

Shell biomineralization and the role of nutritional quality on physiological traits of the marine turban snail *Tegula atra*

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Abstract

Herbivorous molluscs represent one of the greatest grazing impacts on abundance and diversity of primary producers in benthic ecosystems. However, shell biomineralization and the effects of nutritional content of kelp species on the physiological rate of herbivorous snails inhabiting intertidal-subtidal environments have scarcely been evaluated. Here, we investigated the mineralogy and organic composition of the shell on the marine turban snail *Tegula atra*. In addition, we conducted manipulative experiments to compare the nutritional quality of two kelp species, *Lessonia spicata* and *Macrocystis pyrifera*, and their influence on the feeding behavior and physiological rates of this snail species. Our results showed that the shell of *T. atra* consists of an outer calcitic and an inner aragonitic layer. Proteins were the main organic compounds at the inner surface of the shell, which showed an inverse pattern respect to carbonate signal. Experimental results demonstrated that *L. spicata* had the highest nutritional value (i.e., high organic matter and protein content), and was the preferred alga, compared with *M. pyrifera* in a choice experiment. However, no significant differences on consumption rate were found when the snails were maintained on a single algal species. Absorption efficiency and oxygen uptake were significantly higher for snails reared with *L. spicata* than *M. pyrifera*, but growth rate was similar. These results suggest that high metabolic activity was compensated by the high absorption efficiency of *T. atra* when it consumed the preferred *L. spicata*, which could explain the lack of relationship between preference and growth rate.

1. Introduction

In coastal ecosystems, macroalgal productivity and diversity provide habitat and food for many organisms, transferring energy to higher trophic levels (Steneck et al. 2002; Grilo et al. 2019; Fraser et al. 2020). In addition, abundance, and composition of primary producers (e.g., kelp forests) can be strongly controlled by foraging strategies of grazing herbivores (Vásquez and Buchmann 1997; Veliz and Vásquez 2000; Henríquez et al. 2011; Oróstica et al. 2014; Zarco-Perello et al. 2021). Consequently, for several marine organisms that inhabit coastal areas, their feeding preferences, consumption and growth rates may vary following changes in quantity and quality of foods item types (Lawrence 1975; Foster et al. 2015; Quintanilla-Ahumada et al. 2018). Indeed, a consumer experiencing low nutritional quality or inappropriate diet may modify its ingestion, absorption and/or respiration rates (Cross et al. 2005; Cox and Murray 2006; Remy et al. 2017; Grilo et al. 2019). Therefore, the nutritional value of food may impact metabolism, energy balance, and the growth rate of marine organisms (Cross et al. 2005; Duarte et al. 2010, 2011; Remy et al. 2017).

Herbivores are dependent on the nutritive value of macroalgae. The nutritional quality of macroalgae, including carbohydrates, proteins, organic matter, and the C:N ratio, have all been shown to play an important role in the feeding preferences of marine herbivores. Thus, the elevated energy content of food and/or its nutritive value, which increases energy gain, may be the key to survival, growth, reproduction, and fitness (Jormalainen et al. 2001; Cruz- Rivera and Hay 2003; Barile et al. 2004; Duarte et al. 2010, 2011). Therefore, any change in the nutritional quality of macroalgae may alter the physiological

processes and energy balance of herbivores. However, several behavioral feeding strategies have been reported by herbivorous organisms, which allow them to adjust their diet, and maintain and/or enhance their growth rate (e.g., Simpson and Simpson 1990; Barile et al. 2004; Cruz Rivera and Hay 2001, 2003). Among marine herbivorous organisms, the nutritional quality of algae (i.e., especially nitrogen and/or protein content) has been considered one of the most important components that can affect food consumption and food preference (Mattson 1980; Duffy and Hay 1991; Cruz-Rivera and Hay 2000; Barile et al. 2004). Thus, the selection of food has been related to diets with higher nutritional value and are therefore mostly consumed by herbivorous organisms (Cruz-Rivera and Hay 2003; Barile et al. 2004; Duarte et al. 2011). The consumption of preferred diets with high nutritional content have been shown to modify physiological traits but are not always reflected for the highest growth rates of grazers (e.g., Duarte et al. 2011, 2014; Quintanilla-Ahumada et al. 2018).

Along the benthic ecosystem of the Chilean coast, grazing plays an important role in the recovery, spatial structure, and distribution of kelps forest species (e.g., *Macrocystis pyrifera*, *Lessonia spicata*, *Lessonia trabeculata*) (Oróstica et al. 2014; Vásquez and Buchmann 1997; Henríquez et al. 2011). However, shell biomineralization patterns, feeding behaviour and physiological traits of herbivorous gastropods inhabiting intertidal and shallow subtidal areas have been scarcely evaluated, and even less is known about how nutritionally these kelps species can modify their performance. The herbivorous snail *Tegula atra* (Lesson) (Fig. 1), is a marine snail that inhabits the Peruvian coastline from (7° 24' S) to the straits of Magallanes (53° 28' S), and Chilean Patagonia. It has been described as highly dominant in terms of abundance and density on the intertidal and upper subtidal rocky shores of Northern and Southern Chile (Veliz and Vásquez 2000; Vásquez and Buchmann 1997; Vásquez et al. 1998). Along these shores, *T. atra* is associated with two large brown algae species (i.e., *Macrocystis pyrifera* and *Lessonia spicata*) (Veliz and Vásquez 2000; Vásquez and Buchmann 1997; Vásquez et al. 1998), suggesting that it is an important grazer upon *M. pyrifera*, especially in protected areas of Southern Chile (Vásquez and Buchmann 1997). In addition, *T. atra* also consumes *L. spicata* (formerly *Lessonia trabeculata*), and has played an important role in the recovery, spatial structure, and distribution of kelp forest species where they are principally associated, and therefore, (Veliz and Vásquez 2000; Vásquez and Buchmann 1997; Henríquez et al. 2011; Oróstica et al. 2014; Elliott Smith et al. 2021) present a greater contribution to herbivory in these coastal areas. Both kelp species are considered dominant in subtidal and intertidal rocky shore habitats (Veliz and Vásquez 2000; Vásquez and Buchmann 1997). Previous studies have demonstrated that these kelp species differ in their nutritional quality (e.g., Gomez and Westermeier 1995; Duarte et al. 2010), and this may affect the feeding behaviour and physiological traits of *T. atra*. In addition to their ecological relevance, *T. atra* also has commercial importance, with landings that reached 78 t in 2014 (Yañez et al. 2017). However, very few autoecological studies have evaluated the feeding ecology, shell biomineralogy, and the physiological performance of *T. atra* as a model species.

The aim of this study was to describe, for the first time, the shell biomineralization properties of *T. atra*, and to evaluate under experimental laboratory conditions the feeding behaviour and physiological traits of this snail. We hypothesized that *T. atra* will preferentially consume algae with the highest nutritional value (e.g., with high organic matter and protein content), and this will achieve highest absorption

efficiency, and growth rate. Hence, those individuals that fed on this macroalgae species will obtain larger amounts of energy to meet their physiological demands.

2. Materials And Methods

2.1 Animal and kelp collection

Individuals of *T. atra* (~ 2.3 cm \pm 0.01 SE in shell length and 4.8 ± 0.1 g of wet weight) were collected during the summer of 2019 from Calfuco beach, Southern Chile (ca. 39° S). After collection, the snails were transported to the coastal laboratory of the Universidad Austral de Chile (Valdivia, Chile) and maintained in aquaria (50 l) with filtered seawater (ca. 12°C), and constant aeration and a natural light/dark cycle before the start of the experiment. In addition, fresh kelps (i.e., *Lessonia spicata* and *Macrosystis pyrifera*) were collected from adjacent rocky shores and used immediately after collection for the experimental assays. Finally, four *T. atra* were collected to characterize shell mineralogy and organic composition (see below).

Before the acclimation period the experimental snails were randomly assigned to aquaria (replicates; n total = 32) and algae treatments (*L. spicata* and *M. pyrifera*; n = 16 per each algae species). The snails were daily fed with fresh pieces of seaweeds (4-5 g of *L. spicata* and *M. pyrifera* by separated) and maintained at 12° C during a period of 30 days.

