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Abalone under moderate heat stress have elevated metabolic rates and changes to digestive enzyme activities

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ABSTRACT

Abalone around the world are subject to increasing frequency of marine heatwaves, yet we have a limited understanding of how acute high temperature events impact the physiology of these commercially and ecologically important species. This study examines the impact of a 5 °C temperature increase over ambient conditions for six weeks on the metabolic rates, digestive enzyme activities in the digestive gland, and digestive efficiency of Red Abalone (*Haliotis rufescens*) and Pāua (*H. iris*) on their natural diets. We test the hypothesis that abalone digestive function can keep pace with this increased metabolic demand in two separate experiments, one for each species. *H. iris* had higher food intake in the heat treatment. Both species had higher metabolic rates in the heat treatment with $Q_{10} = 1.73$ and $Q_{10} = 2.46$ for *H. rufescens* and *H. iris*, respectively. Apparent organic matter digestibility, protein digestibility, and carbohydrate digestibility did not differ between the heat treatment and the ambient (control) treatment in either experiment. *H. rufescens* exhibited higher maltase, alanine-aminopeptidase, and leucine-aminopeptidase activities in the heat treatment. Amylase, β-glucosidase, trypsin, and alkaline phosphatase activities in the digestive gland tissue did not differ between temperature treatments. *H. iris* exhibited lower amylase and β-glucosidase activities in the heat treatment, while maltase, trypsin, leucine-aminopeptidase, and alkaline phosphatase activities did not differ between treatments. We conclude that over six weeks of moderate heat stress both abalone species were able to maintain digestive function, but achieved this maintenance in species-specific ways.

1. Introduction

1.1. Environmental stressors and digestive outcomes

Digestion and nutrient acquisition are some of the most fundamental ways in which animals interact with their environment and are impacted by it. What animals eat, how they process those nutrients (digestion), and what they excrete determine energy and resource flow in a given ecosystem (Karasov and Martínez del Rio, 2007). This fundamental process of digestion, including the discrete components of it such as individual enzyme activities, is impacted by both temperature and pH (e.g., acidification) in a wide diversity of marine organisms (e.g. Stumpp et al., 2013; Rosa et al., 2016; Khan et al., 2020). Specifically in Red Abalone (*Haliotis rufescens*), traits related to lipid provisioning, metabolism, and growth determine tolerance to low pH caused by ocean acidification (Swezey et al., 2020). It is therefore surprising (and ironic – gastropod means "stomach-foot") that digestion of natural foods and the impact of environmental stressors on these biochemical processes are poorly understood in an ecologically and economically important group of gastropods, abalone. A better understanding of the baseline function of these systems is required to predict how threats like climate change and disease interact to influence digestive function of these animals, especially in the wild (Somero, 2010).

Artificial feeds have been designed and tested for *Haliotis midae* held at an elevated temperature, and growth and mortality have been measured on different feeds (Green et al., 2011). In fact, most digestion

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work has focused on the growth and survival of animals in aquaculture facilities on artificial feeds (Fleming et al., 1996), but very little work has delved into the biochemistry of digestion and how individual components of digestion (e.g., specific digestive enzyme activities) are impacted by environmental stressors. In hybrid *Haliotis laevigata* x *H. rubra*, heat stress led to an increase in haemocyte infiltration and digestive gland damage (Hooper et al., 2014). In *H. rufescens* exposed to the causative agent of withering syndrome (a bacteria *Candidatus* Xenohaliotis californiensis or *Ca*.Xc), the combination of disease, high temperatures, and starvation led to glycogen decrease in the digestive gland (Braid et al., 2005). As expected in ectotherms, feeding rate is impacted by temperature; *H. rufescens* increase their feeding rates progressively as temperatures increase to the highest they normally experience in the wild (about 18 °C), but then decline sharply at higher temperatures, as the animal is stressed (Leighton, 2000). Acute increases in temperature associated with marine heatwaves (Holbrook et al., 2019) can push abalone into physiological stress, leading to precarious conditions for these animals. For example, the extreme heatwave in 2014 in Northern California (Bond et al., 2015) contributed to the reduction of *Nereocystis leutkeana* (bull kelp; a food resource of *H. rufescens*) by more than 90% and caused an 80% increase in mortality rate of *H. rufescens* (Rogers-Bennett and Catton, 2019).

There is clear evidence that physiological stress at temperatures >18 °C, as well as starvation, impacts the allocation of resources to reproduction in *H. rufescens* (Rogers-Bennett et al., 2010; Morash and Alter, 2015). Given that heat stress could impact individual aspects of digestive physiology (e.g., digestive enzyme activities), stress on the gut could have wide-ranging impacts, including on the allocation of resources to reproduction. Generally, digestion on natural diets has been little studied in abalone as most work on digestion is focused on artificial feeds. The lack of information about natural baseline physiology hinders our ability to (a) understand the specific mechanisms by which stressors impact digestive physiology, and (b) apply data from captive animals to wild animals.

1.2. Abalone diet and digestion: Enzyme function and methodological issues

Morphologically, the abalone alimentary tract consists of a mouth with a radula, esophagus, digestive gland/stomach complex, and intestine (Fig. 1; Mclean, 1970; Morton, 1979; Bevelander, 1988). In terms of digestive enzymes, proteases such as trypsin, chymotrypsin, aminopeptidases, and carboxypeptidases have been measured in abalone guts (Garcia-Carreno et al., 2003; Garcia-Esquivel et al., 2007; Hernandez-Santoyo et al., 1998; Serviere-Zaragoza et al., 1997; Erasmus et al., 1997; Groppe and Morse, 1993). With regards to carbohydrate digestion, endogenous amylase, cellulase, alginate lyase, laminarinase, agarase, and carrageenase activity (Erasmus et al., 1997; Enriquez et al., 2001; Suzuki et al., 2003; Lo et al., 2003) have also been found in abalone guts, as well as exogenous (microbially-derived) alginase (Sawabe et al., 1995). These enzymes are all involved in the digestion of brown and red algae (including storage polysaccharides and cell wall constituents) by abalone (Painter, 1983; Matsuhiro and Zambrano, 1990).

Differences in digestive enzyme activities were observed in *H. laevigata* consuming different diets, at different ages, and at different

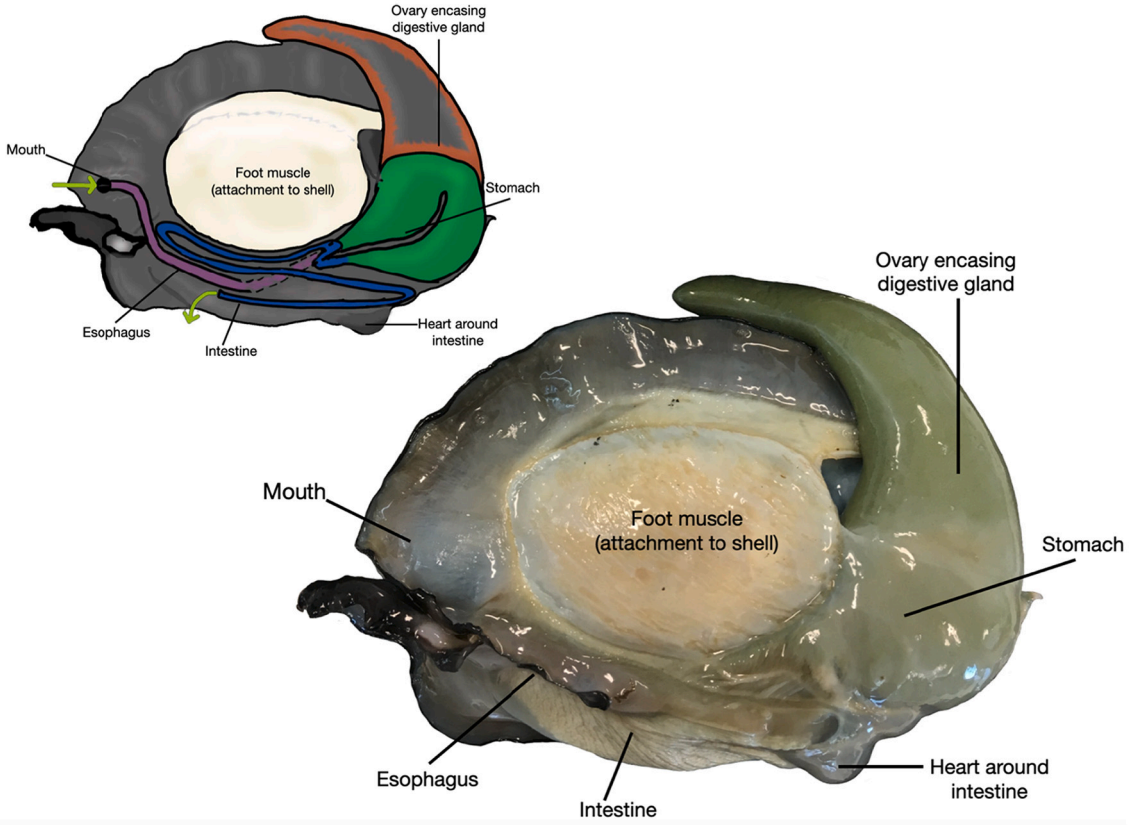

Fig. 1. Anatomy of abalone digestive system. Animal in the photo is *H. iris*, with a dorsal view and shell removed. Only the location of digestive organs are labelled for simplicity, and those organs are obscured by other tissues in the photograph. A colored diagram is shown for clarity, with arrows indicating the intake of food and output of feces. The digestive system runs along the lower portion of the body in the photo, with the large digestive gland housed inside the green horn-shaped ovary on the right of the image. Food enters the mouth via a radula, moves through the esophagus toward the stomach and digestive gland, then exits into the intestine. The intestine stretches from stomach toward the mouth, then wraps back toward the stomach, making one more turn before running through the heart and to the anus. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

temperatures, using the entire gut homogenized as a single sample (Bansemer et al., 2016a; Bansemer et al., 2016b). However, abalone digestive systems have multiple organs with compartmentalized function. For example, *H. fulgens* may have seven different aminopeptidases (Garcia-Carreno et al., 2003), and digestive enzymes have different activity levels in different regions of the *H. rufescens* gut (Garcia-Esquivel and Felbeck, 2006). Thus, abalone may produce a variety of digestive enzymes in different gut regions. Moreover, digestive enzyme activities are frequently measured at temperatures that would be stressful (e.g., 25 °C; Picos-Garcia et al., 2000) or lethal (e.g., 30 °C; Garcia-Carreno et al., 2003) for abalone (Garcia-Esquivel et al., 2007). Since digestive enzyme activity levels indicate an animal's ability to digest specific substrates or nutrients, it is probably best to measure them at the natural environmental temperature range for a given abalone species, and with relevance for the gut region in which the enzyme is active in the animal.

1.3. Digestibility of diets under different conditions

Digestibility, or the proportion of ingested material that is digested or broken down and absorbed by an organism relative to the amount of the same material in the diet, is an important metric for understanding what organisms assimilate from their food. *H. laevigata* held at temperatures from 14 °C to 26 °C, for example, showed no differences in dry matter digestibility or protein digestibility (Currie et al., 2015), suggesting that this species, at least on an artificial diet, can maintain digestibility over a wide range of temperatures. However, measured over 14 °C to 22 °C, the optimum protein content for maximized protein digestibility increases concomitantly with temperature (Stone et al., 2013). In partial contrast, *H. midae* has been demonstrated to have a peak dry matter apparent digestibility at 18 °C, compared to lower digestibility at 15 °C and 22 °C (Dixon, 1992). Most digestibility studies, particularly those examining the role temperature plays in digestion, have been conducted using artificial diets due to the need to optimize feeds for commercial aquaculture (Fleming et al., 1996). Abalone digestive enzymes and growth have been well studied on artificial feeds (Britz et al., 1996; Knauer et al., 1996; Hernandez-Santoyo et al., 1998; Picos-Garcia et al., 2000; Garcia-Esquivel and Felbeck, 2009), but few investigations combine digestive physiology, natural diets, and stressors.

