## Title

Effects of climate change on the growth and chemical composition of primary producers and its impacts on coastal aquaculture



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Effects of Climate Change on the Growth and Chemical Composition of Primary Producers and its Impacts on Coastal Aquaculture

By

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## Abstract

### Effects of climate change on the growth and chemical composition of primary producers and its impacts on coastal aquaculture

Coastal aquaculture has been growing rapidly in the last decades as a response to the global seafood demand and overexploited wild marine populations. As more sustainable food production practices are sought, the attention on the production of low trophic level organisms has increased significantly. Responsible production of primary producers and primary consumers in coastal areas can help meet the ever-increasing seafood demands and ease burdens on natural resources. However, conditions in coastal areas are not easy to control, and changes in ambient factors can impact coastal aquaculture productivity in various ways. Temperature and pH are factors that are already changing globally and are expected to keep changing in response to climate change.

The growth and chemical composition of primary producers, like algae and seaweed, are influenced by the ambient conditions where they grow. Their cells can chemically adapt to the environment, responding to changes in ambient conditions by producing biomass with different nutritional values. Primary producers are the base of the food chain in aquatic ecosystems, serving as a food source for consumers at higher trophic levels. Herbivores and filter feeders directly depend on the availability of good quality primary producers' sources, hence, any changes in the nutrient content and growth of primary producers will affect the ability to produce these species. Therefore, to assure sustainable growth of coastal aquaculture, it is important to understand how species of interest are affected by the changes in ambient conditions predicted due to climate change, and how they interact and relate to each other.

Here, the relationship between environmental conditions, primary producers, and primary consumers was explored. The main goal of this study was to understand how changes in temperature and pH can

influence the production of herbivore species in coastal areas, by changing the nutritional value of their diets. For this, as a first approximation, a microalgae culture system that maintained multiple pH cultures through automatic addition of $CO_2$ to keep the desired pH was designed, and the effects of temperature and pH on the growth and protein content of two species of marine microalgae were explored. The species *Nannochloropsis oculata* and *Chaetoceros gracilis* were selected for this study as they are important species in coastal aquaculture of filter feeder species. The study consisted of growing these two species under two different temperatures, 13°C and 20°C, and two pH levels, 8.2 and 7.6, representing the current and projected ocean conditions, in Northern California, due to climate change, respectively. The highest final cell count, specific growth rate, and protein content were found when both species of algae were grown at 20°C and pH 7.6, indicating the projected conditions caused by climate change did not have negative effects on the marine microalgae tested. The statistical analysis results for all the parameters suggest that temperature has a bigger influence than pH on both species of algae.

Then, the effects of temperature on the growth and nutritional composition of the marine seaweed *Palmaria mollis* (dulse) were studied. Dulse is a red seaweed popular in aquaculture, widely used in the production of red abalone in Northern California. The study consisted of growing dulse under three different temperatures, 13°C (current mean ocean temperature in Northern California), 15°C, and 17°C (representing projected temperatures due to climate change). Dulse was grown for 21 days, and its growth, protein, fatty acids, and carbohydrate content were monitored. The results showed that the growth and protein content of dulse were affected by temperature. A negative correlation between temperature and the growth of this species was observed. Dulse grown at 13°C gained the most biomass during the experimental period; however, it contained the least amount of protein in their dry matter. There were no significant differences in the fatty acids and carbohydrate content among the temperatures tested. However, a significant difference in the distribution of fatty acids was found. Dulse growing at 13°C showed the highest percentage of monounsaturated fatty acids and the lowest percentage of saturated fatty acids. The results suggest that changes in temperature predicted due to climate change will affect the

nutritional value and the availability of *P. mollis*, potentially affecting the production of herbivores that depend on them.

Lastly, the influence of rising ocean temperature on the juvenile red abalone (*Haliotis rufescens*) through changes in its food source was studied. Three different diets were prepared by growing dulse under three different temperatures, 13°C, 15°C, and 17°C. Juvenile red abalone were grown under ambient conditions for 105 days with the prepared dulse as their only food. Abalone growth was measured at Days 0, 40, 75, and 105, and their chemical composition, protein, carbohydrate, and fatty acids, were analyzed at the end of the experiment. Abalone fed dulse growing at 17°C showed higher cumulative growth rates, final condition factor, and specific growth rate. The abalone weight seemed to be more affected by the diets than the shell length. No significant difference was found in their chemical composition across treatments. The results suggest that higher temperatures due to climate change tested in this study do not have negative indirect effects on the juvenile red abalone.

The results of this study suggest that rising ocean temperature and ocean acidification caused by climate change might have positive effects on the protein content and the growth of the marine microalgae studied. Furthermore, temperature seemed to be a more influential factor than pH. In the same way, rising ocean temperature positively affected the protein content of the seaweed studied, however, its growth and condition deteriorated as temperature increased. Therefore, even though dulse growing at 17°C yielded higher growth rates of abalone, keeping this seaweed at higher temperatures will not be sustainable. The different diets used for this study did not affect the nutritional composition of the juvenile red abalone. Finally, higher temperatures due to climate change did not seem to have negative indirect effects on the juvenile red abalone and overall dulse growth rate was the only factor studied that was negatively affected by the predicted conditions due to climate change.

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# Chapter 1: General Introduction

## 1.1 Coastal Aquaculture

Aquaculture is the breeding, rearing, and harvesting under controlled conditions of fish, shellfish, algae, and other aquatic organisms in all types of water environments. Sustainable aquaculture can help meet the ever-increasing food demands and ease burdens on natural resources (FAO, 2020; NOAA, 2021a; USDA, 2022). As a response to the high demand for fish and seafood protein, aquaculture has been growing fast. From 1990 to 2018, global aquaculture production increased by 527% (FAO, 2020). In 2020, 48.2% of all fish production was raised in aquaculture (~ 84.1 million metric tons of fish), and it continues to develop (Shahbandeh, 2022). By 2030, 62% of seafood produced for human consumption is expected to come from aquaculture (GSA, 2019a). Furthermore, as of 2017, the fish stocks that are within biologically sustainable levels had decreased to 65.8%, and 34% of the world's marine fish stocks were already overfished (FAO, 2020). This has caused a tremendous impact on the marine and freshwater environments, not just a scarcity of fish, but also on the ecosystem health in both environments (GSA, 2019a; FAO, 2020).

Aquaculture, thus, has become an answer to supply the fish and seafood demand of the increasing human population. However, aquaculture has its own issues, such as causing negative environmental impacts. Intensive production generates huge amounts of organic matter, which frequently provokes eutrophication and algae blooms, some of them are toxic and negatively affect the production of filter-feeding organisms, like oysters, shellfish, and shrimps, as they filter the toxic substances and retain them in their tissues, and could further affect people who consumed them (Ryther & Dustan, 1971). Other negative environmental impacts may include pollution of the surrounding environment with nutrient wastes and harmful chemicals, destruction of coastal habitat and ecosystems to build aquaculture infrastructure, overuse of

antibiotics and development of bacterial resistance, escapes of non-native species, among others (Klinger & Naylor, 2012; Allsopp et al., 2013).

There are many kinds of aquaculture, from freshwater to marine, land-based to offshore, plants and algae to fish, integrated multi-trophic cultures to monocultures, among others. All have specific advantages and disadvantages (Klinger & Naylor, 2012; Naylor et al., 2021; CEF, 2022). Here, we are focused on coastal aquaculture. Coastal aquaculture covers a broad range, FAO (1992) defines the geographical area covered by the term "coastal" as the "shoreland influenced by the sea, the water column, and the seabed extending to the edge of the continental shelf", and thus "coastal aquaculture" covers land-based and water-based brackish and marine aquaculture practices. Other definitions include the one given by Athithan (2021), who defines coastal aquaculture as the "farming and husbandry of marine aquatic plants and animals in coastal areas of ponds, pen, and/or enclosures in saline and brackishwater under controlled conditions". And coastal area as the "area of land within a distance of 2 km from high tide line of seas, rivers, creeks, and backwaters". Hence, coastal aquaculture includes in-land and inshore (at sea but close to the shore) marine organisms farming. The majority of inshore aquaculture production is made up of low-trophic level organisms like seaweeds, bivalves, and mollusks. For the purpose of this study, we are interested in this kind of coastal aquaculture, which is generally less intensive, requires lower external inputs in the system, does not compete for land or water resources, among many other environmental and economic benefits.

Coastal aquaculture is one of the most popular marine aquaculture systems. It plays an important role in the seafood industry, and it keeps growing to meet market demands; for example, 90% of the world's aquaculture production of mollusks, crustaceans, and seaweeds are produced in coastal areas (FAO, 2016). However, production in these areas is intimately related to environmental conditions, which makes it very susceptible to changes in natural factors, like temperature, pH, $CO_2$ concentration, and nutrients in the water.

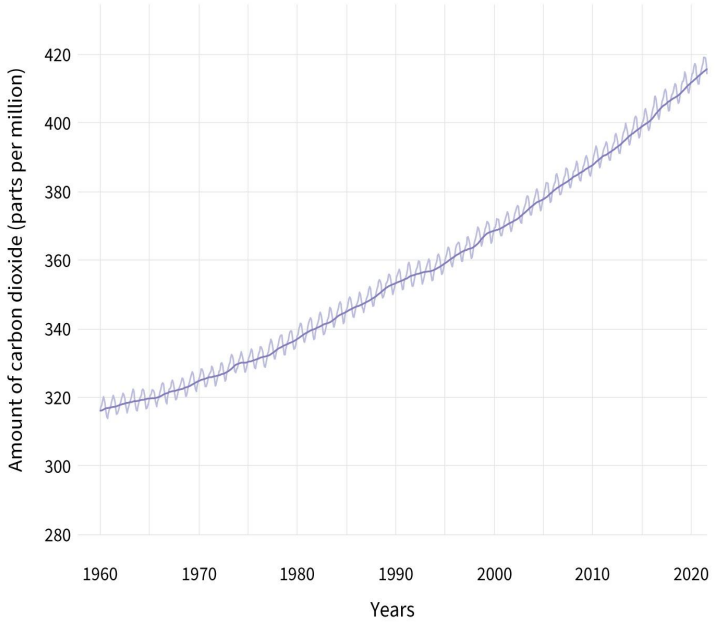
### 1.2 Climate change and coastal aquaculture

In the last two centuries, the world has experienced an unprecedented increase in $CO_2$ concentration ($[CO_2]$) in the atmosphere (about a 50% increase since the start of the industrial revolution in 1750), primarily from fossil fuel emissions and secondarily from changes in net land-use emissions (e.g. conversion of forest into agricultural land). Forests and phytoplankton cannot utilize carbon dioxide fast enough to keep up with the increases in emissions and atmospheric carbon dioxide levels, therefore, the $[CO_2]$ increased dramatically over the past few decades (IPCC, 2013; Jones, 2017; NOAA, 2018, 2021b, 2022b).

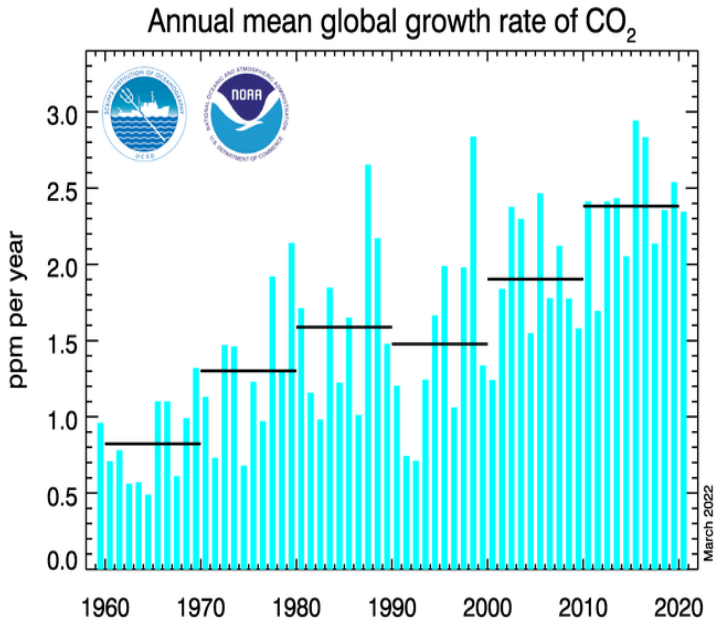
Before the Industrial Revolution, the atmospheric $[CO_2]$ levels were consistently around 280 ppm, in 2011, $[CO_2]$ levels were 391 ppm, and by 2020, the $[CO_2]$ increased to 412.5 ppm (NOAA, 2021b). Data from the last decade show a mean increase rate of $[CO_2]$ of 2.3 ppm/yr (NOAA, 2018, 2021b). Based on the projections for future $[CO_2]$ in the atmosphere, the $[CO_2]$ is expected to rise to 550-1000 ppm by 2100 (IPCC, 2018). Figure 1.2.1 shows the average monthly accumulation of $[CO_2]$ since 1960, the mean growth rate of $[CO_2]$, and different $[CO_2]$ scenarios from the Special Report on Emissions Scenarios (SRES) projected by 2100, the respective scenarios are summarized in Table 1.

Table 1. Description of the Special Report on Emissions Scenarios used in Figure 1.2.1(c) (ACER, 2022).

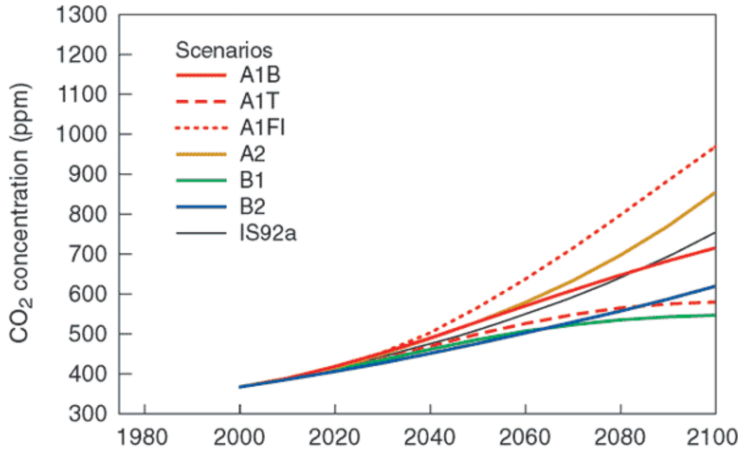
| Scenario | Description | |
|---|---|---|
| A1 | - Very rapid economic growth<br>- Introduction of new technologies<br>- Global population peaking 2050 | B: balanced different energy sources<br>T: non-fossil energy<br>FI: fossil-fuel intensive |
| A2 | - Emphasis in self-reliance and preservation of local identities and economic growth<br>- Global population slowly increasing continuously<br>- Slower, more fragmented technological change | |
| B1 | - Rapid change to service and information economy<br>- Cleaner, more efficient technologies<br>- Global population peaking 2050<br>- Emphasizing global solutions to sustainability<br>- Improved equity | |
| B2 | - Local solutions to economic, social, environmental sustainability<br>- Intermediate levels of economic development<br>- Less rapid technological change<br>- Global population continuously increasing | |
| IS92a | - Prediction from the earlier IPCC Second Assessment Report | |


Amount of carbon dioxide (parts per million)
420
400
380
360
340
320
300
280
1960
1970
1980
1990
2000
2010
2020
Years

(a)


Annual mean global growth rate of CO2
NOAA
ppm per year
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1960
1970
1980
1990
2000
2010
2020
March 2022

(b)



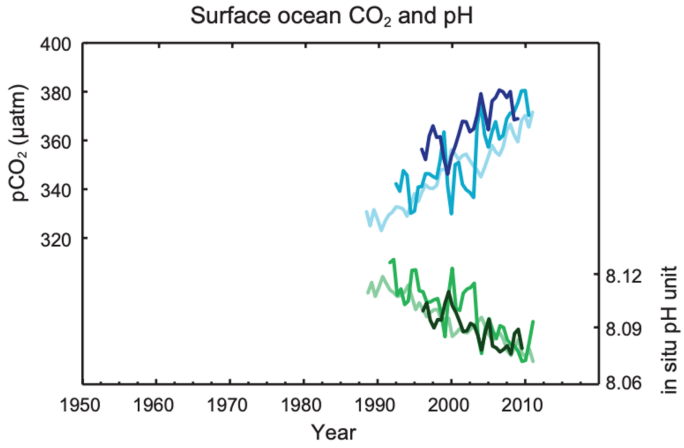

(c)

Figure 1.2.1. (a) Monthly mean atmospheric [$CO_2$] (NOAA, 2021b), (b) annual mean growth rate of [$CO_2$] (NOAA, 2022a), and (c) projected atmospheric [$CO_2$] (IPCC, 2021; ACER, 2022).

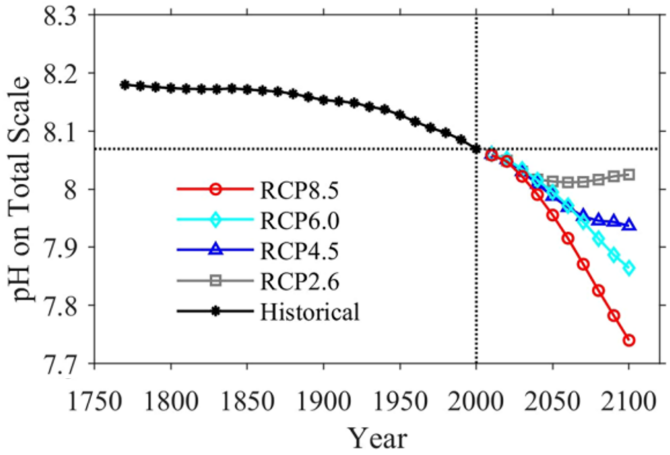
The ocean covers 71% of the Earth's surface, and it acts as a buffer and absorbs atmospheric gases. It has absorbed about 30% of the emitted anthropogenic $CO_2$. However, the absorption of $CO_2$ at a high rate is the direct cause of the drop in pH in the ocean, which is known as ocean acidification (IPCC, 2014). The $CO_2$ dissolves in the water and forms carbonic acid ($H_2CO_3$), which dissociates into a hydrogen ion (H+) and a bicarbonate ion ($HCO_3^-$) (Orr et al., 2005). These hydrogen ions decrease the water's pH, acidifying the ocean. Furthermore, available carbonate ions ($CO_3^{2-}$) bond with excess H+ to form $HCO_3^-$, resulting in fewer carbonate ions available for calcifying organisms, which they need to build and maintain their shells, skeletons, and other calcium carbonate structures (Orr et al., 2005; Roleda & Hurd, 2012; Clements & Chopin, 2017; NOAA, 2020).

The pH of ocean surface water has decreased by 0.1 since the beginning of the industrial revolution, corresponding to a 26% increase in hydrogen ion concentration (IPCC, 2013; NOAA, 2018). The pH of the ocean is expected to keep dropping due to the increase in [$CO_2$], as shown in Figure 1.2.2. Ocean acidification constitutes considerable risks to marine ecosystems, especially affecting polar ecosystems

and coral reefs. Furthermore, a drop in pH impacts the physiology, behavior, and population dynamics of individual species from phytoplankton to animals (IPCC, 2014). Shellfish, such as abalone, may have more difficulties forming their biogenic calcium carbonate ($CaCO_3$) and therefore have difficulty maintaining their external calcium carbonate skeletons (Orr et al., 2005).




(a)




(b)

Figure 1.2.2. (a) Surface ocean [$CO_2$] and pH from 1990 to 2010 (IPCC, 2013) and (b) global ocean surface pH projections to 2100 from the Representative Concentrations Pathway (RCP) scenarios (Jiang et al., 2019).

The rise in the average temperature is directly correlated with the increase in $CO_2$ gas, among other greenhouse gases (IPCC, 2013). Along with the increase in atmospheric temperature, there is a corresponding increase in ocean temperature. The ocean has stored more than 90% of the energy accumulated between 1971 and 2010 (IPCC, 2013), which has rise its temperature. This temperature rise, or ocean warming, is larger near the water surface, with the upper 75 m of the water column warmed by 1.09°C from 2011 to 2020 (IPCC, 2022). Projections also suggest that the temperature on the surface of the ocean will increase 1.5 - 2°C by the year 2100 in the best-case scenario from the Shared Socioeconomic Pathways (SSPs) scenarios, as shown in Figure 1.2.3.

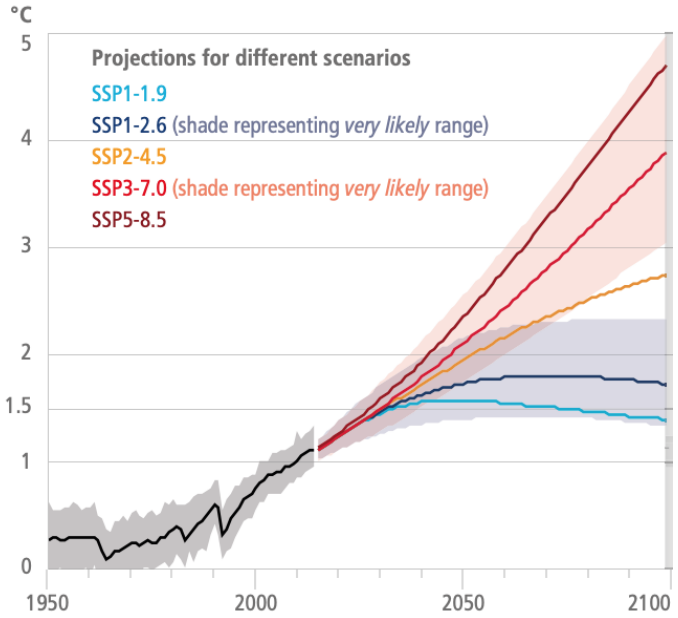



Figure 1.2.3. Global surface temperature change. Increase relative to the period 1850-1900. SSP represents the Shared Socioeconomic Pathways scenarios (IPCC, 2022).

All these changes ($[CO_2]$, temperature, and pH) in the marine environment impact the dynamics and ecosystem in several ways. Many marine species have shifted their geographic ranges, seasonal activities, migration patterns, abundances, and species interactions in response to ongoing climate changes (IPCC, 2014).

Elevated temperature affects life histories, nutrient uptakes, growth, productivity, shifts in geographical ranges (which could cause invasions and extinctions), among others, which change the dynamics and the ecology of marine communities (IPCC, 2014). Moreover, simultaneous factors, such as water warming and ocean acidification, can lead to interactive, complex, and amplified impacts on species and ecosystems (IPCC, 2014).

Since currently there are no ways to control the ocean's environmental conditions, coastal aquaculture is highly affected by these changes in marine conditions. Particularly, these environmental conditions cause direct and indirect impacts on primary producers in the ocean (Stokes, 1986). Algae and other primary producers use the nutrients in the water to photosynthesize and produce organic matter. The chemical composition of these organisms, i.e. lipid, protein, fatty acids, and carbohydrate content, is therefore related to the environmental conditions where they are growing (Stokes, 1986; Wang et al., 2011). At the same time, consumers are impacted by the quality of their food. A change in the chemical composition of the primary producers could impact primary consumers and the food chain. It is important then, to understand the consequences of these changes on primary producers and their effect on the food chain.

### 1.3 Primary producers

Primary producers are organisms capable of transforming inorganic nutrients into organic matter through photosynthesis. They produce organic compounds from atmospheric or dissolved carbon dioxide. Microalgae and seaweeds are at the first trophic level in a food chain and serve as a food source for consumers at higher trophic levels, therefore, they serve as the base for the entire aquatic ecosystem (Krauss & Nies, 2015). Microalgae and seaweeds are also the most abundant primary producers in the ocean, and they fix $CO_2$ with an efficiency ten times greater than terrestrial plants (Tang et al., 2011; Singh & Singh, 2014; Jagtap & Meena, 2022). Furthermore, they are used as a source of food, fuel (oil, biodiesel, bioethanol, biohydrogen, and biogas), stabilizing agents (carrageenan), fertilizer, and in

wastewater treatment as well as in power plants to reduce $CO_2$ emissions and more (Jensen, 1993; FAO, 2003; Singh & Singh, 2014).

Various physical and chemical parameters affect primary producers directly or indirectly, such as light, irradiance, temperature, [$CO_2$], pH, aeration, salinity, nutrients, etc. (FAO, 1996). These parameters also affect their chemical composition like lipid, protein, fatty acid, and carbohydrate content (McConico & Vogt, 2013; Mohsenpour & Willoughby, 2016; Ajjawi et al., 2017; Eloka-Eboka & Inambao, 2017; Hulatt et al., 2017), survival, growth rate, etc. (Eggert, 2012; Raeesossadati, 2014; Negi et al., 2015; Singh & Singh, 2015).

Temperature strongly influences the cellular chemical composition, uptake of nutrients and $CO_2$, productivity, and growth rates for every species of algae and seaweed. Primary producers' growth rates increase with increasing temperature up to a certain limit (Eggert, 2012; Singh & Singh, 2015). However, an increase in biomass does not imply an improvement in their nutritional value (Bottemiller-Evich, 2017). Seaweeds adjust their proteins (qualitatively and/or quantitatively) as well as both lipid composition and degree of unsaturation of fatty acids in response to temperature changes (Eggert, 2012). The response of seaweeds to ocean acidification can be species-specific and may vary depending on their carbon physiology, mode of calcification, morphology (functional growth forms), and life history (Roleda & Hurd, 2012).

### 1.4 Primary consumers

Primary consumers, also known as herbivores, are organisms that feed on primary producers. They make up the second trophic level and are consumed by secondary consumers, tertiary consumers, or apex predators. Zooplankton, herbivore fish and filter feeders are examples of marine primary consumers (Krauss & Nies, 2015; Chandler, 2018).

The production of low trophic level organisms (primary consumers) presents advantages over the production of secondary consumers or higher levels. For example, some of the important environmental problems related to aquaculture come from the feed introduced in the system to grow carnivorous species (Imelda and Rao, 2013; Gökalp, 2019), the constant input of feed for these animals can cause environmental problems, as their farming generates waste and nutrifies the water provoking eutrophication, low oxygenation, among other problems, not to mention feed is one of the highest costs of aquaculture production (Cole et al., 2009; GSA, 2019b). On the other hand, herbivores feed on seaweeds, algae, and other microorganisms living in the water. Consequently, aquaculture of herbivores is a low-cost production activity, depends on more sustainable food sources, and requires little to no external inputs in the system (DeWeerdt, 2020). In fact, it is believed that one of the most significant opportunities to scale sustainable aquaculture solutions is to focus on herbivorous species production (Tasting the future, 2020).

Furthermore, practices like integrated coastal aquaculture systems, where low trophic level animals can be produced together with primary producers, can allow us not only to sustainably produce products for the seafood market but also to improve the coastal conditions, since as discussed before, seaweeds are able to intake nutrients from the water while improving its quality (Biswas et al., 2020; Knowler et al., 2020; Resende et al., 2022).

### 1.5 Project statement

This work explores the relationship between environmental conditions, primary producers, and primary consumers. We initially studied the combined effects of temperature and pH on the growth and nutrient content of two algae species, *Nannochloropsis oculata* and *Chaetoceros gracilis*. These two species were chosen since they are well studied and widely used as food sources for low trophic shellfish aquaculture. To go further, we then studied the effects of temperature on the growth and chemical composition of the marine seaweed *Palmaria mollis*, and tested the indirect effect of the increasing ocean temperature on the

primary consumers by feeding seaweeds cultured at various temperatures to juvenile red abalone, *Haliotis rufescens*, and measuring their growth, food consumption rate, and their chemical compositions.

### 1.6 Objectives

The overall goal of this project is to evaluate the direct effects of the pH and temperature on primary producers' growth and chemical/nutritional composition and evaluate the indirect effects on primary consumers. The objectives are outlined below:

1. Determine the effects of pH and temperature on the growth and chemical/nutritional composition of marine algae *Nannochloropsis oculata* and *Chaetoceros gracilis*.
2. Determine the effects of temperature on the growth and chemical/nutritional composition of seaweed *Palmaria mollis*.
3. Evaluate the effects of the nutritional composition of *Palmaria mollis*, on the juvenile red abalone *Haliotis rufescens* who consume them.

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# Chapter 2: Effects of temperature and pH on the growth and protein content of two marine algae, *Nannochloropsis oculata* and *Chaetoceros gracilis*

## 2.1 Abstract

Production of marine microalgae has become popular in the last decades. These high-value algae are sought after for the production of biogas, wastewater treatment plants, or as feed for farmed species in hatcheries such as filter feeders, like oysters, among other uses. Studies have been conducted in order to optimize the growth of biomass and the production of by-products from these species. However, less attention has been put into understanding how the rise of ocean temperature and ocean acidification could potentially impact them. Furthermore, the combined effects of temperature and pH have been less studied. This study aims to understand the possible effects of the projected temperature and pH of the ocean due to climate change on the growth and nutrient content of marine microalgae.

The species *Nannochloropsis oculata* and *Chaetoceros gracilis* were chosen for the study as they are important species in coastal aquaculture, and several farmers use them as feed for their animals in the hatcheries. The study consisted of growing these two species under two different temperatures, 13°C and 20°C, and two pH levels, 8.2 and 7.6, representing current and projected ocean conditions, in Northern California, due to climate change, respectively. The highest final cell count, specific growth rate, and protein content were found when both species of algae were grown at 20°C and pH 7.6, indicating the projected conditions caused by climate change did not have negative effects on the marine microalgae tested. The ANOVA results for all the parameters suggest that temperature has a bigger influence than pH on both species of algae.

## 2.2 Introduction

Microalgae are the most abundant primary producers in the ocean, and they can fix $CO_2$ with an efficiency ten times greater than terrestrial plants (Singh & Singh, 2014). As primary producers, they are autotroph species capable of transforming inorganic compounds into organic matter through photosynthesis, and therefore, they are the base of a food web and the foundation of an ecosystem. They are also very important species that have been used as a source of food, fuel (oil, biodiesel, bioethanol, biohydrogen, and biogas), stabilizing agents (carrageenan), fertilizers, and in wastewater treatment as well as in power plants to reduce $CO_2$ emissions and more (Jensen, 1993; Singh & Singh, 2014). They are of high importance to maintaining and developing sustainable and strong coastal food production, or coastal aquaculture, as they are the base of the food chain and serve as a food source for consumers at higher trophic levels (Krauss & Nies, 2015).

Coastal areas are very productive and can be great spaces to grow important marine species for the global seafood market (Primavera, 2006). Herbivores and filter feeder species are ideal products of coastal aquaculture, as little to no commercial feed input is needed for growing them (Primavera, 2006; van der Schatte Olivier, 2020). Filter feeders, like oysters and mussels, consume algae and other microorganisms living in the water (Azra et al., 2021). Therefore, they are excellent low-cost options for seafood production, and a solution to an already overfished industry (van der Schatte Olivier, 2020; Azra et al., 2021).

However, the nature of coastal aquaculture makes it virtually impossible to control the water quality for aquaculture, making it highly susceptible to natural environmental conditions, like temperature and pH (Islam et al., 2019). Unfortunately, in the last decades, the ocean has been experiencing rapid changes in its environmental conditions, which have impacted the entire dynamics and biology of the marine ecosystems (Clements & Chopin, 2017). These changes have occurred due to an unprecedented increase

in $CO_2$ concentrations caused by anthropogenic activities, such as agriculture and livestock farming, burning of fossil fuels, deforestation, among others (IPCC, 2013; Concern, 2022).

Increasing $CO_2$, among other greenhouse gases, has increased the average ocean temperature and caused its pH to drop, which is known as ocean acidification (IPCC, 2014). These changes affect directly and indirectly primary producers in the ocean (Stokes, 1986). The way that microalgae and other primary producers use the nutrients in the water to photosynthesize and produce organic matter is influenced by temperature and pH. Therefore, changes in these factors can affect microalgae growth rates (Singh & Singh, 2015), productivity (Raeesossadati et al., 2014), community composition (Tatters et al., 2018), survival, (Hansen, 2002), and nutritional value or chemical composition, i.e. protein, lipid, and carbohydrate content (Stokes, 1986; Wang et al., 2011; Mohsenpour & Willoughby, 2016; Eloka-Eboka & Inambao, 2017; Hulatt et al., 2017). Moreover, changes in the chemical composition of algae, consequently, as consumers depend on the quality of their food, could impact the primary consumers and the complete food chain.

Temperature strongly influences the nutrients and $CO_2$ uptake by the microalgae and further impacts their productivity, growth rates, and chemical composition. Generally, higher temperatures boost the microalgae metabolism increasing growth rates up to a point, or optimal temperature (Ras et al., 2013; Singh & Singh, 2015). After the optimal temperature, enzyme degradation can be caused by heat stress, as well as failure of the photosynthetic system, cell damage, and even death may occur (Ras et al., 2013; Singh & Singh, 2015; Barten et al., 2020). The optimal temperature for most microalgal species is between 20°C and 25°C (Ras et al., 2013) but it varies between species and can even change in relation to other conditions like salinity, light, pH, etc., and it can be as high as 40°C for some species (Ras et al., 2013; Barten et al., 2020). However, temperature can also influence the chemical composition of microalgae, and an increase in biomass due to an increase in temperature could be a trade-off for their nutritional value (Bottemiller-Evich, 2017). For example, when three species of microalgae were cultured

at 20°C and 30°C, the growth rates of all of them were higher at 30°C, however, the protein content of the three species decreased when they were cultured at that temperature (Militão et al., 2019).

Another important factor that influences microalgae is pH. The metabolism of microalgae depends on the pH of the medium where they are grown, given that pH can influence the algal photosynthetic rates and their carbon conversion efficiency, affecting their growth and chemical composition (Chen and Durbin, 1994; Moazami-Goudarzi and Colman, 2012; Galès et al., 2020). Although the increase in $CO_2$ availability related to the drop in pH can potentially stimulate photosynthesis, the stress due to the low pH may be detrimental (Gao et al., 2019). In general, a pH of 8.2 is considered to be the optimal pH for most marine microalgae species (Moazami-Goudarzi and Colman, 2012). Moreover, in many marine systems, pH may be an important factor in regulating algal abundance and distribution (Chen and Durbin, 1994). Studies have shown that the response of algae to pH varies within species (Hinga, 2002) and it could also vary when interacting with other factors, like temperature, salinity, etc. (Gao et al., 2019).

The chemical composition of algae has been generally framed around lipids production for biodiesel and other high-end uses. However, the need for sustainable protein sources in the world has increased rapidly, both for human consumption and livestock and aquaculture production (Khoshnevisan et al., 2022). Algae are great candidates for protein supply. In fact, protein is the principal organic constituent of algae, usually followed by lipids and then carbohydrates. Generally, protein constitutes 12-35% of the dry weight of algae (FAO, 1996). However, some strains of algae can produce as much protein as 70% of their dry weight (Matos, 2019). Furthermore, for animals and humans, proteins represent the primary structural and functional elements of the body and are required for the growth and regeneration of tissues, whereas lipids and carbohydrates serve mainly as fuels, (Hawkins and Bayne, 1991; de la Puerta Fernandez, 2017). However, proteins are usually the most expensive element in animals' diets and cannot be replaced by other elements (de la Puerta Fernandez, 2017). Algae with high protein content might be a great sustainable option to supply this need.