2.2 Shell mineralogy and organic composition

Phase composition, microstructure and texture of *T. atra* shells were measured with backscattered electron diffraction (EBSD). The internal structure of the shells was photographed with FE-SEM imaging, where BSE contrast was used. Shell samples were embedded in epoxy resin, which were subjected to several sequential mechanical grinding and polishing steps. The last two steps consisted of etch-polishing with colloidal alumina in a vibratory polisher and, subsequently, by ion polishing with an Ar beam in an ion polisher. For EBSD measurements the samples were coated with 4 to 6 nm of carbon. Measurements were carried out using a Hitachi SU5000 field emission SEM, equipped with an Oxford EBSD detector. During the measurements the SEM was operated at 20 kV, and Kikuchi patterns were indexed with CHANNEL 5 HKL software. Information obtained from EBSD diffraction was presented as colour-coded phases and crystal orientation maps, the latter with corresponding pole figures giving data density distributions, with a half width of 5° and a cluster size of 3° respectively (see Griesshaber et al. 2013; Checa et al. 2021).

The organic composition at three sections of the *T. atra* shell (Fig. 1d) were measured by Attenuated Total Reflection-Fourier Transform Infrared spectroscopy (ATR-FTIR). The inner and outer surfaces of each shell section (ca. 2x2 mm) were pressed on the ATR diamond crystal and the IR spectra at a 2 cm⁻¹ resolution, and 100 scans were measured using Fourier Transform Infrared (FTIR) spectrometer (model 6200, JASCO Analytical Instrument). The relative amount of principal chemical components (i.e., polysaccharides, proteins, water, lipids and carbonates) were estimated from the main absorption peak

areas associated with the respective functional group (e.g., COC: sugars/polysaccharides, amine: proteins, O-H: water, and C-O: carbonates). Calculated peak areas of the observance spectrum follow procedures described by Rodriguez-Navarro et al. (2013) (see also Checa et al. 2019; García-Huidobro et al. 2020, 2021).

2. 3 Nutritional quality of kelp species

The nutritional quality (e.g., total organic matter and concentration of proteins) of *L. spicata* and *M. pyrifera* were measured. To estimate the percentage of organic matter content from each seaweed species (i.e., *L. spicata* n = 8 and *M. pyrifera* n = 8), samples of 4-5 g were dried at 60° C for 48 h and incinerated in a muffle furnace at 450 ° C for 4 h, and then reweighed with an analytical balance (\pm 0.00001 g precision). The organic fraction of both algae species was then estimated by percentage of weight loss. To estimate the concentration of proteins for each kelp (i.e., *L. spicata* n = 9 and *M. pyrifera* n = 9) we used the bicinchoninic acid method (BCA) from Pierce (BCA Protein Assay Kit) using bovine serum albumin as a standard. Algal samples (5 mg of dry tissue) were mixed with 2 ml of sodium dodecyl sulphate (SDS) (0.5%). Subsequently, samples were sonicated for 1.5 min and centrifuged (Labnet, USA) at 5500 rpm for 35 min. After, 25 μ l of the supernatant was incubated in triplicate with BCA at 37° C for 30 min in the oven, and then read in a microplate with spectrophotometer. The protein concentration was determined colorimetrically by measuring the absorbance at 562 nm. Finally, the protein concentration was expressed in % of dry weight.

2. 4 Preference and food consumption rate

To evaluate the feeding preference (i.e., consumption with choice) of *T. atra*, pieces of algae from both kelp species were offered to the snails at the same time. Groups of 8 snails were maintained in aquaria (9 l), and one piece of each algal species (i.e., 4-5 g of *L. spicata* and *M. pyrifera*) was offered simultaneously. Each treatment was replicated 8 times, and each replicate was associated to their controls that contained only pieces of algae (n = 8 for each algal species). The controls were used to estimate changes in weight of kelp without snails. The trial was performed during 24 h in controlled conditions with filtered seawater (ca.12° C), and constant aeration with 12:12 photoperiod. Consumption rate of snails was measured separately in a no-choice experiment. Here, the experimental animals did not have a choice between the two kelp species. Each treatment (i.e., only algae species) contained 8 replicates and each replicate (i.e., aquaria 9 l) had 4 experimental snails, and 4-5 g of *L. spicata* and *M. pyrifera*, that were offered *ad libitum* to snails. In addition, each experimental aquaria was associated to its control (i.e., containing only algae; n = 8 for each algae species). The experiments of algal consumption were conducted for 24 h in the same controlled conditions as described above. Consumption rate was calculated as the loss of fresh material from the algae offered to the snails, corrected by the losses of non-consumption of its corresponding control replica using an analytical balance (\pm 0.00001 g precision). The food consumption was estimated following the equation (see Roa

1992; Silva et al. 2004): Consumption = (E initial- E final) - (C initial- C final), where E and C denote Experimental and Control algal weights, respectively. The rates of consumption were standardized as amount of algal biomass per day per individual ($\text{mg day}^{-1} \text{ individual}^{-1}$).

2. 5 Absorption efficiency

Absorption efficiency (AE) was derived from the relationship between organic and inorganic matter of ingested food and fecal material, using the method of Conover (1966). AE was calculated according to the following equation: $AE = [(F' - E') / (1 - E') F'] \times 100$, where F' = the proportion of organic matter in the food, and E' = the proportion of organic matter in the feces. Feces of each replicate (i.e., *L. spicata* $n = 16$ and *M. pyrifera* $n = 16$) were collected every 24 h and frozen during the experimental period (30 d) until analysis. Feces were dried at 60°C for 48 h, weighed and then burned in a muffle furnace at 450°C for 4 h, and weighed again with an analytical balance ($\pm 0.00001 \text{ g}$ precision) to determine the organic and inorganic content. The same methodology was used to determinate the percentage of organic matter content of each kelp species.

2. 6 Oxygen uptake and growth rate

To evaluate the oxygen consumption rate of *T. atra* at the end of the experiment, snails ($n = 16$ for each algae species; total $n = 32$) were placed individually in glass chambers (140 ml) containing filtered seawater ($0.45 \mu\text{m}$). The oxygen uptake was measured using a Fiber Optic Oxygen Transmitter (FIBOX 3, PreSens) and oxygen sensor spots (PreSens GmbH, Regensburg, Germany). Oxygen sensors were previously calibrated in anoxic water using a saturated solution of Na_2SO_3 and in saturated water (100%) with oxygen using bubbled air. Data were recorded using the OxyView 3.51 software (PreSens GmbH). During the experiments, glass chambers were placed into a temperature-controlled water bath (Lauda RE112®). All measurements were performed at a controlled temperature of 12°C . In addition, the measurements of dissolved oxygen in seawater were recorded. Additional chambers containing only filtered seawater (i.e., without snails) were used as controls ($n = 3$). For each measurement, the volume of the chambers was corrected by the displaced volume of each experimental snail. Finally, oxygen uptake was calculated by the decrease of oxygen in the chamber per hour per individual ($\text{ml h}^{-1} \text{ individual}^{-1}$).

Growth rate of *T. atra* individuals was estimated from changes in wet weight of the snails reared on a diet of a single algal species (i.e., *L. spicata* or *M. pyrifera*). Therefore, snails were fed daily with 5-6 g of fresh algae pieces ($n = 16$ for each algal species; $n \text{ total} = 32$) during the experimental period of 30 d. Finally, individual growth rates were estimated based on wet weight measurements performed using an analytical balance ($\pm 0.00001 \text{ g}$ precision) on days 1 and 30 of the experiment.

2. 7 Statistical analyses

Differences in organic composition of the shell sections and surfaces were tested using two-way ANOVAs. The feeding behaviour and physiological rates of *T. atra* reared with the two algal species were evaluated using ANOVA models and *t*-tests. Food preferences of snails were assessed using paired *t*-tests. Consumption rate (i.e., without choice), oxygen uptake and growth rate of snails reared with each algal species were compared using one-way ANOVAs. To compare nutritional quality (i.e., protein and organic matter content) of both kelp species, one-way ANOVAs were used. In addition, a repeated-measure ANOVA was used to evaluate the temporal effects of absorption efficiency (i.e., days 10, 20 and 30 of the experimental period) of individuals reared on a diet of a single algal species. The normality and homoscedasticity of the data were tested using Kolmogorov-Smirnov and Bartlett tests, respectively (Zar 1999). All analyses were carried out using the software Minitab v.14, and differences were considered significant at $p < 0.05$.

