Research Article

Consumption and digestion of animal food by rocky intertidal herbivores: an evaluation of digestive flexibility and omnivory in three grazing species

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ABSTRACT. The ingestion of invertebrates by herbivores on rocky intertidal shores is traditionally considered a casual phenomenon. However, a recent study of 29 species in northern Chile shows that animal consumption is widespread, consistent, and important, suggesting that some of these herbivores may actually be omnivores. Therefore, we examined the capability of three common Chilean herbivores (the key-hole limpets *Fissurella limbata* and *Fissurella picta* and the polyplacophoran *Chiton granosus*) to digest animal food. For each species, we conducted no-choice feeding experiments using artificial foods based on either algal or animal tissue from one of their frequent prey (*Ulva rigida*, *Perumytilus purpuratus*). After the feeding trials, we evaluated the total proteolytic activity (availability of free amino acids) in the digestive contents of the species studied and, as a reference, we evaluated this activity in animals obtained directly from the field. We found that all three species were able to eat animal food, and this consumption was not significantly different from that of algal food, suggesting that both foods were not only edible but at least similarly palatable. In addition, we detected comparable levels of proteolytic activity under the three feeding conditions for the three species. No statistical differences were found for *C. granosus*, but activity was significantly higher with animal food in *F. limbata* and with algal food in *F. picta*. Our data show the high digestive flexibility of these species, suggesting their ability for adaptive modulation and the possibility that they are true omnivorous consumers. We discuss the implications of these results for our current view of the structure of rocky intertidal food webs.

Keywords: omnivorous, proteolytic, food web, key-hole limpet, polyplacophora, Chile.

Consumo y digestión de alimento animal por herbívoros del intermareal rocoso: evaluación de flexibilidad digestiva y omnivoría en tres especies de pastoreadores

RESUMEN. La ingestión de invertebrados por herbívoros de costas intermareales rocosas se considera tradicionalmente un fenómeno casual. Sin embargo, un estudio reciente en 29 especies del norte de Chile muestra que el consumo animal es generalizado, consistente e importante, sugiriendo que algunos de estos herbívoros pueden ser realmente omnívoros. Por tanto, se examinó la capacidad de tres herbívoros chilenos comunes para digerir alimento animal: las lapas *Fissurella limbata* y *Fissurella picta*, y el poliplacóforo *Chiton granosus*. Para cada especie, se efectuaron experimentos de alimentación sin elección usando alimentos artificiales basados en tejido animal o algal de algunas de sus presas frecuentes (*Ulva rigida*, *Perumytilus purpuratus*). Luego de los ensayos de alimentación se evaluó la actividad proteolítica total (disponibilidad de aminoácidos libres) en sus contenidos digestivos, y como referencia, se determinó la actividad en animales obtenidos directamente desde condiciones de terreno. Se encontró que las tres especies fueron capaces de comer alimento animal, y este consumo no fue significativamente distinto al de alimento algal, sugiriendo que ambos alimentos fueron no sólo comestibles sino al menos similarmente palatables. Además, se detectaron niveles comparables de actividad proteolítica bajo las tres condiciones de alimentación en las tres especies, sin diferencias estadísticas en *C. granosus*, pero con una actividad significativamente más alta con alimento animal en *F. limbata* y con alimento algal en *F. picta*. Los datos muestran la alta flexibilidad digestiva de estas especies, sugiriendo su capacidad para modulación adaptativa y la posibilidad de que sean verdaderos omnívoros. Se discuten las implicancias de estos resultados sobre la visión actual de la estructura de tramas tróficas del intermareal rocoso.

Palabras clave: omnívoro, proteolítica, trama trófica, lapa, poliplacóforo, Chile.

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INTRODUCTION

Limpets, key-hole limpets, placophorans, and snails are common constituents of invertebrate assemblages on Chilean rocky intertidal shores. These species are traditionally considered to be herbivorous consumers that play a key role in the structure and organization of littoral communities (Moreno & Jaramillo, 1983; Santelices, 1989; Fernández & Castilla, 2005). Although the diet of these species is long recognized to include an important proportion of invertebrate animals (Santelices & Correa, 1985; Osorio *et al.*, 1988), this phenomenon has been largely interpreted as casual or incidental and, thus, the species are usually referred to as primarily herbivorous (López *et al.*, 2003).