Most studies focus on manipulating different environmental factors to optimize algal growth and lipid production. Few studies have focused on understanding the possible changes in algae caused by climate change. In this study, we aim to study the possible changes in growth rates and nutrient content of two strains of microalgae *Nannochloropsis oculata* and *Chaetoceros gracilis* due to the rise in temperature and drop in pH caused by climate change. Both microalgae *N. oculata* and *C. gracilis* were selected for this study because they are important species for aquaculture and the production of important components like biofuels. *N. oculata* is known to have rapid growth rates, to be rich in protein, and polyunsaturated fatty acids content, and can be found in both marine and freshwater environments (Sukarni et al., 2014; Wiktorowicz-Conroy, 2022). *C. gracilis* is widely used as a food source in shrimp and mollusk mariculture and it is a species of interest as a biofactory to produce biofuels and other beneficial metabolites (Ortega-Salas and Reyes-Bustamante, 2013; Tokushima et al., 2016).

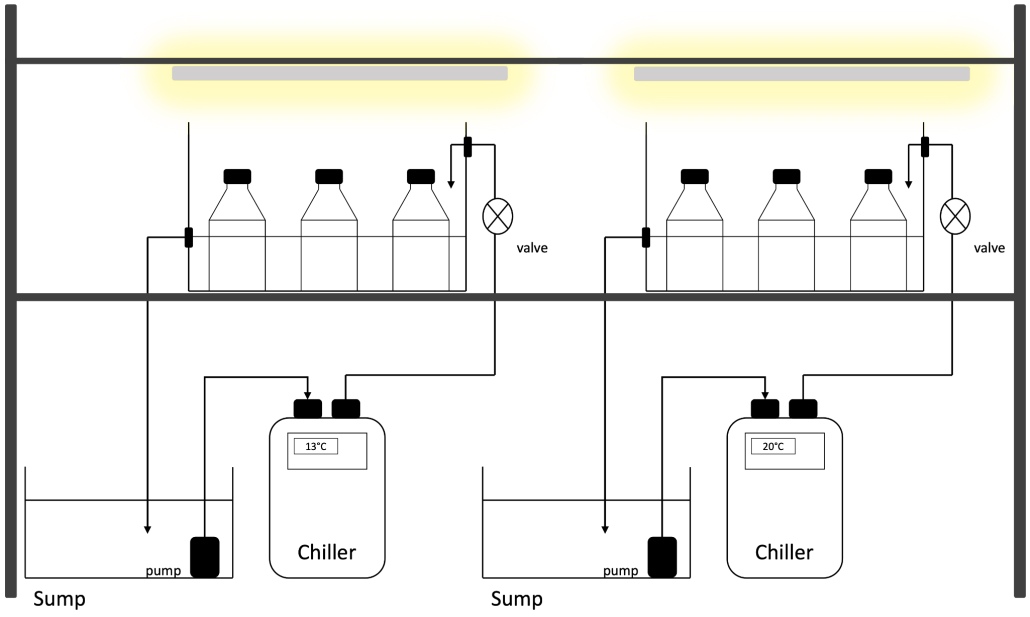
The study consisted of growing these two species for 20 days under two different temperatures, 13°C, and 20°C, and two pH levels, 8.2 and 7.6, representing current ocean conditions and projected conditions by 2100 due to climate change, respectively. The hypothesis for this study was that both strains of algae have higher growth rates at higher temperature and lower ph, but their protein content is lower under the same conditions.

## 2.3 Materials and methods

### *2.3.1 Microalgae cultivation and harvest*

The inoculum of marine microalgae *N. oculata* and *C. gracilis* were purchased from The Culture Collection of Algae at the University of Texas at Austin (UTEX, Texas, USA), UTEX LB 2164 and UTEX LB 2658, respectively. The microalgae were grown in 900-mL flasks in salty water made with Instant Ocean sea salt (Spectrum Brand Inc., Virginia, USA) at a salinity of 32 ppt and enriched with f/2 medium (ProLine®F/2 Algae Food, Aquatic Eco-Systems, Florida, USA). All cultures were inoculated with a density of $1x10^5$ cells/mL in a biological safety cabinet to avoid contamination. The cultures were

exposed to a photoperiod 12 h:12 h (light:dark cycle). A two-temperature (13°C and 20°C) x two-pH (7.6 and 8.2) factorial design with triplicates was applied. A temperature-controlled water bath equipped with a chiller (DBA-075 1/10HP, JBJ Arctica, Missouri, USA) was used to maintain the cultures at the targeted temperatures. The pH of each bottle was assigned randomly within each temperature. Figure 2.3.1 shows a diagram of the water temperature control system utilized for the experiments and the setup of the cultures.




(a)

(b)

Figure 2.3.1. (a) Water temperature control system diagram and (b) setup of the experiment.

The pH was controlled using a pH controller (Mini pH Controller BL931700, Hanna Instruments, Rhode Island, USA) by adding pure $CO_2$ when necessary to drop the pH to the set pH. A detailed description of the pH control system is shown in section 2.3.2. Air was bubbled into the cultures continuously during the entire experiment to assist with mixing the culture to keep cells in suspension, allowing uniformity of conditions for the culture. All cultures were harvested by centrifugation (Centrifuge 5702, eppendorf, Connecticut, USA) at a speed of 4400 rpm for 15 minutes, freeze-dried (Freezone 4.5, Labconco, Missouri, USA) for at least 48 hours and preserved at -80°C. Biomass (cells/ml), specific growth rate ($d^{-1}$), and protein concentration (%DW) were analyzed for each condition.

- *2.3.1.1 Nannochloropsis oculata*

*N. oculata* was cultivated at 13°C for both pHs (7.2 and 8.2) first (Trial 1), and the cultures at 20°C started ten days later (Trial 2). The cultures were carried out in triplicates for 20 days. Each culture was

sampled every two days for cell counting. Cells were counted utilizing a hemocytometer (Bright-Line™, Hausser Scientific, Pennsylvania, USA) under a microscope (Digital Microscope, Celestron, California, USA). On Day 20, all experiments were terminated, and biomass was harvested, freeze-dried, and preserved at -80°C for further analysis.

The biomass obtained from Trial 1 was destroyed during the process of freeze-drying; therefore, another trial (Trial 3) was conducted for T=13°C at both pH levels. However, due to COVID-19 related circumstances, in order to reduce in-person presence in the laboratory, cultures were sampled every five days for cell counting (except for Day 15, when cells were counted on Day 16 instead) instead of every two days. On Day 20, the experiments were terminated and sampled following the same procedures used for Trial 2.

Due to the different sampling frequencies for Trial 3, significant differences in biomass production (cells/ml) throughout the trials were analyzed with data from Trials 1 and 2. Biomass production between Trials 1 and 3 was also compared to determine if there was any significant difference between the trials under the same conditions but with different sampling frequencies.

Likewise, specific growth rate and final cell count were compared between Trials 1 and 2. The specific growth rate was calculated using Equation 1 (Hossain et al., 2019)

$$\boldsymbol{\mu} = \frac{ln(\frac{N_2}{N_1})}{t_2 - t_1} \qquad (1)$$

where,

$\boldsymbol{\mu}$: specific growth rate ($d^{-1}$)

$N_1$ and $N_2$: initial and final cell count, respectively (cells/mL)

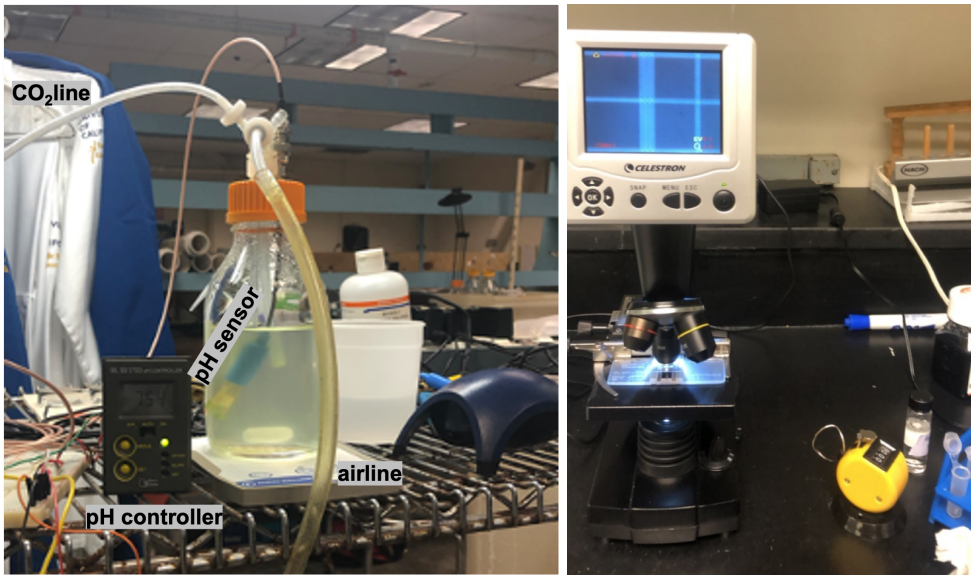
$T_1$ and $t_2$: initial and final time, respectively (days)

The total protein content was analyzed and compared as described in section 2.3.3 for both temperatures and pH from the cultures grown in Trial 2 and 3.

- *2.3.1.2 Chaetoceros gracilis*

*C. gracilis* was cultivated simultaneously for all the treatments in the experiment. The experiments were carried out in triplicates for 20 days. Each culture was sampled every two days for cell count. Cells were counted utilizing the same method described in 2.3.1.1. On day 20, all cultures were harvested, freeze-dried, and preserved at -80°C for further analysis.

A detailed picture of the algae cultures is shown in Figure 2.3.2. Each culture bottle is connected to an air supply, a $CO_2$ supply, and a pH sensor. A picture of the hemocytometer and the microscope used to determine the density of the cultures is also shown in Figure 2.3.2.




(a) (b)

Figure 2.3.2. (a) Detail of algae culture bottle and (b) hemocytometer and microscope used to determine the density of the cultures.

*2.3.2 pH control system*

$CO_2$ gas was added to the system to mimic the ocean acidification caused by climate change. For this purpose, we focused on the maintenance of the pH level. Since algae uptake of inorganic carbon from the culture media can cause the pH to rise significantly (Juneja et al., 2013), the pH in the media was monitored closely and pure $CO_2$ gas was added automatically to the system when the pH rose. For this, a pH controller was connected to a solenoid and a sensor. The sensor was placed in each culture, and the pH controller was also connected to a data logger that was constantly recording pH data every minute during the trial period. Figure 2.3.3 shows a diagram of the pH control system. A detailed diagram shows the connection between all the parts of the pH control system in Figure 2.3.4.

The pH controller sent a signal to the solenoid that was connected to a pure $CO_2$ cylinder (Figures 2.3.3 and 2.3.5). The signal would open the solenoid valve and let the gas in the system whenever the pH rose from the set pH, and close to stop the gas flow when the pH set was reached again. Since the pH reading was sensitive and can change fast, the $CO_2$ gas was added slowly by controlling it with a manifold (Figure 2.3.5). Although sometimes the pH continued to drop even after the gas was stopped and caused some variability in the system, we were able to maintain the pH at the targeted level in the study, as shown in Figures 2.3.6 to 2.3.13.

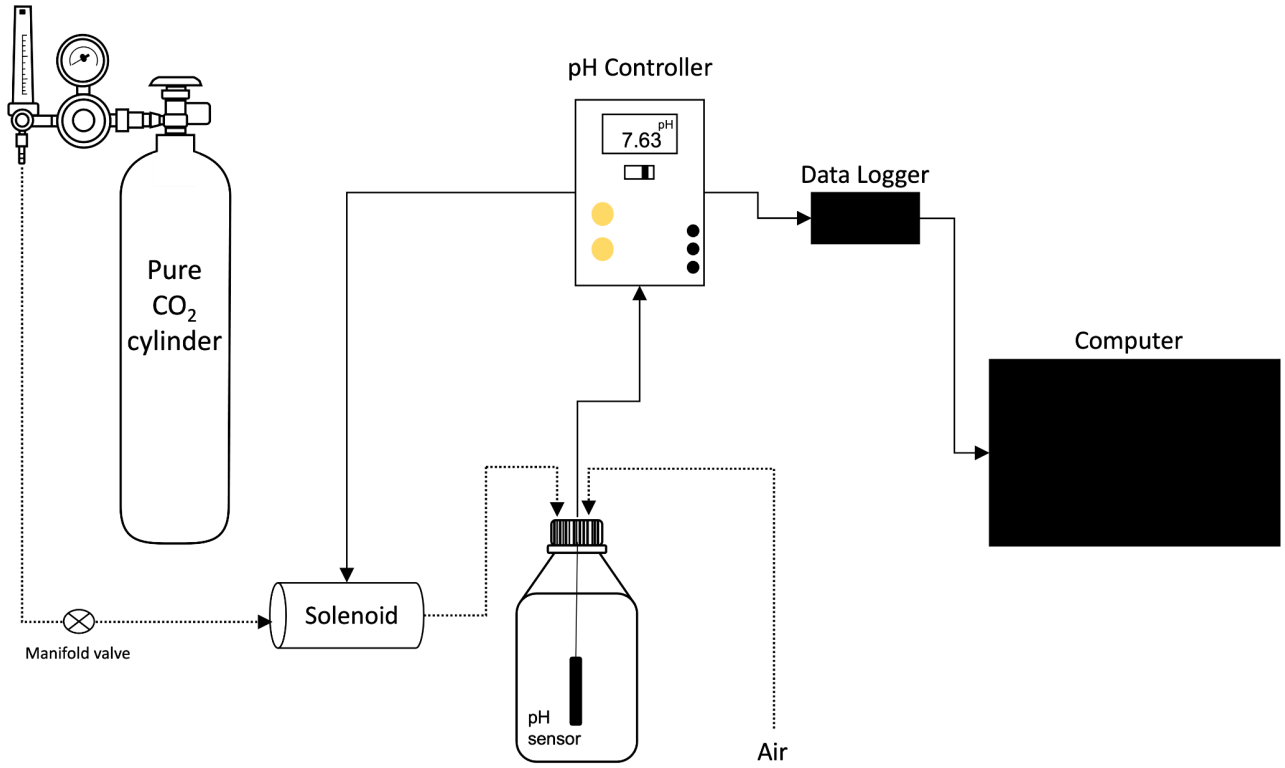



Figure 2.3.3. Diagram showing the pH control system.

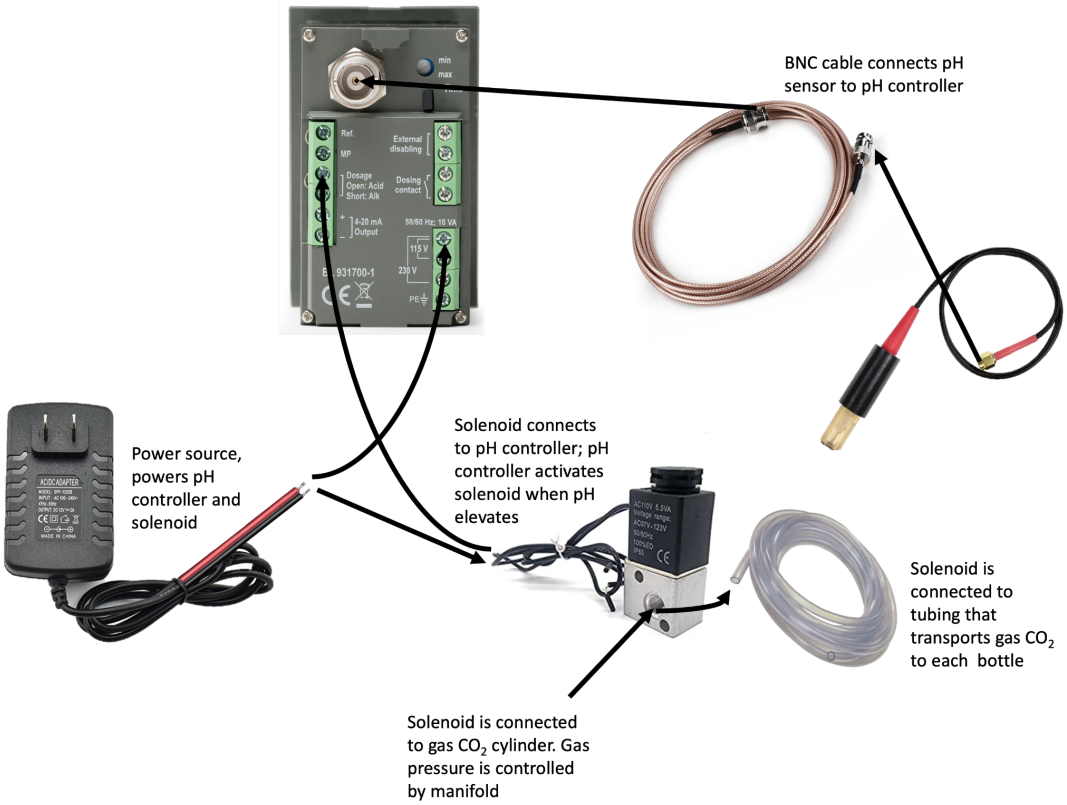
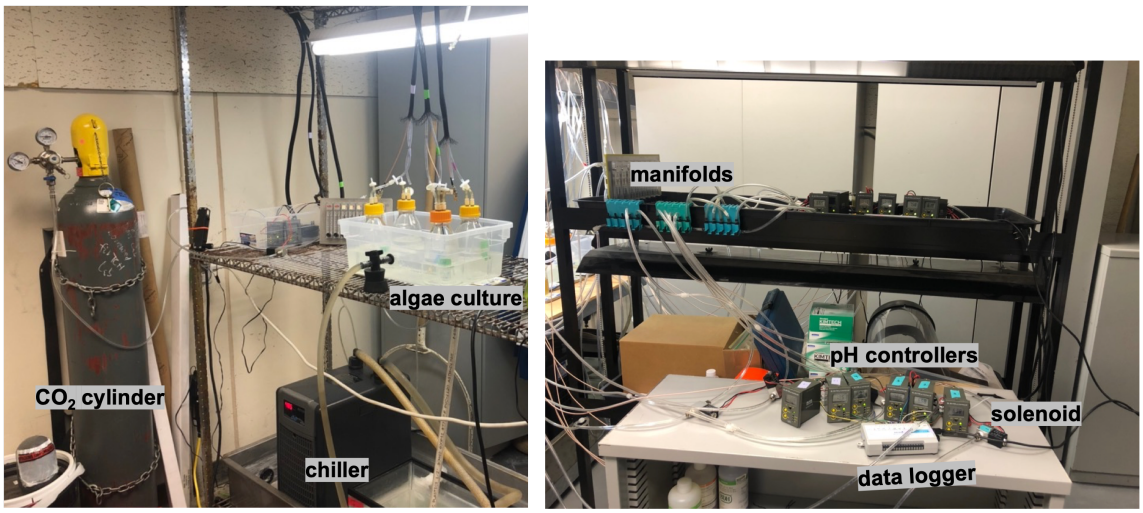



Figure 2.3.4. Representation of the connection between the pH control system parts in detail.




(a) (b)

Figure 2.3.5. (a) System connection to the $CO_2$ cylinder and (b) detailed connections to manifolds, solenoids, pH controllers, and data logger.

*2.3.3 pH system characterization*

The pH was recorded every minute in each culture bottle. Temperatures will be referred to as T1 and T2 as the specific temperature related to each pH culture was not tracked. T1 and T2 are arbitrary labels with no dependence on temperature value. The mean pH throughout the experiments for each treatment and microalgae strain are located in Table 2.3.1.

Table 2.3.1. Mean pH values for each treatment and microalgae.

| Microalgae strain | Treatment | | pH (mean±SE) |
|---|---|---|---|
| *N. oculata* | T1 | pH 7.6 | 7.41±0.001[b] |
| | | pH 8.2 | 7.87±0.0004[a] |
| | T2 | pH 7.6 | 7.51±0.001[b] |
| | | pH 8.2 | 7.79±0.001[a] |
| *C. gracilis* | T1 | pH 7.6 | 7.37±0.001[b] |
| | | pH 8.2 | 8.37±0.005[a] |
| | T2 | pH 7.6 | 7.32±0.002[b] |
| | | pH 8.2 | 8.02±0.001[a] |

Significant differences between pH treatments for each temperature and microalgae strain are indicated by different superscripts.

All pH values were significantly different for *N. oculata* ($p<0.0001$) and *C. gracilis* ($p<0.0001$) experiments. Figures 2.3.6 to 2.3.13 show the change of pH in time of the algae cultures for each pH and each species. Each figure shows the recorded values of each bottle culture, or replication, and the mean values for each treatment.

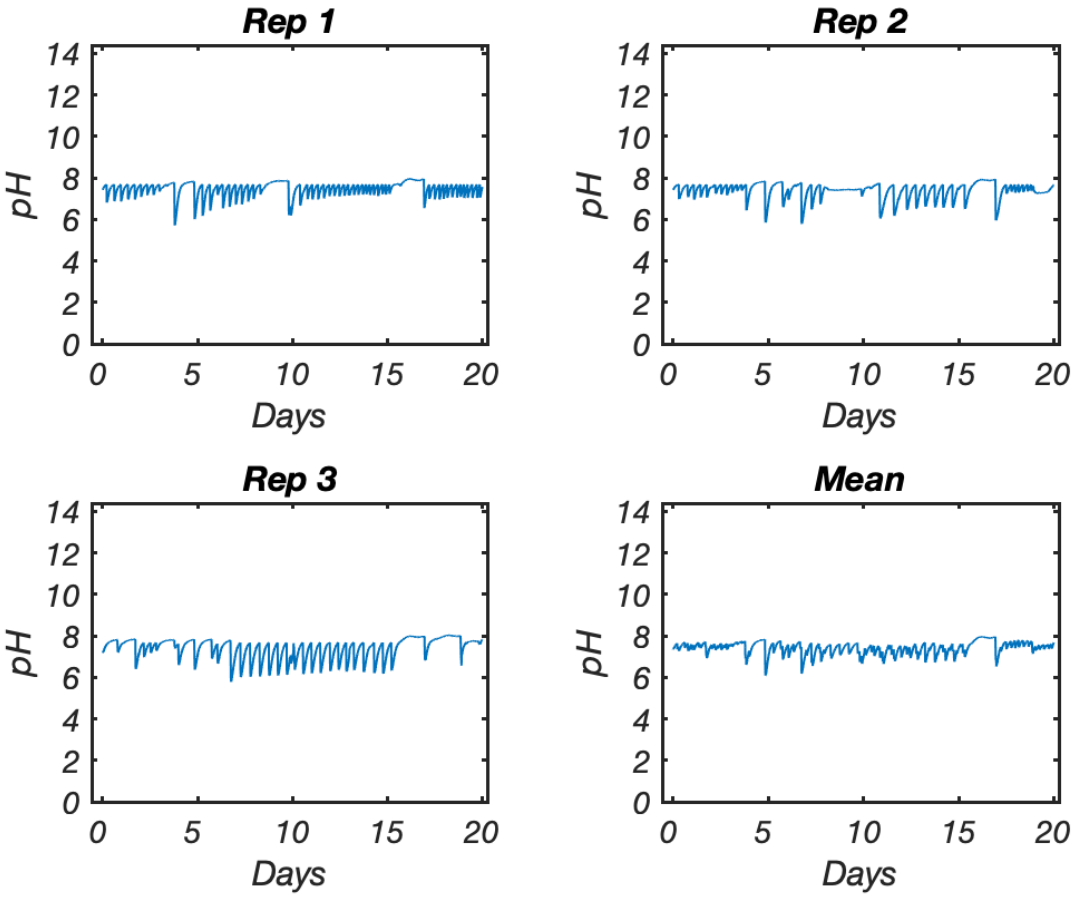



Figure 2.3.6. Change of pH in time for cultures of *Nannochloropsis oculata* set at pH 7.6 for T1.

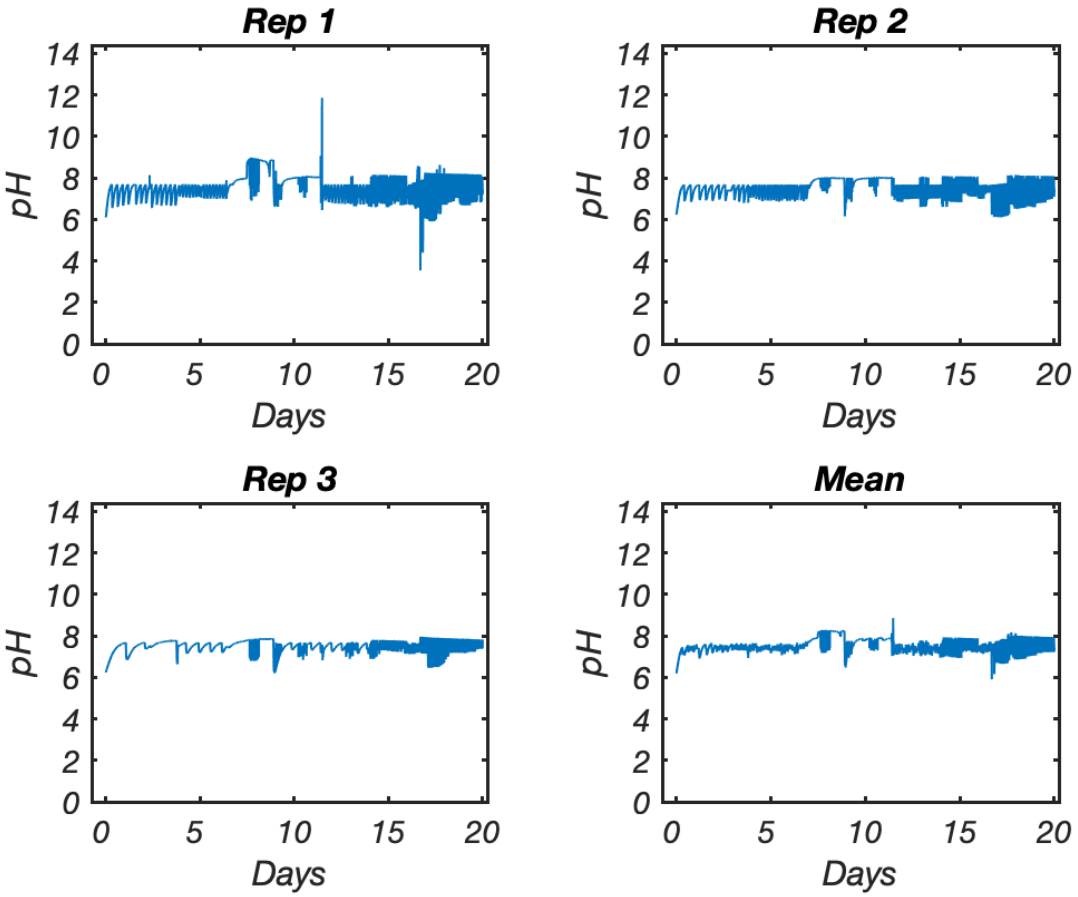



Figure 2.3.7. Change of pH in time for cultures of *Nannochloropsis oculata* set at pH 7.6 for T2.

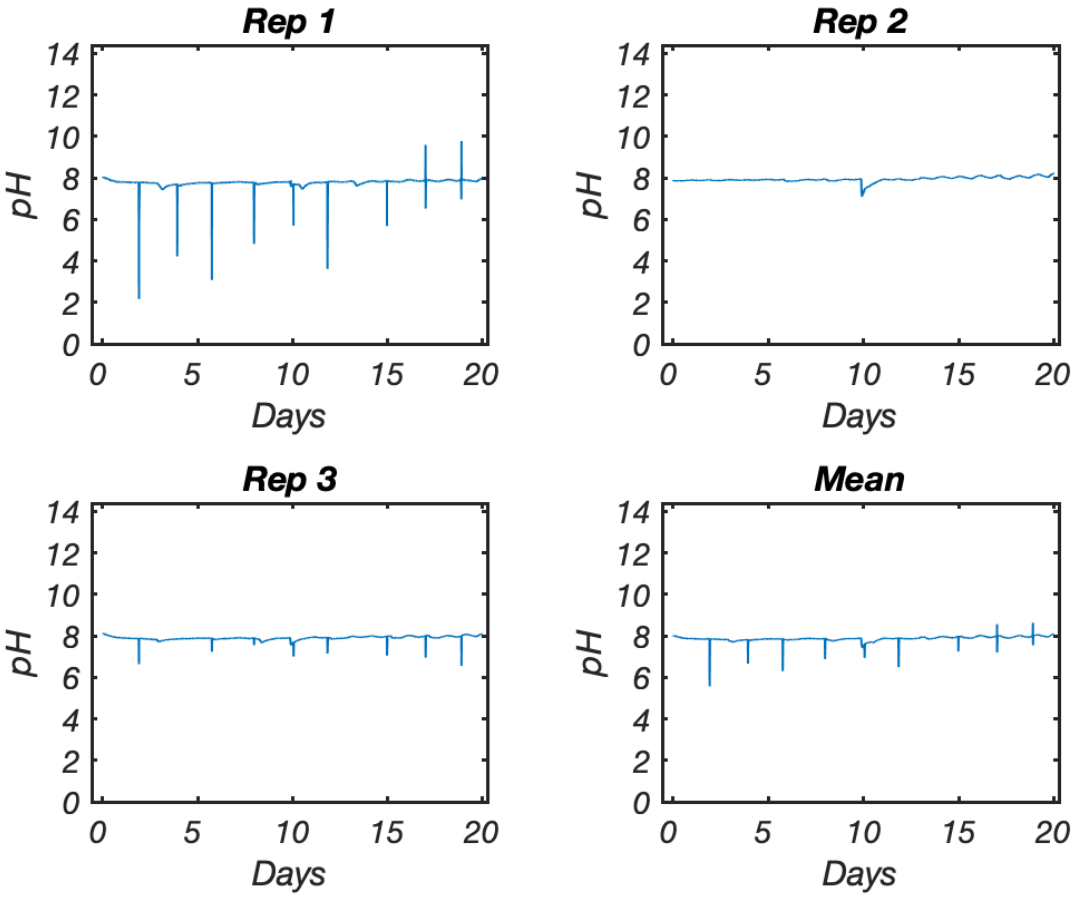



Figure 2.3.8. Change of pH in time for cultures of *Nannochloropsis oculata* set at pH 8.2 for T1.

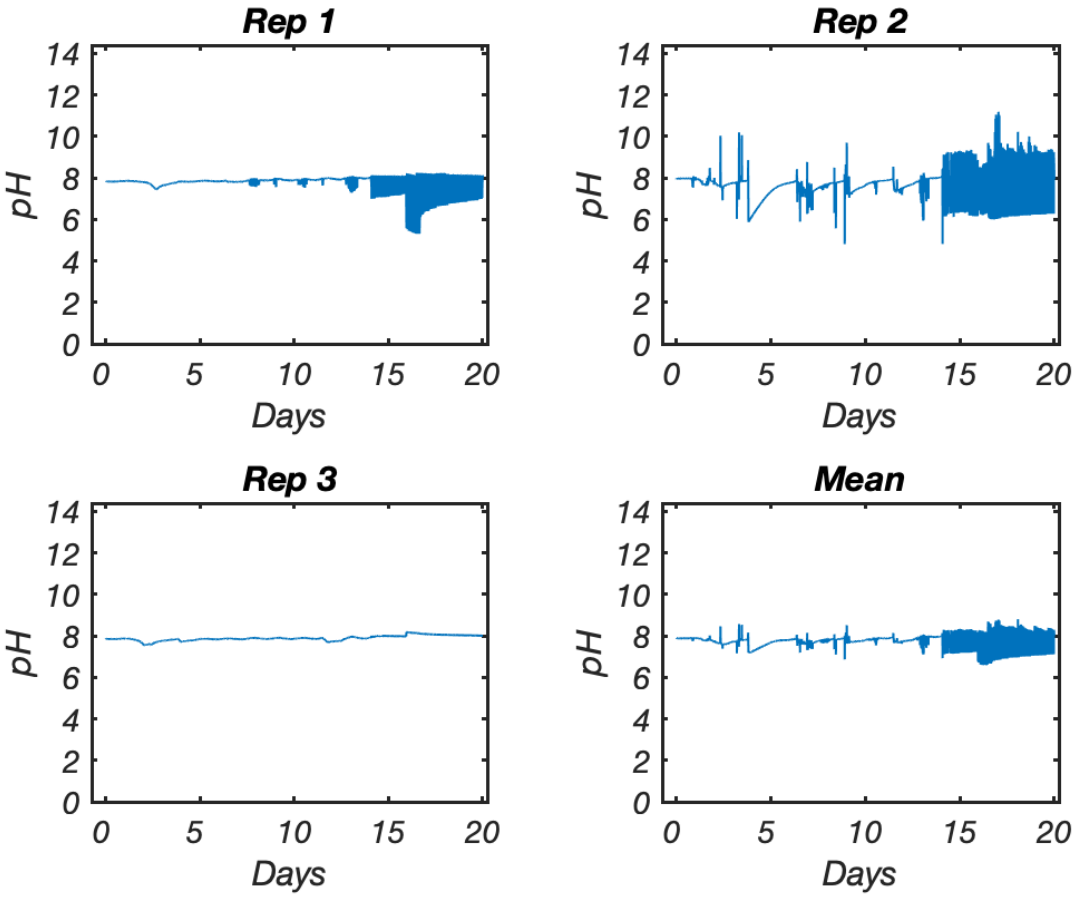



Figure 2.3.9. Change of pH in time for cultures of *Nannochloropsis oculata* set at pH 8.2 for T2.