3. Results

3.1 Shell mineralogy and organic composition

Shells of *T. atra* are mainly formed by aragonite and about one third is calcite. Internal shell structure (Fig. 2), carbonate phase, microstructure and texture are given in Fig. 3. *T. atra* shells are formed by an outer calcitic and an inner aragonitic shell layer (Fig. 2 and 3), differentiated from each other by a marked thin zone formed of granular aragonite (e.g., Fig. 2B, 3A). The aragonitic shell layer has a columnar nacreous microstructure, and the calcitic shell part consists of an assemblage of large, irregularly shaped prisms. At the carbonate polymorph transition, the aragonite is very fine grained, and the nacre tablets were assembled to columns, which are developed further away from the calcite, towards the inner shell sections. Regardless of the microstructure, granular or nacreous, the aragonite has an axial texture with the aragonite crystallites being highly co-oriented (see the pole figure for the aragonite in Fig. 3C). The calcite in the calcitic shell layer shows a weak axial texture and a significantly weaker crystal co-orientation strength, relative to what we observed for the aragonitic portion of the shell (see the pole figure for the calcite in Fig. 3C).

The organic composition at the outer and inner surfaces of the three shell sections of *T. atra* are shown in Fig. 4. Lipids and polysaccharides showed non-significant differences among shell sections and surfaces (Fig. 4a, b; Table 1). However, protein content significantly increased at the inner surface and progressively increased from the shell growing edge, to the mid and apex sections (Fig. 4c; Table 1). In contrast, carbonate signals increased at the outer surface and decreased progressively from the shell growing section to the mid and shell apex sections (Fig. 4d; Table 1).

Table 1

Summary results (two-way ANOVA) of the intensity of ATR-FTIR spectra absorption bands comparing across shell sections (i.e., edge, medium and apex) and surfaces (i.e., inner and outer) of *Tegula atra* individuals. Significant differences are shown in bold.

Variable	Source of Variation	df	F	P-value
Lipids (%)	Section	2, 18	1.43	0.263
	Surface	1, 18	0.24	0.625
	Section · Surface	2, 18	0.51	0.606
Polysaccharides (%)	Section	2, 18	1.67	0.216
	Surface	1, 18	0.18	0.671
	Section · Surface	2, 18	0.15	0.855
Proteins (%)	Section	2, 18	2.76	0.089
	Surface	1, 18	12.81	0.002
	Section · Surface	2, 18	0.23	0.792
CO ₃ (%)	Section	2, 18	8.97	0.001
	Surface	1, 18	20.17	< 0.001
	Section · Surface	2, 18	2.87	0.082

3.2 Nutritional quality of kelp species

Nutritional quality of macroalgae species showed that *L. spicata* had significantly higher organic matter than *M. pyrifera* (one-way ANOVA: $F_{(1,15)} = 31.82$, $p = 0.002$; Fig. 5a). In addition, significantly higher protein content was found in *L. spicata* compared with *M. pyrifera* (one-way ANOVA: $F_{(1,17)} = 18.72$, $p = 0.001$; Fig. 5b). Thus, protein content was 1.4 times higher in *L. spicata* than *M. pyrifera*.

3.3 Feeding behavior

Snails of *T. atra* showed a stronger preference for *L. spicata* compared with *M. pyrifera* when both algal species were simultaneously offered (i.e., with choice experiment). Hence, *L. spicata* was significantly preferred by snails, and was consumed almost 3 times more than *M. pyrifera* (paired *t*-test; $t = 2.71$; $p = 0.03$; Fig. 6a). When the snails were presented a single algal species the consumption rate of individuals was not significantly different (one-way ANOVA: $F_{(1,15)} = 0.04$; $p = 0.85$; Fig. 6b).

3.4 Physiological traits

The absorption efficiency (AE) of snails showed a tendency to increase during the experimental period. Individuals of *T. atra* reared with *L. spicata* had AE that was significantly higher than those reared with *M. pyrifera* on days 10 and 20. However, at the end of the experiment (day 30), the AE of individuals reared

with each algal species was similar. In all treatments, AE was significantly higher after day 30 compared to after days 10 and 20 (interaction days × algae: $F_{(2,30)} = 4.11$, $p = 0.02$; Fig. 7a). On the other hand, oxygen uptake of *T. atra* was significantly higher for individuals reared with *L. spicata* than those fed with *M. pyrifera* (one-way ANOVA: $F_{(1,31)} = 6.00$, $p = 0.02$; Fig. 7b). Therefore, the mean oxygen uptake was 0.39 ± 0.05 ml O₂ h⁻¹ and 0.22 ± 0.03 ml O₂ h⁻¹, respectively. Finally, the growth rate of *T. atra* was not significantly different among those reared with different algal species, during the experimental period: wet weight (one-way ANOVA: $F_{(1,31)} = 0.86$, $p = 0.36$; Fig. 7c).

4. Discussion

Shell mineralogy and organic composition

This is the first study to characterize the calcium carbonate polymorphs and organic composition of *T. atra* shells. Here, we found that *T. atra* has a shell comprising of two calcium carbonate forms (i.e., calcite and aragonite), that consists of a calcite layer at the outer shell portions and a thick aragonite layer at the inner shell area. The results obtained an organic composition of the shell showing that carbonate signals were inversely proportional to relative organic composition content (e.g., proteins). Similar results on organic compounds and carbonate signal relationships have been reported for other mollusc species such as limpets (e.g., García- Huidobro et al. 2020), and scallops (e.g., Lagos et al. 2021). The aragonite content of the mollusc shells has been proposed to be more vulnerable than those with calcite mineral in response to environmental stressors (e.g., low pH/high $p\text{CO}_2$ levels; Barclay et al. 2019). Thus, more studies to evaluate the implications of environmental stressors on shell formation and/or the compensatory mechanisms of herbivore calcifying organisms are needed, and our results suggest that these investigations are warranted (see Lagos et al. 2021). Considering that consuming food of higher nutritional quality can provide more energy for calcifying organisms, the energy intake through food consumption can influence their shell quality/integrity (e.g., organic compounds, resistance to breakage, etc). Therefore, the quality of food becomes even more important to better understand potential vulnerability and/or adaption of organisms under climate change scenarios (e.g., Leung et al. 2019) at different levels of the trophic web (e.g., algae-herbivore interactions). Trade-offs between energetic physiology and skeletal production (i.e., biomineralization) under environmental stressors have been reported in other snail species (e.g., *Littorina littorea*; Melatunan et al. 2013), (e.g., *Eatoniella mortoni*; Leung et al. 2019).

Nutritional quality of kelp species and feeding behavior

Our results show that feeding preference of *T. atra* appears to be based on nutritional quality of kelps. The macroalgae *L. spicata* presented the highest nutritional value (i.e., organic matter and protein content) compared with *M. pyrifera* and was preferentially consumed by *T. atra*. Furthermore, the snails reared with *L. spicata* (i.e., the most nutritious kelp) achieved the highest oxygen uptake and absorption efficiency but not growth rate.

Nutritional quality of macroalgae affects the feeding behavior of marine herbivorous organisms (Pennings et al. 1998; Pennings and Paul 1992; Pansch et al. 2008), and their biochemical components (e.g., protein content), which has been described as one of the most important constituents to influence food preferences (Barile et al. 2004). Thus, the algae that contain the greatest amount of protein are mostly consumed by grazers (e.g., Barile et al. 2004). Preferential feeding on algae with high content of organic matter and/or proteins have also been reported for other herbivorous organisms such as echinoderms (e.g., *Loxechinus albus*; González et al. 2008), amphipods (e.g., *Orchestoidea tuberculata*; Duarte et al. 2010, 2011; *Gammarus mucronatus* and *Elasmopus levis*; Cruz-Rivera and Hay 2000), gastropods (e.g., *Aplysia californica*; Barile et al. 2004; *Diloma nigerrima*; Quintanilla-Ahumada et al. 2018), and another species of *Tegula* genus (e.g., *Tegula funebris*; Steinberg 1985 and *Tegula brunnea*; Thornber et al. 2008). The results obtained in this study agree with these previous studies, as the nutritional quality of macroalgae affected the feeding behavior of *T. atra*, and also, the alga with the highest protein content (i.e., *L. spicata*) was preferred by this snail. In other species of *Tegula* the nutritional quality of algae species, and their influence on feeding preferences, have shown contrasting results. Previous studies have demonstrated a significant influence (e.g., *T. funebris*; Steinberg 1985), influence in part (e.g., *T. brunnea*; Thornber et al. 2008), as well as no influence (e.g., *T. brunnea*, *T. montereyi* and *T. pulligo*; Watanabe 1985) on feeding preferences of grazers. However, herbivore food choice, and algal traits (e.g., nutritional quality, morphology differences etc), that this snail species uses to choose its food has scarcely been documented. Nevertheless, it is important to emphasize here that in addition to nutritional quality, palatability (i.e. morphology, shape and toughness), presence of chemical defences (e.g., secondary metabolites), availability and vertical gradient of macroalgae, and/or the combination of these factors (Thornber et al. 2008), have also been shown to influence the food choices of herbivores (Pennings et al. 1998; Pavia and Toth 2000; Granado and Caballero 2001; Jormalainen et al. 2005; Vergés et al. 2007; Rotini et al. 2018), which could modify the feeding behavior of grazers. Therefore, we do not exclude consideration of these factors which can influence the feeding behavior and performance of *T. atra*.

