EFFECT OF MACROALGAE DIET ON GROWTH RATE AND NUTRITION OF THE PINTO ABALONE, *HALIOTIS KAMTSCHATKANA*

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Abstract

Developing methods for the cultivation of the pinto abalone, *Haliotis kamtschatkana*, has seen increased attention, both by groups using restoration aquaculture to reestablish endangered populations in Washington and British Columbia, and in the State of Alaska, where pinto abalone are viewed as a promising new species for commercial mariculture. To enhance the viability of cultivating *H. kamtschatkana* for commercial and restoration purposes, more information is needed on the optimal macroalgal diet that maximizes abalone growth and nutrition. This study compares the suitability of two commonly cultivated species of macroalgae as feed: *Saccharina latissima* (sugar kelp), a kelp with relatively low protein content, and *Devaleraea mollis* (Pacific dulse), a rhodophyte with comparatively high protein content. Fifty *H. kamtschatkana* specimens, with a mean shell length of 45.25 ± 4.26 mm, were collected from the wild and fed either *S. latissima*, *D. mollis*, or an alternating diet of the two for 28 weeks. Feed consumption was measured weekly, while shell length and weight change were recorded every two months. Despite consuming significantly more *S. latissima* than *D. mollis* ($p < 0.001$, *H. kamtschatkana* showed no significant difference in specific growth rate (SGR) ($p = 0.775$) or linear growth rate (LGR) ($p = 0.746$) among the diets. Feed conversion efficiency (FCE) was significantly higher for *D. mollis* than *S. latissima* ($p < 0.001$), although there was no significant difference in protein efficiency ratio (PER) ($p = 0.129$). Proximate composition analyses of abalone tissue showed no significant difference in protein, lipid, carbohydrate, or caloric content across macroalgae diets. Additionally, sexual dimorphism was observed, with females exhibiting significantly higher daily feed consumption ($p = 0.001$), specific growth rate ($p = 0.003$), and linear growth rate ($p = 0.001$) than males. These results indicate that while both macroalgae species are suitable as feeds for *H. kamtschatkana* cultivation, the benefits of a *D. mollis* diet are

less pronounced compared to other commercially cultivated abalone species. This study provides actionable insights for those interested in cultivating *H. kamtschatkana* for commercial or restoration purposes and adds to our understanding of an environmentally and culturally important species in the Northeast Pacific Ocean.

Introduction

Abalone (*Haliotis* spp.) farming is one of the fastest-growing aquaculture sectors worldwide, with its value increasing from $4 million in 1990 to over $1.8 billion in 2017 (Hernández-Casas et al., 2023). While most of this growth has occurred in China and the Republic of Korea, substantial farming operations have also developed in other nations, such as South Africa, Chile, and Australia (Cook, 2023c). While abalone cultivation in North America has grown at a more modest rate, interest in its expansion has increased in areas, such as Alaska, which has set a goal of growing its mariculture industry to $100 million in economic output by 2040 (State of Alaska, 2018). At the same time, several research efforts, including the White Abalone Restoration Consortium and the Pinto Abalone Restoration Program, have begun to use abalone aquaculture to restore depleted wild populations throughout the west coast of North America (Rogers-Bennett et al. 2016; Sowul et al., 2022).

One of the primary factors dictating the success of farming abalone for commercial or restoration purposes is providing the abalone with a consistent and affordable supply of nutrition, either through formulated feeds or fresh macroalgae (Cook, 2023a). Formulated feeds generally contain a combination of plant-based carbohydrates and animal-based proteins, such as fishmeal or casein (Bullon et al., 2023). While consistent in availability, formulated feeds can be cost prohibitive and use carbon intensive ingredients, lessening the environmental sustainability

claims that can be made by abalone farmers (Ghamkhar & Hicks, 2020). As a result, most farms worldwide still rely on macroalgae, abalone's primary diet in the wild, for feed (Li et al., 2024). In some regions, where wild macroalgae are relatively abundant, such as California, Mexico, and Chile, producers can still meet their feed requirements through harvesting natural beds, primarily of giant kelp (*Macrocystis* spp.) (Flores-Aguilar et al., 2007; Searcy-Bernal et al., 2010). As the industry has continued to scale and climate change has reduced wild kelp populations (Beas-Luna et al., 2020), more farms have turned to macroalgae cultivation to meet feed requirements (Park et al., 2016). In the Republic of Korea, for example, over 60% of the 1.2 million metric tons of farmed kelp the nation produces is used as abalone feed (Hwang et al., 2022).

Macroalgae cultivated as feed for abalone primarily consist of brown algae known as kelps (Phaeophyta: Laminariales), red algae (Rhodophyta), or in rarer cases, green algae (*Ulva)* (Bullon et al., 2023). Preference for either kelps or rhodophytes varies among abalone species (Leighton, 2000). For instance, the Australian abalone species, *Haliotis rubra*, exhibits a distinct preference for rhodophytes, such as *Jeannerettia lobata* and *Laurencia botryoides* over various kelp species, including the giant kelp *Macrocystis pyrifera* (formerly *M. angustifolia*) (Fleming, 1995). Conversely, most abalone species in the northern Pacific Ocean prefer kelps over rhodophytes (Qi et al., 2010; Rhoades et al., 2018). Regardless of preference, numerous studies have highlighted superior growth rates in abalone species when fed rhodophytes rather than kelps, a trend generally attributed to higher nutrient concentrations in rhodophytes, particularly protein (Mai et al., 1996; Mercer et al., 1993; Roussel et al., 2019). For example, research on *Haliotis sorenseni* demonstrated a potential 30% faster linear growth rate when fed *Devaleraea mollis* (formerly *Palmaria* mollis) compared to a *M. pyrifera* diet (Rosen et al., 2000).