However, a recent trophic evaluation of 29 herbivore species in northern Chile, conducted in four communities over 1.5 years, showed that their ingestion of animal items was common and widespread (Camus *et al.*, 2008). These herbivores consumed, on average (mean ± s.e.), a total of 48.2 ± 5.1 items per species, of which 26.2 ± 1.4% were animal items. This proportion was positively related to herbivore body mass and exceeded 60% for particular species at given times and places (e.g., *Fissurella* species, up to 82% in *F. limbata*). In addition, eight of these species ingested heterospecific herbivores that were also potential competitors, thus making such interactions equivalent to the more complex phenomenon of intraguild predation (see Polis *et al.,* 1989) and turning the herbivore trophic level into a sub food web with trophic chains up to three levels in length (see analysis in Camus *et al.*, 2008).

The above results strongly suggest that some of these herbivores could actually be omnivores that use animals as a nutritional source. Such a possibility is far from remote, as recent analyses have demonstrated true omnivory and even carnivory in chiton and fissurellid species from other coasts (Latyshev *et al.*, 2004; Hughes *et al.*, 2005; Grall *et al.*, 2006). In addition, the existence of omnivory may have numerous implications for the understanding of food web structures and dynamics on rocky shores (Navarrete *et al.*, 2008; Sanhueza *et al.*, 2008), and should not be neglected in experimental studies of community organization and regulation. In fact, herbivores were long proposed to be termed predators, as they play an equivalent functional role to carnivores in terms of predator-prey dynamics (Lubchenco, 1978); this suggestion should make more sense if these herbivores indeed turn out to be omnivores. However, the above implications have not been explored so far, and we suggest that the main reason is that we have always treated and referred to these intertidal consumers as herbivores.

In this context, we conducted a non-choice experimental assessment of the capability of rocky intertidal herbivores to consume and digest algal *versus* animal food, selecting three common grazing mollusks widely distributed on the Chilean coast (from 18º to at least 42ºS; Oliva & Castilla, 1986; Hidalgo *et al.*, 2006): the polyplacophoran *Chiton granosus* Frembly, and the key-hole limpets *Fissurella limbata* Sowerby and *Fissurella picta* (Gmelin). These species have a strong potential to be physiological omnivores (Camus *et al.*, 2008) and, therefore, we focused on the detection of specific proteolytic activity (such as the availability of free amino acids) within their digestive tract after ingesting animal food. Such findings have been suggested to offer primary evidence for omnivory (*e.g.*, Clark *et al.*, 1985; Sabapathy & Teo, 1995), although they constitute a necessary albeit insufficient condition for the adequate digestion of animal prey. Some true herbivores exhibit high proteolytic activity when processing algal food (Horn, 1989; Ojeda & Cáceres, 1995; Johnston & Freeman, 2005), so we expect them to exhibit noticeably lower activity when feeding exclusively on animal food. For example, herbivorous fishes may have intensive proteolytic digestion because their long digestive tracts allow a longer exposure of food to enzymatic action, although the proteolytic activity per volume unit of gut content is clearly lower in herbivores than in omnivores or carnivores (Hofer & Schiemer, 1981). For our study species, thus, the detection of comparable proteolytic activity for algal and animal food would be a first suggestion of an omnivorous habit, provided that animal preys are indeed a regular component of their diet. Additionally, such results may be better understood in the frame of the adaptive modulation hypothesis. In this context, omnivores are expected to have the capability to modulate enzymatic activity and nutrient transport depending on the dietary substratum; this cannot be done by specialized animals such as strict herbivores (*e.g.*, Buddington *et al.*, 1987; Sabat *et al.*, 1998). Therefore, the capability of a given herbivore to display at least similar levels of proteolytic activity when consuming algal *versus* animal food will be herein taken as evidence of digestive flexibility and preliminary support for the hypothesis that the species is omnivorous.

