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

Patricio A. Camus¹, Yuri Y. Cid¹, Lilian Cisterna¹ & Cristian W. Cáceres¹
¹Departamento de Ecología Costera, Facultad de Ciencias, Universidad Católica de la Santísima Concepción
Casilla 297, Concepción, Chile

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.

Corresponding author: Patricio A. Camus (pcamus@ucsc.cl)
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 (Latsyhev 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 18° to at least 42°S; Oliva & Castilla, 1986; Hidalgo et al., 2006): the polyplophoran Chiton granosus Frembly, and the key-hoe 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 prey 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 Cocholgúe (36°35'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 14°C 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 P. purpuratus (Lamarck) and the green alga U. 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., M. 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 (-20°C), 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 37°C. 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 (F₁,₁₂ = 4.60, p = 0.053), F. limbata (F₁,₁₀ = 0.177, p = 0.683), or F. picta (F₁,₁₀ =
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 ($\alpha = 0.05$).

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 ($0.082 \leq p \leq 0.114$). 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 ($\mu$g tyrosine min$^{-1}$) obtained by Johnson & Freeman (2005) for six species of crab with varied 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 (α = 0.05).

Figure 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 (α = 0.05).
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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