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Trophic relationships between a Patagonian gastropod and its epibiotic anemone revealed by using stable isotopes and direct observations

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Abstract The carnivorous snail *Adelomelon ancilla* usually carries, attached to its shell, the anemone *Antholoba achates*, which also lives attached to hard substrates in the same area. Interaction between both species was studied by stable isotopes analyses (SIA), direct observations, and analysis of gut contents. Results did not show evidences of dietary overlap between anemones and snails. *A. ancilla* consumed mainly bivalves and secondarily gastropods. The diet of *A. achates* involved sea urchins and echiurids as main prey. The trophic niches of anemones and snails did not overlap; the species had similar $\delta^{15}N$ values but differences in their $\delta^{13}C$ values. Sessile and epibiotic anemones also showed differences in their $\delta^{13}C$ means, revealing access to different resources. Whereas SIA provided information on trophic relationships and structure, observation of feeding events provided details of prey utilization patterns. Combining direct and indirect approaches helps to overtake method weaknesses and greatly improve dietary descriptions.

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Introduction

Understanding the structure and functioning of marine ecosystems requires information on the trophic relationships among species (Paine 1988; Bertness and Leonard 1997; Bertness et al. 1999), because trophic interactions have a direct effect on population dynamics and community structure (Sih 1993; Díaz et al. 2012). Thus, food webs constitute special descriptors of biological communities focusing on the transfer of mass and energy between organisms (Hughes 1993). However, understanding marine food webs is also difficult given the logistic difficulties (Paine 1988). Fortunately, combining direct dietary descriptions (e.g., collecting regurgitations or stomach contents) and indirect approaches (analyzing predator's stable isotope or fatty acids composition) helps to overtake those difficulties and greatly improve dietary description (Schindler and Lubetkin 2004). Furthermore, marine food webs are complex and dynamic structures with trophic linkages that could be influenced by ecological processes such as competition, symbiosis (that could enhance predator efficiency or prey defenses), as well as changes in prey availability (Sih 1993). An example of a complex association is the relationship between gastropod shells occupied by the hermit crab *Pagurus bernhardus* and the hydroid *Hydractinia echinata* (Rees 1967). The possible benefit to the hydroid is an increase in its mobility, enabling it to obtain greater amounts of food and also to avoid adverse physiological conditions (Ross 1974). Meanwhile, in the case of the hermit crabs, protection provided by the nematocysts of the anemone would be a benefit decreasing their mortality rate due to predation (Ross 1960; McLean and Mariscal 1973; Brooks and Mariscal 1986). This is a case of "positive interaction" which can occur when one organism makes the local environment more favorable for

another either directly (e.g., reducing nutrient stress via shading in invertebrates) or indirectly (e.g., by removing competitors) (Stachowicz 2001; Munguia et al. 2009). In any case, it is expected that the particularity of the relationship between epibiotic species and their hosts is reflected in their diets. This type of process and trophic linkages appears to operate between some neogastropods and the anemones attached to their shells.

Neogastropods are top predators of the benthic environment (Taylor et al. 1980) and the majority of them are defined as carnivorous which generally seek out and consume whole prey organisms (Curio 1976). In predatory marine snails, feeding comprises a series of complex behavioral patterns including to search for, to capture, to immobilize, and to penetrate prey and finally to swallow prey tissues (Taylor et al. 1980). This characteristic allows making a good estimation of the frequency of prey occurrence by direct field observation, which is unlikely to be skewed by digestion mechanisms (Hyslop 1980).

The marine snail *Adelomelon ancilla* (Lightfoot, 1786) (Fig. 1) is an edible neogastropod belonging to the family Volutidae and distributed along the southern tip of South America, from 35° S (Brazil) in the South Western Atlantic to Ushuaia in the Beagle Channel (Bigatti personal observation), and northward into the Pacific, reaching central Chile (25° S, Chiloé Island, Castellanos and Landoni 1992). Volutids present large sizes and somatic production, slow growth rate, late sexual maturity, and direct development (e.g., Bigatti et al. 2007). In Golfo Nuevo, northern Patagonia, more than 98 % of the *A. ancilla* snails have at least one anemone *Antholoba achates* (Drayton in Dana, 1846) attached to their shells (Bigatti et al. 2009; Fig. 1), but this anemone also lives attached to hard substrates in the same area (Zabala personal observation). In the SW Atlantic Ocean, this anemone is epibiotic of other gastropods, such as the volutid *Adelomenon brasiliana* (Luzzatto and Pastorino 2006), the nassariid *Buccinanops cochlidium* (Pastorino 1993), and the crab *Libinia espinosa* (Acuña et al. 2003), but it is unknown if there is a trophic relationships with their hosts. When considering the diet of anemones living fixed to hard substrata, they can only feed on individuals living close to them, while specimen living on mobile substrata, as on gastropod shells, can exploit different trophic resources (Rees 1967; Ross 1974; Ross and Kikuchi 1976; Luzzato and Pastorino 2006). The mobile condition enables species to exploit a larger portion of available resources (e.g., space, light, nutrients, and food) and thus could increase the utilization of the fundamental niche space (Bruno et al. 2003). However, for predators belonging to the same trophic pathway, it is possible that the mobile condition differentiates the trophic niche in other aspects than prey items, like for example foraging arenas (Nordström et al. 2010). Therefore,

studying the diet of the snail and the anemone (both the epibiotic and sessile variants) enables the understanding of their interaction.