Despite evidence that diet affects digestive physiology across a diversity of taxa (Brzek et al., 2011; Leigh et al., 2018; Wehrle et al., 2020; Herrera et al., 2022), studies of abalone gut physiology on natural diets are not common. One example examining digestibility of different algae (a natural diet) and diet preference demonstrated that *Haliotis rubra* prefers algal species with high digestible nitrogen content (Fleming, 1995). Indeed, a better understanding of healthy abalone gut function is critical for understanding the impact of stressors, such as disease and temperature, on abalone digestive physiology both in aquaculture and in the wild.

1.4. Study objectives

The objectives of the present study are (1) to characterize digestive enzyme activity and nutrient digestibility of two abalone species on natural diets and (2) to test the hypothesis that abalone digestive function can keep pace with increased metabolic demand at the higher end of temperatures typically experienced in the wild. To address the goals of this study, we conducted two separate experiments with two abalone species. In both experiments, we measured metabolic rates, digestibility, and the activity levels of digestive enzymes relevant to the natural diets of the abalone. We studied Pāua (*Haliotis iris*) and Red Abalone (*H. rufescens*), independently, at temperatures typical of their respective environments, and a species-relevant "stressful" temperature 5 °C above that ambient temperature. We examined these parameters over four weeks, approximating a timescale akin to a short marine heatwave, which are increasing in frequency (Holbrook et al., 2019; Oliver et al., 2021). These taxa were chosen because they live at relatively high latitudes in opposite hemispheres and are experiencing rapidly warming conditions and marine heatwaves that threaten their survival (Rogers-Bennett and Catton, 2019; Thomsen et al., 2019; Hart et al., 2020; Misra et al., 2021).

2. Methods

2.1. Animal collection and husbandry – H. rufescens

Twenty *H. rufescens*, approximately 190 g each, were purchased from The Cultured Abalone Farm (Goleta, CA, USA) where they consumed *Macrocystis pyrifera* as part of their diet. We acknowledge that captivity potentially alters microfilms on algae and the diversity of macroalgae available to abalone; we attempted to use animals as close to wild as possible without harvesting any additional wild animals, given that the recreational fishery for *H. rufescens* closed just before this work began and it remains closed at the time of writing. The animals were transferred to the University of California Irvine (UCI) animal care facility and acclimated to a recirculating filtered seawater system kept in a cold room set at 12.5 °C. This temperature was approximately the average temperature for Northern California sites where *H. rufescens* were found subtidally at the time the study started and is also within range for this species across multiple locations (Rogers-Bennett et al., 2010). At 18 °C, *H. rufescens* becomes overtly stressed (Rogers-Bennett et al., 2010), and accordingly we chose a temperature just below that (17.5 °C) for our heat treatment.

The abalone were held at 12.5 °C for approximately two months while being fed *M. pyrifera* daily. *M. pyrifera* drift kelp is preferred by *H. rufescens* (Leighton, 2000), with *Nereocystis leutkeana* constituting most of the diet in Northern California, where *M. pyrifera* is more seasonally abundant. After the two-month acclimation period, we divided animals into two identical tank systems with individual 3-L tanks for each animal ($N = 10$ per treatment). Each system had its own sump with physical, chemical, biological and UV filtration. The experimental system had heaters placed in the sump to raise the temperature of the water in that system to 17.5 °C (5 °C above the ambient system). Before the experiment started, the water temperature in the heated system was warmed by approximately 1 °C/day until it reached 17.5 °C. Animals were then acclimated to their respective temperature treatments (ambient or heated) for approximately one month before starting a feeding trial and fecal collections.

For the duration of the experiment, water ammonia concentrations and water temperature were monitored daily; ammonia was not detectable throughout the experiment. Oxygen levels were maintained by adding airlines with air stones to each sump. Complete turnover of water in each individual abalone enclosure occurred every 15 min. Food options, availability of food, and water source were kept consistent between treatment groups throughout the experiment. Using the same water source eliminates any issues with different microbes potentially being present in either system. Animals were divided randomly into treatments and measured to ensure that size distribution between treatments did not vary.

2.2. Animal collection and husbandry – H. iris

We collected nine *H. iris* from Breaker Bay and eleven *H. iris* from Princess Beach, Houghton Bay on the south coast of Wellington, New Zealand during the first week of March 2018, and housed the animals in the Victoria University of Wellington Coastal Ecology Lab. The abalone were housed together in a runway of flow-through filtered seawater for 10 days, then randomly divided and transferred to individual buckets with the same flow-through seawater source. Population-level effects were not tested as animals were combined from each collection site before being randomly assigned to treatment. Moreover, Houghton Bay and Breaker Bay are about 3 km apart, each facing south into the ocean

(Cook Strait), and are considered to involve the same population of *H. iris* (Will et al., 2011). The ambient temperature of filtered seawater pumped into the facility averaged 14 °C. To keep the same effects-based (+5 °C) temperature change for *H. iris* that we used for *H. rufescens*, we used 19 °C for the *H. iris* heat treatment temperature. *H. iris* were observed living in the bay where the seawater intake was located, so this was also used as the ambient temperature for the experiment. *H. iris* were allowed to acclimate to their individual containers for another week and were primarily fed *Lessonia variegata* and supplemented with *Ecklonia radiata* (collected from the same locations) ad libitum. Both algal species were present at both *H. iris* collection sites. *H. iris* consumes mostly brown algae, specifically preferring *Lessonia variegata* (Cornwall et al., 2009); we examined digestibility of this species as well as *Ecklonia radiata* (which is slightly less preferred by *H. iris;* Cornwall et al., 2009) when we needed to supplement diet with another algal species. After one week, we used a combined head tank and bath system to raise the water temperature in half of the tanks to 5 °C above ambient by approximately 1 °C/day to achieve the heat treatment (19 °C). We held the animals were held at their temperature treatments for an initial two weeks before the feeding trial and fecal collections began.

Both *H. rufescens* and *H. iris* were fed ad libitum during acclimation periods until 24 h before beginning the experiment. At this point, food was removed and all feces from that 24-h fast were removed from treatment buckets to ensure any fecal material collected during the experiment was from the experimental period only.

For the duration of the experiment, animals remained on filtered seawater from Island Bay, with no recirculation. Temperatures were monitored twice daily to ensure consistency through the experiment, as well as logged 24 h/day to ensure that we did not miss any daily drastic changes in temperature. Oxygen levels were maintained by ensuring that the turnover rate for each bucket was 15–20 min. Food options, availability of food, and water source were kept consistent between treatment groups throughout the experiment. Animals were divided randomly into treatments and measured to ensure that size distribution between treatments did not vary.

2.3. Experimental design

Both experiments were conducted using the same design and methods. We weighed animals and measured their shell length (using standard methods, the longest measurement across the opening of the shell was used) at the start of the feeding trials, and at the end during dissections. Animals were housed individually in 3 L containers in flowing seawater, with turnover rate of at least three times per hour. The entire exposure to treatment temperature in both experiments lasted six weeks, and the feeding trial (fecal collections) was conducted during the last four of those six weeks. Metabolic rates were measured (described below) at the end of the six-week experiment.

Every day, animals were fed brown algae species described above. Algae were rinsed in freshwater and dried at 60 °C to remove any other organisms and preserve algae for the duration of the experiment. Algae was rehydrated in small amounts of seawater before being added to abalone enclosures. Abalone of both species were observed eating algae that had been dried and rehydrated, seemingly without reservation. We fed animals ad libitum, and always offered more food than they would eat within the day. Twenty-four hours after food was added, we removed the orts (uneaten food) and replaced the food with a new piece of rehydrated algae, to prevent food from degrading. For *H. iris* only, we weighed the offered food (dry mass) before being added to buckets. We re-dried and weighed the removed orts and subtracted ort mass from the mass of offered food in order to compare the mass ingested between temperature treatments. We collected fecal material via siphoning, and decanted and discarded the accompanying saltwater. We then rinsed the feces with deionized water and dried them at 60 °C overnight. We combined each day's dried feces with previous days' collections for each abalone, and collected approximately 1-3 g of dried fecal material per animal by the end of the four-week digestibility experiment. At the end of the digestibility experiment, we held animals for less than a week longer at their respective temperatures and continued to feed them respective diets ad libitum. During this final week, we measured the abalone's metabolic rate, final mass and length, and we collected tissues.

2.4. Metabolic rate measurements

After the four-week feeding trials, we recorded respiration rates for each experimental animal during daylight hours following methods from Connor et al. (2016). All measurements were performed at the treatment temperature that an individual had experienced during the feeding trial. Animals were placed in a in a chamber of known volume (1.1–2.3 L chambers, depending on the size and shape of the animal). The chamber was submerged in a cooler of seawater at the same temperature as the animal's assigned treatment (ambient or heated) with air lines to ensure oxygenation. The animal was allowed to acclimate to the chamber for at least thirty minutes before being sealed in the chamber with a stir bar to ensure homogeneity of seawater oxygen saturation. Using an optical dissolved oxygen probe (Neofox, Ocean Optics, Dunedin, FL), we measured the decline in dissolved oxygen over time (measuring every 30s) in the chamber. The sensor was calibrated with aerated (100% O_2) and anoxic (achieved by bubbling N_2 gas into the water within a sealed container) seawater. For each animal, measurements were taken until the oxygen concentration declined by 10% (approximately 15 min), at which time the chamber was reopened and flooded with oxygen-saturated seawater before beginning another measurement on the same animal, with each animal measured three times in sequence (Herrera et al., 2022; Connor et al., 2016). The mass of each animal was recorded, and later corrected by removing the mass of the shell (as the shell has no metabolically active tissue).

Metabolic rate ($\mathbf{M\ O_{2\text{-}A}}$) in mg O_2/min/g was calculated using Eq. 1 (Jew, 2019), where $\mathbf{V_{respirometer}}$ is the volume of space able to be occupied by seawater or respiring animal tissue (not the shell) in the chamber during measurements for a given animal. To determine this volume, the mass of the shell ($\mathbf{M_{shell}}$, grams) was subtracted from the total volume of the chamber. $\mathbf{V_{abalone}}$ is the volume of the entire abalone (liters), estimated using the density of 1 g/ml. $\mathbf{\Delta CwO_2}$ is the change in dissolved oxygen (mg O_2/l), $\mathbf{\Delta t}$ is the change in time in minutes, $\mathbf{M_{abalone}}$ is the mass of the entire abalone (grams), and $\mathbf{M_{shell}}$ is the mass of the shell (grams). Metabolic rate in oxygen consumption was converted to calories/min/g ($\mathbf{M\ O_{2\text{-}B}}$) using Eq. 2 (Elliot and Davison, 1975), then converted to Joules/min/g by multiplying by 4.184 J/cal. The average Q_{10} per treatment, which measures the sensitivity of metabolic rate to an increase in 10 °C, was calculated using Eq. 3, with Ambient and Heat referring to the two temperature treatments for a given species, and averages used for M-$O_{2\text{-}B}$ for each treatment.

$$\boldsymbol{M\ O_{2-A}} = \frac{\left(\boldsymbol{V_{respirometer}} - \boldsymbol{V_{abalone}}\right) \times \boldsymbol{\Delta CwO_2}}{\boldsymbol{\Delta t} \times \left(\boldsymbol{M_{abalone}} - \boldsymbol{M_{shell}}\right)} \quad (1)$$

$$\boldsymbol{M\ O_{2-B}} = \boldsymbol{M\ O_{2-A}} \times 0.35\ cal/mg\ O_2 \quad (2)$$

$$Q_{10} = \left(\frac{M\ O_{2-B}\ Ambient}{M\ O_{2-B}\ Heat}\right)^{\left(\frac{10}{(T_{Heat} - T_{Ambient})}\right)} \quad (3)$$

Within each species, we used the same water sources and refreshed the water between replicate measurements. We assume that the bacteria and microorganisms in the water column did not measurably impact metabolic rate. The *H. iris* shells were not scrubbed before the experiment to reduce handling stress, and thus, the epibionts growth on the shell could have contributed to oxygen consumption in our measurements. However, epibiont growth on all shells was qualitatively similar and since length of the shells did not vary with treatment, we assumed that the relative biomass on the shells compared to the biomass of the abalone was not substantial.

