GROWTH OF JUVENILE RED ABALONE (*Haliotis rufescens*) FED DIFFERENT SEAWEED-BASED DIETS

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ABSTRACT

GROWTH OF JUVENILE RED ABALONE (*Haliotis rufescens*) FED DIFFERENT SEAWEED-BASED DIETS

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The rise of abalone aquaculture has mitigated most of the global demand placed on wild stocks of abalone; however, the current production of abalone relies heavily on naturally harvested kelp. The continued reliance on wild kelp as a feed source further contributes to the disappearance of kelp forests throughout coastal ecosystems. This study aims to better understand how juvenile red abalone *Haliotis rufescens* grow and utilize nutrients from three diets: a control diet of naturally harvested bull kelp *Nereocystis luetkeana,* a formulated commercial diet (ABKelp®), and Pacific dulse *Palmaria mollis* produced using Integrated Multi-Trophic Aquaculture (IMTA). Juvenile abalone greater than 10 mm were reared in a recirculating quarantine system from October 2nd, 2019 to March 23rd, 2020. Each diet treatment had nine replicates with data on weight, shell length and shell depth collected monthly. A mixed-effects model ANOVA indicated that diet treatments had a significant effect on abalone weight ($F_{2,24}$=49, $P < 0.05$), length ($F_{2,24}$=61.7, $P < 0.05$) and depth ($F_{2,24}$=43.2, $P < 0.05$). Abalone grew significantly larger on both macroalgal diets (bull kelp and Pacific dulse) as compared with the formulated feed. Dulse produced the largest abalone; however, post-hoc pairwise comparisons revealed that abalone in the dulse and bull kelp treatments were not significantly different

from one another in terms of final average weight, shell length, and shell depth. Abalone fed dulse had significantly higher weight gain per day and body weight to shell length ratios than the other two treatments. Amino acid profiling demonstrated that the formulated feed had the highest concentration of essential amino acids, but that tissue from abalone fed dulse had the highest protein nitrogen content and amino acids. Fatty acid analysis showed a seasonal increase in Eicosapentaienoic acid (EPA (20:5n3)) for dulse. It is suggested that juvenile red abalone have the potential to biosynthesize Clupanodonic acid (22:5n3) from EPA but may not have the ability to convert it into Docosahexaenoic acid, or DHA (22:6n3), as is seen in other members of the *Haliotis* genus. It is speculated that the formulated fed abalone would have exhibited greater growth and consumption had the presentation of the feed been in a more compressed form. This study further emphasizes the benefits of IMTA cultured algae as a feed source for juvenile red abalone and a sustainable alternative to wild harvest kelp.

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INTRODUCTION

Abalone are a highly sought-after marine gastropod that play an integral role in the ecosystems and cultures of many coastal communities (Hahn 1989). Recreational diving for the mollusk can be traced back to Japan as early as 30 CE (Hahn 1989). Unfortunately, the popularity of abalone as a food source and for ornamental purposes has contributed to a worldwide decline through overfishing of wild stocks (Elliott 2001). Noticeable declines in the abundance of numerous abalone species around the 1980's and 1990's have caused many of the fisheries to be restricted or closed entirely to alleviate fishing pressure on these reduced stock sizes. Specifically, the red abalone *Haliotis rufescens* fishery in Northern California was closed because of overfishing, shifting environmental factors, a decline in kelp forests, and low abalone abundance due to high mortality from starvation (CDFW 2018b). Further analysis of the fishery resulted in an extension of the closure until April 1, 2021, due to a large decline in abalone populations and lack of recovery (CDFW 2018a).

One of the major stressors causing the declines of wild populations is the loss of kelp forests along the California coastline (Friedman et al. 1997). There is currently a global trend in the decline of kelp forests due to multiple factors including loss of top predators and shifting climatic conditions, with California's kelp forests considered noticeably vulnerable to these changes (Krumhansl et al. 2016). The loss of kelp forests as a food source is expected to hinder recovery of wild abalone populations as well as limit kelp utilization for commercial aquaculture as a feed source.

Another detrimental factor in the decline of wild abalone is the prevalence of a disease known as withering syndrome (Friedman et al. 1997, Moore et al. 2000, Friedman et al. 2003). Withering syndrome is associated with a gastrointestinal Rickettsiales-like prokaryote or RLP, *Xenohaliotis californiensis* (Moore et al. 2000). The disease is easily discernible in infected abalone by a noticeable atrophy of foot tissue. The red, white, black, green, and pink abalone of the *Haliotis* genus in California have become depleted, in part, as a response to the introduction and proliferation of the disease in the past 30 years (Moore et al. 2009). Like the loss of kelp forests, one of the major factors influencing the disease is increased water temperature (Friedman et al. 1997). Considering the general global trend of increasing water temperatures, it is assumed that the prevalence of the disease, as well as the loss of kelp forests, will increase. This further necessitates the need for sustainable alternatives.

Commercial aquaculture of red abalone in the United States has relied heavily on wild harvest giant kelp *Macrocystis* spp. or bull kelp *Nereocystis* spp. (Hahn 1989). These operations are located along the eastern Pacific and are situated in regions with abundant wild kelp (Evans and Langdon 2000). Due to this, abalone aquaculture has not been able to expand into regions where kelp is not found or areas that are detached from the coast entirely (Elbert 1992). As such, there is a need to fulfill the demands of commercial aquaculture and mitigate the loss of kelp forests, which can be addressed and alleviated with the implementation of sustainable aquaculture practices.

Aquaculture of abalone has grown in recent years to supplement the demand created by the closure of many fisheries. As of 2018, aquaculture methods were estimated to be producing around 97%, 187,362 metric tons, of the global consumption of abalone (FAO 2020). Abalone are a slow-growing species and take anywhere from three to three and a half years to reach market size, approximately 100mm (Bautista-Teruel et al. 2003). As such, research is critical to determine more efficient ways of growing and culturing them. Some studies have shown that low stocking density as well as feeding Pacific dulse, *Palmaria mollis*, are beneficial for juvenile red abalone larger than 5 mm (Rosen et al. 1999, Vivanco-Aranda et al. 2011). These studies were relatively short in duration (e.g., one week in one case), and did not investigate the effect of varied diets on growth nor the nutrient composition of the diet. Most of the research that has looked at varying diets of macroalgae and other feed sources (e.g., formulated feeds) worked with abalone ranging from 20 mm to 42 mm (Kemp et al. 2015, Garcia-Esquivel and Felbeck 2009).

Integrated Multi-trophic Aquaculture (IMTA) is any system that incorporates multiple trophic levels in an aquaculture setting. These systems involve cultivating organisms in a way that allows for uneaten feed, waste products and dissolved nutrients of one trophic level to be recaptured and converted into energy and fertilizer for the growth of other organisms in lower trophic tiers. Research into IMTA produced abalone using *Palmaria mollis*, *Ulva lactuca* and *Gracilaria chilensis* has shown the effectiveness of co-culture of abalone and macroalgae (Evans and Langdon 2000, Macchiavello and Bulboa 2014). However, these studies did not investigate the potential of finfish co-culture as the nutrient source for the growing macroalgae. There exists a need to

understand the effectiveness of finfish produced feed coupled with differential diets and how they affect 10 mm abalone, the stage when they begin to primarily consume macroalgae.

Proper nutrition is critical for abalone producers to ensure optimal growth of their cultured species. Protein nitrogen has been seen to be a crucial nutrient and that insufficient levels can hinder growth of abalone (Fleming 1995, Britz and Hecht 1997). Amino acids are the building blocks of proteins and are critical for the growth and health of all organisms. There are two broad categories of amino acids: non-essential and essential amino acids. Non-essential amino acids are those that are synthesized at sufficient levels in the body to ensure optimum health and efficient growth. Essential amino acids are the amino acids that are not produced at sufficient levels and must be incorporated into the diet of animals (Wu et al. 2012). The essential and non-essential amino acids for red abalone have been outlined by the research done by Allen and Kilgore (1975) and reviewed by Fleming et al. (1996). By investigating the different amino acid profiles of different diets, abalone producers can make easier decisions on which diets to feed to their cultured species.

Lipids and fatty acids are one of the less studied aspects of abalone nutrition since they are a relatively low percentage of the diet and they are typically met through supplementation of fish and soybean meal (Durazo-Beltran et al. 2003). It is generally understood that abalone should be fed diets that are higher in omega-3 and omega-6 fatty acids to expedite growth (Mai et al. 1996). Research on fatty acids in abalone has shown that they have the potential to synthesize fatty acids not found in their diet by elongation

of short-chain fatty acids into long-chain fatty acids as well as desaturation of long-chain fatty acids into short-chain substituents (Uki et al. 1986, Mai et al. 1996, Xu et al. 2004). This is a critically important aspect of abalone nutrition in that different species have been able to perform this biosynthesis to different extents (Uki et al. 1986, Mai et al. 1996, Xu et al. 2004). By understanding which fatty acids different species can synthesize and which ones are essential for growth, nutritionists and farmers can supplement the fatty acids that they are not able to create to ensure optimal nutrition.

There are many interacting aspects of aquaculture that play a pivotal role in optimal growth of cultured abalone. It has been shown that there are many interacting variables that affect abalone growth rates, such as temperature, genetics, water exchange rate and stocking density, but diet is considered to be particularly impactful in improving growth in these animals (Lapota et al. 2000, Elliott 2001). Amino acids and Essential Fatty Acids (EFA) play a vital role in the growth and feed efficiency of abalone (Daume et al. 2003, Xu et al. 2011). Proper nutrition is critical in successfully culturing abalone and increasing growth efficiency to shorten the time it takes to reach market weight (Naidoo et al. 2006). This study aims to better understand how juvenile red abalone, grow, and utilize nutrients from three diets: a control diet of naturally harvested bull kelp *Nereocystis luetkeana*, a formulated commercial diet (ABKelp®), and IMTA produced Pacific dulse. The study had 3 primary objectives:

1. Evaluate the effects of three algae-based diets on abalone size and daily growth based on weight, shell length and shell depth.

2. Determine the amino acid and nitrogen content of the diets and how readily they are deposited in the tissues of the abalone.
3. Determine the fatty acid profile of the diets and investigate the synthesis of long-chain polyunsaturated fatty acids in juvenile red abalone.