Physiological traits and growth rate

The increase in the quality and/or quantity of food items may increase the metabolic demands of gastropods (e.g., McSkimming et al. 2015). Shumway et al. (1993). These studies showed that the respiration rate of periwinkles *Littorina littorea* and *Littorina obtusata* increased by 40-60% when fed their preferred algae. More recently, Remy et al. (2017) demonstrated that amphipods *Gammarus aequicauda* reared with food with high nutritional quality showed higher respiration and absorption rates, and enhanced fitness, than amphipods reared with food items with low nutritional value. In this study, oxygen uptake of individuals reared on a diet of a single algal species was significantly different. More specifically, snails reared with *L. spicata* (the preferred kelp species) had significantly higher oxygen uptake than those fed with *M. pyrifera*. The higher oxygen uptake of *T. atra* reared with *L. spicata* was supported by high absorption efficiency that corresponded to high values of energy intake. Thus, snails fed with *L. spicata* incorporated more energy to take advantage of the high nutritional quality of this kelp species. Furthermore, individuals reared with *M. pyrifera* reduced their respiration rate to cope with an

unsuitable food source (i.e., low nutritional value of these food items), which could be a response aimed at improving energy balance. Therefore, the reduction of metabolism could be interpreted as a compensatory mechanism of *T. atra* to maintain its growth rate under diets of low nutritional value.

A higher growth rate of individuals fed with the most preferred algae with the highest nutritional value would have been expected. However, in this study, the feeding preference of *T. atra* on *L. spicata* did not allow for an increase in growth rate compared to those snails fed with *M. pyrifera*. We hypothesized that the additional energetic cost of consuming *L. spicata*, and/or their processing capacity, could be greater than consuming *M. pyrifera*. For example, post-ingestive mechanisms of processing food, change of digestive enzymes, and increases in absorption efficiency of nutrients, have been demonstrated as some physiological adjustments involved to optimize nutrient extraction and utilization by herbivorous organisms (Foster et al. 1999; Cox and Murray 2006; Secor 2009). Hence, our results show that those individuals reared with the most nutritious alga, indicated that the energetic cost of consuming this alga was high, which would be reflected in its metabolic rate (i.e., highest oxygen consumption). In addition, the mechanical cost and digestive process associated with consuming dietary food that has a macronutrient composition can increase energy expenditure. For example, carbohydrates have more complex molecules and may require a higher energetic cost to process and be digested by *T. atra*. In addition, snails did not increase consumption of food items of comparatively lower nutritional quality (i.e., *M. pyrifera*), to achieve optimal growth in the absence of better-quality food. Our results also suggest that a decrease in metabolic activity was the behavioral/physiological mechanism used for snails to compensate for the low nutritional value of *M. pyrifera*, to maintain a positive growth rate. Consequently, our results showed no compensatory feeding strategy for *T. atra*. We do not know if the compensatory physiological performance (i.e., decreased metabolic rate) observed in *T. atra* fed with food of low nutritional quality is sustainable for longer periods of time, and/or if it would affect the fitness of the snail. It is important to emphasize that *T. atra* had high absorption efficiency, and acclimation capacity, in the experimental conditions used. Our results indicate that individuals reared with both kelp species present positive and similar values for growth. Thus, we demonstrated that *T. atra* had high behavioral/physiological plasticity in response to nutritional quality by consuming the two kelp species studied here. This susceptibility for plasticity makes *T. atra* an ideal model for studying such plastic responses and associated trade-offs. The results of this study could help to better understand physiological traits of *T. atra* upon consuming kelp species (that differ in their nutritional quality). These kelp species and their grazers, commonly inhabit intertidal-subtidal areas of the Chilean coast and are important ecologically and economically. Considering that the impacts of global stressors (e.g., increasing seawater temperature and pCO_2 levels) in marine environments will most likely increase, the net physiological demands on calcifying organisms. In addition, as herbivores are dependent on the nutritional quality of seaweeds to meet their energetic demands, any alterations in their palatability and/or nutritional content, due to the influence of these environmental stressors (or their interactions), may affect in a complex way their physiological traits (Duarte et al. 2016; Leung et al. 2019; Kinnby et al. 2021a, b), and also their fitness. Calcifying organisms have been shown to be particularly vulnerable to changing environments and because of this, understanding how molluscs construct and maintain their

shells can help to better comprehend potential vulnerability and/or adaptative mechanisms of marine organisms to confront climate change (Barclay et al. 2020; Lagos et al. 2021). In coastal environments, grazer-seaweed interactions may be facing one of their biggest challenges, as environmental stress imposed on these relationships could influence community structure in the future (e.g., Jellison and Gaylord 2019; Grilo et al. 2019; Fieber and Bourdeau 2021; Kinnby et al. 2021a; Burnam et al. 2022). This highlights the need to understanding and to predict how rocky-shore systems may change in the near-future, and how these effects can modify marine herbivore-algae interactions.

Declarations

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Conflict of interest

The authors declare that there are not conflicts of interest regarding the publication of this manuscript.

Author contributions

Conceptualization: Samanta Benítez, Jorge M Navarro, Nelson A Lagos. Data curation: Samanta Benítez, Jorge López, Paola Villanueva, Felipe Moscoso. Formal analysis: Samanta Benítez, Paola Villanueva, Nelson A Lagos. Investigation: Samanta Benítez, Jorge M. Navarro, Nelson Lagos, Methodology: Samanta Benítez, Jorge López, Paola Villanueva, Felipe Moscoso, Erika Griesshaber

Supervision and Validation: Jorge M Navarro, Nelson A Lagos. Writing ± original draft: Samanta Benítez, Jorge M Navarro, Nelson A Lagos, Erika Griesshaber. Writing ± review & editing: Samanta Benítez, Jorge M Navarro, Nelson A Lagos, Erika Griesshaber. All authors read and approved the final manuscript.

Ethic approval

No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with unregulated invertebrate species. Therefore, no protected or endangered species were involved in this study.

Data availability

Datasets generated and analysed during the current study are available from the corresponding author.

References

1. Barclay KM, Gingras MK, Packer ST, Leighton LR (2020) The role of gastropod shell composition and microstructure in resisting dissolution caused by ocean acidification. *Mar Environ Res* 162:105105
2. Barile P, Lapointe B, Capo T (2004) Dietary nitrogen availability in macroalgae enhances growth of the sea hare *Aplysia californica* (Opisthobranchia: Anaspidea). *Journal of Experimental Marine Biology and Ecology* 303:65–78
3. Burnham KA, Nowicki RJ, Hall ER, Pi J, Page H, N (2022) Effects of ocean acidification on the performance and interaction of fleshy macroalgae and a grazing sea urchin. *J Exp Mar Biol Ecol* 547:151662
4. Conover RJ (1966) Assimilation of organic matter by zooplankton. *Limnol Oceanogr* 11:338–345
5. Cox TE, Murray SN (2006) Feeding preferences and the relationships between food choice and assimilation efficiency in the herbivorous marine snail *Lithopoma undosum* (Turbinidae). *Mar Biol* 148(6):1295–1306
6. Checa AG, Salas C, Rodríguez-Navarro AB, Grenier C, Lagos NA (2019) Articulation and growth of skeletal element in balanid barnacles (Balanidae, Balanomorpha, Cirripedia). *R Soc Open sci* 6:190458
7. Checa AG, Linares F, Grenier C, Griesshaber E, Rodríguez-Navarro AB, Schmahl WW (2021) The argonaut constructs its shell via physical self-organization and coordinated cell sensorial activity. *Iscience* 24(11):103288
8. Cross WF, Benstead JP, Frost PC, Thomas SA (2005) Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. *Freshw Biol* 50:1895–1912
9. Cruz-Rivera E, Hay ME (2000) Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. *Ecology* 81:201–219
10. Cruz-Rivera E, Hay ME (2001) Macroalgal traits and the feeding and fitness of an herbivorous amphipod: the roles of selectivity, mixing, and compensation. *Mar Ecol Prog Ser* 218:249–266
11. Cruz-Rivera E, Hay ME (2003) Prey nutritional quality interacts with chemical defences to affect consumer feeding and fitness. *Ecol Monogr* 73:483–506
12. Duarte C, Navarro JM, Acuña K, Gómez I (2010) Feeding preferences of the sandhopper *Orchestoidea tuberculata*: the importance of algal traits. *Hydrobiologia* 651:291–303
13. Duarte C, Acuña K, Navarro JM, Gomez I (2011) Intra-plant differences in seaweed nutritional quality and chemical defenses: importance for the feeding behaviour of the intertidal amphipod *Orchestoidea tuberculata*. *J Sea Res* 66:215–222

