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The Impact of Temperature on the Nutritional Composition of Dulse (Devaleraea mollis) and its Influence on Juvenile Red Abalone (Haliotis rufescens)

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# The Impact of Temperature on the Nutritional Composition of Dulse (*Devaleraea mollis*) and its Influence on Juvenile Red Abalone (*Haliotis rufescens*)

By

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## Abstract

The world is experiencing changes in weather patterns that are affecting not only ecosystems but agricultural systems and bringing uncertainty to food production. The research continues to grow on the impacts of climate change and developing solutions to mitigate its harmful effects on our industrial food systems is imperative for food security. Humans rely on a global food system spanning vegetation and terrestrial animals, with increasing growth in aquaculture. Understanding how changes in temperature and increases in carbon dioxide change our foods allows for mitigation and regulations to be implemented. It is well documented in agriculture how plants adapt to climate change and its effect on the nutrition of produce. With the growth of the seaweed farm sector, it is essential to understand how increasing temperatures change seaweeds and how these changes impact natural ecosystems. Changes in primary producers such as seaweed can reverberate throughout the food chain, impacting higher trophic levels, inducing stress, and degrading ecosystem health.

To determine how the changes in nutritional composition that could result from climate change affect the growth of herbivorous animals, the direct effects of temperature on seaweeds needs to be understood. This project aimed to determine the changes in protein and carbohydrate concentration in a primary producer in varying temperature ranges and its influence on the growth of an herbivorous animal. The seaweed dulse, *Devaleraea mollis,* was cultured at three different temperatures (13°C, 15°C and 17°C) which was fed to juvenile red abalone (*Haliotis rufescens*) of two different size classes (small 15.6±1.76mm, and medium 42.86±4.65mm), and maintained in ambient seawater separate from the culture system.

For the small size class, a total of 288 abalone were divided into 24 rearing troughs, (trough = experimental unit) with twelve abalone per trough, and randomly assigned to a diet treatment group. The medium size class totaled 63 abalone divided into 21 rearing troughs, with three abalone per trough, and randomly assigned a diet treatment group. Initial and final length and weight measurements were taken on the abalone and used to evaluate the condition factor (CF) and change in body weight ratio (BWR) as an indication of overall health. Seaweed samples were also collected and sent to the UC Davis Analytical Laboratory for nutritional analysis. Significant pairwise tests on linear mixed effects regressions on CF, BWR of the small class abalone, and protein and total nitrogen percentage in the dulse were found. The results showed condition factor significantly differed between the small size class of abalone fed the 13°C to the 15°C and 17°C cultured dulse, and change in body weight ratio showed significant differences between the small class fed 13°C and 17°C cultured dulse. These results indicate that juvenile red abalone in the small size class fed the 17°C cultured dulse had a significantly better physiological status evaluated by their condition factor and change in body weight ratio than other diets. The 17°C cultured dulse also had significantly higher protein percentages than the 13°C and 15°C cultures. Linear mixed models for the carbohydrate analysis (total glucose, starch and total non-structural carbohydrates) showed no differences between the treatment groups. In conclusion, our study indicates that temperature may affect protein synthesis in dulse, which could influence the growth of abalone. Further research to extrapolate the acclimation period of dulse grown with excess nutrients in different temperatures is needed to fully understand its influence on abalone.

Keywords: Abalone, Dulse, Seaweed Nutrition, Temperature Effects, Aquaculture

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## Introduction: Climate Change and Agriculture

Climate change, through changing temperature and weather patterns, causes significant changes in crop yields, soil composition, and plant growth cycles, which in turn impacts agriculture in numerous ways. According to the Intergovernmental Panel on Climate Change (IPCC), global warming has led to an increase in heatwaves, droughts, and extreme precipitation events (IPCC, 2022). To adapt to these challenges, new technologies are being developed and research related to the effects of climate change on specific crops is ongoing (Drebenstedt et al., 2023; Farooq et al., 2023; Kim & Mendelsohn, 2023). Large changes in rainfall patterns lead to water stress affecting plant growth rates and losses overall (FAO, 2022). The stressors plants may experience under climate change conditions not only affect crop yields but also lead to molecular, biochemical, and physiological changes. Drought conditions often lead to decreased photosynthetic activity, causing less available energy or generation of toxic metabolites that could lead to plant death (Ahuja et al., 2010). Disruption of protein and carbohydrate distribution has also been observed in vital crops like wheat in drought conditions (Winning et al., 2008). Commonly, environmental changes modify plant proteins through protein hydrolysis or oxidative inhibition of protein synthesis (Nemati et al., 2018). Carbon dioxide ($CO_2$) is tied to plant productivity and as levels are increasing in our atmosphere, plant nutrient concentrations are changing (Dietterich et al., 2015). Elevated levels of $PCO_2$ threaten human nutrition when leguminous crops have decreased levels of protein and undergo ‘carbohydrate dilution’ that dilutes plant nutritional components (Myers et al., 2014). Essential elements such as zinc and iron have also had significant decreases when grown with increased levels of $PCO_2$ (Myers et al., 2014). Greenhouse gasses, like $CO_2$, trap heat from the sun leading to an increase in global temperatures (Collins et al., 2007). The rising temperatures impact terrestrial ecosystems and agricultural land with an even greater impact on oceans. The specific heat capacity of water allows for significant absorption of the excess heat that leads to a range of impacts (IPCC, 2022). Although extensive research has been done regarding elevated temperature effects on terrestrial systems, there is less known about how rising ocean temperatures could affect the growing seafood industry.

## Aquaculture Significance

As human populations continue to develop and grow (Gu et al., 2021), the need for protein sources produced sustainably is necessary. The demand for aquaculture is becoming increasingly important as wild fisheries become more restricted with regulation or simply decline due to overfishing (Roheim et al., 2018). Agriculturally, seafood is the fastest-growing market, increasing by 5% annually (FAO, 2020). Diversifying the aquaculture sector, by growing fish, mussels, crabs, oysters, and seaweeds creates more opportunities for food options and can improve ecosystem health (Metian et al., 2020). While aquaculture continues to grow, understanding how farms react to climate changes can alleviate large production losses and lead to the mitigation of potential environmental stressors (Barange et al., 2015). Many aquaculture species are shelled organisms that rely on optimal water chemistry conditions for proper growth and growth is known to be directly impacted by ocean acidification, or the acidification of seawater due to increased absorption of $CO_2$ (Fernández et al., 2019). Ocean acidification can also hinder metabolic functions by affecting the uptake pathways of nutrients and minerals needed. Acidification can have negative effects on reproduction by inhibiting gonad growth in many aquatic animals (Dworjanyn & Byrne, 2018). While aquaculture grows in popularity, it is imperative to develop sustainable aquaculture approaches by developing systems that have lower carbon footprints, use renewable materials and energy when possible, and evaluate the trade-offs of aquatic food production. Understanding climate change impacts and mitigating these changes will propel the aquaculture system forward with sustainability at the forefront.

## Seafood Nutrition

Along with an increase in seafood production, awareness of the benefits of consuming seafood in a regular diet grows. Globalization allows for more locally-grown species to be transported through the world and the popularity of certain nutritional seafood products can increase the demand for aquaculture (Belton et al., 2020). Healthy doses of n-3 polyunsaturated fats (PUFAs) including docosahexaenoic acid (DHA) and eicosatetraenoic acid (EPA), vitamins D and B12, selenium, and iodine are often attributed to fish consumption, although many of these nutrients fish obtain through their diets (Ebm et al., 2021). There has also been a growing interest in seaweed consumption in the Western world (Young et al., 2022), leading to an increased interest in seaweed farming. Seaweeds are rich in essential vitamins and minerals and can be farmed efficiently, with limited resources (Sanjeewa et al., 2018). Research on nutrition and essential vitamin and mineral content is continually expanding and the ability to provide these nutrients through aquaculture sustainably in the face of a changing climate is essential for ecosystem protection (Roheim et al., 2018). One efficient method for growing seaweeds and invertebrates, such as abalone and mussels, is through the use of integrated multi-trophic aquaculture (IMTA). Farming with IMTA combats ocean acidification for shelled organisms such as oysters, mussels, and abalone. Growing these organisms in pens located near seaweed farms buffers the pH of the surrounding water because seaweed can absorb $CO_2$ which benefits shell growth. IMTA also provides essential nutrient exchange between species as seaweeds. benefits from organic metabolic waste from the mollusks (Demetropoulos & Langdon, 2004; Fernández et al., 2019; Rosen et al., 2000).

## Seaweed Nutrition

Seaweed cultivation is growing rapidly, with an estimated growth rate of 9.1% over the next five years and was valued at 10.1 billion dollars in 2022 (Grand View Research on Global Commercial Seaweed Markets, 2020). Seaweeds are considered a functional ingredient (Peñalver et al., 2020) because of their ample amounts of proteins, carbohydrates, minerals, and vitamins useful for human diets (Hardjani et al., 2017; Polat et al., 2021). Red seaweeds (class *Rhodophyceae*) have the highest protein content compared to other commonly consumed brown (class *Phaeophyceae*) or green seaweeds (class *Chlorophyceae*), except the commonly consumed Wakame (*Undaria pinnatifida)* which can produce up to 24% protein content (Fleurence et al., 2018). In contrast, the red seaweed *Palmaria palmata* has been cultured with a crude protein content of up to 35% (Morgan et al., 1980). In addition to high protein content, all essential amino acids are found in seaweeds throughout the seasons with the most abundant amino acids being aspartic and glutamic acids (Dawczynski et al., 2007). While the digestibility of seaweed protein trapped in the cellular matrix is undetermined, the bioavailability can be increased through fermentation (Galland-Irmouli et al., 1999).

Carbohydrate content also plays an important role in the nutritional benefits of seaweeds. Carbohydrates are classified into monosaccharides, disaccharides, and polysaccharides with seaweeds presenting richness in polysaccharides due to their cell wall composition (Stiger-Pouvreau et al., 2016). Although this leads to indigestibility in humans, some studies have found the presence of several seaweed carbohydrate enzymes in the human microbiota (Cantarel et al., 2012; Cian et al., 2015). The mineral content of seaweeds changes due to environmental factors but largely includes potassium, phosphorus, magnesium, sodium, and calcium and include the micronutrients iodine, zinc, copper, iron, and manganese (Fleurence et al., 2012). These minerals are important to human metabolic processes proving essential for optimal nutrition (Mišurcová et al., 2011). Seaweeds also directly use sunlight for vitamin formation, specifically vitamins A, B, C, and E (MacArtain et al., 2008), and can be a non-animal source of vitamin B12 (Watanabe et al., 2014).

## Mollusk Nutrition

Aquaculture of marine invertebrates is a growing sector, reaching 8.3 million tonnes in 2022 (FAO, 2022). Aquaculture is less nutrient intensive than finfish aquaculture while providing similar nutritional benefits (Ghafoor et al., 2020; Prato et al., 2019). The majority of molluscan aquaculture produces clams, oysters, mussels, scallops, and abalone (Guo, 2009). The Mediterranean diet is often referred to as a balanced healthy diet that includes regular consumption of shellfish (Kios et al., 2023, p. 10). A commonly consumed mussel, *Mytilus galloprovincialis*, has high protein content (17.4-19.9g /100g) while also containing beneficial PUFAs biochemically available for human consumption. There are also beneficial levels of vitamins A, D, and E (Bulgaria. et al., 2020; Panayotova, 2020). Abalone (*Haliotis spp.*) are another popularly consumed mollusk, found throughout the world. China boasts over 300 operating abalone farms (Cook, 2014). In California, abalone have been harvested for thousands of years by Indigenous tribes (Vellanoweth et al., 2006) and have been an important wild fishery for California commercial and recreational divers until its complete closure in 2018 which was valued at $44 million (Reid et al., 2016). All seven Californian endemic species (*H. rufescens, H. corrugate, H.*

*fulgens, H. cracherodii, H. sorenseni, H. kamtschatkana, H. kamtschatkana assimilis),* are currently all listed as endangered or critically endangered are set to be reevaluated in 2026 (IUCN, 2022). Abalone are a great candidate for IMTA where they can be grown alongside their seaweed food source allowing for more successful growth. In multiple studies, abalone grew significantly larger when grown in a co-culture with dulse, *Devaleraea mollis*, which naturally buffers pH levels (Fang et al., 2018; Hamilton et al., 2022).