Locally known as the pinto or northern abalone, *Haliotis kamtschatkana* is the sole abalone species consistently found north of Oregon along the Pacific coast (Washington Department of Fish and Wildlife, 2024). The species has been depleted throughout its range and is listed as endangered in both Washington and British Columbia, though not in Alaska, where healthy populations still exist regionally (Neuman et al., 2018). In the past several decades, restoration groups have developed protocols allowing for the consistent spawning and cultivation of early-stage *H. kamtschatkana* for outplanting in the wild (Carson et al., 2019). The burgeoning macroalgae farming industry in Alaska, combined with its extensive sheltered coastline, presents an ideal environment for ocean-based abalone cultivation (McDowell Group, 2017). *H. kamtschatkana* is seen as the primary abalone species with the potential to be cultivated in Alaska, both because of regulations restricting the farming of non-native species (Article 2: Aquatic Farming, 2023), and its relatively fast growth rate in cold waters (Hoshikawa et al., 1998). This latter trait has also made *H. kamtschatkana* a target for hybridization experiments attempting to increase growth rates at low temperatures in commonly cultivated species, such as *H. discus hannai* (Lafarga-De la Cruz and Gallardo-Escárate, 2011). Nonetheless, key knowledge gaps still exist in *H. kamtschatkana* cultivation, particularly for later-stage growth to a marketable size of 30 g or larger (Cook, 2023b).

One such knowledge gap is understanding the optimal diet that maximizes *H. kamtschatkana* growth and nutrition, while minimizing the amount of macroalgae required as feed. Previous studies have suggested that *H. kamtschatkana* growth rates are comparable to commercially cultivated abalone species, such as *H. discus hannai* and *H. rufescens*, indicating its potential competitiveness in the market (Paul et al., 1977; Paul and Paul, 1980; Hoshikawa et al., 1998). These early studies utilized a mixed diet of wild-harvested macroalgae. However,

many of these macroalgal species could not be harvested at the amounts needed for scaled cultivation. Additionally, these authors did not compare consumption rates across macroalgae, an important factor in understanding the ability of different algal species to be assimilated into abalone growth (A. Paul et al., 1977; A. J. Paul & Paul, 1980).

To further the successful cultivation of *H. kamtschatkana*, producers must better understand the growth patterns, feed requirements, and resultant nutritional profiles, when abalone are fed a diet composed of commonly cultivated macroalgae. As such, this study provides insight into the suitability of utilizing two cultivated macroalgal species, *Saccharina latissima* (commonly referred to as sugar kelp), and *Devaleraea mollis* (commonly referred to as Pacific dulse), as feed for *H. kamtschatkana*. Based on macroalgae nutritional profiles and research in other species of abalone, it was expected that *H. kamtschatkana* fed *D. mollis* would have similar consumption levels but higher growth rates and protein composition than abalone fed either a diet of *S. latissima* or an alternating diet of the two (Mai et al., 1996; Mercer et al., 1993; Rosen et al., 2000; Roussel et al., 2019).

Methods

Abalone Collection and Culture System

This study assessed the effects of three diets on the growth and composition of the pinto abalone, *Haliotis kamtschatkana*. The diets consisted of *Devaleraea mollis* (Rhodophyta), *Saccharina latissima* (Phaeophyceae), and an alternating diet of each macroalgae exchanged every two weeks. Abalone growth was assessed every eight weeks, using weight and shell length to calculate specific and linear growth rates (SGR and LGR). In addition, feed consumption was

measured weekly to estimate average daily feed consumption (DFC), feed conversion efficiency (FCE), feed conversion ratios (FCR), and protein efficiency ratios (PER).

Abalone (n = 50) were collected from ten rocky subtidal zones in Sitka, Alaska (n = 5 per site). Collections were conducted by SCUBA or free diving to a depth not greater than 10 m and using a metal spatula to remove abalone from rocky substrates. At the surface, animals were moved to a cooler with ambient seawater ranging from 4 to 6 °C that was refreshed after 2 h. Once at the dock, all abalone were placed in aquaria at 4 to 6 °C, where they remained for 24 h before being transported to the NOAA Ted Stevens Marine Research Institute in Juneau, AK. There, each abalone was placed in a l ½ L Scotty Vented Bait Jar, and each jar was placed in an individual 9 L tank on an Aquaneering Z-rack array.

Experimental feeds were cultivated mimicking how each macroalgae is farmed in a commercial setting, with *D. mollis* grown in nutrient-controlled land-based systems and *S. latissima* grown on ocean-based farms exposed to natural nutrient fluctuations. Culture of *D. mollis* occurred in two recirculating 360 L tumble cultures under artificial light (100 ± 10 mol photons m^{-2} day^{-1}). UV-filtered seawater was replaced weekly, and 2 mL of F/2 nutrient media (Guillard, 1975) was added for every 6 L seawater (120 mL F/2 per water change). Culture of *S. latissima* occurred in a 500 L Living Stream tank (Frigid Units Inc.) under flow-through conditions. Sand-filtered seawater was replaced at a rate of 2000 L h^{-1}

Consumption and Growth Assessment

At the start of the experiment, all abalone were labeled with a unique identifier, photographed, and sexed. Sexing occurred visually using gonad coloration and was later verified for twenty dissected animals with a 100% success rate. Residual water was removed with a paper towel and abalone were weighed with a digital balance to the nearest 0.01 g as a baseline

measurement. Length of the longest shell axis was recorded with a vernier caliper to the nearest 0.01 mm. Collected abalone had a mean shell length of 45.25 ± 4.26 mm and a mean weight of 12.64 ± 3.74 g. Of the 50 abalone collected, 28 were males and 22 were females.

Abalone were divided randomly into three feed groups. Fifteen abalone were given *S. latissima*, 15 abalone were given *D. mollis,* and 15 abalone were given an alternating diet of *D. mollis* for two weeks followed by *S. latissima* for two weeks, until the end of the experiment. The remaining five abalone were sacrificed for proximate analysis. The *D. mollis* group included seven females and eight males with a mean shell length of 44.95 ± 4.71 mm and a mean weight of 13.15 ± 5.34 g. The *S. latissima* group included six females and nine males with a mean shell length of 44.59 ± 3.84 mm and a mean weight of 12.61 ± 3.16 g. The alternating group included six females and nine males with a mean shell length of 44.46 ± 4.04 mm and a mean weight of 12.10 ± 3.44 g. There was no significant difference between either shell length ($H(1) = 0.12$, $p = 0.726$) or weight ($H(1) = 0.00$, $p = 0.977$) between groups .