14. Duarte C, Acuña K, Navarro JM, Gómez I, Jaramillo E, Quijón P (2014) Variable feeding behavior in *Orchestoidea tuberculata* (Nicolet 1849): exploring the relative importance of macroalgal traits. *J Sea Res* 87:1–7
15. Duarte C, López J, Benítez S, Manríquez PH, Navarro JM, Bonta CC, Torres R, Quijón P (2016) Ocean acidification induces changes in algal palatability and herbivore feeding behavior and performance. *Oecologia* 180(2):453–462
16. Duffy JE, Hay ME (1991) Food and shelter as determinants of food choice by an herbivorous marine amphipod. *Ecology* 72(4):1286–1298
17. Elliott Smith E, Harrod EA, Docmac C, Newsome F S. D (2021) Intraspecific variation and energy channel coupling within a Chilean kelp forest. *Ecology* 102(1):e03198
18. Foster GG, Hodgson AN, Boyd CS (1999) Polysaccharolytic activity of the digestive enzymes of the macroalgal herbivore, *Turbo sarmaticus* (Mollusca: Vetigastropoda: Turbinidae). *Comp Biochem Physiol B: Biochem Mol Biol* 122(1):47–52
19. Foster MC, Byrnes JE, Reed DC (2015) Effects of five southern California macroalgal diets on consumption, growth, and gonad weight, in the purple sea urchin *Strongylocentrotus purpuratus*. *PeerJ* 3:e719
20. Fieber AM, Bourdeau PE (2021) Elevated pCO₂ reinforces preference among intertidal algae in both a specialist and generalist herbivore. *Mar Pollut Bull* 168:112377
21. Fraser KM, Lefcheck JS, Ling SD, Mellin C, Stuart-Smith RD, Edgar GJ (2020) Production of mobile invertebrate communities on shallow reefs from temperate to tropical seas. *Proceedings of the Royal Society B* 287(1941): 20201798
22. García-Huidobro MR, Aldana M, Varas O, Pulgar J, García-Herrera C, Obarca-Ortega A, Grenier C, Rodríguez-Navarro AB, Lagos NA (2020) Geographical variability and parasitism n body size, reproduction and Shell characteristics of the limpet *Fissurella crassa* (Mollusca: Vetigastropoda). *Mar Environ Res* 161:105060
23. García-Huidobro MR, Poupin MJ, Urrutia C, Rodríguez-Navarro AB, Grenier C, Vivanco JF, Ramajo L, Benjumeda I, Lagos NA, Lardies MA (2021) An intrapopulational study of organic compounds and biochemical properties of the Shell of the Antarctic bivalve *Laternula elliptica* (P. P. King, 1832) at King George Island. *Polar Biol* 44:1343–1352
24. Gómez I, Westermeier R (1995) Energy contents and organic constituents in Antarctic and south Chilean marine brown algae. *Polar Biol* 15(8):597–602
25. González SJ, Cáceres WC, Ojeda FP (2008) Feeding and nutritional ecology of the edible sea urchin *Loxechinus albus* in the northern Chilean coast. *Rev Chil Hist Nat* 81:575–584
26. Granado I, Caballero P (2001) Feeding rates of *Littorina striata* and *Osilinus atratus* in relation to nutritional quality and chemical defenses in seaweeds. *Mar Biol* 138(6):1213
27. Griesshaber E, Wolfgang W, Schmahl HS, Ubhi, Huber J, Nindiyasari F, Maier B, Ziegler A (2013) Homoepitaxial meso- and microscale crystal co-orientation and organic matrix network structure in *Mytilus edulis* nacre and calcite. *Acta Biomaterialia* Volume 9:9492–9502

28. Grilo TF, Repolho T, Rosa R, Cardoso PG (2019) Performance and herbivory of the tropical topshell *Trochus histrio* under short-term temperature increase and high CO₂. *Mar Pollut Bull* 138:295–301
29. Henríquez LA, Buschmann AH, Maldonado MA, Graham MH, Hernández-González MC, Pereda SV, Bobadilla MI (2011) Grazing on giant kelp microscopic phases and the recruitment success of annual populations of *Macrocystis pyrifera* (Laminariales, Phaeophyta) in southern Chile. *J Phycol* 47:252–258
30. Jellison BM, Gaylord B (2019) Shifts in seawater chemistry disrupt trophic links within a simple shoreline food web. *Oecologia* 190(4):955–967
31. Jormalainen V, Honkanen T, Heikkilä N (2001) Feeding preference and performance of a marine isopod on seaweed host: cost of habitat specialization. *Mar Ecol Prog Ser* 220:219–230
32. Jormalainen V, Honkanen T, Vesakoski O, Koivikko R (2005) Polar extract of the brown alga *Fucus vesiculosus* (L.) reduce assimilation efficiency but do not deter the herbivorous isopods *Idotea baltica* (Pallas). *J Exp Mar Biol Ecol* 317:143–157
33. Kinnby A, Toth GB, Pavia H (2021) a Climate change increases susceptibility to grazers in a foundation seaweed. *Frontiers in Marine Science* 8:729
34. Kinnby A, White JC, Toth GB, Pavia H (2021) b Ocean acidification decreases grazing pressure but alters morphological structure in a dominant coastal seaweed. *PLoS ONE* 16(1):e0245017
35. Lagos NA, Benítez S, Grenier C, Rodríguez-Navarro A, B, García-Herrera C, Abarca-Ortega A, Lardies MA (2021) Plasticity in organic composition maintains biomechanical performance in shells of juvenile scallops exposed to altered temperature and pH conditions. *Sci Rep* 11(1):1–13
36. Lawrence JM (1975) On the relationships between marine plants and sea urchins. *Oceanography and Marine Biology Annual Review* 13:213–286
37. Leung JY, Doubleday ZA, Nagelkerken I, Chen Y, Xie Z, Connell SD (2019) How calorie-rich food could help marine calcifiers in a CO₂-rich future. *Proceedings of the Royal Society B* 286(1906): 20190757
38. Mattson WJ (1980) Herbivory in relation to plant nitrogen content. *Annu Rev Ecol Syst* 11:119–161
39. Melatunan S, Calosi P, Rundle SD, Widdicombe S, Moody AJ (2013) Effects of ocean acidification and elevated temperature on shell plasticity and its energetic basis in an intertidal gastropod. *Mar Ecol Prog Ser* 472:155–168
40. McSkimming C, Russell BD, Tanner JE, Connell S, D (2015) A test of metabolic and consumptive responses to local and global perturbations: enhanced resources stimulate herbivores to counter expansion of weedy species. *Marine and Freshwater Research* 67(1):96–102
41. Oróstica MH, Aguilera MA, Donoso GA, Vásquez JA, Broitman BR (2014) Effect of grazing on distribution and recovery of harvested stands of *Lessonia berteriana* kelp in northern Chile. *Mar Ecol Prog Ser* 511:71–82
42. Pansch C, Gómez I, Rothäusler E, Veliz K, Thiel M (2008) Species-specific defense strategies of vegetative versus reproductive blades of the Pacific kelp *Lessonia nigrescens* and *Macrocystis integrifolia*. *Mar Biol* 155:51–62