## Temperature Effects

Recent models indicate weather patterns due to climate change bring temperature changes as well as changes in water movement. In the Eastern Pacific, El Niño warming events are increasing in frequency and duration, causing large warm anomalies in the ocean (Cai et al., 2018). These patterns affect pelagic fish migrations altering plankton distributions and changes in spawning recorded in Pacific sardine (*Sardinops sagax*) and Pacific hike (*Merluccius productus*) (Auth et al., 2018). Primary producers, organisms at the base of the food web, like microalgae and seaweeds, (Pinkerton et al., 2021) are also affected by increases in temperature leading to change in the rates of seaweed enzymatic activity and affecting the rate of nutrient uptake in active transport (Roleda & Hurd, 2019). Throughout seasons, variations in amino acid profiles can occur changing the distribution of available enzymes to the organism (Galland-Irmouli et al., 1999). Although some species are adapted to seasonal variation (Morgan et al., 1980), long-term stress diminishes cellular metabolism and degrades organism health. Under short-term thermal stress, a higher amount of reactive oxygen species (ROS) are present (Kumar et al., 2020). In response to these reactive molecules, seaweeds begin polymerizing available sugars and storing starches (Cian et al., 2015). Temperature also affects protein synthesis and enzymatic activity by influencing the rate of reactions and nutrient uptake (Harnedy & FitzGerald, 2011). Seaweed cell walls represent a large portion of polysaccharides, composing at least 50% of the dry weight. Furthermore, the cell wall is important in maintaining an osmotic balance with the surrounding environment and if the temperature is altered, the composition can be compromised (Stiger-Pouvreau et al., 2016). Temperature also plays a role in osmolarity which is regulated by the cell wall (Torres et al., 2021). Additional stress from hypo or hypersaline environments can change internal cellular conditions harming turgor but also creating an accumulation of stress proteins to protect and renature any damages (Stiger-Pouvreau et al., 2016).

## Abalone and Temperature

Abalone farming is popular throughout the world, with many farms relying on coastal waters for their systems. Sensitive to their environment, abalone show reduced growth rates when thermal stress is applied, which also makes them more susceptible to disease (Morash & Alter, 2016). Metabolic increases require more energy and more oxygen to maintain physiological fitness (Takami et al., 2008). Abalone are ectotherms with temperature influencing much of their metabolic processes with increasing temperature increasing metabolic rate but only to a critical thermal maximum, after which survival is limited (Frederich & Pörtner, 2000). Moreover, abalone prefer different temperatures throughout their developmental stages. For example, the red abalone, *H. rufescens*, was found to prefer warmer temperatures until reaching a shell length (SL) of 30mm and then adjusted their temperature preference to lower temperatures as SL increased (Steinarsson & Imsland, 2003). For aquaculture farms, it is important to understand the shift in metabolic rates that results from changes in temperature and that more energy is required once stressful levels of temperature are reached. With thermal stress, the animal's fuel source from carbohydrates to protein can shift (Somero, 2002), possibly altering the taste of the abalone (Duan et al., 2020).

## Conclusion

As climate change continues to influence ecosystems, understanding how these fluctuations in environmental conditions impact marine ecosystems is essential for protecting them. The future of human food security lies in mitigation against thermal stress on aquaculture systems and shedding light on the adaptations natural communities exhibit during these warming events. The growth in aquaculture continues and investing in sustainable systems that protect aquaculture stocks and create hardy breeding populations can provide farmers security in their production.  Furthermore, abalone aquaculture in California is an important factor in protecting these endangered animals, and creating a market to purchase aquacultured abalone protects against poaching. The ability to successfully grow healthy stocks ensures the continued conservation efforts for endangered species. Growing abalone along with protecting other important aquaculture species against climate change in aquaculture alleviates the pressure on wild fisheries that are also experiencing these climatic changes. Further research into how temperature changes the nutritional content of primary producers is necessary to predict what changes wild ecosystems might endure. Moreover, studying the indirect effects of the nutritional changes on herbivorous primary consumers provides farmers with efficient feeds that allow animals to grow healthy and sustainably.

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## Chapter 2: The Impact of Temperature on the Nutritional Composition of Dulse (*Devaleraea mollis*) and its Influence on Juvenile Red Abalone (*Haliotis rufescens*)

### Introduction

Changing ocean conditions, particularly ocean warming, have a significant impact on algal primary producers, which are vital to maintaining healthy ocean ecosystems and play a crucial role in the food web (Gao et al., 2012). Ocean warming events, mainly attributed to anthropogenic greenhouse gas concentrations, are becoming more frequent and extended in duration (Cheng et al., 2022). Record-breaking temperatures were recorded along the Sonoma Coastline in 2014 with nearshore waters reaching a new record of 17.4°C (Rogers-Bennett, L., & Catton, C., 2019). Warming conditions degrade food web components through losses of nutrient density in primary producers (Gao et al., 2012) and shifts in metabolic processes across several food web hierarchical levels of organization (Brown et al., 2004). Thus, an understanding of the primary producer's physiological response to changing ocean temperatures can inform how community structure will be influenced (Takami et al., 2008).

The North Eastern Pacific Ocean has experienced two significant marine heatwaves in the past decade with long-lasting ecological impacts (Scannell et al., 2020). These El Niño warming events in the NE Pacific, also known as the “blob”, contributed to warm anomalies in the ocean (Cai et al., 2018) and continue to influence ocean conditions impacting ecosystems and weather systems (Cornwall, 2019). In 2014, large losses in chlorophyll levels were observed in phytoplankton communities in response to the “blob”, which displaced top trophic-level predators that rely on abundant primary producer populations to maintain food webs (Bond et al., 2015; Whitney, 2015). Unusual changes in ocean temperatures continue to be monitored by satellite imagery (Su et al., 2022), and coupling this technology with knowledge of how temperature change impacts species allows for mitigation against climate change in coastal aquaculture farm food systems. Similar ocean warming events collapsed scallop fisheries in Western Australia (Caputi et al., 2016), furthering the importance of understanding what causes these heat waves to happen and how they will impact important fishery species.

Seaweed primary producers, when exposed to increases in ocean temperature, experience altered rates of enzymatic activity and nutrient uptake (Roleda & Hurd, 2019). A multi-year study observing ocean warming throughout an ecosystem recorded less nutrient availability causing a decrease in photosynthetic rates of the brown seaweed, *Fucus vesiculosus*, with the largest responses occurring in the summer (Wahl et al., 2020). A brown seaweed, *Sargassum oligocystum*, was studied over three distinct seasons and was found to vary in protein and lipids (Praiboon et al., 2018) while green seaweeds post-monsoon season, had a lower amino acid content (Vinuganesh et al., 2022). Prolonged exposure to thermal stress can have adverse effects on the health of seaweeds, even though some species have adaptations for seasonal variations. When faced with short-term thermal stress, seaweeds respond by generating reactive oxygen species that stimulate the synthesis of sugars and the storage of starches (Cian et al., 2015). However, this stress also compromises the integrity of seaweed cell walls, which play a crucial role in maintaining osmotic balance with the surrounding environment (Stiger-Pouvreau et al., 2016). Seaweeds living in hypo- or hypersaline environments experience additional stress due to fluctuations in internal cellular conditions, which affect turgor and trigger the accumulation of stress proteins to safeguard against cellular or subcellular damage (Kumar et al., 2020).

In addition to the direct effect of increased temperature on primary producers, altered ocean temperature influences coastal habitats like the rocky shoreline and impacts the recruitment of larval invertebrates affecting the community dynamic of animals and algae alike (Hawkins et al., 2008; Kavousi et al., 2022). Increases in ocean temperatures around the globe have impacted many invertebrate species. In Western Australia, after an abnormal warming event, a reduction of 99% recruitment of the Roei (*H.* roei) abalone was observed along with significant decreases in other fishery species such as king (*Penaeeus latisulcatus*) and tiger prawns (*P. esculentus*) and swimmer crabs (*Portunus armatus*) (Caputi et al., 2016). Invertebrate larvae are particularly affected by warming temperatures, with decreased survival observed in gastropods (Davis et al., 2013), smaller larvae and decreased survival observed in bivalves (Talmage & Gobler, 2011), and increased larval and embryonic mortality in echinoderms (Byrne et al., 2009, 2010). Similarly, in laboratory experiments, abalone larvae display increased tissue anomalies and declines in swimming abilities under increased temperature (Kavousi et al., 2022).

Abalone have been a popular recreational fishery for decades, and the farming of abalone dates back to the 1970s (Oakes & Ponte, 1996), initiated by the decline in wild populations associated with ecological warming events, sea urchin population growth, and overfishing (Cook, 2014). As ocean temperatures rise, abalone are more susceptible to the spread of diseases like withering foot syndrome, *Candidatus* Xenohaliotis californiensis. Withering foot syndrome causes weight loss, weakness, and foot atrophy that ultimately leads to high mortality that has further accelerated the loss of abalone in the wild and captivity (Moore et al., 2002; Di et al., 2016; Crosson & Friedman, 2018; Frederick et al., 2022). Red abalone exhibit higher metabolic rates when under heat stress, shortening the duration of time that gut enzymes can interact with food overall, affecting their health (Frederick et al., 2022). Additionally, red abalone exhibit poor health above 16°C indicated by reduced gamete production and growth (Rogers-Bennett et al., 2010). Red abalone is essential to coastal California’s ecosystems and aquaculture industry (Woolford, 2009). Understanding the threat to aquaculture by increased temperatures is imperative for abalone's continued farming and conservation efforts.

The relationship between abalone and macroalgae represents a consumer-resource interaction in a food web. There can be direct effects of the environmental change on the resource that result in changes in the consumer, which are referred to as indirect effects of environmental change (Brose et al., 2012). Warming can affect species positively or negatively and trigger a cascade on populations of lower or higher trophic levels. The cascade of temperature influencing the growth of a resource can be observed to increase the growth of the consumer although this can be species-specific (Rall et al., 2010). The trend of ecosystem warming has led to many indirect effects throughout the food web including increases in metabolism in consumers (Shurin et al., 2012).

Knowing how temperature changes influence growth of an organism through indirect effects on prey allows for a better understanding of these cascades throughout abalone ecosystems and allows the aquaculture industry to mitigate potential climate change conditions in their farms. My M.S. thesis was designed to evaluate growth in two size classes of juvenile red abalone, cultured under ambient water conditions fed dulse, *D. mollis,* cultured different thermal regimes. The three different temperature treatments for the dulse cultures were selected based on current and projected ocean temperatures and preliminary studies showing changes in dulse nutritional density (Mata, 2022, *PhD Dissertation*) that may influence the growth of juvenile abalone. The research in my M.S. provides aquaculture farms with insight into diet and growth optimization in abalone farming.

## Materials and Methods

### *Experimental Design*

Juvenile red abalone of two size classes (small and medium) were fed dulse grown at three different temperatures (13°C, 15°C, 17°C) from August - November 2022 at the UC Davis Bodega Marine Laboratory (BML) (Bodega Bay, CA). Diet treatments were applied to 45 random troughs with juvenile abalone (n=8 small, n=7 medium)) for 92 days. Each trough received ambient seawater and was fed three times a week *ad libitum.* Abalone length and weight measurements were taken at the beginning (day=1) and end of the experiment (day=92). Samples of dulse were dried from each feeding and were analyzed by the UC Davis Analytical Laboratory, Davis, CA.

### *Seaweed Cultures*

*Devaleraea mollis* was chosen for diet tumble cultures because of its use in California aquaculture, ease of growth through fragmentation, and high palatability to abalone (Naidoo et al., 2006; Mulvaney, 2016; Grote, 2019). Dulse cultures were grown at the experimental temperature for at least 21 days before being used in the experimental diet. The dulse culture tanks, consisting of two culturing tanks per temperature treatment were separate systems from the abalone cultures. Every 21 days, a new batch of dulse was placed in the upper culture tank to adjust to the selected temperature treatment. The stocking density of the dulse cultures increased over the duration of the experiment as the abalone increased in feeding amounts (500-1200g/184-L tank). Each temperature culture was set up identically, composed of three 184-L fiberglass tanks (two culture tanks and one sump) insulated and stacked vertically, a head tank, a UV sterilizer (Pentair Smart UV 40 Watts, Minneapolis, MN, USA), a chiller (DBA-075 1/10HP, JBJ Arctica, Missouri, USA), LED lighting (Aqueon OptiBright LED Light, 850 Lumens, 21 umol/s, Franklin, WI, USA) and a pump (EcoPlus Eco 1056 Water Pump, 1000 GPH, Hawthorne, BC, CAN) totaling to a 560-L recirculating aquaculture system. Water quality (API water test colorimetry kit, Los Angeles, CA, USA) was measured weekly while performing a 35% water change on the seaweed cultures using incoming seawater pumped from the BML ocean water intake. The seaweed tank cultures were labeled cold (13°C), medium (15°C) and warm (17°C). The water temperature of the cultures was monitored every six hours for the duration of the experiment by a submerged temperature logger (HOBO Tidbits v2, Onset, Bourne, MA, USA).  Seaweed culture tanks were also scrubbed by hand every three weeks during water changes to remove epiphytic growth. By the completion of the study, five batches of seaweed were cultured and subsampled to analyze for significant differences between the protein and carbohydrate levels of the dulse cultures.