MATERIALS AND METHODS

Study system design

The study was conducted at the Humboldt State University Marine Laboratory located in Trinidad, CA. Data collection began on October 2nd, 2019 and ended early on March 23rd, 2020. The proposed schedule was to end on April 2nd to have 6 full months of data. However, access to campus facilities was restricted due to COVID-19, therefore the experiment was ended 11 days early. Three table tanks within a wet laboratory were converted into a recirculating quarantine system. Each table tank (114 cm x 53 cm x 27 cm) housed nine smaller treatment tanks (Lee's Critter Keeper Medium 30 cm x 17 cm x 20 cm). Three table tanks were utilized with a total of 27 smaller treatment tanks. This allowed for the three treatments to have nine replicates throughout the system. The individual treatment tanks held roughly 10.4 liters of water. Including the 189-liter sump and the plumbing, the system had a capacity of approximately 568 liters.

Each table tank was connected to the main water supply for the closed system. The water supply was then divided into the treatment tanks within each table tank via a manifold system distributing separate flows into each small tank. This allowed for a singular, separate source of water to each treatment tank. The flow rate of each manifold tube was approximately 4 mL/s which resulted in 34 tank replacements per day to ensure proper water quality within treatment tanks.

The purpose of isolated water supplies was to eliminate any bias in olfactory cues of other diets. It has been seen in previous studies that abalone may have food preferences based on chemical cues in nearby feed (Harada and Hirano 1983). These cues could potentially bias the study in that abalone fed one diet might have consumed more if presented with the olfactory cues of another diet, stimulating them to feed (Sakata et al. 1991). Individual tanks had fitted lids to ensure no escapement of abalone from their tanks into the main table tanks. The treatment tanks drained out of the slits in the tank lids to remove effluent and suspended particulate. This water drained into the main table tanks before collection into a sump for biofiltration, sterilization, and recirculation.

The system was designed to be a quarantine system because the abalone that were used in this study are from a geographic location documented to have withering syndrome. As such, the system was set up as a closed, recirculating system with a 120-Watt classic AquaUltraviolet sterilizer with a flow rate less than 5,148 liters per hour. This ensured optimal contact time with the UV bulb and water, at least 90,000 $\mu W/cm^2$. Following the recommendations from the California Department of Fish and Wildlife (CDFW), there was posted signage and restricted access to the system coupled with ozonation of water removed from the system, for further biosecurity controls. These measures were critical in safeguarding the potential for contamination and preventing introduction of the pathogen into coastal waters.

Experimental treatments

Three algae-based diets were tested in the experiment. The diet treatments were assigned randomly to the treatments tanks to minimize placement bias (Figure 1). Treatment 1 consisted of naturally harvested bull kelp collected from Trinidad State Beach to serve as a control which has been shown to support "normal" abalone growth rates in the wild and in laboratory settings (Trevelyan et al. 1998). Treatment 2 was a formulated commercial feed, ABKelp®, designed for high abalone growth rate. This feed was provided by AlgaMar, a seaweed harvesting and production company out of Ensenada, Mexico. The ABKelp® that was provided was presented in a pelleted, extruded form with pellets averaging 10 mm in length. Treatment 3 was IMTA cultured macroalgae Pacific dulse, harvested from a recirculating Integrated Multi Trophic Aquaculture system of sablefish *Anoplopoma fimbria* and macroalgae at the Humboldt State University Marine lab. This system included two separate 379-liter rectangular tanks housing 19 sablefish each with fish averaging 22 cm in length. The sablefish wastewater then flowed through mechanical and bio-filtration before being delivered to one of two 379-liter cylindrical tanks housing Pacific dulse.

Dulse has been shown to be nutritionally advantageous to other macroalgae and can increase growth rates due to an increased protein content resulting from fish effluent co-culture (Buchal et al. 1998, Naidoo et al. 2006). Treatments 1 and 2 were chosen to represent the industry standards for cultured abalone: Bull or giant kelp and formulated

feed. Treatment 3, the IMTA-produced Pacific dulse, was tested as a more sustainable macroalgal alternative to the industry standards.

Abalone (average of 12.4 ± 1 mm shell length) were acquired from The Cultured Abalone Farm, 9580 Dos Pueblos Canyon Rd, Goleta, CA 93117. This is the stage of their life cycle in which they begin to consume macroalgae as a primary constituent of their diet (Hahn 1989). Six individual juvenile abalone were randomly assigned to each of the 27 treatment tanks and reared for the duration of the experiment. Once assigned to a treatment tank, individual abalone were uniquely marked with small plastic colored beads adhered to their shells using an ethyl cyanoacrylate ester (CA) coral glue CorAffix (Two Little Fisheries Inc.). These beads allowed individual growth to be tracked for the duration of the study.

Aquaculture system design can greatly impact the growth and wellbeing of cultured animals. As such, factors such as temperature and access to food should be considered for the species being grown. Based on the recommendations from The Cultured Abalone farm for optimal growth, the system was built with a heat pump which maintained the temperature of the system at 15°C, slightly warmer than the typical temperature range for the marine lab of 12-13°C. To increase the contact time between the abalone and the feed, and provide refugia from light, all tanks had a pvc structure added (comprised of 30 cm diameter pvc pipe (0.635 cm thick by 15.25 cm long) that was bisected lengthwise). These inserts gave the abalone shelter and a central location for feed placement to encourage them to consume the treatment diets. Other research has indicated that abalone at this stage of their life can consume anywhere from 10-30% of

their body weight in algae daily (Hahn 1989). Based on recommendations from AlgaMar and The Cultured Abalone Farm, abalone were fed *ad libitum* feed replenished every two days to ensure optimal growth, to be consistent with the industry standards, and to reduce the potential for competition among individuals. Uneaten algae and feed were removed every two days to prevent decomposition and promote good water quality within each treatment tank.

It was anticipated that there would be premature mortality due to handling stress and placement into treatment tanks. To alleviate this, excess abalone were kept for two weeks to serve as a replacement line for premature mortalities. The replacement abalone were fed dulse during the two-week window and displayed minimal consumption during this time. A total of two abalone were used to replace premature mortalities. Any abalone that perished after this two-week window weas removed from the tank with no replacement.

Data collection

Abalone weight, shell length, and shell depth were measured monthly throughout the experiment. Abalone weight was measured hydrostatically using a Mettler Toledo AB204-S Digital scale. Shell length and depth were determined using a Pittsburgh® 6” Composite Digital Caliper. Shell length was measured from the midpoint of the abalone from the posterior end of the shell to the anterior end. Shell depth was measured from the top of the spire to the bottom lip of the shell on the posterior end of the shell. Shell length and depth measurements were performed by multiple observers throughout the course of

the study. Aside from the monthly data collection, abalone remained inside their treatment tank without handling.

The consumption rate of different diets is important in that it helps explain the differences seen in growth. If a diet is over- or under-consumed, it may be indicative of poor nutrition or unpalatability. The consumption rate of the three diets was monitored in all tanks at three different feedings to determine if there was a significant difference in consumptive behaviors that might alter the growth rates seen at the end of the study. The three feeding windows were consistent with the feeding schedule of every two days and were collected on the 5^{th}, $7^{th,}$ and 9^{th} of January. The consumption was calculated by measuring the feed that went into each tank and the feed that was removed after 2 days. Two control tanks with no abalone were set up for each diet and used to measure the decrease in feed weight due to natural degradation and not consumption. For each feed type, the mean decrease in feed weight from the control tanks were subtracted from the estimates from the tanks with abalone to calculate consumption rates (reported as grams feed consumed·grams abalone^{-1}·day^{-1}).

Water quality was assessed on a biweekly basis to ensure proper conditions for the growing abalone. Specifically, data on nitrate, nitrite, and salinity were collected by testing sump water with a Handheld Hach DR 900 Multiparameter Colorimeter and the associated Hach pillow reagents. The system was designed with an uncovered sump with the treatment tanks draining into a larger table tank before entering the plumbing system. This resulted in a high surface area to volume ratio which caused high evaporation rates

in the system. This resulted in an increase in salinity from 32 ppt to 39 ppt at the beginning of January. After working on solutions to this issue, a drip system was installed to compensate for the evaporation of water out of the system. This allowed for fresh sea water intrusion whenever the sump fell below a certain volume and fixed any issues associated with elevated salinity.

Data analysis

Daily and monthly growth rates were calculated using the data collected throughout the course of the study. Daily growth rates (D) are expressed as body weight (BW), shell length (SL), or shell depth (SD) gained per day and were calculated as follows:

$$D = (X - X_0)/t$$

Where X is the final individual measurement (BW, SL, or SD), X_0 is the initial individual measurement, and t is total time in days.

Monthly growth rates (G) are expressed as BW, SL, and SD gained per day and were calculated as follows for each of the three variables:

$$G = ((X_i - X_{i-1}))/t$$

Where X_i is individual measurement (BW, SL, or SD) for month i, X_{i-1} is the previous month's individual measurement, and t is time in days since the last measurement.

The body weight-to-shell length ratio (BW/SL) was calculated for all individuals and averaged by treatment. This metric of relative condition is a useful tool for cultured abalone because some diets produce higher weight (i.e., meat yield) for the same market

size (shell length of approximately 100 mm). A higher BW/SL ratio indicates that abalone had an increased somatic weight gain per unit of shell increase.

A mixed-effects analysis of variance (ANOVA) was used to test the effect the three feed treatments had on final weight, length, depth, growth rates and consumption. This test was used instead of a traditional two-way analysis of variance (ANOVA) because there were multiple subsamples (i.e. abalone) within a treatment tank that were not independent of one another. The mixed-effects approach was carried out using the lmer function in the lme4 package in R (Bates et al. 2015). "Tank" was modeled as a random variable and the diet treatment was modeled as a fixed effect. Following the results of significant tests, post-hoc pairwise comparisons were conducted using the difflsmeans function in the lmerTest package (Kuznetsova et al. 2017) to assess the pairwise significance among the three treatment levels. Model residuals were assessed graphically to verify model assumptions of normality and homogeneity of variance. For the shell weight analysis, examination of the model residuals indicated a violation of the assumption of homogeneity of variance, therefore shell weights were log-transformed, and the model was refit.

To visualize the abalone growth trajectory, a weight by length relationship was created for all treatments. This data was modeled using an allometric growth curve ($BW = aSL^b$) where BW is the weight, SL is the shell length, a is a constant and b is the growth coefficient. The model was fit using a mixed-effects analysis of covariance (ANCOVA) after log transforming both the BW and SL. This analysis treated log(SL) and diet treatment as fixed effects to predict log(BW). Random slopes and intercepts for

each individual abalone were estimated as random effects to account for the repeated measurements of abalone size through time. Model predictions were back transformed and bias-corrected (Sprugel 1983) for plotting on the original scale. The model was fit using the lme4 package in R (Bates et al. 2015).