44. Pavia H, Toth G (2000) Inducible chemical resistance to herbivory in the brown seaweed *Ascophillum nodosum*. *Ecology* 81:3212–3225
45. Pennings S, Carefoot T, Siska E, Chase M, Page T (1998) Feeding preferences of a generalist salt-marsh crab: relative importance of multiple plant traits. *Ecology* 79:1968–1979
46. Pennings SC, Paul VJ (1992) Effect of plant toughness, calcification, and chemistry on herbivory by *Dolabella auricularia*. *Ecology* 73:1606–1619
47. Quintanilla-Ahumada D, Quijón PA, Navarro JM, Pulgar J, Duarte C (2018) Living on a trophic subsidy: Algal quality drives an upper-shore herbivore's consumption, preference and absorption but not growth rates. *PLoS ONE* 13(4):e0196121
48. Remy F, Darchambeau F, Melchior A, Lepoint G (2017) Impact of food type on respiration, fractionation and turnover of carbon and nitrogen stable isotopes in the marine amphipod *Gammarus aequicauda* (Martynov, 1931). *J Exp Mar Biol Ecol* 486:358–367
49. Rodríguez-Navarro AB, Dominguez-Gasca N, Muñoz A, Ortega-Huertas M (2013) Change in the chicken eggshell cuticle with hen age and egg freshness. *Poult Sci* 92:3026–3035
50. Rotini A, Tibbetts IR, Migliore L, Rossini RA (2018) The trade-off between digestibility and phenol content influences the food choice of the obligate seagrass-feeding neritid snail *Smaragdia souverbiana*. *Journal of Molluscan Studies* 84(1):12–18
51. Roa R (1992) Design and analysis of multiple-choice feeding-preference experiments. *Oecologia* 8:509–515
52. Secor SM (2009) Specific dynamic action: a review of the postprandial metabolic response. *Journal of Comparative Physiology B-Biochemical Systemic and Environmental Physiology* 179:1–56
53. Shumway SE, Lesser MP, Crisp DJ (1993) Specific dynamic action demonstrated in the herbivorous marine periwinkles, *Littorina littorea* L. and *Littorina obtusata* L. (Mollusca, Gastropoda). *Comparative Biochemistry and Physiology Part A: Physiology* 106(2):391–395
54. Silva J, Larraín A, Bay-Schmith E, Roa R (2004) Feeding regime experiments to enhance gamete production in the carnivorous sea urchin *Arbacia spatuligera*. *Aquaculture* 321:279–291
55. Simpson SJ, Simpson CL (1990) The mechanisms of nutritional compensation by phytophagous insects. In: Bernays EA (ed) *Insect–Plant Interactions*, 2. CRC Press, Boca Raton, Florida, USA, pp 111–160
56. Steneck RS, Graham MH, Borque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environ Conserv* 29:436–459
57. Steinberg PD (1985) Feeding preferences of *Tegula funebris* and chemical defenses of marine brown algae. *Ecol Monogr* 55(3):333–349
58. Thornber CS, Jones E, Stachowicz JJ (2008) Differences in herbivore feeding preferences across a vertical rocky intertidal gradient. *Mar Ecol Prog Ser* 363:51–62
59. Vergés A, Becerro MA, Alcoverro T, Romero J (2007) Variation in multiple traits of vegetative and reproductive seagrass tissues influences plant–herbivore interactions. *Oecologia* 151:675–686

60. Veliz D, Vásquez JA (2000) La Familia Trochidae (Mollusca: Gastropoda) en el norte de Chile: consideraciones ecológicas y taxonómicas. *Revista chilena de historia natural* 73(4):757–769
61. Vásquez JA, Buchmann AH (1997) Herbivore-kelp interaction in Chilean subtidal communities: A review. *Revista Chilena de Historia Natural* 70:41–52
62. Vásquez JA, Camus PA, Ojeda FP (1998) Diversidad, estructura y funcionamiento de ecosistemas costeros del Norte de Chile. *Revista Chilena de Historia Natural* 71:479–499
63. Watanabe JM (1984) The influence of recruitment, competition, and benthic predation on spatial distributions of three species of kelp forest gastropods (Trochidae: Tegula). *Ecology* 65(3):920–936
64. Yañez E, Nelson A, Lagos R, Norambuena C, Silva J, Letelier K-P, Muck GS, Martin S, Benítez B, Broitman H, Contreras C, Duarte S, Gelcich FA, Labra MA, Lardies, Patricio H, Manríquez P, Quijon (2017) Laura Ramajo, Exequiel Gonzalez, Renato Molina, Allan Gómez Aravena, Luis Soto, Aldo Montecino, María Angela Barbieri, Francisco Plaza, Felipe Sánchez, Antonio Aranís, Claudio Bernal, Gabriela Böhm, Impacts of Climate Change on Marine Fisheries and Aquaculture in Chile. A global analysis, *Climate change impacts on fisheries and aquaculture*, pp 239–231
65. Zarco-Perello S, Bosch NE, Bennett S, Vanderklift MA, Wernberg T (2021) Persistence of tropical herbivores in temperate reefs constrains kelp resilience to cryptic habitats. *J Ecol* 109(5):2081–2094
66. Zar JH (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, Upper Saddle River

Figures

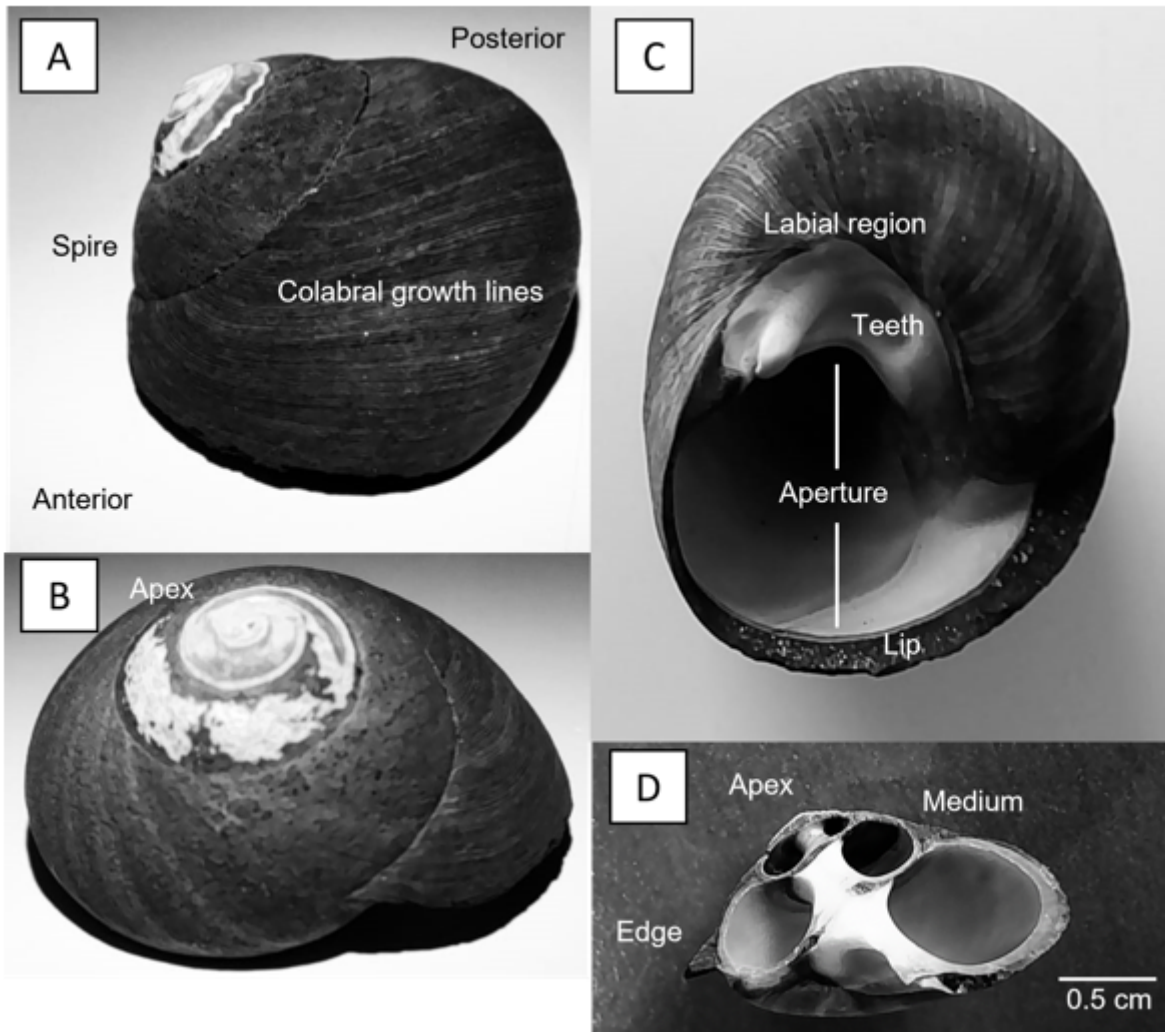


Figure 1

Outer view of *Tegula atra* shell showing globose form shell with spire short, almost flat base, and external surface smooth with oblique colabral growth lines and feeble spiral lines (A). Lateral view showing the common erosion of the shell apex (B). Inner view showing the labial region with one prominent tooth in a columellar fold (C). View showing the outer and inner surfaces of the three shell sections used to measure organic composition (determined by ATR-FTIR normalized signal) (D)