### *Nutritional Analysis*

The culturing timeline of the study resulted in five different batches of dulse throughout the experiment. Seaweed samples (3.5g wet weight) were collected from both the adjustment and feeding tanks three times a week, dried in a 60°C oven, and stored in an airtight container. Each batch consisted of the three designated temperatures with twelve samples from weeks 3-5 sent to the UC Davis Analytical Lab except batches #1 and #2 which only had nine samples, three from each temperature treatment from weeks that had five. Due to the large volume of samples collected and constraints on financial resources, this subset of samples was chosen as representative of the dulse nutrition on particular feeding days and used to evaluate the progression of nutrients over the culture time. Protein was analyzed by thermal conductivity and infrared (IR) detection (AOAC Official Method 972.43) and total crude protein (AOAC Official Method 990.03) was calculated from the nitrogen content of the seaweed sample with the protein factor of 6.25. Free sugars glucose, fructose, and sucrose were analyzed with high-performance liquid chromatography (HPLC) with mass selective detection. Total glucose was calculated by enzymatic hydrolyzation at 55 °C with an aminoglycoside for 12 hours and then analyzed by HPLC with mass selective detection. Total non-structural carbohydrates (TNC) were analyzed by the total glucose, fructose, and sucrose. Starch was calculated by subtracting the free glucose multiplied by 0.9 from the total glucose (Smith, 1969).

### *Abalone Culture*

Cultured red abalone are often used as a proxy for wild abalone experimentation since they are commonly farmed in California, easy to obtain and much of the reproduction and nutrition research can be applied to other species (Peters et al., 2021). Abalone farms rely heavily on efficient diets that range from pellets to live macroalgae because abalone are slow-growing, requiring a minimum of 24 months to reach the smallest possible market size (80mm) (Steinarsson & Imsland, 2003; Heasman & Savva, 2007).

Small juvenile red abalone (n=288) were shipped overnight to the BML from The Cultured Abalone Farm (Goleta, CA, USA) in July 2022 and the medium-size class (n=63) were shipped from the same supplier, and maintained in the laboratory since July 2021. All abalone were tagged (Floy Tags, FTF-69 size ⅛"x ¼", Seattle, WA), measured for shell length by calipers, weighed, and randomly distributed into troughs across three treatment groups with a random number generator (n = 8 replicates for small abalone and n= 7 replicates for medium abalone). Weights and lengths were only measured at the beginning (day=1) and end of the experiment (day=92) to calculate the change in growth. The sampling events were limited to an initial and final measurement to alleviate stress on the juvenile animals and to investigate whether the diets had different effects on growth. The replicate troughs were housed inside cutlery trays (Rubbermaid®, 21.5”x11.5”x3.75”), replicating industrial abalone rearing facility methods, with each trough holding 3.7 L of seawater at a flow rate of ~0.5 L/min of ambient seawater. Each trough was lined with AstroTurf® and clear acrylic lids to deter escapement.

Condition factor (CF) is an index reflecting the relationship between the weight (W) of abalone per unit of SL and provides information on the relative condition of the abalone compared to others of its size (Britz et al., 1996). The CF was calculated using the following formula:

$$CF = W/SL^{\wedge}2.99 * 5575$$

Where W was wet weight (g) and SL was shell length (mm). To calculate the change in body weight ratio (BWR), first, the change in weights ($W_c$) and lengths ($L_c$) were calculated. For the change in weights, the initial weight ($W_i$) was subtracted from the final weight ($W_f$). For the length change the initial length ($L_i$) was subtracted from the final length ($L_f$).

$$W_c = W_f - W_i$$

$$L_c = L_f - L_i$$

The change in body weight ratio (BWR) is an index taking the calculated weight change ($W_c$) and dividing it by the calculated length change ($L_c$).

$$BWR = W_c / L_c$$

*Feeding and consumption*

Before the start of the experiment, consumption rates were collected to determine the appropriate mass of dulse to feed per trough. The abalone were fed *ad libitum* with regard to their consumption rates to prevent overfeeding and the waste of seaweed stock. Trough replicates were fed identical amounts at all three feedings (Monday, Wednesday, and Friday), with each size class receiving an appropriate amount with respect to their consumption which increased throughout the experiment. Consumption was measured weekly on the Wednesday feeding day.

To measure consumption, each trough was siphoned with clear tubing into a fine mesh netting (200 µm) into a 2L container. The excess dulse and the netting material were secured with a rubber band and spun in a salad spinner for 50 rotations to remove excess water. Daily food consumption (DFC) was calculated using the following formula:

$$\mathrm{DFC} = F_m - (W_f - W_n)$$

The weight (±0.01 g) of the netting + dulse was measured ($W_f$), the dulse was removed and the netting was weighed again ($W_n$). DFC was calculated by subtracting the difference of $W_n$ from the $W_f$ from the feeding amount given at the Monday feeding ($F_m$). Fecal samples were also collected from the water container of each trough. Fecal matter was pooled by trough for the duration of the experiment to examine the apparent digestibility in a future study. Feeding amounts increased throughout the experiment as the abalone grew and were adjusted accordingly.

### *Statistical Analysis*

All data sets were analyzed in R Studio (Version 2022.12.0+353) and evaluated for normality and homoscedasticity using a Shapiro-Wilk and Breusch-Pagan test respectively (CRAN.R-project.org/packages=performance). The significance for all analyses were set to $P<0.05$. A linear mixed effects regression (LMER) was used for all statistical testing to incorporate variation among day and batch sampled for the protein and carbohydrate analysis or trough for the abalone condition factor model. R was used to investigate these effects using the 'lmer' package

(CRAN.R-project.org/packages=lmer). All pairwise comparisons with Tukey's method were used to determine differences between treatments using the 'emmeans' package (CRAN.R-project.org/package=emmeans). Differences between size classes were not compared due to the uncertainty and differences in age, stocking density husbandry and culturing methods prior to experimentation.

## Results

### *Survival*

Abalone survival was almost 100% from the acclimation period through the end of the experiment for both abalone size classes. While the medium abalone had been acclimated to our system for a year prior to the start of the experiment, medium and small abalone experienced the same handling stress associated with weighing, measuring tagging and distributing across troughs for four weeks prior to experimentation and exhibited 100% survival. During experimentation, the small size class had one mortality in one trough in the 17°C treatment group resulting in a 99% mean survival across all diets. The medium-size class had a plumbing failure in one trough, that resulted in three abalone mortalities from the 13°C treatment group. This replicate was removed from all analyses. All medium size class abalone exhibited 100% mean survival across all diets.

### *Seaweed Cultures*

All three temperatures of the seaweed cultures were statistically different from each other throughout the experiment (ANOVA) with the average temperatures recorded as cold (12.7°C±0.43), medium (14.9°C±0.48), and warm (16.7°C±0.92). Water quality did not differ between the replicate cultures (ANOVA).

### *Protein Analysis*

The protein percentages were 15.6, 16.1, and 17.4 % for the 13°C, 15°C, and 17°C cultures respectively. There were significant differences in the linear mixed effects pairwise comparison (Tukey's HSD) of 13°C to17°C (P=0.005, df=46, SE±0.525 and 15°C to 17°C (P=0.04, df=46, SE±0.525) temperatures, with no statistical difference between the 13°C and 15°C (P=0.72, df=46, SE±0.525). Average nitrogen levels were 2.51, 2.57, and 2.78% in the 13°C, 15°C, and 17°C cultures respectively. Significant differences in the nitrogen levels of 13°C -17°C (P=0.004, df=39.4, SE±0.08) and 15°C -17°C dulse cultures (P=0.03, df=39.4, SE±0.08) were also found (Figure 1). Both linear regression mixed models aimed to examine the effect of temperature on protein or nitrogen percentage while accounting for the variation across different sampling days and batches that were analyzed.

### *Carbohydrate Analysis*

There were no differences in total glucose, TNC, and starch between the seaweed cultures grown at different temperatures. All three measurements were integrated into linear regression mixed models that accounted for variation across different sampling days and batches along with a pairwise comparison using Tukey's HSD analysis (Figure 1). Many free glucose, fructose, and sucrose values returned from the nutritional analysis recorded as <0.2 so a model could not be determined for these values since the potential range of anything less than two has too much variation (Appendix G).

### *Abalone Growth and Condition Factor*

Abalone were measured for initial and final lengths and weights to use in the condition factor analysis (Table 1). Condition factor models included trough as a random effect to control for variation despite the identical design. At the start of the experiment, there were no differences in the condition factor between treatment groups in the small class (P= 0.96 13°C -15°C, P=0.95 13°-17°C, P=0.84 15°C -17°C). At the end of the 13 weeks, *H. rufescens* in the small size class fed the 13°C cultured dulse diet had a lower condition factor when compared to those fed with

15°C and 17°C cultured dulse with differences in condition factors between the 13°C -15°C (P=0.033, df=21, SE±0.06) and 13°C -17°C treatment groups (P=0.015, df=20.9, SE±0.06) (Figure 2). There were no significant differences in the medium-size class in the initial or final CF calculations (Figure 1).

### *Abalone Growth and Change in Body Weight Ratio*

The weight and length measurements were also used in an analysis of the body-weight ratio (Table 1). These calculations used the change in weight and change in length to standardize the growth measurements for differences found in the initial measurements between troughs. Evaluating the body weight ratio with respect to the changes in weights and lengths observed in the abalone treatment groups gives a quantitative comparative analysis between individuals of differing sizes. A linear mixed effect model included trough as a random variable and a Tukey's post hoc all pairwise comparisons test were used to assess changes in body weight ratio across treatments. The small size class showed a significant difference in the change of body weight ratio between the abalone fed the 13°C and 17°C dulse (P=0.005, df=20.7, SE±0.017) (Table 1). In the medium-size class, no significant differences were found for BWR between treatment groups (Table 1).

### *Dulse consumption over time*

Over the 13-week experiment, there were no significant differences in consumption between the treatment groups when examining the total weeks measured for either of the size classes. A linear mixer effects model with trough as a random variable was made for each size class and evaluated with Tukey's post hoc comparison (Figure 2 and Figure 3). No significance was found between temperature treatment groups for the small size class (P= 0.93 df=21 13°C -15°C, P=0.55 df=21 13°C -17C, P=0.76 df=21 15°C -17°C) or the medium (P= 0.66 df=18 13°C -15°C, P=0.83 df=18 13°-17°C, P=0.34 df=18 15°C -17°C).

## Discussion

Warming ocean temperatures, among other factors, are affecting the performance of organisms directly and indirectly through the food web. Optimal temperature ranges for the optimal growth of species through direct experimentation have been determined for some species; however, a gap exists in understanding the indirect effects of temperature on primary and secondary consumers. In this study, we examined how juvenile red abalone, *H. rufescens* reared at ambient ocean temperatures, were impacted by the direct effect of temperature on dulse, *D. mollis*, their food supply. From previous pilot experimentation, we predicted that abalone fed the warm (17°C) cultured dulse would have higher growth during the experiment due to the higher protein content availability in their food. Dulse grown at 17°C, outside the optimal growth range for this macroalgae, would activate the anticipatory cellular stress response leading to elevated protein levels and more nutrient density in the feed. Our results suggest that temperature does lead to an increase in protein levels in dulse and that diet does affect the growth of juvenile abalone. Moreover, the growth in abalone was not due to higher consumption throughout the treatments suggesting the nutrient density of the dulse did impact the growth.

### *Abalone Dulse Consumption*

It is well known that consumption rates can significantly impact the growth of organisms. To ascertain the effects of the diet on juvenile abalone growth and to reduce the confounding factor of increased food intake leading to increased growth, food consumption was measured weekly. Our results showed no difference in consumption between the three treatments fed *ad libitum* over the experiment eliminating the possibility that the growth in the group that fed on 17°C dulse was due to more consumption. All of the abalone were subjected to the same ambient water temperatures evenly distributing to the effect of temperature on metabolic rates (Secor, 2009).