Samples of abalone tissue and the three feeds were analyzed to investigate nutrient assimilation and composition. Body tissue from multiple abalone (excluding the shell) were combined to obtain sufficient mass (10 g per sample) for fatty acid and amino acid analysis. The bull kelp treatment yielded two tissue samples (each a composite of 25 individual abalone), the formulated feed treatment yielded one tissue sample (a composite of all 42-surviving abalone), and the dulse treatment yielded two tissue samples (a composite of 25 and 26 abalone). In addition to the five abalone tissue samples, seven samples of the diets (three bull kelp, three Pacific dulse, and one from the formulated) were sent to the University of Missouri for fatty acid profiling and amino acid profiling. The different analyses investigated the nutrient assimilation and composition of the abalone from the treatments as compared to the nutrients found in the treatment diets. All abalone tissue samples were collected on March 23rd. To test for seasonal variation of the nutrients in the fresh macro algae diets, samples were collected at three different times: October 2nd, January 26th, and March 23rd. Only one feed sample was sent off for the formulated feed because it was expected to be consistent and not vary across time. The seasonality difference of bull kelp and dulse for amino acids are reported in Appendix A with the fatty acids reported in Appendix B.

Essential amino acids and fatty acids are those that are required in the diet as they cannot be synthesized in an organism at a rate to fulfill the individual's demand. The amino acids that were reported from the analysis were classified as being essential for red abalone based on the research of Allen and Kilgore (1975). The literature on abalone regarding essential fatty acids (EFAs) suggests that linoleic, linolenic, arachidonic, eicosapentaenoic (EPA) as well as other n-3/n-6 fatty acids are critical for growth (Uki et al. 1986, Hanna and Sinclair 1996, Mai et al. 1996, Dunstan et al 1996, Nelson et al. 2002 and Duraza-Beltran et al. 2003). As such, these EFAs are the only fatty acids reported from the analysis. A nonparametric Wilcoxon rank sum test was used to test for a significant difference in medians between bull kelp and dulse treatments for each nutritional measure. The formulated feed treatment could not be included because there was no replication (n=1) for those samples. The Wilcoxon tests were conducted on all essential fatty and amino acids for the diet and tissue samples. The Wilcoxon test was used instead of a two-sample t test as a more conservative test because of the low sample sizes for diet tissues (n=2 for each treatment) and feed sampled (n=3 for each treatment). All non-essential amino acids and fatty acids are reported in Appendix C and Appendix D, respectively.

RESULTS

Diets and Growth

The abalone used in this study had an initial mean weight of 0.34 ± 0.08 g (mean ± SD), shell length of 12.4 ± 1.0 mm, and depth of 2.9 ± 0.4 mm (measured on October 2). There was no significant difference in starting weight ($F_{2,24}$=0.09, P = 0.92), length ($F_{2,24}$=0.02, P = 0.98) and depth ($F_{2,24}$=0.40, P = 0.67) among the three treatments (Table 1).

The abalone that were fed dulse experienced better water clarity since the dulse was actively proliferating and removing organics from the water column. The bull kelp and formulated tanks required more maintenance and cleaning due to decomposition of the feeds which could have contributed to adverse water quality conditions.

A replacement line of abalone was used to replace two abalone that experienced premature mortality. Following the replacement period, all mortality was recorded without replacement. Abalone in the formulated treatment had the greatest mortality at 22% at the completion of the study while the bull kelp and dulse had markedly less mortality at 7.4% and 5.6%, respectively (Figure 2).

Abalone fed dulse had a final mean weight of 3.17 ± 1.04 g, length of 27.44 ± 3.24 mm, and depth of 4.64 ± 0.54 mm (Table 2, Figures 3-5). The abalone on the two macroalgae diets grew at relatively the same pace for the first half of the study (Figures 3 and 4). After month 4 (January 2), the abalone in the dulse treatment begin to separate

slightly from the bull kelp treatment and started out-performing (on average) the other treatments for all variables.

Diet treatment had a significant effect on the final body size variables (weight, length, depth) based on the mixed-effects model ANOVA (Table 1). Results from post-hoc pairwise comparisons indicated that abalone in the formulated feed treatment were significantly smaller in weight ($P < 0.05$), length ($P < 0.05$) and depth ($P < 0.05$) than both the bull kelp and Pacific dulse treatments (Table 1). Abalone in the dulse treatment were not significantly different from bull kelp abalone in terms of final weight, final shell length or final shell depth (Table1).

The abalone on the formulated feed were significantly smaller than those on the macroalgae feeds. Abalone on the formulated feed had significantly less growth in weight gain per day ($P < 0.05$), length gain per day ($P < 0.05$), and depth gain per day ($P < 0.05$) than those in the bull kelp and dulse treatments (Table 1, 3). Post-hoc pairwise comparisons showed that abalone fed dulse had significantly higher ($P < 0.05$) weight gain per day (16.3 ± 5.9 mg per day) compared to both bull kelp and formulated feed treatments (Table 1). The dulse treatment yielded slightly higher growth per day in terms of shell length (86.9 ± 18.4 μm per day) and shell depth (10.1 ± 3.3 μm per day) than the dull kelp treatment (Table 3), but these differences were not significant. Abalone in the dulse treatment had a significantly higher ($P < 0.05$) body weight to shell length ratio at 0.11 ± 0.03 $g \cdot mm^{-1}$ as compared to the formulated and bull kelp treatments. Thus, abalone in the dulse treatment had a higher average tissue mass per unit of shell length.

The dulse and bull kelp treatments had their lowest monthly growth rates in terms of weight gain for the first-month interval, but the monthly growth rateincreased as the study progressed (Figure 6). Bull kelp abalone had the highest initial length change for the first month, 106.8 $\mu m \cdot day^{-1}$, but displayed more of a decrease for subsequent intervals than dulse-fed abalone. Dulse-fed abalone had the highest final differential growth values for weight change, 25.6 $mg \cdot day^{-1}$, and length change, 84 $\mu m \cdot day^{-1}$. The fresh macroalgae treatments showed similar trends for monthly length change decreasing over the length of the study (Figure 6). The abalone in the formulated treatment did not display significant increases in length change by monthly intervals.

Weight by length relationships indicated differential growth patterns across treatments (Figure 7). From the growth curves and the higher growth parameter, b, dulse and bull kelp were seen to outperform the formulated abalone. The results of the mixed-effects ANCOVA for log BW indicated that the formulated treatment had a significantly lower slope or growth parameter b ($P < 0.05$) than the bull kelp or dulse treatments. Dulse had the highest growth parameter of 2.79 while the formulated treatment had the lowest growth parameter of 2.56 (Table 4). This indicates that formulated-fed abalone growth in weight per unit length was significantly lower as compared with the other treatments. When the formulated data was excluded from the analysis, it was determined that the slopes (i.e., b parameter) for the dulse and bull kelp treatments were not significantly different from one another. After forcing the slopes for the dulse and bull kelp treatments to be equal, model results indicated that abalone in the dulse treatment were 3% heavier

at a given SL than those in the bull kelp treatment, but the difference was marginally not significant (P=0.060).

Consumption of diets was calculated by measuring the feed that went into each tank and the feed that was removed after 2 days. For consumption of diets across the three treatments, the formulated feed was significantly under-consumed (0.08 ± 0.13 g·g^{-1}·day^{-1} ; ANOVA, $F_{2,24}=87.3$, $P < 0.05$) when compared with the macroalgae diets (Figure 8). Bull kelp consumption rate averaged at 0.63 ± 0.25 g·g^{-1}· day^{-1} while dulse abalone consumed 0.71 ± 0.26 g·g^{-1}·day^{-1}. There was no significant difference in consumption between the abalone fed bull kelp or those fed dulse.

Amino Acids

The total essential amino acids and total amino acids for the abalone tissue and feed samples (Table 5) highlighted the effectiveness of abalone to uptake various nutrients. All amino acids that were tested and reported through the analysis are included in Appendix C. At the end of the experiment, there was only enough biomass for one tissue sample for the formulated feed treatment, so there is only one value for all formulated tissue samples with no standard deviation. Dulse and bull kelp abalone tissues (two replicates) and diet samples (three replicates) were tested for significance differences in the essential amino acids reported. The results from the Wilcoxon non-parametric test indicated that there was no significant difference between the treatments for any of the reported essential amino acids. This is most likely reflective of the low

sample sizes that greatly reduced the power of the statistical test; however, comparisons of amino acids can still be informative of nutritional differences.

Abalone tissue samples in the dulse treatment had elevated levels of all essential amino acids (EAA) (Table 5). Formulated tissue samples were lower in all essential amino acids, with bull kelp serving as an intermediate between dulse and formulated. dulse tissue samples had the highest percentages of total essential amino acids, 55 ± 0.4 %, as well as total amino acids, 21.7 ± 0.2%. Conversely, the formulated tissues were lowest in total essential amino acids, 19.4%, and total amino acid counts, 50% (Table 5). Abalone tissue samples from the formulated feed treatment had the highest level of crude fat, 5.4%, and the lowest level of crude protein, 52.7%. Dulse tissues were the lowest for crude fat at 4.2 ± 0.1%, with the highest crude protein at 55 ± 1.8 % (Table 5).

Contrasting what was seen in the tissue samples, the formulated feed had higher total essential amino acids, 9.1%, and total amino acids, 23.2% (Table 5). Bull kelp was notably lower in total essential amino acids, 4.6 ± 0.7 %, and total amino acids at 10.7 ± 1.3% with dulse seen as an intermediate between bull kelp and formulated feed. Bull kelp was considerably lower in all essential amino acids than the values for the other feeds. The formulated feed was higher in Leucine at 2.5% which was noticeably higher than bull kelp, 0.8 ± 0.1%, and dulse, 1.3 ± 0.2. Dulse showed elevated values for Lysine, 1.5 ± 0.1%, as compared with the bull kelp, 0.7 ± 0.1%, as well as the formulated feed, 0.8% (Table 5). Bull kelp had a higher amount of crude fat, 3.1 ± 0.9%, with the lowest crude protein, 12.5 ± 1.3%. The formulated pellets had the lowest level of crude fat, 0.7%, and highest level of crude protein at 24.3% (Table 5).

Fatty Acids

The varying levels of fatty acids as seen in the diets compared to the abalone tissues (Table 6) demonstrate how readily abalone incorporate the fatty acids of their treatment diet into their tissues. Appendix D contains all essential and non-essential fatty acids tested during the analysis. Bull kelp and dulse abalone tissues (two replicates) and diet samples (three replicates) were tested for significant differences in essential fatty acids (Table 6). Results from the non-parametric analysis showed that there was no significant difference between the treatments for tissue or diet analysis regarding any of the essential fatty acids (Table 6). The lack of statistical significance is once again indicative of too small of a sample size for appropriate statistical evaluation, but comparisons are still informative.