Previous direct dietary observations showed that bivalves were the main prey item of *A. ancilla* (Bigatti et al. 2009). However, stomach content, although providing high resolution and detailed results, may be limited in terms of identifying the diet composition due to differential digestion rates (Hyslop 1980; Duffy and Jackson 1986) and the difficulty of linking ingested and assimilated food (Dubois et al. 2007; Newsome et al. 2009). However, the ratios of naturally occurring stable isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in consumer tissues provide insight into food web structure that can complement conventional dietary assessments (e.g., Sydeman et al. 1997). For this reason, stable isotope analysis (hereafter SIA) of C and N has become a widely used tool in the assessment of trophic relationships, providing valuable evidence to define time-integrated feeding relationships of predators with their prey (Vander Zanden et al. 1999; Schindler and Lubetkin 2004; Layman et al. 2012). Thus, combining SIA method with direct observations was selected as the right tool to evaluate the diet of species like *A. ancilla* and the anemone *A. achates* attached to their shells.

The goals of this paper are to (1) describe diet composition of the neogastropod *A. ancilla* and the anemone *A. achates* by means of direct and indirect methods and (2) evaluate dietary differences between sessile and epibiotic anemones, and between epibiotic anemones and their hosting snail comparing isotopic signatures. Finally, we used our results to compare and contrast direct and indirect dietary description discussing their utility to understand benthic community relationships.

Methods

Direct methods

Direct observations on the diet of the snail *A. ancilla* were done between April 2008 and June 2011 at Playa Paraná and Storni Harbor, both sites located at Golfo Nuevo, Argentina (42°48′S; 64°54′W; Fig. 2). Field sampling was performed monthly by SCUBA diving at depths ranging from 5 to 9 m during both daylight and night hours. Playa Paraná site is characterized by mixed bottoms (sand and gravel) without hard structures in contrast to Storni Harbor, which presents mixed bottoms with many small hard artificial structures (pieces of steel, wood, and rubber wasted from port activities). *A. ancilla* (Fig. 1a) was usually observed buried in the substrate and was easily recognizable from above the sediment by its epibiotic sea anemone
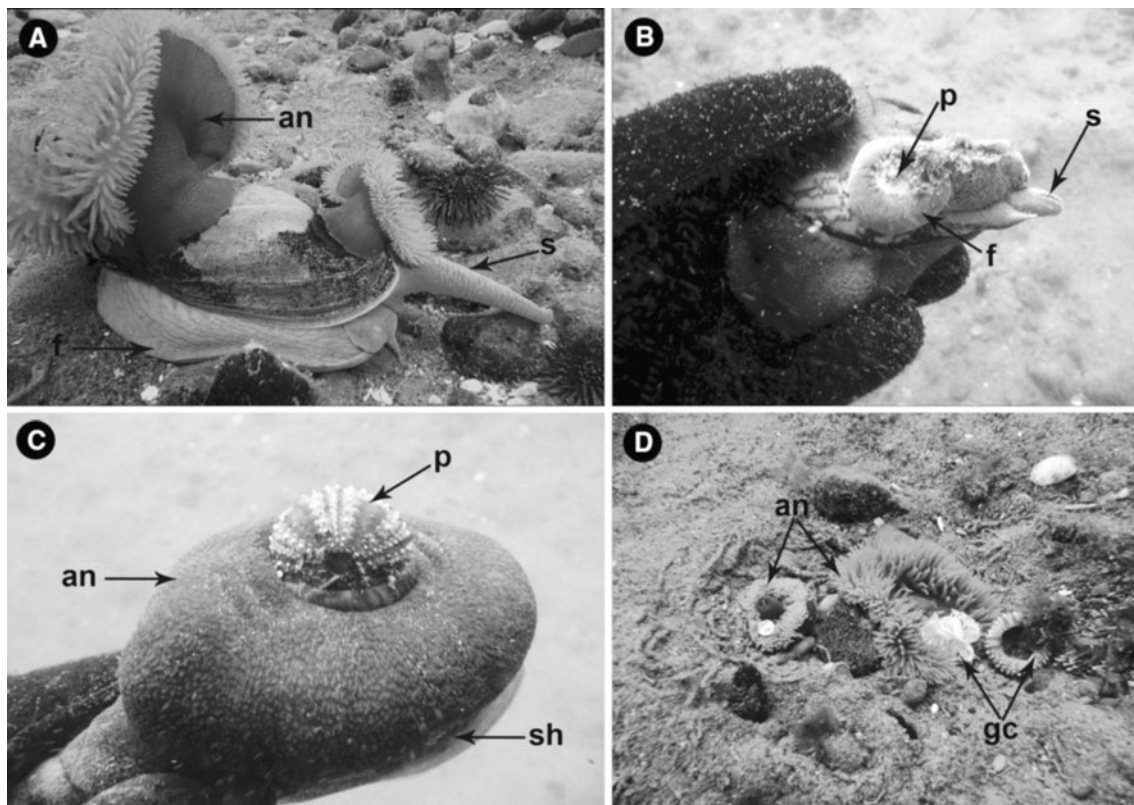
Fig. 1 a *Adelomelon ancilla* in its natural environment (mixed bottoms of gravel and sand) at Golfo Nuevo, Patagonia. Note the *bottom* and the sea urchins *A. dufresnii*. **b** Feeding event of *A. ancilla*. *Arrow* shows the prey (bivalve) engulfed by the foot. Note the *A. achates* anemones fixed to the shell. **c** Feeding event of *A. achates*. *Arrow* shows the prey (sea urchins: *A. dufresnii*) engulfed by the gastric cavity. **d** Epibiotic anemone (over a buried snail) exposing its gut content (sea urchin). Note that the anemone is at the bottom level. *An* anemone, *f* foot, *gc* gut content, *p* prey, *s* siphon, *sh* gastropod shell

