

Influence of maturity condition and habitat type on food resources utilization by *Octopus tehuelchus* in Atlantic Patagonian coastal ecosystems

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Received: 31 December 2015 / Accepted: 20 July 2016
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Abstract This study evaluates differences in *Octopus tehuelchus* diet in relation to sex and maturity condition (juvenile, mature, post-spawning) in two contrasting habitat types (sandy vs. rocky). Muscle of *O. tehuelchus* and of its associated food sources were analyzed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. There was no correlation between $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ and octopus size. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were similar between sexes indicating similar diets. The Bayesian mixing model (MixSIAR) showed that the proportional contribution of the sources varied with the maturity condition of octopuses, and between habitat types. Our results show differences in the proportions of alternative prey in the diet of *O. tehuelchus* related to their physiological condition and the habitat characteristics. The differential food source utilization may be related to differential prey energy contribution and could explain the differences in the growth pattern and life span, as well as in the reproductive traits and recruitment success of *O. tehuelchus* in sandy and rocky habitats. Our results highlight the importance of considering such differences in octopus diet, since they may have consequences

for population dynamics, but also in the species' trophic niche and its functional role in communities.

Introduction

In coastal ecosystems, trophic relationships can be highly complex and food webs can show important differences even over small scales (Mallela and Harrod 2008; Schaal et al. 2011). Individual variation in food source utilization is an important aspect to consider in food web studies (Bolnick et al. 2011), since it has implications in terms of species' trophic niche and community dynamics. Differences in habitat use, behavior and diet could be due to sex- or size-specific differences (Forero et al. 2002; Ruiz-Cooley et al. 2010). Particularly in cephalopods, where semelparity greatly affects the physiology and behavior of individuals, the maturity condition of individuals may cause diet shifts and variation in the utilization of food sources, therefore affecting its trophic role in the ecosystem.

In the intertidal and shallow subtidal areas of North Patagonia, San Matías Gulf (41° S; Argentina), *Octopus tehuelchus* d'Orbigny supports a historic artisanal fishery with regional importance (Iribarne 1991; Narvarte et al. 2006). This small octopus (up to 150 g total weight) is an active and opportunistic predator, which feeds mainly on small mussels (e.g., *Brachidontes rodriguezii*, *Perumitilus purpuratus*), crabs (*Neohelice granulata*, *Corystoides chilensis* and *Rochinia gracilipes*), fishes, polychaetes, snails and small crustaceans (Iribarne et al. 1991; Ré and Gómez Simes 1992; Storero 2010). In addition, this species plays an important ecological role as prey for sea lions (Koen Alonso et al. 2000) and penguins (Forero et al. 2002). In this area, *O. tehuelchus* is found in two contrasting environments: Rocky substrates where natural shelters are highly

Responsible Editor: C. Harrod.

Reviewed by J. Newton and an undisclosed expert.

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available, and in muddy-sandy bottoms where shelters are scarce and mainly consist of empty shells of bivalves and gastropods and artificial shelters (Iribarne 1990; Narvarte et al. 2013). Octopuses in rocky and sandy substrates show differences in their life span and growth (Storero et al. 2010), size-frequency distribution (Storero et al. 2013a), reproductive traits (Storero et al. 2012) and densities (Storero et al. 2013b). These differences could be due to the annual cycle of seawater temperature, jointly with differences in the abundance and diversity of prey, influencing the growth rates and size at maturity of octopuses in different sites (Storero et al. 2012). However, predation pressure and/or high parasitic infection, which differentially affect the populations, may be further factors influencing the life history characteristics in each environment (Storero and Narvarte 2013). These factors and others (e.g., fishing pressure, shelter availability) acting together with the inherent life history of cephalopods were suggested as drivers of the population dynamics of *O. tehuelchus* in the Patagonian coast. Here, we hypothesize that octopus' diet differs between rocky and sandy shores, which may drive the observed variability in the life history characteristics of this species.