### *Dulse Protein*

The optimal temperature range for dulse is between 12°C -15°C (Stevent et al., 2023), which suggests that the warm culture (17°C) dulse may have experienced suboptimal temperatures and possibly thermal stress, which likely contributed to significantly higher protein and nitrogen when compared to the dulse cultured at 13°C. Seaweeds maintain metabolic processes, including photosynthesis along a temperature response curve with a decline in rates when nearing the upper-temperature threshold (Wahl et al., 2020). The moderate elevation of temperature can up-regulate protein synthesis causing a more efficient use of carbon and increases in growth (Zhang et al., 2022). The protein percentages found in our study for 13°C, 15°C and 17°C cultures (15.6, 16.1, and 17.4%, respectively) are within the range measured at 11-22% protein through the annual seasonal shifts of the related species *Palmaria palmata*. The levels of total nitrogen (2.51, 2.57 and 2.78%, respectively) were at the lower ranges for published values (2.4-5.7%) in seasonal shifts for *P. palmata* (Morgan et al., 1980), which could be due to the low nitrogen availability in the form of ammonia ($NH_4^+$) or nitrate ($NO_3^-$) which were consistently measured below detection in our water quality measurements. Acclimating to a range of temperatures allows for the reallocation of resources necessary for survival and reproductive success (Cian et al., 2015; Galland-Irmouli et al., 1999; Kumar et al., 2020; Roleda & Hurd, 2019). Seasonally, $NO_3^-$ and $NH_4^+$ range as low as 5 uM (Boyd & Hurd, 2009), and might have been undetectable with the testing kit. Although the water was changed weekly, there were no recordable amounts of $NO_3^-$ or $NH_4^+$ in the water samples, indicating that the dulse might have been nutrient-limited (Harrison

& Hurd, 2001). Both elements are necessary for growth in seaweeds (Roleda & Hurd, 2019). Furthermore, all the dulse cultures experienced the same water quality parameters, yet higher protein levels were found in the warm culture, suggesting that short-term heat stress activated the cellular stress response by increasing protein concentrations. Altering temperature in dulse culture tanks could lead to more nutrient-dense dulse and increase abalone growth rates. Further research is necessary to determine when the dulse could no longer sustain the short-term heat stress. Although protein in the 17°C culture was significantly higher than the 15°C and 13°C, the mean protein percentage by batch decreased over time over the duration of the experiment (Figure 2). This decreasing trend of protein from the starting cultures indicates that the temperature is likely exceeding thermal tolerances.

### *Dulse Carbohydrates*

Seaweeds use starch to store excess energy as carbohydrates (Costa et al., 2022) and when evaluating our cultures, the possible deficiency in $NO_3^-$ and $NH_4^+$ could impact the seaweeds' ability to store excess nutrients since they were unavailable. Carbohydrates are not as necessary for protecting seaweeds from heat stress, with structure and energy storage as their main function. When experiencing heat stress, the seaweed was less inclined to store excess nutrients and instead build protective proteins (Garcia-Jimenez et al., 2020). In the current study, we found no differences in carbohydrates in dulse cultured at three different temperatures indicating that the acclimation to the temperatures mostly affected the protein percentages instead of carbohydrates. The diversity of carbohydrates found in seaweed species and their common storage as recalcitrant polysaccharides makes it especially difficult for analysis. These structural components are necessary for seaweed integrity and are most resilient to changes in temperature (Tagliapietra et al., 2023). Furthermore, red seaweeds have an abundance of anhydro-galactose and glucose and the biochemical tests to analyze these molecules are limited (van Hal et al., 2014). Changes that might have occurred in our cultures could be undetectable or non-existent as the seaweed did not have excess nutrients to store as starch and allocated energy to protective proteins under stressful conditions. A build-up of proteins, as observed, could also indicate the seaweed was preparing enzymes to catabolize some structural carbohydrates (Bäumgen et al., 2021) but further analysis would be necessary.

### *Abalone Condition Factor*

Evaluating the condition factor of an abalone allows for an overall examination of its health status taking into account measured shell length and body weight when compared to an ideal growth curve across life stages. An abalone growing within the calculated healthy range will have a score close to one (Britz et al., 1996). It is expected that a cultured juvenile abalone should grow optimally, with respect to the established ratio, expending most of its energy on growth without stressors they would experience in a natural environment, such as food limitation or predation (Fraser & Rogers, 2007). The small abalone size class had a final CF of 0.69, 0.88, 0.94 for 13°C, 15°C, and 17°C respectively. These CF are similar to those reported for green lip abalone (0.74 to 0.77) in a 12-week diet study with animals reared at 14°C and were higher than the CF reported for the abalone reared at 22°C (0.61-0.63) measured on similarly sized juveniles, 23.31±0.03mm versus 15.8±1.63mm in this study (Stone et al., 2014). In the small-size red abalone, statistically significant increases in CF changes, from the initial to the final were recorded in the abalone fed the 15°C and 17°C cultured dulse. Juvenile abalone need a balanced diet consisting of protein, carbohydrates, and lipids (Naidoo et al., 2006) that changes rapidly during their younger life stages that is difficult to replicate in an all-encompassing artificial diet (Bautista-Teruel & Millamena, 1999). The smaller condition factor recorded indicates the cold diet was not sufficient in supplying the necessary nutrients to continue growth. A similar green lip abalone size class was also evaluated akin to the medium size class, 56.64±0.08mm versus 43.48±3.79 in this study. Final CF values were higher in the medium red abalone size class, 0.94, 0.97, 0.92, for 13°C, 15°C and 17°C respectively, in comparison to a range of 0.71-0.78 for the green lip abalone (Stone et al., 2014). The medium-size class showed smaller CF rates in contrast to studies examining *Haliotis midae* fed macroalgae diets consisting of *Macrocystis pyrifera* and *Ecklonia maxima* (Fleischman et al., 2012). *M. pyrifera* has produced greater growth in juvenile white abalone when compared to *Palmaria mollis* (McCormick et al., 2016), which could have influenced larger CF scores in the study of *H. midae*. A study that took measurements of abalone of varying age classes to model predicted lengths over time in years found that the growth curves become less steep as time progresses indicating abalone growth slows as they age (Rogers-Bennett et al., 2007).

### *Abalone Body Weight Ratio*

Using a metric such as body weight ratio to quantify growth in invertebrates allows for an estimation of how available energy is being stored in relation to their shell and body mass (Eklöf et al., 2017). Allocating energy into shell growth in juvenile abalone is advantageous for protection from predation but evaluating just a single metric, such as shell length, is incomplete in terms of understanding how the abalone are utilizing nutrients from their diet (Griffiths, A.M., & Gosselin, L.A., 2008), necessitating the evaluation of mollusks with a body weight ratio. When compared to juvenile green lip abalone fed five different macroalgae diets varying in nitrogen content, the abalone fed the diet with nitrogen content similar to our dulse cultures had higher body weight ratios. Interestingly, the study also found that nitrogen-enriched *Ulva rigida* cultures did not produce significant differences in growth rates of green abalone (Taylor, M., & Tsvetnenko, E. 2004). Another 13-week diet study found low growth rates in juvenile red abalone (SL= <5mm) fed a strictly *Ulva ohnoi* diet with the best growth rates from a mixed-macroalgae diet consisting of *M. pyrifera, U. ohnoi and Navicula incerta* (Cicala et al., 2023). Further analysis on the body composition of juvenile abalone would be useful for understanding how the protein content among the three diets in this study was utilized during active growth.

Studies examining juvenile abalone tissue from those fed dulse diets showed high protein and nitrogen content concentrations compared to formulated feeds or collected bull kelp, *Neocystis luetkeana* (Wulffson, 2020). The protein analysis of dulse cultured at 17°C indicated higher protein content that could produce more effective nitrogen uptake in the abalone, resulting in a higher growth measured by body weight ratio. Other abalone feeding experiments found that increased protein intake leads to higher growth due to protein availability in the feed that facilitates growth (Fraser & Rogers, 2007). Creating a more nutrient-dense feed that increases animal growth is sought after in the aquaculture industry, especially for those culturing slow-growing organisms. Mai et al., (1995) reported ideal lipids content for *H. tuberculata* and *H. discus hannai* to be at 3.11% and 3.11-7.09% respectively for weight gain while observing soft-body protein decrease as the lipid content of the diets increased. Lipids are sometimes substituted for protein in feed to decrease aquaculture feed costs but can have negative effects (Jannathula et al., 2019). Creating a high protein diet with a palatable food source such as dulse through thermal manipulation of the cultures could be advantageous for farmers- reducing supply costs and increasing abalone growth. Similar experiments have cultured dulse with higher protein content allowing for more efficient use of resources and better growth in juvenile abalone. Rosen et al. (2000) altered light illumination and water exchanges in *D. mollis* to determine ideal photoperiods and water exchange balance for culturing the most nutrient-rich dulse. Rosen et al. (2000) found that the most protein-dense dulse was cultured in tanks with the largest amount of water exchange and highest supplemental photoperiods (35 volume changes $d^{-1}$, 24h $d^{-1}$). Similar studies by Demetropoulos & Langdon, (2004) identified optimal nutritional additions of nitrogen, phosphorus, and trace elements to improve yield, producing dulse with a protein content of up to 35% dry weight. There is very little literature examining solely the effects of temperature on dulse. Combining this knowledge in an aquaculture setting allows farmers to potentially reduce costs by growing more nutrient-rich dulse resulting in more sustainably produced and healthier abalone.

## Conclusion

In summary, this study supports that juvenile red abalone growth is affected by the nutritional content of their diet, with a higher protein diet leading to larger growth. The different culture temperatures directly affected the dulse with the warmest culture temperature (17°C) exhibiting higher protein content than dulse grown at lower temperatures. This higher protein content translated into a faster-growing abalone population with condition factors and body weight ratios similar to other studies that exhibited good physical health. The results from this research can lead to climate-proofing aquaculture for the continued growth of abalone and nutrient optimization for farms in the face of climate change. Future directions should investigate further the effect of the nutritional differences in the diet on abalone reproductive and metabolic health.

Table 1. Initial and final measurements for both abalone size classes fed *D. mollis* cultured at three temperatures. Values are presented as means ± standard deviations. Significant differences are noted by superscripts (P=0.05).

| | **Small Abalone** | | | **Medium Abalone** | | |
|---|---|---|---|---|---|---|
| | **13°C** | **15°C** | **17°C** | **13°C** | **15°C** | **17°C** |
| **Initial Length (mm)** | 15.6±1.76 | 15.53±1.48 | 16.41±1.50 | 42.86±4.65 | 44.90±2.51 | 42.67±3.77 |
| **Final Length (mm)** | 25.4±2.70 | 23.63±2.84 | 24.24±2.45 | 52.04±5.63 | 54.36±4.37 | 53.44±5.43 |
| **Length Change** | 9.80±1.84 | 8.10±2.10 | 7.83±1.80 | 9.18±2.47 | 9.47±2.65 | 10.77±2.81 |
| **Initial Weight (g)** | 0.531±0.18 | 0.52±0.14 | 0.63±0.17 | 14.16±5.22 | 16.04±2.86 | 14.26±3.72 |
| **Final Weight (g)** | 2.06±0.70 | 2.04±0.57 | 2.34±0.58 | 23.56±8.02 | 26.92±5.72 | 24.54±6.91 |
| **Weight Change** | 1.53±0.56 | 1.51±0.45 | 1.71±0.44 | 9.40±3.79 | 10.88±3.43 | 10.28±4.18 |
| **Initial Condition Factor** | 0.79±0.16 | 0.79±0.06 | 0.80±0.05 | 1.00±0.09 | 1.02±0.09 | 1.05±0.15 |
| **Final Condition Factor** | 0.70±0.10$^{a}$ | 0.89±0.17$^{b,c}$ | 0.94±0.16$^{b,c}$ | 0.94±0.09 | 0.97±0.12 | 0.91±0.08 |
| **Body Weight Ratio Change** | 0.15±0.05$^{a}$ | 0.19±0.05$^{a}$ | 0.22±0.06$^{b}$ | 1.03±0.31 | 1.17±0.27 | 0.97±0.32 |

