitat, which was 16°C, and respiration increased steadily with increasing temperature, though this needs to be studied further to understand better why this is the case.

All of this suggests three things. First is that temperature is more of a factor when discussing their production and consumption of oxygen compared to acidification. The second is that photosynthesis functions its best at the current temperatures whereas respiration will continue to rise. Lastly, their ability to form their skeletons will be greatly impacted by increased acidification, especially during nighttime and winter months when there is very little or no light available.

## 7. APPENDIX 2

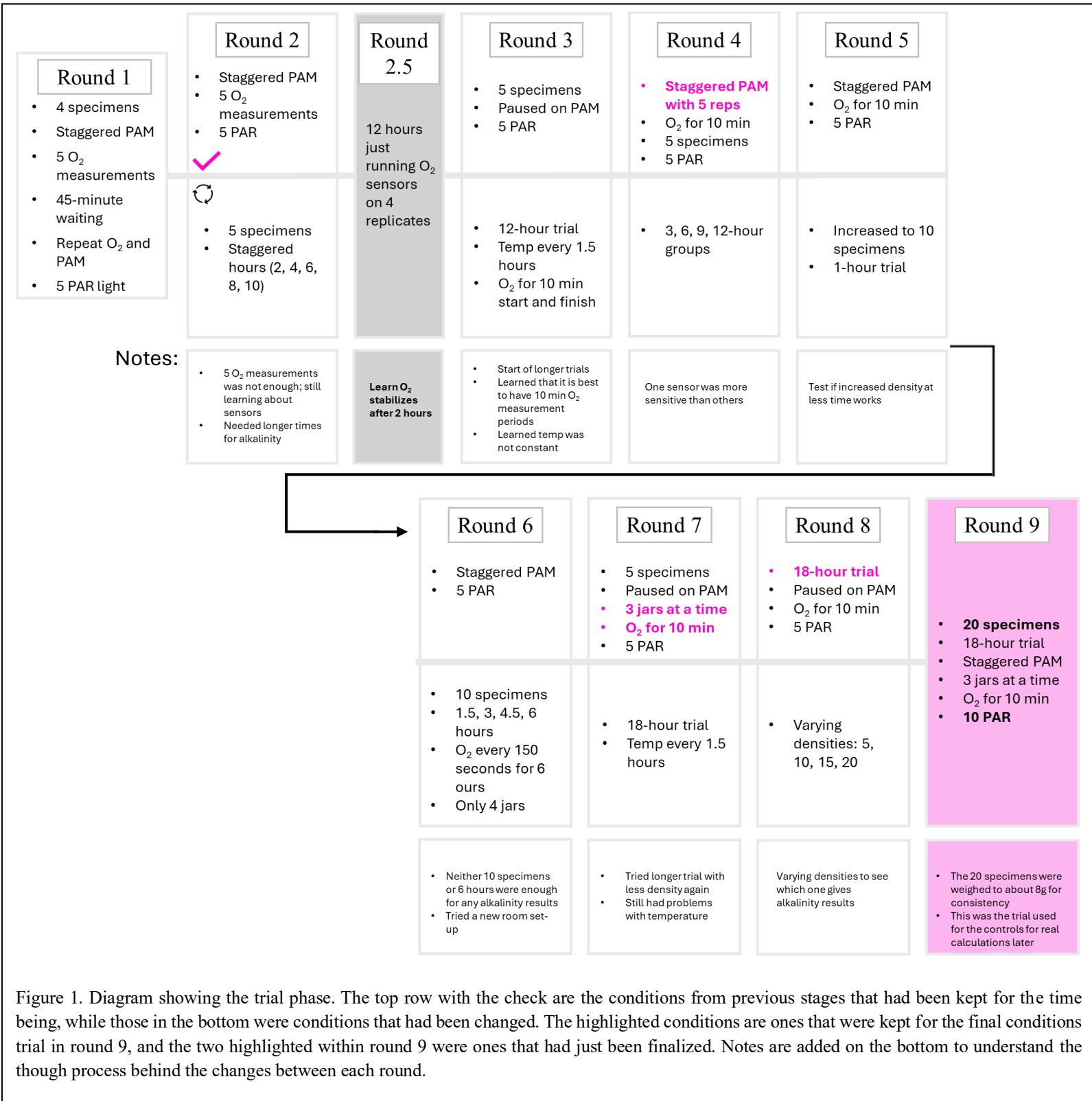


Figure 1. Diagram showing the trial phase. The top row with the check are the conditions from previous stages that had been kept for the time being, while those in the bottom were conditions that had been changed. The highlighted conditions are ones that were kept for the final conditions trial in round 9, and the two highlighted within round 9 were ones that had just been finalized. Notes are added on the bottom to understand the though process behind the changes between each round.