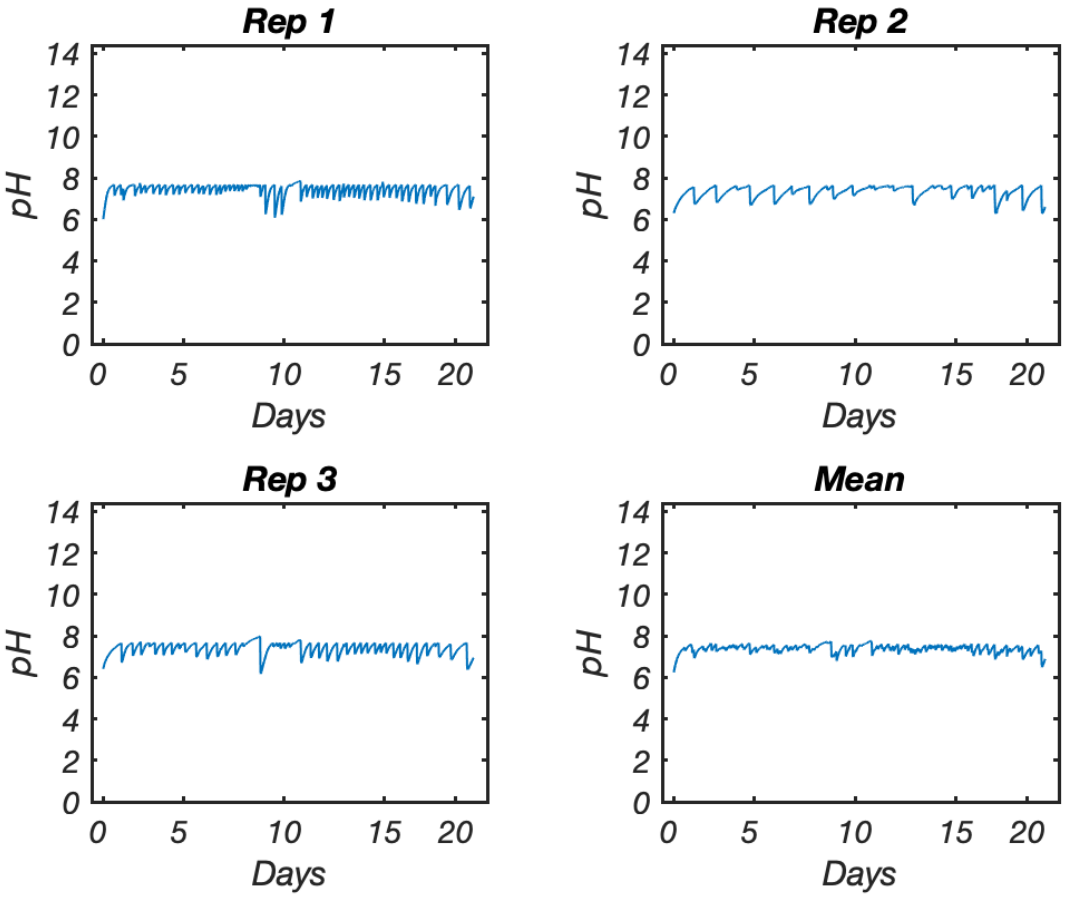



Figure 2.3.10. Change of pH in time for cultures of *Chaetoceros gracilis* set at pH 7.6 for T1.

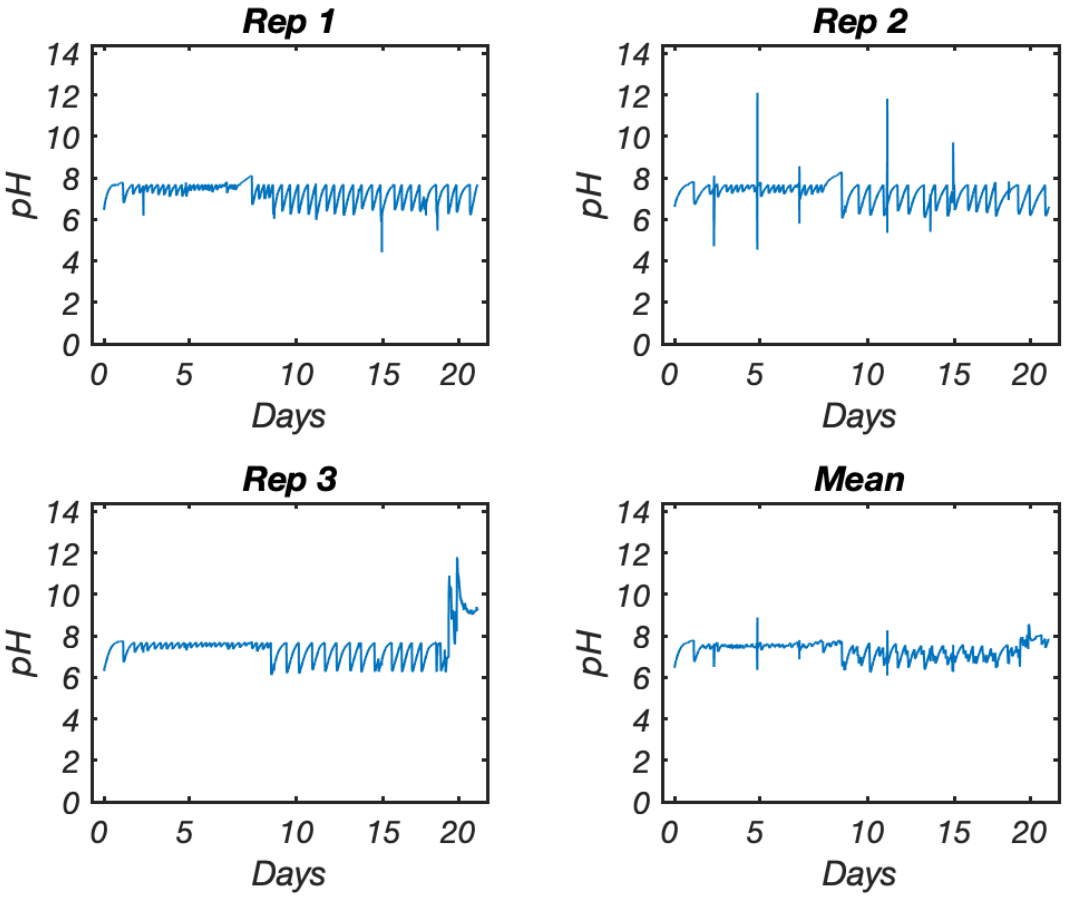



Figure 2.3.11. Change of pH in time for cultures of Chaetoceros gracilis set at pH 7.6 for T2.

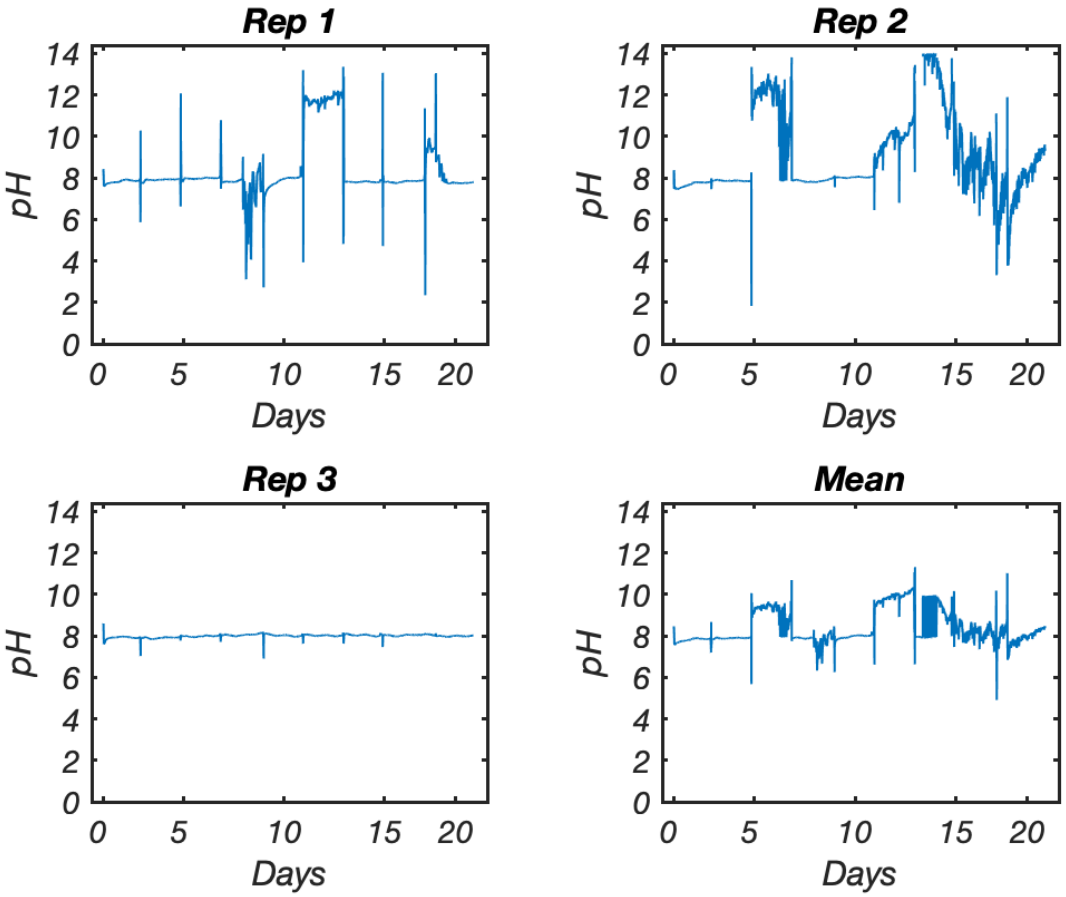



Figure 2.3.12. Change of pH in time for cultures of *Chaetoceros gracilis* set at pH 8.2 for T1.

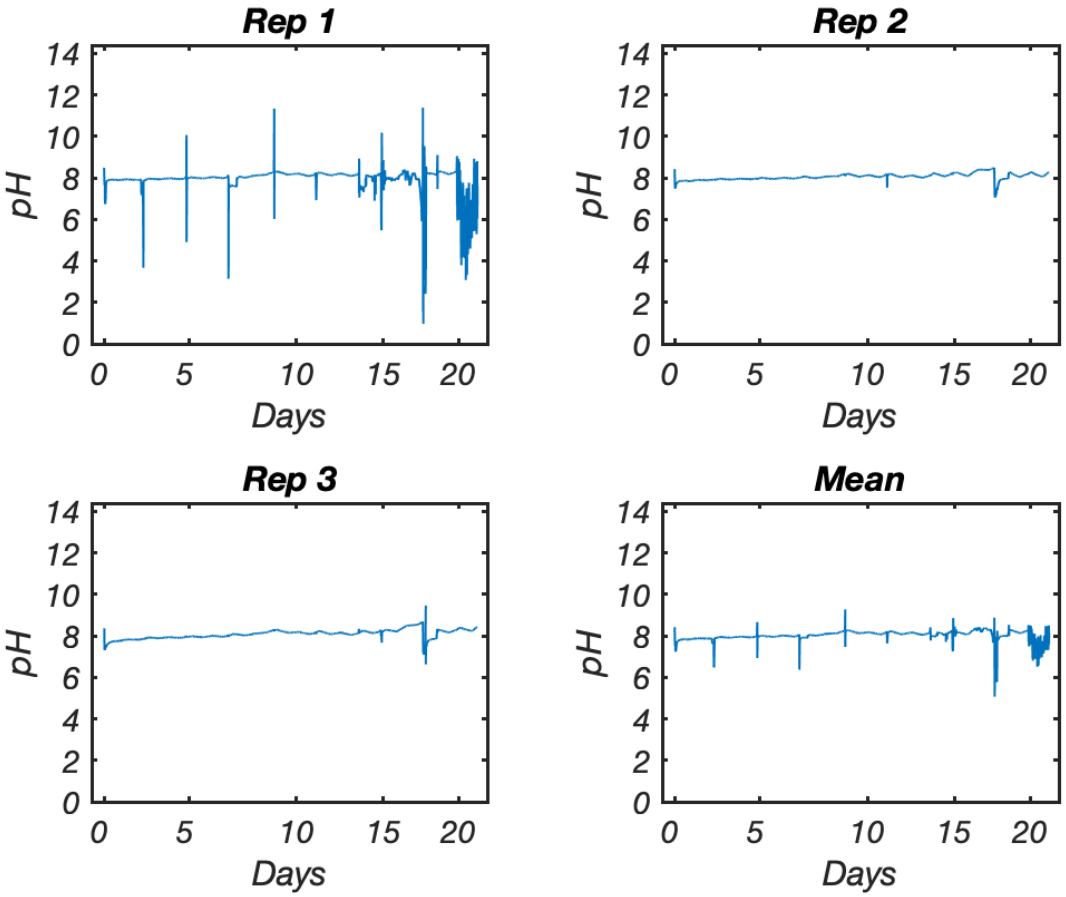



Figure 2.3.13. Change of pH in time for cultures of Chaetoceros gracilis set at pH 8.2 for T2.

*2.3.4 Protein extraction and quantification*

Protein extraction from the harvested algae biomass followed Slocombe et al. (2013) and was adapted to utilize the Bio-Rad DC Protein Assay (BIO-RAD, California, USA) for quantification. For each microalgae species, 2.5 mg of freeze-dried microalgae material was weighed out and vortexed in 100 mL 24% (w/v) trichloroacetic acid (TCA). Homogenates were incubated at 95°C for 15 minutes, in screw-capped microcentrifuge tubes and allowed to cool down to room temperature (25°C). Samples were then diluted to 6% TCA (w/v) with the addition of 300 mL of distilled water. The samples were centrifuged at 15,000 rpm for 20 min at 4°C, and their supernatants were discarded. The pellets were resuspended in 250 mL Bio-Rad DC Protein Assay reagent A' (20 mL reagent S for each mL of reagent

A) and incubated at 55°C for 3 hours. The Bio-Rad DC Protein Assay's reagents S and A are chemical mixtures. Reagent S is a surfactant solution that contains sodium dodecyl sulphate (5-<10%) and reagent A is an alkaline copper tartrate solution that contains sodium hydroxide (2.5-<5%) and disodium tartrate (0.1-1%). Samples were then cooled down to room temperature and spun at 15,000 rpm for 20 minutes at 25°C, the supernatant was retained and preserved at -80°C for further analysis. For protein quantification, the Bio-Rad DC Protein Assay was utilized. A standard was prepared with bovine serum, and six dilutions were prepared to have concentrations of protein from 0.2 mg/mL to 1.5 mg/mL, a standard curve was obtained every time we ran the assay, with $R^2 = 0.990 \pm 0.002$ (mean±SE). Figure 2.3.14 is an example of one of the standard curves obtained for the assay. The samples and standard dilutions were prepared with distilled water and analyzed following the Bio-Rad DC Protein Assay protocol. To quantify the protein of the microalgae samples, 30 mL of the sample was placed in the microplate followed by 200 mL of reagent B. For the standard curve, 5 mL of each dilution was placed in the microplate, then 25 mL of reagent A' was added followed by 200 mL of reagent B (Figure 2.3.15). The microplate was then placed in a slow shaker in the dark for 15 min. After the shaker, the plate was placed in a microplate reader (Infinite M200, Tecan, Zürich, Switzerland) and the absorbance was then read at 750 nm.

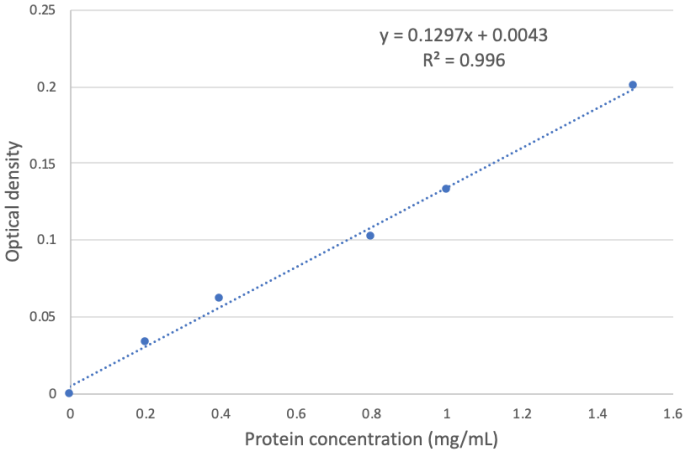
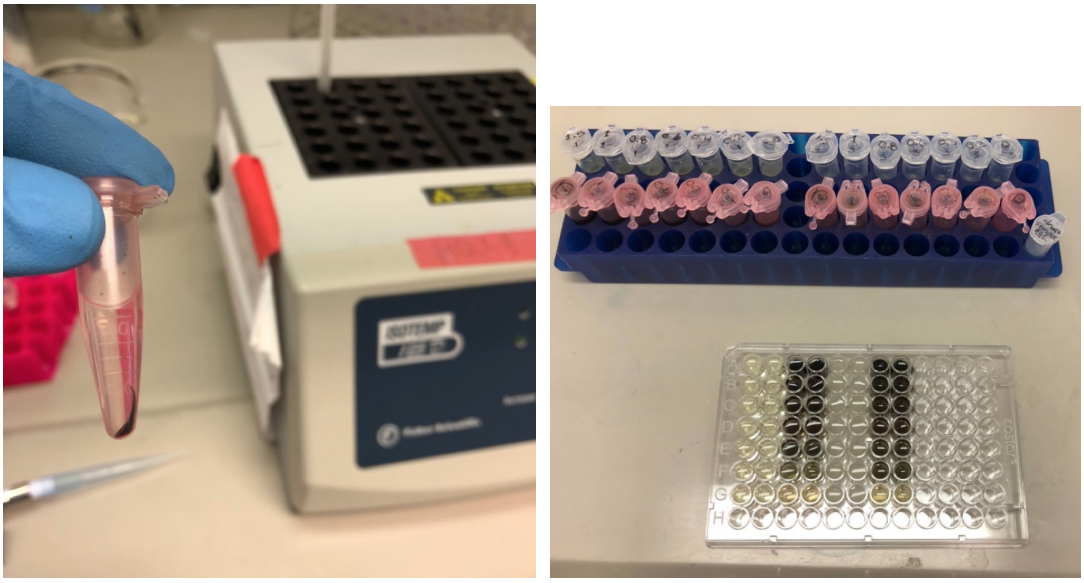



Figure 2.3.14. Example of the standard curve obtained for every microplate used to read the protein extractions.



(a) (b)

Figure 2.3.15. (a) Picture of a pellet obtained as part of the protein extraction protocol and (b) picture of a microplate ready to be placed in the shaker for further reading.

*2.3.5 Statistical analysis*

The results were analyzed for statistical differences using the SAS Enterprise Guide (SAS, North Carolina, USA) analytical software. The effects of pH, temperature, and their interaction were analyzed using linear models. To analyze the biomass vs time curve, a 2-way ANOVA analysis was performed with pH and temperature as the independent variables and time as a continuous variable. When the analysis was performed some heteroskedasticity problems were found, and a log data transformation was performed to fix them. To analyze the final cell count, specific growth rate, and protein content a 2-way ANOVA analysis was performed with pH and temperature as the independent variables. Differences were considered significant when $p<0.05$.

## 2.4 Results

### *2.4.1 Effects of temperature and pH on the growth of microalgae*

The growth of *N. oculata* under various conditions is shown in Figure 2.4.1. The results show a significant effect of temperature on the growth of *N. oculata* ($p < 0.0001$) as well as the pH ($p = 0.0009$). However, the temperature-pH interaction effect was not significant ($p = 0.9$). The highest final biomass was obtained when the algae were grown at 20°C and pH 7.6 ($1.47 \times 10^7$ cells/ml) and the lowest one was at 13°C and pH 8.2 ($3.68 \times 10^6$ cells/ml). In addition, no significant difference ($p = 0.12$) was found between trials with the same conditions but different sampling frequencies (Trials 1 and 3), as shown in Figure 2.4.2.

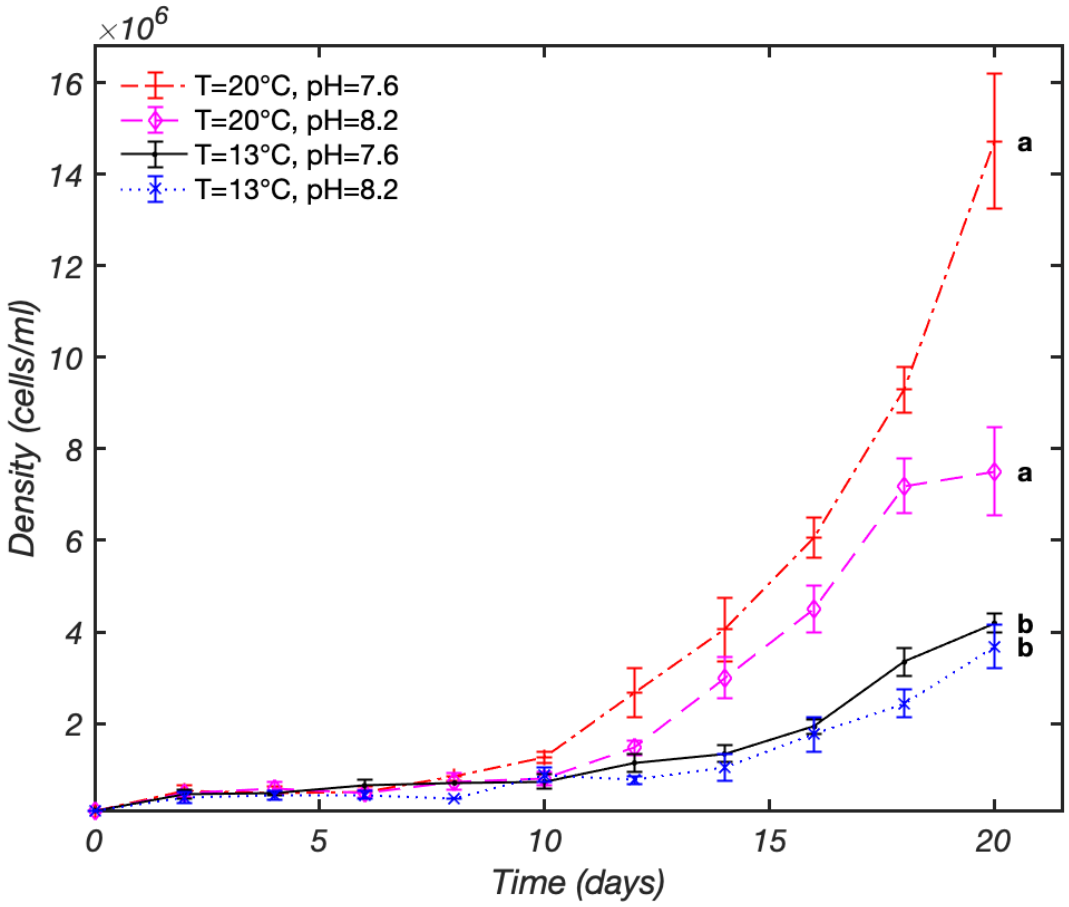
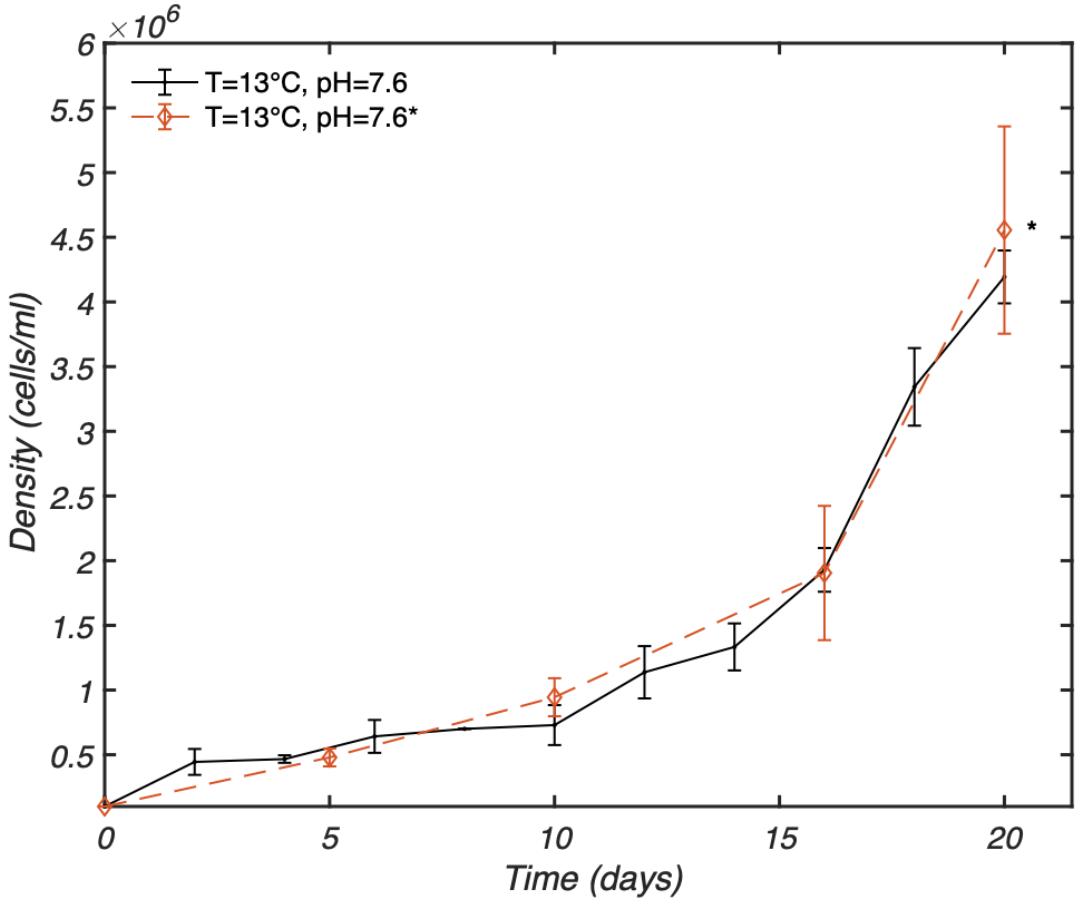



Figure 2.4.1. *Nannochloropsis oculata* growth under different pH and temperature conditions (n=3). Error bars indicate standard errors. Superscripts indicate significant differences between curves.


×10^6
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
Density (cells/ml)
T=13°C, pH=7.6
T=13°C, pH=7.6*
*
0
5
10
15
20
Time (days)

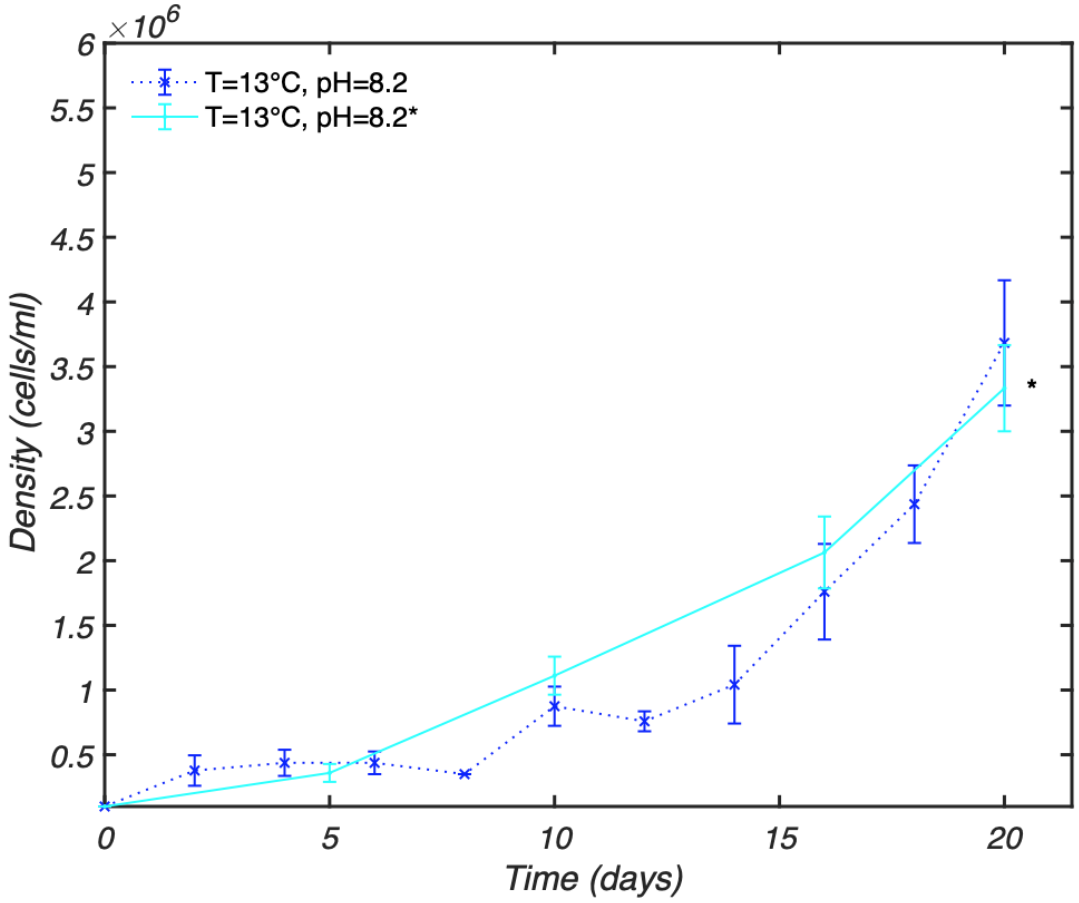



Figure 2.4.2. *Nannochloropsis oculata* growth for treatments at the same temperature for both pH but with different sampling frequencies (Trial 1 and Trial 3). Error bars indicate standard errors (n=3). * was for data from Trial 3. No significant differences were found between curves with the same pH.

A significant difference was found in the growth of *C. gracilis* at different temperatures ($p < 0.0001$) but not at different pH ($p = 0.15$). The temperature-pH interaction also showed no significant effect on the growth of *C. gracilis* ($p = 0.3$). The highest final cell count was obtained when the algae were grown at 20°C and pH 7.6 ($3.02 \times 10^6$ cells/ml) and the lowest at 13°C and pH 8.2 ($7.09 \times 10^5$ cells/ml), as shown in Figure 2.4.3.

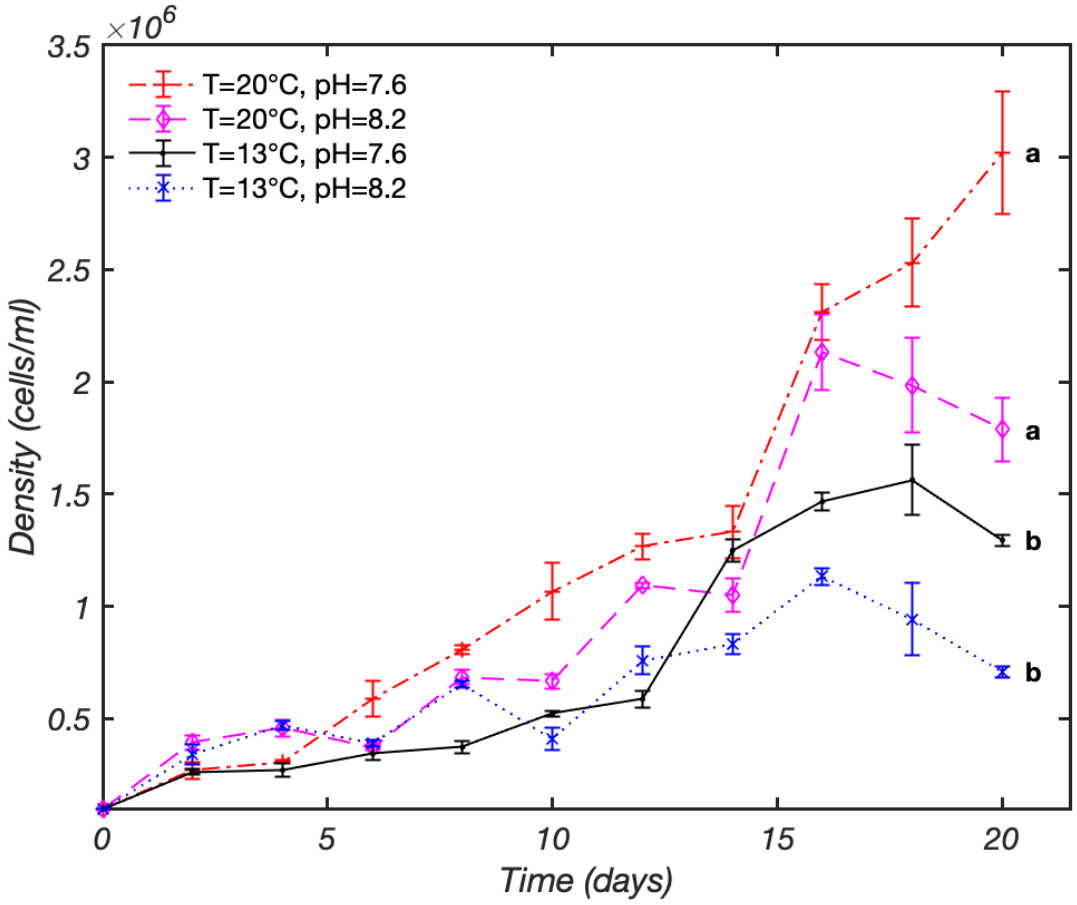



Figure 2.4.3. *Chaetoceros gracilis* growth under different pH and temperatures (n=3). Error bars indicate standard errors. Superscripts indicate significant differences between curves.

A significant difference was found in the specific growth rate (**μ**) of *N. oculata* due to temperature ($p < 0.0001$), pH ($p = 0.0058$), and their interaction ($p = 0.043$). The highest specific growth rate was obtained when algae were grown at 20°C and pH 7.6 (**μ**=0.249 $d^{-1}$) and the lowest when grown at 13°C and pH 8.2 (**μ**=0.179 $d^{-1}$). A significant difference was found in the specific growth rate of *C. gracilis* due to temperature ($p < 0.0001$) and pH ($p < 0.0001$), but not due to their interaction ($p = 0.5$). The highest specific growth rate was observed when algae were grown at 20°C and pH 7.6 (**μ**=0.170 $d^{-1}$) and the lowest when grown at 13°C and pH 8.2 (**μ**=0.098 $d^{-1}$).