A. achates (Fig. 1b). All *A. ancilla* individuals found were observed in situ, and whenever they were feeding, the snail and its prey were collected in separate labeled bags for later identification and measurements. Most individuals were measured in the field on board of a boat and released to the water; a complementary subset ($n = 30$) of snails were euthanized to conduct gut content analysis in the laboratory under a stereoscopic microscope. Direct observations on the diet of the anemone *A. achates* (both epibiotic and sessile specimens) were done only at Playa Paraná, where SCUBA was used to register and identify all prey.

To test whether sample size was representative, cumulative trophic diversity curves were used to assess whether the number of direct field data was enough to provide a precise description of the diet and to perform comparative analyses between sites. It is important to remark that, in contrast to cumulative curves generated using stomach contents, direct observations used here only identify links between the predator and a single prey at a given time. Curves were constructed by calculating the Brillouin diversity index (IHz) using the pooled quadrate method (Pielou 1966). For each site, 100 cumulative curves were constructed by random permutation of the sampled direct field data. Diversity curves were considered asymptotic if at least the last two values were within ±0.05 of the overall diversity. Frequency of occurrence (O %), defined as the percentage of snail feeding on each prey item observed, was calculated for the entire dataset and for both sites. Values of 95 % CI were generated for the three O % by nonparametric bootstrap simulations (Tirasin and Jorgensen 1999). Calculations were done with the R software version 2.15.3 (R Development Core Team) using the percentile method implemented in the package Boot (Ripley 2005) performing 9999 bootstrap replicates.

Indirect methods

A total of 19 snails with their epibiotic anemones were collected by SCUBA during February 2010. Sampling was performed at Playa Paraná (Fig. 1) in a time frame of 2 weeks to avoid temporal variability (Kurle et al. 2011). We chose this site for logistic purposes; however, the prey items sampled commonly occur at both study

Fig. 2 Sampling sites (*1* Playa Paraná and *2* Storni Harbor) in Golfo Nuevo, Argentina
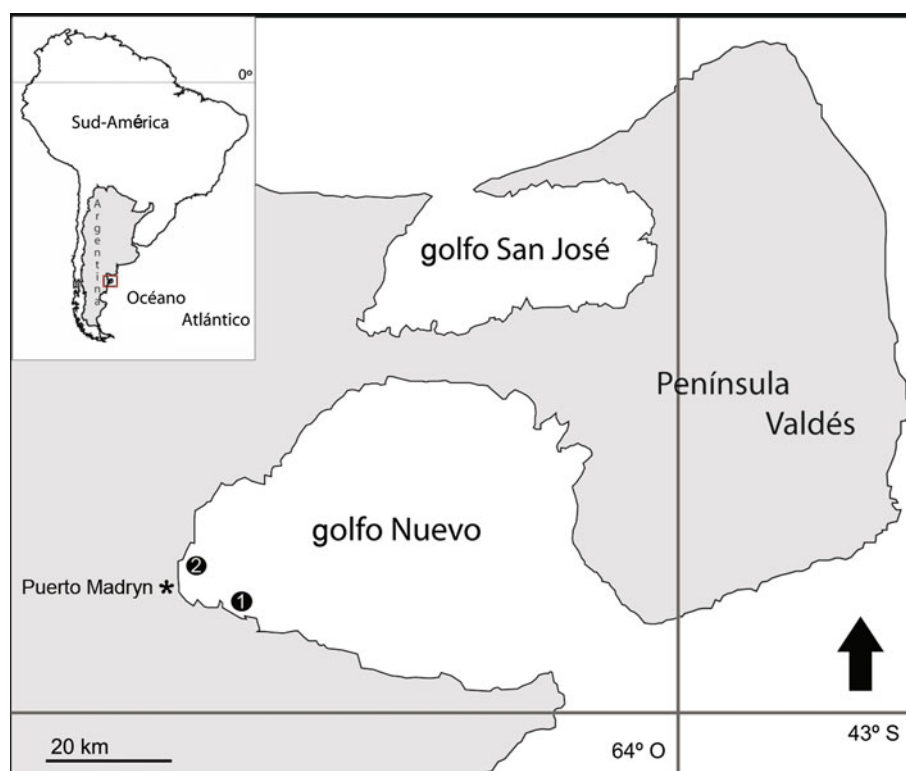
sites. A small piece (1 cm^3) of predator's foot muscle was cut in the field without killing the snail. The tissue cutting was done onboard the boat immediately after diving and individuals were released. Each piece of tissue was stored in individual bags and preserved cooled for posterior processing. The same procedure was performed for epibiotic ($N = 19$) and sessile anemones ($N = 16$) using the exterior portion of the column tissues. Anemones were collected detaching them from the snails or small stones and hard substrata in the same place at the same time. Six potential prey items (Table 1) were collected based on our observations and published data (Bigatti et al. 2009) that covered the spectrum of main consumed prey at the population level in Golfo Nuevo (see "Results"). Once in the laboratory, all samples (snail muscle, anemones, and prey tissues) were cleaned with tap water and the inedible debris and digestive systems removed (i.e., anemones and prey's digestive system), rinsed with water and weighed, before drying at 60 °C until weight stabilized (~48 h), and then grounded using a hand mortar.

Carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope values were determined by mass spectrometry (see Lajtha and Michener 1994 for details) at the University of California-Davis Stable Isotope Facility. Carbon and nitrogen stable isotopic ratios were expressed in δ notation according to the following equation:

$$\delta X (‰) = 1,000 \left[(R_{sam}/R_{std}) - 1 \right],$$

where X is ^{13}C or ^{15}N and R_{sam} and R_{std} are the ^{13}C:^{12}C or ^{15}N:^{14}N ratios of the sample and standard, respectively. The standards are Vienna-Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N$_2$ for nitrogen (Peterson and Fry 1987). The units are expressed as parts per thousand or per mil (‰). Internal standards used were G-11 nylon, G-13 bovine liver, G-17 USGS-41 glutamic acid, and G-9 glutamic acid.

To estimate the approximate contribution of each prey to the snail diet, we used the mixing model SIAR (Parnell et al. 2010). Mixing models estimate the proportional contribution of sources (dietary items) within a mixture (consumer tissue) and thereby infer diet composition (Phillips and Gregg 2003; Inger et al. 2006; Samelius et al. 2007; Newsome et al. 2009). In particular, SIAR is a Bayesian-mixing model that accounts for variation in predator, isotopic discrimination and sources values, and allows the input of prior information on prey importance. (Jackson et al. 2008; Moore and Semmens 2008; Parnell et al. 2010). We performed a mixing model assuming five functional prey (sampled bivalves were pooled because their isotopic signatures were similar; Table 1). To correct prey isotope data for trophic discrimination and plot consumers in mixing space, we used as specific as possible discrimination values. We assumed Δ^{13}C = 2.6 ± 0.3 ‰ (McKnight 2009) because this value was calculated for a gastropod, and in addition, other Δ^{13}C calculated for mollusks (e.g., Δ^{13}C = 2; Dubois et al. 2007) is similar. Regarding Δ^{15}N, in the absence of specific values

Table 1 Mean and standard deviation values of carbon and nitrogen stable isotopic values and C:N elemental ratios in predator, anemones, and prey items collected at Playa Paraná site, February 2010

Taxon/species	Feeding behavior	n (N)	δ^{13}C (‰)	δ^{15}N (‰)	C:N
Predator					
A. ancilla	Carnivorous	19 (1)	-13.92 ± 0.35	16.74 ± 0.36	3.18 ± 0.09
Prey items					
Bivalves					
Diplodonta patagonica	Filter feeder	7 (5)	-17.96 ± 0.39	13.12 ± 0.37	4.33 ± 0.09
Bushia rushii	Filter feeder	3 (1)	-17.92	12.61	4.28
Gastropods					
T. patagonica	Herbivorous	5 (5)	-15.59 ± 0.24	12.71 ± 0.24	3.72 ± 0.03
N. isabelleana	Carnivorous	3 (1)	-15.65 ± 0.49	14.77 ± 0.10	3.80 ± 0.06
Sea urchins					
A. dufresnii	Omnivorous	5 (5)	-17.30 ± 0.94	12.04 ± 0.31	4.41 ± 0.26
Echiurids					
P. chilensis	Detritivorous	4 (1)	-15.77 ± 0.22	13.91 ± 0.16	3.41 ± 0.10
Anemones					
A. achates (epibiotic)	Omnivorous	19 (1)	-15.82 ± 0.51	16.57 ± 0.33	4.35 ± 0.29
A. achates (sessile)	Omnivorous	16 (1)	-16.38 ± 0.72	16.44 ± 0.26	4.23 ± 0.28

n number of samples, *N* number of pooled individuals in each sample

published for gastropod, we assumed the general value Δ^{15}N $= 3.4 \pm 0.4$ ‰ (Vander Zanden and Rasmussen 2001) which is similar to discrimination values estimated for other mollusks (e.g., Δ^{15}N $= 3.6$; Dubois et al. 2007). Uncertainty included in SIAR around discrimination values was chosen to avoid that corrections were large compared to the spread of the consumers and the probability that predators' signature laid outside the prey polygon was high (see Galván et al. 2012 for details). The model was run twice, without prior information and inputting possible dietary proportions based on our observations and published data (Bigatti et al. 2009). SIAR allows users to incorporate information about possible mean proportions for each dietary source and the standard deviation of one of them (Parnell et al. 2010); in this case, we inserted the following possible prey proportions: bivalves: 0.45 ± 0.10; herbivorous gastropods: 0.20; carnivorous gastropods: 0.20; echiurids: 0.10; sea urchins: 0.05.