MATERIAL AND METHODS

Apparently, no published information exists on the particular characteristics of digestion in *C. granosus*, *F. limbata*, or *F. picta*. However, the three species

have similar and structurally simple digestive systems, in accordance with the generalized model of mollusks (Ruppert & Barnes, 1994); the main components are the esophagus, stomach, and intestine. Buccal salivary glands secrete mucus to facilitate the transport of food into the esophagus, where esophageal (pharyngeal) glands release enzymatic secretions for carbohydrate digestion. Proteolytic activity is largely extracellular and occurs in the stomach, where the food mixes with enzymatic secretions released into the lumen by digestive glands (Fretter & Graham, 1994; Ruppert & Barnes, 1994; Sasaki, 1998). Although some digestion may occur in the anterior intestine of chitons, the main role of the intestine is the compaction of feces. The rate of food passage through the digestive tract depends, to a large extent, on the intestinal length, which can vary between closely related species. As some *Fissurella* species from Venezuela exhibit food passage rates of 6 to 12 h (Franz, 1990), we used this range as a reference to establish the acclimation periods and the duration of feeding trials in our experiments.

In winter and spring 2007, individuals of *C. granosus*, *F. limbata*, and *F. picta* were collected from the intertidal zone of Caleta Cocholgüe (36º35'S), on the coast of Concepción, Chile, and then transported to the laboratory in containers with seawater and aeration. We then conducted parallel evaluations of the three species following the same protocol. For each species, the collected individuals were separated into three experimental groups of six individuals each (seven in the case of *C. granosus*). The first group of individuals was sacrificed immediately after collection to obtain a reference level of the proteolytic activity of animals feeding on a field diet. Prior to the experiments, the remaining individuals were maintained in aquaria (separated by species) with circulating seawater at 14ºC and constant aeration for three days, without food. Such conditions allowed the acclimation of individuals for subsequent experiments, and the complete elimination of contents from their digestive tract. After acclimation, animals were separated into two groups in different aquaria, placed in individual chambers, and fed for six days with artificial food (either animal or algal tissue) (10%) homogenized in an agar matrix (1%) and diluted in filtered seawater (see description below). Each day of the experiment, individual animals were left for 5 h in a Petri dish covered with a layer of 5 g of food; this was done to measure the amount of food consumed each day. These results are referred to as daily consumption, although we note that they correspond to a 5-h period.

Artificial foods were prepared with powdered tissue of the mussel *Perumytilus purpuratus* (Lamarck)

and the green alga *Ulva rigida* C. Agardh, hereafter referred to as animal and algal food, respectively. These two species are among the most common organisms on Chilean rocky intertidal shores (Camus & Andrade, 1999; Prado & Castilla, 2006), and both of them are usually ingested in the field by the three herbivores studied (Camus, unpublished results). The protein content of *U. rigida* is, on average, approximately 18% (dry weight), showing temporal variations between *ca*. 11 and 24% (Rouxel *et al.*, 2001). We did not find equivalent information for *P. purpuratus* in the literature, although a recent estimation indicates a protein content of nearly 55% (Figueroa & Fariña, unpublished data), similar to the amount reported for other mussel species (*e.g.*, *Mytilus trossulus* Gould, with an average of *ca*. 50%, ranging from *ca*. 44 to 57%; Kreeger, 1993).

At the end of feeding experiments, individuals were dissected and their digestive tract (from esophagus to anus) was removed to obtain its content (food along with enzymes secreted into the lumen) and measure its total proteolytic activity. The digestive content was centrifuged (13,000 rpm for 20 min), the supernatant was blended (2:1) with a solution of cold acetone (-20ºC), and then it was centrifuged again (13,000 rpm for 12 min). Later, 1.0 mg of the digestive extract was diluted in 10 mL phosphate buffer (pH 7); 1 mL of this solution was added to 2 mL bovine serum albumin (BSA) and incubated for 1 hour at 37ºC. Proteolytic activity was assessed in terms of amino acid released and using the ninhydrin method (Troll & Cannan, 1953); it was measured at 570 nm in a spectrophotometer and compared against an l-alanine calibration curve. The results were expressed as mg alanine mL^{-1} h^{-1}. Both food consumption and proteolytic activity were evaluated separately for each species by means of one-way ANOVA, excepting one case that did not meet the assumptions and was evaluated with a Kruskal-Wallis test (Siegel & Castellan 1988).