After a four-week conditioning period at ambient seawater temperatures of approximately 6 °C, a heating unit was added, and tank temperatures were increased by 1 °C every week until a range of 9 to 12 °C was reached, approximating water temperatures throughout the *H. katmschatkana* range in the summer months (National Oceanic and Atmospheric Association 2024). As abalone growth was negligible during the conditioning period, day zero was determined as the first week when water temperature exceeded 8 °C, a minimum level at which *H. kamtschatkana* growth has been shown to be optimized (Paul and Paul, 1980). Light levels were subject to a fluctuating natural photoperiod and did not exceed 10 mol photons m^{-2} day^{-1}. Sand-filtered seawater was added to each tank at a rate of 40 L h^{-1} to ensure consistent pH, oxygen concentration, and salinity.

Macroalgae were patted dry with paper towels until excess external water was removed, then added to each jar at an approximate rate of 0.5 g macroalgae g^{-1} abalone, an amount sufficient for each abalone to have as much feed as they would consume. Each week, unconsumed macroalgae in each jar were removed, patted dry, and weighed to the nearest 0.01 g. Differences from initial weight were used to calculate average consumption. Feed was replenished, and the same feeding cycle was repeated for 28 consecutive weeks. Macroalgae of each type were also weighed, added, and replaced weekly in two empty control jars to account for any weight gain or loss due to macroalgal growth or deterioration independent of consumption.

Using these metrics, daily feed consumption (DFC) was calculated as:

$$DFC\ (mg\ seaweed\ g^{-1}\ abalone\ day^{-1}) = \frac{\left[\frac{F_g - F_u}{t}\right]}{W}$$

where F_g was the amount of feed given to each abalone in g, F_u was the amount of feed uneaten in g and removed from each abalone, W was the mean wet weight in g (with shell) of the abalone during the experimental period assuming linear growth, and t is the time elapsed in days.

Individual abalone weight and shell growth were measured every eight weeks to minimize stress by handling. Two metrics, specific growth rate (SGR) and linear growth rate (LGR) were used to assess abalone growth. SGR was calculated as

$$SGR\ (\%\ day^{-1}) = \frac{100\left(ln\ W_f\ - ln\ W_i\ \right)}{t}$$

and LGR was calculated as

$$LGR\ (m\ shell\ growth\ day^{-1}) = \frac{SL_f - SL_i}{t}$$

where W_f was the final wet weight of each abalone in g including the shell, W_i was the initial (day zero) weight of each abalone in g including the shell, SL_f was the final shell length of each

abalone in µm, SL_i was the initial (day zero) shell length of each abalone in µm, and t was a given experimental time period in days.

Using a combination of consumption and growth metrics, three metrics were used to estimate the ability of abalone to assimilate each diet into growth. Feed conversion ratio (FCR) was calculated as

$$FCR = \frac{F_g - F_u}{W_f - W_i}$$

feed conversion efficiency (FCE) was calculated as

$$FCE = \frac{100\ (W_f - W_i)}{F_g - F_u}$$

and protein efficiency ratio (PER) was calculated as

$$PER = \frac{W_f - W_i}{P}$$

where F_g was the amount of feed given to each abalone in g, F_u was the amount of feed uneaten and removed from each abalone in g, and P was the dry weight of protein consumed over the experimental period in g (based on macroalgae nitrogen content, see below).

Macroalgae and Abalone Proximate Composition Analyses

Samples of *P. mollis* and *S. latissima* were removed from culture weekly, weighed, and dried in a 40 °C oven for 72 h. A dry to wet weight comparison was used to calculate weekly moisture content. Dried macroalgae from each week were homogenized using a Mini-Beadbeater (BioSpec).

Five abalone at the start of the conditioning period and five abalone from each diet post-experiment were randomly selected to be sacrificed while ensuring a representative division of sex. The ratio of males to females of sacrificed abalone was 2:3 for the day zero group, 3:2 for the *D. mollis* group, 3:2 for the *S. latissima* group, and 3:2 for the alternating group. Soft tissue

was divided into the foot and the remaining viscera, and their proximate composition analyzed separately. Abalone were freeze-dried for 36 h and moisture content was calculated from the wet to dry weight difference. Abalone were pulverized using a mortar and pestle, and the resultant powder was used for the remaining analyses.

Nitrogen and carbon concentrations of both macroalgae and abalone were obtained using continuous-flow isotope ratio mass spectrometry. This method utilized a Thermo Scientific Flash 2000 elemental analyzer and Thermo Scientific Conflo IV interfaced with a Thermo Scientific DeltaV Plus Mass Spectrometer. In addition to nitrogen and carbon content, stable isotope ratios were obtained and reported in δ notation as parts per thousand deviations from the international standards VPDB (carbon) and Air (nitrogen). Protein content was estimated by multiplying nitrogen concentration by a conversion factor of 5.0 for macroalgae (Angell et al., 2016) and 5.8 for abalone (Gnaiger & Bitterlich, 1984).

Abalone ash content was determined by weighing dry tissue before and after combustion in a muffle furnace at 550 °C for 8 h.

Abalone lipid extraction occurred using a modified Folch extraction (Folch et al., 1957). Approximately, 0.1 g of tissue per abalone was suspended in 30 mL of 2:1 chloroform and methanol with 0.01% butylated hydroxytoluene and left at 4 °C overnight. Solids were filtered from the solution using 202 creped filter paper, and 7.1 mL of 0.88% sodium chloride solution was added. The mixture was centrifuged for 30 min to create a biphasic system, and the non-lipid phase was discarded. Lipids were again filtered into a vial containing anhydrous sodium sulfate to fully dehydrate the solution. This solution was deposited into a pre-weighed vial, and a nitrogen stream evaporated chloroform. The remaining mass was weighed to the nearest 0.1 mg to calculate total lipids extracted.