Previous knowledge of octopus diet in this environment is through direct observations or stomach content analysis (Iribarne et al. 1991; Ré and Gómez Simes 1992; Storero 2010). Although very useful, these methods have limitations and specific problems for cephalopods due to the absence or minimal amount of food in stomachs, the difficulty of identifying prey consumed without the hard parts, which are usually rejected (e.g., crustacean integument, skeleton), and the differences in digestibility among prey (Rodhouse and Nigmatullin 1996). To avoid these drawbacks, naturally occurring stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) are widely used in the evaluation of trophic relationships and the transport of organic matter along food chains (e.g., Peterson and Fry 1987, Michener and Kaufman 2007). Stable isotope analysis (hereafter SIA) is based on the premise that carbon and nitrogen isotope ratios of a consumer reflect the isotopic values of the prey consumed (DeNiro and Epstein 1978, 1981; Peterson and Fry 1987), providing information on diet integrated over a period of time (Tieszen et al. 1983). Previous experiments on turn over rates of muscle in squids showed that after 10 days of feeding, stable isotopes values tended to reflect those of their prey (Stowasser et al. 2006).

In this study, we use SIA to evaluate sources of variation in the diet of *O. tehuelchus* and prey utilization, in order to evaluate the stated hypothesis. Given that feeding shifts occur from juveniles to adults (Ré and Gómez Simes 1992; Rodhouse and Nigmatullin 1996; Cherel et al. 2009), and there are evidences that this species continue feeding (at a lower rate) after spawning (pers. obs.), we compared

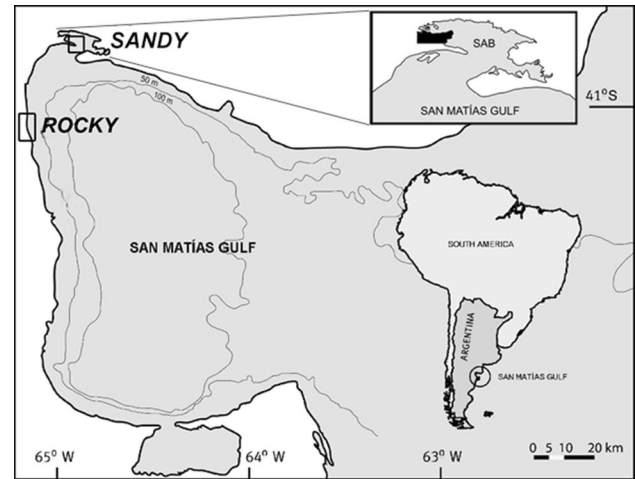


Fig. 1 Study sites in San Matías Gulf (Argentina). San Antonio Oeste city is marked in *black*. SAB San Antonio Bay

the isotope composition of *O. tehuelchus* of different sizes and maturity condition. Thus, the main objectives here are to: (1) evaluate diet differences in *O. tehuelchus*, due to sex and maturity condition (juvenile, mature and post-spawning) and (2) assess and compare the food sources associated to *O. tehuelchus* in two intertidal ecosystems (sandy vs. rocky) with contrasting environmental characteristics.

Materials and methods

Study site

The study was carried out in two intertidal areas of San Matías Gulf (40°–42° S, Fig. 1). The west coast of the gulf shows extended rocky intertidal shores and has been the traditional fishing area of *O. tehuelchus* since 1950s (Iribarne 1991; Narvarte et al. 1996). The rocky intertidal habitat (Fig. 1) is characterized by tidal pools with the small mussel *B. rodriguezii* and *P. purpuratus* as the dominant species, and the presence of various algae, crabs, echinoderms and gastropods (Narvarte et al. 2006). The area is regularly visited during spring/summer months by fishers who collect octopuses using a ~40-cm-long iron gaff or by turning over rocks (Iribarne 1991).