RESULTS

Food consumption

As expected, the three herbivore species were all able to consume algal food, but our results also showed that they had no impediment to consume animal food. Figure 1 shows the mean daily consumption rate, which, on average, varied from 0.24 (*F. limbata*) to 0.418 (*C. granosus*) g day^{-1} of algal food, and from 0.21 (*F. picta*) to 0.49 (*C. granosus*) g day^{-1} of animal food. The consumption rates did not differ significantly for *C. granosus* ($F_{1,12} = 4.60$, p = 0.053), *F. limbata* ($F_{1,10} = 0.177$, p = 0.683), or *F. picta* ($F_{1,10} =$

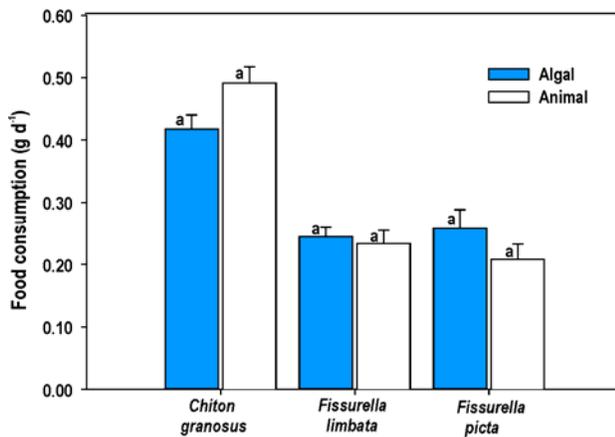**Figure 1.** Daily consumption rate (mean ± s.e.) of two artificial foods by three common herbivorous species from Chilean rocky intertidal shores. Foods were offered separately (no choice), and consisted of powdered tissue of the green alga *Ulva rigida* (algal) and the mussel *Perumytilus purpuratus* (animal) homogenized in an agar matrix. a: indicates no significant differences between species (α = 0.05).

Figura 1. Tasa de consumo diario (media ± e.e.) de dos alimentos artificiales por tres especies herbívoras comunes de costas intermareales rocosas de Chile. Los alimentos fueron ofrecidos por separado (sin elección), y consistieron en tejido pulverizado del alga verde *Ulva rigida* (algal) y el mitílido *Perumytilus purpuratus* (animal) homogenizado en una matriz de agar. a: indica que no hay diferencias significativas entre las especies (α = 0,05).

1.656, p = 0.227). Although these results might be partly attributed to the effect of starvation, no individual showed signs of avoiding the animal food, and the lack of difference in the consumption of both foods indicates that they had a similar edibility for the herbivores.

Proteolytic activity

The digestive tract of the three species exhibited proteolytic activity under the three conditions examined (field, animal, and algal diets). The average enzymatic activity in *C. granosus* (Fig. 2a) ranged from 0.13 (both for field and animal diet) to 0.16 (algal diet) mg alanine mL^{-1} h^{-1}. In spite of the higher activity with algal food, no significant differences were found among the three conditions (Kruskall Wallis; H = 0.636, p = 0.728, n = 18). This result was particularly important because it suggests that the consumption of animal food by *C. granosus* does not convey a reduction of its digestive capability.

Somewhat different results were obtained for the *Fissurella* species. *F. limbata* (Fig. 2b) showed a higher variation in proteolytic activity ranging from 0.12 (algal) to 0.17 (animal) mg alanine mL^{-1} h^{-1}, with significant differences between the three conditions ($F_{1,19}$ = 9.70, p = 0.0013). *A posteriori* comparisons revealed significantly higher activity for animal than for algal food (Tukey; p = 0.00095), without differences among the remaining comparisons (0.082 ≤ p ≤ 0.114). In contrast, proteolytic activity in *F. picta* (Fig. 2c) ranged from 0.12 (field) to 0.16 (algal) mg alanine mL^{-1} h^{-1}, with significant differences between the three conditions ($F_{1,19}$ = 9.03, p = 0.0018), although with significantly higher activity for algal food as compared with animal food (Tukey; p = 0.0051) and the field diet (p = 0.0071), but with no difference between these latter two (p = 0.987). As with *C. granosus*, the proteolytic activity of the two *Fissurella* species fed with animal food was never lower than with the other diets.