Abalone bomb calorimetry analysis was undertaken using a Parr 6725 Semimicro Calorimeter (Parr), in which approximately 50 mg samples from each abalone were pelletized and combusted in a 22 mL oxygen bomb to measure a temperature differential. Finally, the approximate percentage of carbohydrates in abalone tissues was estimated from measured caloric content using the generally accepted ratios of 9 kcal g^{-1} of lipids (measured), 4 kcal g^{-1} of protein (measured), and 4 kcal g^{-1} of carbohydrates (Schmidt-Nielsen 1975; Food and Agriculture Organization of the United Nations, 2003). The equation below was used, where carbohydrates, proteins, and lipids are expressed in mg g^{-1} abalone tissue and calories are expressed as kcal g^{-1} abalone tissue.

$$Carbohydrates = \frac{Calories - Protein * 4 - Lipids * 9}{4}$$

Statistical Analyses

Diet parameters were tested for normal distribution of residuals and homogeneity of variance among diets using Shapiro-Wilk and Levene's tests, respectively. If error term assumptions were met, differences among diet groups and sexes were analyzed using one-way ANOVA at the $p < 0.05$ level and Tukey's HSD (honest significant difference) post-hoc tests. When error term assumptions were violated, non-parametric Kruskal-Wallace ANOVAs at the $p < 0.05$ level with Dunn's post-hoc tests were used. Values are reported as mean ± 1SD unless otherwise noted.

Results

Abalone Survival

One abalone from the alternating diet treatment and two abalone from the *D. mollis* diet treatment died during the experiment on weeks one, five, and five, respectively. However, all

three deaths were attributed to plumbing failures, and no discernible health abnormalities were present prior to mortality. All remaining abalone appeared healthy throughout the experiment and unsacrificed abalone have remained so post-experiment.

Daily Feed Consumption

Abalone fed solely with *D. mollis* had significantly lower daily feed consumption (DFC) (63% lower) than those fed only with *S. latissima* ($F(2,36) = 25.3$, $p < 0.001$; Table 1, Figure 1). The abalone fed an alternating diet followed this same trend, consuming significantly less on weeks, when they were fed *D. mollis* (130% less) than on weeks when they were fed *S. latissima* ($F(3, 49) = 53.9$, $p < 0.001$; Table 1, Figure 1). Abalone on the alternating diet consumed significantly more on weeks when they were fed *S. latissima* (6.50 ± 3.10 mg macroalgae g^{-1} abalone day^{-1}) than abalone on the non-alternating *S. latissima* diet (5.27 ± 2.63 mg macroalgae g^{-1} abalone day^{-1}) ($F(3, 49) = 53.9$, $p < 0.001$; Figure 2). The DFC was significantly higher for females (4.80 ± 2.77 mg macroalgae g^{-1} abalone day^{-1}) than for males (4.21 ± 2.76 mg macroalgae g^{-1} abalone day^{-1}) ($H(1) = 11.7$, $p = 0.001$; Figure 3).

Abalone Growth Rates

Specific growth rates (SGR) of abalone fed *D. mollis*, *S. latissima*, or the alternating diet were not significantly different ($F(2, 38) = 0.3$, $p = 0.775$; Table 2, Figure 2). Likewise, linear growth rates (LGR) were not significantly different across diets ($F(2, 38) = 0.3$, $p = 0.746$; Table 2, Figure 2). However, female abalone had a significantly higher SGR by 42% ($0.27 \pm 0.07\%$ day^{-1}) than male abalone ($0.19 \pm 0.06\%$ day^{-1}) when grouped across all three diets ($F(1, 39) = 10.0$, $p = 0.003$; Figure 3). LGR was significantly higher by 66% in females (34.66 ± 13.68 μm day^{-1}) than males (20.89 ± 13.15 μm day^{-1}) ($F(1, 39) = 10.7$, $p = 0.002$; Figure 3).

Feed Utilization

Feed conversion efficiency (FCE) was significantly higher for *D. mollis* (57% higher) than for *S. latissima* but not the alternating diet ($H(2) = 12.1$, $p = 0.002$; Table 2). Likewise, feed conversion ratio (FCR) was significantly lower (44% lower) for *D. mollis* than for *S. latissima* but not the alternating diet ($H(2) = 12.1$, $p = 0.002$; Table 2). The protein efficiency ratio (PER) did not significantly differ among diets ($F(2, 38) = 2.2$, $p = 0.129$; Table 2). There was no significant difference between FCE ($H(2) = 1.7$, $p = 0.189$), FCR ($F(1, 30) = 1.6$, $p = 0.133$), or PER ($F(1, 30) = 2.6$, $p = 0.115$) between males and females.

Proximate Composition Analyses

Mean carbon concentration was significantly higher, by 6.39%, in *D. mollis* than in *S. latissima* ($F(1, 48) = 65.5$, $p < 0.001$; Table 1). Mean nitrogen concentration was significantly higher, by 0.75%, in *D. mollis* than in *S. latissima* ($F(1, 48) = 29.7$, $p < 0.001$; Table 1). Consequently, dry weight protein content was significantly higher, by 3.50%, in *D. mollis* than *S. latissima* ($F(1, 48) = 29.7$, $p < 0.001$; Table 1). Moisture content was significantly lower, by 4.90%, in *D. mollis* than in *S. latissima* ($F(1, 52) = 15.0$, $p < 0.001$; Table 1). Carbon, nitrogen, and protein content differences were more noticeable in wet than dry macroalgae, with concentrations for *D. mollis* being roughly twice those for *S. latissima*.

No significant difference was found in moisture, ash, protein, lipids, carbohydrates, or calories across diets when comparing abalone feet and viscera (Table 3).

Discussion

Understanding the effects of different macroalgal diets on the growth and composition of *Haliotis kamtschatkana* is critical for the continued development of abalone farming for

commercial and restoration purposes in the northeast Pacific Ocean. This study tested whether feeding *H. kamtschatkana* with the protein-rich rhodophyte, *Devaleraea mollis,* would result in animals exhibiting faster growth and better nutritional quality than specimens fed with the kelp, *Saccharina latissima,* or an alternating diet. Such a trend has been demonstrated in other abalone species, including *H. discus hannai*, *H. tuberculata*, and *H. sorenseni*, where specific growth rate increased by up to 30% when fed with *D. mollis* or *Palmaria palmata* (both commonly referred to as dulse) rather than kelps, such as *S. latissima* (Mercer et al., 1993; Mai et al., 1996; Rosen et al., 2000; Roussel et al., 2019). Contrary to expectations, results in this study showed no significant difference in abalone growth rates regardless of their diet.