Formulated abalone tissues were noticeably higher in Linoleic (18:2n6) and 3n-Arachidonic (20:4n3) and were the only tissue samples that had any levels of Docosahexaenoic acid or DHA (22:6n3). Conversely, these tissues were the lowest in levels of Arachidonic (20:4n6), Eicosapentaenoic acid or EPA (20:5n3), and Clupanodonic (22:5n3). Dulse tissues had elevated EPA, 13.5 ± 0.5%, and Clupanodonic acid, 9.1 ± 0.5% , at almost double the values seen in the other tissue samples (Table 6). Dulse tissues were lower in C:18 fatty acids as compared with the other treatments. Bull kelp tissues were seen to have the highest values for Linolenic (18:3n3) and Arachidonic acid.

Dulse was lower in all C:18 fatty acids when compared with the other diets (Table 6). The formulated pellets had a drastically higher proportion of Linoleic, 28.8%, as compared to bull kelp, 7.8 ± 1.1 %, and Dulse, 0.6 ± 0.1%. Formulated feed was devoid of Stearodonic (18:4n3) and 3n-Arachidonic acid. Bull kelp and dulse were not found to have any trace of 3n-Arachidonic, Clupanodonic, or DHA (Table 6). Dulse had substantially higher values for Arachidonic, 12.4 ± 2.6%, and EPA, 24.2 ± 14.3%. The large standard deviation for the EPA values of dulse was due to the highly variable seasonality as it increased in percentage throughout the duration of the study. The initial dulse had 13.2% EPA as total fat, with the final sample having 40.4% EPA. This was the only fatty acid that had this large of a seasonal discrepancy across any tissue or feed samples. As in the tissue samples, bull kelp had the highest values for Linolenic and Stearodonic acid (Table 6). Bull kelp tissue samples had the highest proportion of n-3 and n-6 fatty acids, 46.4%, while the bull kelp diet samples were the lowest at 32.2%. The formulated diet had the highest percentage of n-3 and n-6 fatty acids, 45.7%, with the formulated abalone tissue samples the lowest of the treatments at 41.7%.

Table 1: P-values for average body weight (BW), shell length (SL), and shell depth as well as growth rates and BW/SL ratio reported for statistical significance in this study. A mixed-effects model ANOVA test was carried out with 26 degrees of freedom for the final and initial variables. Post-hoc pairwise comparisons were conducted to assess the significance among the three treatment levels. Pairwise comparisons are denoted by letters for each treatment: B indicates bull kelp, D indicates dulse and F indicates formulated. All significant values at $P < 0.05$ are bolded. All values listed as <<0.001 were less than 1.0e-5.

Test		Weight (g)	Length (mm)	Depth (mm)	Weight gain (mg/day)	Length gain (μm/day)	Depth gain (μm/day)	BW/SL Ratio
ANOVA	Final	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**
	Initial	0.879	0.976	0.362				
Pairwise	B - D	0.059	0.082	0.120	**0.047**	0.075	0.443	**0.0386**
	B - F	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**
	D - F	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**	**<<0.001**

Table 2: Final mean variable values (± standard deviation) for abalone in the bull kelp, dulse, and formulated treatments . Body weight to shell length (BW/SL) ratio is shown. Significant differences ($P < 0.05$) in a column are indicated by different superscripts.

Treatment	Weight (g)	Shell Length (mm)	Shell Depth (mm)	BW/SL (g/mm)
Bull Kelp	2.72 ± 1.04^{a}	26 ± 4.17^{a}	4.45 ± 0.61^{a}	0.10 ± 0.03^{a}
Formulated	1.07 ± 0.37^{b}	18.96 ± 2.62^{b}	3.54 ± 0.44^{b}	0.05 ± 0.013^{b}
Dulse	3.17 ± 1.04^{a}	27.44 ± 3.24^{a}	4.64 ± 0.54^{a}	0.11 ± 0.03^{c}

Table 3: Mean daily growth values (± standard deviation) for abalone in bull kelp, dulse, and formulated treatments. Significant differences ($P < 0.05$) in a column are indicated by different superscripts.

Treatment	Weight (mg/day)	Shell Length (μm/day)	Shell Depth (μm/day)
Bull Kelp	13.8 ± 5.8^{a}	78.9 ± 22.9^{a}	9.4 ± 3.8^{a}
Formulated	4.2 ± 2^{b}	37.9 ± 14.5^{b}	3.9 ± 3.3^{b}
Dulse	16.3 ± 5.9^{c}	86.9 ± 18.4^{a}	10.1 ± 3.3^{a}

Table 4: Parameter estimate for the allometric growth curve for all bull kelp, dulse, and formulated treatments ± standard error.

Treatment	Constant (a)	Growth Parameter (b)
Bull Kelp	-7.98	2.72
Formulated	-7.55	2.56
Dulse	-8.14	2.79

Table 5: Amino acid analysis of abalone tissue and diet with individual essential amino acids (EAA), total EAA, total amino acids, crude fat, and crude protein for bull kelp, dulse, and formulated feed treatments. All values expressed as % (± standard deviation). Crude protein = %N x 6.25. Wilcoxon rank sum test results indicated that bull kelp and dulse diet and tissue samples were not significantly different for any of the essential amino acids. Standard deviations listed as 0 represent values <0.05. Superscript of T indicates tissue sample while D is for diet samples.

	Bull Kelp^{T}	**Dulse^{T}**	**Formulated^{T}**	**Bull Kelp^{D}**	**Dulse^{D}**	**Formulated^{D}**
Threonine	2.1 ± 0	2.2 ± 0	2	0.5 ± 0.1	0.9 ± 0.1	0.8
Valine	2.2 ± 0	2.3 ± 0	2	0.7 ± 0.1	1.2 ± 0.1	1.1
Methionine	1 ± 0	1.1 ± 0	1	0.2 ± 0	0.4 ± 0.1	0.4
Isoleucine	1.9 ± 0	2 ± 0	1.8	0.5 ± 0.1	0.8 ± 0.1	0.9
Leucine	3.2 ± 0.1	3.5 ± 0	3.1	0.8 ± 0.1	1.3 ± 0.2	2.5
Phenylalanine	1.7 ± 0	1.9 ± 0	1.6	0.5 ± 0.1	0.9 ± 0.1	1.2
Lysine	3.1 ± 0.1	3.3 ± 0	2.9	0.7 ± 0.1	1.5 ± 0.1	0.8
Histidine	0.8 ± 0	0.9 ± 0	0.8	0.2 ± 0	0.4 ± 0	0.4
Arginine	4.3 ± 0.1	4.6 ± 0.1	4.2	0.5 ± 0.1	1.3 ± 0.1	1
Total EAA	20.4 ± 0.3	21.7 ± 0.2	19.4	4.6 ± 0.7	8.7 ± 0.9	9.1
Total AA	51.5 ± 0.3	55 ± 0.4	50	10.7 ± 1.3	19.8 ± 1.4	23.3
Crude Fat	5.3 ± 0.7	4.2 ± 0.1	5.4	3.1 ± 0.9	2 ± 0.7	0.7
Crude Protein	55 ± 1.8	57.6 ± 1.2	52.7	12.5 ± 1.3	20.6 ± 0.8	24.3

Table 6: Fatty acid analysis of abalone tissue and diet (expressed as percent of total fat) for bull kelp, dulse, and formulated treatments ± standard deviation. The percentage of n-3 and n-6 polyunsaturated fatty acids for the lipid content is reported. Wilcoxon rank sum test results indicated that bull kelp and dulse diet and tissue samples were not significantly different for any of the essential fatty acids. Standard deviations listed as 0 represent values <0.05. Superscript of T indicates tissue sample while D is for diet samples.

	Bull Kelp^{T}	**Dulse^{T}**	**Formulated^{T}**	**Bull Kelp^{D}**	**Dulse^{D}**	**Formulated^{D}**
Linoleic (18:2n6)	3.8 ± 0.4	2 ± 0	9.2	7.8 ± 1.1	0.6 ± 0.1	28.8
Linolenic (18:3n3)	3 ± 0.1	0.4 ± 0.1	1.2	3.7 ± 0.8	0 ± 0.1	1.7
Stearidonic (18:4n3)	3.3 ± 0.2	0.1 ± 0.1	1.7	4.7 ± 1.2	0.1 ± 0.1	0
Arachidonic [20:4n6]	10.8 ± 0.2	8.3 ± 0	4.6	7.9 ± 0.7	12.4 ± 2.6	1.4
3n-Arachidonic (20:4n3)	4.4 ± 0.2	3.7 ± 0.2	5.5	0	0	0
EPA (20:5n3)	7.3 ± 0.3	13.5 ± 0.5	6.4	4 ± 0.2	24.2 ± 14.3	4.5
Clupanodonic (22:5n3)	5.9 ± 0.3	9.1 ± 0.5	4.6	0	0	0.5
DHA (22:6n3)	0	0	2	0	0	5.8
N-3/N-6 %	46.4	45.2	41.7	32.2	43.7	45.7

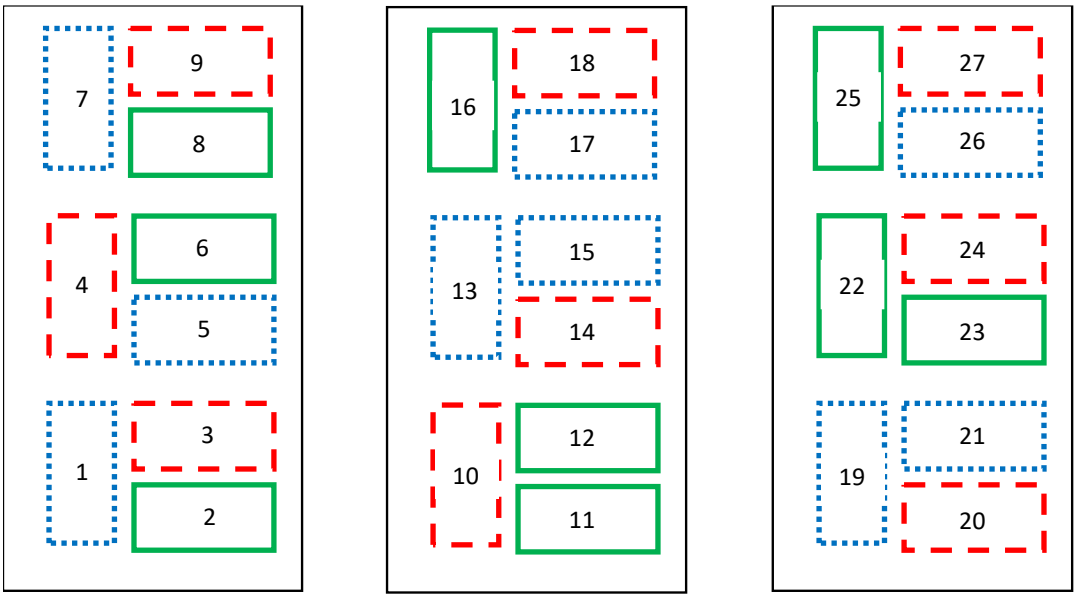

Figure 1: Layout of varied treatment tanks (smaller, colored rectangles) distributed among three table tanks (large black rectangles) at the Humboldt State Marine Laboratory. Green (solid lines) indicating treatment 1, naturally harvested kelp as a control. Blue (dotted lines) indicating treatment 2, ABKelp® formulated diet for abalone. Red (dashed lines) indicating treatment 3, IMTA produced macroalgae *Palmaria mollis*. Arrangement of treatment tanks was randomly assigned within each table tank to avoid bias.