DISCUSSION

Our results are, to the best of our knowledge, the first data suggesting the existence of true (physiological) omnivory in Chilean invertebrate consumers previously typified as herbivorous, and we give particular importance to the consistent response of the three species. The experiments did not test for food preference but, based on the natural diet of these species (Camus *et al.*, 2008), we expected them to consume a greater amount of algal than animal food. Therefore, the results also suggest that both algal and animal food were not only edible but at least similarly palatable for the three species, even for *C. granosus*, which consumed nearly twice as much food as *F. limbata* and *F. picta*. The variable levels of proteolytic activity exhibited by individuals that consumed a natural diet were expected because they are unlikely to eat exactly the same resources in the field. Thus, it is important to note that such levels were always comparable to those recorded with artificial foods, suggesting that our data closely reflect what animals do in nature.

The levels of proteolytic activity recorded for experimental or field diets ranged from 0.12 to 0.17 mg alanine mL^{-1} h^{-1}, and are difficult to compare with published data because of the variety of units and calibrations used by different authors. As a reference, thus, we converted the data on total protease activity (μg tyrosine min^{-1}) obtained by Johnson & Freeman (2005) for six species of crab with varyied feeding habits into comparable units (mg h^{-1}). The transformed values for crab species fluctuated between a minimum of 0.03 mg h^{-1} for the herbivorous *Nectocarcinus in*
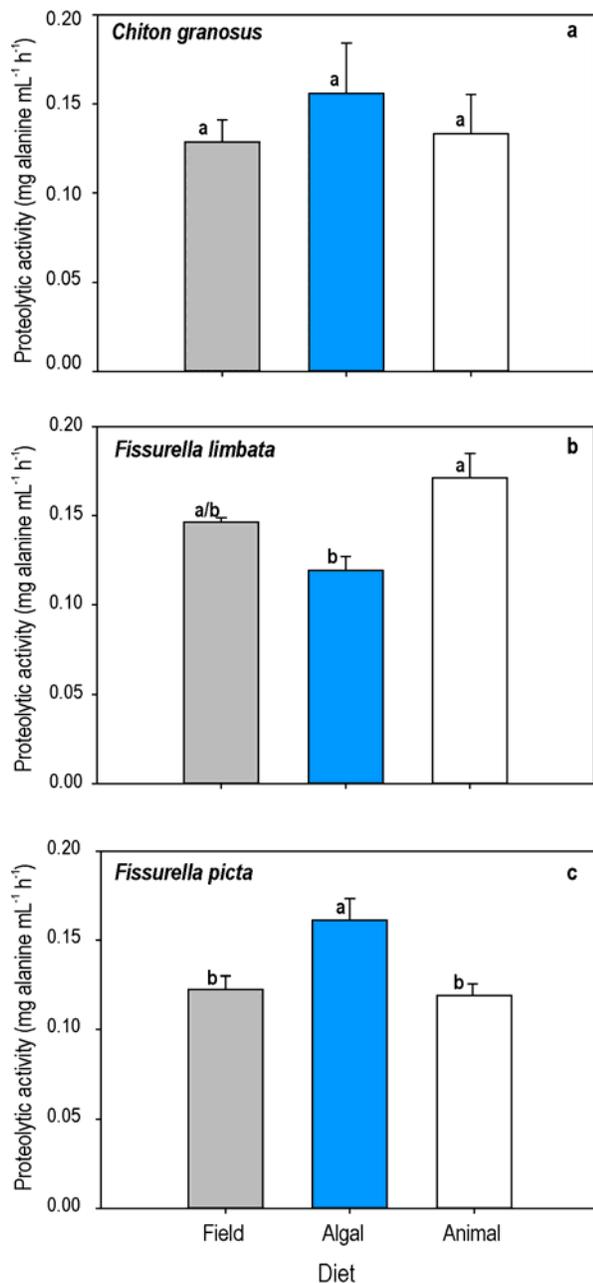
Figure 2. Proteolytic activity (mean ± s.e.) measured in the digestive tract of three herbivore species under three feeding conditions: natural diet ingested in the field, and artificial diets (algal *vs* animal food) ingested in the laboratory. Comparison within species: the letters between bars indicate statistical differences ($\alpha = 0.05$).