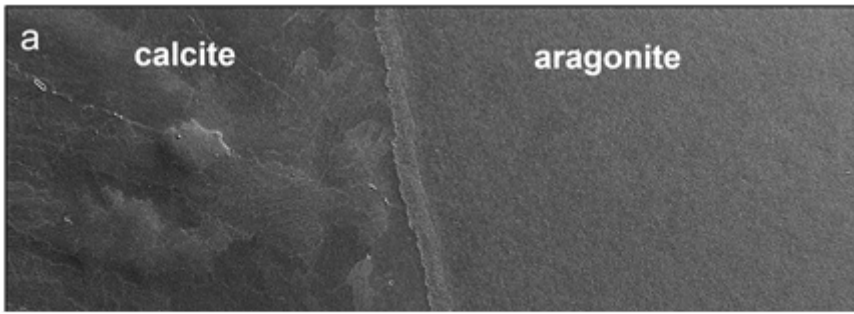


Figure 2

EBSD images of cross-sections through the shell of *Tegula atra* depicting the presence of two shell layers (a) an inner aragonitic and an outer calcitic layer (b) as well as a thin transitional section between the two layers (a and b)

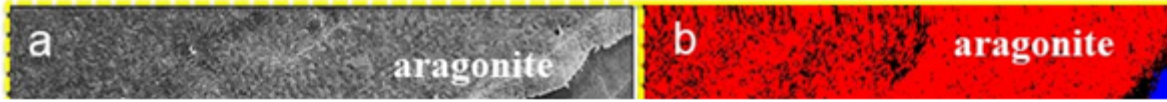


Figure 3

Internal structure (a) phase (b) microstructure and texture (c) of the different shell layers of *Tegula atra*. The aragonite is developed as columnar nacre and as granular aragonite, the calcite comprises differently sized and shaped prisms (c)

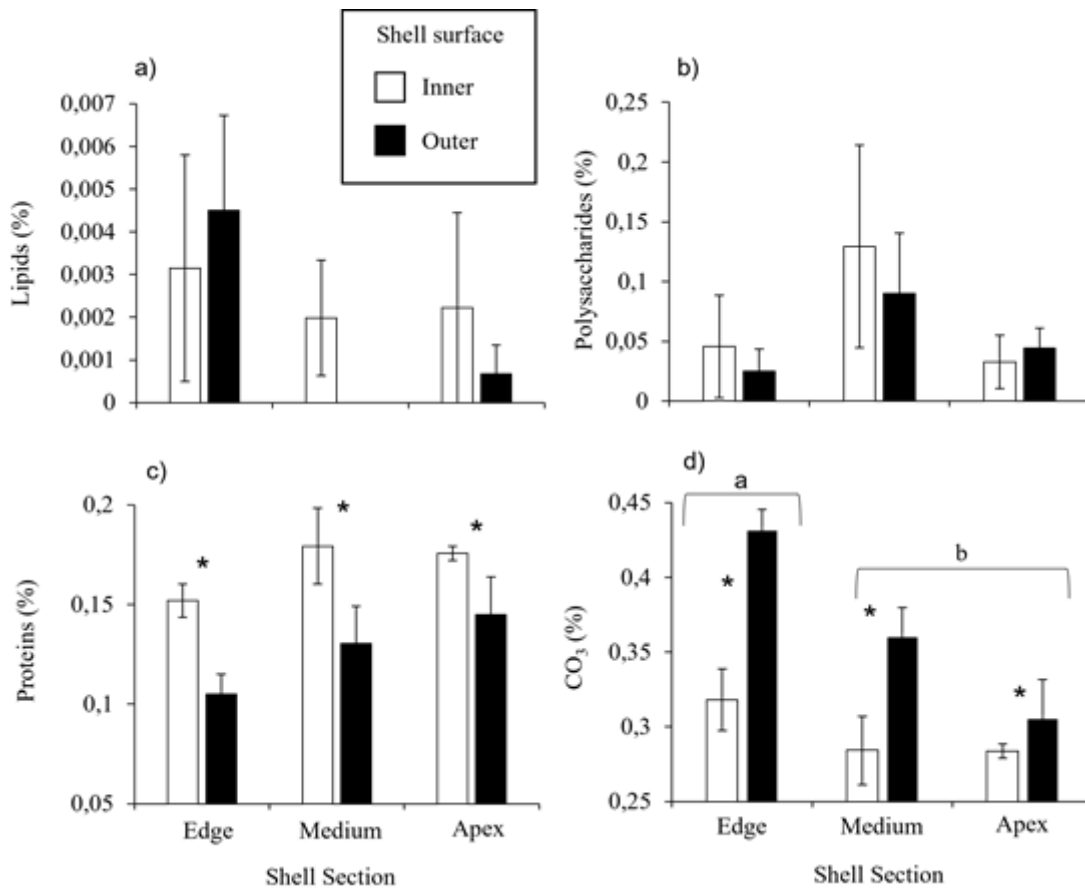


Figure 4

Normalized intensity of main absorption bands of ATR-FTIR spectra (lipids, polysaccharides, proteins and carbonates) recorded across shell sections (i.e., edge, medium and apex) and shell surface (i.e., inner and outer) of *Tegula atra*. Bars correspond to means (± 1 SE). Asterisks and different letters indicate significant differences using a post hoc Tukey's HSD test