An equally surprising result of this study was that *H. kamtschatkana* consumed *D. mollis* at a significantly lower rate than *S. latissima* in alternating and non-alternating diets (Table 1). This result contrasts with studies comparing consumption of rhodophytes (particularly those classified as dulse) and kelps by other species of abalone. One study found that *H. tuberculata* consumed *Palmaria palmata*, a closely related species to *D. mollis*, at a higher rate than *S. latissima,* and *H. discus hannai* consumed the two species at an equal rate (Mercer et al., 1993). Another study on *H. rufescens* found no significant difference between their consumption rates of Pacific dulse *D. mollis* and the bull kelp *Nereocystis luetkeana* (Wulffson, 2020). While further research is necessary to determine the cause of relatively low rates of *D. mollis* consumption by *H. kamtschatkana*, one possibility is that *H. kamtschatkana* has a decreased preference for *D. mollis* compared to other species of abalone. Differences in chemosensory (McShane et al., 1994) and textural (Lee & Kim, 2013) preference in macroalgae have been documented across various species of abalone. Given the extreme northern range of *H.*

kamtschatkana and difference in natural macroalgae populations there (Campbell et al., 2003), an adapted shift in feed preference from other species of Pacific abalone is plausible.

A second explanation is that the abalone cultivated in this experiment met their maximum nutritional uptake levels in all three diets. As poikilotherms, the digestion rates of abalone are limited by the surrounding water temperature (Dahlhoff & Somero, 1993). In this study, the relatively cold mean culture temperature of 10.6 ºC may have limited abalone metabolism to the point where sufficient macroalgae consumption was possible from all three diets to maximize nutrient uptake. This explanation is supported by the lack of a significant difference in protein efficiency ratios among diets, demonstrating that a similar level of protein was consumed regardless of whether abalone were offered *D. mollis*, *S. latissima*, or an alternating diet (Table 1). In studies that have demonstrated similar consumption and increased growth in abalone provided with dulse over kelps, culture temperatures of 14 ºC or higher were used (Mercer et al., 1993; Mai et al., 1996; Rosen et al., 2000; Roussel et al., 2019). Additionally, several recent studies in a *H. rubra* x *H. laevigata* hybrid have clearly demonstrated that the ability for abalone to utilize feeds with higher protein content increases with water temperature (Hassan et al., 2023, 2024). These studies indicate that at higher temperatures, abalone may not be able to consume macroalgae at a rate sufficient to maximize their digestion and nutrient uptake, even when it is presented to them *ad libitum.* As a result, the advantages of consuming the more nutrient-dense dulse than kelp are likely maximized at temperatures where consumption rate rather than digestion rate is the limiting factor for abalone growth.

Even with reduced benefits compared to other studies, this experiment still demonstrates advantages of utilizing *D. mollis* as a feed for *H. kamtschatkana*, given that its feed conversion efficiency was significantly higher than for *S. latissima* or the alternating diet (Table 1). In other

words, almost half as much additional dry weight and over twice as much additional wet weight of *S. latissima* compared to *D. mollis* was required to achieve equivalent amounts of abalone growth. This difference is important for commercial production when evaluating the tradeoffs of producing macroalgae for abalone feed. While *S. latissima* is generally considered cheaper to produce than *D. mollis* (Redmond et al., 2014; Stévant et al., 2023), the decision to cultivate one species versus the other will likely depend on farm location and cultivation style. The feed conversion ratios determined here should help *H. kamtschatkana* producers evaluate the most cost-effective strategy for macroalgal production as abalone feed.

Previous studies have indicated that higher concentrations of protein in feed can translate into higher protein levels in abalone composition (Mercer et al., 1993; Mai et al., 1995; Tung and Alfaro, 2011). However, in this study, elevated protein levels in *D. mollis* were insufficient to produce a noticeable change in abalone proximate composition, including protein, lipid, carbohydrate, and overall caloric content (Table 3). Likewise, no clear difference was seen in proximate compositions of abalone fed an alternating diet. It is important to note that other factors beyond proximate composition may vary as a result of the different diets, including amino acid composition, fatty acid composition, or enzymatic makeup in the digestive system. Exploring these factors further may be important in understanding the benefits of different diets beyond growth, including resistance to disease or environmental fluctuations that are increasingly attributed to abalone fed mixed macroalgal diets rather than a single species (Kroeker et al., 2021; Sun et al., 2021).

For this study, more males than females were collected (28:22), aligning with the observed sex ratio in the wild, where sex ratios are 1:1 or males slightly outnumber females (Sloan et al., 1988; Campbell et al., 2003). Remarkably, outcomes herein report the first evidence

of sexual size dimorphism for *H. kamtschatkana*, with females exhibiting significantly higher daily feed consumption and growth, including an increase in shell growth rate by over 65%. While sexual size dimorphism with females being larger than males is considered common across gastropods (Ng et al., 2019), its occurrence in abalone has not been broadly documented. In *H. discus hannai,* where dimorphism has been the most studied, differences in size between males and females are not as pronounced as found in *H. kamtchatkana* in this study (Park et al., 2016). For gastropods where sexual size dimorphism is present, the explanation for its cause is unclear. One possible explanation is male-initiated sexual selection, as numerous studies in gastropods have found that males preferentially follow mucus trails of larger females to reach a mate (Saltin et al., 2013; Ng and Williams, 2014). Another explanation is that larger female size is a genetic biproduct of genes important for other sexual characteristics. Studies on *H. discus hannai,* for example, suggest that genes important for female gonadal development, such as the zp4 gene, which enhances the development of the extracellular matrix of eggs, may incidentally contribute to increased growth in females relative to males (Choi et al., 2021). While numerous other theories have been hypothesized, including higher energetic costs for males than for females (Rolán-Alvarez et al., 2015), niche partitioning (Shine, 1989), and fecundity selection (Riascos & Guzman, 2010), it is clear that further research is necessary to determine the cause of sexual size dimorphism in *H. kamtschatkana* and how it compares to other species of abalone.