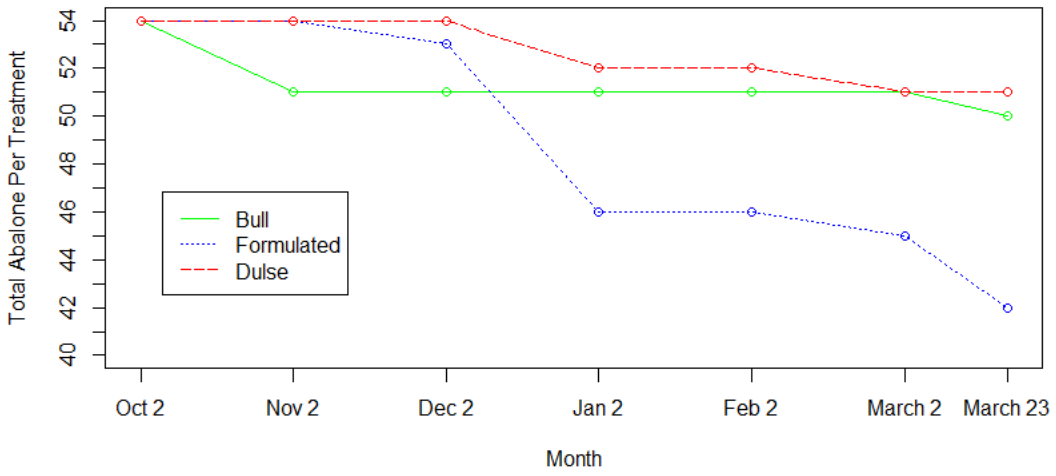

Figure 2: Total living abalone per treatment across the duration of the study. Abalone survival was checked at every monthly interval.

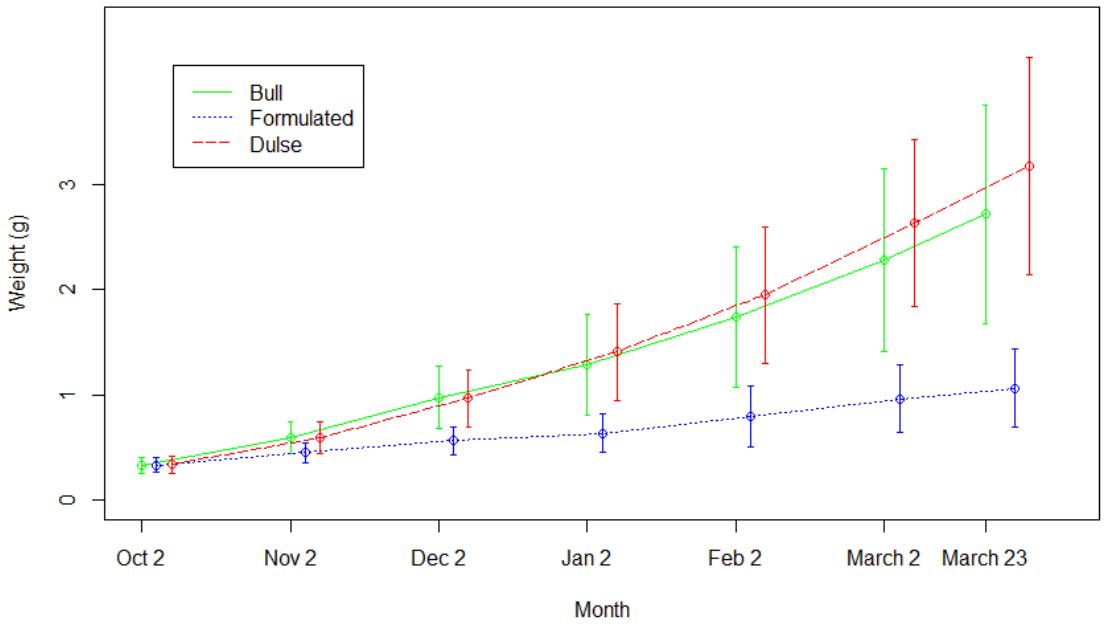

Figure 3: Weight of juvenile red abalone (*Haliotis rufescens*) in grams ± SD for all three treatments over an almost 6-month period. Data points are shifted to better visualize the standard deviation between treatments.

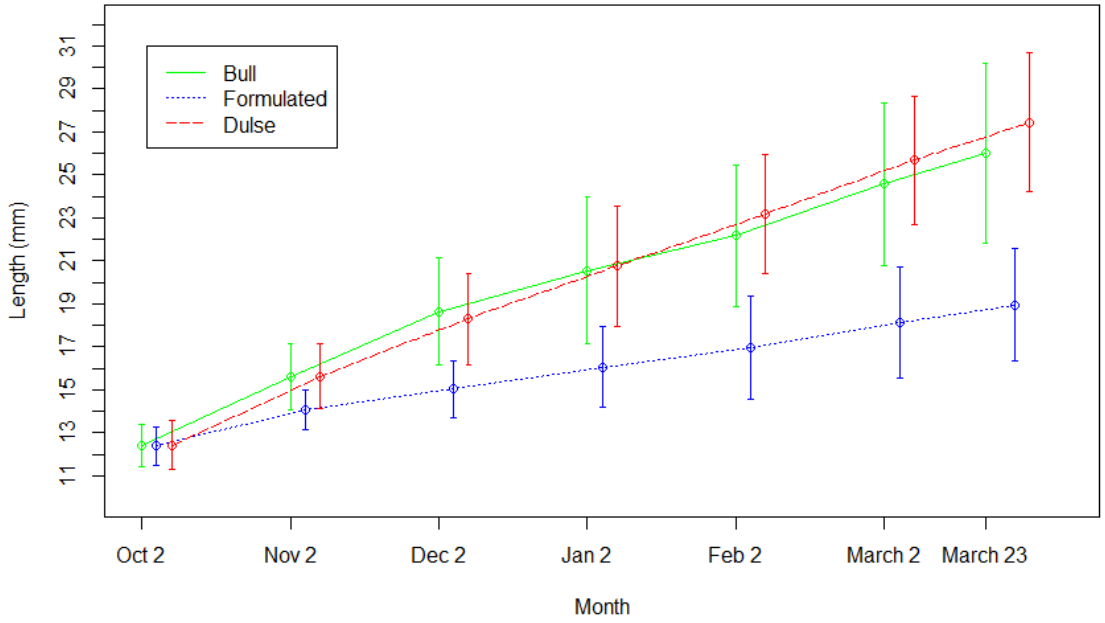

Figure 4: Length of juvenile red abalone (*Haliotis rufescens*) in millimeters ± SD for all three diet treatments over an almost 6-month period. Data points are shifted to better visualize the standard deviation between treatments.

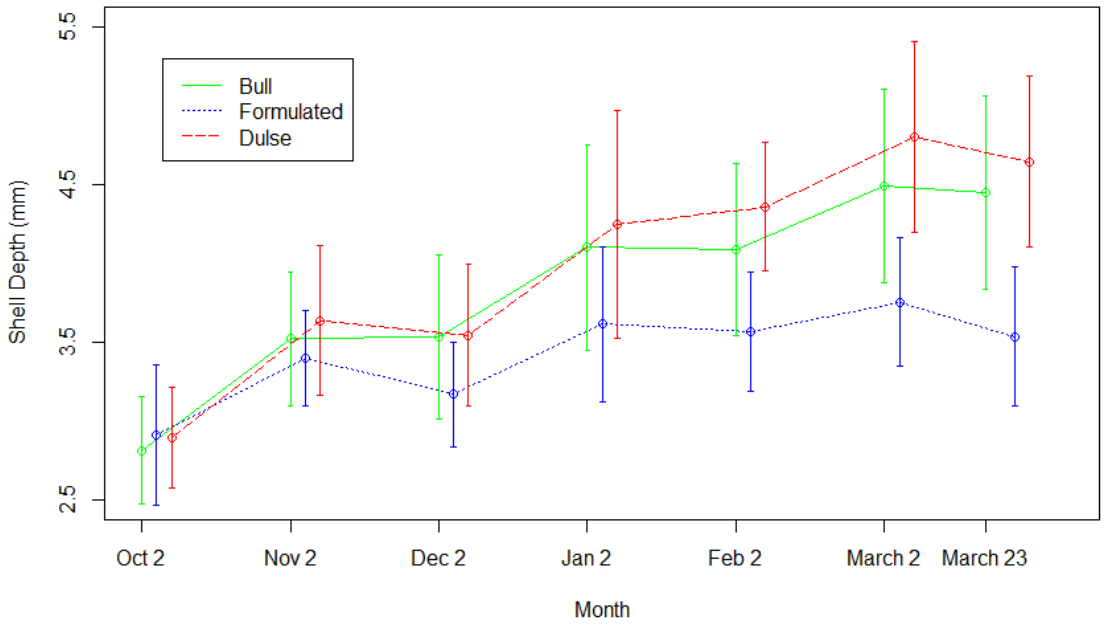

Figure 5: Depth of juvenile red abalone (*Haliotis rufescens*) depth in millimeters ± SD for all three treatments over an almost 6-month period. Data points are shifted to better visualize the standard deviation between treatments.