For each of the strains of microalgae, a linear regression analysis was used to obtain an empirical equation, in the form of Equation 2, that describes the growth rate as a function of temperature and pH for algae growing under the same conditions used in this study, and between 13°C and 20°C, and pH 7.6 and 8.2.

$$\boldsymbol{\mu} = m(f_{pH}) * T + b(f_{pH}) \qquad (2)$$

For this, first the specific growth rate was expressed as a function of temperature for each pH by plotting the specific growth rate of *N. oculata* obtained for each treatment in terms of temperature (Figure 2.4.4). The equations obtained are shown below,

$$\boldsymbol{\mu}(pH = 7.6) = 0.0086 * T + 0.0786$$

$$\boldsymbol{\mu}(pH = 8.2) = 0.0057 * T + 0.1057$$

These results show that both the slope and interception terms of the linear regression equation are dependent on the pH. Thus, to determine these terms as a function of pH, the slope (m) and the interception (b) terms obtained for each equation were plotted in terms of pH (Figures 2.4.5 and 2.4.6.). The results are shown below,

$$m = -0.0048 * pH + 0.0453$$

$$b = 0.0452 * pH - 0.2647$$

Therefore, the specific growth rate change of *N. oculata* as a function of temperature and pH can be described as,

$$\boldsymbol{\mu} = (-0.0048 * pH + 0.0453) * T + 0.0452 * pH - 0.2647$$

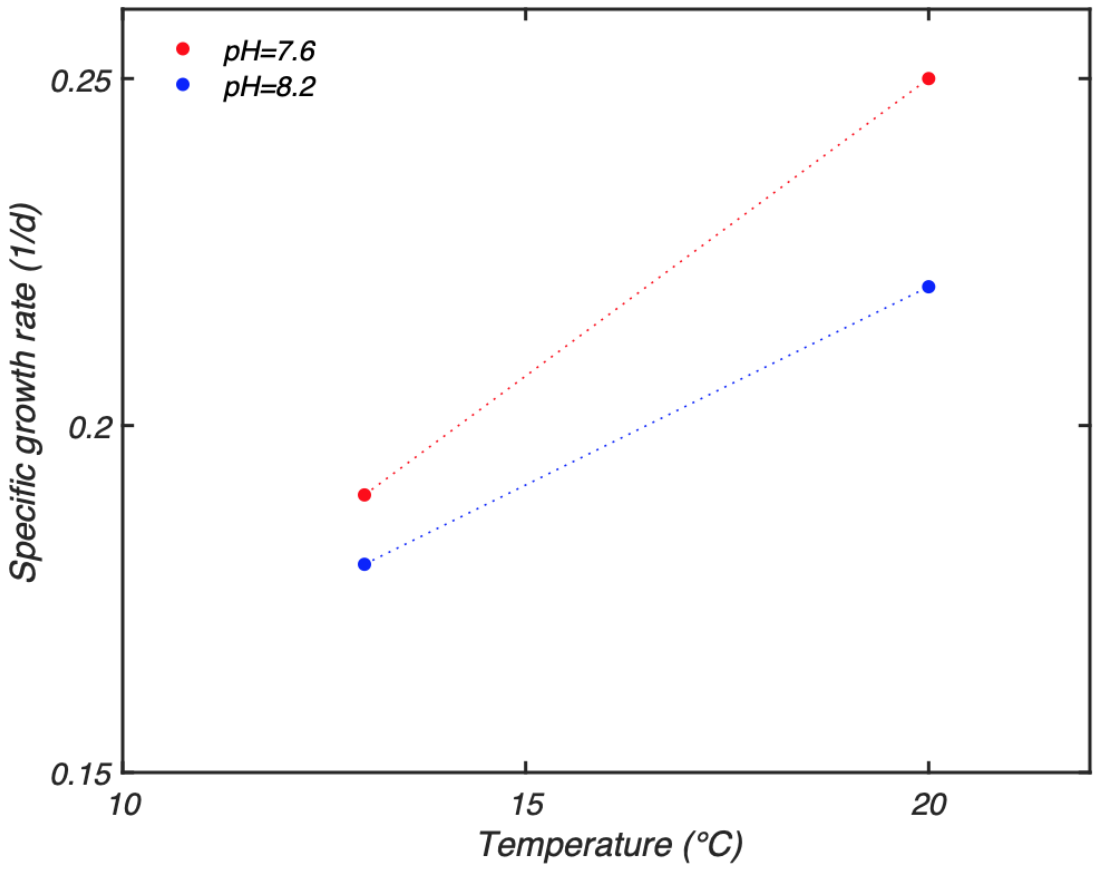



Figure 2.4.4. Effect of temperature and pH on *Nannochloropsis oculata* specific growth rate.

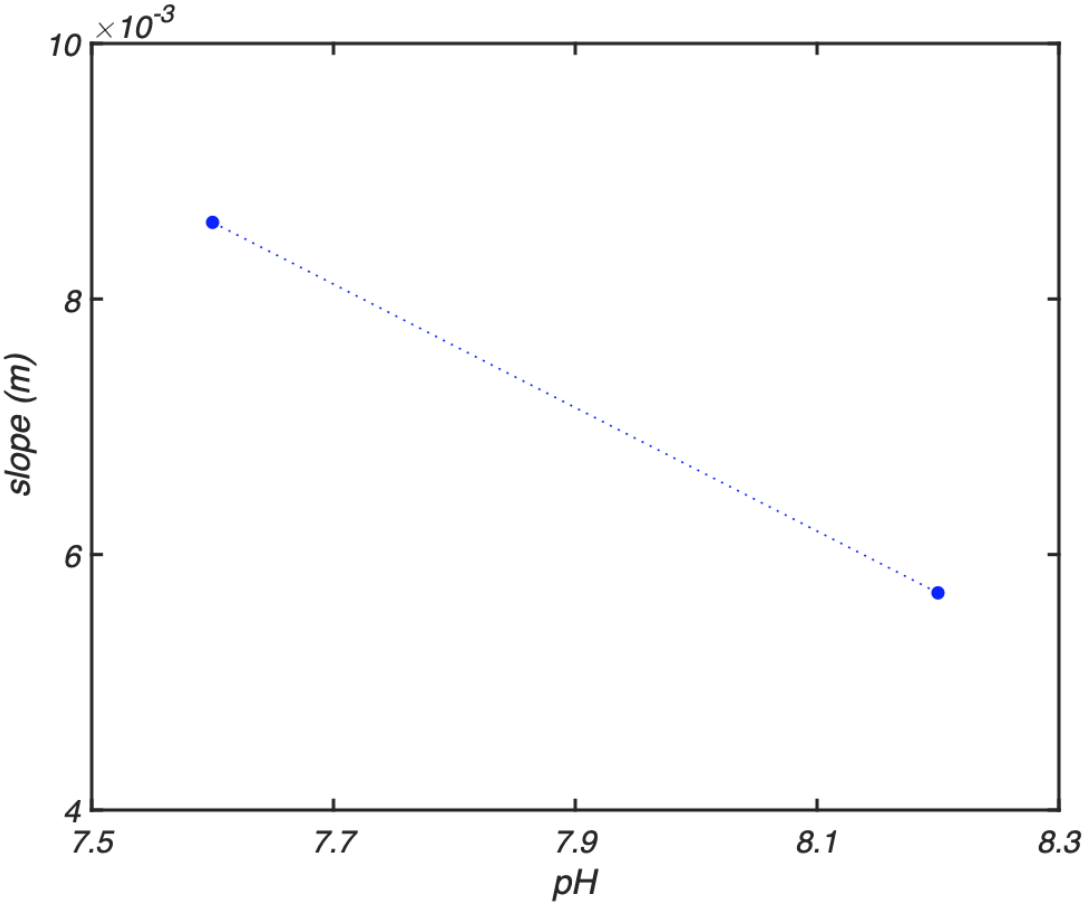



Figure 2.4.5. Influence of pH on the slope of the specific growth rate of *Nannochloropsis oculata.*

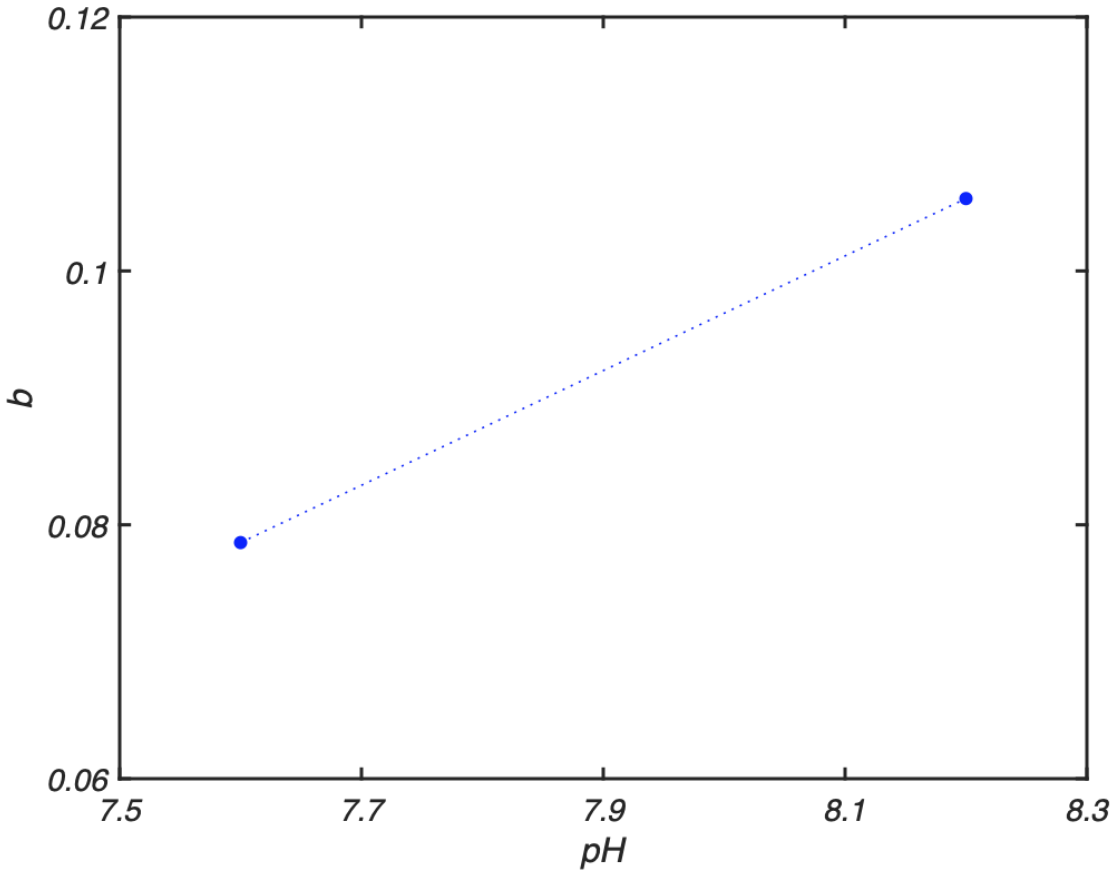



Figure 2.4.6. Influence of pH on the intercept of the specific growth rate of *Nannochloropsis oculata.*

The specific growth rate of *C. gracilis* for each treatment in terms of temperature is shown in Figure 2.4.7. The specific growth rate curves for each pH in terms of temperature are shown below,

$$\boldsymbol{\mu}(pH = 7.6) = 0.0057 * T + 0.0557$$

$$\boldsymbol{\mu}(pH = 8.2) = 0.0057 * T + 0.0257$$

These results show that only the interception term of the linear regression equation is dependent on the pH. This term was obtained by plotting the interception term of each equation in terms of pH (Figure 2.4.8). The result is shown below,

$$b = -0.05 * pH + 0.4357$$

Therefore, the specific growth rate change of *C. gracilis* in terms of temperature and pH can be described as,

$$\boldsymbol{\mu} = 0.0057 * T - 0.05 * pH + 0.4357$$

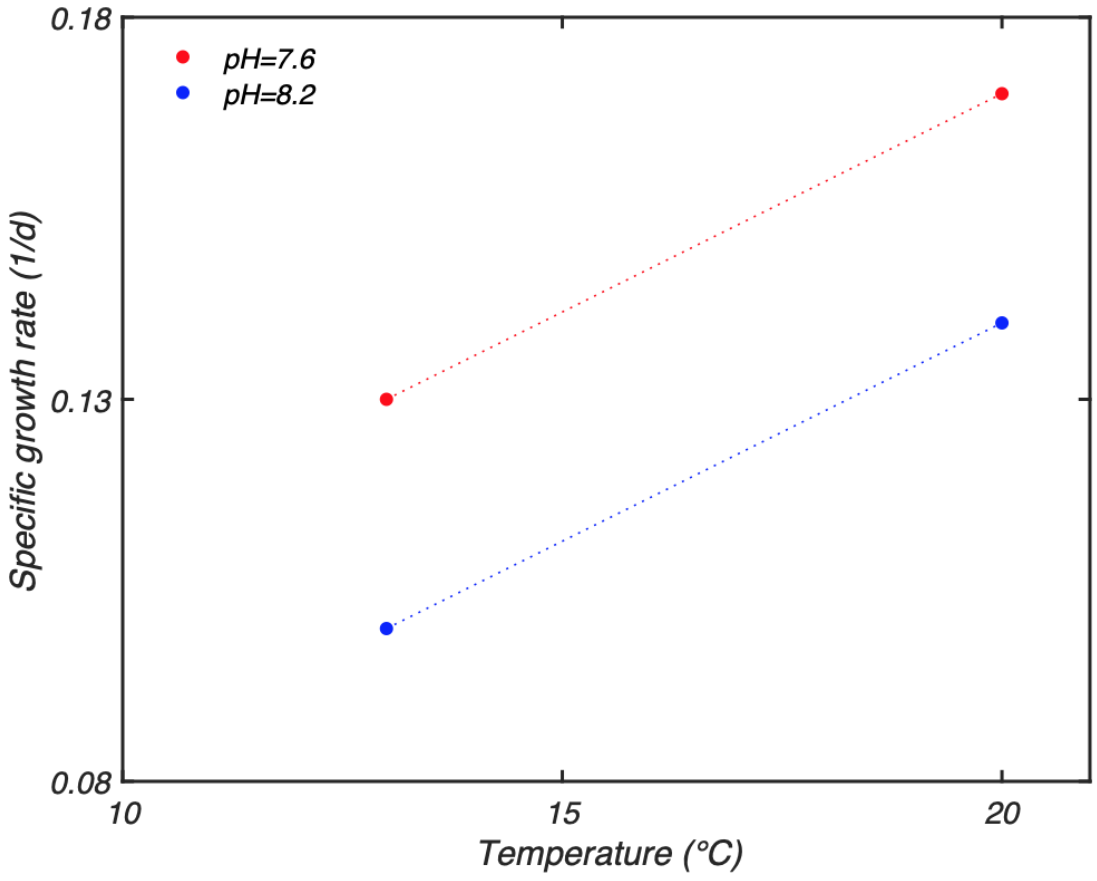



Figure 2.4.7. Effect of temperature and pH on *Chaetoceros gracilis* specific growth rate.

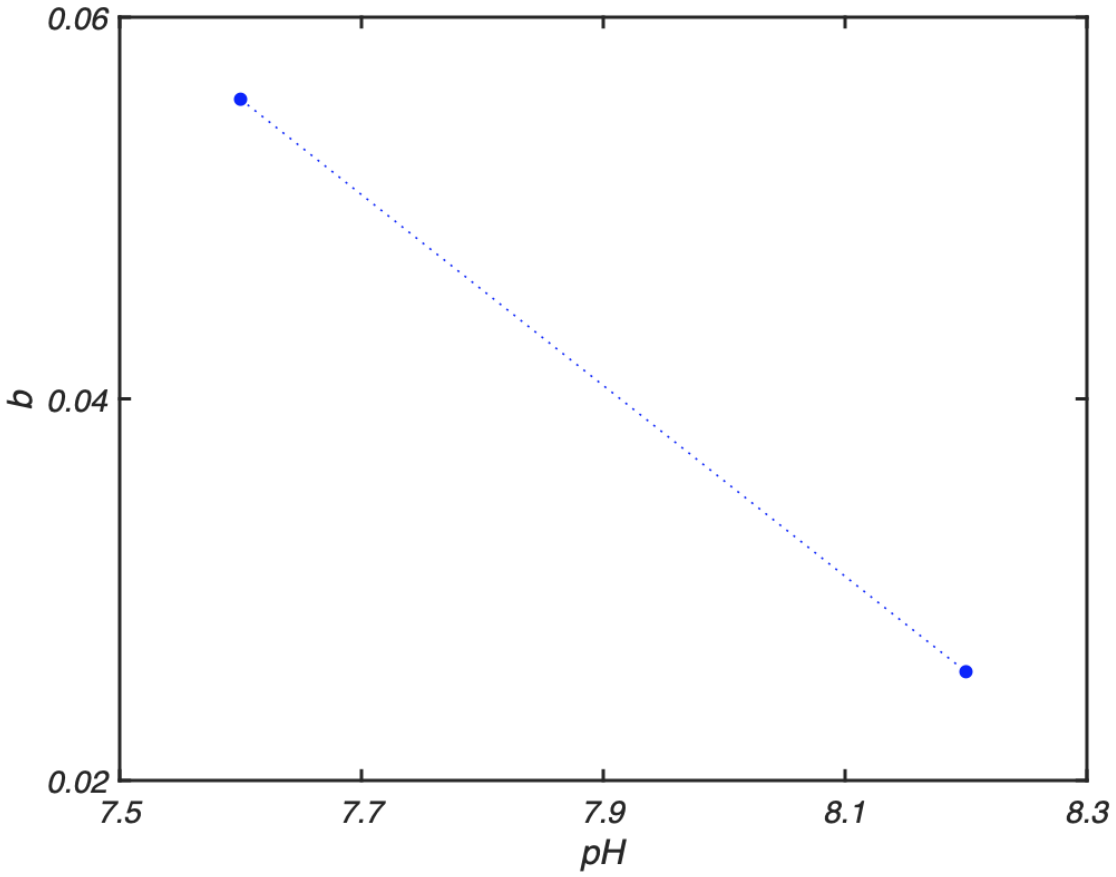



Figure 2.4.8. Effect of temperature and pH on *Chaetoceros gracilis* specific growth rate.

*2.4.2 Effects of temperature and pH on the protein content of marine microalgae*

A significant difference was found in the protein content of *N. oculata* only due to temperature (p=0.002) but not pH (p=0.28) or the temperature-pH interaction (p=0.15). The highest protein concentration was found when algae were grown at a higher temperature (20°C) and lower pH (pH=7.6), where 38.63% protein of dry matter was obtained. A significant difference was found in the protein content of *C. gracilis* due to temperature (p<0.0001) and pH (p=0.01), no effect was found due to their interaction (p=0.30). The higher protein content was observed when *C. gracilis* was grown under higher temperature and lower pH, where 46.1% protein of dry matter was obtained.

All the values obtained for specific growth rate, final biomass, and protein content are shown below in Table 2.4.1.

Table 2.4.1. Specific growth rate, final cell counting, and protein content for both strains of microalgae.

| Microalgae strain | Treatment | | Specific growth rate ($d^{-1}$) | Final cell counting (cell/mL) | Protein content (%DW) |
|---|---|---|---|---|---|
| *N. oculata* | T=20°C | pH=7.6 | $0.25\pm4.91x10^{-3a}$ | $1.47x10^{7}\pm1.47x10^{6a}$ | $38.63\pm3.04^{a}$ |
| | | pH=8.2 | $0.22\pm6.54x10^{-3b}$ | $7.50x10^{6}\pm9.62x10^{5b}$ | $26.87\pm7.55^{a,b}$ |
| | T=13°C | pH=7.6 | $0.19\pm2.40x10^{-3c}$ | $4.19x10^{6}\pm2.04x10^{5b}$ | $13.02\pm1.46^{b}$ |
| | | pH=8.2 | $0.18\pm7.12x10^{-3c}$ | $3.68x10^{6}\pm4.84x10^{5b}$ | $14.85\pm4.42^{b}$ |
| *C. gracilis* | T=20°C | pH=7.6 | $0.17\pm4.39x10^{-3a}$ | $3.02x10^{6}\pm2.72x10^{5a}$ | $46.07\pm2.60^{a}$ |
| | | pH=8.2 | $0.14\pm4.09x10^{-3b}$ | $1.79x10^{6}\pm1.40x10^{5b}$ | $41.96\pm2.26^{a}$ |
| | T=13°C | pH=7.6 | $0.13\pm9.53x10^{-4c}$ | $1.30x10^{6}\pm2.44x10^{4b,c}$ | $20.23\pm1.59^{b}$ |
| | | pH=8.2 | $0.10\pm1.76x10^{-3d}$ | $7.09x10^{5}\pm2.44x10^{4c}$ | $11.89\pm0.70^{b}$ |

Data expressed as mean±SE, n=3. Significant differences in a column for each microalga are indicated by different superscripts.

## 2.5 Discussion

The results suggest that *N. oculata* and *C. gracilis* benefit from the higher temperature and lower pH tested in this study. The highest growth rates of both strains were obtained when they were grown at 20°C and pH 7.6. Both strains of algae produced more protein under these conditions as well. Furthermore, the results suggest that temperature has a bigger impact than pH on growth and protein production. The only significant effect due to the interaction of temperature and pH found was in the *N. oculata* specific growth rate.

These results were somewhat expected, higher temperatures boost algae metabolism increasing growth rates when the temperature is increased up to the specific optimal temperature for the cultured species (Ras et al., 2013; Singh & Singh, 2015; Barten et al., 2020). The optimal temperature for *N. oculata* has been reported to be 25°C (Cho et al., 2007) and *C. gracilis* can grow well at temperatures as high as 30°C (Tokushima et al., 2016). The optimal temperatures for both species are higher than the highest temperature tested in this study, therefore higher growth rates at this temperature were expected. Likewise, pH has been shown to influence the growth rates of algae. Studies have shown that algae can adapt to a broad range of pH, but the maximum algal growth occurs around neutral pH (Juneja et al., 2013). Therefore, higher growth rates due to elevated temperature (20°C) and lower pH (pH=7.6) were expected in this study.

However, some studies have shown a decrease in protein content when certain species of microalgae were cultivated at higher temperatures (Teoh et al., 2010; Militão et al., 2019). These results vary broadly depending on the cultured species and the temperature tested. Other microalgal species produce more protein at higher temperatures (Teoh et al., 2010). Furthermore, studies like Latsos (2022) showed lower protein content despite higher productivity in some strains of marine algae when grown at lower pH. Therefore the effects of temperature and pH on the protein content of microalgae are variable across the literature and seem to be species-specific. The protein content obtained in *N. oculata* in this study ranged

from 13% to 38%, which is within the range of the protein content reported the for this same species in the literature, which ranges from 14% to 55% (Hulatt et al., 2017; Zanella and Vianello, 2020). The protein content obtained in *C. gracilis* in this study ranged from 11% to 46%, protein content for this species was reported to be between 15% and 19.5% (Khairy et al., 2014) and as high as 50% in other *Chaetoceros* species (Araújo and Garcia, 2005; Raghavan et al., 2008).

The empirical equations obtained here to describe the specific growth rate change as a function of temperature and pH for each species of algae studied assume a linear relationship between the factors. This assumption was made since only two temperatures and two pH levels were tested, and therefore there were not enough data to use a different mathematical model, for example, the Arrhenius-type equation which has been commonly used to describe the growth of algae (Mayo, 1997; Sozmen et al., 2022). Therefore, more temperatures and pH should be tested to obtain a better, more accurate description of the relationship between the specific growth rate of algae and the temperature and pH of the media, and other mathematical models should be considered.

The purpose of this study was to understand the possible climate change effects on these marine algae strains in the ocean and coastal ecosystems. From our results, both strains of algae *N. oculata* and *C. gracilis* could benefit from the increase in ocean temperature and decrease in pH, at least to the projected levels for 2100. Consequently, it could benefit herbivores and filter feeder species that fed on them, since protein demands could be met more efficiently. However, other important considerations must also be taken into consideration, for example, other factors such as light and nutrients availability and/or their interactions, the possible change of dominant strains in natural communities, and the lipids, fatty acids, and carbohydrate content.

## 2.6 References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# Chapter 3. Effects of temperature on the growth and chemical composition of marine seaweed *Palmaria mollis*

## 3.1 Abstract

Understanding the effects of rising ocean temperature due to climate change on primary producers is essential for the sustainable growth of aquaculture. Temperature is known to be one of the most important factors that influence photosynthetic and growth rates of seaweed, as well as their macronutrients, i.e. protein, lipids, and carbohydrates. Seaweeds are in the first trophic level in a food chain, and serve as a food source for consumers at higher trophic levels. Therefore, it is empirical to understand potential changes in their growth and/or chemical composition due to the predicted temperature rise caused by climate change.

The seaweed *Palmaria mollis*, known as Pacific dulse (dulse in this document for simplicity), a red seaweed popular in aquaculture, was selected for this study. The study consisted of growing dulse under three different temperatures, 13°C (current mean ocean temperature in Northern California), 15°C, and 17°C (projected temperatures by 2100). Seaweed was grown for 21 days, and its growth, protein, fatty acids, and carbohydrate content were monitored. The results showed that growth and protein content were affected by temperature. Dulse grown at 13°C gained the most biomass during the experimental period; however, it contained the least percentage of protein in their dry matter. There were no significant differences in the fatty acids and carbohydrate content among the temperatures tested. However, a significant difference in the distribution of fatty acids was found. Dulse growing at 13°C showed the highest percentage of monounsaturated fatty acids and the lowest percentage of saturated fatty acids. The results suggest that changes in temperature predicted due to climate change will affect the nutritional value and the availability of *P. mollis*, potentially affecting the production of herbivores that depend on them.

### 3.2 Introduction

Seaweeds, also known as macroalgae, are autotrophic organisms that serve a critical role in marine ecosystems as primary producers, converting the energy from sunlight into organic matter through photosynthesis. As autotrophic organisms, macroalgae constitute the basis of the food chain in aquatic ecosystems and serve as food, shelter, and habitat for a variety of species, including invertebrates, fish, and marine mammals (Marine Aquaculture, 2019; García-Poza et al., 2020). Traditionally, seaweeds have been harvested and used by coastal communities, especially in some Asian countries like China and Indonesia, as a source of food, medicine, fertilizers, among other uses (Teagasc, 2012; Chopin & Tacon, 2021). Furthermore, seaweeds have attracted attention worldwide, as studies have shown their potential health benefits. Along with nutritional benefits, seaweed applications for biofuel, bioremediation, and as feed supplements for agricultural animals are growing in popularity, and their full potential continues to be explored making them important for both ecological and economic reasons. (Chopin & Tacon, 2021).

As the applications for seaweed are expanding, it is no longer sustainable to solely depend on wild seaweed harvesting (Monagail et al., 2017). Fortunately, farming seaweed is relatively easy, accessible, and has a low environmental impact. In fact, it has been shown that farming seaweed can have a positive impact on the ecosystem where it is grown (Hasselström et al., 2018; NOAA, 2020; Duarte et al., 2022). As the world continues to value sustainable practices, commercial seaweed farming has been developing. Global seaweed production has grown yearly at a rate of 6.2% since 2018 (Duarte et al., 2022).

Most seaweed farms are located in coastal areas, creating economic opportunities for coastal communities and providing ecosystem services, such as improving water quality by uptaking nutrients like nitrogen and phosphorus (Kim et al., 2017; NOAA, 2020). However, as autotrophic organisms, environmental conditions directly influence the growth and chemical composition of seaweeds, since they adapt to natural changes in ambient water conditions by changing their chemical composition and altering their protein, carbohydrate, and lipid content (Eggert, 2012; Harley et al., 2012). Furthermore, how water

quality and environmental changes affect seaweed depends on each species' sensitivity and adaption mechanisms (Longphuirt et al., 2013; Kim et al., 2016).

Temperature is an important factor that highly affects seaweed growth and nutritional composition. Both growth and photosynthetic rates of seaweeds increase with temperature, rapidly declining after their upper critical temperature (Eggert, 2012). Seaweeds use different physiological strategies to adapt to changes in temperature, and their response also varies according to the species, the scale of temperature changes, and how often they experience temperature fluctuations. One of the most important adaptation mechanisms in seaweed to temperature is the change in enzyme concentration or protein content (Eggert, 2012; Baweja et al., 2016). Seaweeds also adapt to increases in temperature by adjusting their fatty acids composition, where at higher temperatures the proportion of polyunsaturated fatty acids (PUFA) decreases relative to their saturated fatty acids (SFA) (Eggert, 2012; Britton et al., 2020). In the same way that temperature affects the protein and fatty acids in seaweed, their carbohydrate content has been found to increase at higher temperatures (Olsson et al., 2020).

Predictions of climate change indicate a global mean sea surface temperature increase of 2°C in the next decades (IPCC, 2019). For coastal communities that rely on seaweed production, understanding how seaweeds react to these changes assists in their sustainable production and provides information needed to develop and manage their farming successfully.

*Palmaria mollis*, or dulse, is a red seaweed that is popular for its ability to be farmed and its nutritional properties, it has been shown that *P. mollis* can support high growth rates of herbivore animals like abalone (Evans and Langdon 2000; Demetropoulos and Langdon 2004; Langdon et al. 2004). Dulse is also effective as a biofilter for contaminated waters (Demetropoulos and Langdon 2004) and is a good source of food and food ingredients for human consumption (Fleurence et al., 2018). *Palmaria* species have gained worldwide attention due to their chemical composition. They are well known for their high

protein content, which has been determined to be a good alternative to animal-sourced protein, and have been linked to health benefits (Dumay et al., 2022; Fidelis e Moura et al., 2022). However, less attention has been paid to their fatty acids and carbohydrate content.

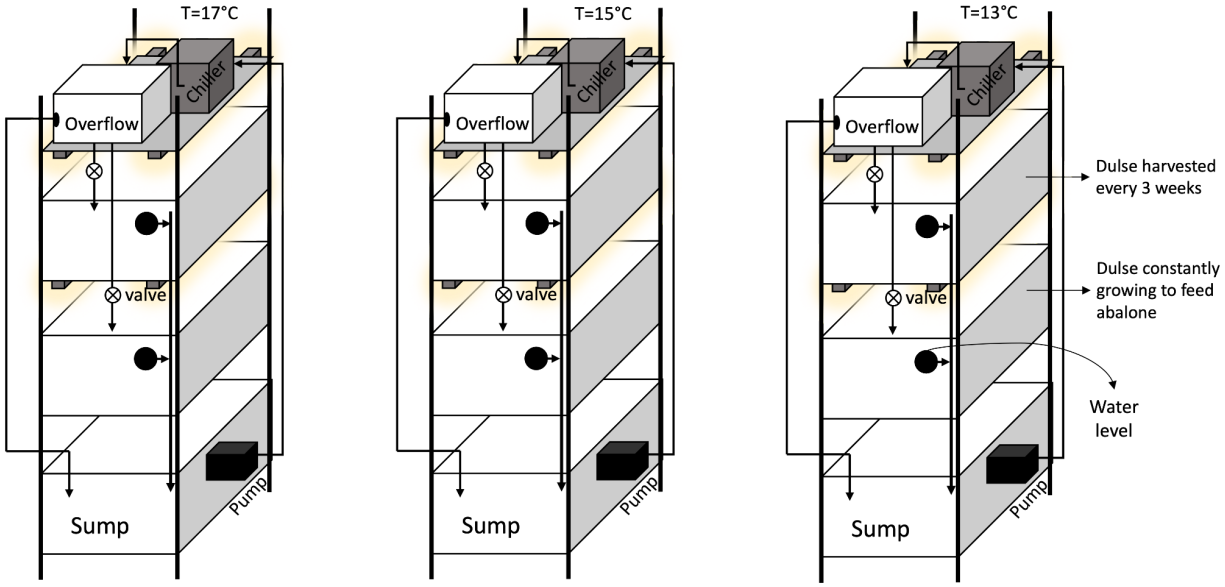
In this study, the growth and the protein, carbohydrate, and fatty acids content of *P. mollis* were investigated under two projected ocean warming scenarios temperatures by 2100 for Northern California. The hypothesis for this study was that the temperature has a positive effect on the growth rate of *P. mollis* as temperature increases. However, higher temperatures were expected to decrease the protein content, increase the carbohydrate content and increase the percentage of saturated fatty acids while decreasing the percentage of polyunsaturated fatty acids, therefore temperature has a negative effect on the chemical composition of *P. mollis* as temperature increases, resulting in more biomass but less nutritious matter when higher temperatures.

### 3.3 Materials and methods

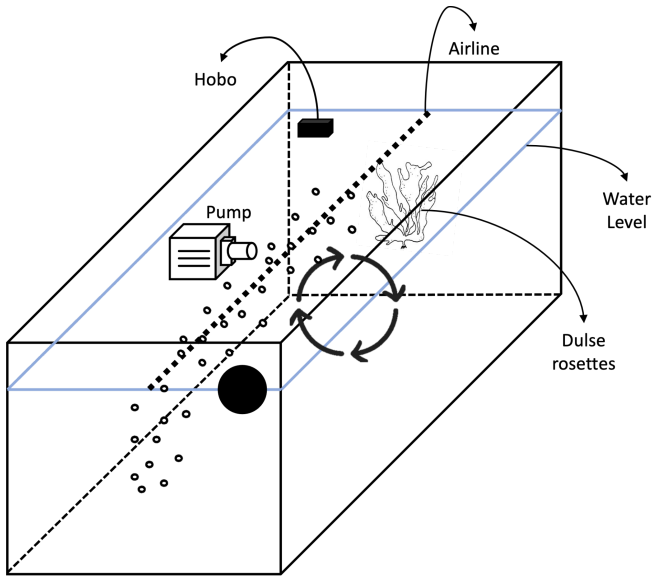
#### *3.3.1 Seaweed culture*

Cultured *P. mollis* rosettes at the UC Davis Bodega Marine Laboratory, Bodega Bay, California, USA, for the White Abalone Recovery Program collected from an outdoor tumble-culture tank with flow-through filtered ambient seawater were used for all experiments. The initial *P. mollis* stocking density was 500 g per 184 L water volume tank (Figure 3.3.1b). The seaweed was tumble-cultured in the tanks in a recirculating system filled with filtered seawater, all seawater was exchanged every week. The tumbling for the cultures was created by pumping water with a submerged pump (Rio plus Aqua Power Head Pump 1400, 420 GPH, Taipei, Taiwan). Additionally, aeration was provided with an air pump (Whisper AP150, Tetra, Virginia, USA) and a submergible bubble wall air diffuse hose to each tank to ensure better movement and oxygenation. The seaweeds were cultured under 2 LED lights at full light spectrum with a 12 h:12 h light-dark photoperiod.

A three-temperature (13°C, 15°C, and 17°C) single-factor design with six replicates was applied. Fresh seawater was initially added to each system and was maintained at 13°C, 15°C, and 17°C using chillers (DBA-075 1/10HP, JBJ Arctica, Missouri, USA), the setup of the system is shown in Figure 3.3.1. To prevent biofouling, water was disinfected by low-pressure ultraviolet light. The temperature was monitored and recorded with a floating data logger (Onset HOBO Pendant® MX, Massachusetts, USA) on an hourly basis.




(a)




(b)

(c)

Figure 3.3.1. Diagrams of (a) seaweed culture systems, (b) each seaweed culture tank, the data logger (Hobo) was floating as the seaweed and (c) a picture of the start day of a culture of the seaweed.

All seaweed was harvested weekly, spin-dried in a salad spinner (Geedel, California, USA), and weighed to track biomass. While seaweed was being weighed, all tanks were emptied and scrubbed, then water

was replaced with fresh, filtered seawater. All seaweed was placed back in the tanks afterward. Dulse was grown at each temperature for three weeks to reach a constant biochemical composition, as suggested by Cook and Kelly (2007). After three weeks, all seaweed was harvested, wet weighed, oven-dried at 60°C for at least 24 hours, until constant weight, and preserved at -80°C before being submitted to the labs for the protein, carbohydrate, and fatty acid analyses. A picture of one of the harvested seaweed at week 3 is shown in Figure 3.3.2.

Figure 3.3.2. A picture of the harvested seaweed at week 3.

*3.3.2 Chemical composition*

Dried seaweed samples were sent to the UC Davis analytical lab (ANLAB) for protein and carbohydrate analyses. The total amount of nitrogen in the samples was determined by the Total Nitrogen Combustion Method (SOP 522, in Anlab, UC Davis(a)) to calculate their protein content. The total crude protein was determined by multiplying a protein factor of 6.25 to the nitrogen content (SOP 625, in Anlab, UC Davis(b)).