A key objective of this study was to calculate growth and consumption metrics, allowing producers to compare the competitiveness of *H. kamtschatkana* with other commercially cultivated species of abalone, such as *H. rufescens* and *H. discus hannai*. Relative consumption and growth rates decline curvilinearly as abalone increase in size, making it important to compare abalone of similar size classes (Shepard et al., 2000; Venter et al., 2018). Additionally,

abalone are known to grow faster in warmer waters within their range of thermal tolerance (Venter et al., 2018). Hence, comparing studies of abalone cultivated at similar temperatures is equally as important. The *H. kamtschatkana* in this study had a higher linear growth rate and similar feed conversion ratio to *H. rufescens* of a similar size class fed *Macrocystis pyrifera* at 15.2 ± 0.1 ºC (Garcia-Esquivel & Felbeck, 2009). However, *H. discus hannai* of a similar size raised at 10 ºC had slightly higher SGRs and FCRs when fed a diet of *Saccharina japonica* (Fang et al., 2018). While metrics can vary significantly with experimental design, these comparisons indicate that growth and consumption rates of *H. kamtschatkana* lie within the range of other successfully commercially cultivated species.

While comparing growth rate to temperature was not the primary focus of this study, only negligible growth was observed during the acclimation period in which culture temperatures remained below 8 ºC. Likewise, a clear decrease in daily feed consumption was detected as culture temperatures declined towards the end of the experiment. Even though acceptable levels of growth and DFC were demonstrated at an average temperature of 10.6 ºC, it is relevant to highlight that this is higher than the yearly mean of ocean temperatures in many parts of the northeast Pacific Ocean (NOAA, 2024). To minimize time to market and maximize profitability, or to minimize the transitioning time from the nursery to the wild, producers should consider water temperature as a factor in selecting a suitable site for cultivation.

Several experiments are planned to further our understanding of the effect of *D. mollis* and *S. latissima* on *H. kamtschatkana* growth and development. While the time-intensive determination of DFC rates was limited to a 28-week period, remaining abalone from this experiment have been maintained at their respective diets for an additional 30 weeks, and continued monitoring will enable determination of whether differences in growth rates are

apparent over a longer period. Beyond growth rates, macroalgae diets have also resulted in differences in gonadal development (Demetropoulos & Langdon, 2004; Roussel et al., 2019). To determine any impact of *D. mollis* and *S. latissima* diets in this respect, remaining abalone from this experiment will be used as broodstock, and fecundity and survival rates of offspring will be compared. As seawater temperature may be a highly determinate factor in the success of Alaskan abalone farms, these offspring will also be used to study the combined effect of feed type and culture temperature on the growth of *H. kamtschatkana*.

Future research should include more fine-scale testing into the textural or chemosensory factors determining abalone feed preference, and whether certain cultivation styles can create macroalgal phenotypes in *D. mollis* that increase *H. kamtschatkana* growth and consumption. While this study focused on *D. mollis* and *S. latissima* as the most widely cultivated species of rhodophytes and kelps, respectively, other species, such as *Alaria marginata, Macrocystis pyrifera,* and *Nereocystis luetkeana* are being increasingly cultivated, and future research should examine their suitability as feeds for *H. kamtschatkana* as well (Camus et al., 2018; Stekoll et al., 2024). As abalone feed preference varies at different life stages (Barkai & Griffiths, 1986), future research should examine if the trends identified here hold for *H. kamtschatkana* of different size and age classes. Finally, given the size dimorphism indicated in this study, future research should investigate if feed preference and consumption rates vary by sex and if varying diets produce differential benefits in males versus females.

Conclusion

This study developed a baseline understanding of the potential of farming *H. kamtschatkana* using cultivated *D. mollis* and *S. latissima* as feed. Results indicated that both macroalgal species

are acceptable sources of nutrition for producing growth comparable with other commercially cultivated species of abalone. While feed conversion efficiency was significantly higher for *D. mollis* than for *S. latissima*, this study demonstrated the surprising result that *D. mollis* did not result in faster growth of *H. kamtschatkana* than *S. latissima,* as has been demonstrated in other species of abalone. Lastly, this study provided the first known evidence of sexual size dimorphism in *H. kamtschatkana.* Together, these results provide actionable insights for individuals interested in farming *H. kamtschatkana* for commercial or restoration purposes and add to the fundamental understanding of the physiology of a key species in the ecosystem of the northeast Pacific Ocean.