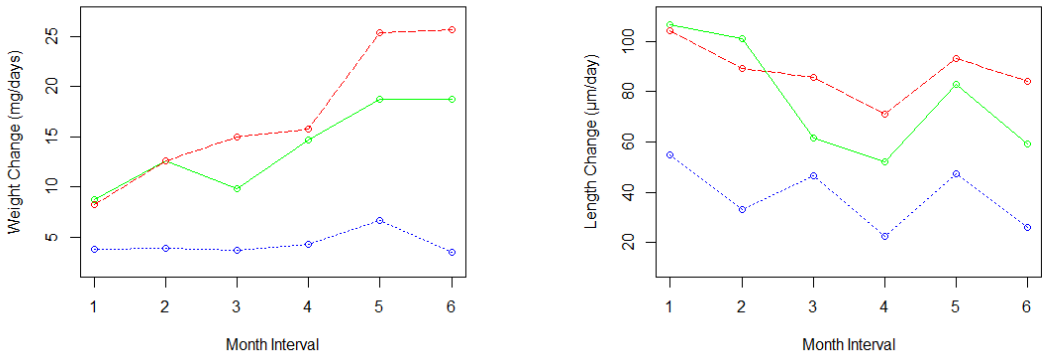

Figure 6: Average growth rates in terms of weight change per day (mg·day-1) and length change per day (µm·day-1) by treatment for subsequent month intervals. The left plot represents weight change while the right plot is length change.

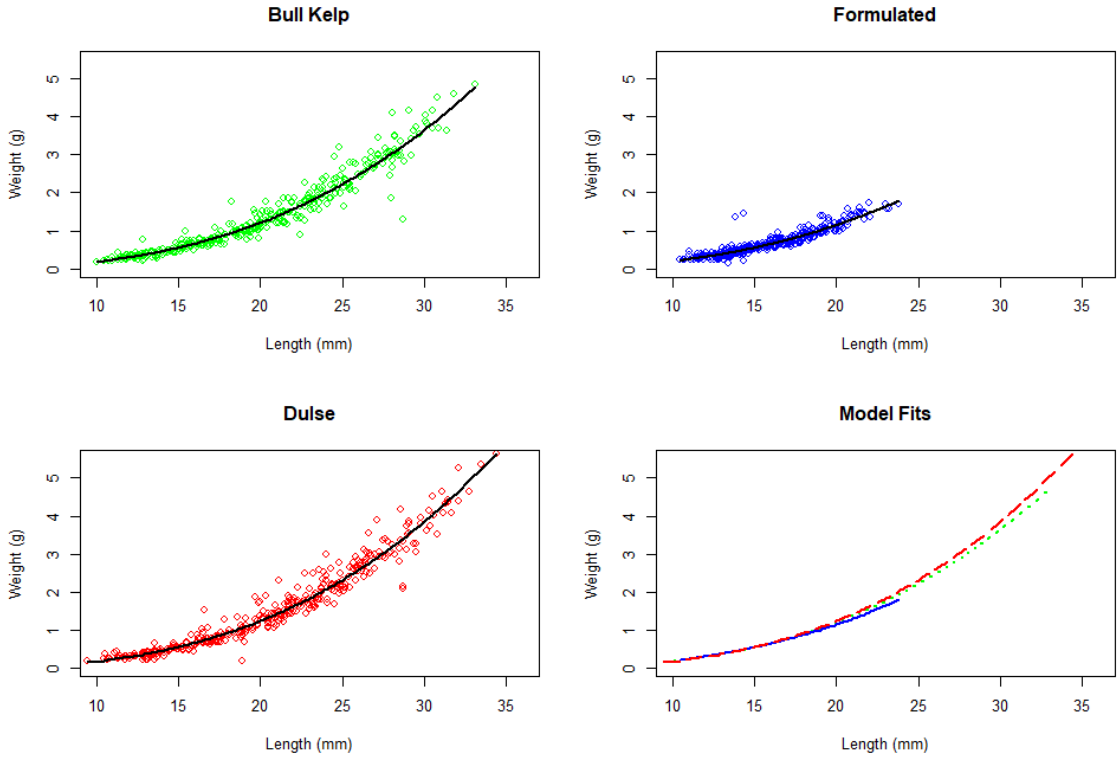

Figure 7: Weight (g) by Shell Length (mm) growth curves for the three treatments (bull kelp, dulse, and formulated). The lines represent an allometric growth model curve for each treatment. The points represent repeated measurements of all abalone throughout the length of the study. The lower right panel is a comparison between the model fits for the three treatments.

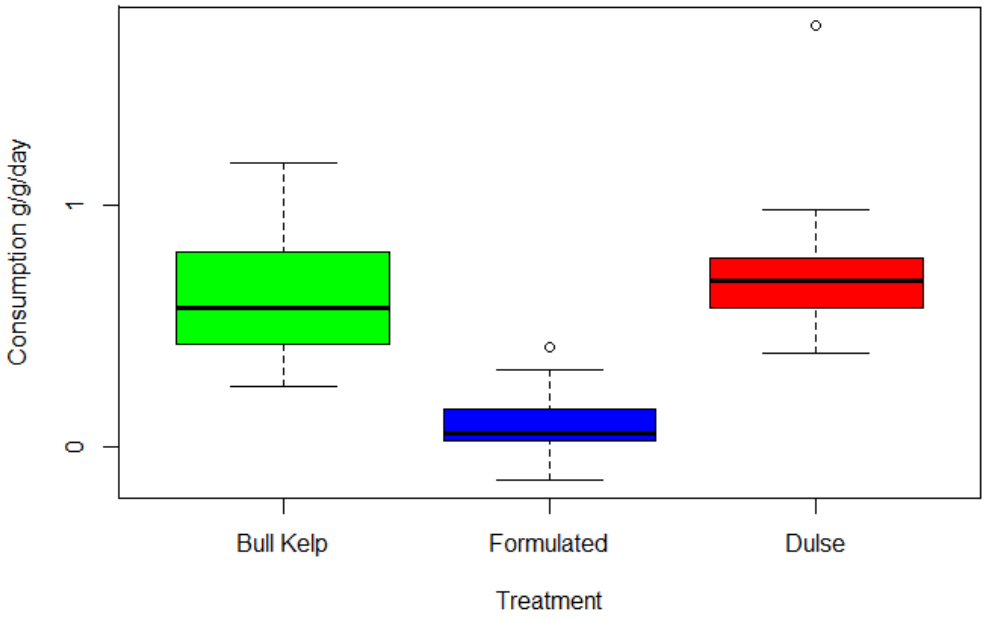

Figure 8: Feed consumed by abalone in bull kelp, formulated, and dulse treatments across the three sampling periods (grams consumed·grams abalone-1·day-1).

DISCUSSION

Diets and Growth

Commercial aquaculture of abalone relies on high growth rates to ensure that cultured abalone attain market size within a timeframe that is economically viable. As such, understanding the growth rates based on feed sources has been a topic of significant importance. This study confirms what other research has shown in that dulse supports faster growth in abalone as compared bull kelp (Uki et al. 1986, Rosen et al. 2000). The abalone grew as well, if not greater, on dulse than bull kelp. Not only did they exhibit greater weight gain per day, but the abalone that were fed dulse had a significantly higher BW/SL ratio, and thus could have more economic value. The similar growth between the macroalgae diets early on may be due to the abalone becoming acclimatized to the novel feed source. Research has shown that, when given the option, red abalone prefer brown algae (bull kelp) over red (dulse) (Tenore 1976). Since the abalone were accustomed to a brown algae diet, it might have taken them time to fully accept the dulse. Given the trend for dulse fed abalone becoming larger than those fed bull kelp, had the study gone on longer, there might have been more significant discrepancies in final average variable measurements.

While the growth between the two macroalgae diets were not significantly different for all measured variables, the dulse was produced entirely without relying on wild populations of seaweed while the bull kelp was collected from the wild. The dulse

was grown in an IMTA system that supported efficient utilization of nutrients such that the waste products of Sablefish acted as fertilizer and reduced the overall waste produced by the system. Commercial aquaculture has been reliant on wild populations of bull kelp as a feed source and continue to harvest them in large quantities (Elbert 1992). Wild kelp populations are dwindling (Kruhamsal et al. 2016) and kelp forests are susceptible to considerable declines from natural events such as El Niño (Elbert 1992). The implementation of sustainably produced dulse for abalone supports growth equivalent to wild-harvested bull kelp, and it would alleviate pressure on wild kelp populations and allow abalone production to exist in regions where kelp is not abundant. While there has been research on the effectiveness of IMTA-produced algae (Evans and Langdon 2000, Macchiavello and Bulboa 2014), the potential for finish integration into these systems has not been fully explored. The current study used macroalgae that was produced using sablefish effluent; future research needs to evaluate the nutrient analysis of dulse and other macroalgae produced with effluent from other finfish to verify if this system is successful with other species.

The present study demonstrates that formulated diets in an extruded pellet form may not be a suitable feed source for juvenile red abalone of this size class. The formulated feed that was used was specifically formulated for juvenile abalone, and the size of the extruded pellets (10 mm) was recommended by AlgaMar. Many previous studies have shown that formulated diets can produce various *Haliotis* spp. as efficiently or more efficiently than fresh macroalgal diets with the potential to drastically reduce the time it takes to reach a marketable size (Viana et al. 1996, Chen and Lee 1999, Garcia-

Esquivel and Felback 2007, Jackson et al. 2007, Vivanco-Aranda et al. 2011). The primary reason why abalone fed formulated pellets did not grow as efficiently as those on macroalgal diets due to significantly less consumption and not due to poor nutritional quality. These pellets were formulated to support abalone growth, as evidenced by their nutrient composition; however, the size or consistency of the feed may have been the reason that this treatment experienced the lowest growth rates. The pellets, 10 mm in length, were roughly the same size of the abalone at the start of the experiment and were in a drastically different form from their normal diet (fresh giant kelp). If the formulated feed was compressed into a thinner presentation, the abalone of this size may have found it more eatable This shape would allow for abalone to crawl over the surface and scrape away food, as was seen in the macroalgae diets. A thinner form (i.e., sheet or flakes) with greater surface area may have not only encouraged feeding but could have drastically reduced the amount of mortality that this treatment experienced. While the nutrition of the diet was sound, it is always critical to understand the way that cultured animals interact with food to support optimal growth for all life stages.