Carbohydrates were determined in the form of total glucose, total nonstructural carbohydrates (TNC), and starch. The total glucose for TNC and starch method was used. The samples were enzymatically hydrolyzed at 55°C with amyloglucosidase for 12 hours and analyzed using high performance liquid chromatography with mass selective detection to determine total glucose. TNC is the sum of total glucose, free fructose, and free sucrose. Starch was calculated by subtracting the free glucose from the total glucose and multiplying it by 0.9 (SOP 690, in Anlab, UC Davis (c)).

Another portion of dried seaweed samples were sent to the UC Davis West Coast Metabolomics Center for fatty acid analysis. The lab uses the Matyash protocol (MTBE method) for lipid extraction, where lipids are found in the top layer of the separation, ensuring that extracts are not contaminated by proteins or polar compounds, the extractions then are analyzed by chromatography to acquire data, and finally, the data are processed in four stages to find peaks and identify lipids (West Coast Metabolomics Center, UC Davis).

*3.3.3 Water quality*

To confirm that the water quality was maintained throughout the treatments, the pH, ammonia, nitrite, and nitrate were tested by colorimetry at the beginning and the end of each weekly water exchange with the Saltwater master test kit (API®, Chicago, USA). Carbon dioxide and alkalinity were tested by titration with the Marine Test Kit HI3823 (Hanna instruments, Quebec, Canada) and salinity was measured with a refractometer (Imagitarium, Saltwater refractometer, California, USA). Ammonia and nitrate were qualitative measurements since the color scales provided by the kit were not sensitive enough for our values. We determine the levels for ammonia to be between 0-0.25 ppm, and for nitrate between 0-5 ppm. Since the kits to measure nutrient (ammonia and nitrate) levels in the water were not sensitive enough to track nutrient depletion in each tank, a complete water exchange in each system (460 L) was conducted weekly. The water quality data for all the treatments are shown in Table 3.3.1.

Table 3.3.1. Water quality values of seaweed culture across all experiments for each treatment.

| Factor | Treatment | | | p-value |
|---|---|---|---|---|
| | 13°C | 15°C | 17°C | |
| pH | 8.09±0.02 | 8.09±0.02 | 8.09±0.02 | 0.97 |
| nitrite (ppm) | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | - |
| carbon dioxide (ppm) | 12.00±1.90 | 11.91±1.75 | 12.91±1.93 | 0.92 |
| alkalinity (ppm as $CaCO_3$) | 125.47±4.49 | 125.65±4.00 | 126.88±3.80 | 0.97 |
| salinity (ppt) | 34.13±0.30 | 34.25±0.31 | 34.25±0.31 | 0.95 |
| ammonia (ppm) | 0 > x > 0.25 | 0 > x > 0.25 | 0 > x > 0.25 | - |
| nitrate (ppm) | 0 > x > 5 | 0 > x > 5 | 0 > x > 5 | - |

Data expressed as mean±SE, n=18. No significant differences were found in the water quality parameters monitored.

The temperatures were generally maintained at the targeted temperature and significantly different from each other ($p<0.0001$). The mean and range temperature for each treatment is shown in Table 3.3.2. The temperature profile for each treatment and each replicate can be seen in Figure 3.3.3.

Table 3.3.2. Mean and range temperature for each treatment tank

| Temperature | Treatment | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| Mean±SE (°C) | $12.95\pm0.01^{c}$ | $15.22\pm0.01^{b}$ | $16.96\pm0.01^{a}$ |
| Lowest (°C) | 11.96 | 12.70 | 14.71 |
| Highest (°C) | 15.31 | 20.46 | 17.97 |

Significant differences are indicated by different superscripts.

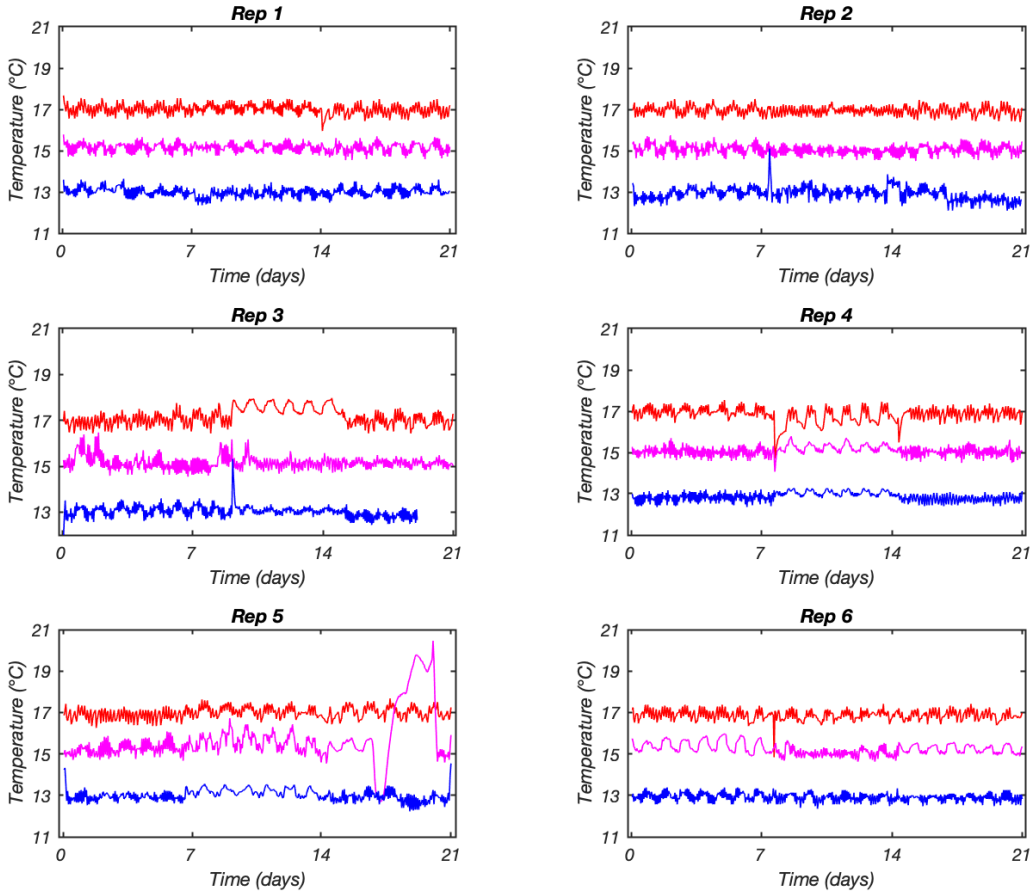



Figure 3.3.3. Temperature profiles for each treatment and replicate.

While running experiments for the fifth replicate, the chiller used to keep the 15°C temperature failed overnight, causing the temperature to spike. Statistical analysis indicates the temperature difference was consistent between all replicates despite the spike.

*3.3.4 Statistical analysis*

The results were analyzed statistically using the analytical software SAS Enterprise Guide (SAS, North Carolina, USA). The growth was treated as a function of time, and a linear-model ANOVA was carried out. In this case, differences in biomass were analyzed using a one-way ANOVA, with time as a

continuous variable. Post-hoc pairwise comparisons with Tukey adjustment were conducted to assess the significance among the three treatment levels.

The differences in biochemical composition (protein, total glucose, TNC, starch, and fatty acids) were tested using a mixed-effects model ANOVA. To account for the seasonal variability, time was considered a block, and the model was run with time as a random effect. Post-hoc pairwise comparisons with Tukey adjustment were conducted to assess the significance among the three treatment levels. Differences were considered significant when $p<0.05$ for all the analyses.

## 3.4 Results

### *3.4.1 Temperature effect on the growth of seaweed*

The results show a significant difference in the growth of dulse between all temperature treatments ($p<0.0001$), as shown in Figure 3.4.1. We also observed that dulse growing at 17°C was decomposing after the second week in every trial, becoming mushy and discolored by Week 3 when it was harvested.

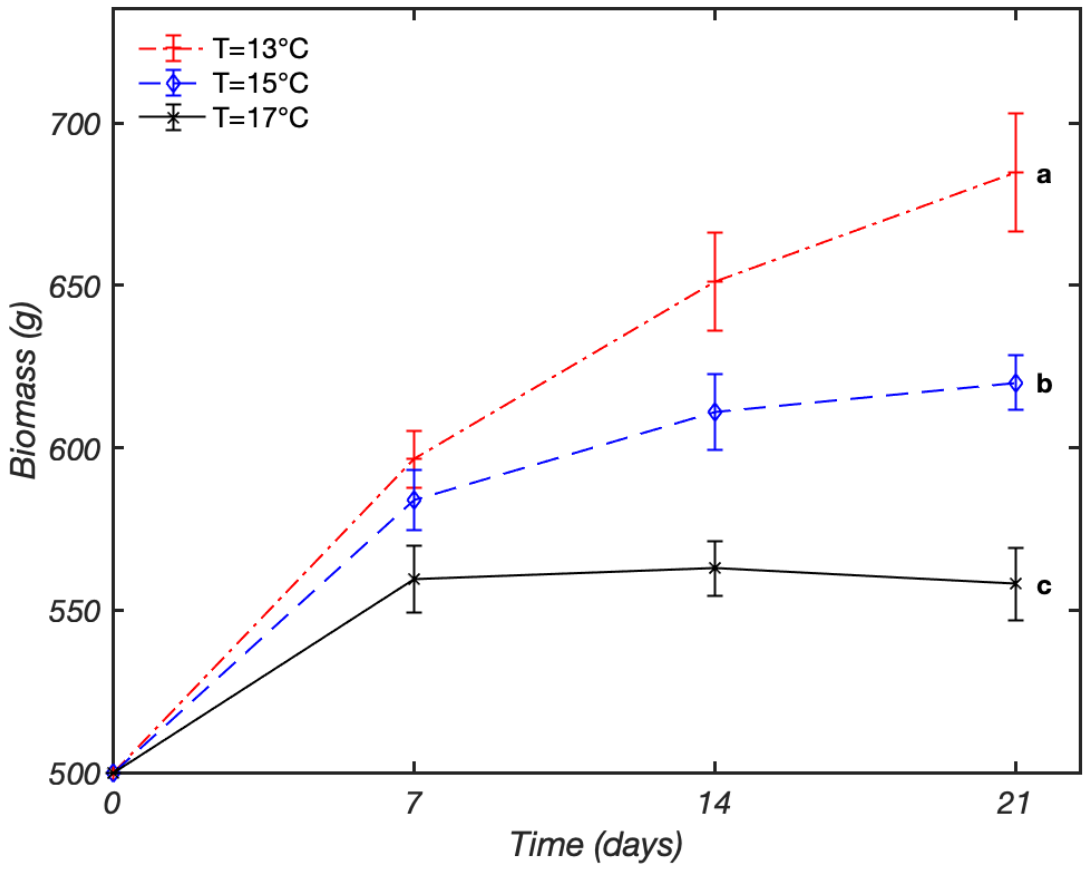



Figure 3.4.1. Temperature effect on the growth of dulse. Data are shown as mean±SE, n=6. Significant differences between curves are indicated by different letters.

*3.4.2 Temperature effect on the nutritional composition of seaweeds*

Temperature also had a significant effect on the protein content (% of dry matter) of *P. mollis* (p=0.0062). The *P. mollis* growing at 15°C and 17°C had significantly higher protein content than *P. mollis* growing at 13°C, as shown in Figure 3.4.2. However, the carbohydrates, in the form of total glucose, TNC, and starch, showed no significant differences between treatments (p=0.42, 0.43, and 0.47, respectively), as shown in Figure 3.4.3. The complete results are summarized in Table 3.4.1.

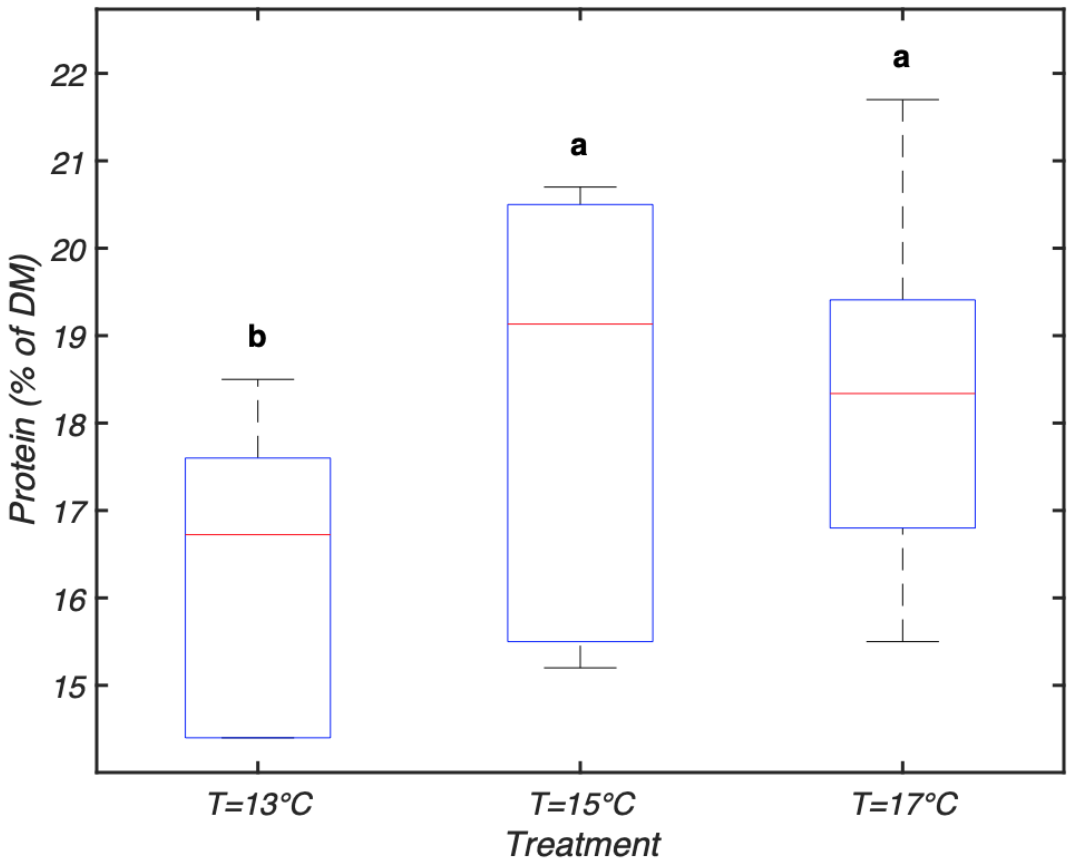



Figure 3.4.2. Protein content (% DM) of *P. mollis* growing under different temperatures (n=6). IQR=box (27-75th percentiles), the central mark indicates the median, and whiskers extend to the most extreme data points not considered outliers. Significant differences are indicated by different letters.

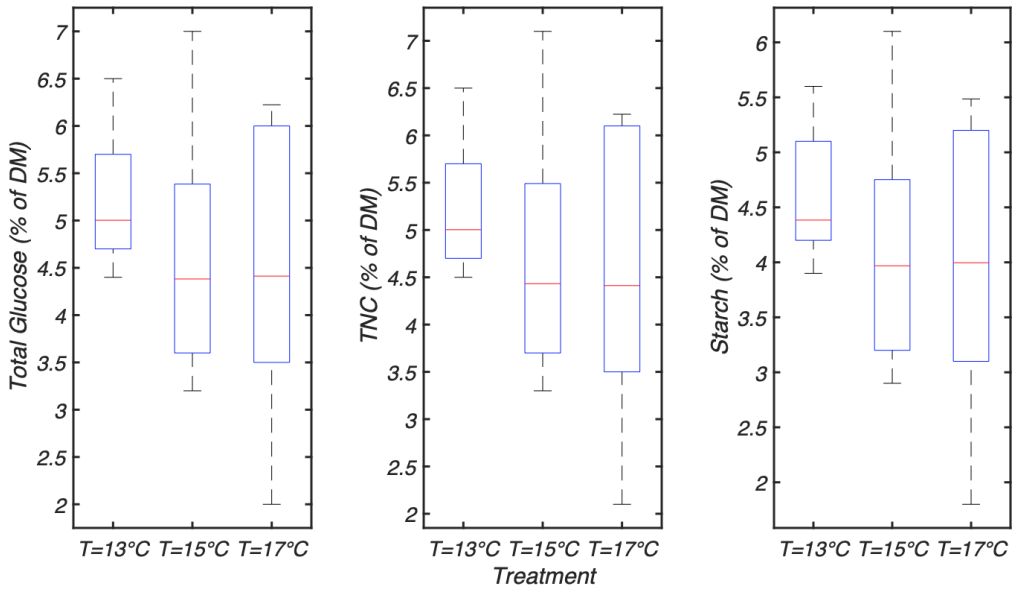



Figure 3.4.3. Total glucose, total nonstructural carbohydrates (TNC), and starch content (% DM) of *P. mollis* growing under different temperatures (n=6). IQR=box (27-75th percentiles), the central mark indicates the median, and whiskers extend to the most extreme data points not considered outliers.

Table 3.4.1. *P. mollis* protein and carbohydrate content for each temperature condition. All data is presented as % of dry matter (DM).

| Temperature | Total Nitrogen | Protein | Total glucose | TNC | Starch |
|---|---|---|---|---|---|
| 13°C | 2.62±0.11[b] | 16.39±0.71[b] | 5.22±0.31 | 5.23±0.30 | 4.59±0.26 |
| 15°C | 2.94±0.16[a] | 18.36±1.00[a] | 4.66±0.57 | 4.74±0.57 | 4.15±0.48 |
| 17°C | 2.93±0.14[a] | 18.35±0.91[a] | 4.42±0.69 | 4.46±0.68 | 3.93±0.59 |

Data are expressed as mean±SE, n=6. Significant differences are indicated by different superscripts.

The total fatty acids composition of seaweeds from all treatments consisted of 41-48% of saturated fatty acids (SFAs), 21-24% of monounsaturated fatty acids (MUFAs), and 30-34% of polyunsaturated fatty acids (PUFAs). Significant differences were found in the percentage of SFA (p=0.0198) and the

percentage of MUFA (p=0.0266); however, the differences were not significant if they were presented as the amounts (ng/mg), as shown in Figure 3.4.4. Detailed data are presented in Tables 3.4.2 and 3.4.3.

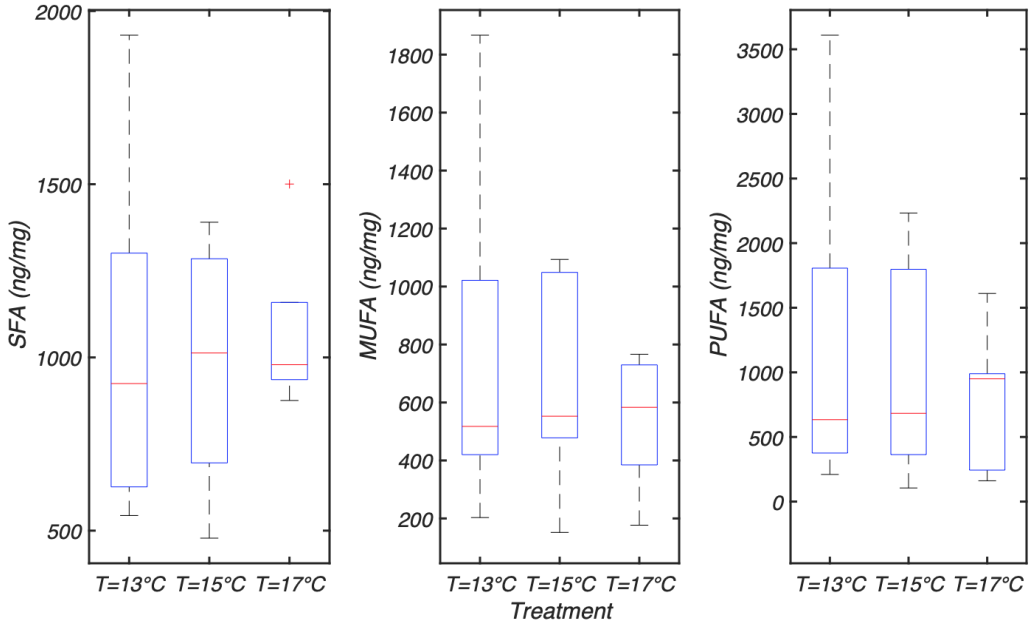



Figure 3.4.4. Abundance of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (ng/mg) of *P. mollis* growing under different temperatures (n=6). IQR=box (27-75th percentiles), the central mark indicates the median, whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually using the '+' marker symbol.

Essential fatty acids (EFA), are those PUFA that humans and other animals cannot synthesize, but are necessary for the correct functioning of their bodies and good health; therefore, they must be provided through diet (Di Pasquale, 2009; Kaur et al., 2014). These fatty acids are divided into omega-3 (n-3) and omega-6 (n-6) fatty acids. The most important EFA for human and animal nutrition are considered to be fatty acids 18:2 (n-6), linoleic acid (LA), and 18:3 (n-3), α-linolenic acid (ALA); in addition, fatty acids 20:4 (n-6), arachidonic acid (ARA), 20:5 (n-3), eicosapentaenoic acid (EPA), and 22:6 (n-3),

docosahexaenoic acid (DHA), are also important as they are hard to synthesize from LA and ALA due to the low conversion efficiency (Higdon, 2003; Di Pasquale, 2009; Glencross, 2009). In this study, we focus on these EFA over the complete list of fatty acids therefore the statistical analysis was performed only on these five fatty acids. However, a complete list of fatty acids can be found in Tables 3.4.3 and 3.4.4.

Our results showed a higher percentage of these EFA in *P. mollis* grown at 13°C, except for DHA, which was highest at 15°C. However, no significant differences were found between all the treatments. Data for all of these five EFA are presented in Figure 3.4.5. A complete list of the fatty acids analyzed is shown in Tables 3.4.2 and 3.4.3. In general, there was no significant difference found between treatments other than the percentage of SFA and MUFA.

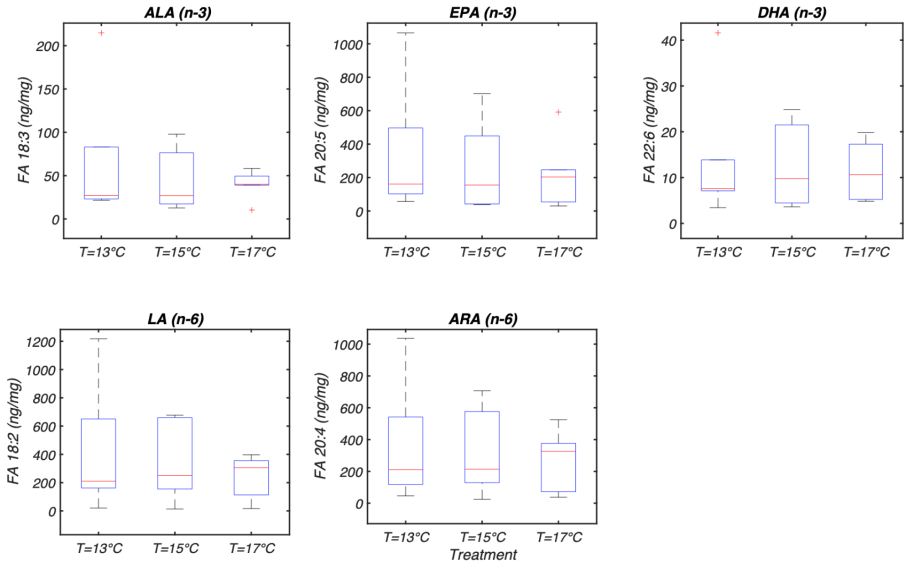



Figure 3.4.5. Abundance of essential fatty acids (ng/mg) in *P. mollis* growing under different temperatures (n=6). FA indicates fatty acids. IQR=box (27-75th percentiles), the central mark indicates the median, whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually using the '+' marker symbol.

Table 3.4.2. Fatty acid profile of seaweed growing under different temperatures. Values are expressed as the percentage of total fatty acids (%).

| Structure | Fatty acid content (% of total fatty acids) | | |
|---|---|---|---|
| | 13°C | 15°C | 17C° |
| 12:0 | 0.02±0.01 | 0.03±0.01 | 0.02±0.005 |
| 13:0 | 0.01±0.002 | 0.01±0.005 | 0.01±0.001 |
| 14:0 | 3.52±0.91 | 3.40±0.70 | 3.38±0.68 |
| 15:0 | 0.34±0.03 | 0.32±0.03 | 0.31±0.03 |
| 16:0 | 23.95±2.59 | 25.53±3.16 | 23.74±2.35 |
| 17:0 | 0.16±0.05 | 0.16±0.03 | 0.18±0.04 |
| 18:0 | 12.95±4.31 | 13.79±3.89 | 20.23±5.42 |
| 19:0 | 0.03±0.01 | 0.04±0.01 | 0.05±0.01 |
| 24:0 | 0.24±0.09 | 0.30±0.12 | 0.32±0.09 |
| 14:1 | 0.02±0.003 | 0.02±0.002 | 0.02±0.003 |
| 15:1 | 0.02±0.01 | 0.02±0.01 | 0.005±0.001 |
| 16:1 | 8.34±0.76 | 6.91±0.82 | 6.25±0.55 |
| 17:1 | 0.94±0.75 | 0.46±0.25 | 0.39±0.23 |
| 18:1 | 14.58±1.36 | 16.17±1.54 | 13.56±1.59 |
| 19:1 | 0.27±0.19 | 0.21±0.08 | 0.17±0.06 |
| 20:1 | 0.11±0.03 | 0.14±0.04 | 0.11±0.02 |
| 21:1 | 0.01±0.002 | 0.01±0.003 | 0.01±0.002 |
| 22:1 | 0.14±0.04 | 0.15±0.04 | 0.13±0.02 |
| 23:1 | 0.02±0.01 | 0.04±0.03 | 0.18±0.10 |
| 24:1 | 0.30±0.14 | 0.34±0.13 | 0.38±0.09 |
| 25:1 | 0.01±0.004 | 0.03±0.02 | 0.05±0.03 |
| 26:1 | 0.10±0.03 | 0.16±0.07 | 0.16±0.04 |
| 18:2(n-6)* | 11.08±2.23 | 10.75±2.06 | 9.19±1.86 |
| 18:3(n-3)* | 1.91±0.29 | 1.59±0.26 | 1.73±0.36 |
| 19:2 | 0.02±0.01 | 0.02±0.01 | 0.02±0.004 |
| 20:2(n-6) | 0.07±0.01 | 0.09±0.01 | 0.07±0.01 |
| 20:3(n-6) | 0.04±0.004 | 0.04±0.01 | 0.04±0.01 |

| Structure | Fatty acid content (% of total fatty acids) | | |
|---|---|---|---|
| | 13°C | 15°C | 17C° |
| 20:4(n-6)* | 10.20±1.62 | 9.97±1.90 | 10.12±2.18 |
| 20:5(n-3)* | 9.59±1.78 | 8.05±2.01 | 8.11±2.38 |
| 21:2 | 0.001±0.0004 | 0.003±0.002 | 0.002±0.0005 |
| 21:3 | 0.001±0.0004 | 0.001±0.001 | 0.001±0.0003 |
| 21:4 | 0.01±0.001 | 0.01±0.002 | 0.01±0.001 |
| 22:2(n-6) | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 |
| 22:3 | 0.01±0.01 | 0.02±0.01 | 0.01±0.01 |
| 22:4(n-6) | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 |
| 22:5 | 0.12±0.03 | 0.13±0.03 | 0.14±0.01 |
| 22:6(n-3)* | 0.43±0.07 | 0.50±0.13 | 0.46±0.06 |
| 24:2 | 0.02±0.01 | 0.02±0.01 | 0.01±0.01 |
| 24:4(n-6) | 0.003±0.001 | 0.001±0.001 | 0.004±0.002 |
| 24:5 | 0.003±0.001 | 0.005±0.003 | 0.004±0.001 |
| 26:2 | 0.30±0.11 | 0.39±0.12 | 0.28±0.08 |
| 26:3 | 0.08±0.04 | 0.09±0.03 | 0.06±0.02 |
| 26:5 | 0.002±0.001 | 0.003±0.001 | 0.003±0.001 |
| 32:6 | 0.0004±0.0004 | 0.0004±0.0003 | 0.001±0.0003 |
| 32:7 | 0.0002±0.0002 | 0.0001±0.0001 | 0.00±0.00 |
| 34:7 | 0.00005±0.00005 | 0.0004±0.0002 | 0.0002±0.0002 |
| 34:8 | 0.001±0.0005 | 0.001±0.0005 | 0.001±0.0004 |
| 36:8 | 0.001±0.0004 | 0.001±0.0003 | 0.001±0.0003 |
| 36:9 | 0.002±0.001 | 0.002±0.001 | 0.002±0.001 |
| 38:10 | 0.001±0.0002 | 0.003±0.001 | 0.002±0.0003 |
| 38:9 | 0.004±0.001 | 0.01±0.01 | 0.03±0.02 |
| ∑SFA | 41.22±5.39$^{b}$ | 43.59±5.83$^{ab}$ | 48.24±6.89$^{a}$ |
| ∑MUFA | 24.84±1.28$^{a}$ | 24.65±0.95$^{ab}$ | 21.41±1.73$^{b}$ |
| ∑PUFA | 33.94±5.12 | 31.76±5.58 | 30.35±5.95 |
| ∑n-3 | 11.93±2.05 | 10.15±2.25 | 10.29±2.50 |
| ∑n-6 | 21.43±3.79 | 20.91±3.84 | 19.47±3.94 |

| Structure | Fatty acid content (% of total fatty acids) | | |
|---|---|---|---|
| | 13°C | 15°C | 17C° |
| ∑FA | 100 | 100 | 100 |

Data are expressed as mean±SE, n=6. Significant differences are indicated by different superscripts. The fatty acids with a * are considered essential fatty acids.

Table 3.4.3. Fatty acid profile of seaweeds (ng/mg) growing under different temperatures

| Structure | FA content (ng/mg) | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| 12:0 | 0.42±0.03 | 0.55±0.12 | 0.49±0.08 |
| 13:0 | 0.25±0.03 | 0.25±0.01 | 0.30±0.03 |
| 14:0 | 148.82±81.45 | 107.77±42.00 | 91.87±24.58 |
| 15:0 | 10.77±3.69 | 8.30±1.67 | 7.45±1.20 |
| 16:0 | 634.29±139.69 | 595.32±00.94 | 551.15±72.17 |
| 17:0 | 3.44±0.90 | 3.07±0.37 | 3.77±0.38 |
| 18:0 | 238.64±28.08 | 258.16±10.69 | 409.02±57.17 |
| 19:0 | 0.74±0.12 | 0.81±0.05 | 1.04±0.09 |
| 24:0 | 4.38±0.59 | 4.89±0.45 | 6.38±0.68 |
| 14:1 | 0.55±0.17 | 0.43±0.10 | 0.39±0.10 |
| 15:1 | 0.38±0.13 | 0.35±0.21 | 0.12±0.04 |
| 16:1 | 257.22±95.18 | 183.33±57.01 | 152.06±25.01 |
| 17:1 | 18.98±13.17 | 9.65±4.27 | 7.85±3.94 |
| 18:1 | 461.78±157.16 | 431.53±98.37 | 350.37±71.67 |
| 19:1 | 4.78±3.38 | 3.86±1.42 | 3.45±1.04 |
| 20:1 | 2.13±0.44 | 2.53±0.33 | 2.45±0.24 |
| 21:1 | 0.12±0.04 | 0.17±0.03 | 0.27±0.04 |
| 22:1 | 2.70±0.67 | 2.73±0.44 | 2.90±0.33 |
| 23:1 | 0.36±0.14 | 0.90±0.60 | 3.86±2.06 |
| 24:1 | 6.24±2.82 | 6.87±2.76 | 8.63±2.33 |
| 25:1 | 0.33±0.08 | 0.58±0.32 | 1.16±0.50 |

| Structure | FA content (ng/mg) | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| 26:1 | 2.20±0.66 | 3.30±1.33 | 3.82±1.13 |
| 18:2(n-6)* | 411.99±182.85 | 334.48±111.49 | 248.96±61.56 |
| 18:3(n-3)* | 66.15±31.22 | 43.06±14.47 | 39.54±6.59 |
| 19:2 | 0.50±0.09 | 0.49±0.08 | 0.49±0.09 |
| 20:2(n-6) | 1.98±0.51 | 2.04±0.29 | 1.82±0.33 |
| 20:3(n-6) | 1.05±0.26 | 0.83±0.16 | 0.81±0.09 |
| 20:4(n-6)* | 360.47±152.00 | 310.54±110.41 | 277.36±76.49 |
| 20:5(n-3)* | 340.61±158.76 | 257.10±109.45 | 221.56±82.21 |
| 21:2 | 0.02±0.01 | 0.03±0.01 | 0.04±0.01 |
| 21:3 | 0.02±0.01 | 0.01±0.01 | 0.01±0.01 |
| 21:4 | 0.18±0.05 | 0.17±0.05 | 0.19±0.04 |
| 22:2(n-6) | 0.29±0.06 | 0.29±0.05 | 0.33±0.04 |
| 22:3 | 0.17±0.13 | 0.16±0.10 | 0.17±0.08 |
| 22:4(n-6) | 0.51±0.07 | 0.55±0.08 | 0.68±0.08 |
| 22:5 | 2.68±0.34 | 2.81±0.51 | 3.32±0.51 |
| 22:6(n-3)* | 13.53±5.78 | 12.33±3.84 | 11.40±2.50 |
| 24:2 | 0.26±0.08 | 0.26±0.05 | 0.27±0.07 |
| 24:4(n-6) | 0.04±0.01 | 0.04±0.02 | 0.08±0.02 |
| 24:5 | 0.05±0.01 | 0.07±0.02 | 0.08±0.01 |
| 26:2 | 8.72±2.90 | 9.73±2.27 | 7.80±2.59 |
| 26:3 | 2.16±0.79 | 2.13±0.53 | 1.66±0.59 |
| 26:5 | 0.04±0.01 | 0.06±0.02 | 0.05±0.01 |
| 32:6 | 0.01±0.004 | 0.01±0.01 | 0.01±0.01 |
| 32:7 | 0.004±0.004 | 0.003±0.003 | 0.00±0.00 |
| 34:7 | 0.004±0.004 | 0.01±0.01 | 0.004±0.004 |
| 34:8 | 0.01±0.01 | 0.02±0.005 | 0.01±0.01 |
| 36:8 | 0.01±0.01 | 0.02±0.01 | 0.03±0.01 |
| 36:9 | 0.04±0.01 | 0.03±0.004 | 0.04±0.01 |
| 38:10 | 0.01±0.005 | 0.05±0.01 | 0.04±0.01 |

| Structure | FA content (ng/mg) | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| 38:9 | 0.08±0.01 | 0.22±0.13 | 0.55±0.33 |
| ∑SFA | 1041.74±208.72 | 979.12±141.42 | 1071.46±94.68 |
| ∑MUFA | 757.77±247.48 | 646.23±147.78 | 537.34±92.79 |
| ∑PUFA | 1211.59±531.75 | 977.53±346.09 | 817.32±220.36 |
| ∑n-3 | 420.29±195.39 | 312.49±127.02 | 272.51±89.51 |
| ∑n-6 | 776.33±335.42 | 648.76±221.36 | 530.05±137.03 |
| n-3:n-6 | 0.71±0.25 | 0.59±0.19 | 0.66±0.23 |
| DHA:EPA | 0.05±0.01 | 0.07±0.01 | 0.08±0.02 |
| EPA:ARA | 1.03±0.26 | 0.84±0.18 | 0.82±0.16 |
| ∑FA | 3011.11±978.08 | 2602.88±616.94 | 2426.12±328.07 |

Data are expressed as mean±SE, n=6. The fatty acids with a * are considered essential fatty acids.