Figure Descriptions

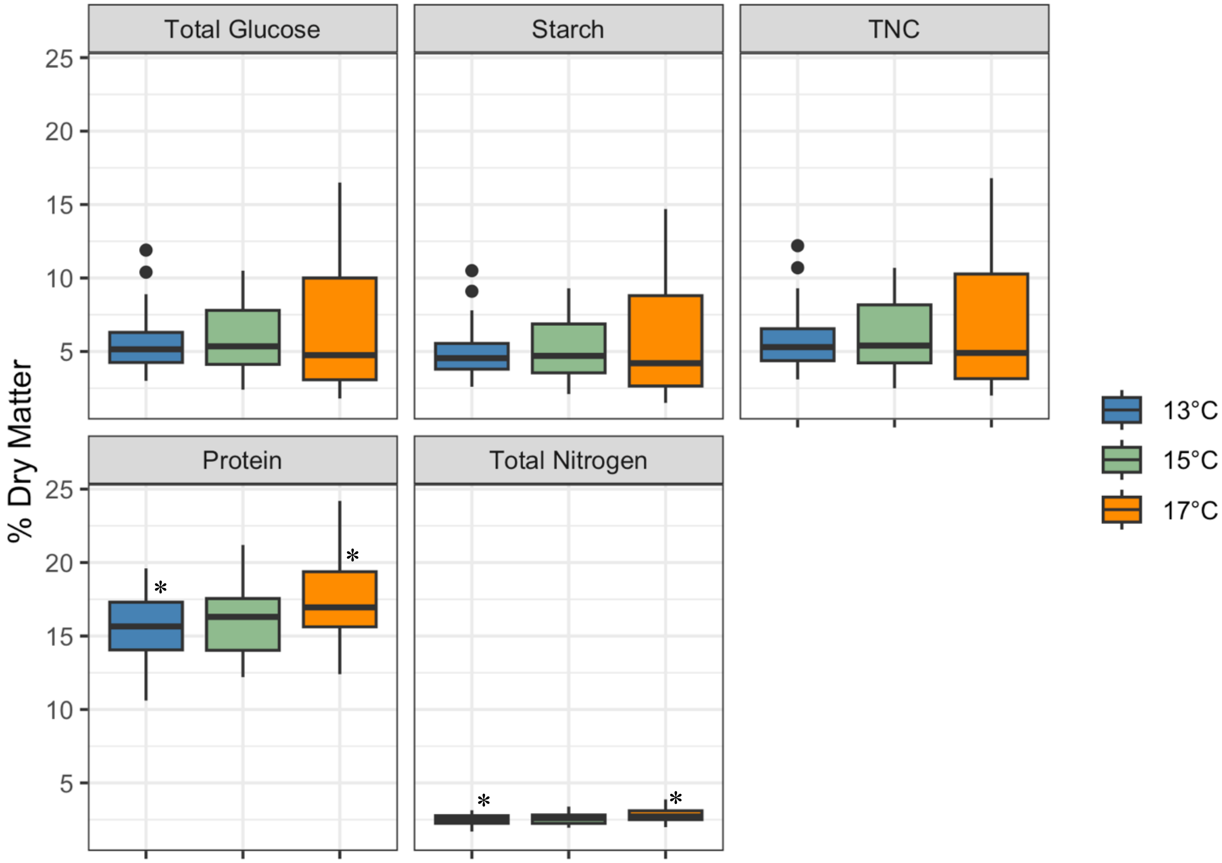
Figure 1: Mean nutritional composition of *D. mollis* by temperature treatment. Protein and total nitrogen had significant differences, indicated by an asterisk, between the 13°C -17°C (P=0.005, df=39.4, SE±0.525) and 15°C -17°C (P=0.04, df=39.4, SE±0.525). Samples were collected weekly for the duration of the experiment and a subset from each three-week batch (n=6 weeks) was analyzed for each treatment. Boxes represent the interquartile range and the whiskers extending represent the upper and lower quartile range with the closed circles representing the outliers.

Figure 2: Protein percentages for the seaweed cultures over time in the three different treatment groups. Dulse samples were dried at every feeding and a subset were analyzed. Each circle represents the mean protein percentage for each treatment at that sampling time point. A linear regression model was used and no differences were found between the treatment groups loss of protein over time.

Figure 3: Feed consumption for the small abalone size class fed three different dulse diets at three water temperatures for 13-weeks. Each circle represents mean dulse consumption of the treatment groups for each week (n=mean of 8 troughs). A linear regression model was used and there were no significant differences between the abalone consumption rates by temperature treatment experiment's duration.

Figure 4: Feed consumption for the medium abalone size class fed three different dulse diets at three water temperatures for 13-weeks. Each circle represents mean dulse consumption of the treatment groups for each week (n=mean of 7 troughs). A linear regression model was used and there were no significant differences between the abalone consumption rates by temperature treatment experiment's duration.

Figure 1.



Total Glucose
Starch
TNC
Protein
Total Nitrogen
% Dry Matter
25
20
15
10
5
25
20
15
10
5
*
*
*
*
13°C
15°C
17°C

Figure 2.

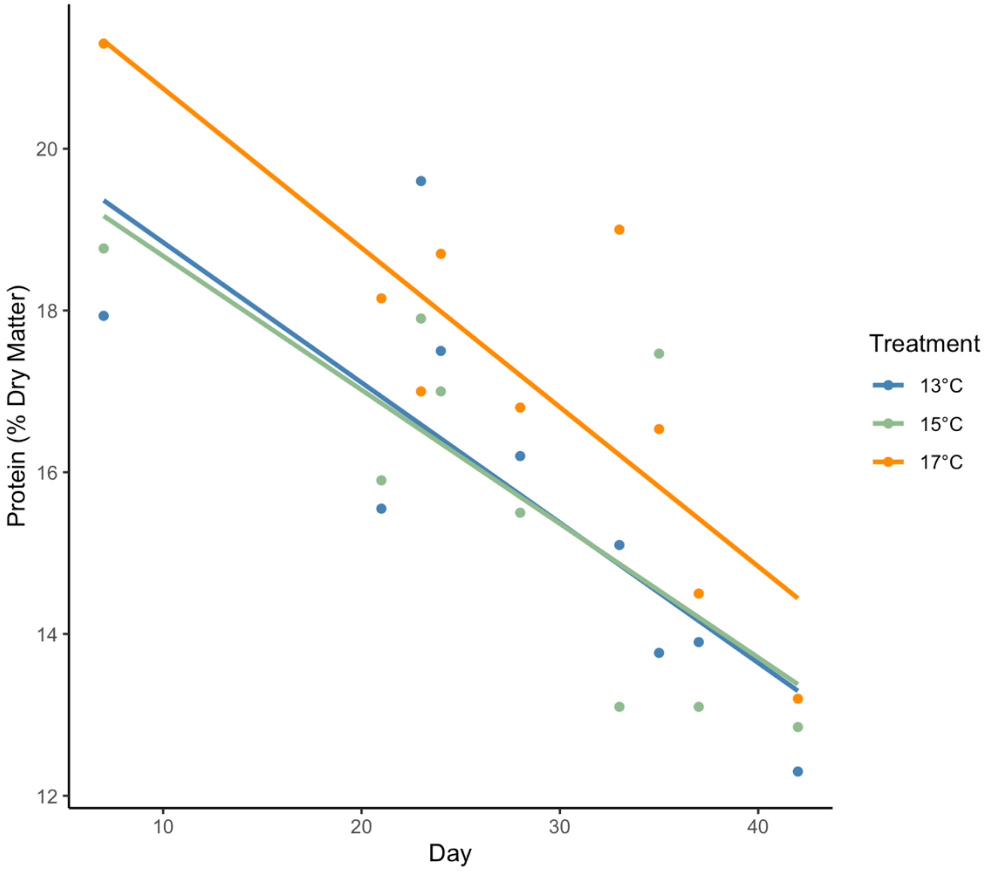
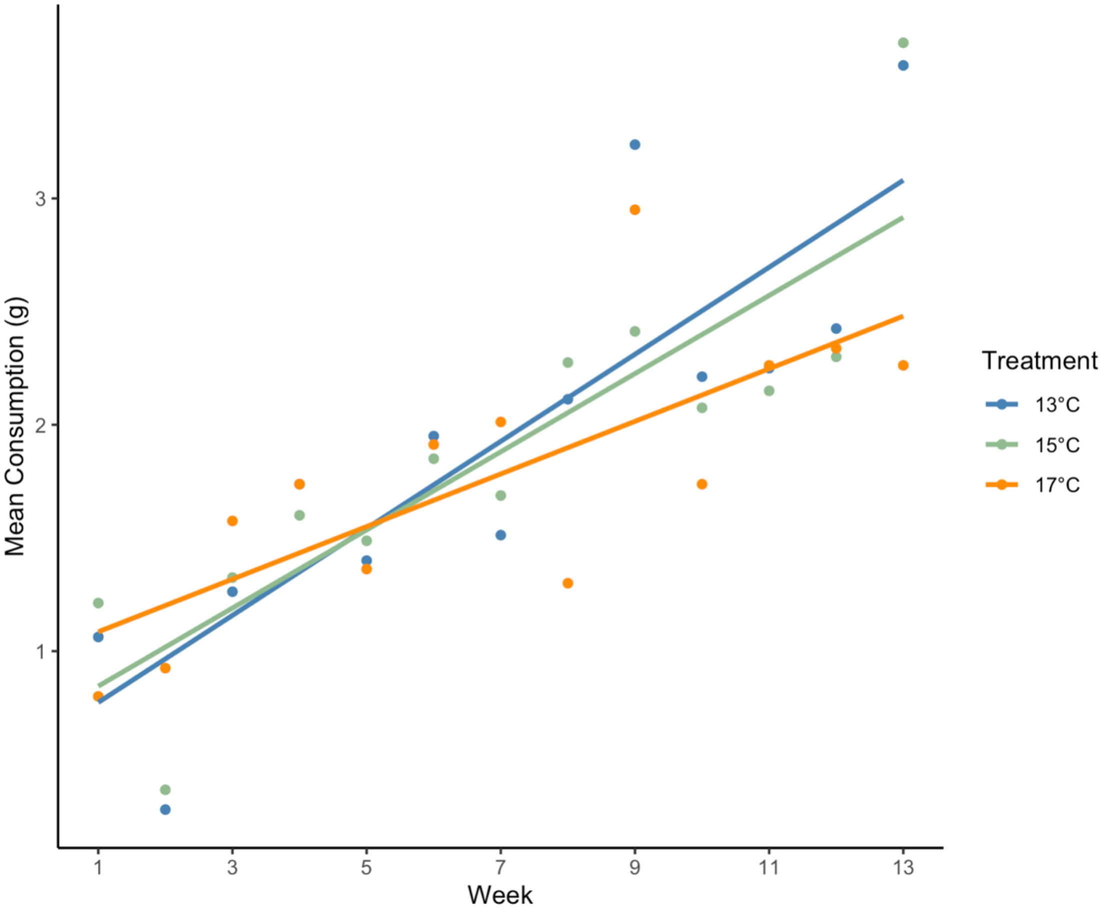
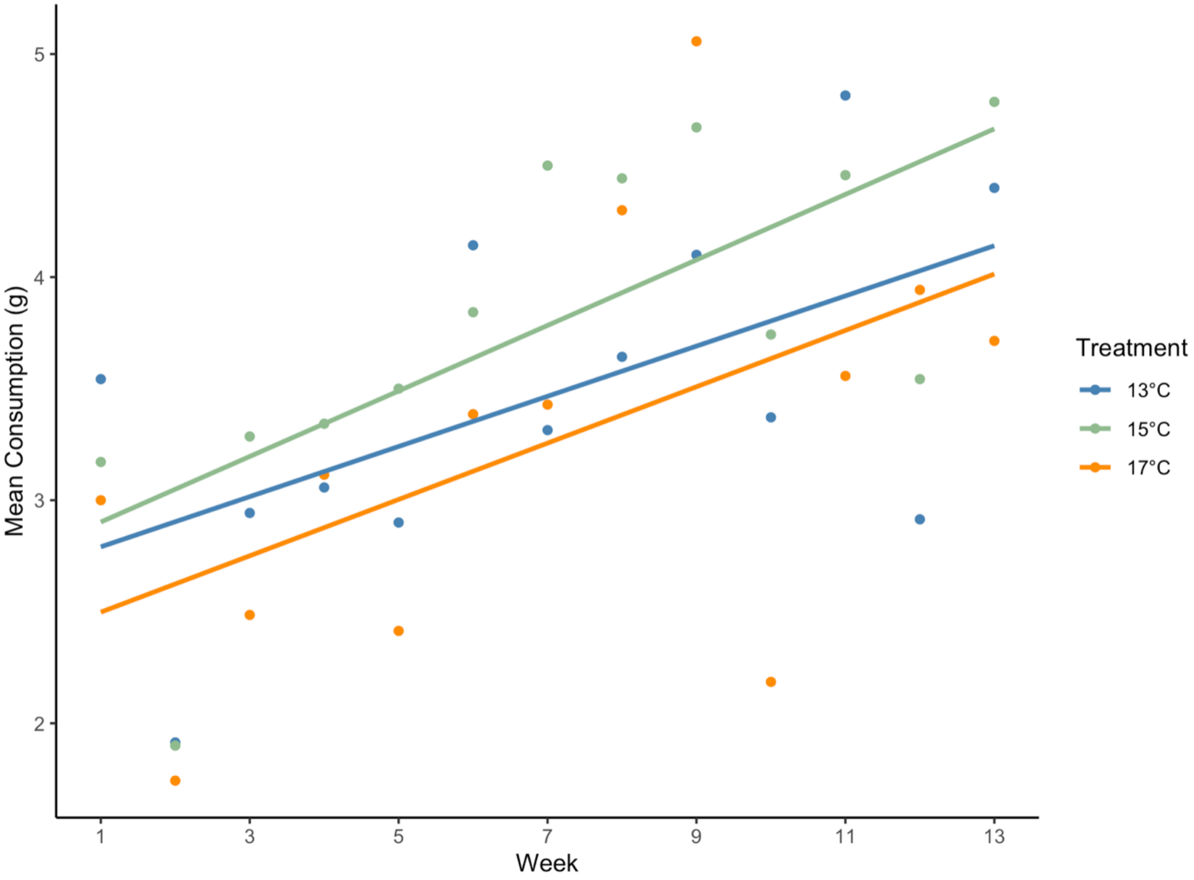

Protein (% Dry Matter)
20
18
16
14
12
10
20
30
40
Day
Treatment
13°C
15°C
17°C

Figure 3.