2.5. Dissections and tissue sample preparation for biochemical assays

After metabolic measurements, animals were returned to their enclosures to reduce handling stress and to allow them to eat for at least 48 h before dissections. Animals were placed in ice until unresponsive, as this appeared to be the least stressful and most humane method to euthanize the abalone. Ice as an anesthetic also slows enzymatic action, so we expect this to minimally impact digestive enzyme measurements. Animals were then removed from their shell by severing the foot muscle attachment with a spatula. We performed dissections on a sterilized metal tray filled with ice. The digestive organs were removed and digestive gland tissue near the stomach region was isolated (Fig. 1). We measured the pH of each sample with litmus paper to determine appropriate pH for later homogenization buffers. Gut contents were removed from the digestive tissue. All gut tissue samples were immediately frozen in 1.5 ml vials, either in liquid nitrogen for *H. rufescens* or in a − 80 °C freezer for *H. iris*. Samples were transported to UC Irvine (in liquid nitrogen for *H. rufescens*, and on dry ice for *H. iris*) and stored at −80 °C until used.

We homogenized digestive gland samples in ice-cold citric acid sodium citrate buffer (pH 5.6) using a Polytron homogenizer (Brinkman Instruments; Westbury, New York) for 30-s pulses. Any connective tissue was removed and discarded. Homogenized samples were centrifuged at 9400*g* for 2 min at 4 °C, then the supernatant was stored in 100–130 μl aliquots at −80 °C until used in assays (German et al., 2015).

2.6. Nutrient content sample preparation and assays

All algae were rinsed in DI water, then dried at 60 °C for 24–48 h. Algal subsamples were taken from each algal blade (approximately one square centimeter). Algal samples were then combined within species, ground with liquid nitrogen using a mortar and pestle, then homogenized on ice in 25 mM Tris-HCl pH 7.0. We centrifuged samples at 9400*g* for 2 min at 4 °C, then stored the supernatant in 50–200 μl aliquots at −80 °C until used in assays. Fecal homogenates were prepared using the same methods.

Soluble carbohydrate content of diet and fecal homogenates was measured using colorimetric methods developed by Dubois et al. (1956). Homogenates were divided into triplicate technical replicates of 62.5 μl and boiled for 30 min to hydrolyze larger polysaccharides, then cooled and combined with 156 μl of 5% phenol. Sulfuric acid (781 μl, 18 M) was added to produce the colorimetric reaction. After a ten-minute 22 °C incubation, vials were shaken at 30 °C for 40 min and 100 μl was read in technical duplicate at 490 nm using a Biotek Synergy H1 microplate reader (Agilent, Santa Clara, CA, USA) with standard curve slopes of 0.0014–0.0029 abs/nmol glucose. The values from the six technical replicates (three homogenate replicates and two colorimetric readings per homogenate replicate) were averaged for each sample. We measured protein content via the colorimetric bicinchoninic acid assay (Smith et al., 1985) using a Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, Illinois), and samples were diluted to fit within the standard curve.

Organic content of fecal and algal samples was compared using an ash-corrected digestibility measurement because total fecal collections were impossible given the way that abalone scrape algae, producing tiny fecal particles. Hence, small amounts of feces were lost during the experiment no matter which method of fecal collection was used. In this ash-corrected method, ash, the leftover product of combustion, is used as an indigestible marker for the amount of material consumed and excreted (German, 2011). To determine the percentage of fecal or algal material that is organic content, samples were dried thoroughly at 105 °C, and weighed intermittently (approximately 180 mg per sample was used) to ensure that all water content has evaporated. Samples were combusted on ceramic crucibles at 550 °C for 3 h, and the mass of leftover indigestible marker, ash, was recorded. Apparent organic matter digestibility was calculated using Eq. 4 where $\mathbf{a_d}$ and $\mathbf{a_f}$ are the amount of indigestible ash in the diet and feces, and $\mathbf{m_d}$ and $\mathbf{m_f}$ are the mass of organic matter (dry matter – ash) in the diet and feces, respectively (German, 2011).

$$\textit{Apparent Organic Matter Digestibility}\ (\%) = \left[1 - \left(\frac{a_d}{a_f}\right) \times \left(\frac{m_f}{m_d}\right)\right] \times 100 \quad (4)$$

2.7. Digestive enzyme assays

The biochemical activity levels of three carbohydrases (amylase, maltase, and β-glucosidase), three proteases (trypsin, alanine-aminopeptidase, leucine-aminopeptidase), and alkaline phosphatase were measured using digestive gland homogenates, following the methods of German et al. (2004), Horn et al. (2006), and German et al. (2015). Every enzyme assay except for trypsin activity was run at pH 5.6, the same pH measured in the digestive gland during dissections. Trypsin was measured at pH 7 because the substrate precipitated out of solution at a lower pH. The temperatures used for the assay incubations were the same as the treatment temperature the abalone experienced, such that digestive enzyme activities are as reflective as possible of the activity levels in vivo at the time of dissection.

2.8. Statistical analysis

Shell lengths between treatments were compared using Welch's two-sample *t*-tests to ensure that both treatments had similar average sized animals within each species. Shell lengths did not change over the 4-week feeding trial, so only final lengths were considered for this comparison. We compared metabolic rates, apparent organic matter digestibility percentages, carbohydrate and protein nutrient content, and enzyme activities between the two temperature treatments within each species. Species were not compared to one another statistically because the experiments were performed at different times (separated by several months) and using different water sources (among other variables). One heat treatment *H. iris* was removed from all analyses because its metabolic rate measured an order of magnitude above any other animal measured, its tissue was comparatively pale, and it rarely ate compared to all other animals, indicating that it may not have been healthy at the start of the experiment. Another *H. iris* in the heat treatment died during the experiment due to a water line blockage which resulted in its enclosure unit reaching lethally high temperatures. Thus, the heated *H. iris* used for analysis had a sample size of $N = 8$. For the *H. rufescens* carbohydrate digestibility group, one measurement was removed because technical replicates were extremely variable within the sample, despite multiple attempts at measurement. The average fell within the range of the other animals in this treatment, but we were not comfortable including it in analysis because of the extreme variability within technical replicates. For the *H. iris* β-glucosidase group, one animal was removed from analysis because it also had high variability in its technical replicates. The average of the value fell within the range of the other samples in the group, but because of the extreme variability in technical replicate measurements, we conservatively did not include it in further analysis.

Prior to all parametric tests, a Bartlett's test for homoscedasticity was performed on the data, and a Shapiro-Wilk test for normality was performed on the residuals of all models. Where necessary to meet normality assumptions, log or square-root transformations were used before analysis (Table 1). Ingested algal mass (collected for *H. iris* only) was standardized for each animal as dry algal mass consumed (mg) per gram of abalone soft tissue, and averaged for daily consumption. Data for apparent organic matter digestibility in *H. iris* and maltase activity in *H. rufescens* failed to meet the assumption of homoscedasticity but met normality assumptions, so a Welch's t-test for unequal variances was used on those two datasets (Table 2). Data for amylase activity in *H. rufescens* and protein digestibility in *H. iris* did not come from a

Table 1
Summary statistics for all normally distributed and homoscedastic data. Sample sizes are for analyzed samples in each treatment and assay. Mean ± standard deviation within each heat treatment is shown, with F-statistic, degrees of freedom (treatment, residuals), and *p*-value for every ANOVA. When data were transformed before creating the model, the transformation is indicated. The mean and standard deviations for categories that underwent transformation are means and standard deviations of the raw data, not the transformed data.

		Sample size (n)		Mean ± SD						ANOVA			
Metric	Species	Ambient	Heat	Ambient			Heat			F	df	p	Transformation
M-O_2 (J/min/g)	*H. rufescens*	10	10	0.0223	±	0.0063	0.0293	±	0.0053	7.22	1, 18	0.015	–
	H. iris	10	8	0.0095	±	0.0051	0.0149	±	0.0076	3.67	1, 16	0.074	log
Apparent organic matter digestibility (%)	*H. rufescens*	10	10	68.03	±	4.64	62.93	±	8.12	2.98	1, 18	0.102	–
Carbohydrate digestibility (%)	*H. rufescens*	9	10	52.15	±	10.04	55.34	±	9.28	0.52	1, 17	0.481	–
	H. iris	10	8	85.85	±	4.71	86.17	±	2.70	0.03	1, 16	0.864	–
Protein digestibility (%)	*H. iris*	10	8	79.64	±	3.38	76.41	±	5.96	2.11	1, 16	0.166	–
Amylase activity (μmol min^{-1} g^{-1})	*H. iris*	10	8	4.11	±	1.78	2.81	±	0.74	3.76	1, 16	0.070	log
Maltase activity (μmol min^{-1} g^{-1})	*H. iris*	10	8	1.73	±	0.68	2.16	±	0.37	2.63	1, 16	0.125	–
β-glucosidase activity (nmol min^{-1} g^{-1})	*H. rufescens*	10	10	57.27	±	18.61	49.35	±	11.45	1.32	1, 18	0.266	–
	H. iris	10	7	27.56	±	18.78	13.38	±	7.55	3.56	1, 15	0.079	sqrt
Trypsin activity (μmol min^{-1} g^{-1})	*H. rufescens*	10	10	0.23	±	0.09	0.19	±	0.05	1.06	1, 18	0.318	log
	H. iris	10	8	0.43	±	0.30	0.29	±	0.12	1.25	1, 16	0.279	log
Alanine-aminopeptidase activity (μmol min^{-1} g^{-1})	*H. rufescens*	10	10	35.59	±	16.57	55.47	±	27.93	3.75	1, 18	0.069	–
Leucine-aminopeptidase activity (nmol min^{-1} g^{-1})	*H. rufescens*	10	10	97.96	±	30.66	155.65	±	41.42	14.56	1, 18	0.001	log
	H. iris	10	8	76.36	±	42.22	69.16	±	40.9	0.133	1, 16	0.720	–
Alkaline phosphatase activity (nmol min^{-1} g^{-1})	*H. rufescens*	9	10	138.38	±	40.27	111.22	±	18.85	2.87	1, 17	0.109	log
	H. iris	10	8	104.14	±	38.69	78.71	±	28.27	2.41	1, 16	0.140	–
Algal mass consumed (mg/day/g)	*H. iris*	10	8	2.52	±	0.86	3.48	±	1.30	3.54	1, 16	0.078	–

Table 2
Summary statistics for all data that either were non-normally distributed (Mann-Whitney U test) or had unequal variances (Welch's t-test). Sample sizes are for analyzed samples in each treatment and assay. Mean ± standard deviation within each heat treatment is shown, with test statistic and p-value.