Trophic comparisons between sessile and epibiotic anemones, and between epibiotic anemones and snails were done via testing differences in their δ^{13}C and δ^{15}N isotope signatures. First, mean isotope values were compared using Student's t tests after testing for homogeneity of variance among samples using the Fisher's *F* test (Crawley 2007). Second, trophic niches were compared using the hypothesis-testing framework proposed by Turner et al. (2010) and the approach based on multivariate ellipse metrics (Jackson et al. 2011). Differences in centroid location, which provide information on trophic niche position, and eccentricity, which provides insight into differences in the underlying distribution of δ^{13}C and δ^{15}N data, were tested using nested linear models and residual permutation procedures (see Turner et al. 2010 for statistical details). Niche width was estimated for each group using multivariate ellipse-based metrics (Jackson et al. 2011). The analysis generates standard ellipse areas (SEA) which are bivariate equivalents to standard deviations in univariate analysis. We used SEA values corrected for small sample size (SEA$_C$) to calculate niche overlap and generated Bayesian estimates of SEA (SEA$_B$) to test differences in trophic niche by comparing their 95 % credible limits (see Jackson et al. 2012; Thomson et al. 2012 for examples). Finally, correlation analyses were done to test for possible relationships between individual snails and its particular epibiotic anemone. Calculations were done with the R software version 2.15.3 (R Development Core Team) using the package SIAR (Jackson et al. 2011) and the scripts provided by Turner et al. (2010); additional details on calculations and statistical framework can be found in the previous references.

Results

Direct methods

The feeding for a total of 68 snails was registered with only eight prey items consumed. The cumulative trophic diversity curves showed that the number of direct observations for each site was adequate to obtain a precise description of the snail diet (Fig. 3). The prey items captured included mainly clams and herbivorous gastropods with a smaller proportion of carnivorous gastropods and rarely echiurids, scallops, or mytilids (Fig. 4; see Table 2 for complete list of prey items consumed). The main prey

Fig. 3 Trophic diversity accumulation curves of **a** both sites together, **b** Playa Paraná site and **c** Storni Harbor site. *Horizontal lines* show Brillouin diversity index (Hz) values (Hz $\pm$ 0.05 Hz) and the *vertical line* shows a value $n - 2$ (n number of direct field data)
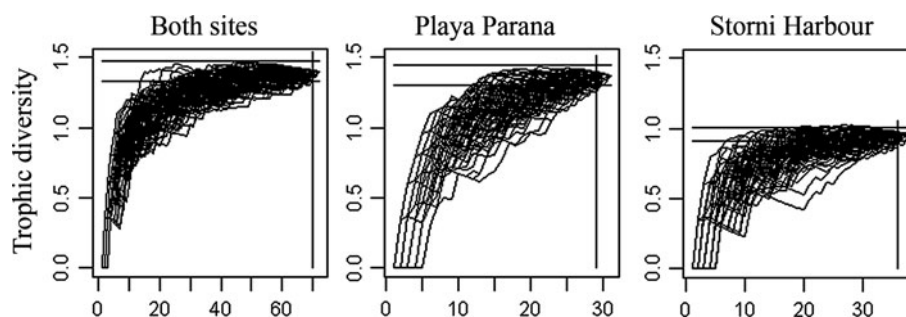
item varied between study sites. Bivalves were the main group consumed at Playa Paraná (with 57 O %); meanwhile, in Storni Harbor site, bivalves represented 28 O % being the second in importance (Fig. 4). The herbivorous gastropod *Tegula patagonica* showed the most relevant difference between sites, whereas at Playa Paraná, the frequency of occurrence was very low (3 %); in Storni Harbor, *T. patagonica* was the main prey item (55 O %) (Fig. 4). Regarding the carnivorous gastropods *Notocochlis isabelleana*, scarce differences were found between sites (P. Paraná, 33 O % and S. Harbor, 17 O %). Only 2 cases of *A. ancilla* were observed feeding on the echiurid *Pinuca chilensis*, at Playa Paraná (6 O %). Meanwhile, sea urchins (i.e., *Arbacia dufresnii*) were not registered (0 O %) as *A. ancilla* prey.

The gut content analysis of snails did not add information about the diet composition. From all the stomachs analyzed ($n = 30$), only three cases contained an unidentified mucous mass and the others were empty.

The diet of epibiotic anemones (Fig. 1c, d) comprised the sea urchins *A. dufresnii*, (27 feeding events) as the main prey item, followed by crabs (*Peltarion spinosolum* and *Leurocyclus tuberculosus*) in three cases and snails (*N. isabelleana*). Sometimes, more than one individual was observed inside the anemone's stomach (Fig. 1d). On the other hand, the frequency of occurrence for sessile anemones was minor; the prey items involved were the echiurids (*P. chilensis*) and the sea urchins (*A. dufresnii*) as the main prey (four and three cases, respectively).

Indirect methods

The potential prey selected and collected, comprised multiple trophic guilds (Fig. 5), and showed isotopic compositions different enough to allow its analysis as different prey items. The predator isotopic values were contained within the mixing space after application of discrimination corrections (Fig. 5).

Histograms representing the estimated contributions of prey to *A. ancilla* diet differed depending on the addition or not, of prior information to the model solved. Model's output without prior information showed a similar importance and contribution (0.2 % approximately) for all prey

items. However, large differences were observed about the inclusion of zero as a possible contribution for some prey. For bivalves and carnivorous gastropods (*N. isabelleana*), the histograms were more precise and did not include the 0 as possible solution; meanwhile, the rest of the prey items (herbivorous gastropods, echiurids, and sea urchins) had broader ranges and included zero with a high probability of occurrence (Fig. 4).