Mean Consumption (g)
3
2
1
1
3
5
7
9
11
13
Week
Treatment
13°C
15°C
17°C

Figure 4.



5
4
3
2
Mean Consumption (g)
1
3
5
7
9
11
13
Week
Treatment
13°C
15°C
17°C

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## Appendix A

Code for Data Analysis in R

```
library(tidyverse)
library(ggplot2)
library(mgcv)
library(dplyr)
library(lme4)
library(emmeans)
library(lmerTest)

small <- read.csv("SmallAbs.csv")
glimpse(small)
small <- small %>% select(-ID)
glimpse(small)
small <- na.omit(small)
mean(small$length_initial)
sd(small$length_initial)
mean(small$weight_initial)
sd(small$weight_initial)
colnames(small) <- tolower(colnames(small))

hist(small$length_final)
hist(small$length_initial)
sd_table <- small %>%
  select(-trough) %>%
  group_by(treatment) %>%
  summarise(across(everything(),list(mean=mean, sd=sd),na.rm=T))
write.table(sd_table, file = "small_table_sd.csv", sep = ",",row.names = F)


means_small <- small %>%
  select(-trough) %>%
  group_by(treatment) %>%
  summarise_all(mean, na.rm=T)
means_small
write.table(means_small, file="small_means.csv", sep = ",", row.names = FALSE)

df <- data.frame(
  treatment = c("13°C", "15°C", "17°C"),
  length_initial = c(15.6121622, 15.5297297, 16.4089744),
  length_final = c(25.4135135, 23.6256757, 24.2410256),
  length_change = c(9.80135135, 8.09594595, 7.83205128),
  weight_initial = c(0.53133784, 0.5245, 0.62798718),
  weight_final = c(2.06093243, 2.03645946, 2.3389359),
```

```
  weight_change = c(1.53441892, 1.51195946, 1.71094872),
  cfi = c(0.79006072, 0.78594821, 0.79513866),
  cff = c(0.6976854, 0.88847394, 0.94131479),
  length_initial_se = c(0.2, 0.3, 0.1),
  length_final_se = c(0.3, 0.2, 0.1),
  weight_initial_se = c(0.1, 0.2, 0.3),
  weight_final_se = c(0.2, 0.1, 0.3),
  cfi_se = c(0.1, 0.2, 0.3),
  cff_se = c(0.2, 0.3, 0.1)
)

length_aov <- aov(length_initial ~trough, data=small)
summary(length_aov)
TukeyHSD(length_aov)

weight_aov <- aov(weight_initial ~trough, data = small)
summary(weight_aov)
TukeyHSD(weight_aov)

#bw ratio - weight/length **** does not include random effects from trough******
small <- small %>% mutate(bw_ratio = weight_final/length_final)
glimpse(small)
small <- small %>% mutate(bw_raiocha=weight_change/length_change)

#######USE THIS WITH LMERTEST PACKAGE!!!!
###final/final
ratio_lm_s <- lmer(bw_ratio~treatment+(1|trough), data = small)
summary(ratio_lm_s)
ratio_means_s <- emmeans(ratio_lm_s, ~treatment)
ratio_comp_s <- pairs(ratio_means_s, adjust="tukey")
ratio_comp_s

####bw change
bwc_lm <- lmer(bw_raiocha~treatment+(1|trough), data = small)
summary(bwc_lm)
raiocha_means <- emmeans(bwc_lm, ~treatment)
raiocha_comp <- pairs(raiocha_means, adjust="tukey")
raiocha_comp

#boxplot of bw ratio by treatment
ggplot(small,   aes(x=treatment,   y=bw_ratio,   color=treatment))+geom_boxplot()+ggtitle("Body
Weight Ratio of Treatment Groups")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+geom_jitter()

#stat summary models
post_pre <- lmer(length_change~treatment +(1|trough),data = small)
```

```
lnsmch <- emmeans(post_pre, ~treatment)
lnsmchp <- pairs(lnsmch)
lnsmchp


changem <- lmer(length_final~treatment + length_initial +(1|trough),data = small)
summary(post_pre)
summary(changem)

cf <- function(weight, length){weight/(length^2.99)*5575}
#initial CF
small$cfi <- cf(small$weight_initial, small$length_initial)
str(small)

#final CF
small$cff <- cf(small$weight_final, small$length_final)
str(small)

#CF final model
cfm <- lmer(cff~treatment +(1|trough)+cfi, data=small) #addition of cfi to adjust for baseline
summary(cfm)
model_meanscff <- emmeans(cfm, ~treatment)
model_compacff <- pairs(model_meanscff, adjust="tukey")
model_meanscff
model_compacff

cfim <- lmer(cfi~treatment+(1|trough), data = small)
summary(cfim)
model_meanscfi <- emmeans(cfim, ~treatment)
model_compacfi <- pairs(model_meanscfi, adjust="tukey")
model_meanscfi
model_compacfi

######running cf with changes
cf <- function(weight, length){weight/(length^2.99)*5575}

#ratio CF
small$cf_change <- cf(small$weight_change, small$length_change)
str(small)

cf_ratio <- lmer(cf_change~treatment+(1|trough), data = small)
summary(cf_ratio)
means_cfchange <- emmeans(cf_ratio, ~treatment)
model_cf_pairs <- pairs(model_meanscfi, adjust="tukey")
model_cf_pairs
```

```
small_w_change <- lmer(weight_change~treatment + (1|trough), data = small)
means_smwech <- emmeans(small_w_change, ~treatment)
model_smwech <- pairs(means_smwech)
model_smwech

post_pre <- lmer(length_change~treatment +(1|trough),data = small)
lnsmch <- emmeans(post_pre, ~treatment)
lnsmchp <- pairs(lnsmch)
lnsmchp

med %>%
  ggplot(aes(x=treatment, y=bw_raiocha, fill=treatment))+geom_boxplot()+
  scale_fill_manual(values = c("steelblue","azure2","cadetblue"))+
  scale_fill_manual(values = c("steelblue","azure2","cadetblue"))+labs(x="Treatment", y="Body
Weight Ratio", color="Treatment")+
  scale_fill_manual(values = c("steelblue","azure2","cadetblue"),
                    labels = c("13°C", "15°C",
"17°C"))+theme_bw()+scale_x_discrete(label=temp_labels)

small_long <- small %>%
  pivot_longer(cols = c(cfi, cff),
               names_to = "variable",
               values_to = "condition_factor")

ggplot(small_long, aes(x = treatment, y = condition_factor, fill = variable)) +
  geom_boxplot(position = position_dodge(width = 0.8)) +
  ggtitle("Condition Factors per Treatment Group") +
  labs(x = "Treatment", y = NULL, fill = NULL) +
  scale_fill_manual(values = c("steelblue", "darkseagreen")) +
  theme_bw() +
  scale_x_discrete(labels = temp_labels)
#####GOOD COMBINED PLOT CF
small_long <- small %>%
  pivot_longer(cols = c(cfi, cff),
               names_to = "variable",
               values_to = "condition_factor") %>%
  mutate(variable = factor(variable, levels = c("cfi", "cff")))

ggplot(small_long, aes(x = treatment, y = condition_factor, fill = variable)) +
  geom_boxplot(position = position_dodge(width = 0.8)) +
  ggtitle("Condition Factors per Treatment Group") +
  labs(x = "Treatment", y = NULL, fill = NULL) +
  scale_fill_manual(values = c("azure2", "cadetblue")) +
  theme_bw() +
  scale_x_discrete(labels = temp_labels)
```

```
#start of consumption data
con <- read.csv("small_con.csv")
colnames(con) <- tolower(colnames(con))
glimpse(con)

con <- con %>%
  pivot_longer(starts_with("week_"), names_to = "week", values_to = "grams_consumed") %>%
  mutate(week= str_replace(week, "week_([0-9])$", "week_0\\1"))


##consumption by trough
con %>%
  ggplot(aes(x=week, y=grams_consumed,
color=treatment))+geom_point()+facet_wrap(~trough)+geom_smooth(method = "lm", se=T)+
  ggtitle("Consumption by Trough over 13 Weeks")+theme(axis.text.x = element_text(angle = 50,
hjust=8))+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+labs(x="Week",
y="Consumption (g)")
##wrapped by treatment boxplot
con %>% group_by(treatment) %>%
  ggplot(aes(x=week, y=grams_consumed,
color=treatment))+geom_boxplot()+facet_wrap(~treatment)+ggtitle("Seaweed Consumption by
Treatment over 13 Weeks")+
  theme(axis.text.x = element_text(angle = 45, hjust=1))+scale_color_manual(values =
c("steelblue","darkseagreen","darkorange"))
##wrapped by treatment point
con %>% group_by(treatment) %>%
  ggplot(aes(x=week, y=grams_consumed,
color=treatment))+geom_point()+facet_wrap(~treatment)+ggtitle("Seaweed Consumption by
Treatment over 13 Weeks")+
  theme(axis.text.x = element_text(angle = 45, hjust=1))+scale_color_manual(values =
c("steelblue","darkseagreen","darkorange"))+labs(x="Week", y="Consumption (g)")

##good graph and labeled
con %>%
  ggplot(aes(x=week, y=grams_consumed, color=treatment)) +
  geom_point() +
  facet_wrap(~trough) +
  geom_smooth(method = "lm", se=T) +
  ggtitle("Consumption by Trough over 13 Weeks") +
  theme(axis.text.x = element_text(angle = 50, hjust=8)) +
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange")) +
  labs(x="Week", y="Consumption (g)", color="Treatment")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
                     labels = c("Cold","Medium","Warm"))
```

```
con %>%
  group_by(treatment) %>%
  summarise(mean(grams_consumed))+
  ggplot(aes(x=treatment, y=mean(grams_consumed)))+geom_col()

con %>%
  filter(treatment=="c") %>%
  group_by(week) %>% summarise(mean(grams_consumed))

con %>%
  group_by(week) %>%
  mutate(mean_c = mean(grams_consumed))


#consumption boxplot
con %>%
  ggplot(aes(x=week, y=grams_consumed, color= treatment))+geom_boxplot()+ggtitle("Seaweed
Consumption by Treatment over 13 Weeks")+theme_minimal()+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
                     name = "Treatment",
                     labels = c("Cold", "Medium", "Warm"))+
  labs(x="Week", y="Consumption (g)")+
  theme(axis.text.x = element_text(angle = 45, hjust=1))

####MEAN CONSUPTION
con_means <- con %>%
  group_by(week, treatment) %>%
  summarise(mean_grams_consumed = mean(grams_consumed))

#####mean dot plot of consumption
ggplot(con_means, aes(x = week, y = mean_grams_consumed, color = treatment,
group=treatment)) +
  geom_point() +
  geom_smooth(method = "lm", se=F)+
  ggtitle("Mean Seaweed Consumption by Treatment over 13 Weeks") +
  theme_minimal() +
  labs(x = "Week", y = "Mean Consumption (g)") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
                     name = "Treatment",
                     labels = c("Cold", "Medium", "Warm"))

con_means$week <- as.numeric(gsub("week_", "", con_means$week))
ggplot(con_means, aes(x = week, y = mean_grams_consumed, color = treatment, group =
treatment)) +
```

```
  geom_point() +
  geom_smooth(method = "lm", se = FALSE) +
  theme_minimal() +
  labs(x = "Week", y = "Mean Consumption (g)") +
  theme(axis.text.x = element_text(angle = 0, hjust = 1)) +
  scale_color_manual(values = c("lightskyblue3", "paleturquoise3", "slategray2"),
                     name = "Treatment",
                     labels = c("13°C", "15°C", "17°C")) +
  scale_x_continuous(breaks = seq(min(con_means$week), max(con_means$week), by = 2))


con_model2 <- lmer(grams_consumed~treatment+(1|trough),data=con)
summary(con_model2)
means_conchange <- emmeans(con_model2, ~treatment)
model_conpairs <- pairs(means_conchange, adjust="tukey")
model_conpairs

#function for specific growth rate and anova
sgr <- function(initial_size, final_size){log(final_size/initial_size)/92}
small$sgr <- sgr(small$weight_initial, small$weight_final)
small$sgr_l <- sgr(small$length_initial, small$length_final)
str(small)
##weights?
sgr_m <- lmer(sgr~treatment+(1|trough), data = small)
summary(sgr_m)
check_model(sgr_m)
means_sgr <- emmeans(sgr_m, ~treatment)
model_sgr <- pairs(means_sgr, adjust="tukey")
model_sgr
means_sgr

##length
sgr_l <- lmer(sgr_l~treatment*class+(1|trough), data = small)
summary(sgr_l)
check_model(sgr_l)
means_sgr_w <- emmeans(sgr_w, ~treatment*class)
model_sgr_w <- pairs(means_sgr_w, adjust="tukey")
model_sgr_w
means_sgr_w


####weight change
wt_change <- lmer(weight_change~treatment+(1|trough), data = small)
summary(wt_change)
means_wt <- emmeans(wt_change,~treatment)
model_wt <- pairs(means_wt)
```

```
model_wt

med <- read.csv("R_data_med_wl.csv")
glimpse(med)
med <- med %>% select(-Treatment)
med <- na.omit(med)
colnames(med) <- tolower(colnames(med))

glimpse(med)

med_table <- med %>%
  select(trough, length_initial, length_final, weight_initial, weight_final, cfi,cff) %>%
  group_by(trough) %>%
  summarise(across(everything(),list(mean=mean),na.rm=T))

write.table(med_table, file = "med_table2.csv", sep = ",", row.names = FALSE)

#initial CF
med$cfi <- cf(med$weight_initial, med$length_initial)
str(med)

#final CF
med$cff <- cf(med$weight_final, med$length_final)
str(med)


cfmed <- lmer(cff~treatment +(1|trough)+cfi, data=med) #addition of cfi to adjust for baseline
summary(cfmed)
model_meanscffmed <- emmeans(cfmed, ~treatment)
model_compacffmed <- pairs(model_meanscffmed, adjust="tukey")
model_meanscffmed
model_compacffmed

cfimed <- lmer(cfi~treatment+(1|trough), data = med)
summary(cfimed)
model_meanscfimed <- emmeans(cfimed, ~treatment)
model_compacfimed <- pairs(model_meanscfimed, adjust="tukey")
model_meanscfimed
model_compacfimed

###graphs

med %>%
  ggplot(aes(x=treatment, y=bw_ratio, color=treatment))+geom_boxplot()+ggtitle("Initial
Condition Factor per Treatment Group")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+
```

```
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+labs(x="Treatment",
y="Body Weight Ratio", color="Treatment")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
            labels = c("13°C", "15°C",
"17°C"))+theme_bw()+scale_x_discrete(label=temp_labels)

###graphing boxplot CF final
med %>%
  ggplot(aes(x=treatment, y=cff, color=treatment))+geom_boxplot()+ggtitle("Final Condition
Factor per Treatment Group")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+labs(x="Treatment",
y="Final Condition Factor", color="Treatment")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
            labels = c("13°C", "15°C",
"17°C"))+theme_bw()+scale_x_discrete(label=temp_labels)

glimpse(med_long_s)

med_long_s <- med_long %>%
  select(variable, condition_factor, treatment)
med_long_s %>%
  group_by(variable) %>%
  summarise_all(mean,na.rm=F)


ggplot(med_long_s,aes(x=treatment, y=condition_factor, fill=variable))+
  geom_boxplot()

glimpse(med)
library(tidyr)

# Reshape the data into a longer format
med_long <- med %>%
  pivot_longer(cols = c(cfi, cff),
           names_to = "variable",
           values_to = "condition_factor")

# Plot the boxplots
ggplot(med_long, aes(x = treatment, y = condition_factor, fill = variable)) +
  geom_boxplot() +
  ggtitle("Initial and Final Condition Factors per Treatment Group") +
  scale_fill_manual(values = c("steelblue", "darkseagreen")) +
  labs(x = "Treatment", y = "Condition Factor") +
  scale_x_discrete(labels = temp_labels) +
```

```
  theme_bw()+scale_fill_manual(values = c("cadetblue", "azure2"),
                               labels=c("Initial Condition Factor", "Final Condition Factor"))+
  guides(fill=guide_legend(title = NULL))
glimpse(med_long)

med_long <- med %>%
  pivot_longer(cols = c(cfi, cff),
               names_to = "variable",
               values_to = "condition_factor") %>%
  mutate(variable = factor(variable, levels = c("cfi", "cff")))

ggplot(med_long, aes(x = treatment, y = condition_factor, fill = variable)) +
  geom_boxplot(position = position_dodge(width = 0.8)) +
  labs(x = "Treatment", y = "Condition Factor", fill = NULL) +
  scale_fill_manual(values = c("azure2", "cadetblue"),labels = c("Initial Condition Factor", "Final
Condition Factor")) +
  theme_bw() +
  scale_x_discrete(labels = temp_labels)

labels <- c("Cold", "Medium", "Warm")


##########MEDIUM CONSUMPTIONDATA
conmed <- read.csv("med_con.csv")
colnames(conmed) <- tolower(colnames(conmed))
glimpse(conmed)

conmed <- conmed %>%
  pivot_longer(starts_with("week_"), names_to = "week", values_to = "grams_consumed") %>%
  mutate(week= str_replace(week, "week_([0-9])$", "week_0\\1"))

con_meansmed <- conmed %>%
  group_by(week, treatment) %>%
  summarise(mean_grams_consumed = mean(grams_consumed))

con_meansmed$week <- as.numeric(gsub("week_", "", con_meansmed$week))

ggplot(con_meansmed, aes(x = week, y = mean_grams_consumed, color = treatment, group =
treatment)) +
  geom_point() +
  geom_smooth(method = "lm", se = FALSE) +
  theme_minimal() +
  labs(x = "Week", y = "Mean Consumption (g)") +
  theme(axis.text.x = element_text(angle = 0, hjust = 1)) +
  scale_color_manual(values = c("lightskyblue3", "paleturquoise3", "slategray2"),
                     name = "Treatment",
```

```
                    labels = c("13°C", "15°C", "17°C")) +
  scale_x_continuous(breaks = seq(min(con_meansmed$week), max(con_meansmed$week), by =
2))

ggplot(con_meansmed, aes(x = week, y = mean_grams_consumed, color = treatment,
group=treatment)) +
  geom_point() +
  geom_smooth(method = "lm", se=F)+
  ggtitle("Mean Seaweed Consumption by Treatment over 13 Weeks") +
  theme_minimal() +
  labs(x = "Week", y = "Medium Size Mean Consumption (g)") +
  theme(axis.text.x = element_text(angle = 45, hjust = 1))+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
                    name = "Treatment",
                    labels = c("Cold", "Medium", "Warm"))


###med con models
medconmod <- lmer(grams_consumed~treatment+(1|trough),data=conmed)
means_conchangemed <- emmeans(medconmod, ~treatment)
model_conpairsmed <- pairs(means_conchangemed, adjust="tukey")
model_conpairsmed

sd(small$length_initial)
mean(small$length_initial)
###by week
medconmodd <- lmer(grams_consumed~week+(1|trough),data=conmed)
means_conchangemedd <- emmeans(medconmodd, ~week)
model_conpairsmedd <- pairs(means_conchangemedd, adjust="tukey")
model_conpairsmedd

nut %>%
  group_by(treatment) %>%
  summarise(mean(n_total),sd(n_total))

##bwratio
med <- med %>% mutate(bw_ratio = weight_final/length_final)
med <- med %>% mutate(weight_change = weight_final-weight_initial)
med <- med %>% mutate(length_change = length_final-length_initial)
med <- med %>% mutate(bw_raiocha=weight_change/length_change)

med_bw <- lmer(bw_raiocha~treatment+(1|trough), data = med)
medbwmean <- emmeans(med_bw, ~treatment)
medbwpairs <- pairs(medbwmean)
medbwpairs
```

```
###graph BWR
med %>%
  ggplot(aes(x=treatment, y=bw_raiocha, color=treatment))+geom_boxplot()+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"))+labs(x="Treatment",
y="Body Weight Ratio", color="Treatment")+
  scale_color_manual(values = c("steelblue","darkseagreen","darkorange"),
            labels = c("13°C", "15°C",
"17°C"))+theme_bw()+scale_x_discrete(label=temp_labels)

med_tables <- med %>%
  select(-trough) %>%
  group_by(treatment) %>%
  summarise(across(everything(),list(mean=mean, sd=sd),na.rm=T))
write.table(med_tables,file = "medium_table.csv", sep = ",", row.names = FALSE)
```