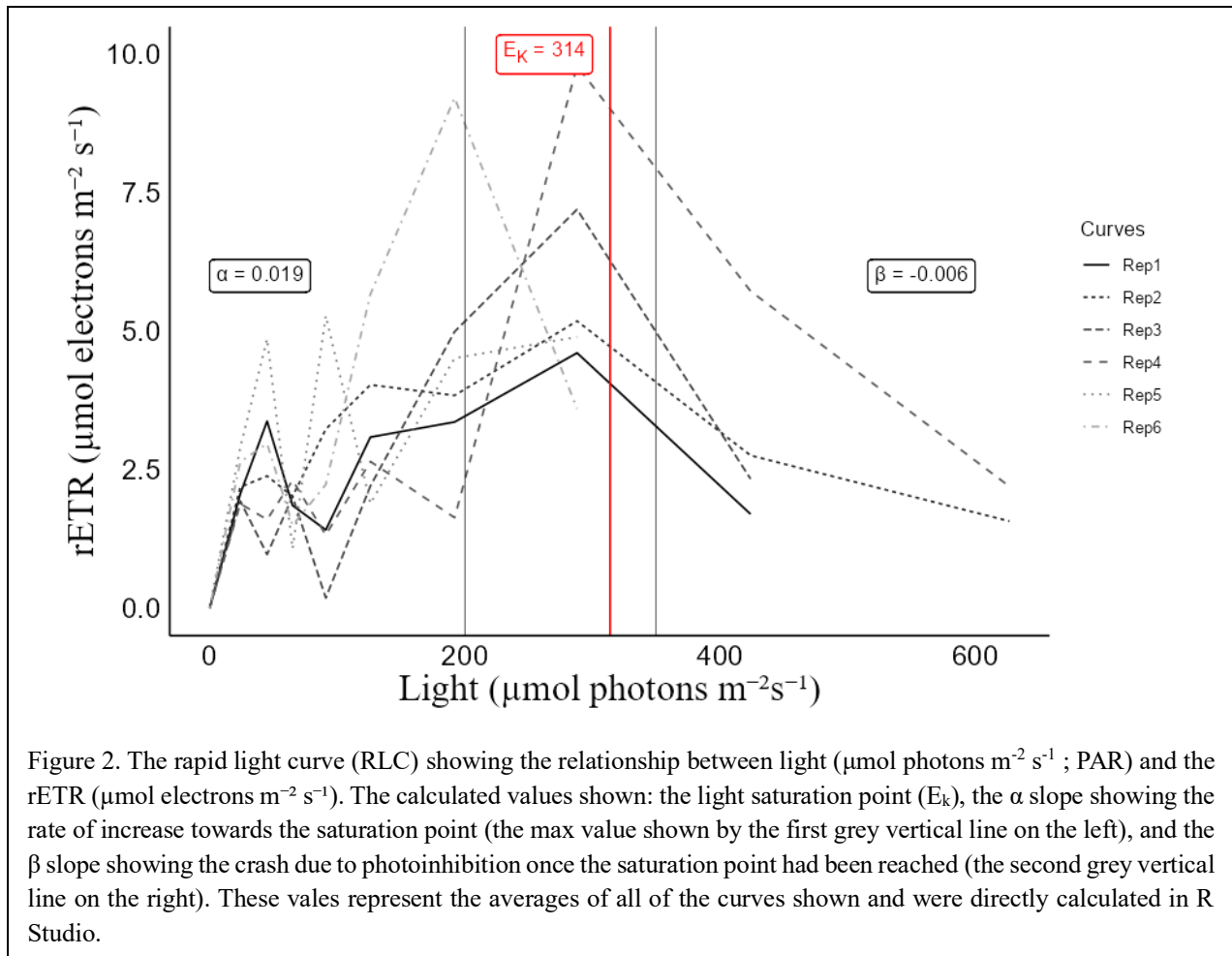


Table 1. Maximum photosynthetic efficiency pos hoc test results from start values and t.test start~end test results.