The sandy intertidal habitat is located 55 km northward, within San Antonio Bay in the north-west sector of San Matías Gulf. The bay is 160 km² in area and is a shallow water system with semidiurnal tides between 6 and 9 m in range. The bay is characterized by channels and extensive tidal flats, with sandy-pebbly bottoms, surrounded by salt marshes (Isacch et al. 2006; Carbone et al. 2007). The crab *Neohelice granulata* is the most conspicuous macrofaunal species in the exposed intertidal and forms extensive crab

beds. During low tides, people from the surrounding urban area collect octopuses from empty valves, shells and artificial shelters (usually bottles, pipes, bricks, etc.). In addition, this sandy habitat is influenced by the coastal city of San Antonio Oeste. High nutrient concentrations and macroalgae blooms have been reported near the city (Teichberg et al. 2010), and the discharge of wastewater from the city has been suggested as the source of land-derived nutrients which may drive to eutrophication in this site (Martinetto et al. 2010). Terrestrial-derived nitrogen is assimilated by macroalgae, which are enriched in ^{15}N (Martinetto et al. 2010).

Oceanographic characteristics also show important differences between sandy and rocky shores. The annual cycle of seawater temperature shows that San Antonio Bay has larger fluctuations, being 2–4 °C warmer (during summer) and colder (during winter) than the rocky intertidal (Storero et al. 2010).

Sampling and data analysis

In order to evaluate diet shifts and to identify the main items prey and trophic relationships of *O. tehuelchus*, we used SIA. Five samples of *O. tehuelchus* of different sex (female, male), in each maturity condition (juvenile, mature, post-spawning) were randomly collected in sandy and rocky habitats, with a total of 5×2 (sex) \times 3 (maturity condition) \times 2 (sites) = 60 samples. Samples of consumers and potential prey (five replicates of each prey) were collected simultaneously during spring-summer months in order to avoid variability between seasons. The prey species sampled are common species in the habitat of *O. tehuelchus* at both sites, and are cited as potential prey of this octopus (Iribarne et al. 1991; Ré and Gómez Simes 1992; Storero 2010). The prey species collected were: *Brachidontes rodriguezii*, *Tegula patagonica*, *Chaetopleura isabellei*, *Cyrtograpsus* sp., *Exosphaeroma* sp., *Siphonaria lessoni* (only in rocky), *Buccinanops globulosus* and *Neohelice granulata* (only in sandy). When individuals were too small for single measurements, aggregate samples including at least three individuals were made to ensure that each species was represented correctly and to obtain an adequate amount for the analysis. For all species, SIA was carried out using muscle samples. Before the analyses, octopuses were measured (dorsal mantle length—DML—, in mm, total mass—TW—in g) and sexed. Maturity condition was defined according to Pujals (1986) and Storero et al. (2012): Juvenile (<37 mm DML, immature gonads), Mature (\geq 37 mm DML, females with mature ovaries and large volume, males with visible spermatophores within the spermatophoric complex), Post-spawning (\geq 37 mm DML, flaccid gonads with gray-violet coloration). Shells were removed from mollusks and their muscle tissue dissected.

The claws of crabs were used for the analysis trying to remove most of the exoskeleton to prevent that the presence of chitin reach tissues influence the $\delta^{15}\text{N}$ composition of muscle tissue. Isopods and crabs were treated with 1 N HCl using the drop-by-drop technique to remove inorganic carbonates before the analysis.

Samples were dried at 60 °C for 48 h and then grounded into fine powder with mortar and pestle. Determination of C and N percentages and stable isotope analyses were performed by an isotope ratio mass spectrometer in the stable isotope facility of the University of California, Davis. Carbon and nitrogen stable isotopes ratios were expressed in δ notation as part per thousand (‰) according to the following relationship:

$$\delta X (\text{‰}) = \left[\left(R_{\text{sample}} / R_{\text{standard}} \right) - 1 \right] \times 1000$$

where X is ^{13}C or ^{15}N , and R_{sample} and R_{standard} are the $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$ ratios of the sample and the standard respectively (McKinney et al. 1950). The standard reference for carbon is Pee Dee Belemnite (PDB) and atmospheric N_2 for nitrogen. Laboratory internal standards used were G11 (Nylon), G13 (Bovine Liver), G17 (USGS-41 Glutamic Acid) and G9 (Glutamic Acid). Replicate measurements of internal laboratory standards indicate measurement standard deviations lower than 0.2 and 0.09 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively.