**3.5 Discussion**

Contrary to some other species of seaweeds that benefit from higher temperatures, *P. mollis* in this study showed a negative correlation between rising temperature and its growth. *Palmaria* genus is considered to be a cold temperature species (Demetropoulus and Langdon, 2004; Dumay et al., 2022), many studies have determined the optimal temperature range of *P. palmata* to be 6-14°C. Although not many studies have been done to determine the optimal temperature for the growth of *P. mollis*, Demetropoulus and Langdon (2004) determined its optimal temperature to be 12°C at low light intensities (specific light density (SLD) = 0.010 mol photons/g/d) and 14-18°C at high light intensities (SLD = 0.021mol photons/g/d). Our results suggest that the *P. mollis* used for this study appears more sensitive to elevated temperatures. Furthermore, significant reductions in growth rates of *P. palmata* were found at 18°C (Demetropoulus and Langdon, 2004; Grote, 2019), which is consistent with our results of decreased growth with increasing temperature and the observation of dulse decomposing and becoming mushy and discolored after the third week of culture at 17°C. This observation could be related to the response of the fluidity of seaweed cell membranes to temperature (Eggert, 2012; Britton et al., 2020), which could affect

their cell walls and consequently their structure, however, this is an interesting observation but was out of the scope of our study.

*Palmaria* is considered to be a high protein content seaweed genus (Fleurence et al., 2018; Fidelis e Moura et al., 2022), with high variability of protein content ranges that are likely to be affected by factors such as seasons and temperature (Gadberry et al., 2018). The protein content of *P. palmata* has been reported as ranging from 8% to 35% DM (Fleurence et al., 2018; Dumay et al., 2022). Wulffson (2020) reported a 20.6% DM protein content was found in the cultured *P. mollis* tested. Our study shows the protein content crossing treatments ranges from 14.4-18.5% DM when growing at 13°C, 15.2-20.7% DM when growing at 15°C, and 15.5-21.7% DM when growing at 17°C. The variability within each temperature group can be explained by naturally seasonal variability and factors in the ocean that we were unable to control, such as nutrients and pollutants. Furthermore, the protein content was significantly different when dulse was grown at 13°C only. Protein content was not significantly different between dulse growing at 15°C and 17°C. The lowest protein content was found in *P. mollis* growing at 13°C, despite its higher growth. In general, for all treatments, the protein content of *P. mollis* was within a normal range for *Palmata* species.

No significant difference was found in total glucose, TNC, or starch between all the treatments. This could indicate that protein in seaweed is more susceptible to temperature than carbohydrates. In fact, Eggert (2012) described how protein is especially sensitive to temperature in seaweeds. Even though no significant differences were found for these factors, data showed a pattern where the higher protein content was related to a lower total glucose and TNC content.

The partition of fatty acids found in this study was in agreement with previous studies for *Palmaria* species, where the highest percent of the total fatty acids was SFA, followed by PUFA and MUFA. *P. palmata* has been reported to have a fatty acid repartition of 43.8-63.5% of SFA, 20.4-52.8% of PUFA,

and 4.9-16.1% of MUFA (Dumay et al., 2022). Our results are within the similar ranges found in *P. palmata*; however, we found a higher percentage (21.4-24.8%) of MUFA in our samples compared to those found in *P. palmata*.

As described by Eggert (2012), seaweed membranes can undergo changes in degrees of unsaturation of fatty acids in response to temperature changes, where at lower temperatures, the unsaturation of fatty acids is expected to increase, since the unsaturation of fatty acids improves membrane fluidity. We confirmed this behavior in our results, where the seaweed growing at 13°C had a significantly higher percentage of MUFA than seaweed growing at 17°C, and consequently a lower percentage of SFA. No significant difference was found in the percentage of PUFA for any of the treatments, suggesting that the bigger change in the degree of saturation due to temperature happened within the MUFA and not their PUFA.

The percentage range of omega-3 in *P. palmata* has been found to be 25.52-51.90% and omega-6 at 2.14-7.30% (Dumay et al., 2022). Our results showed a different behavior, the highest mean percentage of omega-3 was 11.93%, and the highest mean omega-6 was 21.43%. Therefore, our samples contained a higher percentage of omega-6 than omega-3. Although both omega-6 and omega-3 are very important for human and animal health, western diets are higher in omega-6, and deficient in omega-3, therefore products higher in omega-3 are preferred (Simopoulos, 2002). Moreover, no significant difference was found between treatments in either of the percentages of omega-3 or omega-6. Although no significant difference was found between treatments for any of the EFA considered in this study, it is important to note that *P. mollis* used in this study contained all of the important fatty acids, highlighting the omega-6 (linoleic acid and arachidonic acid) and the omega-3 (eicosapentaenoic acid).

In conclusion, rising ocean temperature highly negatively affected *P. mollis* growth, suggesting that the farming of this species where the mean ocean temperature elevates to the predicted conditions due to

climate change will depend on more controlled culture systems where the temperature can be maintained under 13°C. Additionally, rising ocean temperature affected the chemical composition of the *P. mollis* used in this study by increasing their protein content and their percentage of saturated fatty acids, however, their carbohydrate content and their percentage of polyunsaturated fatty acids were not influenced by the temperatures tested here.

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# Chapter 4. Influence of rising ocean temperature on juvenile red abalone *Haliotis rufescens* growth and chemical composition through changes in its food source, marine seaweed *Palmaria mollis*


## 4.1 Abstract

The red abalone (*Haliotis rufescens*) are a high-value species important in food production and conservation aquaculture in California. They are susceptible to environmental factors, like temperature and pH, and changes in their diet. The effect of changes in the diet due to predicted rising ocean temperature due to climate change on juvenile red abalone was studied. Three different diets were prepared by growing red seaweed Pacific dulse (*Palmaria mollis*) under three different temperatures, 13°C, 15°C, and 17°C. Juvenile red abalone (23.76±0.15 mm and 1.82±0.04 g (mean±SD)) were grown under ambient conditions for 105 days with the prepared dulse as their only food. Abalone growth was measured at Days 0, 40, 75, and 105, and their chemical composition, protein, carbohydrate, and fatty acids, were analyzed at the end of the experiment. Abalone fed dulse growing at 17°C showed higher cumulative growth rates, final condition factor, and specific growth rate. The abalone weight seemed to be more affected by the diets than the shell length. No significant difference was found in their chemical composition across treatments. The results suggest that higher temperatures due to climate change tested in this study do not have negative indirect effects on the juvenile red abalone.

## 4.2 Introduction

Red abalone (*Haliotis rufescens*) is the largest species of edible sea snails, they can be found in the Eastern Pacific from Oregon, USA to Bahia Tortugas, Mexico. They are slow-growing herbivores that feed on seaweeds like kelp and dulse. This species is the largest abalone in the world and the most common abalone found in Northern California (Haaker et al., 2001; MARINe, 2021). Abalone have been sought for human consumption prehistorically; however, for several reasons including overfishing, predation (mostly by sea otters), and the fatal bacterial disease Withering Syndrom, caused by *Candidatus Xenohaliotis californiensis*, wild populations of abalone have been decimated (California Department of fish and wildlife, 2021).

Abalone has significant importance to mariculture, around 85000 mt of abalone is produced annually across the world (FAO, 2016). To meet the high market demand and as a response to the wild population decline, the culture of abalone in California began in the 1960s (Woolford, 2019; California Department of fish and wildlife, 2020). Red abalone are a valuable aquaculture product in California that can exceed over $100/lb. The high market price of this species is related to their slow growth nature, it takes three to four years to grow them from larvae to market sizes (~7.6 to 8.9 cm shell length) (FAO, 1990; Lutz, 2022). They are produced in coastal areas, where is hard to control environmental conditions like pH and temperature (FAO, 1990; Kadvany, 2019).

Environmental factors related to climate change such as pH and temperature in the ocean have been shown to directly impact abalone reproduction, early development, and survival. Studies have shown that ocean water conditions with pH $\leq$ 7.6 negatively effects abalone shell formation, decreases growth rates, and reduces embryonic developmental success (Li et al., 2013; Swezy et al., 2020). Thermal tolerance studies have shown that abalone sperm production is reduced with increasing water temperature, as well as the susceptibility and subsequent mortality to Withering Syndrome disease at 18°C or higher (Rogers-Bennett et. al. 2010; Crosson, 2020).

While the direct effects of climate change have been broadly studied in abalone, studies on the indirect effects have been frequently overlooked. For example, pH and temperature are factors that affect seaweed growth and chemical composition. Temperature has been shown to be one of the most influential factors that alter the chemical composition, and consequently the nutritional quality of seaweeds (Eggert, 2012). Abalone are sensitive to the quality of their food, which alters their growth rates and the quality of their meat (Langdon et al., 2004); therefore, changes in the quality of seaweeds due to climate change can have an added impact on abalone farming in cultures where these factors cannot be controlled.

This study aims to investigate the effects of changes in the seaweed nutritional quality cultured in predicted ocean warming conditions on the juvenile red abalone growth, and nutritional composition, as a potential indirect impact of ocean warming. The hypothesis for this study was that the changes in the seaweed nutritional quality cultured at the higher predicted temperatures will yield lower growth rates in the juvenile red abalone, as well as decrement their nutritional value.

## 4.3 Materials and methods

### *4.3.1 Acclimatization*

In June 2021, 215 juvenile red abalone were shipped overnight to the UC Davis Bodega Marine Laboratory (BML), Bodega Bay, California, USA, from The Cultured Abalone Farm located in Rancho Dos Pueblos on the coast of Santa Barbara County, California, USA. Upon arrival, all abalone were acclimated for 30 days at 10°C in a 184 L water volume tank filled with UV-filtered ambient seawater in dark conditions (Figure 4.3.1). Oxygenation was provided with an air pump and the abalone were fed ad libitum *P. mollis* cultured at the BML for the White Abalone Recovery Program collected from an outdoor tumble-culture tank with flow-through filtered ambient seawater. All uneaten *P. mollis* was taken out of the tank twice a week and replaced with fresh seaweed. Once a week the tank was completely emptied, rinsed, and refilled with filtered seawater. Abalone survival during acclimation was 94.4%.

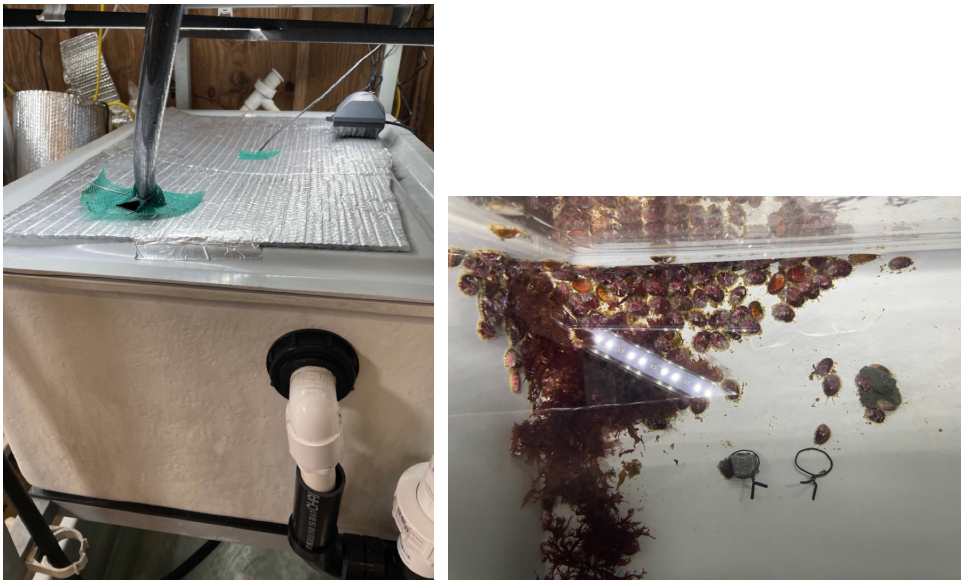


Figure 4.3.1 First acclimatization setup for the juvenile red abalone.

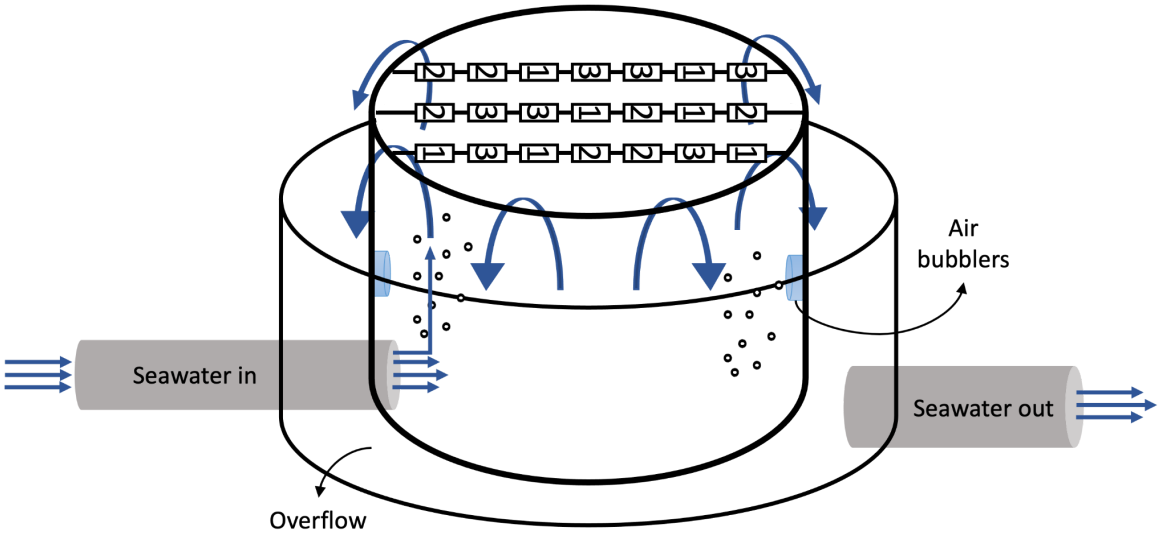
At the completion of the 30 days acclimatization process, abalone were placed in cages (15.24x15.24x3.21cm) with a density of five abalone per cage. The cages were made with plastic containers, a hole was made at the bottom and at the lid of the container and a mesh that allowed water, waste, and gas exchange, but was small enough to prevent escapement, was glued to the surface (Figure 4.3.2). All cages were placed in a flow-through filtered ambient seawater tank (Figure 4.3.3) in near-dark conditions, and abalone kept being fed ad libitum the same *P. mollis* described above, replaced on a weekly basis. Air was pumped into the tank for oxygenation at two opposite sides of the tank as shown in Figure 4.3.3 (b). Abalone were held in these culture containers for an additional 30 days prior to the start of the experiment. During this acclimatization period, abalone survival was 89.2%.

(a)

(b)

Figure 4.3.2. (a) Picture of a cage made to contain abalone during the experiment and (b) picture of a cage in the experiment.

(a)




(b)

Figure 4.3.3. (a) Picture of experiment setup and (b) diagram with details of the experimental setup.

*4.3.2 Experimental design*

For the experiment, a total of 21 cages were hung in three lines (seven cages per line), and a diet treatment was randomly assigned to each cage (seven replicate cages per diet) for a complete randomized block experimental design. The details of the treatment assignment are shown in Figure 4.3.4. Three days

before the start of the experiments, the feeding was stopped to normalize the initial conditions of each individual. On August 23rd, 2021, a day before the start of the experiments, the juvenile red abalone from each culture container were weighed and measured by calipers to standardize between containers and treatments. Abalone had an initial mean weight of 1.82±0.04 g and a mean shell length of 23.76±0.15 mm (mean±SE). No significant differences in the starting weight ($p=0.41$) and shell length ($p=0.30$) were found among the three treatments.

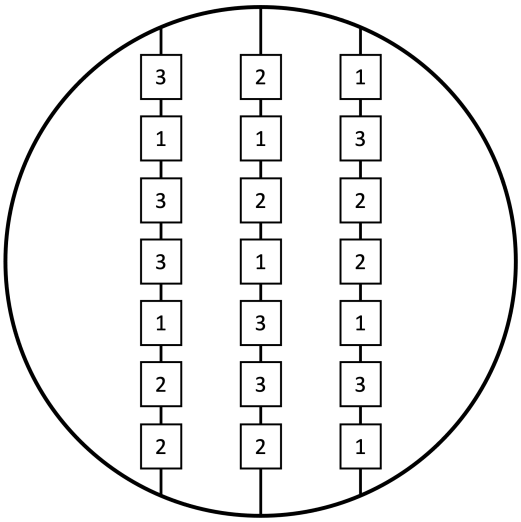
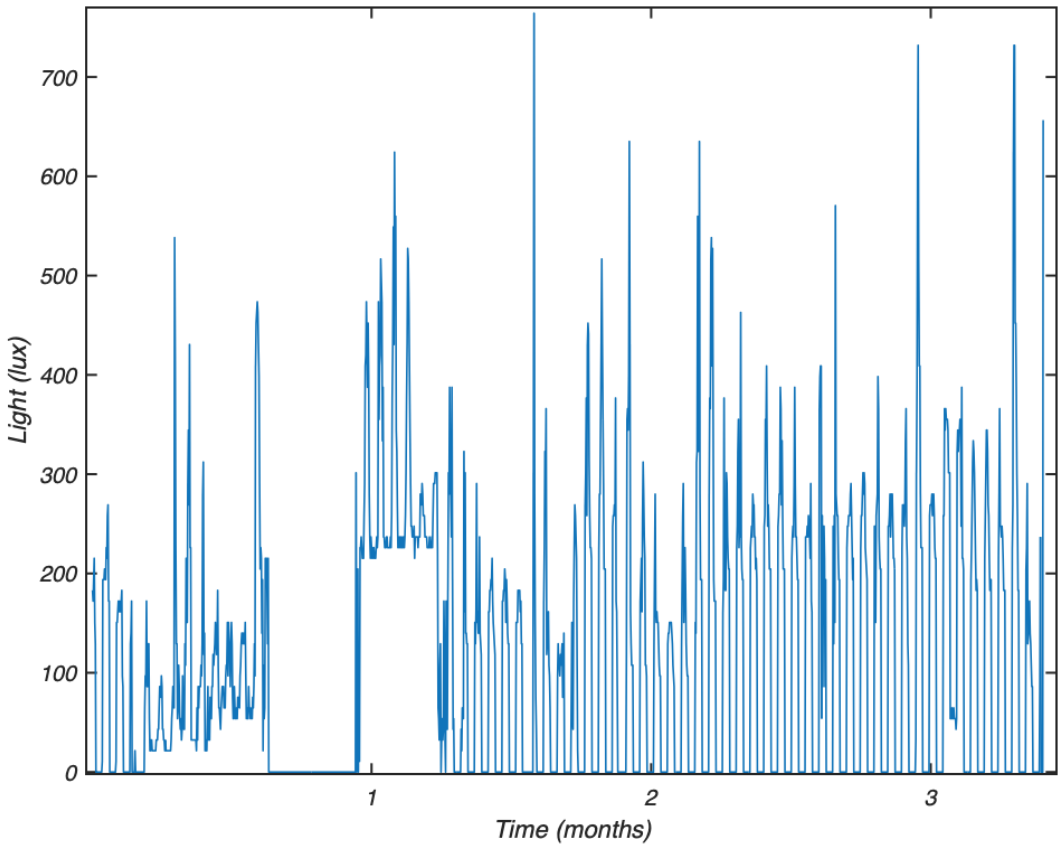
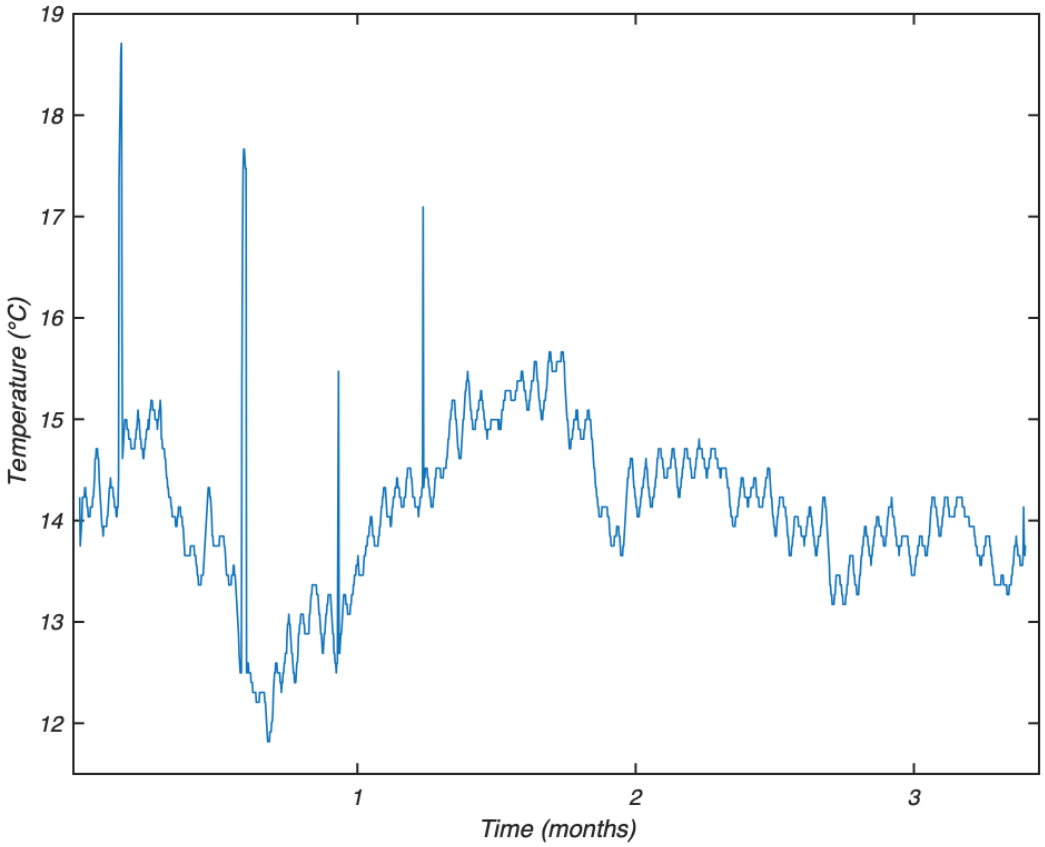



Figure 4.3.4. Abalone treatment arrangement. Numbers 1, 2, and 3 correspond to each diet treatment as follows, treatment 1: dulse growing at 17°C, treatment 2: dulse growing at 15°C, and treatment 3: dulse growing at 13°C.

During the experimental period (105 days), abalone were fed ad libitum on a weekly basis. All uneaten *P. mollis* were removed each week and replaced with fresh rosettes. The water temperature (14.11°C±0.02, mean±SE) and the light intensities (115.47±3.37 lux, mean±SE) were recorded with a floating data logger (Onset HOBO Pendant® MX, Massachusetts, USA) every hour. Light and temperature profiles during the experimental period are shown in Figure 4.3.5. Abalone were checked once per week, and any abalone found dead were removed. No abalone was replaced throughout the period of the trial.




(a)




(b)

Figure 4.3.5. (a) light and (b) temperature data for the abalone culture throughout the study.

*4.3.3 Preparation of diets*

The cultured *P. mollis* from the acclimatization period were used as the initial stock population for the different diets. To prepare the three diets, *P. mollis* was grown by tumble culture in 184 L water volume rectangular tanks in a recirculating system filled with UV-filtered seawater (460 L total water volume) under three different temperatures (13°C, 15°C, and 17°C) for three weeks prior to the start of the experiment, and maintained under the same conditions for the entirety of the trial. The *P. mollis* tumbling culture was created by pumping water with a pump (Rio plus Aqua Power Head Pump 1400, 420 GPH, Taipei, Taiwan). Additionally, aeration was provided with an air pump (Whisper AP150, Tetra, Virginia, USA) to the tanks to ensure better movement and oxygenation. The seaweeds were cultured under 2 LED

lights at full light spectrum with a 12 h:12 h light-dark photoperiod. The water temperature was maintained using chillers (DBA-075 1/10HP, JBJ Arctica, Missouri, USA) and recorded every hour with a floating data logger (Onset HOBO Pendant® MX, Massachusetts, USA).

An initial *P. mollis* stocking density of 500 g per 184 L water volume (Figure 3.3.1) was placed in each tank. Every seven days all seaweed was harvested to clean the seaweed culture tanks and to feed the abalone. During seaweed harvest, the total volume of seawater was replaced in each system and the abalone culture containers were cleaned. The remaining seaweed, after feeding the abalone, was weighed and returned to their respective treatment tanks. Every 21 days, samples of every treatment were collected and oven-dried for future analysis of their chemical composition, and 500 g of each seaweed treatment was returned to their specific tanks.

A complete list of the water quality parameters monitored in the seaweed cultures is shown in table 4.3.1. The pH, ammonia, nitrite, and nitrate were tested by colorimetry at the beginning and the end of each weekly water exchange with the Saltwater master test kit (API®, Chicago, USA). The carbon dioxide and alkalinity were tested by titration with the Marine Test Kit HI3823 (Hanna instruments, Quebec, Canada) and salinity was measured with a refractometer (Imagitarium, Saltwater refractometer, California, USA). Ammonia and nitrate were qualitative measurements since the color scales provided by the kit were not sensitive enough for our values.

Table 4.3.1. Water quality values of seaweed cultures.

| Factor | Treatment | | | p-value |
|---|---|---|---|---|
| | 13°C | 15°C | 17°C | |
| pH | 8.09±0.02 | 8.09±0.02 | 8.09±0.02 | 0.97 |
| nitrite (ppm) | 0.00±0.00 | 0.00±0.00 | 0.00±0.00 | - |
| carbon dioxide (ppm) | 12.00±1.90 | 11.91±1.75 | 12.91±1.93 | 0.92 |
| alkalinity (ppm as $CaCO_3$) | 125.47±4.49 | 125.65±4.00 | 126.88±3.80 | 0.97 |
| salinity (ppt) | 34.13±0.30 | 34.25±0.31 | 34.25±0.31 | 0.95 |
| ammonia (ppm) | 0 > x > 0.25 | 0 > x > 0.25 | 0 > x > 0.25 | - |
| nitrate (ppm) | 0 > x > 5 | 0 > x > 5 | 0 > x > 5 | - |
| Temperature (°C) | $12.95\pm0.01^{c}$ | $15.22\pm0.01^{b}$ | $16.96\pm0.01^{a}$ | p<0.0001 |

Data expressed as mean±SE. Significant differences are indicated by different superscripts.

The nutritional values of *P. mollis* cultured under the three temperatures were obtained in the previous chapter (chapter 3), where seaweed were growing under the same conditions but every three weeks all seaweed was harvested, and a new culture was started with the *P. mollis* cultured outdoors described in section 4.3.1. The seaweed used to feed the abalone in this chapter was grown in the indoor culture systems only.

*4.3.4 Data collection*

To reduce potential stress effects of handling on juvenile abalone growth and survival, abalone shell lengths (SL) and wet weights (W) were recorded on Days 0, 40, 75, and 105. SL was determined by measuring the longest shell axis (±0.1 mm) with calipers (Figure 4.3.6). On Day 105, abalone were weighed (wet weight), SL measured, shucked, body tissues dried at 45°C for 72 h, and weighed again to obtain their dry weight.

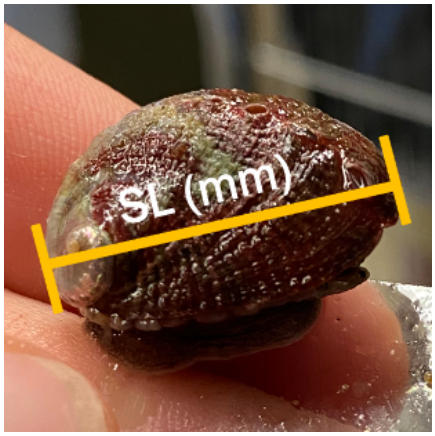



Figure 4.3.6. Position of calipers for measurement of abalone shell lengths.

Abalone linear growth rate (LGR, $\mu$m/day) was determined using Equation 1 (Demetropoulos and Langdon, 2004)

$$LGR = \frac{SL_f - SL_i}{t} \qquad (1)$$

where LGR was the shell growth rate ($\mu$m/day) $L_i$ and $L_f$ were the initial and final SL ($\mu$m) and t was time in days.

Weight gain per day was calculated using Equation 2 (Wulffson, 2020).

$$\Delta W = \frac{W_f - W_i}{t} \qquad (2)$$

where $\Delta$W was the change in weight per day (mg/day), $W_i$ and $W_f$ were the initial and final weight (mg), and t was time in days.

Cumulative growth rates (G) were calculated using the weight and shell length gained per day, as shown in Equation 3 (Wulffson, 2020).

$$G = \frac{X_i - X_{i-1}}{t} \qquad (3)$$

Where G was the change in SL or W ($\mu$m/day; mg/day), $X_i$ was the SL ($\mu$m) or W (mg) for Day i, $X_{i-1}$ was the previous SL or W measurement, and t was the time in days since the last measurement.

Specific growth rate (SGR) was determined using Equation 4 (Evans and Langdon, 2000).

$$SGR = 100x\frac{lnW_f - lnW_i}{t} \qquad (4)$$

where SGR was the specific growth rate (% /day), $W_i$ and $W_f$ were the initial and final wet weights (g), and t was the total time in days.

The relationship between the weight of the abalone per unit shell length, known as condition factor (CF), was calculated using Equation 5 (Britz et al., 1997).

$$CF = \frac{W}{SL^{2.99}}x\,5575 \qquad (5)$$

where W was the body weight (g) and SL was the shell length (mm).

*4.3.5 Feed consumption*

Uneaten seaweed were collected after the feeding regime and weighed at three sampling points (Weeks 6, 11, and 15) during the experiment to determine the daily feed consumption rates (DFC). The collected

uneaten *P. mollis* were damp weighed after all the excess water was removed using a salad spinner. Three additional cages with seaweed only (one for each treatment), sans abalone, were added to the abalone culture tank to serve as a control to account for any loss of seaweed mass by natural degradation.

DFC was calculated using Equation 6 (Demetropoulos and Langdon, 2004)

$$DFC = \frac{F-R-D}{Wt} \qquad (6)$$

where DFC was the daily consumption ($g_{P.\ mollis}$ /$g_{abalone}$ /day), F was the damp weight (g) of *P. mollis* offered during the experimental period, R was the damp weight of uneaten *P. mollis*, D was the weight loss obtained from the control, W was the mean damp weight (assuming linear growth) of abalone during the experimental period, and t was the time in days of feeding trial.

*4.3.6. Statistical analysis*

The results were analyzed for statistical differences using the SAS Enterprise Guide (SAS, North Carolina, USA) analytical software. Mixed-effects analysis of variance (ANOVA) tests were carried out to test the effect of the diet treatments on the growth and biochemical composition of juvenile abalone. A randomized complete block design was used for this study (Figure 4.3.4), each line was considered a block and each cage was the experimental unit since the abalone were not marked individually. The random effect of each cage was considered, and the interaction of time and cage as a random effect was considered for measurements taken over time. Post-hoc pairwise comparisons with Tukey adjustment were conducted to assess the significance among the three treatment levels. Differences were considered significant when $p<0.05$.

Shell length and weight gain over time, cumulative growth rate, condition factor over time, and daily food consumption were analyzed with time as one of the main effects and the interaction of time and cage as a random effect. Initial and final shell length and weight, daily growth rates (LGR and ΔW), specific growth rate, initial and final condition factor, and biochemical composition (protein, total glucose, TNC, starch, and fatty acids) were analyzed with cage as a random effect.

## 4.4 Results

### *4.4.1 Diet composition*

The chemical composition (dry matter, acid detergent fiber (ADF), total nitrogen, protein, total digestible nutrients (TDN), crude fat, ash, total glucose, total non-structural carbohydrates (TNC), starch and fatty acids profile) of the Pacific dulse used to feed the abalone in this experiment is shown in Tables 4.4.1 to 4.4.3. Samples were collected every three weeks for a total of 6 samples per diet treatment.

Table 4.4.1 Biochemical composition of *Palmaria mollis* grown under different temperatures. All data is presented as % of dry matter (DM). Data are expressed as mean±SE.

| *P. mollis* temperature | Dry Matter | ADF-Reflux | Total Nitrogen | Protein | TDN-Reflux |
|---|---|---|---|---|---|
| 13°C | 94.00±0.24 | 9.12±0.89 | 2.25±0.17 | 14.09±1.08 | 72.68±0.55 |
| 15°C | 93.34±0.19 | 8.85±0.97 | 2.47±0.19 | 15.41±1.21 | 73.45±0.68 |
| 17°C | 93.34±0.19 | 8.49±0.75 | 2.64±0.15 | 16.49±0.94 | 73.69±0.48 |

Table 4.4.2 Biochemical composition of *Palmaria mollis* grown under each temperature. All data are presented as % of dry matter (DM). Data are expressed as mean±SE.

| *P. mollis* temperature | Crude Fat | Ash | Total Glucose | TNC | Starch |
|---|---|---|---|---|---|
| 13°C | 1.04±0.14 | 33.55±1.40 | 7.38±0.73 | 7.40±0.73 | 6.50±0.64 |
| 15°C | 0.81±0.08 | 31.83±1.62 | 8.21±0.46 | 8.25±0.47 | 7.20±0.42 |
| 17°C | 0.74* | 31.99±1.00 | 8.18±0.71 | 8.24±0.70 | 7.30±0.62 |

*Five of the six samples for seaweed grown at 17°C did not have enough material to evaluate the crude fat; therefore, crude fat at 17°C shown here was the content of the last sample only.