		Sample size (n)		Mean ± SD						Mann-Whitney U Test	
Metric	Species	Ambient	Heat	Ambient			Heat			W	p
Amylase activity (μmol min^{-1} g^{-1})	*H. rufescens*	10	10	1.59	±	0.97	1.65	±	0.37	37	0.353
Protein digestibility (%)	*H. rufescens*	10	10	70.31	±	9.58	66.51	±	15.11	37	0.853

		Sample size (n)		Mean ± SD						Mann-Whitney U Test	
Metric	Species	Ambient	Heat	Ambient			Heat			W	p
Maltase activity (μmol min^{-1} g^{-1})	*H. rufescens*	10	10	2.15	±	0.32	3.21	±	1.12	−2.88	0.016
Apparent organic matter digestibility (%)	*H. iris*	10	8	45.49	±	6.12	37.92	±	13.47	1.47	0.174

normal distribution, even after transformation, so a non-parametric Mann-Whitney *U* Test was used to compare means in those two datasets (Table 2). Otherwise, individual two-way ANOVAs were conducted, fitting a linear model in R Studio (R v4.1.1). We used an alpha level of 10% for all significance determinations. We chose this significance cutoff because of the limitations in number of animals available for this work; with this sample size, we risk missing important differences between treatments with biologically meaningful effect sizes if we used a more stringent significance cutoff. For all comparisons with $p < 0.1$, we included the percent difference to demonstrate the effect sizes (Halsey, 2019). Boxplots were generated in R Studio (R v4.1.1; R Core Team, 2021) using package ggplot2 (Wickham, 2016); whisker extremes are 1.5 times the interquartile range.

Principal components analysis was conducted on 11 dependent variables (metabolic rates, digestibility percentages, and each enzyme activity), using scaled data. The first two principal components were plotted with vectors representing factor loadings. For three missing values (outlined in results), averages of the remaining values within each treatment x species group were substituted, and zeroes were assigned to alanine-aminopeptidase activities in *H. iris* because this enzyme was undetected and therefore not measured in that species. The PCA is the only statistical analysis that compares both species to one

another.

3. Results

3.1. Animal size

H. iris lengths in the ambient treatment were 127.2 ± 12.4 mm (average ± standard deviation), not significantly different from the average of 130.1 ± 11.2 mm in the heat treatment ($p = 0.61$). Final masses of *H. iris* did not differ between treatments, being 294.9 ± 105.9 g in the ambient treatment and 291.1 ± 57.4 g in the heat treatment ($p = 0.92$).

H. rufescens lengths were similar in both treatments, with 104.8 ± 3.2 mm in the ambient treatment and 104.3 ± 3.1 in the heat treatment ($p = 0.72$). Final mass of *H. rufescens* was 139.7 ± 17.4 g in the ambient treatment and 126.7 ± 12.3 g in the heat treatment ($p = 0.07$). This mass difference between the two *H. rufescens* treatment groups was driven by one "large" outlier whose mass was greater than other animals in the ambient treatment. However, no other measurements for this animal (including mass-specific metabolic rate) were outliers compared with other animals in the group. It was statistically significantly larger than the others because there was very little variability in their lengths (averages between groups are within 1 mm of one another). Therefore, it was not excluded from analysis.

3.2. Metabolic and food ingestion rates

H. iris consumed 38% more algal dry mass in the heat treatment compared to the ambient treatment ($p = 0.078$; Table 1, Fig. 2). The routine metabolic rate of *H. iris* in the heat treatment was 57% higher than in the ambient treatment ($p = 0.074$; Table 1, Fig. 3). Q_{10}, a measurement of sensitivity of metabolic rate to an increase in temperature, was 2.46 for *H. iris*.

Although we did not measure intake quantitatively for *H. rufescens*, it appeared that the heat-treated group consumed and defecated more than the ambient treatment animals (*pers. obs.*). The routine metabolic rate of *H. rufescens* in the heat treatment was 32% higher than in the ambient treatment ($p = 0.015$; Table 1, Fig. 3). Q_{10} was 1.73 for *H. rufescens*.

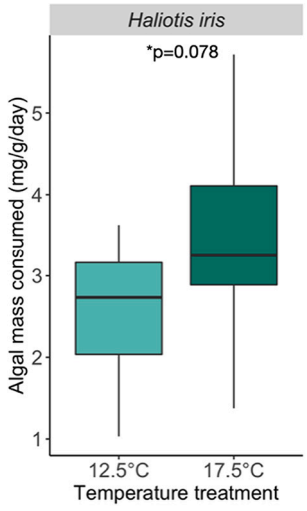

Fig. 2. Mass of algae consumed (mg) per mass of animal soft tissue (g) per day by *Haliotis iris*, averaged over the entire 4-week feeding trial. Boxplot whiskers span 1.5 times the IQR. Comparison was made with an ANOVA, with statistical values and data summarized in Table 1.

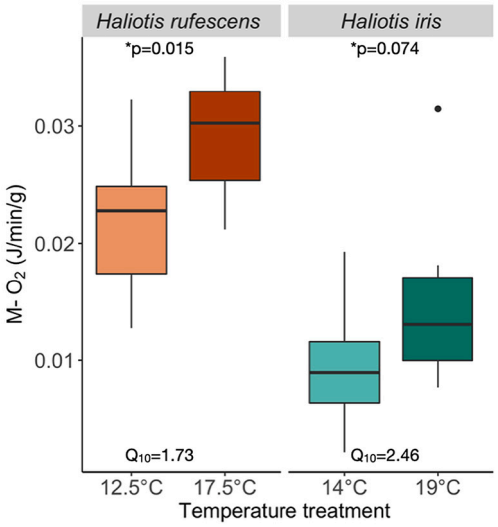

Fig. 3. Metabolic rates measured at treatment temperature in all treatments, in Joules per minute per gram of soft tissue. Boxplot whiskers span 1.5 times the IQR, with individual point(s) representing outliers. Comparison within species were conducted via ANOVA, with statistical values and data summarized in Table 1. Metabolic rate of Red Abalone in the heat treatment (*H. rufescens*, $n = 10$) was significantly higher than in the ambient treatment (n = 10; $p = 0.015$). Päua (*H. iris*) metabolic rate in the heat treatment ($n = 8$) was significantly higher than in the ambient treatment (n = 10; $p = 0.074$). Q_{10} was 1.73 for *H. rufescens*, and 2.46 for *H. iris*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Nutrient digestibility and kelp composition

For *H. iris*, ambient treatment apparent OM digestibility did not differ significantly from the heated treatment ($p = 0.174$; Table 2, Fig. 4A). In *H. iris*, protein digestibility and carbohydrate digestibility did not differ between temperature treatments (protein: $p = 0.166$; carbohydrate: $p = 0.864$; Table 1, Fig. 4B and C).

For *H. rufescens*, apparent OM digestibility in the ambient treatment differed marginally from the heated treatment ($p = 0.102$; Table 1, Fig. 4A). In *H. rufescens*, protein and carbohydrate digestibility did not differ between temperature treatments (protein: $p = 0.174$; carbohydrate: $p = 0.481$; Tables 1 and 2, Fig. 4B and C).

Lessonia variegata contained 70.1 ± 2.0% (mean ± standard deviation) organic matter by dry mass. *Lessonia variegata* contained 22.90 ± 2.60 mg protein per gram of organic matter and 62.34 ± 5.96 mg of carbohydrate (measured as mg of glucose liberated) per gram of organic matter. *M. pyrifera* contained 62.6 ± 0.6% (mean ± standard deviation) organic matter by dry weight. *M. pyrifera* contained 9.55 ± 2.70 mg protein per gram of organic matter and 43.14 ± 1.85 mg of carbohydrate per gram of organic matter.

3.4. Digestive enzyme activities

Amylase activity in *H. iris* in the heat treatment was 32% lower than in the ambient temperature treatment ($p = 0.070$; Table 1, Fig. 5A). Maltase activity in *H. iris* did not differ significantly by temperature treatment, but trended toward being higher in the heat treatment ($p = 0.125$; Table 1, Fig. 5B). In *H. iris,* the β-glucosidase activity was 51% lower when heat treated ($p = 0.079$; Table 1, Fig. 5C). Trypsin activity in *H. iris* did not differ significantly between the temperature treatments ($p = 0.279$; Table 1, Fig. 5D). Alanine-aminopeptidase was not detectable in *H. iris* and leucine-aminopeptidase activity did not differ between treatments ($p = 0.7209$; Table 1, Fig. 5F). Alkaline phosphatase did not differ significantly between the temperature treatments for *H. iris* ($p = 0.140$; Table 1, Fig. 5G).

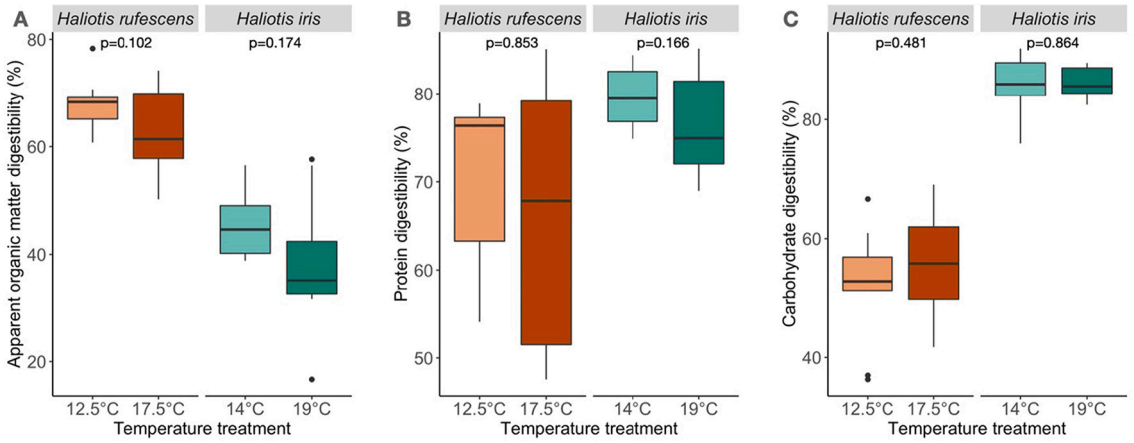

Fig. 4. A. Apparent (ash-corrected) organic matter digestibility, B. protein digestibility, and C. carbohydrate digestibility (displayed as percent) in each treatment. Boxplot whiskers span 1.5 times the IQR, with individual point(s) representing outliers. Comparison within species were conducted via ANOVA for all metrics except the following: protein digestibility in *H. rufescens* was compared with a Mann-Whitney non-parametric test; and apparent organic matter digestibility in *H. iris* was compared with a Welch's *t*-test. Statistical test values and data are summarized in Table 1 and Table 2.