## Appendix B

A supplementary table of small abalone size class replicate raw data.

| **Treatment** | **Initial Length (mm)** | **Final Length (mm)** | **Initial Weight (g)** | **Final Weight (g)** | **Initial Condition Factor** | **Final Condition Factor** |
|---|---|---|---|---|---|---|
| **c** | 15.467 | 25.158 | 0.523 | 2.041 | 0.84 | 0.725 |
| **c** | 16.375 | 25.983 | 0.552 | 2.161 | 0.72 | 0.659 |
| **c** | 14.517 | 24.567 | 0.439 | 1.74 | 0.81 | 0.669 |
| **c** | 17.34 | 28.33 | 0.719 | 2.787 | 0.762 | 0.684 |
| **c** | 15.275 | 25.4 | 0.505 | 2.028 | 0.801 | 0.709 |
| **c** | 14.963 | 22.913 | 0.477 | 1.609 | 0.805 | 0.734 |
| **c** | 15.35 | 24.2 | 0.494 | 1.794 | 0.76 | 0.724 |
| **c** | 15.3 | 25.975 | 0.527 | 2.238 | 0.829 | 0.719 |
| **m** | 16.386 | 26.643 | 0.598 | 2.506 | 0.756 | 0.755 |
| **m** | 15.3 | 25.611 | 0.489 | 2.14 | 0.771 | 0.716 |
| **m** | 15.217 | 25.225 | 0.49 | 1.993 | 0.788 | 0.698 |
| **m** | 14.533 | 23.8 | 0.423 | 1.716 | 0.788 | 0.743 |
| **m** | 16.364 | 24.018 | 0.61 | 2.359 | 0.781 | 0.961 |
| **m** | 15.383 | 21.833 | 0.523 | 1.789 | 0.816 | 0.979 |
| **m** | 15.042 | 21.292 | 0.484 | 1.874 | 0.787 | 1.085 |
| **m** | 15.486 | 21.571 | 0.518 | 1.802 | 0.79 | 1.022 |
| **w** | 15.8 | 25.433 | 0.538 | 2.142 | 0.77 | 0.724 |
| **w** | 15.85 | 25 | 0.547 | 1.894 | 0.782 | 0.697 |
| **w** | 15.533 | 26.2 | 0.479 | 2.116 | 0.726 | 0.677 |
| **w** | 17.009 | 24.409 | 0.672 | 2.548 | 0.771 | 1.001 |
| **w** | 16.425 | 23.583 | 0.661 | 2.349 | 0.839 | 1.017 |
| **w** | 15.958 | 23.375 | 0.578 | 2.254 | 0.791 | 0.993 |
| **w** | 16.858 | 24.25 | 0.688 | 2.466 | 0.801 | 0.987 |
| **w** | 16.858 | 23.667 | 0.7 | 2.497 | 0.818 | 1.078 |

**Appendix C**

A supplementary table of the medium abalone size class replicate raw data.