pH	$F_v/F_m$ - Start (Mean $\pm$ SE)	Tukey Group	$F_v/F_m$ - End (Mean $\pm$ SE)	t-value	df	p-value	Sig.
8.035	0.59 $\pm$ 0.026	ab	0.54 $\pm$ 0.010	-1.94	5.22	0.11	ns
7.9	0.64 $\pm$ 0.012	ab	0.56 $\pm$ 0.017	-3.60	7.26	0.0083	**
7.8	0.60 $\pm$ 0.029	ab	0.57 $\pm$ 0.027	-0.85	7.96	0.42	ns
7.7	0.59 $\pm$ 0.008	a	0.55 $\pm$ 0.037	-1.01	4.41	0.37	ns
7.6	0.67 $\pm$ 0.013	ab	0.58 $\pm$ 0.022	-3.62	6.45	0.0098	**
7.5	0.63 $\pm$ 0.004	ab	0.56 $\pm$ 0.009	-7.24	5.88	0.00039	***
7.45	0.68 $\pm$ 0.025	b	0.57 $\pm$ 0.013	-3.87	5.68	0.0083	**
7.42	0.62 $\pm$ 0.016	ab	0.58 $\pm$ 0.012	-2.03	7.28	0.08	ns
7.4	0.65 $\pm$ 0.029	ab	0.58 $\pm$ 0.011	-2.32	5.2	0.066	ns

\*\*\* indicates  $p < 0.001$ , \*\* indicates  $p < 0.01$ , \* indicates  $p < 0.05$ , ns indicates no significance

Table 2. Net Photosynthesis (NP), Gross Photosynthesis (GP) and GP : Respiration (R) ratio values with their respective post hoc groups to indicate significance drivers across temperatures.

Temp (°C)	NP (Mean ± SE)	Tukey Group	GP (Mean ± SE)	Tukey Group	GP:R (Mean ± SE)	Tukey Group
6	0.00014 ± 1.58E-05	ab	1.06E-04 ± 2.06E-05	ab	6.03 ± 2.08	a
10	9.17E-05 ± 1.60E-05	b	3.46E-05 ± 2.81E-05	b	3.45 ± 1.08	ab
14	0.00014 ± 1.29E-05	ab	7.33E-05 ± 1.53E-05	ab	3.13 ± 0.28	ab
16	0.00021 ± 1.58E-05	a	1.25E-04 ± 1.70E-05	a	3.50 ± 0.24	ab
20	0.00016 ± 4.39E-05	ab	6.20E-05 ± 6.34E-05	ab	3.33 ± 0.87	ab
24	0.00012 ± 1.31E-05	ab	2.76E-05 ± 2.77E-05	ab	2.81 ± 0.68	ab
27	5.87E-05 ± 2.83E-05	b	-1.33E-04 ± 6.42E-05	b	1.60 ± 0.33	b

\*letters across temperature that are the same indicate no significant differences while different letters indicate significance.

Table 3. Respiration ( $R_d$ ) values with their respective post hoc groups to indicate significance drivers across pH levels.

pH	$R_d$ (Mean ± SE)	Tukey Group
8.035	-3.33E-09 ± 4.00E-10	a
7.9	-1.85E-09 ± 4.53E-10	ab
7.8	-1.14E-09 ± 3.84E-10	ab
7.7	-9.72E-09 ± 6.43E-10	b
7.6	-1.31E-09 ± 2.54E-10	ab
7.5	-2.73E-09 ± 4.57E-10	ab
7.45	-1.49E-09 ± 1.14E-09	ab
7.42	-1.40E-09 ± 3.84E-10	ab
7.4	-1.53E-09 ± 2.95E-10	ab

\*letters across Temperature that are the same indicate no significant differences while different letters indicate significance.

Table 4. Respiration ( $R_d$ ) values with their respective post hoc groups to indicate significance drivers across temperatures.