After adding prior information into the mixing models, prey importance shifted: bivalves were the main item, followed by herbivorous gastropods (*T. patagonica*) and by carnivorous gastropods. None of these three groups included the zero as solution; moreover, the contribution of each item increased with respect to the result without prior data. In contrast, the contribution respect to *P. chilensis* (echiurids) was low including zero as a possible solution; meanwhile, sea urchins (*A. dufresnii*) were negligible and zero contribution appears with high probability of occurrence (Fig. 4).

The three groups showed differences in their niche locations, and these results were based on the observed differences in their centroid locations (sessile vs. epibiotic anemones: $P = 0.004$; epibiotic anemone vs. snails; $P < 0.001$ and sessile anemone vs. snails; $P < 0.001$; using 1,000 permutations). These results were mainly due to differences in their $\delta^{13}C$ signatures (sessile vs. epibiotic anemones: t test, $t_{17.8} = -2.34$, $P = 0.03$; and epibiotic anemone vs. snails: t test, $t_{32.1} = 13.3$, $P < 0.001$). In contrasts, anemones and snails showed similar $\delta^{15}N$ mean values (Fig. 6). In accordance, estimated snails' SEA_C did not overlap with anemones' SEA_C but sessile and epibiotic anemones overlap their estimated $SEA_C \sim 40$ % (Fig. 6). Both sessile and epibiotic anemones showed similar spread in their isotopic composition expressed as no differences neither in their SEA_B, ($P = 0.98$) nor eccentricity ($P = 0.58$). The mean SEA_B values and their 95 % Bayesian credible intervals (CI) were for sessile anemones $SEA_B = 1.28$ (95 % CI 0.73–2.22) and for epibiotic anemones $SEA_B = 0.87$ (95 % CI 0.56–1.36). Neither $\delta^{15}N$ nor $\delta^{13}C$ values of the sessile anemones were correlated with its particular hosting snail ($\delta^{15}N$: Pearson's correlation, $r_p = 0.1$, $N = 38$, $P = 0.67$; $\delta^{13}C$: Pearson's correlation, $r_p = -0.01$, $N = 38$, $P = 0.94$).
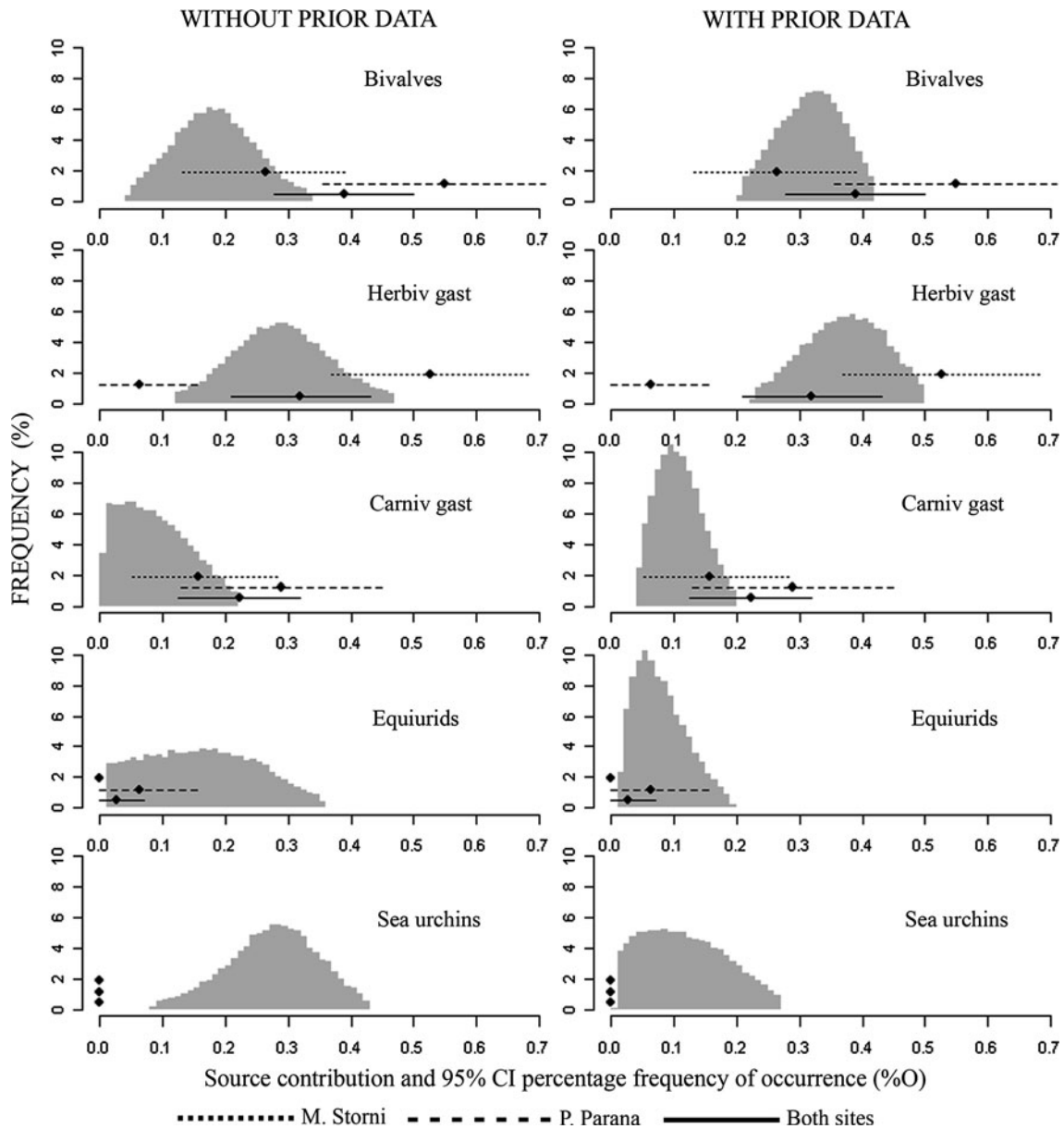
Fig. 4 Distributions of feasible contributions of several prey and mean ± 95 % CI percentage frequency of occurrence (O %) of direct field data in the different sites