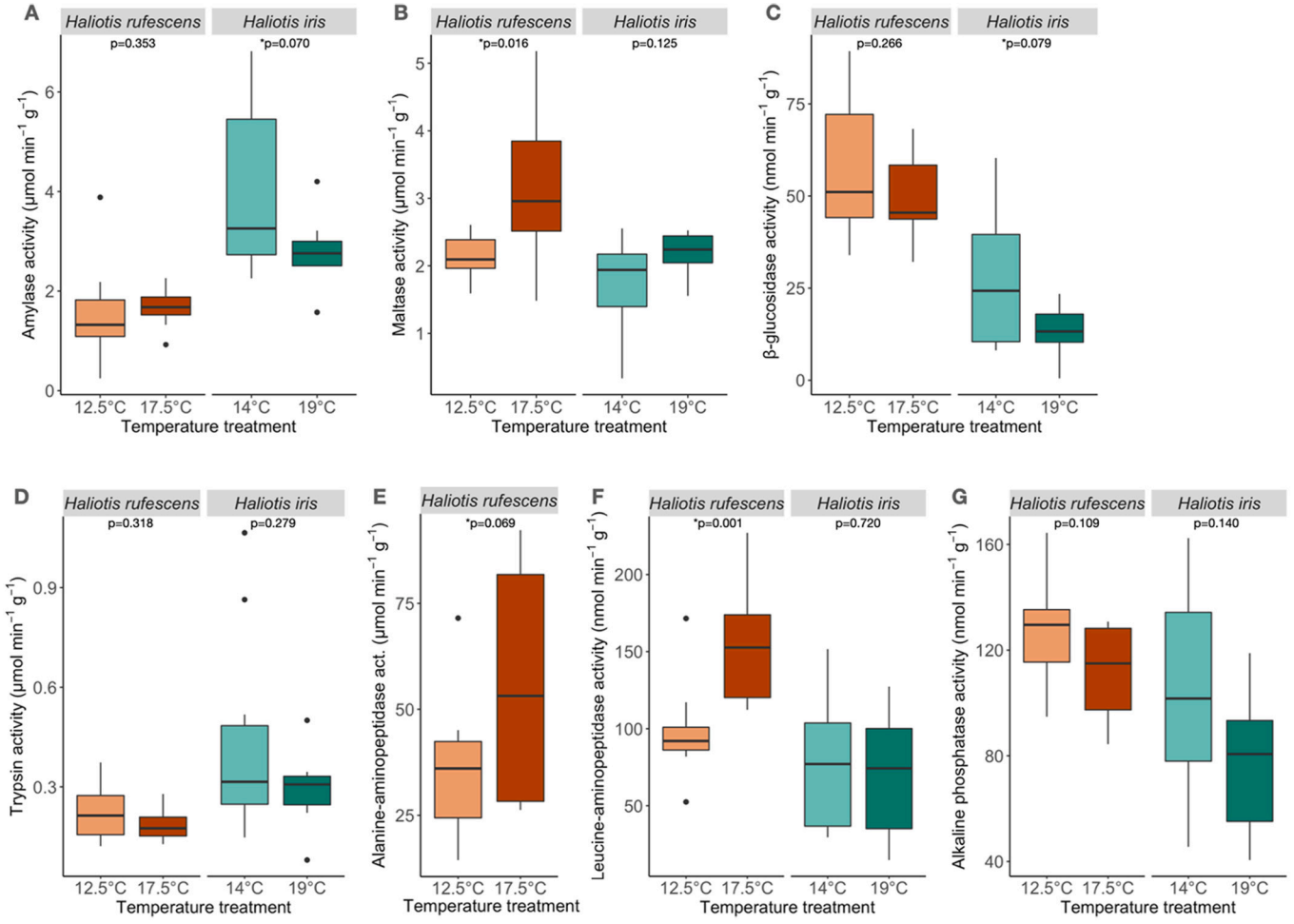

Fig. 5. Digestive enzyme activities (per gram of tissue analyzed) in the digestive gland in both species at each temperature, with comparisons made within a species only via ANOVA except for the following tests: maltase in *H. rufescens* was compared with a Mann-Whitney nonparametric test; and maltase in Red Abalone was compared with a Welch's t-test. Data and statistical tests are summarized in Tables 1 and 2. Boxplot whiskers span 1.5 times the IQR, with individual point(s) representing outliers A. Amylase activity (μmol min^{-1} g^{-1}), B. Maltase activity (μmol min^{-1} g^{-1}), C. β-glucosidase activity (nmol min^{-1} g^{-1}), D. Trypsin activity (μmol min^{-1} g^{-1}), E. Alanine-aminopeptidase activity (μmol min^{-1} g^{-1}), F. Leucine-aminopeptidase activity (μmol min^{-1} g^{-1}). G. Alkaline phosphatase activity (nmol min^{-1} g^{-1}). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Amylase activity in *H. rufescens* did not differ significantly between the temperature treatments ($p = 0.353$; Table 2, Fig. 5A). Maltase activity in heat-treated *H. rufescens* was 49% higher than in the ambient treatment ($p = 0.016$; Table 2, Fig. 5B). β-glucosidase activity in *H. rufescens* did not differ by temperature treatment ($p = 0.266$; Table 1, Fig. 5C). Trypsin activity in *H. rufescens* did not differ significantly between the temperature treatments ($p = 0.318$; Table 1, Fig. 5D). In *H. rufescens,* alanine-aminopeptidase activity was 56% higher and leucine-aminopeptidase activity was 59% higher in the heat treatment (alanine-: $p = 0.069$; Table 1, Fig. 5E; leucine-: $p = 0.001$; Table 1, Fig. 5F). Lastly, alkaline phosphatase did not differ significantly between the temperature treatments for *H. rufescens* ($p = 0.109$; Table 1, Fig. 5G).

3.5. Principal components analysis

A principal components analysis was used to visualize relationships among the physiological metrics measured, temperature treatments, and species (Fig. 6). The first principal component (PC1) explained 48.05% of the variation in the data, and PC2 explained 13.43% of the variation in the data. The PCA plot shows the species separated along the PC1 axis, and, much like the individual parameters we measured, that each species is impacted by heat in different ways. *H. rufescens* has a larger spread along the PC2 axis, with more separation by temperature treatment, whereas *H. iris* shifts in a more positive direction along PC1 in response to the heat treatment. Different types of digestibility and some enzymes explain how the species differ, with trypsin and amylase activities providing some predictive power in the heat response in *H. rufescens*. Although trypsin and amylase activities also appear to affect the heat response in *H. iris*, β-glucosidase and alkaline phosphatase also look to be driving this relationship.

4. Discussion

This study's two main goals were to (1) characterize the digestive enzyme activities and digestive efficiencies of two abalone species on natural diets; and (2) test the hypothesis that abalone digestive function can keep pace with increased metabolic demand at temperatures elevated 5 °C above ambient. *H. iris* in the heat treatment consumed more food than in the ambient treatment. Although metabolic rates of both species increased during the heat treatment, digestibility was not significantly changed. Enzyme activities followed individual species patterns, with some increases and decreases in the heat treatment compared to ambient treatment for some enzymes that explain some, but not all, of the digestibility patterns.

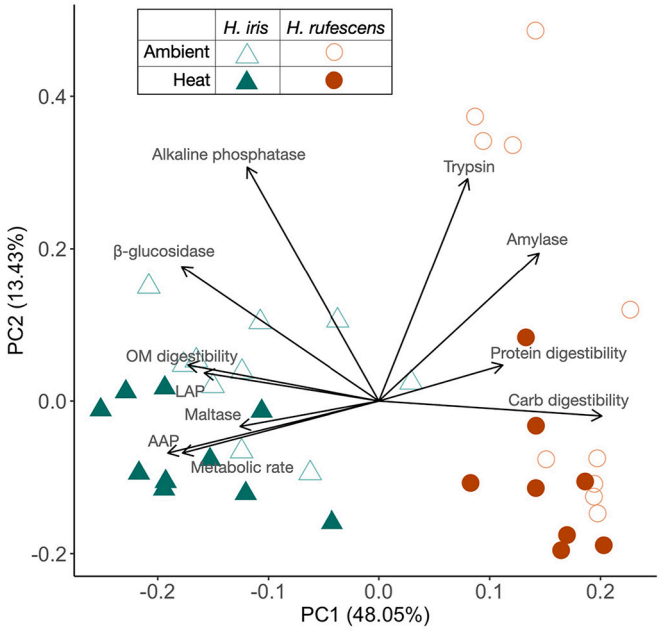

Fig. 6. Scatterplot indicating species and heat treatment on the first two principal components that describe >61% of the variation in this principal component analysis of scaled metabolic rate, enzyme activity levels, and digestibility percentages, with arrows representing factor loadings for those variables. "LAP" is leucine-aminopeptidase, and "AAP" is alanine-aminopeptidase.

4.1. Metabolic rates

Metabolic rates in heat treated *H. rufescens* and *H. iris* were significantly higher than individuals of the same species in the ambient treatment (32% and 57% increases, respectively; Table 1, Fig. 1). *H. iris* shells did contain epibiont growth, though the growth appeared very evenly distributed based on qualitative visual assessment. The *H. rufescens* contained little to no epibiont growth because they originated at a farm. These results confirm that the high temperature treatment group is experiencing elevated metabolic demand relative to animals at ambient temperatures. Temperatures similar to those used in this experiment, combined with a reduction in food availability, induced differences in metabolomic profiles that suggest those conditions are stressful, but not lethal, for *H. rufescens* (Rosenblum et al., 2005). Our metabolic results also demonstrate that each species of abalone can respond differently to the same effective increase in temperature, as was also previously seen in *H. rufescens*, *H. fulgens*, and *H. corrugata* (Dahlhoff and Somero, 1993). In other studies of invertebrates, higher metabolic rates are documented in species from cooler habitats (Addo-Bediako et al., 2002; Vorhees et al., 2013). We found similar patterns, wherein the *H. rufescens* (with a colder ambient temperature) qualitatively have higher metabolic rates, even at cooler ambient temperatures, than *H. iris* (Fig. 3). Although higher intake of food can lead to an increased metabolic rate through the thermodynamic heat increment of feeding or specific dynamic action (Secor, 2009), elevated temperature raises the rates of reactions, and thus the metabolic rate of ectothermic animals (Karasov and Martínez del Rio, 2007). Increased metabolic demand in turn leads ectothermic animals to eat more food to meet those demands. Although we cannot say how much the increased metabolic rate in the abalone was caused by digestion versus a pure temperature effect (we only measured routine metabolic rate), temperature is the ultimate driving force here since it increases metabolic demand in the first place. Thus, the increased metabolic rates of the abalone are not simply the impact of the heat increment of feeding (Secor, 2009) and reflect increased metabolic demands placed on the abalone through warming.

Metabolic rate Q_{10} was 1.73 for *H. rufescens* and 2.46 for *H. iris*, showing that *H. iris* metabolisms are nearly one and a half times more sensitive to temperature than those of *H. rufescens.* M O_2 Q_{10} values we measured are within the range of other Haliotids measured. M O_2 Q_{10} for *H. rufescens* is similar to the $Q_{10} = 1.79$ measured in *H. laevigata*, and M O_2 Q_{10} for *H. iris* is more similar to the $Q_{10} = 2.11$ measured in *H. laevigata* x *H. rubra* hybrids (Alter et al., 2021). If *H. iris* were close to its critical thermal maximum temperature, this might explain their lower Q_{10}; an animal near its maximum cannot withstand higher temperatures. However, critical thermal maxima for both *H. rufescens* and *H. iris* have been measured around 27–28 °C (Díaz et al., 2000; Searle et al., 2006), so this is unlikely to explain the differences we observed in metabolic rate Q_{10}. Q_{10} differences can also be explained by thermal stability of the natural environment, as has been shown in mosquitoes (Vorhees et al., 2013), where higher environmental variability leads to less sensitivity (lower metabolic rate-temperature slope) in metabolic rate. In intertidal animals such as limpets, oxygen consumption rates are more variable in subtidal species across a wide range of temperatures, but intertidal species subjected to the same temperature range show less variable oxygen consumption (Branch et al., 2015). In oysters, subtidal *Ostrea chilensis* had a high Q_{10} during a few weeks of high temperature

exposure while the intertidal *Crassostrea gigas* had a lower Q_{10} during this period (Dunphy et al., 2006).