Table 4.4.3 Fatty acid profile of *Palmaria mollis* grown under each temperature. Values are presented as the percentage of total fatty acids (%). Data are expressed as mean±SE. The fatty acids with a * are considered essential fatty acids. Total fatty acids are presented as ng/mg at the end of the table.

| Structure | Fatty acid content (% of total fatty acids) | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| 12:0 | 0.02±0.004 | 0.03±0.01 | 0.05±0.01 |
| 13:0 | 0.01±0.002 | 0.01±0.002 | 0.02±0.002 |
| 14:0 | 3.16±0.84 | 3.12±0.83 | 2.59±0.61 |
| 15:0 | 0.33±0.03 | 0.29±0.03 | 0.28±0.02 |
| 16:0 | 24.60±1.94 | 22.14±2.30 | 23.10±2.96 |
| 17:0 | 0.15±0.04 | 0.17±0.05 | 0.27±0.11 |
| 18:0 | 19.76±5.52 | 14.58±3.91 | 23.83±4.17 |
| 19:0 | 0.04±0.01 | 0.05±0.01 | 0.06±0.01 |
| 24:0 | 0.28±0.12 | 0.32±0.11 | 0.41±0.13 |
| 14:1 | 0.01±0.002 | 0.02±0.002 | 0.02±0.001 |
| 15:1 | 0.02±0.01 | 0.02±0.01 | 0.02±0.01 |
| 16:1 | 7.38±0.92 | 9.11±1.40 | 5.30±0.43 |
| 17:1 | 0.38±0.20 | 0.23±0.04 | 0.25±0.09 |
| 18:1 | 13.21±1.87 | 14.54±2.12 | 12.60±1.85 |

| Structure | Fatty acid content (% of total fatty acids) | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| 19:1 | 0.11±0.06 | 0.11±0.03 | 0.11±0.03 |
| 20:1 | 0.11±0.04 | 0.15±0.04 | 0.15±0.03 |
| 21:1 | 0.01±0.003 | 0.01±0.003 | 0.02±0.002 |
| 22:1 | 0.13±0.05 | 0.15±0.04 | 0.20±0.05 |
| 23:1 | 0.02±0.01 | 0.02±0.003 | 0.03±0.01 |
| 24:1 | 0.26±0.07 | 0.26±0.08 | 0.26±0.08 |
| 25:1 | 0.01±0.004 | 0.02±0.01 | 0.02±0.01 |
| 26:1 | 0.10±0.03 | 0.13±0.05 | 0.13±0.06 |
| 18:2(n-6)* | 10.14±2.66 | 11.16±3.00 | 9.43±2.49 |
| 18:3(n-3)* | 1.89±0.11 | 2.08±0.24 | 3.02±1.65 |
| 19:2 | 0.02±0.01 | 0.03±0.01 | 0.02±0.01 |
| 20:2(n-6) | 0.07±0.01 | 0.09±0.01 | 0.09±0.01 |
| 20:3(n-6) | 0.03±0.005 | 0.05±0.02 | 0.04±0.01 |
| 20:4(n-6)* | 8.66±1.85 | 10.05±1.52 | 9.61±2.32 |
| 20:5(n-3)* | 8.18±1.33 | 9.95±1.25 | 7.21±1.67 |
| 21:2 | 0.002±0.001 | 0.002±0.0005 | 0.002±0.001 |
| 21:3 | 0.0005±0.0003 | 0.0002±0.0001 | 0.002±0.001 |
| 21:4 | 0.01±0.001 | 0.01±0.001 | 0.01±0.001 |
| 22:2(n-6) | 0.03±0.02 | 0.04±0.02 | 0.03±0.01 |
| 22:3 | 0.03±0.02 | 0.03±0.02 | 0.03±0.01 |
| 22:4(n-6) | 0.03±0.01 | 0.05±0.02 | 0.05±0.02 |
| 22:5 | 0.11±0.03 | 0.16±0.03 | 0.13±0.01 |
| 22:6(n-3)* | 0.35±0.09 | 0.46±0.07 | 0.31±0.03 |
| 24:2 | 0.03±0.01 | 0.03±0.01 | 0.03±0.01 |
| 24:4(n-6) | 0.003±0.002 | 0.003±0.001 | 0.004±0.001 |
| 24:5 | 0.005±0.003 | 0.01±0.002 | 0.004±0.001 |
| 26:2 | 0.23±0.10 | 0.25±0.11 | 0.23±0.09 |
| 26:3 | 0.06±0.03 | 0.06±0.02 | 0.04±0.02 |
| 26:5 | 0.004±0.002 | 0.003±0.001 | 0.004±0.002 |

| Structure | Fatty acid content (% of total fatty acids) | | |
|---|---|---|---|
| | 13°C | 15°C | 17°C |
| 32:6 | 0.0002±0.0002 | 0.001±0.0005 | 0.001±0.001 |
| 32:7 | 0.0004±0.0004 | 0.0004±0.0002 | 0.0005±0.0005 |
| 34:7 | 0.00±0.00 | 0.001±0.0004 | 0.0004±0.0004 |
| 34:8 | 0.0003±0.0003 | 0.001±0.001 | 0.001±0.001 |
| 36:8 | 0.001±0.0003 | 0.001±0.001 | 0.002±0.001 |
| 36:9 | 0.002±0.001 | 0.003±0.001 | 0.005±0.002 |
| 38:10 | 0.001±0.0002 | 0.0002±0.0001 | 0.0002±0.0002 |
| 38:9 | 0.003±0.001 | 0.01±0.001 | 0.01±0.003 |
| ∑SFA | 48.35±6.59 | 40.72±5.26 | 50.61±6.28 |
| ∑MUFA | 21.76±1.96 | 24.76±1.05 | 19.09±1.87 |
| ∑PUFA | 29.89±5.47 | 34.52±4.77 | 30.30±5.69 |
| ∑n-3 | 10.42±1.38 | 12.49±1.41 | 10.55±2.57 |
| ∑n-6 | 18.97±4.43 | 21.44±4.43 | 19.24±4.74 |
| ∑FA | 100 | 100 | 100 |
| ∑FA (ng/mg) | 3067.36±678.80 | 2274.87±454.98 | 1597.96±339.28 |

*4.4.2 Abalone survival and growth*

Abalone survival during the experiment was high, with 89.5% survival across all treatments. Survival in 13°C, 15°C, and 17°C diet treatments was 91.4%, 88.6%, and 88.6%, respectively (Figure 4.4.1).

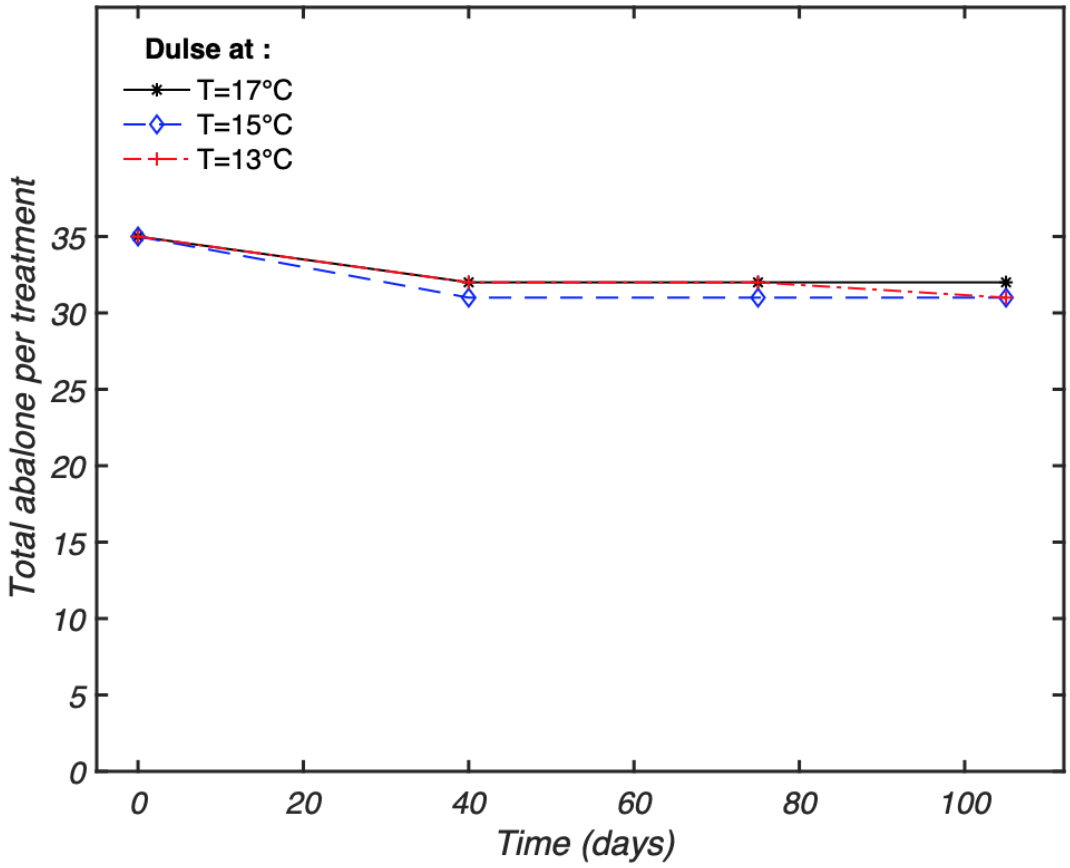



Figure 4.4.1. Survival of abalone across the duration of the study. Abalone survival was checked once a week.

The SL and weight measurements are shown in Table 4.4.4. No significant difference in the final SL was found between treatments (p=0.17). However, a significant difference was found between diet treatments in the rate of SL gained when growth curves were compared (p=0.0012). Furthermore, pairwise comparisons indicated abalone feeding on dulse cultured under 17°C had a significantly higher growth

curve for SL gain than those feeding on dulse cultured under 13°C and 15°C. Figure 4.4.2 shows the change in the SL of abalone throughout the trials.

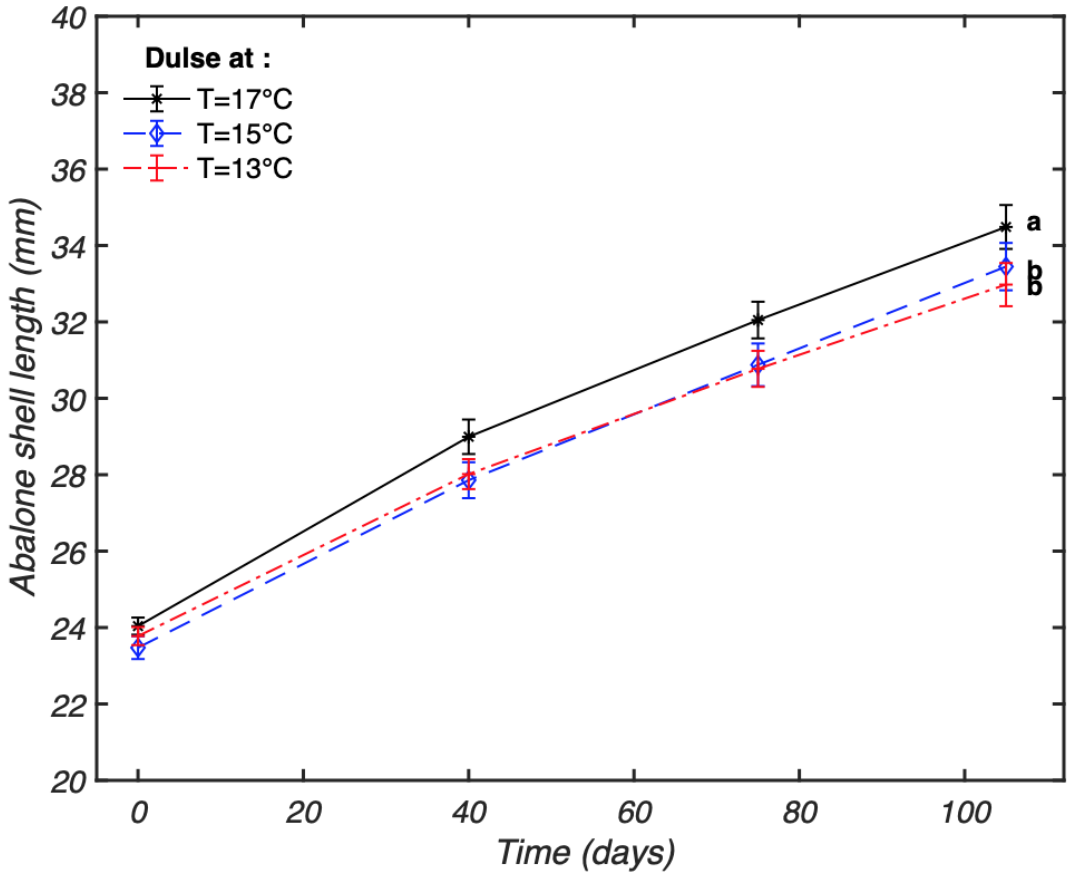



Figure 4.4.2. Abalone shell length growth over time. Data are expressed as mean±SE, n=31-35, based on the mortality. Significant differences between growth curves are indicated by different letters.

A significant difference in the final weight was found between treatments (p=0.006). Abalone feeding on dulse cultured under 17°C showed a significantly higher final weight than those feeding on dulse cultured under 13°C, and a higher final weight than those feeding on dulse cultured under 15°C, although the difference was not significant, as shown in Table 4.4.4. When the growth curves were compared, a significant difference in weight gain was found among the treatment groups (p=0.0002). Similar to the SL, abalone feeding on dulse cultured under 17°C had a significantly higher growth curve for weight gain

than those feeding on dulse cultured under 13°C and 15°C. Figure 4.4.3 shows the change in the weight of abalone throughout the trials.

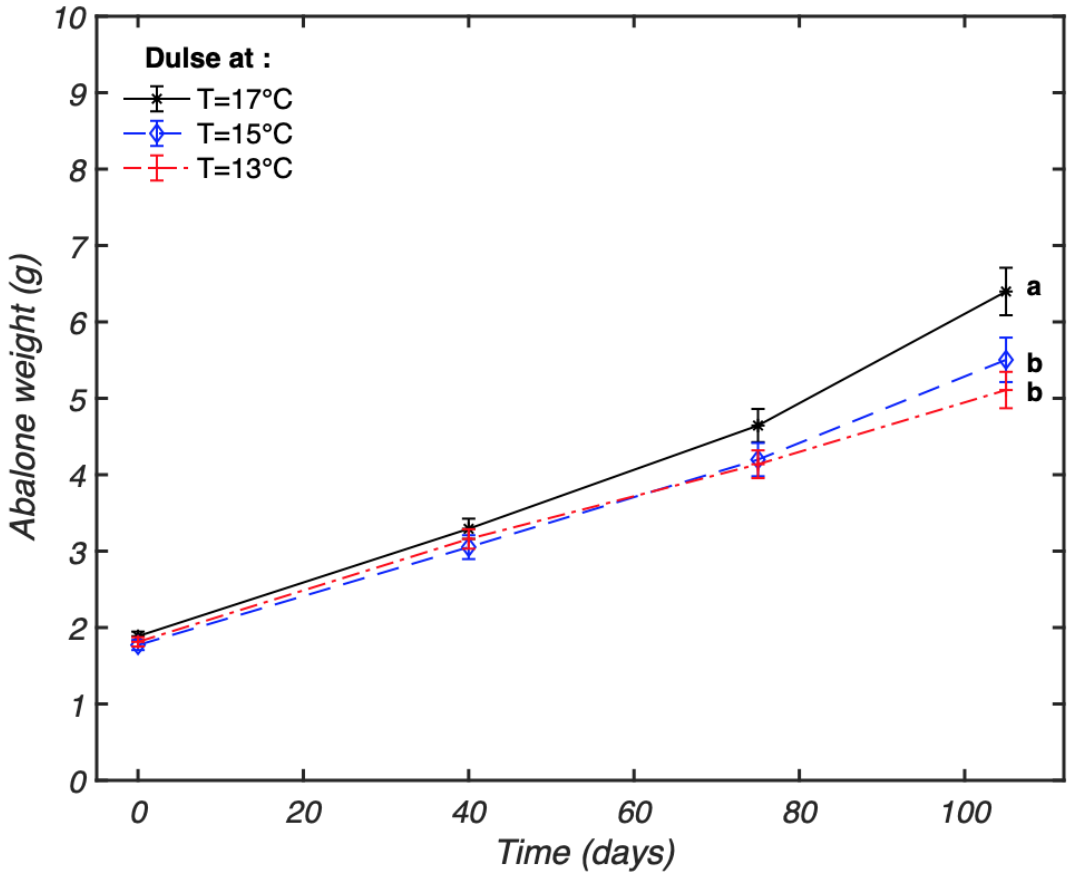



Figure 4.4.3. Abalone weight gain over time. Data are expressed as mean±SE, n=31-35, based on the mortality. Significant differences between growth curves are indicated by different letters.

Table 4.4.4. Initial and final SL and W measurements for all treatments. Data expressed as mean±SE. Significant differences in a column are indicated by different superscripts.

| Treatment | Initial Weight (g) | Initial Shell Lenght (mm) | Final Weight (g) | Final Shell Length (mm) |
|---|---|---|---|---|
| Dulse@13°C | 1.81±0.06 | 23.78±0.24 | 5.11±0.24[b] | 32.98±0.57 |
| Dulse@15°C | 1.77±0.07 | 23.47±0.30 | 5.50±0.29[a,b] | 33.45±0.62 |
| Dulse@17°C | 1.89±0.06 | 24.04±0.22 | 6.40±0.31[a] | 34.49±0.57 |

A significant difference in the daily growth rate in the form of weight gain per day was found (p=0.009). The abalone feeding on dulse cultured under 17°C showed a significantly higher weight gain per day than those feeding on dulse cultured under 13°C, and a higher weight gain per day than those feeding on dulse cultured under 15°C, although the difference was not significant, as shown in Table 4.4.5. Abalone feeding on dulse cultured under 17°C showed the highest LGR, despite no significant difference found between treatments (p=0.10) (Table 4.4.5). Furthermore, a significant difference between treatments was found for SGR (p=0.015). The abalone feeding on dulse cultured under 17°C showed a significantly higher SGR than those feeding on dulse cultured under 13°C, and a higher SGR than those feeding on dulse cultured under 15°C, although the difference was not significant, as shown in Table 4.4.5. There was a significant difference in the cumulative SL gain per day (p=0.0001) (Figure 4.4.4) and weight gain per day (p=0.022) (Figure 4.4.5). Abalone feeding on dulse cultured under 15°C had a significantly higher growth curve for cumulative SL gain per day than those feeding on dulse cultured under 13°C, and a higher growth curve for cumulative SL gain per day than those feeding on dulse cultured under 17°C, although the difference was not significant, as shown in Figure 4.4.4. Furthermore, abalone feeding on dulse cultured under 17°C had a significantly higher growth curve for cumulative weight gain per day than those feeding on dulse cultured under 13°C and 15°C, as shown in Figure 4.4.5.

Table 4.4.5. Abalone daily growth values (mean±SE) for all treatments. Significant differences are indicated by different superscripts.

| Diet Treatment | Weight change (mg/day) | LGR ($\mu$m/day) | SGR (% wet weight/day) |
|---|---|---|---|
| Dulse@13°C | 31.65±1.22$^{b}$ | 87.70±3.38 | 0.99±0.03$^{b}$ |
| Dulse@15°C | 36.12±1.51$^{a,b}$ | 96.57±1.57 | 1.09±0.02$^{a,b}$ |
| Dulse@17°C | 41.80±2.39$^{a}$ | 97.27±4.38 | 1.14±0.04$^{a}$ |

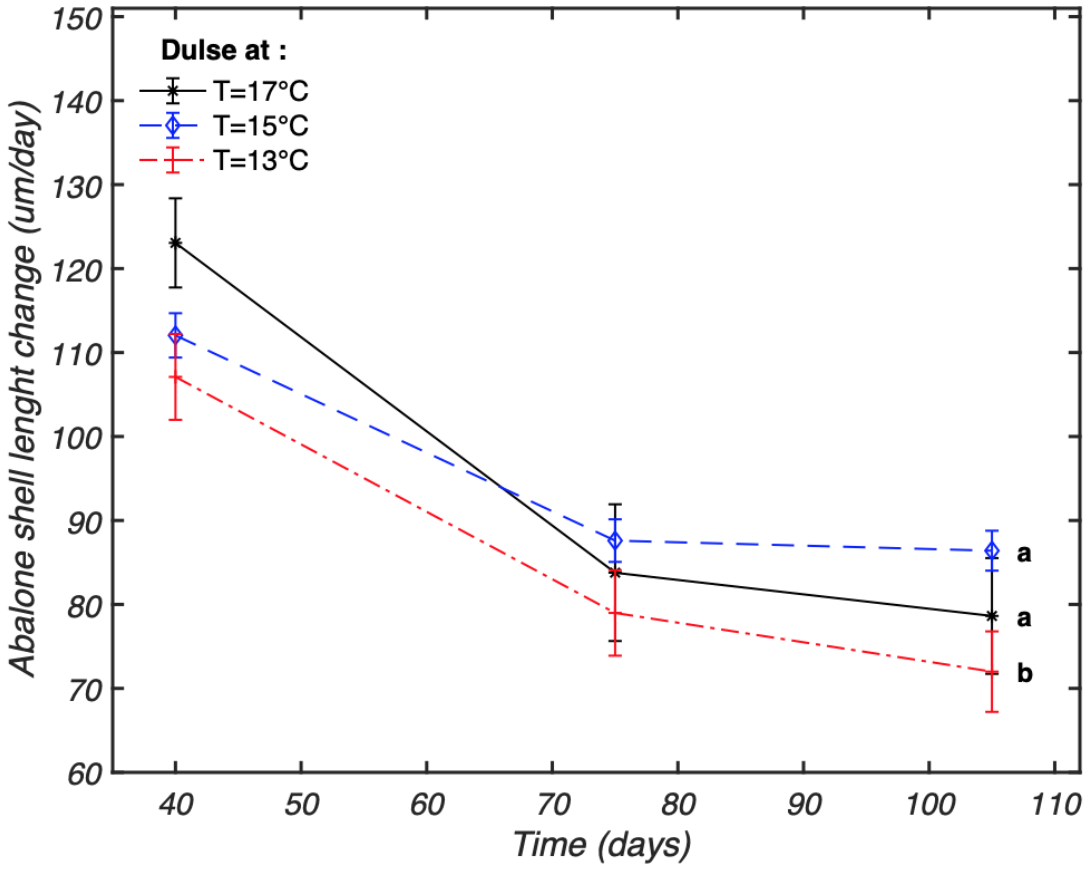



Figure 4.4.4. Abalone cumulative growth in terms of shell length gain per day (µm/day), n=7. Error bars indicate standard errors. Significant differences between growth curves are indicated by different letters.

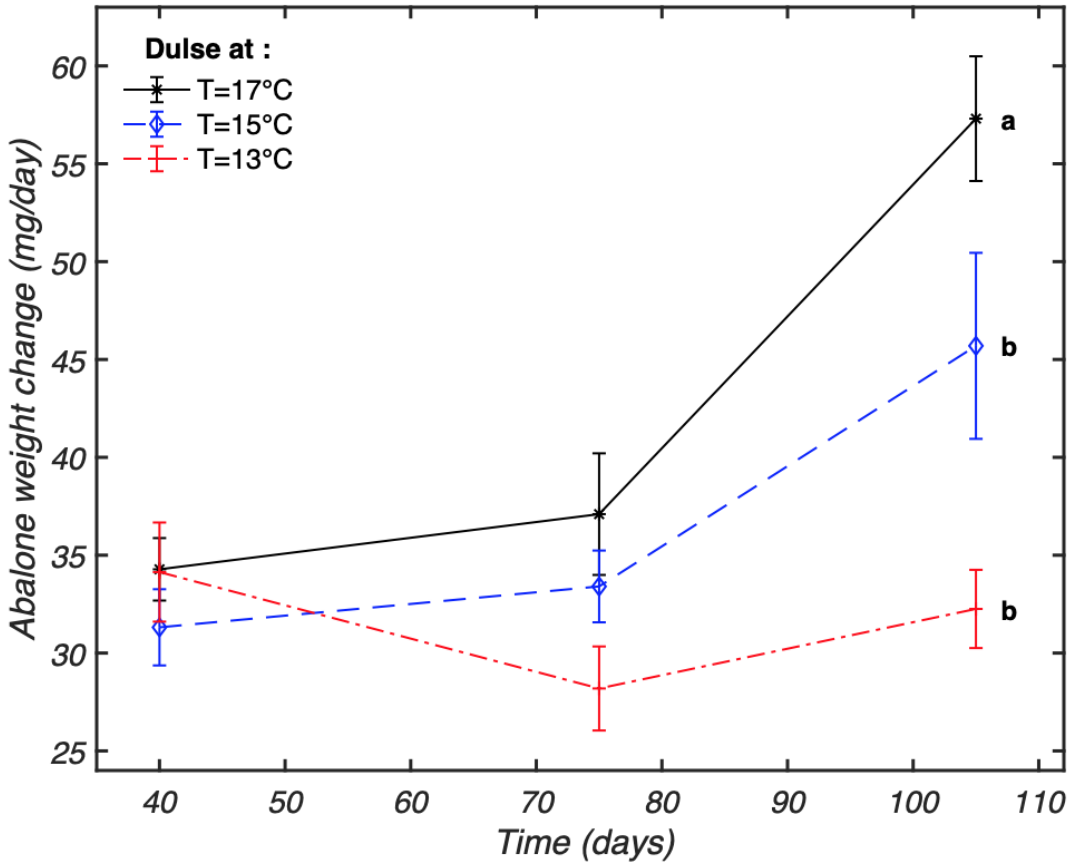



Figure 4.4.5. Abalone cumulative growth in terms of weight gain per day (mg/day), n=7. Error bars indicate standard errors. Significant differences between growth curves are indicated by different letters.

No significant difference was found in the condition factor (CF) over time between the treatments ($p=0.68$), as shown in Figure 4.4.6. However, a significant difference was found in the final condition CF between treatments ($p=0.007$). Abalone feeding on dulse cultured under 17°C showed a significantly higher final CF than those feeding on dulse cultured under 13°C, and a higher final CF than those feeding on dulse cultured under 15°C, although the difference was not significant, as shown in Figure 4.4.7.

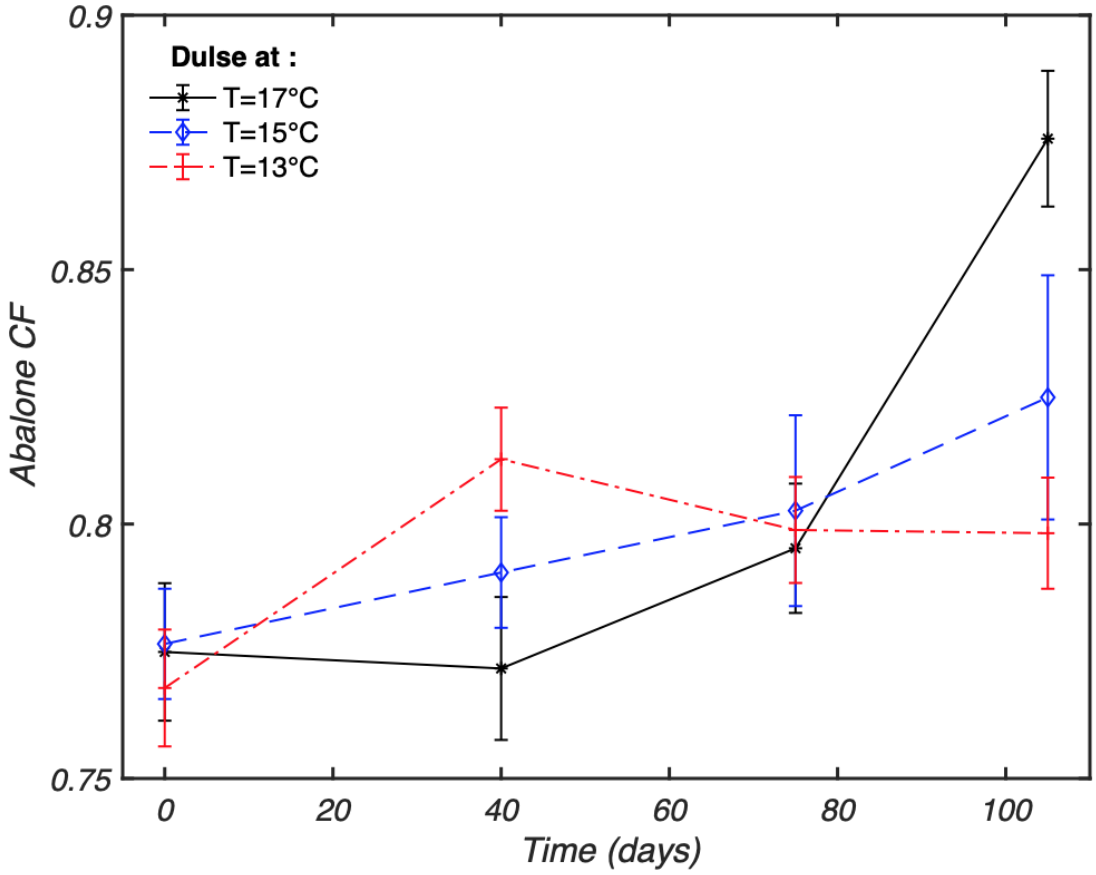



Figure 4.4.6. Abalone CF changes over time. Error bars indicate standard errors. n=31-35 based on the mortality.

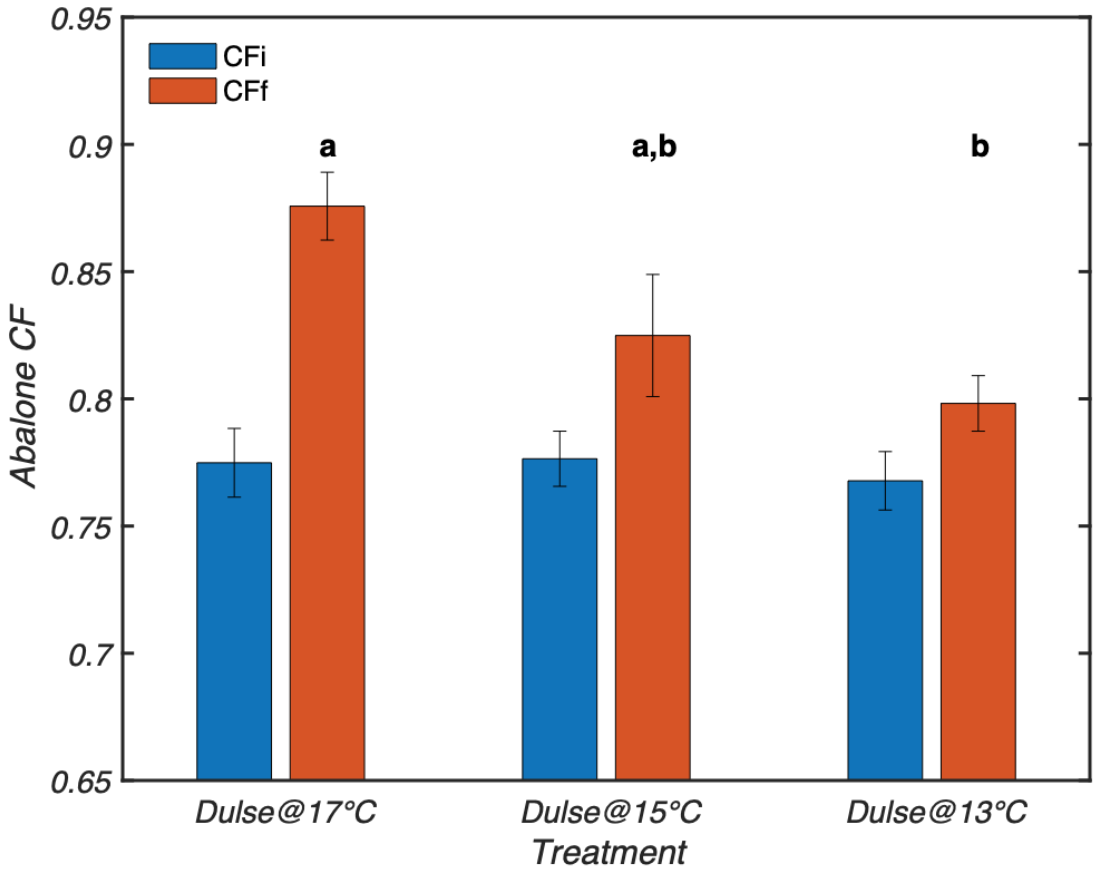



Figure 4.4.7. Initial (blue) and final (red) CF for each treatment. Error bars indicate standard errors. n=31-35 based on the mortality. Significant differences between treatments are indicated by different letters.

*4.4.3 Abalone daily food consumption*

No significant difference was found between treatments in the daily food consumption (DFC) of abalone ($p=0.14$). However, a significant difference was found in the DFC in time ($p=0.007$). The DFC was significantly lower on Week 15 than on Weeks 6 and 11, as shown in Figure 4.4.8. Indicating a lower consumption of seaweed on that week across all treatments.

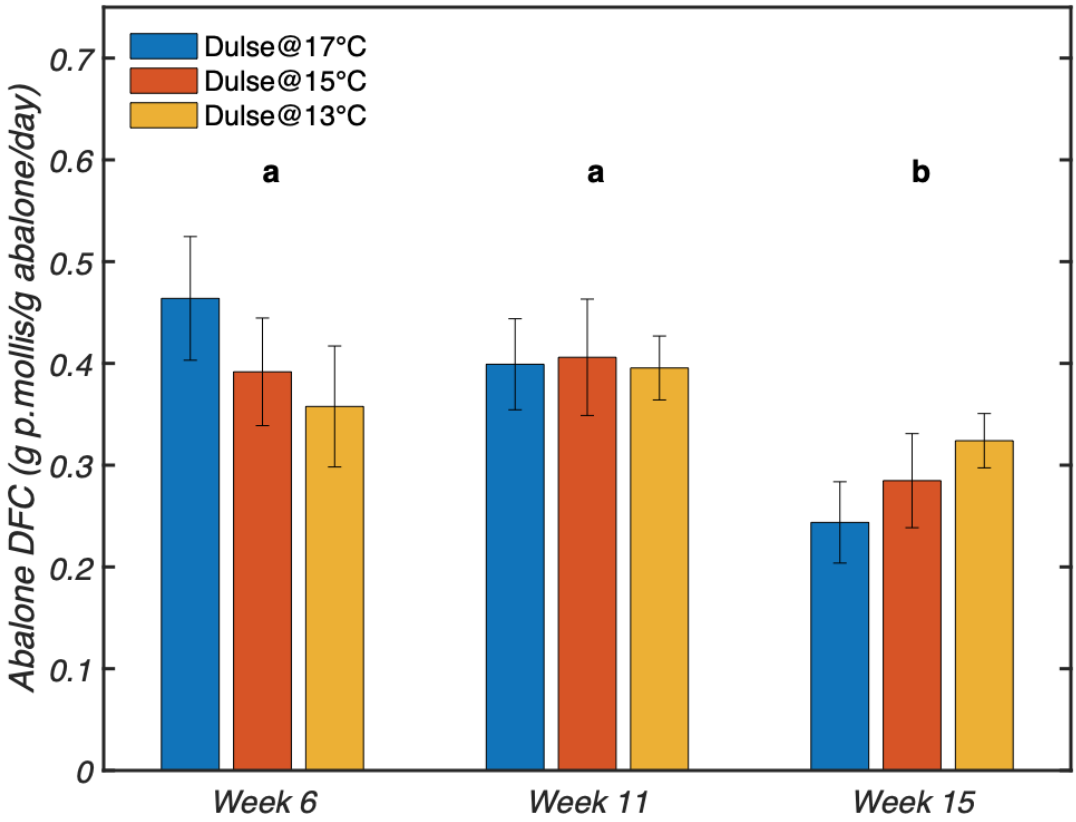



Figure 4.4.8. DFC of abalone in three different weeks throughout the experiments (n=7). Error bars indicate standard errors. Significant differences across trials on DFC are indicated by different letters.