Table 2 Prey items consumed by *A. ancilla* in Golfo Nuevo

Taxon	Family	Specie (item prey)	No. total cases	Total O %
Bivalvia			27	40
	Veneridae	*Protothaca antiqua*	5	18
	Veneridae	*Eurhomalea exalbida*	16	59
	Ungulinidae	*Diplodonta patagonica*	4	15
	Pectinidae	*Aequipecten tehuelchus*	1	4
	Mytilidae	*Aulacomya atra*	1	4
Gastropoda			39	57
	Trochidae	*T. patagonica*	22	32
	Naticidae	*N. isabelleana*	17	25
Echiurida	Pinucidae	*P. chilensis*	2	3

O % (frequency of occurrence)

Fig. 6 δ^{15}N and δ^{13}C for *A. ancilla* (gray points) and *A. achates* anemones, epibiotic (*black triangles*) and sessile (*gray triangles*). *S* snail, *EA* epibiotic anemone, *SA* sessile anemone. Isotopic niches are represented as the *standard ellipses* used to calculate SEAc without discrimination discount
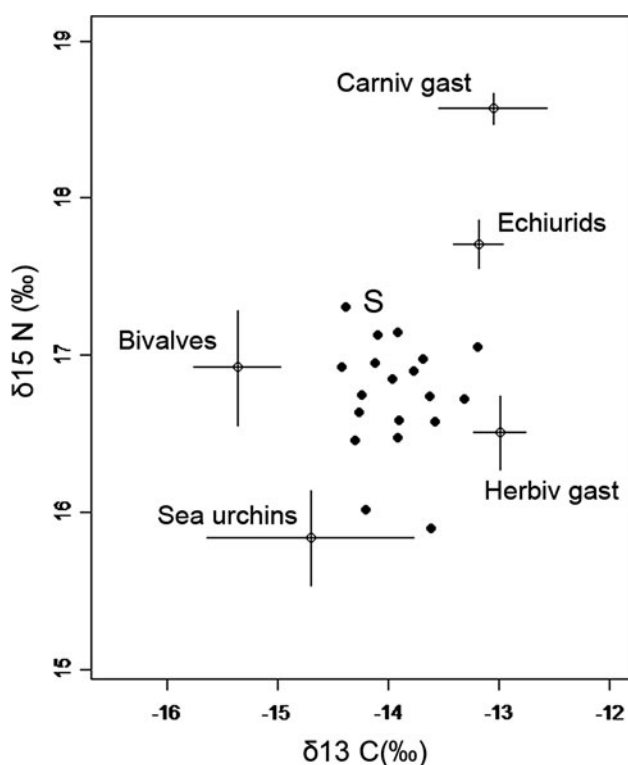
Fig. 5 Mean ± S.E. δ^{15}N and δ^{13}C for: Bivalves (*D. patagonica, B. rushii*); Carnivorous gastropod (*N. isabelleana*); Sea urchins (*A. dufresnii*); Echiurids (*P. chilensis*); Herbivorous gastropods (*T. patagonica*). *Black dots* (S) correspond to *A. ancilla* snail data, after trophic discrimination was discounted on prey values

Discussion

Our results combining direct and indirect approaches revealed that *A. ancilla* consumes mainly bivalves, secondarily herbivorous gastropods, followed by carnivorous gastropods and occasionally they feed on echiurids. Both sessile and epibiotic individuals of *A. achates* showed similar spread in their isotopic niches (i.e., similar SEA$_B$ values), but niche locations differed (i.e., different centroid locations). There was no evidence of an important overlap among the diets of anemones and snails. The diet of *A. achates* involved sea urchins and echiurids as the main prey items and crabs and snails, in minor proportions.