Temperature and environmental variation impact both abalone species examined in this study. There have been declines in *H. rufescens* reproductive parameters and success in wild populations in recent years during interannual climate driven periods of kelp disappearance (Rogers-Bennett et al., 2021), emphasizing that these animals, which can live for decades, are exposed to variable environments during their lifetimes. *H. iris* has been demonstrated to have growth rates and length at maturity that are inversely related to sea surface temperature (Naylor et al., 2006). The *H. iris* animals used for this experiment were collected from populations in the very shallow (1-4 m) subtidal zone. Even so, the results also suggest that the *H. iris* populations studied here have had more stability in their thermal environment than did the *H. rufescens* acquired from aquaculture. Measuring metabolic rate across a broader temperature regime would help clarify where on the metabolic rate-temperature curve these samples were taken.

4.2. Nutrient digestibility

In both species, apparent organic matter digestibility, carbohydrate digestibility, and protein digestibility were not significantly altered in heat treatment individuals. Apparent organic matter digestibility in *H. rufescens* alone trended toward being lower in heat treatment animals relative to ambient (Fig. 4A). These data indicate that both species maintain the same digestive efficiency at both temperatures, despite the increased metabolic demand, and increased intake (as observed in *H. iris* in this study). Indeed, in a study measuring digestive efficiency in *H. laevigata* across a 12 °C gradient, animals had a faster digesta transit rate at 26 °C vs 14 °C, but the animals maintained the same digestibility of their diet (Currie et al., 2015). When animals have a faster gut transit rate, the digesta has less time to interact with enzymes, the biochemical agents of digestion. A possible explanation for this somewhat counter-intuitive observation might be increased digestive enzyme activities, which we observed in some enzyme classes in this work (discussed below). We didn't measure the activity levels of alginate lyase or laminarinase. These enzymes degrade the main structural polysaccharide and storage polysaccharide, respectively, of kelps (Painter, 1983), and these enzymes should be the focus of future studies on the temperature sensitivity of digestion in abalone. Similar conclusions can be drawn on a variety of proteolytic enzymes. Alternatively, especially for the enzymes that did not experience increased activity even when their substrate class digestibility was maintained, the elevated temperatures may be closer to the temperature optimum for those particular enzymes than the ambient temperature was (Somero, 1978). For example, prickleback fishes (family Stichaeidae) reside at temperatures well below the "optimum" for their amylase (German et al., 2016).

4.3. Digestive enzyme activities

In this study, we focused on carbohydrases that break down components of the cell wall (i.e., cellulose) or storage polysaccharides (i.e., starch) of green algae (Painter, 1983), which are commonly consumed by *H. rufescens* (Leighton, 2000). Starch is also relevant for red algae (Painter, 1983). *H. rufescens* showed no difference in amylase activity or β-glucosidase activity between temperature treatments (Fig. 5). Amylase breaks down starch into maltose and glucose, and β-glucosidase breaks down β-glucosides (from cellulose breakdown) and other oligosaccharides into glucose. Maltase helps break down maltose into glucose molecules (Karasov and Douglas, 2013), and *H. rufescens* demonstrates significantly higher maltase activity in the heat treatment relative to the ambient treatment ($p = 0.016$). Because carbohydrate digestibility stayed constant in this species in both temperature treatments, the increase in maltase activity may be part of the mechanism for the maintenance of digestibility of carbohydrates at a higher metabolic rate. By increasing the activity of maltase (either by producing more maltase or by producing a different version of the enzyme that catalyzes carbohydrate breakdown more efficiently), potentially in addition to other parameters not measured in this study for *H. rufescens* (e.g. digesta transit rate, intake), this species is able to maintain carbohydrate digestion across these two temperatures. Further exploration into which of the mentioned potential mechanisms underlie that activity difference in maltase is an important next research step.

H. iris, on the other hand, showed significantly reduced amylase and β-glucosidase activities in the heat treatment compared to the ambient treatment, and no change in maltase activity (Tables 1 and 2; Fig. 5). Despite these declines, *H. iris* maintained carbohydrate digestibility across both treatments. We didn't measure the enzymatic activity levels against laminarin, the main storage polysaccharide in brown algae (Painter, 1983), which may have shown more temperature variability, although β-glucosidase can cleave the beta 1–3 bonds of laminaribiose (Kim et al., 2018) produced as part of the laminarin digestive process, and β-glucosidase activity did decline with the temperature increase. Mannitol is also known to be a major nutritive agent of brown algae for herbivorous fishes that consume kelps (White et al., 2010), but little is known of mannitol absorption or fermentation in mollusks, and *Haliotis discus* is not attracted to mannitol in diet choice experiments (Harada et al., 1994). *H. iris* consumed about 38% more algae (mg of dry algae per gram of abalone soft tissue daily) at the elevated temperature than was consumed at ambient temperatures (Table 1, Fig. 2). Ingesting more food per unit time is the likely mechanism by which an animal can acquire sufficient nutrients from their diet, although higher intake means faster digesta transit rate, and generally, lower overall digestibility (German, 2011). Hence, maintaining digestibility could be linked to some perhaps unmeasured digestive enzyme activity in *H. iris*. For example, like maltase in *H. rufescens*, *H. laevigata* increases amylase activity in response to an 8 °C temperature increase (Bansemer et al., 2016b). This suggests that the response of digestion of carbohydrates in *Haliotis* is species-specific, which can have ramifications for any restoration and fishery work applying the physiological responses seen in some *Haliotis* species to others.

Protease activities followed similar patterns, in that *H. rufescens* experienced some increases at higher temperature and *H. iris* experienced no change, yet neither species experienced changes in protein digestibility. More specifically, *H. rufescens* showed no change in trypsin activity, but an increase in alanine-aminopeptidase and leucine-aminopeptidase activities at higher temperature. On the other hand, *H. iris* experienced no changes in protease activities at higher temperatures. Interestingly, *H. iris* lacked any measurable alanine-aminopeptidase activity, even in the ambient treatment. Alanine-aminopeptidase was also unmeasurable in wild *H. iris* (AM Lee et al., *in prep*), which suggests they may not express the gene for this protein. For *H. rufescens*, the maintenance of protein digestibility may be explained by the increase in the enzyme activities of the two aminopeptidases measured. For *H. iris*, despite higher intake at a higher temperature, animals maintained their protein digestibility at their higher metabolic rate without increasing the protease activities for the enzymes measured in this study. Similarly, trypsin activity in *H. laevigata* was not affected by a change in temperature from 14 to 22 °C (Bansemer et al., 2016b), although digestibility was not measured for *H. laevigata*. There are multiple possible explanations for this maintenance of digestibility despite increased intake and no change in enzyme activities. Trypsin, aminopeptidases, and other proteases vary in their activity in different gut regions (Garcia-Esquivel and Felbeck, 2006), though most enzymes peak in the digestive gland, and this study examined the digestive gland only. Perhaps protein digestion was increased in the intestine or another gut region, and our data did not capture that. Again, our results show species-specific patterns in how discrete aspects of abalone digestive physiology (in this example, protease activities) respond to increased temperature. Future work should explore other enzymes and nutrient classes. For example, galactolipids are prevalent in the algae that abalone consume, making it likely that these animals would have

galactolipases to break down these molecules (Sahaka et al., 2020), but specific lipases have not been well characterized in abalone.

4.4. Principal component analysis and other considerations

Both species were impacted by heat (Fig. 6), as there is a distinction between the animals in ambient versus heat treatments along the y-axis (PC2 explained 13.43% of the variation in the data, with *H. rufescens* having a larger and more variable impact from heat than *H. iris*). PC1 explained 48.05% of the variation in data, and this axis is where we see a distinct separation of species. Based on the vectors, β-glucosidase and alkaline phosphatase are driving some of the heat response for *H. iris* while trypsin and amylase activities drive the response in *H. rufescens*. This further supports the notion that digestive functions and their responses to thermal stress are species-specific, despite similar types of habitat and diets among *H. rufescens* and *H. iris*. Perhaps these differences are instead the result of their evolutionary histories, as these two species are not closely related (Lee and Vacquier, 1995; Coleman and Vacquier, 2002; Estes et al., 2005; Frederick, 2019).

While abalone experience shifts in digestive function during thermal stress, the availability and nutritional quality of their wild diets are also changing due to climate change. Marine heatwaves have led to the decimation of bull kelp forests in *H. rufescens* habitat by sea urchins (Rogers-Bennett and Catton, 2019). Similarly, climate change has led to the replacement of *M. pyrifera* beds with urchin barrens in eastern Tasmania (Johnson et al., 2011). In southern California, *M. pyrifera* is not only declining in abundance, but also declining in nutritional quality, posing an additional threat to animals whose diets are largely composed of kelp (Lowman et al., 2021). Changes in the nutritional quality of kelp will be an important area of future work to better understand whether abalone digestive systems can keep pace with the multitude of changes to their diet.

4.5. Conclusions and future directions

This study focused on the digestive enzyme activities in the digestive gland, but abalone digestive systems have multiple organs, with a radula, esophagus, stomach, digestive gland, and at least two morphologically distinct sections of intestines. In addition, there is strong evidence that digestive enzymes vary among region, specifically in *H. rufescens* (Garcia-Esquivel and Felbeck, 2006), but across many other taxa as well (Teo and Woodring, 1994; Martin et al., 2011; Santos et al., 2018; Wehrle et al., 2020). Further work exploring how each region of the gut responds to temperature and other stressors is essential for developing a clear understanding of how the entire digestive physiology is impacted in a changing ocean. In addition, we examined a limited scope of digestive enzymes. As we saw, alanine-aminopeptidase was detectable in *H. rufescens* but not *H. iris*. An expansion of this work examining additional digestive enzymes is important for further clarifying how each mechanism of digestion is changed at different temperatures, and exploring the genome for presence or absence of different digestive enzyme genes. Lastly, the gut microbiome may be a contributor to digestion but we did not differentiate between endogenous and exogenous enzyme production here; gut microbial communities have been studied in relation to changing temperature, age, disease, and antibiotic treatments in Haliotids (Erasmus et al., 1997; Enriquez et al., 2001; Cicala et al., 2018; Gobet et al., 2018; Parker-Graham et al., 2020; Villasante et al., 2020; Danckert et al., 2021). However, endogenous alginate lyase, carboxymethylcellulase, laminarinase, agarase, and carrageenase have been measured in the digestive gland (referred to as hepatopancreas) of *H. midae*, where bacteria were not found (Erasmus et al., 1997). Exploration of the specific contributions of the gut microbiome to digestion in these species, whether those communities contribute to digestion and in what ways, and how those communities might shift during higher metabolic demands at higher temperatures may be an important area for further study. Lastly, histology of the gut would be a useful area for further study to determine whether there are any morphological gut changes during heat stress.