Amino Acids and Nitrogen Content

Nitrogen content, as protein, is recognized as determining and limiting growth of herbivorous animals and is a critical aspect for diet formulation of abalone (Mattson 1980, Shpigel 1999). Insufficient nitrogen from the diet can limit abalone growth when fed macroalgae diets (Fleming 1995, Britz and Hecht 1997). Composition analysis of abalone body tissues and feed is a useful proxy to understand the amino acids that

animals need in their diet (NRC 2011). The proportion of amino acids in the diet as compared to what is seen in the tissues helps to better understand which amino acids are limiting for abalone growth. The tissue analysis showed that dulse-fed abalone had the highest amount of essential amino acids and the highest total amount of amino acids, including non-essential ones. Dulse has been seen to have higher protein content than other macroalgae fed to abalone (Rosen et al. 2000). When grown in an IMTA system, it has elevated protein levels due to increased nitrogen from waste products (Evans and Langdon 2000, Macchiavello and Bulboa 2014). The increased protein content of the algae as well as increased protein due to IMTA culture helps explain why the dulse and bull kelp were so different in terms of protein content and nitrogen.

Optimal dietary protein levels for abalone diets have been seen to range from 27-35% (Uki and Watanabe 1992, Mai et al. 1995, Coote et al. 2000). The closest diet to this optimum was the formulated feed, followed by the dulse. While the formulated feed had the highest protein content and essential amino acids, it was still seen to produce the lowest quality abalone tissues with lower essential amino acids than both macroalgae diets. The discrepancy between the nutrient levels in the feed as compared with the tissue samples is most likely a result of a lack of consumption of the formulated feed.

Research on amino acids and abalone has indicated that some amino acids can be limiting in the diets of abalone. In terms of essential amino acids, lysine, histidine, and methionine have been documented to be limiting in algal diets (Brown 1991, Angel et al. 2012). These three essential amino acids were lowest in the bull kelp diet, which might further explain why the abalone that were fed bull kelp were not as large as those fed

dulse. Dulse and formulated pellets were similar in concentrations of these potentially limiting amino acids. Dulse had nearly double the concentration of Lysine than the other two diets (1.5% vs. 0.7-0.8%). Lysine has been seen to be particularly limiting in algae feeds of abalone (Brown 1991). The noticeably elevated levels of Lysine in dulse further show that IMTA produced Dulse is a nutritionally advantageous food source for juvenile red abalone in that coculture with finfish compensates for nutrients that are typically insufficient in the diet.

Fatty Acids

Proper nutrition is critical to ensure optimal growth for cultured abalone. Previous studies on abalone investigating essential fatty acids support that n-3 and n-6 C,18 poly unsaturated fatty acids, as well as C20 and C22 highly unsaturated fatty acids are critical for abalone growth (Uki et al. 1986, Hanna and Sinclair 1996, Mai et al. 1996, Dunstan et al. 1996, Nelson et al. 2002 and Duraza-Beltran et al. 2003). There was a noticeable lack of C18 fatty acids in both the diet and tissue samples for the dulse treatment. This coupled with the fact that the dulse abalone were seen to be larger on average, suggests that C18 fatty acids are less relevant for weight gain and growth for juvenile red abalone. This would confirm what other studies have shown in *H. discus hannai* on the greater importance for n-3 highly unsaturated fatty acids to improve abalone growth (Mai et al. 1996).

Abalone have been shown to biosynthesize differential fatty acids through desaturation and elongation with the ability to convert short-chain fatty acids into longer

chain fatty acids (Uki et al. 1986, Mai et al. 1996, Xu et al. 2004). The significantly higher levels of clupanodonic acid in the dulse-fed abalone tissues, while it was not present in the dulse feed, suggest that juvenile red abalone can biosynthesize this fatty acid from EPA due to the structural similarity of the fatty acids. Some research on *H. discus hannai* has suggested that abalone can synthesize DHA (22:6n-3) from EPA (20:5n-3) (Xu et al. 2011). The formulated feed was the only feed to have DHA present, which was seen in the corresponding tissue samples for that feed. None of the fresh macroalgal diets, nor their corresponding tissue samples, had any DHA present. Since the dulse and bull kelp feeds had EPA present, it is possible that juvenile red abalone do not have the mechanism to convert EPA or shorter-chain polyunsaturated fatty acids into DHA. The greater importance of EPA and the lack of DHA in the macroalgae diets support previous studies that suggest that abalone require 20:5n-3 in the diet rather than 22:6n-3 and that DHA may not be appropriate for abalone growth (Uki et al. 1986, Viana et al. 1993). Future research into juvenile red abalone and sustainably produced macroalgae should incorporate fresh seaweed diets with and without DHA to see if this is an essential fatty acid for this species.

CONCLUSIONS

This study confirms that IMTA produced Pacific dulse *Palmaria mollis* is a good feed source for juvenile red abalone greater than 10 mm shell length in an aquaculture setting. The feasibility of large-scale macroalgae production must be addressed if IMTA systems are to be implemented in current or future abalone farms such that they produce feed efficiently enough to remove reliance on wild macroalgae harvest. The formulated feed that was used, ABKelp®, was nutritionally developed to support juvenile abalone. While it was more nutritionally balanced compared to dulse, the pelleted form in which the formulated feed was presented resulted in low consumption rates and poor growth. Had the diet been prepared in a more desirable form for juveniles, this treatment would have likely seen greater growth and less mortality. Should this study be replicated, the employment of diets of different species of macroalgae might show interesting results. Other studies have shown that mixed algae diets can be beneficial because different feed sources provide different essential nutritional components. Further research is required to investigate the ability of juvenile red abalone to biosynthesize DHA from other fatty acid substituents and the overall importance of DHA for this life stage for this species of abalone.

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APPENDIX A

Appendix A: All amino acids as well as crude fat and crude protein reported in the analysis from the University of Missouri for the bull kelp and dulse diet samples. The nutrients from the three samples of bull kelp and dulse are reported as they changed throughout the length of the study as a response to seasonality. Fresh samples were collected and stored for analysis on October 17th, 2019, January 26th , 2020, and March 23rd, 2020. All values expressed as %. Crude protein*= %N x6.25.

Nutrient	Bull Kelp 10/17/19	Bull Kelp 1/26/20	Bull Kelp 3/23/20	Dulse 10/17/19	Dulse 1/26/20	Dulse 3/23/20
Taurine	0.13	0.13	0.14	0.09	0.10	0.09
Hydroxyproline	0.08	0.06	0.08	0.02	0.07	0.06
Aspartic Acid	1.32	0.96	1.09	2.68	2.78	2.91
Threonine	0.59	0.46	0.49	0.94	0.78	0.92
Serine	0.54	0.43	0.46	1.11	0.88	1.00
Glutamic Acid	1.57	1.25	1.49	2.09	1.71	1.96
Proline	0.50	0.41	0.43	0.87	0.75	0.93
Lanthionine	0.00	0.00	0.00	0.00	0.00	0.01
Glycine	0.68	0.53	0.59	1.31	1.23	1.36
Alanine	1.53	1.33	1.53	1.65	1.38	1.68
Cysteine	0.20	0.14	0.15	0.52	0.70	0.80
Valine	0.76	0.57	0.61	1.29	1.09	1.30

Nutrient	Bull Kelp 10/17/19	Bull Kelp 1/26/20	Bull Kelp 3/23/20	Dulse 10/17/19	Dulse 1/26/20	Dulse 3/23/20
Methionine	0.24	0.19	0.22	0.41	0.31	0.42
Isoleucine	0.56	0.43	0.44	0.90	0.73	0.89
Leucine	0.94	0.74	0.77	1.49	1.14	1.40
Tyrosine	0.33	0.23	0.20	0.78	0.77	0.89
Phenylalanine	0.63	0.47	0.47	0.91	0.75	0.89
Hydroxylysine	0.00	0.00	0.00	0.00	0.00	0.00
Ornithine	0.01	0.00	0.00	0.01	0.02	0.02
Lysine	0.76	0.58	0.64	1.38	1.41	1.64
Histidine	0.22	0.16	0.16	0.36	0.42	0.45
Arginine	0.60	0.46	0.53	1.29	1.17	1.33
Total AA	12.19	9.53	10.49	20.10	18.19	20.95
Crude Protein	13.51	11.04	12.90	20.01	20.26	21.55
Crude Fat	3.40	2.12	3.76	1.83	1.40	2.77

APPENDIX B

Appendix B: All fatty acids reported in the analysis from the University of Missouri for the bull kelp and dulse diet samples. The nutrients from the three samples of bull kelp and dulse are reported as they changed throughout the length of the study as a response to seasonality. Fresh samples were collected and stored for analysis on October 17th, 2019, January 26th , 2020, and March 23rd, 2020. All values expressed as W/W%= grams per 100 grams of sample.

Nutrient	**Bull Kelp 10/17/19**	**Bull Kelp 1/26/20**	**Bull Kelp 3/23/20**	**Dulse 10/17/19**	**Dulse 1/26/20**	**Dulse 3/23/20**
C14:0	7.90	8.02	8.56	11.69	10.12	8.13
Myristoleic (9c-14:1)	0.00	0.00	0.00	0.00	0.00	0.00
C15:0	1.55	1.16	0.79	1.47	1.48	1.10
C15:1n5	0.00	0.00	0.00	0.00	0.00	0.00
Palmitic (16:0)	23.28	25.91	25.89	32.16	27.15	19.42
Palmitoleic (9c-16:1)	1.32	0.90	0.79	0.46	0.00	0.00
Palmitoleic (9c-16:1)	1.73	1.35	1.17	1.55	2.43	1.16
Margaric (17:0)	0.73	0.79	0.42	0.00	0.00	0.00
10c-17:1	0.16	0.00	0.00	0.00	0.00	0.00
Stearic (18:0)	1.61	2.08	2.94	0.72	0.35	0.45

Nutrient	Bull Kelp 10/17/19	Bull Kelp 1/26/20	Bull Kelp 3/23/20	Dulse 10/17/19	Dulse 1/26/20	Dulse 3/23/20
Elaidic (9t-18:1)	0.00	0.00	0.00	0.00	0.00	0.00
Oleic (9c-18:1)	17.46	20.56	22.53	1.43	1.17	0.95
Vaccenic (11c-18:1)	0.00	0.00	0.00	3.46	4.80	2.34
Linoelaidic (18:2t)	0.00	0.00	0.00	0.00	0.00	0.00
Linoleic (18:2n6)	7.25	7.08	9.00	0.56	0.63	0.53
Linolenic (18:3n3)	4.50	3.75	2.86	0.00	0.13	0.00
g-Linolenic [C18:3n6]	0.00	0.00	0.00	0.00	0.00	0.00
Stearidonic (18:4n3)	5.69	4.96	3.39	0.19	0.00	0.00
Arachidic (20:0)	0.47	0.60	0.73	0.00	0.19	0.20
Gonodic (20:1n9)	0.14	0.00	0.00	0.36	0.49	0.38
C20:1n11	0.00	0.00	0.00	0.00	0.00	0.00
C20:2	0.00	0.00	0.00	0.00	0.00	0.00
Homo-a-linolenic(20:3n3)	0.00	0.00	0.00	0.00	0.00	0.00
C20:3n6 Homo-g-Linolenic	0.00	0.00	0.00	0.00	0.00	0.00
Arachidonic [20:4n6]	8.19	7.07	8.30	13.67	14.09	9.33
3n-Arachidonic (20:4n3)	0.00	0.00	0.00	0.00	0.00	0.00
EPA (20:5n3)	4.02	4.26	3.85	13.24	19.00	40.35