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Research Data

Data collected for this project will be available publicly at www.nprb.org

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Figures and Tables

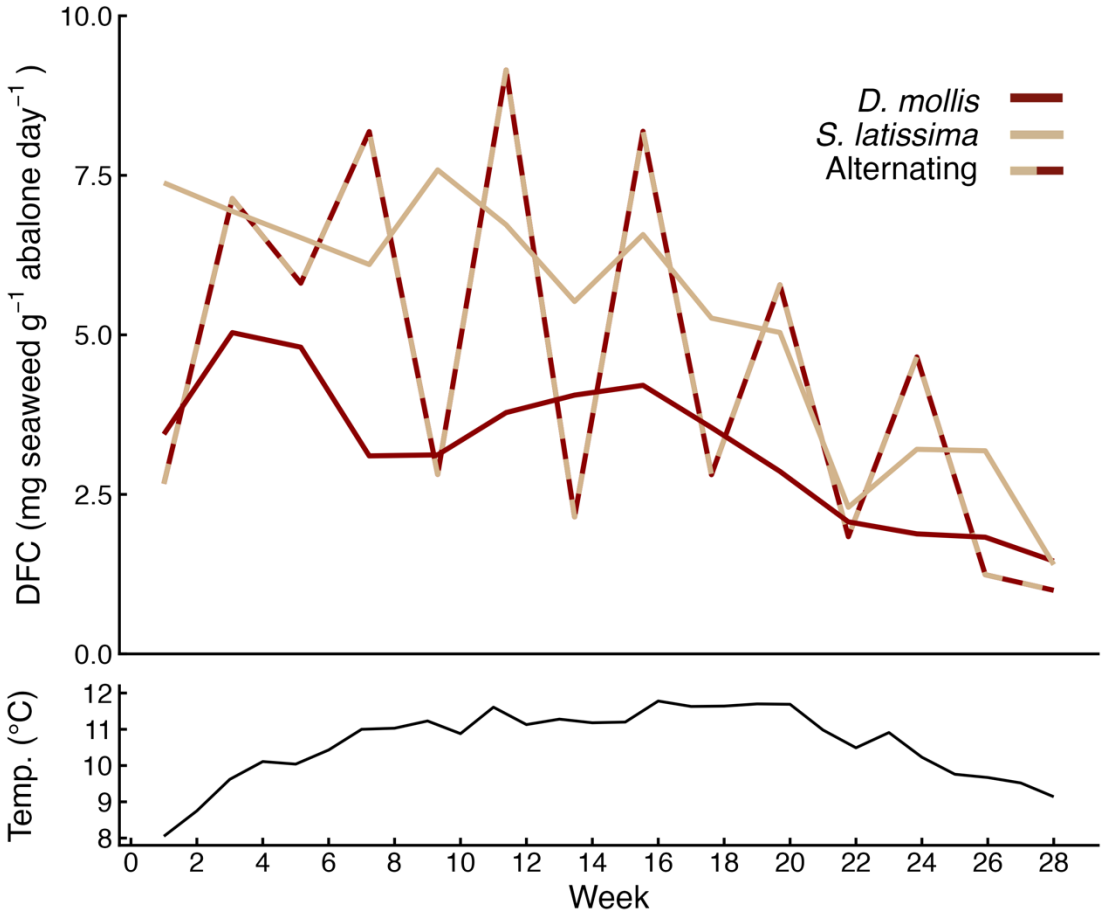

Figure 1 Average daily feed consumption (DFC) and temperature for *Haliotis kamtschatkana* fed with *Devaleraea mollis* (n = 13), *Saccharina latissima* (n = 15), or a biweekly alternating diet of the two (n = 14) for 28 weeks. Datapoints for daily feed consumption represent two-week averages, and data for temperature represent weekly averages. Error bars represent mean for each period ± 1SD. For the alternating diet, abalone were given *D. mollis* for weeks 1 and 2, *S. latissima* for weeks 3 and 4, and continued at this pattern.

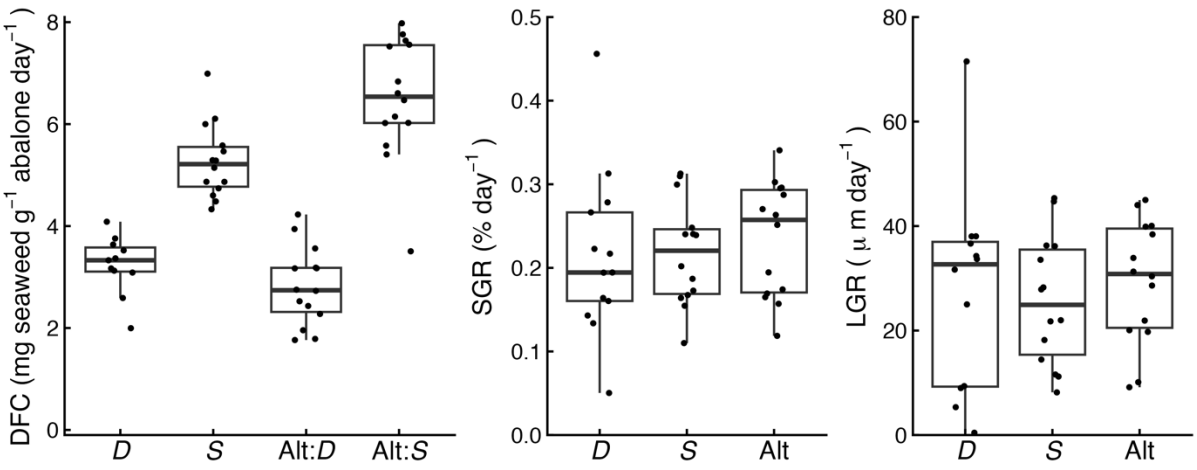

Figure 2 Daily feed consumption (DFC), specific growth rate (SGR), and linear growth rate (LGR) of *Haliotis kamtschatkana* fed *Devaleraea mollis* (*D*), *Saccharina latissima* (*S*), or a biweekly alternating diet of the two (Alt) for 28 consecutive weeks. Alt:*D* and Alt:*S* represent weeks when abalone on the alternating diet were fed *D. mollis* or *S. latissima* respectively. Abalone fed *D. mollis* (*D*) had significantly lower daily feed consumption than abalone fed *S. latissima* (*S*) or the alternating diet (Alt) ($p < 0.001$). Abalone fed the alternating diet consumed significantly more on weeks, when they were fed *S. latissima* (Alt:*S*) than abalone fed *S. latissima* weekly (*S*) ($p < 0.001$). No significant difference was detected in SGR ($p = 0.775$) or LGR ($p = 0.746$). Boxplot represents the median, first and third quartiles, minimum and maximum values, and outliers that are more than 1.5x the interquartile range from the first and third quartiles.