*4.4.4 Abalone chemical composition*

No significant differences were found in any of the chemical compositions analyzed for abalone among the trials. All the chemical compositions of the abalone meat are shown in Tables 4.4.6 to 4.4.8 and Figures 4.4.9 and 4.4.10. The fatty acids 18:2 (n-6), linoleic acid (LA), 18:3 (n-3), α-linolenic acid (ALA), 20:4 (n-6), arachidonic acid (ARA), 20:5 (n-3), eicosapentaenoic acid (EPA), and 22:6 (n-3), docosahexaenoic acid (DHA) are considered essential fatty acids (marked with a * in tables 4.4.7 and 4.4.8) and were the only fatty acids analyzed for statistical differences. In addition, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-3 (n-3),

omega-6 (n-6), and the ratios of n-3/n-6, EPA/ARA and DHA/EPA were analyzed for statistical differences as well.

Table 4.4.6. Abalone protein and carbohydrate content for each diet treatment. All data were presented as % of dry matter (DM).

| Diet Treatment | Total Nitrogen | Protein | Total glucose | TNC | Starch |
|---|---|---|---|---|---|
| Dulse@17°C | 11.74±0.03 | 73.33±0.14 | 7.93±0.32 | 7.94±0.33 | 7.06±0.29 |
| Dulse@15°C | 11.69±0.06 | 73.04±0.29 | 8.43±0.23 | 8.43±0.23 | 7.51±0.23 |
| Dulse@13°C | 11.73±0.03 | 73.20±0.16 | 7.79±0.29 | 7.79±0.29 | 6.93±0.25 |

Data are expressed as mean±SE, n=7.

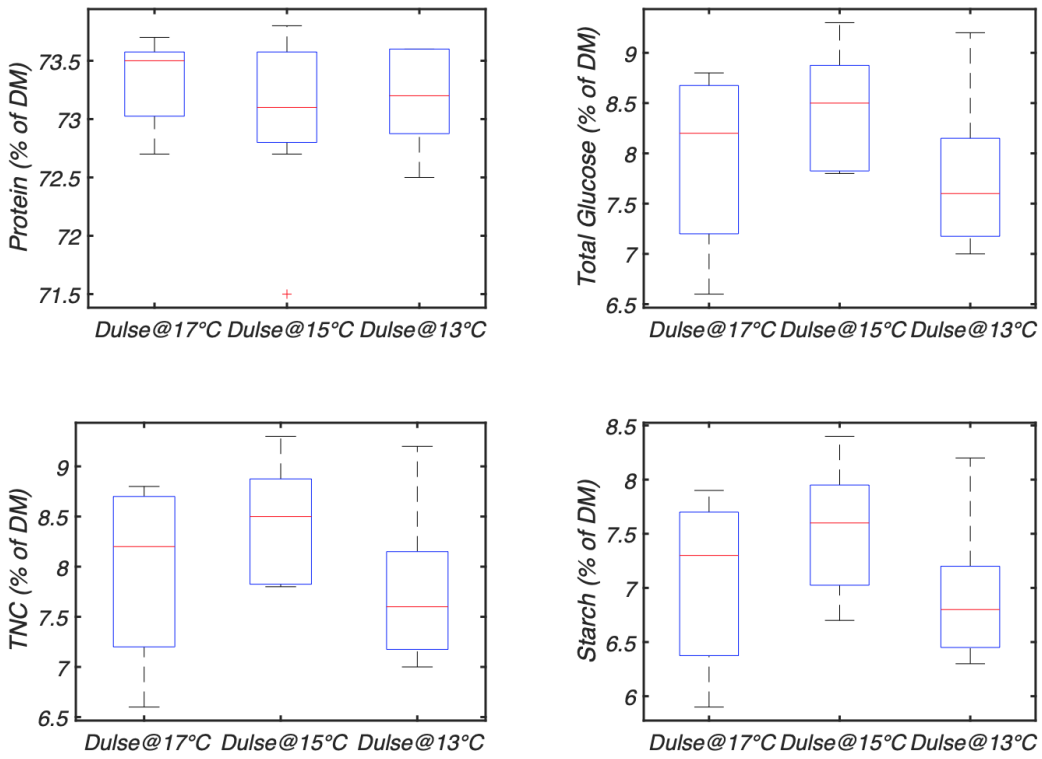



Figure 4.4.9. Protein, total glucose, total nonstructural carbohydrates, and starch content (% DM) of *H. rufences* from different diet treatments (n=7). IQR=box (27-75th percentiles), the central mark indicates the median, whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually using the '+' marker symbol.

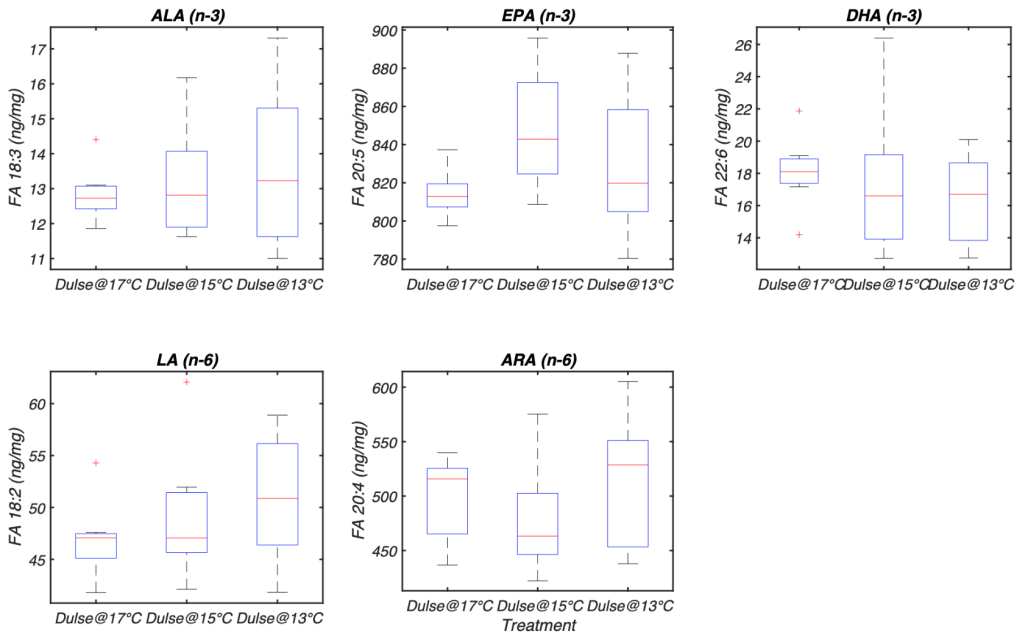



Figure 4.4.10. Abundance of important essential fatty acids (ng/mg) of *H. rufences* growing under different diet treatments (n=7). IQR=box (27-75th percentiles), the central mark indicates the median, whiskers extend to the most extreme data points not considered outliers, and outliers are plotted individually using the '+' marker symbol.

Table 4.4.7. Fatty acid profile of abalone growing under different diet treatments. Values expressed as a percentage of total fatty acids (%).

| Structure | Fatty acids content (% of total fatty acids) | | |
|---|---|---|---|
| | Dulse@13°C | Dulse@15°C | Dulse@17°C |
| 12:0 | 0.01±0.002 | 0.01±0.002 | 0.01±0.001 |
| 13:0 | 0.01±0.0004 | 0.01±0.0004 | 0.01±0.0004 |
| 14:0 | 1.89±0.03 | 1.83±0.06 | 1.84±0.05 |
| 15:0 | 0.29±0.01 | 0.28±0.01 | 0.28±0.01 |
| 16:0 | 9.80±0.18 | 9.87±0.13 | 9.69±0.20 |
| 17:0 | 0.57±0.02 | 0.55±0.01 | 0.56±0.02 |
| 18:0 | 8.38±0.24 | 8.65±0.23 | 7.96±0.36 |
| 19:0 | 0.11±0.01 | 0.11±0.01 | 0.12±0.01 |
| 24:0 | 0.03±0.002 | 0.04±0.002 | 0.03±0.002 |
| 14:1 | 0.03±0.001 | 0.03±0.001 | 0.03±0.001 |
| 15:1 | 0.08±0.002 | 0.07±0.003 | 0.07±0.002 |
| 16:1 | 1.14±0.03 | 1.05±0.03 | 1.06±0.03 |
| 17:1 | 0.29±0.004 | 0.29±0.01 | 0.29±0.01 |
| 18:1 | 10.32±0.12 | 9.60±0.07 | 9.98±0.13 |
| 19:1 | 0.34±0.01 | 0.33±0.01 | 0.34±0.01 |
| 20:1 | 7.71±0.12 | 8.01±0.17 | 7.88±0.19 |
| 21:1 | 0.15±0.00 | 0.15±0.01 | 0.15±0.01 |
| 22:1 | 0.15±0.004 | 0.15±0.01 | 0.15±0.002 |
| 23:1 | 0.17±0.01 | 0.17±0.01 | 0.19±0.01 |
| 24:1 | 0.04±0.003 | 0.05±0.003 | 0.05±0.002 |
| 25:1 | 0.04±0.002 | 0.04±0.002 | 0.04±0.002 |
| 26:1 | 0.02±0.002 | 0.03±0.001 | 0.03±0.003 |
| 18:2(n-6)* | 1.01±0.03 | 0.98±0.02 | 0.96±0.03 |
| 18:3(n-3)* | 0.27±0.01 | 0.26±0.01 | 0.26±0.01 |
| 19:2 | 0.04±0.001 | 0.04±0.001 | 0.04±0.001 |
| 20:2(n-6) | 0.8610.02 | 0.89±0.02 | 0.87±0.01 |

| Structure | Fatty acids content (% of total fatty acids) | | |
|---|---|---|---|
| | Dulse@13°C | Dulse@15°C | Dulse@17°C |
| 20:3(n-6) | 0.18±0.01 | 0.21±0.05 | 0.16±0.01 |
| 20:4(n-6)* | 10.17±0.25 | 9.49±0.15 | 10.12±0.17 |
| 20:5(n-3)* | 16.62±0.32 | 16.89±0.48 | 16.64±0.32 |
| 21:2 | 0.33±0.01 | 0.36±0.02 | 0.35±0.02 |
| 21:3 | 0.02±0.001 | 0.02±0.001 | 0.02±0.001 |
| 21:4 | 0.03±0.001 | 0.04±0.001 | 0.04±0.001 |
| 22:2(n-6) | 9.91±0.21 | 10.49±0.27 | 9.83±0.31 |
| 22:3 | 0.43±0.01 | 0.43±0.01 | 0.43±0.01 |
| 22:4(n-6) | 2.64±0.09 | 2.43±0.08 | 2.86±0.18 |
| 22:5 | 14.70±0.31 | 14.96±0.27 | 15.43±0.22 |
| 22:6(n-3)* | 0.33±0.02 | 0.34±0.03 | 0.37±0.02 |
| 24:2 | 0.02±0.001 | 0.02±0.002 | 0.02±0.001 |
| 24:4(n-6) | 0.03±0.002 | 0.03±0.001 | 0.03±0.001 |
| 24:5 | 0.33±0.01 | 0.33±0.01 | 0.34±0.01 |
| 26:2 | 0.003±0.0003 | 0.003±0.0001 | 0.003±0.0003 |
| 26:3 | 0.0004±0.0001 | 0.0005±0.00005 | 0.0003±0.0001 |
| 26:5 | 0.09±0.01 | 0.09±0.002 | 0.09±0.004 |
| 32:6 | 0.02±0.001 | 0.01±0.001 | 0.02±0.001 |
| 32:7 | 0.02±0.001 | 0.02±0.001 | 0.02±0.0005 |
| 34:7 | 0.02±0.002 | 0.02±0.001 | 0.02±0.001 |
| 34:8 | 0.05±0.003 | 0.04±0.001 | 0.05±0.002 |
| 36:8 | 0.14±0.01 | 0.13±0.005 | 0.13±0.004 |
| 36:9 | 0.02±0.001 | 0.02±0.001 | 0.02±0.001 |
| 38:10 | 0.03±0.001 | 0.03±0.001 | 0.03±0.001 |
| 38:9 | 0.10±0.004 | 0.10±0.003 | 0.10±0.005 |
| ∑SFA | 21.10±0.34 | 21.36±0.26 | 20.51±0.56 |
| ∑MUFA | 20.49±0.20 | 19.98±0.26 | 20.26±0.29 |
| ∑PUFA | 58.41±0.25 | 58.67±0.33 | 59.23±0.41 |
| ∑n-3 | 17.22±0.31 | 17.50±0.46 | 17.27±0.32 |

| Structure | Fatty acids content (% of total fatty acids) | | |
|---|---|---|---|
| | Dulse@13°C | Dulse@15°C | Dulse@17°C |
| ∑n-6 | 24.80±0.40 | 24.51±0.46 | 24.82±0.45 |
| ∑FA | 100 | 100 | 100 |

Data are expressed as mean±SE, n=7. The fatty acids with a * are the essential fatty acids.

Table 4.4.8. Fatty acid profile of abalone growing under different diet treatments (ng/mg).

| Structure | FA content (ng/mg) | | |
|---|---|---|---|
| | Dulse@13°C | Dulse@15°C | Dulse@17°C |
| 12:0 | 0.48±0.09 | 0.47±0.07 | 0.39±0.04 |
| 13:0 | 0.60±0.03 | 0.56±0.04 | 0.54±0.02 |
| 14:0 | 94.97±3.52 | 92.50±4.86 | 90.49±4.23 |
| 15:0 | 14.53±0.71 | 14.30±0.93 | 13.91±0.71 |
| 16:0 | 491.32±17.77 | 496.97±17.40 | 475.12±10.51 |
| 17:0 | 28.70±1.34 | 27.76±1.55 | 27.68±1.28 |
| 18:0 | 419.82±16.04 | 436.36±20.41 | 389.77±17.32 |
| 19:0 | 5.63±0.21 | 5.68±0.48 | 6.12±0.48 |
| 24:0 | 1.72±0.10 | 1.78±0.148 | 1.70±0.10 |
| 14:1 | 1.55±0.04 | 1.39±0.09 | 1.44±0.06 |
| 15:1 | 4.02±0.16 | 3.63±0.22 | 3.69±0.16 |
| 16:1 | 56.95±2.33 | 53.16±3.09 | 51.99±2.31 |
| 17:1 | 14.75±0.55 | 14.42±0.89 | 14.09±0.72 |
| 18:1 | 516.71±13.07 | 483.83±18.18 | 489.94±13.63 |
| 19:1 | 17.14±0.87 | 16.85±1.17 | 16.73±0.57 |
| 20:1 | 385.79±9.77 | 405.09±22.46 | 387.53±15.43 |
| 21:1 | 7.48±0.28 | 7.85±0.77 | 7.50±0.41 |
| 22:1 | 7.74±0.16 | 7.80±0.69 | 7.27±0.23 |
| 23:1 | 8.46±0.43 | 8.72±0.58 | 9.21±0.31 |
| 24:1 | 2.13±0.14 | 2.35±0.19 | 2.34±0.11 |
| 25:1 | 1.86±0.09 | 1.92±0.12 | 2.06±0.08 |

| Structure | FA content (ng/mg) | | |
|---|---|---|---|
| | Dulse@13°C | Dulse@15°C | Dulse@17°C |
| 26:1 | 1.24±0.10 | 1.30±0.05 | 1.51±0.15 |
| 18:2(n-6)* | 50.75±2.31 | 49.32±2.44 | 47.00±1.43 |
| 18:3(n-3)* | 13.65±0.87 | 13.18±0.61 | 12.85±0.30 |
| 19:2 | 1.83±0.11 | 1.91±0.11 | 1.83±0.08 |
| 20:2(n-6) | 43.10±1.37 | 44.93±2.34 | 42.89±1.36 |
| 20:3(n-6) | 9.01±0.67 | 11.24±3.47 | 7.94±0.65 |
| 20:4(n-6)* | 510.76±23.68 | 477.99±19.36 | 496.79±14.71 |
| 20:5 (n-3)* | 830.62±14.09 | 846.33±11.97 | 814.53±4.72 |
| 21:2 | 16.80±0.66 | 18.52±1.84 | 17.30±1.18 |
| 21:3 | 0.99±0.07 | 1.18±0.07 | 1.11±0.08 |
| 21:4 | 1.63±0.08 | 1.77±0.08 | 1.74±0.09 |
| 22:2(n-6) | 496.77±17.53 | 530.46±31.59 | 483.09±21.65 |
| 22:3 | 21.47±1.03 | 21.83±0.91 | 21.28±0.92 |
| 22:4(n-6) | 132.64±7.31 | 122.59±7.19 | 141.01±11.25 |
| 22:5 | 735.16±18.50 | 750.81±14.33 | 756.14±11.93 |
| 22:6(n-3)* | 16.34±1.05 | 17.46±1.75 | 18.11±0.86 |
| 24:2 | 1.14±0.06 | 1.24±0.12 | 1.04±0.06 |
| 24:4(n-6) | 1.41±0.11 | 1.31±0.07 | 1.35±0.05 |
| 24:5 | 16.40±0.42 | 16.84±0.62 | 16.59±0.46 |
| 26:2 | 0.16±0.02 | 0.15±0.01 | 0.16±0.02 |
| 26:3 | 0.02±0.01 | 0.02±0.003 | 0.02±0.004 |
| 26:5 | 4.49±0.29 | 4.41±0.15 | 4.23±0.20 |
| 32:6 | 0.78±0.04 | 0.73±0.03 | 0.76±0.03 |
| 32:7 | 0.81±0.05 | 0.76±0.03 | 0.78±0.03 |
| 34:7 | 1.10±0.08 | 0.98±0.03 | 0.98±0.04 |
| 34:8 | 2.51±0.14 | 2.23±0.08 | 2.24±0.10 |
| 36:8 | 7.24±0.33 | 6.34±0.23 | 6.52±0.21 |
| 36:9 | 1.10±0.04 | 0.94±0.04 | 0.99±0.04 |
| 38:10 | 1.47±0.04 | 1.29±0.05 | 1.40±0.05 |

| Structure | FA content (ng/mg) | | |
|---|---|---|---|
| | Dulse@13°C | Dulse@15°C | Dulse@17°C |
| 38:9 | 5.15±0.19 | 4.80±0.22 | 4.84±0.19 |
| ∑SFAs | 1057.76±35.01 | 1076.38±41.94 | 1005.72±30.09 |
| ∑MUFAs | 1025.83±24.82 | 1008.29±47.24 | 995.29±31.20 |
| ∑PUFAs | 2925.40±71.56 | 2951.57±85.69 | 2905.50±56.51 |
| ∑n-3 | 860.61±13.86 | 876.97±11.68 | 845.49±4.31 |
| ∑n-6 | 1244.44±48.22 | 1237.85±64.13 | 1220.06±43.37 |
| n3:n6 | 0.70±0.02 | 0.72±0.03 | 0.70±0.02 |
| EPA:ARA | 1.64±0.06 | 1.78±0.06 | 1.65±0.05 |
| DHA:EPA | 0.02±0.001 | 0.02±0.002 | 0.02±0.001 |
| ∑FA | 5008.99±123.97 | 5036.23±172.14 | 4906.51±95.18 |

Data are expressed as mean±SE, n=7. The fatty acids with a * are the essential fatty acids.

*4.4.5 Abalone and seaweed chemical composition*

A summary of the biochemical composition of abalone tissue and each diet is presented in Tables 4.4.9 and 4.4.10.

Table 4.4.9. Summary of protein and carbohydrates of abalone tissue and diet. Values expressed as % of dry matter (DM). Data expressed as mean±SE. Superscript of T indicates tissue sample while D is for diet samples.

| | Dulse@13°C$^{T}$ | Dulse@15°C$^{T}$ | Dulse@17°C$^{T}$ | Dulse@13°C$^{D}$ | Dulse@15°C$^{D}$ | Dulse@17°C$^{D}$ |
|---|---|---|---|---|---|---|
| Protein | 73.20±0.16 | 73.04±0.29 | 73.33±0.14 | 14.09±1.08 | 15.41±1.21 | 16.49±0.94 |
| Total Glucose | 7.79±0.29 | 8.43±0.23 | 7.93±0.32 | 7.38±0.73 | 8.21±0.46 | 8.18±0.71 |
| TNC | 7.79±0.29 | 8.43±0.23 | 7.94±0.33 | 7.40±0.73 | 8.25±0.47 | 8.24±0.70 |
| Starch | 6.93±0.25 | 7.51±0.23 | 7.06±0.29 | 6.50±0.64 | 7.20±0.42 | 7.30±0.62 |

Table 4.4.10. Summary of fatty acids of abalone tissue and diet. Values expressed as a percentage of total fatty acids (%). Data expressed as mean±SE. Superscript of T indicates tissue sample while D is for diet samples.

| | Dulse@13°C$^{T}$ | Dulse@15°C$^{T}$ | Dulse@17°C$^{T}$ | Dulse@13°C$^{D}$ | Dulse@15°C$^{D}$ | Dulse@17°C$^{D}$ |
|---|---|---|---|---|---|---|
| LA (18:2n-6) | 1.01±0.03 | 0.98±0.02 | 0.96±0.03 | 10.14±2.66 | 11.16±3.00 | 9.43±2.49 |
| ALA (18:3n-3) | 0.27±0.01 | 0.26±0.01 | 0.26±0.01 | 1.89±0.11 | 2.08±0.24 | 3.02±1.65 |
| ARA (20:4n-6) | 10.17±0.25 | 9.49±0.15 | 10.12±0.17 | 8.66±1.85 | 10.05±1.52 | 9.61±2.32 |
| EPA (20:5n-3) | 16.62±0.32 | 16.89±0.48 | 16.64±0.32 | 8.18±1.33 | 9.95±1.25 | 7.21±1.67 |
| DHA (22:6n-3) | 0.33±0.02 | 0.34±0.03 | 0.37±0.02 | 0.35±0.09 | 0.46±0.07 | 0.31±0.03 |
| ∑SFA | 21.10±0.34 | 21.36±0.26 | 20.51±0.56 | 48.35±6.59 | 40.72±5.26 | 50.61±6.28 |
| ∑MUFA | 20.49±0.20 | 19.98±0.26 | 20.26±0.29 | 21.76±1.96 | 24.76±1.05 | 19.09±1.87 |
| ∑PUFA | 58.41±0.25 | 58.67±0.33 | 59.23±0.41 | 29.89±5.47 | 34.52±4.77 | 30.30±5.69 |
| ∑n-3 | 17.22±0.31 | 17.50±0.46 | 17.27±0.32 | 10.42±1.38 | 12.49±1.41 | 10.55±2.57 |
| ∑n-6 | 24.80±0.40 | 24.51±0.46 | 24.82±0.45 | 18.97±4.43 | 21.44±4.43 | 19.24±4.74 |

**4.5 Discussion**

The slow growth rates of abalone make them a hard species to cultivate. They need high-quality food that supports not only faster growth rates but also maintains optimal health. Fresh macroalgae have been shown to support good growth rates and also to improve their feeding activity, health, and marketability. Specifically, Pacific dulse has been proven to be a good candidate for feed for abalone (Bansemer et al., 2016). However, dulse is very susceptible to changes in the environment where they grow, the rising of the ocean temperature could modify the biochemical composition of dulse and compromise its suitability to grow abalone.

We tested the effects of rising temperature on juvenile red abalone through their diets (dulse growing at 13°C, 15°C, and 17°C). From the previous experiment (chapter 3), we found that the main changes in the biochemical composition of dulse cultured under different temperatures were in protein and the percentage of saturated fatty acids. As protein is one of the main nutritional factors for the growth of abalone (Baek and Cho, 2021), understanding the magnitude of the effects of changes in *P. mollis* due to rising temperature on the growth of the abalone is important.

Survival of abalone in this study was very high across all treatments. Out of the 105 abalone used, only a total of 11 abalone died during the study, indicating the experimental conditions and all diet used was generally acceptable for the growth of the juvenile red abalone. Juvenile red abalone fed dulse growing at 17°C showed higher growth rates, final wet weight, and final shell length. However, the weight of abalone seemed to be more affected by the diets than the shell length did, since significant differences in shell length were only observed when the shell length gain over time was analyzed and not when the final shell length was compared.

Abalone grew at a rate of 31.65-41.80 mg/d and 87.70-97.27 $\mu$m/d in our study. In Wulffson's (2020) study, the growth rates observed in the juvenile red abalone from the same source of this study that was fed the Pacific dulse cultured in an Integrated Multi Trophic Aquaculture system with sablefish *Anoplopoma fimbria* diet where 16.3 mg/d and 86.9 $\mu$m/d. The diet used in our study contained 14-16.5% protein compared with 20.6% of protein content in the seaweed used in the Wulffson study. Furthermore, Demetropoulos and Langdon (2004) showed linear growth rate ranges of 169-198 $\mu$m/d and specific growth rates of 1.47-1.72 %/d for juvenile red abalone fed nutrified cultured *P. mollis* (19-30.3% protein content), whereas we obtained linear growth rate ranges of 87.70-97.27 $\mu$m/d and specific growth rates of 0.99-1.14 %/d. The protein content in the diets used in Demetropoulos and Langdon's (2004) study could explain the higher growth rates. However, differences between studies could be due to experimental conditions other than diet, such as initial conditions of the animals (size, weight, source, etc.), stocking

density, water quality and/or flow, experimental length, among others. Overall, our results are comparable with these studies and showed good growth rates for juvenile red abalone.

The cumulative growth rates show a decrease in shell length gain in time across treatments, while the weight gain is slower at the beginning of the experiment but then increases in time, except for abalone fed dulse cultured at 13°C where the weight gain rate decreased in the first half of the study but increased again following the behavior of the other treatments. Overall, the same behavior was observed in Wulffson's (2020) study for cumulative growth rates of the abalone fed dulse, where shell length gain per day decreases in time while their weight gain change per day increases in time. Abalone fed dulse cultured at 15°C and 17°C showed the highest final cumulative growth values for shell length (86.4 and 78.6 $\mu$m/day, respectively) and weight gain (45.7 and 57.3 mg/day, respectively).

The condition factor was higher for the abalone fed dulse growing at 17°C, supporting the higher growth rates observed. The results suggest that CF in abalone is significantly different as time progresses, which could be interesting to look into in more detail in the future as most studies only looked into the final CF. The condition factor of *Haliotis* species has been shown to be between 0.89-1.31 (Britz et al., 1997; Green et al., 2011; De Guzman and Creencia, 2014) depending on the species and culture conditions. The final condition factors in this study ranged from 0.80 to 0.88, which is at the lowest end of the range found in the literature, this could be related to the species since these condition factors found in the literature are for different *Haliotis* species than the one study here.

The daily food consumption does not appear to be affected by the types of diet tested in this study; however, it seems that all abalone ate less at the end of the experiments regardless of their diet. Therefore, differences in growth rates seem to be due to the biochemical composition of *P. mollis* and not by underfed or overfed in any treatment. Furthermore, DFC range from 0.24 to 0.46 $g_{P.\ mollis}$ /$g_{abalone}$ /day across treatments and trials in our study, which is lower than 0.71 $g_{P.\ mollis}$ /$g_{abalone}$ /day reported by

Wulffson (2020). However, the DFC difference between studies could be due to experimental conditions as discussed before.

Protein content is a very important factor in abalone nutrition, and high protein content diets are recommended for abalone (Baek and Cho, 2021). Optimal dietary crude protein levels for abalone growth are ranging from 27% to 47% depending on the species and culture conditions (Bansemer et al., 2016; Baek and Cho, 2021). The protein content in the diets in this study was between 14%-17%, which proved to be enough to support good growth rates for the juvenile red abalone. This is important since protein is the most expensive element of abalone nutrition (Baek and Cho, 2021). Furthermore, protein content in our abalone tissue was ~73%, considerably higher than the 57.6% reported by Wulffson (2020).

Fatty acids play an important role in abalone nutrition as well. Essential fatty acids like ARA, EPA, and DHA are necessary to obtain good growth rates and development (Glencross, 2009). Furthermore, for *Haliotis* spp. PUFA are the most digestible and better assimilated fatty acids compared to SFA and MUFA (Boles, 2020). All EFA were found in the dulse used in this study for all treatments in percentages that proved to be beneficial for the growth of juvenile red abalone. Moreover, ARA and EPA were found in percentages from 7 to 10%. Furthermore, although the highest fatty acids found in the dulse samples were SFA (40 to 50%), PUFA were found in high percentages too (29 to 35%).

There was no significant difference in any of the biochemical compositions of abalone in this study, despite differences in growth rates. However, when compared with the biochemical composition of their diets (Table 4.4.10), it was interesting to see a lower percentage of LA (18:2 n-6) and ALA (18:3 n-3) in abalone than in their diets. The EPA (20:5 n-3) was higher than those in their diets, and ARA (20:4 n-6) and DHA (22:6 n-3) were consistent between abalone and their diet. Furthermore, abalone samples showed higher concentrations of PUFA than SFA and MUFA, despite higher SFA in *P. mollis*. A higher concentration of PUFA in abalone could be beneficial since this kind of fatty acids distinctly influences their meat flavor (Bansemer, 2016).

In conclusion, contrary to what was hypothesized, *P. mollis* growing at 17°C supported higher growth rates, and the changes in seaweed growing under different temperatures did not have any effects on the juvenile abalone's nutritional composition. Suggesting that the temperatures predicted due to ocean warming tested in this study will not have an added negative effect on the growth or nutritional composition of the juvenile red abalone throughout their diet.

## 4.6 References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## Chapter 5. Summary, conclusion, and recommendations

Aquaculture industry keeps growing at a high rate in response to an unstable and overfished wild fish supply, and when done correctly, it can be an answer to meet the global seafood demand. However, there are several concerns about its environmental impacts and sustainability. Some of the big problems of this activity are the occupancy of land and the contamination of the water due to excess food and the use of drugs, which could potentially create problems like eutrophication. A possible solution for this is to focus on culturing low trophic level organisms inshore. Seaweed, herbivores, and filter feeders are excellent candidates to be farmed inshore since little to no input is necessary to be added to the system. In addition, seaweed can improve water quality by removing excess nutrients from it. However, this kind of aquaculture has its own problems. Ambient factors like temperature and pH are key factors to the growth and quality of this low trophic level species, and moreover, the ambient factors are almost impossible to manipulate. Therefore, to assure sustainable production in coastal areas, it is important to understand how species of interest are affected by the changes in ambient conditions that are predicted in the near future. Particularly, temperature and pH are factors that are already changing globally and are expected to keep changing in response to climate change.

In this study, the effects of temperature and pH on the growth and protein content of two marine microalgae, *Nannochloropsis oculata* and *Chaetoceros gracilis*, were explored. This study provides insights into the aquaculture of filter feeders, like oysters and mussels, that consume microalgae. The results showed that both the growth and protein content of the marine microalgae were influenced by temperature and pH. The highest specific growth rate and protein content were obtained when the microalgae were cultured under the conditions predicted under climate change effects (20°C and pH 7.6). For microalgae production under controlled conditions, higher temperature and lower pH is beneficial. However, other factors need to be considered if we want to predict the growth of microalgae in the natural

environment. For example, competition and community composition, other species of microalgae might adapt better and grow faster in the new environment and might dominate the community. Studies mimicking natural conditions are important and needed to understand possible scenarios beyond laboratory conditions.

The interaction of temperature and pH does not seem to have an impact on the growth and protein content of the microalgae. Moreover, temperature was determined to be a more influential factor than pH. Therefore, to continue looking into the effects of climate change on inshore or coastal aquaculture, the effects of temperature on the growth and chemical composition of marine seaweed *Palmaria mollis* (dulse) were studied. Dulse is widely used in aquaculture to feed herbivore species like abalone, and any changes in biomass and/or chemical composition and growth rates could be crucial for the survival and aquaculture of herbivores.

The chemical composition, such as the protein, total glucose, total nonstructural carbohydrates (TNC), starch content (as a percentage of dry matter), and fatty acids profile, of dulse was analyzed. The results showed that temperature affected growth and protein content mainly. No significant differences in the total glucose, TNC, and starch content were found between treatments. The biomass produced was negatively correlated to higher temperatures. Furthermore, dulse cultured at 17°C was decolored and mushy at the time of harvesting. Protein content was the lowest in dulse cultured at 13°C and higher at 15°C and 17°C, where no difference was found. Fatty acids did not seem to be affected by temperature except in the percentage of total fatty acids of saturated fatty acids and monounsaturated fatty acids. No significant differences in the total fatty acids and fatty acids profile were found between dulse cultured at different temperatures. These results indicate that more protein can be obtained in dulse at higher temperatures; however, biomass obtained at those temperatures was significantly lower. The dulse started to degrade after two weeks cultured under high temperature (17°C), and they might have completely died

if the culture was kept longer. Therefore, higher temperatures are not recommended for the culture of *P. mollis*.

The seaweed *P. mollis* is one of the favorite seaweed species to feed cultured abalone; therefore, the effects of different chemical compositions (mainly in protein content) in *P. mollis* due to rising ocean temperature on the juvenile red abalone growth and chemical composition were studied. Abalone showed higher growth rates when fed *P. mollis* cultured at 17°C, which corresponds to the seaweed with higher protein content, but also was mushy and decomposing, it is possible that the state of the seaweed might have helped with the digestibility and/or bioavailability of nutrients, however, this needs to be explored. Furthermore, these abalone showed the highest percentage of polyunsaturated fatty acids, which could be important for their taste. No other significant difference was observed in their chemical composition or daily food consumption. From these results, higher temperatures due to climate change did not seem to have negative indirect effects on the juvenile red abalone. However, the biomass of *P. mollis* showed to be negatively affected by higher temperatures, and if the biomass of *P mollis* is affected to a point of the extinction of wild populations, higher temperatures would have an indirect negative effect on abalone, since one of their main diets would no longer be available unless a constant production under controlled conditions is obtained. Furthermore, the production of *P. mollis* should be under low temperatures to obtain optimal production.

This study is only a small part of the big picture in the coastal aquaculture industry. More studies are needed to have a deeper understanding of possible scenarios that farmers around the world will have to handle. For example, the effects of temperature on primary producers have been shown to be species-specific; therefore, it is important to understand the effects for the different species of interest. Study of the amino acids profile for species of interest is important as well, protein is an expensive dietary component not only in abalone production but in all other farmed species, and in addition to the level of crude protein, the optimal level of amino acids in formulated diets is a key factor to reduce feed cost and

increase production. Also, it is important to look into more dramatic temperature changes, as well as the interaction of temperature with other factors that are shifting as a result of climate change and other conditions in the ocean and coastal areas, such as salinity, nutrient levels, light, among others.