Figura 2. Actividad proteolítica (media ± e.e.) medida en el tracto digestivo de tres especies herbívoras bajo tres condiciones de alimentación: dieta natural ingerida en terreno, y dietas artificiales (alimento algal *vs* animal) ingeridas en el laboratorio. Comparaciones dentro de especies: letras distintas indican diferencias estadísticas entre las barras ($\alpha = 0,05$).

tegrifrons (Latreille) and maxima of 0.059 mg h^{-1} for the carnivorous *Plagusia chabrus* (Linnaeus) and 0.072 mg h^{-1} for the omnivorous *Leptograsus variegatus* (Fabricius). Johnson & Freeman (2005) suggested that the higher total activity in *L. variegatus* with respect to *P. chabrus* was due to the ingestion (likely incidental) of some red algae by the latter species, which nonetheless showed the highest trypsin activity (ca. 1.5 times that of *L. variegatus*), in accordance with its carnivorous status. However, our results are not directly comparable because Johnston & Freeman (2005) used a homogenate of the digestive glands after crab species had consumed their natural diet in the field, whereas we used gastric contents and our species were experimentally fed with a single type of food. In this regard, we note that our three study species displayed high proteolytic activity under all feeding conditions (including field diet) and, in spite of the statistical differences detected in *Fissurella* species, they all evidenced high digestive flexibility and the capability to display adaptive modulation (Buddington *et al.*, 1987; Sabat *et al.*, 1998). Although we failed to test a third experimental food (mixing algal and animal tissue), we predict that combining the high protein content of both foods should result in a higher proteolytic activity than that observed for each food separately, as expected in the case of omnivorous species. Nonetheless, we note that our results are not a demonstration of the many aspects and implications of omnivory, and they should also be verified employing more accurate techniques (*e.g.*, see Latyshev *et al.*, 2004). Notwithstanding, this study highlights the potential of the three studied species to switch between animal and algal foods, an ability that allows omnivores to take advantage of a wide variety of resources in the field and exploit nutritionally unpredictable environments (*e.g.*, Moyle *et al.*, 1995).

Therefore, we suggest that similar analyses should be conducted for other intertidal consumers, particularly mollusk and echinoderm species, considering that the most important Chilean herbivores are also strong candidates to be omnivores (Camus *et al.*, 2008). An evaluation of the existence and prevalence of omnivory may lead to drastic changes in our perception of the functional role of herbivore consumers both from ecophysiological and predator-prey perspectives, with at least two immediate consequences. Once a given species is demonstrated to be omnivorous, a logical step is to assess the relative importance of algal *versus* animal food for its nutritional and energetic balance, and to relocate its trophic position within the community food web. On the other hand, the evaluation of real food webs often relies on the assignation of the omnivorous status based on species

diets; without acknowledging the distinction between eating and feeding. Some commonly used concepts of omnivory refer to feeding at more than one trophic level (Pimm & Lawton, 1978), being linked to a basal species at more than one link in a trophic chain (Yodzis, 1984), or eating basal (plant) and intermediate (animal) species (Arim & Marquet, 2004). In the case of herbivores, however, the incidental consumption of animals is indeed a common phenomenon and, therefore, omnivory should not be readily assumed from dietary composition.

In a different context, the consumption of animals by herbivores, even if incidental, may be important as a potential source of indirect effects on their prey (Camus *et al.*, 2008). However, it may turn out to be especially significant if true omnivory is involved, particularly for Chilean rocky shores where omnivores are usually assumed to be absent. In this context, the eventual demonstration of omnivory should force a reappraisal of the structure, dynamics, and stability of rocky intertidal food webs (Navarrete *et al.*, 2008; Sanhueza *et al.*, 2008), particularly with regard to top-down and bottom-up cascading effects. Actually, Chilean intertidal herbivores are highly connected species contributing a high number of weak trophic links (Camus *et al.*, 2008). Thus, changing their status from herbivorous to omnivorous would not only involve the existence of longer food chains but also the addition of many omnivore links (Yodzis, 1984), closed omnivore loops (Sprules & Bowerman, 1988), and a higher incidence of intraguild predation with potential importance for food web dynamics (see Arim & Marquet, 2004). The fact that such a reappraisal can be triggered from a simple assessment of the capacity to digest animal food should, thus, be considered an incentive for conducting research in that direction.

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