Diet of *Adelomelon ancilla*

There were differences between sites. In Playa Paraná, bivalves were the main prey, whereas in Storni Harbor, *A. ancilla* mostly fed on the herbivorous snail *T. patagonica*. Prey proportions in Playa Paraná were coincident with previous studies on this snail (Bigatti et al. 2009), but differed with the dietary proportions provided by stable isotopes on snails captured in the same site. Playa Paraná has a mixed bottom (sand and gravel) without hard
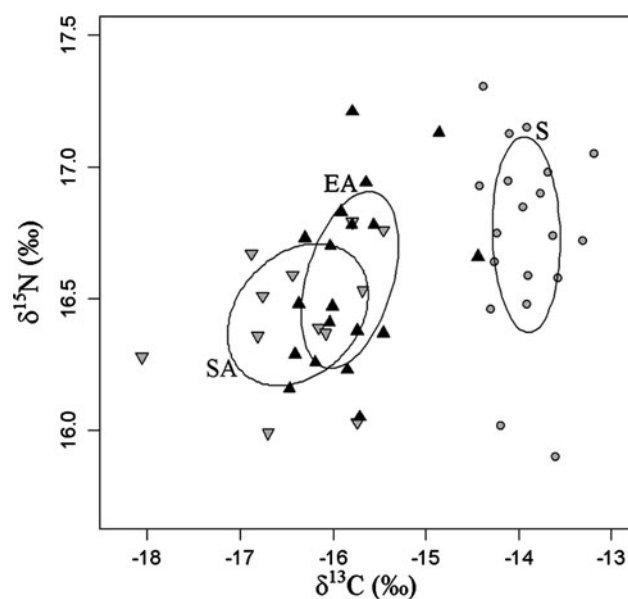
structures, while Storni Harbor has mixed bottom with many small hard artificial structures (pieces of steel, wood, and rubber produced by harbor activities). *T. patagonica* is an herbivore gastropod that lives mostly on hard substrata, being more common in the Storni Harbor area. Thus, it is possible that Storni Harbor data were biased by the presence of artificial bottom structures overestimating herbivorous gastropods consumption in natural habitats, but revealing dietary preferences. However, the overall direct observation results (Storni Harbor plus Playa Paraná) were coincident with stable isotope data (Fig. 4), showing that *T. patagonica* is more important as prey item than it was previously described (Bigatti et al. 2009). In concordance, *A. ancilla* inhabiting the continental shelf-break of the SW Atlantic was described using stable isotope data, as predator of the bivalve *Zygochlamys patagonica* and the gastropod *Fusitriton oregonensis* (Botto et al. 2006). Taking into account our data, we conclude that *A. ancilla* consumes mainly living mollusks and could change the dietary main item consumed depending on the species composition of the site where it inhabits.

Trophic niches and resource partitioning

Based on the δ^{15}N values, we observed that snails and anemones (epibiotic and sessile ones) shared the same trophic level. However, δ^{13}C values showed that snail and their epibiotic anemones feed on different kinds of prey. Flaherty and Ben-David (2010) showed that predators with

generalist's diet preferences and habitat use could have narrow isotopic niches. In the present study, this was the case of both the anemone and the snail. This isotopic niche interpretation might erroneously infer the potential overlapping on diet resources between anemones and snails. However, our observations in situ demonstrated that there is no overlapping among their diet preferences. The anemone fed on epifaunal organisms (sea urchins and crabs), while the snail fed mostly on infaunal invertebrates (bivalves and infaunal gastropods). Direct results support the differences in prey selection between the anemones and the snail. Additionally, most *A. ancilla* usually have marks of previous anemones attached on their shells, and in some *A. ancilla* individuals, from which anemones were removed by hand, a shell recolonization by at least one anemone was observed (S. Zabala unpublished results) within 15 days. This fact shows a possible dynamic plasticity in the form of life of the anemones, fluctuating between sessile and epibiotic condition through time. For this reason, it is not surprising that the C isotopic values between epibiotic and sessile anemones showed some overlapping individuals (Fig. 6). Sessile and epibiotic anemones showed differences in the location of their isotopic niches; however, it is not possible to unequivocally assign this difference to prey items consumed or spatial differences in the isotopic signature of the same prey items (Nordström et al. 2010). Nevertheless, given that snails are common on sandy bottoms near reefs (in terms of meters) and that all samples were collected in the same area, the hypothesis that sessile or epibiotic anemones had different diets is more plausible.

Methodological issues

Although indirect dietary techniques, such as stable isotope and fatty acid analysis, offer manageable alternatives for elucidating and understanding dietary patterns in numerous species and trophic relationships among species (Newsome et al. 2009; Paradis and Ackman 1977; Cahu et al. 1995; Brett and Müller-Navarra 1997), computational models used to analyze our results do not incorporate factors such as prey availability and palatability or competitive interactions. Therefore, there is no reason to expect that means will accurately index the unique solution that scientists seek (Benstead et al. 2006). However, direct data as stomach content analysis or direct field data to elucidate diet composition do not offer information about the nutritional input of each prey, as stable isotopes do (Cheng-Tze et al. 2007). Thus, whereas SIA provides information on trophic relationships and structure, and conventional dietary assessments provide details of prey utilization patterns, both techniques used together, provide a powerful means of detecting trophic relationships patterns in marine ecosystems. This point was illustrated by our results, because results of the model without prior information showed that echiurids and sea urchins were important prey, while the consumption of *N. isabelleana* was most probably near zero or negligible (Fig. 4). Echiurids were observed being fed by snails in rare occasions (two times), and sea urchins were reported by Bigatti et al. (2009) as rare snail prey. Thus, both can be considered rare prey. However, given the isotopic composition of sea urchins and its location in the mixing polygon (Fig. 5), it is arithmetically more probable that they were taken as important sources, although they seem to be rare prey. This case exemplifies how erroneous conclusions could arise when stable isotopes have been used without solid information on the studied system.

In conclusion, our results support the use of different dietary resources between the neogastropod *A. ancilla* and its epibiotic anemones, and the interaction between *A. ancilla-* and *A. achates*-driven niche differences, in terms of prey items or foraging areas, between sessile and epibiotic anemones, and reinforce the importance of use SIA and conventional dietary assessments methods in conjunction with understand and elucidate diet composition and trophic relationships in marine ecosystems.

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