Temp	$R_d$ (Mean ± SE)	Dunn Group
6	-3.77E-05 ± 8.26E-06	a
10	-5.71E-05 ± 1.40E-05	ab
14	-6.85E-05 ± 5.25E-06	ab
16	-8.41E-05 ± 2.54E-06	ab
20	-9.80E-05 ± 2.31E-05	ab
24	-9.59E-05 ± 1.59E-05	ab
27	-0.00019 ± 4.30E-05	b

\*letters across pH levels that are the same indicate no significant differences while different letters indicate significance.

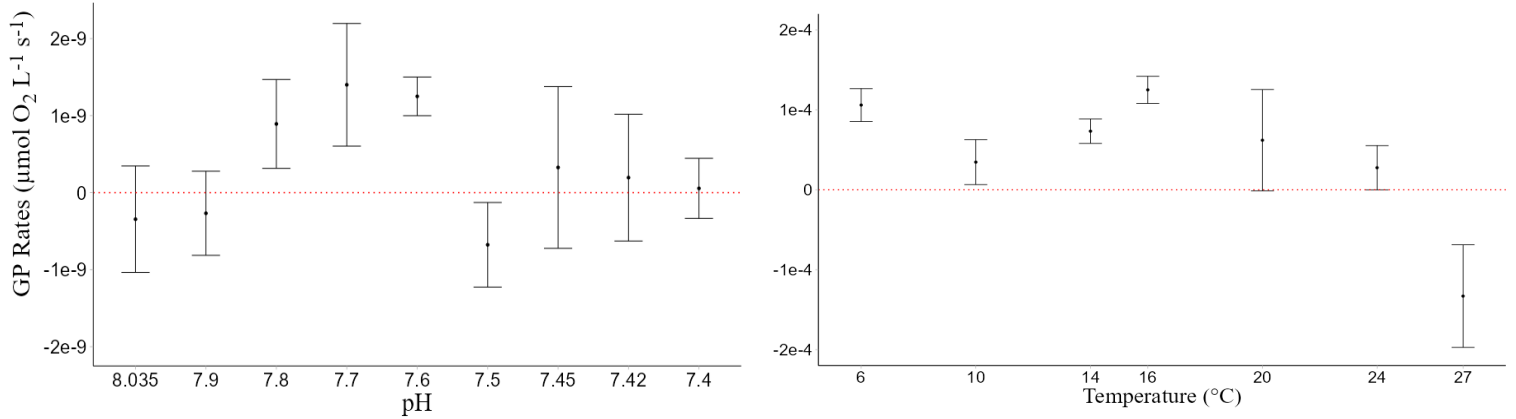


Figure 3. The gross photosynthetic (GP) rates of maerl after exposure to varying pH levels and temperatures. There is a red line at 0 as an indication for the shift between photosynthetically active and inactive (positive and negative) systems. Values are averages with SE lines.

Table 5. Calcification rate t-test results between light and dark conditions across pH levels.

pH	Light (Mean ± SE)	Dark (Mean ± SE)	t-value	df	p-value	Sig.
8.035	1.17 ± 0.11	0.72 ± 0.026	3.88	4.4	0.015	*
7.9	1.02 ± 0.10	0.27 ± 0.069	5.99	6.58	0.00069	***
7.8	0.91 ± 0.11	-0.44 ± 0.24	5.11	5.64	0.0026	**
7.7	0.70 ± 0.017	-0.20 ± 0.12	7.75	4.18	0.0013	**
7.6	0.78 ± 0.48	-0.21 ± 0.38	1.63	7.57	0.14	ns
7.5	0.81 ± 0.12	-0.62 ± 0.056	11.42	5.92	0.000029	***
7.45	0.56 ± 0.068	-0.85 ± 0.13	9.57	6.02	0.000073	***
7.42	0.0082 ± 0.072	-1.41 ± 0.068	14.28	7.97	0.00000058	***
7.4	-0.24 ± 0.063	-2.04 ± 0.20	8.39	4.76	0.0005	***

\*\*\* indicates  $p < 0.001$ , \*\* indicates  $p < 0.01$ , \* indicates  $p < 0.05$ , ns indicates no significance

Table 6. Calcification rate values with their respective post hoc groups to indicate significance drivers across temperatures.