Nutrient	Bull Kelp 10/17/19	Bull Kelp 1/26/20	Bull Kelp 3/23/20	Dulse 10/17/19	Dulse 1/26/20	Dulse 3/23/20
C21:0	0.00	0.00	0.00	0.00	0.20	0.11
Behenoic (22:0)	0.00	0.00	0.00	0.38	0.32	0.31
Erucic [22:1n9]	0.00	0.00	0.00	0.43	0.56	0.43
C22:1n11	0.00	0.00	0.00	0.00	0.00	0.00
C22:2n6	0.52	0.44	0.33	0.00	0.00	0.18
Adrenic [C22:4n6]	0.28	0.37	0.29	0.00	0.00	0.00
C22:5n6	0.00	0.00	0.00	0.00	0.00	0.00
Clupanodonic (22:5n3)	0.00	0.00	0.00	0.00	0.00	0.00
DHA (22:6n3)	0.00	0.00	0.00	0.00	0.00	0.00
C23:0	0.00	0.00	0.00	0.00	0.00	0.00
Lignoceric (24:0)	0.00	0.00	0.00	0.23	0.00	0.11
Nervonic (24:1n9)	0.16	0.18	0.00	1.91	1.28	1.49

APPENDIX C

Appendix C: All essential and non-essential amino acids produced by the analysis performed at the University of Missouri. All values expressed as %. Crude protein*= %N x6.25. Superscript of T indicates tissue sample while D is for diet samples.

	Bull Kelp^{T}	**Dulse^{T}**	**Formulated^{T}**	**Bull Kelp^{D}**	**Dulse^{D}**	**Formulated^{D}**
Taurine §	6.09 ± 0.04	6.13 ± 0.03	6.23	0.13 ± 0.01	0.09 ± 0.01	0.17
Hydroxyproline	0.87 ± 0.02	1 ± 0.05	0.90	0.07 ± 0.01	0.05 ± 0.03	0.45
Aspartic Acid	4.6 ± 0.04	4.94 ± 0.04	4.49	1.12 ± 0.18	2.79 ± 0.12	1.72
Threonine	2.14	2.24	1.99	0.51 ± 0.07	0.88 ± 0.09	0.82
Serine	2.29 ± 0.02	2.48 ± 0.04	2.22	0.48 ± 0.06	1 ± 0.12	0.97
Glutamic Acid	6.65 ± 0.13	7.07 ± 0.11	6.48	1.44 ± 0.17	1.92 ± 0.19	4.30
Proline	2.05 ± 0.045	2.23 ± 0.06	2.01	0.45 ± 0.05	0.85 ± 0.09	1.95
Lanthionine §	0.01 ± 0.01	0.01 ± 0.01	0.00	0	0	0.01
Glycine	3.59 ± 0.13	4.08 ± 0.11	3.50	0.6 ± 0.08	1.3 ± 0.07	1.49
Alanine	2.56 ± 0.02	2.74 ± 0.04	2.51	1.46 ± 0.12	1.57 ± 0.17	1.75
Cysteine	0.75 ± 0.01	0.74 ± 0.01	0.65	0.16 ± 0.03	0.67 ± 0.14	0.40
Valine	2.16 ± 0.02	2.26 ± 0.02	2.01	0.65 ± 0.1	1.23 ± 0.12	1.10
Methionine	1	1.05 ± 0.01	0.94	0.22 ± 0.03	0.38 ± 0.06	0.42

	Bull Kelp^{T}	Dulse^{T}	Formulated^{T}	Bull Kelp^{D}	Dulse^{D}	Formulated^{D}
Isoleucine	1.91 ± 0.04	2.02 ± 0.01	1.83	0.48 ± 0.07	0.84 ± 0.1	0.92
Leucine	3.24 ± 0.06	3.45 ± 0.01	3.14	0.82 ± 0.11	1.34 ± 0.18	2.54
Tyrosine	1.52 ± 0.01	1.67 ± 0.02	1.34	0.25 ± 0.07	0.81 ± 0.07	0.78
Phenylalanine	1.73 ± 0.01	1.85 ± 0.01	1.58	0.52 ± 0.09	0.85 ± 0.09	1.17
Hydroxylysine	0.18 ± 0.01	0.21 ± 0.01	0.18	0	0	0.08
Ornithine §	0.03	0.03	0.03	0	0.02 ± 0.01	0.04
Lysine	3.07 ± 0.09	3.27 ± 0.03	2.92	0.66 ± 0.09	1.48 ± 0.14	0.77
Histidine	0.83 ± 0.01	0.91 ± 0.01	0.79	0.18 ± 0.04	0.41 ± 0.05	0.44
Arginine	4.34 ± 0.1	4.62 ± 0.08	4.23	0.53 ± 0.07	1.26 ± 0.08	0.96

APPENDIX D

Appendix D: All essential and non-essential fatty acids produced by the analysis performed at the University of Missouri. All values expressed as W/W%= grams per 100 grams of sample. Superscript of T indicates tissue sample while D is for diet samples.

	Bull Kelp^{T}	**Dulse^{T}**	**Formulated^{T}**	**Bull Kelp^{D}**	**Dulse^{D}**	**Formulated^{D}**
C14:0	5.56 ± 0.07	4.9 ± 0.19	3.55	8.16 ± 0.352	9.98 ± 1.784	4.17
Myristoleic (9c-14:1)	0	0	0.00	0	0	0.00
C15:0	0.18 ± 0.05	0.8 ± 0.01	0.33	1.17 ± 0.38	1.35 ± 0.217	0.34
C15:1n5	0	0.11 ± 0.028	0.00	0	0	0.00
Palmitic (16:0)	15.33 ± 0.36	15.67 ± 0.856	13.81	25.03 ± 1.513	26.24 ± 6.418	19.88
Palmitoleic (9c-16:1)	0.41 ± 0.01	0.31	0.55	1 ± 0.28	0.15 ± 0.2666	3.25
Palmitoleic (9c-16:1)	0.71 ± 0.05	0.92 ± 0.071	1.60	1.42 ± 0.286	1.71 ± 0.651	0.00
Margaric (17:0)	6.51 ± 0.03	6.85 ± 0.148	7.12	0.65 ± 0.199	0	0.33
10c-17:1	0.05 ± 0.06	0.17 ± 0.141	0.06	0.05 ± 0.092	0	0.21
Stearic (18:0)	4.88 ± 0.18	4.6 ± 0.318	5.41	2.21 ± 0.674	0.51 ± 0.191	3.13
Elaidic (9t-18:1)	0.17 ± 0.06	0.16 ± 0.021	0.34	0	0	0.00
Oleic (9c-18:1)	4.35 ± 0.71	2.1 ± 0.226	5.00	20.18 ± 2.556	1.18 ± 0.24	15.73

	Bull Kelp^{T}	Dulse^{T}	Formulated^{T}	Bull Kelp^{D}	Dulse^{D}	Formulated^{D}
Vaccenic (11c-18:1)	4.96 ± 0.01	7.04 ± 0.113	6.97	0	3.53 ± 1.232	1.64
Linoelaidic (18:2t)	0	0	0.00	0	0	0.00
Linoleic (18:2n6)	3.83 ± 0.39	2.02 ± 0.035	9.16	7.78 ± 1.063	0.57 ± 0.051	28.83
Linolenic (18:3n3)	3.01 ± 0.13	0.42 ± 0.12	1.17	3.7 ± 0.82	0.04 ± 0.07	1.74
g-Linolenic [C18:3n6]	0	0	0.00	0	0	0.00
Stearidonic (18:4n3)	3.29 ± 0.21	0.07 ± 0.1	1.69	4.68 ± 1.18	0.06 ± 0.11	0.00
Arachidic (20:0)	0.2 ± 0.02	0.05	0.12	0.6 ± 0.13	0.13 ± 0.11	0.42
Gonodic (20:1n9)	2.72 ± 0.09	0.71 ± 0.13	1.60	0.05 ± 0.08	0.41 ± 0.07	0.78
C20:1n11	2.03 ± 0.04	3.13 ± 0.12	3.70	0	0	0.00
C20:2	0	0	0.00	0	0	0.21
Homo-a-linolenic(20:3n3)	0	0	0.00	0	0	0.09
C20:3n6 Homo-g-Linolenic	0	0	0.00	0	0	0.00
Arachidonic [20:4n6]	10.8 ± 0.21	8.34 ± 0.02	4.62	7.85 ± 0.68	12.36 ± 2.64	1.41
3n-Arachidonic (20:4n3)	4.4 ± 0.19	3.7 ± 0.21	5.52	0	0	0.00
EPA (20:5n3)	7.31 ± 0.32	13.47 ± 0.47	6.37	4.04 ± 0.21	24.2 ± 14.29	4.53
C21:0	0	0	0.00	0	0.1 ± 0.1	0.00
Behenoic (22:0)	0.06 ± 0.09	0	0.23	0	0.34 ± 0.04	0.00

	Bull Kelp^{T}	**Dulse^{T}**	**Formulated^{T}**	**Bull Kelp^{D}**	**Dulse^{D}**	**Formulated^{D}**
Erucic [22:1n9]	0.15 ± 0.01	0.13 ± 0.01	0.11	0	0.47 ± 0.08	0.10
C22:1n11	0	0	0.00	0	0	0.00
C22:2n6	1.11 ± 0.08	0.41 ± 0.1	0.23	0.43 ± 0.01	0.06 ± 0.1	0.00
Adrenic [C22:4n6]	2.11 ± 0.05	1.95 ± 0.14	0.74	0.31 ± 0.05	0	0.00
C22:5n6	0	0	0.00	0	0	0.00
Clupanodonic (22:5n3)	5.94 ± 0.28	9.09 ± 0.49	4.59	0	0	0.47
DHA (22:6n3)	0	0	2.03	0	0	5.78
C23:0	0	0	0.00	0	0	0.09
Lignoceric (24:0)	0	0	0.00	0	0.11 ± 0.12	0.27
Nervonic (24:1n9)	0	0.17 ± 0.07	0.05	0.11 ± 0.1	1.56 ± 0.32	0.36