The work described here emphasizes that, at least over a short timescale (six weeks), abalone were able to maintain digestibility of their diets during elevated metabolic demand caused by heat stress in species-specific ways. Understanding how each species alters digestive strategies and mechanisms to cope with changes in metabolic demand is important for efficient maintenance of aquaculture of these important species, especially as we may be able to formulate species-informed diets for animals in aquaculture during marine heatwaves. When focused on the restoration of abalone species (e.g., both *H. sorenseni* and *Haliotis cracherodii* are listed as federally Endangered species in the United States), an understanding of the specific ways by which each restoration-targeted species undergoes nutritional and metabolic stress during marine heatwaves is essential for management of these species, in addition to how their nutrition and digestive needs must be considered in captive rearing. Our data suggest that we should not apply data from other species to other Haliotids without studying each species directly.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Addo-Bediako, A., Chown, S.L., Gaston, K.J., 2002. Metabolic cold adaptation in insects: a large-scale perspective. Funct. Ecol. 16, 332–338.

Alter, K., Morash, A.J., Andrewartha, S.J., Andrew, S., Clark, T.D., Elliott, N.G., Frappell, P.B., 2021. Aerobic and anaerobic movement energetics of hybrid and pure parental abalone. J. Comp. Physiol. B. 191, 1111–1124.

Bansemer, M.S., Qin, J.G., Harris, J.O., Duong, D., Currie, K.L., Howarth, G.S., Stone, D. A.J., 2016a. Dietary inclusions of dried macroalgae meal in formulated diets improve the growth of greenlip abalone (*Haliotis laevigata*). J. Appl. Phycol. 28, 3645–3658.

Bansemer, M.S., Qin, J.G., Harris, J.O., Schaefer, E.N., Wang, H.R., Mercer, G.J., Howarth, G.S., Stone, D.A.J., 2016b. Age-dependent response of digestive enzyme activities to dietary protein level and water temperature in greenlip abalone (*Haliotis laevigata*). Aquaculture 451, 451–456.

Bevelander, G., 1988. Abalone: Gross and Fine Structure. Boxwood Press.

Bond, N.A., Cronin, M.F., Freeland, H., Mantua, N., 2015. Causes and impacts of the 2014 warm anomaly in the NE Pacific. Geophys. Res. Lett. 42, 3414–3420.
Braid, B.A., Moore, J.D., Robbins, T.T., Hedrick, R.P., Tjeerdema, R.S., Friedman, C.S., 2005. Health and survival of red abalone, *Haliotis rufescens*, under varying temperature, food supply, and exposure to the agent of withering syndrome. J. Invertebr. Pathol. 89, 219–231.
Branch, G.M., Borchers, P., Brown, C.R., Donnelly, D., 2015. Temperature and food as factors influencing oxygen consumption of intertidal organisms, particularly limpets. Am. Zool. 28, 137–146.
Britz, P.J., Hecht, T., Knauer, J., 1996. Gastric evacuation time and digestive enzyme activity in abalone *Haliotis midae* fed a formulated diet. South African J. Mar. Sci. Suid-Afrikaanse Tydskrif Vir Seewetenskap 17, 297–303.
Brzek, P., Kohl, K.D., Caviedes-Vidal, E., Karasov, W.H., 2011. Fully reversible phenotypic plasticity of digestive physiology in young house sparrows: lack of long-term effect of early diet composition. J. Exp. Biol. 214, 2755–2760.
Cicala, F., Cisterna-Céliz, J.A., Moore, J.D., Rocha-Olivares, A., 2018. Structure, dynamics and predicted functional role of the gut microbiota of the blue (*Haliotis fulgens*) and yellow (*H. corrugata*) abalone from Baja California Sur, Mexico. PeerJ 6, e5830.
Coleman, A.W., Vacquier, V.D., 2002. Exploring the phylogenetic utility of ITS sequences for animals: a test case for abalone (*Haliotis*). J. Mol. Evol. 54, 246–257.
Connor, K.M., Sung, A., Garcia, N.S., Gracey, A.Y., German, D.P., 2016. Modulation of digestive physiology and biochemistry in *Mytilus californianus* in response to feeding level acclimation and microhabitat. Biol Open 5, 1200–1210.
Cornwall, C.E., Phillips, N.E., McNaught, D.C., 2009. Feeding preferences of the abalone *Haliotis iris* in relation to macroalgal species, attachment, accessibility and water movement. J. Shellfish Res. 28 (589–597), 589.
Currie, K.L., Lange, B., Herbert, E.W., Harris, J.O., Stone, D.A.J., 2015. Gastrointestinal evacuation time, but not nutrient digestibility, of greenlip abalone, *Haliotis laevigata* Donovan, is affected by water temperature and age. Aquaculture 448, 219–228.
Dahlhoff, E., Somero, G.N., 1993. Effects of temperature on mitochondria from abalone (genus *Haliotis*) - adaptive plasticity and its limits. J. Exp. Biol. 185, 151–168.
Danckert, N.P., Wilson, N., Phan-Thien, K.-Y., Stone, D.A.J., 2021. The intestinal microbiome of Australian abalone, *Haliotis laevigata* and *Haliotis laevigata* × *Haliotis rubra*, over a 1-year period in aquaculture. Aquaculture 534, 736245.
Díaz, F., del Rio-Portilla, M.A., Sierra, E., Aguilar, M., Re-Araujo, A.D., 2000. Preferred temperature and critical thermal maxima of red abalone Haliotis rufescens. J. Therm. Biol. 25, 257–261.
Dixon, M.G., 1992. The Effect of Temperature and Photoperiod on the Digestive Physiology of the South African Abalone *Haliotis midae*. Faculty of Science, Ichthyology and Fisheries Science.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Dunphy, B.J., Wells, R.M.G., Jeffs, A.G., 2006. Oxygen consumption and enzyme activity of the subtidal flat oyster (*Ostrea chilensis*) and intertidal Pacific oyster (*Crassostrea gigas*): responses to temperature and starvation. N. Z. J. Mar. Freshw. Res. 40, 149–158.
Elliot, J.M., Davison, W., 1975. Energy equivalents of oxygen consumption in animal energetics. Oecologia 19, 195–201.
Enriquez, A., Viana, M.T., Vasquez, C., Shimada, A., 2001. Digestion of cellulose by stomach homogenates of green abalone (*Haliotis fulgens*). J. Shellfish Res. 20, 297–300.
Erasmus, J.H., Cook, P.A., Coyne, V.E., 1997. The role of bacteria in the digestion of seaweed by the abalone *Haliotis midae*. Aquaculture 155, 377–386.
Estes, J.A., Lindberg, D.R., Wray, C., 2005. Evolution of large body size in abalones (*Haliotis*): patterns and implications. Paleobiology 31, 591–606.
Fleming, A.E., 1995. Digestive efficiency of the Australian abalone *Haliotis rubra* in relation to growth and feed preference. Aquaculture 134, 279–293.
Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: a review and future directions. Aquaculture 140, 5–53.
Frederick, A.R., 2019. How do warming temperatures and disease affect abalone? Examining digestive physiology and withering syndrome in *Haliotis*. Ecology and Evolutionary Biology. University of California, Irvine.
Garcia-Carreno, F.L., del Toro, M.A.N., Serviere-Zaragoza, E., 2003. Digestive enzymes in juvenile green abalone, *Haliotis fulgens*, fed natural food. Comp. Biochem. Physiol. B 134, 143–150.
Garcia-Esquivel, Z., Felbeck, H., 2006. Activity of digestive enzymes along the gut of juvenile red abalone, *Haliotis rufescens*, fed natural and balanced diets. Aquaculture 261, 615–625.
Garcia-Esquivel, Z., Felbeck, H., 2009. Comparative performance of juvenile red abalone, *Haliotis rufescens*, reared in laboratory with fresh kelp and balanced diets. Aquac. Nutr. 15, 209–217.
Garcia-Esquivel, Z., Montes-Magallon, S., Gonzalez-Gomez, M.A., 2007. Effect of temperature and photoperiod on the growth, feed consumption, and biochemical content of juvenile green abalone, *Haliotis fulgens*, fed on a balanced diet. Aquaculture 262, 129–141.
German, D.P., 2011. Digestive efficiency. In: Farrell, A.P.J.J.C., Richards, J.G., Stevens, E.D. (Eds.), Encyclopedia of Fish Physiology, from Genome to Environment. Elsevier, San Diego, CA.
German, D.P., Horn, M.H., Gawlicka, A., 2004. Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. Physiol. Biochem. Zool. 77, 789–804.
German, D.P., Sung, A., Jhaveri, P., Agnihotri, R., 2015. More than one way to be an herbivore: convergent evolution of herbivory using different digestive strategies in prickleback fishes (Stichaeidae). Zoology (Jena, Germany) 118, 161–170.
German, D.P., Foti, D.M., Heras, J., Amerkhanian, H., Lockwood, B.L., 2016. Elevated gene copy number does not always explain elevated amylase activities in fishes. Physiol. Biochem. Zool. 89, 277–293.
Gobet, A., Mest, L., Perennou, M., Dittami, S.M., Caralp, C., Coulombet, C., Huchette, S., Roussel, S., Michel, G., Leblanc, C., 2018. Seasonal and algal diet-driven patterns of the digestive microbiota of the European abalone *Haliotis tuberculata*, a generalist marine herbivore. Microbiome 6, 60.
Green, A.J., Jones, C.L.W., Britz, P.J., 2011. The protein and energy requirements of farmed South African abalone *Haliotis midae* cultured at optimal and elevated water temperatures. Aquac. Res. 42, 1653–1663.
Groppe, J.C., Morse, D.E., 1993. Molluscan chymotrypsin-like protease: structure, localization, and substrate specificity. Arch. Biochem. Biophys. 305, 159–169.
Halsey, L.G., 2019. The reign of the p-value is over: what alternative analyses could we employ to fill the power vacuum? Biol. Lett. 15, 20190174. https://doi.org/10.1098/rsbl.2019.0174.
Harada, K., Miyasaki, T., Tamura, Y., 1994. Chemoattractant effects of sugars and their related compounds on black abalone *Haliotis discus*. Comp. Biochem. Physiol. A Physiol. *109* (1), 111–115. https://doi.org/10.1016/0300-9629(94)90316-6.
Hart, L.C., Goodman, M.C., Walter, R.K., Rogers-Bennett, L., Shum, P., Garrett, A.D., Watanabe, J.M., O'Leary, J.K., 2020. Abalone recruitment in low-density and aggregated populations facing climatic stress. J. Shellfish Res. 39.
Hernandez-Santoyo, A., Hernandez-Arana, A., Arreguin-Espinosa, R., Rodriguez-Romero, A., 1998. Purification and characterization of several digestive proteases from the blue abalone, *Haliotis fulgens*. Aquaculture 159, 203–216.
Herrera, M.J., Heras, J., German, D.P., 2022. Comparative transcriptomics reveal tissue level specialization towards diet in prickleback fishes. J. Comp. Physiol. B. 192, 275–295.
Holbrook, N.J., Scannell, H.A., Sen, Gupta A., Benthuysen, J.A., Feng, M., Oliver, E.C.J., Alexander, L.V., Burrows, M.T., Donat, M.G., Hobday, A.J., Moore, P.J., Perkins-Kirkpatrick, S.E., et al., 2019. A global assessment of marine heatwaves and their drivers. Nat. Commun. 10, 2624. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6570771/.
Hooper, C., Day, R., Slocombe, R., Benkendorff, K., Handlinger, J., Goulias, J., 2014. Effects of severe heat stress on immune function, biochemistry and histopathology in farmed Australian abalone (hybrid *Haliotis laevigata* x *Haliotis rubra*). Aquaculture 432, 26–37.
Horn, M.H., Gawlicka, A.K., German, D.P., Logothetis, E.A., Cavanagh, J.W., Boyle, K.S., 2006. Structure and function of the stomachless digestive system in three related species of New World silverside fishes (Atherinopsidae) representing herbivory, omnivory, and carnivory. Mar. Biol. 149, 1237–1245.
Jew, C.J., 2019. Living in a high CO2 world: aquaculture and fish that breathe air. In: *UC Irvine*. ProQuest ID: Jew_uci_0030D_15584. Merritt ID: ark:/13030/m5xh4rdx. Retrieved from. https://escholarship.org/uc/item/95w310jd.
Johnson, C.R., Banks, S.C., Barrett, N.S., Cazassus, F., Dunstan, P.K., Edgar, G.J., Frusher, S.D., Gardner, C., Haddon, M., Helidoniotis, F., Hill, K.L., Holbrook, N.J., et al., 2011. Climate change cascades: shifts in oceanography, species' ranges and subtidal marine community dynamics in eastern Tasmania. J. Exp. Mar. Biol. Ecol. 400, 17–32.
Karasov, W.H., Douglas, A.E., 2013. Comparative digestive physiology. Compr Physiol 3, 741–783.
Karasov, W.H., Martínez del Rio, C., 2007. Why Study Digestion Physiological Ecology. Princeton University Press, p. 117.
Khan, F.U., Hu, M., Kong, H., Shang, Y., Wang, T., Wang, X., Xu, R., Lu, W., Wang, Y., 2020. Ocean acidification, hypoxia and warming impair digestive parameters of marine mussels. Chemosphere 256, 127096.
Kim, D.H., Kim, D.H., Lee, S.H., Kim, K.H., 2018. A novel β-glucosidase from Saccharophagus degradans 2-40T for the efficient hydrolysis of laminarin from brown macroalgae. Biotechnology for Biofuels 11 (1), 64. https://doi.org/10.1186/s13068-018-1059-2.
Knauer, J., Britz, P.J., Hecht, T., 1996. Comparative growth performance and digestive enzyme activity of juvenile South African abalone, Hal*iotis* midae, fed on diatoms and a practical diet. Aquaculture 140, 75–85.
Lee, Y.H., Vacquier, V.D., 1995. Evolution and systematics in Haliotidae (Mollusca: Gastropoda): inferences from DNA sequences of sperm lysin. Mar. Biol. 124, 267–278.
Leigh, S.C., Nguyen-Phuc, B.Q., German, D.P., 2018. The effects of protein and fiber content on gut structure and function in zebrafish (*Danio rerio*). J. Comp. Physiol. B. 188, 237–253.
Leighton, D.L., 2000. The Biology and Culture of California Abalones. Dorrance Publishing Co., Inc., Pittsburgh, PA.
Lo, N., Watanabe, H., Sugimura, M., 2003. Evidence for the presence of a cellulase gene in the last common ancestor of bilaterian animals. P R Soc B 270, S69–S72.
Lowman, H.E., Emery, K.A., Dugan, J.E., Miller, R.J., 2021. Nutritional quality of giant kelp declines due to warming ocean temperatures. Oikos. 1–14. https://doi.org/10.1111/oik.08619.
Martin, G.G., Martin, A., Tsai, W., Hafner, J.C., 2011. Production of digestive enzymes along the gut of the giant keyhole limpet *Megathura crenulata* (Mollusca: Vetigastropoda). Comparat. Biochem. Physiol. A-Mol. Integr. Physiol. 160, 365–373.
Matsuhiro, B., Zambrano, D.M., 1990. Carbohydrate constituents of *Lessonia trabeculata*. J. Appl. Phycol. 2, 183–185. https://doi.org/10.1007/BF00023381.
Mclean, N., 1970. Digestion in *Haliotis rufescens* Swainson (Gastropoda-Prosobranchia). J. Exp. Zool. 173, 303.
Misra, R., Sérazin, G., Meissner, K.J., Sen, Gupta A., 2021. Projected changes to Australian marine Heatwaves. Geophys. Res. Lett. 48.