pH	Light (Mean ± SE)	Tukey Group	Dark (Mean ± SE)	Tukey Group
8.035	1.17 ± 0.11	a	0.72 ± 0.026	a
7.9	1.02 ± 0.10	a	0.27 ± 0.069	ab
7.8	0.91 ± 0.11	a	-0.44 ± 0.24	bc
7.7	0.70 ± 0.017	ab	-0.20 ± 0.12	bc
7.6	0.78 ± 0.48	ab	-0.21 ± 0.38	bc
7.5	0.81 ± 0.12	ab	-0.62 ± 0.056	bcd
7.45	0.56 ± 0.068	abc	-0.85 ± 0.13	cd
7.42	0.0082 ± 0.072	bc	-1.41 ± 0.068	de
7.4	-0.24 ± 0.063	c	-2.04 ± 0.20	e

\*letters across temperature that are the same indicate no significant differences while different letters indicate significance.

Table 7. Carbonate production t-test results between light and dark conditions across pH levels.

pH	Light (Mean ± SE)	Dark (Mean ± SE)	t-value	df	p-value	Sig.
8.035	556.05 ± 53.94	341.78 ± 12.16	3.88	4.4	0.015	*
7.9	489.56 ± 50.00	135.17 ± 34.30	5.85	6.68	0.00075	***
7.8	457.28 ± 55.69	-217.93 ± 118.86	5.14	5.68	0.0025	**
7.7	350.88 ± 8.59	-99.69 ± 56.37	7.9	4.19	0.0012	**
7.6	390.53 ± 240.08	-107.29 ± 188.47	1.663	7.57	0.14	ns
7.5	388.55 ± 53.68	-309.73 ± 28.15	11.52	6.05	0.000024	***
7.45	280.65 ± 34.08	-420.77 ± 64.70	9.59	6.06	0.000069	***
7.42	4.15 ± 36.54	-704.53 ± 34.07	14.19	7.96	0.0000062	***
7.4	-123.58 ± 32.44	-1006.42 ± 101.05	8.32	4.82	0.00049	***

\*\*\* indicates  $p < 0.001$ , \*\* indicates  $p < 0.01$ , \* indicates  $p < 0.05$ , ns indicates no significance

Table 6. Carbonate production values with their respective post hoc groups to indicate significance drivers across pH levels.

pH	Light (Mean ± SE)	Tukey Group	Dark (Mean ± SE)	Tukey Group
8.035	556.05 ± 53.94	a	341.78 ± 12.16	a
7.9	489.56 ± 50.00	a	135.17 ± 34.30	ab
7.8	457.28 ± 55.69	a	-217.93 ± 118.86	<del>abc</del>
7.7	350.88 ± 8.59	ab	-99.69 ± 56.37	<del>abc</del>
7.6	390.53 ± 240.08	ab	-107.29 ± 188.47	<del>bc</del>
7.5	388.55 ± 53.68	ab	-309.73 ± 28.15	<del>bcd</del>
7.45	280.65 ± 34.08	<del>abc</del>	-420.77 ± 64.70	cd
7.42	4.15 ± 36.54	<del>bc</del>	-704.53 ± 34.07	de
7.4	-123.58 ± 32.44	c	-1006.42 ± 101.05	e

\*letters across temperature that are the same indicate no significant differences while different letters indicate significance.

Table 8. Carbonate production ( $\text{CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ ) over each pH level in light and dark conditions based on averages of replicates ± SE.

pH	Condition	Carbonate Production ( $\text{g CaCO}_3 \text{ m}^{-2} \text{ y}^{-1}$ )
8.035 ± 0.01	Light	556 ± 54
	Dark	342 ± 12
7.900 ± 0.005	Light	490 ± 50
	Dark	135 ± 34
7.800 ± 0.005	Light	457 ± 56
	Dark	-218 ± 119
7.700 ± 0.005	Light	389 ± 39
	Dark	-100 ± 56
7.600 ± 0.005	Light	391 ± 240
	Dark	-107 ± 188
7.500 ± 0.005	Light	389 ± 54
	Dark	-310 ± 28
7.450 ± 0.005	Light	281 ± 34
	Dark	-421 ± 65
7.420 ± 0.002	<b>Light</b>	<b>4.2 ± 37</b>
	Dark	-705 ± 34
7.400 ± 0.005	Light	-124 ± 32
	Dark	-1006 ± 101