| Trough | Initial length (mm) | Final length (mm) | Initial weight (g) | Final weight (g) | Initial CF | Final CF |
|---|---|---|---|---|---|---|
| A1 | 36.9 | 41.1 | 8.14 | 12.49 | 0.937 | 1.041 |
| A2 | 34.1 | 42.8 | 6.48 | 12.08 | 0.944 | 0.892 |
| A3 | 43.9 | 54.7 | 14.27 | 26.37 | 0.976 | 0.935 |
| A4 | 47.6 | 60 | 18.47 | 34.77 | 0.992 | 0.935 |
| A6 | 40.8 | 50.5 | 13.11 | 21.00 | 1.117 | 0.946 |
| A7 | 49.3 | 61.2 | 16.71 | 40.55 | 0.808 | 1.028 |
| A8 | 44.2 | 55.4 | 15.39 | 28.47 | 1.032 | 0.972 |
| B1 | 53 | 63.4 | 27.32 | 39.92 | 1.065 | 0.910 |
| B2 | 42.6 | 55.4 | 14.75 | 22.66 | 1.104 | 0.774 |
| B4 | 46.2 | 55.8 | 18.35 | 28.40 | 1.078 | 0.949 |
| B5 | 42.2 | 46.9 | 10.78 | 16.58 | 0.830 | 0.931 |
| B6 | 44.8 | 55.7 | 13.82 | 30.37 | 0.890 | 1.020 |
| B7 | 45.3 | 54.4 | 19.28 | 31.90 | 1.201 | 1.150 |
| C1 | 45.8 | 54.5 | 16.37 | 24.62 | 0.987 | 0.883 |
| C2 | 45.2 | 55.8 | 16.49 | 27.01 | 1.034 | 0.902 |
| C3 | 48.7 | 58.7 | 21.01 | 35.07 | 1.054 | 1.007 |
| C4 | 44.1 | 54.7 | 17.41 | 25.05 | 1.176 | 0.888 |
| C5 | 45.5 | 57.4 | 18.16 | 29.91 | 1.117 | 0.918 |
| C6 | 41.9 | 54.7 | 11.04 | 23.53 | 0.868 | 0.834 |
| C7 | 45 | 52.8 | 14.35 | 27.58 | 0.912 | 1.087 |

## Appendix D

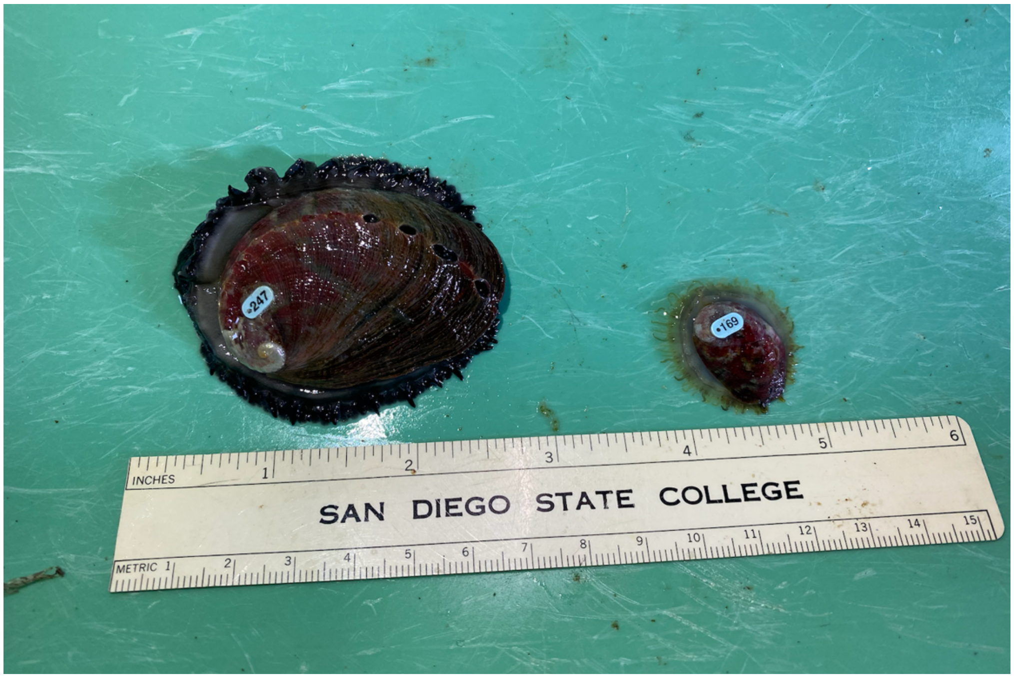
Photos of abalone replicate troughs

## Appendix E

Photo of dulse culture

## Appendix F

Photo of medium and small abalone size class at start of the experiment.



## Appendix G

Nutritional Analysis data from the UC Davis Analytical Lab

| Number | Tank | Treatment | Batch | Day | N (Total) | Protein | Glucose | Fructose | Sucrose | Glucose-Tot | TNC | Starch |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | T1 | w | 1 | 28 | 3.17 | 19.8 | <0.2 | <0.2 | <0.2 | 12.9 | 13.1 | 11.5 |
| 2 | T2 | m | 1 | 28 | 2.82 | 17.6 | <0.2 | 0.2 | <0.2 | 6.7 | 6.9 | 5.9 |
| 3 | T3 | c | 1 | 28 | 2.63 | 16.4 | 0.2 | 0.3 | <0.2 | 6.4 | 6.7 | 5.6 |
| 4 | T1 | w | 2 | 23 | 2.72 | 17 | <0.2 | <0.2 | <0.2 | 2.8 | 3 | 2.4 |
| 5 | T2 | m | 2 | 23 | 2.86 | 17.9 | <0.2 | <0.2 | <0.2 | 7.4 | 7.6 | 6.6 |
| 6 | T3 | c | 2 | 23 | 3.14 | 19.6 | <0.2 | <0.2 | <0.2 | 3.4 | 3.5 | 3 |
| 7 | T1 | w | 3 | 21 | 2.69 | 16.8 | <0.2 | <0.2 | <0.2 | 4.6 | 4.7 | 4.1 |
| 8 | T2 | m | 3 | 21 | 2.5 | 15.6 | <0.2 | <0.2 | <0.2 | 4.1 | 4.2 | 3.5 |
| 9 | T3 | c | 3 | 21 | 2.38 | 14.9 | <0.2 | 0.3 | <0.2 | 4.4 | 4.6 | 3.8 |
| 10 | T1 | w | 4 | 24 | 2.99 | 18.7 | <0.2 | <0.2 | <0.2 | 3.8 | 4 | 3.3 |
| 11 | T2 | m | 4 | 24 | 2.72 | 17 | <0.2 | <0.2 | <0.2 | 4.2 | 4.3 | 3.7 |
| 12 | T3 | c | 4 | 24 | 2.8 | 17.5 | <0.2 | 0.2 | <0.2 | 5.2 | 5.4 | 4.6 |
| 14 | T1 | w | 5 | 7 | 3.88 | 24.2 | <0.2 | 0.2 | <0.2 | 1.8 | 2 | 1.5 |
| 15 | T2 | m | 5 | 7 | 3.39 | 21.2 | <0.2 | <0.2 | <0.2 | 2.4 | 2.5 | 2.1 |
| 16 | T3 | c | 5 | 7 | 2.9 | 18.1 | <0.2 | <0.2 | <0.2 | 5.1 | 5.2 | 4.5 |
| 20 | T1 | w | 5 | 21 | 3.12 | 19.5 | <0.2 | <0.2 | <0.2 | 4.1 | 4.2 | 3.6 |
| 21 | T2 | m | 5 | 21 | 2.59 | 16.2 | <0.2 | <0.2 | <0.2 | 3 | 3.2 | 2.6 |
| 22 | T3 | c | 5 | 21 | 2.6 | 16.2 | <0.2 | <0.2 | <0.2 | 4.8 | 4.9 | 4.2 |
| 23 | T1 | w | 5 | 28 | 2.71 | 16.9 | <0.2 | <0.2 | <0.2 | 5.5 | 5.6 | 4.9 |
| 24 | T2 | m | 5 | 28 | 2.4 | 15 | <0.2 | <0.2 | <0.2 | 3.5 | 3.6 | 3 |
| 25 | T3 | c | 5 | 28 | 2.78 | 17.4 | <0.2 | <0.2 | <0.2 | 4.2 | 4.3 | 3.8 |
| 26 | T1 | w | 1 | 35 | 2.56 | 16 | <0.2 | <0.2 | <0.2 | 8 | 8.2 | 7.1 |
| 27 | T2 | m | 1 | 35 | 2.62 | 16.4 | <0.2 | 0.3 | <0.2 | 7.8 | 8.1 | 6.9 |
| 28 | T3 | c | 1 | 35 | 2.24 | 14 | 0.2 | 0.4 | <0.2 | 5.6 | 6 | 4.8 |
| 29 | T1 | w | 2 | 35 | 2.72 | 17 | <0.2 | <0.2 | <0.2 | 10.4 | 10.6 | 9.3 |
| 30 | T2 | m | 2 | 35 | 3.02 | 18.9 | 0.2 | <0.2 | <0.2 | 8.4 | 8.5 | 7.4 |
| 31 | T3 | c | 2 | 35 | 1.97 | 12.3 | 0.2 | 0.3 | <0.2 | 11.9 | 12.2 | 10.5 |
| 32 | T1 | w | 3 | 28 | 2.48 | 15.5 | <0.2 | 0.3 | <0.2 | 16.5 | 16.8 | 14.7 |
| 33 | T2 | m | 3 | 28 | 2.19 | 13.7 | <0.2 | 0.2 | <0.2 | 10.5 | 10.7 | 9.3 |
| 34 | T3 | c | 3 | 28 | 2.27 | 14.2 | <0.2 | 0.3 | <0.2 | 6.6 | 7 | 5.8 |
| 35 | T1 | w | 4 | 28 | 2.41 | 15 | <0.2 | 0.2 | <0.2 | 4.9 | 5.1 | 4.3 |
| 36 | T2 | m | 4 | 28 | 2.51 | 15.7 | <0.2 | 0.2 | <0.2 | 4.9 | 5.1 | 4.2 |
| 37 | T3 | c | 4 | 28 | 2.69 | 16.8 | <0.2 | <0.2 | <0.2 | 3.4 | 3.6 | 2.9 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | T1 | w | 5 | 33 | 3.04 | 19 | <0.2 | <0.2 | <0.2 | 3 | 3.1 | 2.6 |
| 39 | T2 | m | 5 | 33 | 2.09 | 13.1 | <0.2 | <0.2 | <0.2 | 5.1 | 5.1 | 4.5 |
| 40 | T3 | c | 5 | 33 | 2.42 | 15.1 | <0.2 | <0.2 | <0.2 | 6 | 6.1 | 5.4 |
| 41 | T1 | w | 1 | 42 | 2.24 | 14 | 0.2 | 0.2 | <0.2 | 9.7 | 9.9 | 8.5 |
| 42 | T2 | m | 1 | 42 | 2.16 | 13.5 | 0.2 | 0.4 | <0.2 | 9.9 | 10.3 | 8.7 |
| 43 | T3 | c | 1 | 42 | 2.24 | 14 | 0.2 | 0.4 | <0.2 | 8.9 | 9.3 | 7.8 |
| 44 | T1 | w | 2 | 42 | 1.99 | 12.4 | 0.2 | 0.4 | <0.2 | 12.1 | 12.5 | 10.7 |
| 45 | T2 | m | 2 | 42 | 1.95 | 12.2 | 0.2 | 0.3 | <0.2 | 10.4 | 10.7 | 9.2 |
| 46 | T3 | c | 2 | 42 | 1.69 | 10.6 | 0.3 | 0.3 | <0.2 | 10.4 | 10.7 | 9.1 |
| 47 | T1 | w | 3 | 37 | 2.31 | 14.5 | 0.2 | 0.3 | <0.2 | 10.1 | 10.4 | 8.9 |
| 48 | T2 | m | 3 | 37 | 2.1 | 13.1 | 0.2 | 0.4 | <0.2 | 7.8 | 8.2 | 6.8 |
| 49 | T3 | c | 3 | 37 | 2.23 | 13.9 | <0.2 | 0.3 | <0.2 | 5.7 | 6 | 4.9 |
| 50 | T1 | w | 4 | 35 | 2.65 | 16.6 | <0.2 | <0.2 | <0.2 | 2.1 | 2.2 | 1.7 |
| 51 | T2 | m | 4 | 35 | 2.73 | 17.1 | <0.2 | <0.2 | <0.2 | 3.9 | 4 | 3.5 |
| 52 | T3 | c | 4 | 35 | 2.4 | 15 | <0.2 | 0.3 | <0.2 | 3.8 | 4.1 | 3.2 |
| 53 | T1 | w | 3 | 7 | 3.18 | 19.9 | <0.2 | <0.2 | <0.2 | 3 | 3 | 2.6 |
| 54 | T2 | m | 3 | 7 | 2.83 | 17.7 | <0.2 | <0.2 | <0.2 | 4.3 | 4.5 | 3.8 |
| 55 | T3 | c | 3 | 7 | 2.73 | 17 | <0.2 | <0.2 | <0.2 | 4.6 | 4.7 | 4 |
| 56 | T1 | w | 4 | 7 | 3.16 | 19.8 | <0.2 | <0.2 | <0.2 | 3.3 | 3.3 | 2.8 |
| 57 | T2 | m | 4 | 7 | 2.79 | 17.4 | <0.2 | <0.2 | <0.2 | 5.6 | 5.7 | 4.9 |
| 58 | T3 | c | 4 | 7 | 2.99 | 18.7 | <0.2 | <0.2 | <0.2 | 3 | 3.1 | 2.6 |