Morash, A.J., Alter, K., 2015. Effects of environmental and farm stress on abalone physiology: perspectives for abalone aquaculture in the face of global climate change. Rev. Aquac. 7, 1–27.
Morton, J.E., 1979. Feeding and Digestion Molluscs. Hutchinson & Co., Ltd, London, pp. 97–123.
Naylor, J.R., Andrew, N.L., Kim, S.W., 2006. Demographic variation in the New Zealand abalone *Haliotis iris*. Mar. Freshw. Res. 57, 215–224.
Oliver, E.C.J., Benthuysen, J.A., Darmaraki, S., Donat, M.G., Hobday, A.J., Holbrook, N. J., Schlegel, R.W., Sen, Gupta A., 2021 Jan. Marine heatwaves. Annu. Rev. Mar. Sci. 13, 313–342.
Painter, T.J., 1983. Algal polysaccharides. In: Aspinall, G.O. (Ed.), The Polysaccharides, Volume 2. Academic Press Inc, New York, NY, pp. 196–285.
Parker-Graham, C.A., Eetemadi, A., Yazdi, Z., Marshman, B.C., Loeher, M., Richey, C.A., Barnum, S., Moore, J.D., Soto, E., 2020. Effect of oxytetracycline treatment on the gastrointestinal microbiome of critically endangered white abalone (*Haliotis sorenseni*) treated for withering syndrome. Aquaculture 526, 735411.
Picos-Garcia, C., Garcia-Carreno, F.L., Serviere-Zaragoza, E., 2000. Digestive proteases in juvenile Mexican green abalone, *Haliotis fulgens*. Aquaculture 181, 157–170.
R Core Team, 2021. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Rogers-Bennett, L., Catton, C.A., 2019. Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. Sci. Rep. 9, 15050.
Rogers-Bennett, L., Dondanville, R.F., Moore, J.D., Vilchis, L.I., 2010. Response of red abalone reproduction to warm water, starvation, and disease stressors: implications of ocean warming. J. Shellfish Res. 29, 599–611.
Rogers-Bennett, L., Klamt, R., Catton, C.A., 2021. Survivors of climate driven abalone mass mortality exhibit declines in health and reproduction following kelp forest collapse. Front. Mar. Sci. 8.
Rosa, R., Pimentel, M., Galan, J.G., Baptista, M., Lopes, V.M., Couto, A., Guerreiro, M., Sampaio, E., Castro, J., Santos, C., Calado, R., Repolho, T., 2016. Deficit in digestive capabilities of bamboo shark early stages under climate change. Mar. Biol. 163.
Rosenblum, E.S., Viant, M.R., Braid, B.M., Moore, J.D., Friedman, C.S., Tjeerdema, R.S., 2005. Characterizing the metabolic actions of natural stresses in the California red abalone, Haliotis rufescens using H-1 NMR metabolomics. Metabolomics 1, 199–209.
Sahaka, M., Amara, S., Wattanakul, J., Gedi, M.A., Aldai, N., Parsiegla, G., Lecomte, J., Christeller, J.T., Gray, D., Gontero, B., Villeneuve, P., Carrière, F., 2020. The digestion of galactolipids and its ubiquitous function in Nature for the uptake of the essential α-linolenic acid. Food Funct. 11, 6710–6744.
Santos, A.J., Frederick, A.R., Higgins, B.A., Carrillo, A., Carter, A.L., Dickson, K.A., German, D.P., Horn, M.H., 2018. The beach-spawning California grunion *Leuresthes tenuis* eats and digests conspecific eggs. J. Fish Biol. 93, 282–289.
Sawabe, T., Oda, Y., Shiomi, Y., Ezura, Y., 1995. Alginate degradation by bacteria isolated from the gut of sea-urchins and abalones. Microb. Ecol. 30, 193–202.
Searle, T., Roberts, R.D., Lokman, P.M., 2006. Effects of temperature on growth of juvenile Blackfoot abalone, Haliotis iris Gmelin. Aquac. Res. 37, 1441–1449.
Secor, S.M., 2009. Specific dynamic action: a review of the postprandial metabolic response. J. Comp. Physiol. B 179, 1–56. https://doi.org/10.1007/s00360-008-0283-7.
Serviere-Zaragoza, E., Navarrete del Toro, M.A., Garcia-Carreno, F.L., 1997. Protein-hydrolyzing enzymes in the digestive systems of the adult Mexican blue abalone, *Haliotis fulgens* (Gastropoda). Aquaculture 157, 325–336.
Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150, 76–85.
Somero, G.N., 1978. Temperature adaptation of enzymes: biological optimization through structure-function compromises. Annu. Rev. Ecol. Syst. 9, 1–29.
Somero, G.N., 2010. The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol. 213, 912–920.
Stone, D.A.J., Harris, J.O., Wang, H.R., Mercer, G.J., Schaefer, E.N., Bansemer, M.S., 2013. Dietary protein level and water temperature interactions for Greenlip abalone *Haliotis laevigata*. J. Shellfish Res. 32, 119–130.
Stumpp, M., Hu, M., Casties, I., Saborowski, R., Bleich, M., Melzner, F., Dupont, S., 2013. Digestion in sea urchin larvae impaired under ocean acidification. Nat. Clim. Chang. 3, 1044–1049.
Suzuki, K., Ojima, T., Nishita, K., 2003. Purification and cDNA cloning of a cellulase from abalone Haliotis discus hannai. Eur. J. Biochem. 270, 771–778.
Swezey, D.S., Boles, S.E., Aquilino, K.M., Stott, H.K., Bush, D., Whitehead, A., Rogers-Bennett, L., Hill, T.M., Sanford, E., 2020. Evolved differences in energy metabolism and growth dictate the impacts of ocean acidification on abalone aquaculture. Proc. Natl. Acad. Sci. U. S. A. 117, 26513–26519.
Teo, L.H., Woodring, J.P., 1994. Comparative total activities of digestive enzymes in different gut regions of the house cricket, *Acheta domesticus*. (Orthoptera: Gryllidae). Ann. Entomol. Soc. Am. 87, 886–890.
Thomsen, M.S., Mondardini, L., Alestra, T., Gerrity, S., Tait, L., South, P.M., Lilley, S.A., Schiel, D.R., 2019. Local extinction of bull kelp (*Durvillaea* spp.) due to a marine heatwave. Front. Mar. Sci. 6.
Villasante, A., Catalan, N., Rojas, R., Lohrmann, K.B., Romero, J., 2020. Microbiota of the digestive gland of red abalone (*Haliotis rufescens*) is affected by withering syndrome. Microorganisms 8.
Vorhees, A.S., Gray, E.M., Bradley, T.J., 2013. Thermal resistance and performance correlate with climate in populations of a widespread mosquito. Physiol. Biochem. Zool. 86, 73.
Wehrle, B.A., Herrel, A., Nguyen-Phuc, B.Q., Maldonado Jr., S., Dang, R.K., Agnihotri, R., Tadic, Z., German, D.P., 2020. Rapid dietary shift in *Podarcis siculus* resulted in localized changes in gut function. Physiol. Biochem. Zool. 93, 396–415.
White, W.L., Coveny, A.H., Robertson, J., Clements, K.D., 2010. Utilisation of mannitol by temperate marine herbivorous fishes. J. Exp. Mar. Biol. Ecol. 391 (1–2), 50–56. https://doi.org/10.1016/j.jembe.2010.06.007.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. ISBN 978-3-319-24277-4. https://ggplot2.tidyverse.org.
Will, M., Hale, M.L., Schiel, D.R., et al., 2011. Low to moderate levels of genetic differentiation detected across the distribution of the New Zealand abalone *Haliotis iris*. Mar. Biol. 158, 1417–1429.