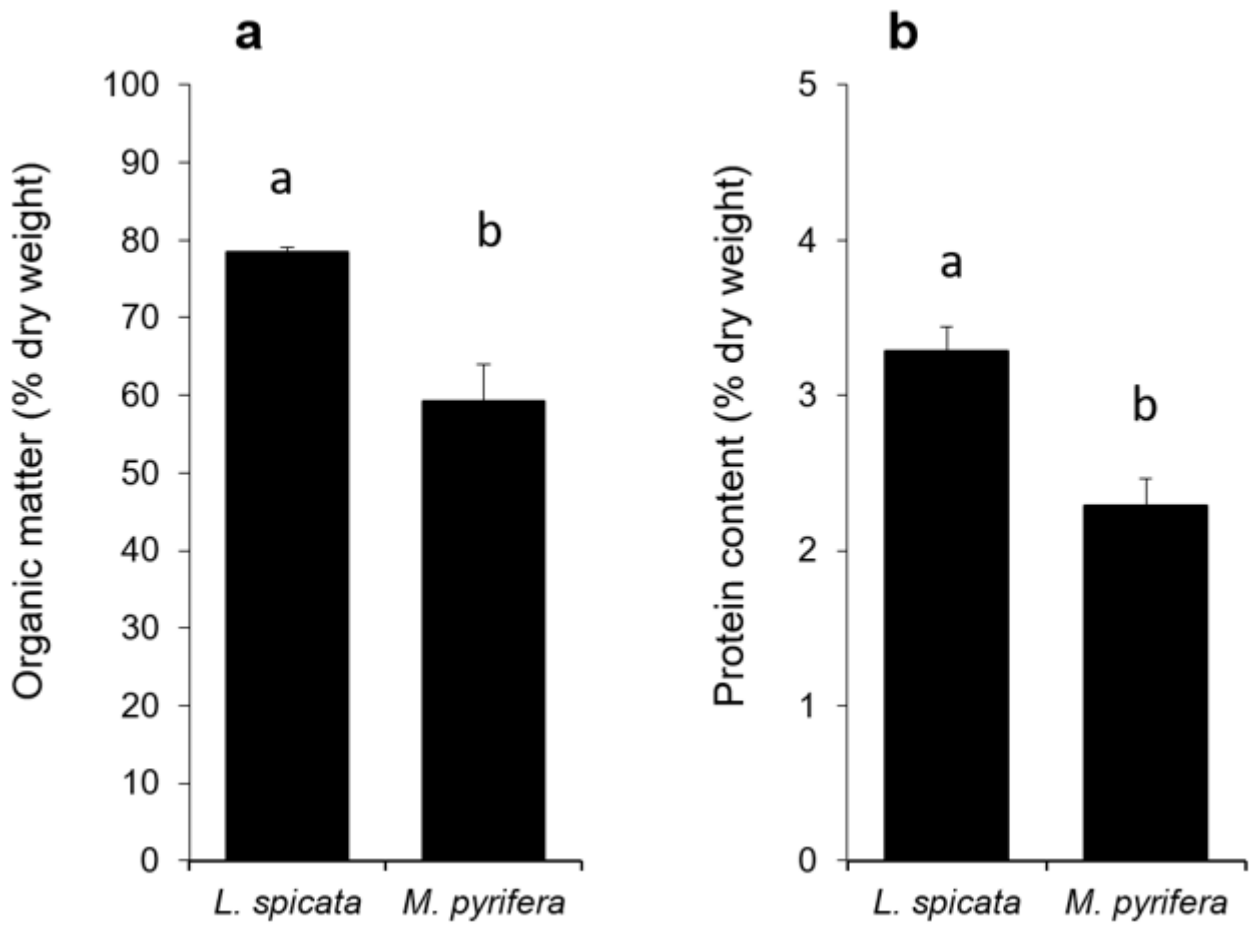


Figure 5

Organic matter (a) and protein content (b) of kelps *Lessonia spicata* and *Macrocystis pyrifera*. Bars correspond to means (± 1 SE). Different letters indicate significant differences

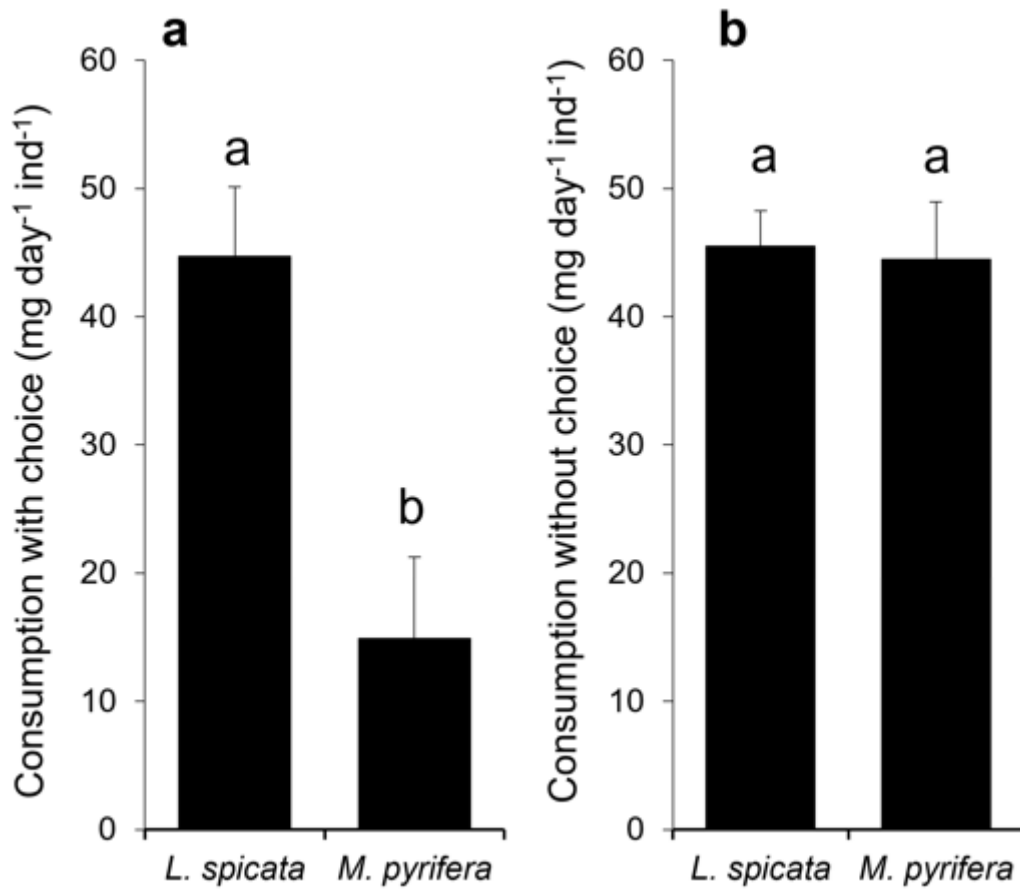


Figure 6

Feeding preference exhibited by snails *Tegula atra* grazing on seaweeds *Lessonia spicata* and *Macrocystis pyrifera* (with choice) (a) consumption rate on each alga individually (without choice) (b). Bars correspond to means ($\pm 1SE$). Different letters indicate significant differences

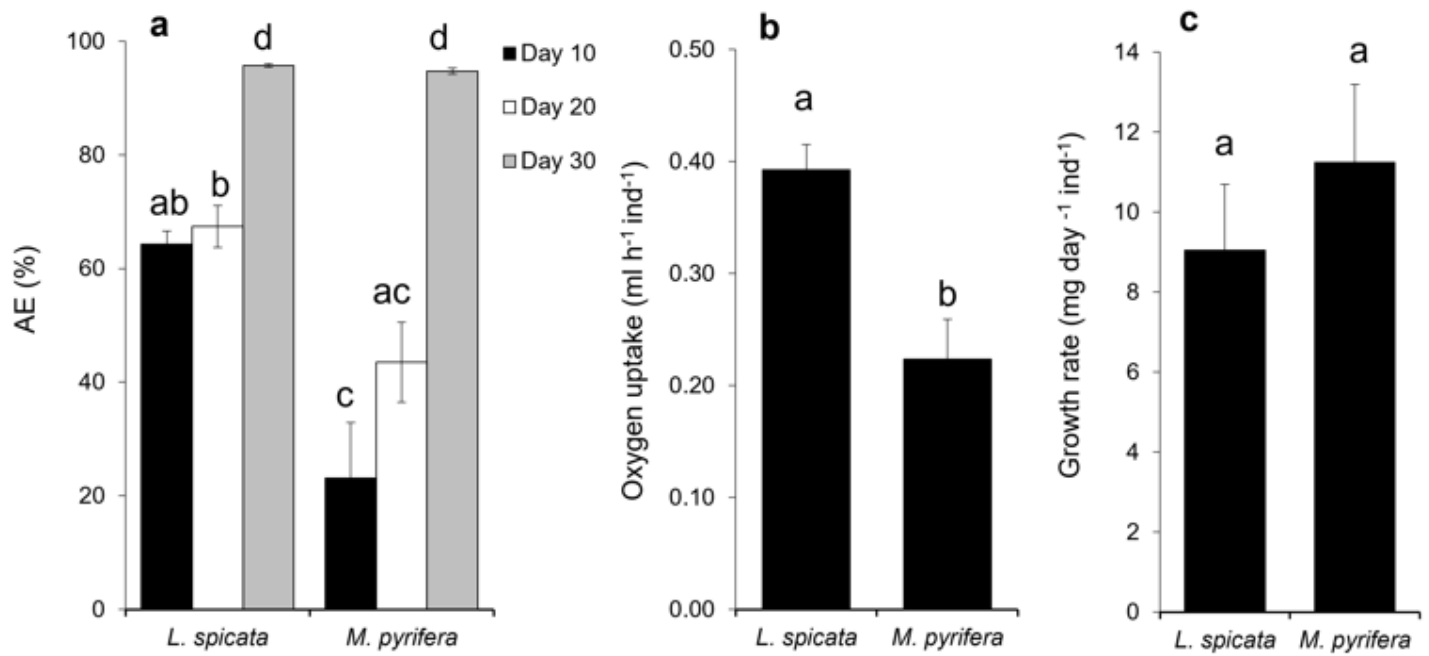


Figure 7

Absorption efficiency (AE) (a) oxygen uptake (b) and growth rate (c) of snail *Tegula atra* reared on a diet of a single kelp species (i.e., *Lessonia spicata* and *Macrocystis pyrifera*) during the experimental period (30 d). Bars correspond to means (± 1 SE). Different letters indicate significant differences among treatments using a post hoc Tukey's HSD test