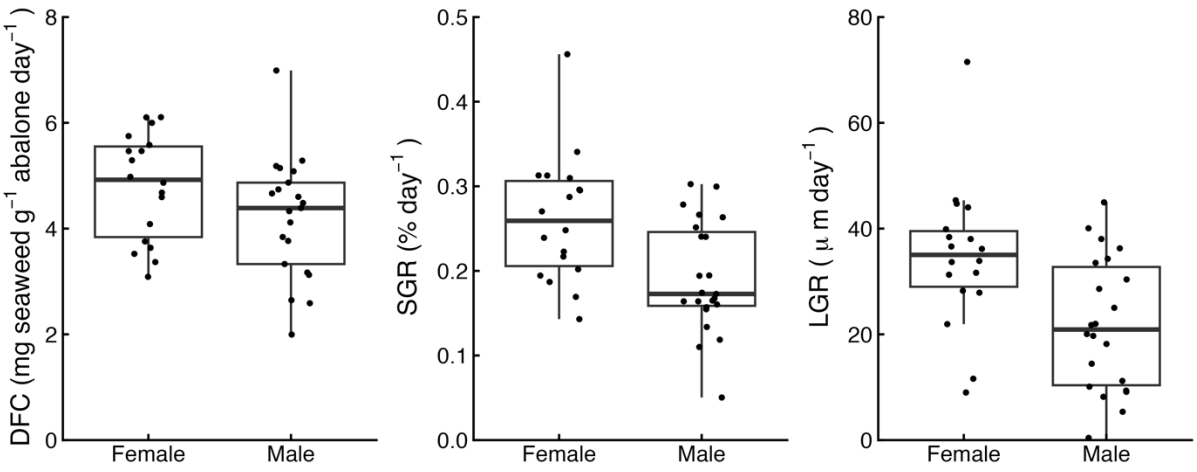

Figure 3 Daily feed consumption (DFC), specific growth rate (SGR) and linear growth rate (LGR) of *Haliotis kamtschatkana* by sex when pooled across three experimental diets, including *Devaleraea mollis*, *Saccharina latissima*, or a biweekly alternating diet of the two, for 28 consecutive weeks. Significant differences were detected in DFC ($p < 0.001$), SGR ($p = 0.003$), and LGR ($p = 0.002$). Boxplot represents the median, first and third quartiles, minimum and maximum values, and outliers that are more than 1.5x the interquartile range from the first and third quartiles.

Table 1 Consumption and growth parameters for *Haliotis kamtschatkana* fed *Devaleraea mollis, Saccharina latissima,* or a biweekly alternating diet of the two for 28 consecutive weeks (mean ± 1SD). Values in the same row with different letters indicate results that were significantly different from each other ($p < 0.05$). Daily food consumption (DFC), specific growth rate (SGR), linear growth rate (LGR), feed conversion ratio (FCR), feed conversion efficiency (FCE), and protein efficiency ratio (PER), are displayed.

	D. mollis	*S. latissima*	Alternating
DFC ($mg_{dry\ macroalgae}\ g^{-1}_{abalone}\ day^{-1}$)	3.24 ± 1.67^a	5.27 ± 2.63^b	4.66 ± 3.25^b
SGR ($\%\ day^{-1}$)	0.21 ± 0.10^a	0.22 ± 0.06^a	0.23 ± 0.07^a
LGR ($\mu m\ day^{-1}$)	25.56 ± 20.39^a	25.67 ± 12.31^a	29.46 ± 11.78^a
FCR	1.62 ± 0.74^a	2.33 ± 0.52^b	1.90 ± 0.35^b
FCE (%)	70.38 ± 23.15^a	44.77 ± 9.44^b	54.23 ± 10.28^b
PER ($g_{weight\ gain}\ g^{-1}_{protein\ intake}$)	3.92 ± 1.30^a	3.16 ± 0.66^a	3.59 ± 0.67^a

Table 2 Mean weekly nitrogen, carbon, protein, and moisture content of *Devaleraea mollis Saccharina latissima* grown over 28 consecutive weeks (mean ± 1SD). Wet mass nitrogen, carbon, and protein content were calculated as *Dry mass concentration* * (1 – *Moisture %).* Values in the same row with different letters indicate results that were significantly different from each other ($p < 0.05$).

	D. mollis	*S. latissima*
Dry mass nitrogen (%)	3.57 ± 0.38^{a}	2.82 ± 0.53^{b}
Dry mass carbon (%)	30.78 ± 3.09^{a}	24.31 ± 2.74^{b}
Dry mass protein* (%)	17.88 ± 1.91^{a}	14.03 ± 2.63^{b}
Moisture (%)	84.97 ± 5.91^{a}	89.87 ± 2.30^{b}
Wet mass nitrogen (%)	0.55 ± 0.24^{a}	0.29 ± 0.09^{b}
Wet mass carbon (%)	4.73 ± 2.18^{a}	2.49 ± 0.74^{b}
Wet mass protein* (%)	2.72 ± 1.37^{a}	1.44 ± 0.48^{b}

*Calculated by multiplying nitrogen concentration by a conversion factor of 5.0 (Angell et al., 2016)

Table 3 Proximate composition of *Haliotis kamtschatkana* tissues (foot and viscera) fed *Devaleraea mollis, Saccharina latissima*, a biweekly alternating diet of the two for 28 weeks, and baseline abalone sacrificed at the start of the experiment. Data are reported as mean ± 1SD for abalone (n = 5 per treatment). No significant difference in wet mass was seen among diets in either the foot or the viscera.

	Foot				Viscera			
	D. mollis	*S. latissima*	Alternating	Baseline	*D. mollis*	*S. latissima*	Alternating	Baseline
Moisture (%)	22 ± 1	23 ± 2	21 ± 3	22 ± 2	21 ± 5	26 ± 1	23 ± 2	18 ± 5
Ash (%)	7 ± 1	6 ± 1	7 ± 2	7 ± 2	8 ± 2	7 ± 2	9 ± 1	9 ± 2
Protein[1] (%)	67 ± 8	70 ± 1	68 ± 4	68 ± 4	62 ± 10	58 ± 10	59 ± 8	52 ± 4
Lipids (%)	4 ± 2	3 ± 5	3 ± 3	5 ± 2	16 ± 5	16 ± 7	16 ± 5	17 ± 4
Carbohydrates[2] (%)	38 ± 8	40 ± 12	39 ± 8	36 ± 6	27 ± 19	35 ± 10	30 ± 18	33 ± 12
Energy density (kcal g^{-1})	4.5 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.0	5.0 ± 0.5	5.2 ± 0.4	5.0 ± 0.1	4.9 ± 0.2

[1]Calculated by multiplying nitrogen concentration values by a conversion factor of 5.8 (Gnaiger and Bitterlich, 1984)

[2]Derived from protein, lipid, ash, and caloric concentrations as described in *Methods,* Equation 2.7