Regression analysis was used to examine trends of stable isotope composition with octopus size (TW). To evaluate differences in the diet of *O. tehuelchus* between sex and maturity condition, isotopic compositions were compared with ANOVA. Normal distribution and homogeneity of variances were checked before the analyses, and the Tukey's test was used for a posteriori comparisons (Zar 1999). Differences in the isotopic composition of each prey and octopus were compared between sites (sandy vs. rocky) with *t* test, after correcting for non-homogenous variances as necessary.

In order to estimate the proportional contribution of the potential sources to octopus diet a Bayesian stable isotope mixing model was used (MixSIAR, Stock and Semmens 2013). MixSIAR is a hierarchical version of the mixing model formulation that incorporates individual (and group-level) diet variability among predators. The MixSIAR GUI interface was used to analyze stable isotope data using the MixSIAR model framework (Parnell et al. 2013; Stock and Semmens 2013). The GUI and model code are written in the open source languages R (R Core Team 2013) and JAGS.

Site (rocky, sandy) and maturity condition (juvenile, mature, post-spawning) were used as main effect, considering condition within site. Individual effects were not included in the analysis. $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ of each individual octopus were used as mixture data. The mean and standard

deviation values of only five prey items (the small mussel *B. rodriguezii*, the gastropod *T. patagonica*, the chiton *C. isabellei*, the isopod *Exosphaeroma* sp., the crab *Cyrtograpsus* sp.) were used as source data, together with their C and N concentration data. Sources were selected because they appear to be the most likely prey based on their respective stable isotope compositions, considering their presence and relative abundance in both sites (Storero 2010), and previous dietary analysis by stomach contents (Ré and Gómez Simes 1992; Storero 2010). In addition, two species in rocky (*S. lessoni* and *B. rodriguezii*) were grouped together because they were not significantly different in their $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (t test, $p > 0.05$, Phillips et al. 2014). Discrimination values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were provided as 0.4 ± 1.3 and 3.4 ± 0.9 ‰ (mean \pm SD), respectively, following commonly accepted theoretical values (DeNiro and Epstein 1981; Post 2002), and similar isotopic discrimination measures for soft tissues in cuttlefish ($\Delta^{15}\text{N}$ 3.3 ‰, Hobson and Cherel 2006).

Given that no differences in lipid concentration among preys were expected, lipids were not extracted from tissues. However, given that C:N ratios were higher than 3.5 in three sources (see Table 3), which may indicate the presence of ^{13}C -depleted lipids, we applied on prey and predator the mathematical normalization of $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{norm}}$) suggested by Post et al. (2007) before running the MixSiar model. Markov Chain Monte Carlo parameters were set as follows, chain length = 300,000; burn in = 200,000; thin = 100, number of chains = 3. Trace plots and the result of Gelman-Rubin, Heidelberger-Welch and Geweke diagnostic tests were used to confirm that the model had converged, and the DIC (Deviance Information Criterion) was used to choose the best model.

Results

O. tehuetchus $\delta^{13}\text{C}$ values ranged between -18.6 and -13.9 ‰, and $\delta^{15}\text{N}$ values ranged between 13.5 and 16.1 ‰. The regression analysis did not show any relationship between $\delta^{13}\text{C}$ and log TW in either site (sandy $R^2 = 0.12$, $F = 3.95$, $P = 0.06$, rocky $R^2 = 0.014$, $F = 0.4$, $P = 0.53$). Similarly, there was no evidence for a relationship between $\delta^{15}\text{N}$ and log TW in neither site (sandy $R^2 = 0.03$, $F = 0.82$, $P = 0.37$, rocky $R^2 = 0.04$, $F = 1.11$, $P = 0.30$).

In rocky habitats $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not show differences between sexes, but differences were significant between maturity conditions (Table 1). Post-spawning octopuses were depleted in ^{13}C , and mature octopuses were depleted in ^{15}N (Table 1; Fig. 2). In sandy habitats, the interaction between sex and maturity condition was significant. Mature octopuses were depleted in ^{13}C , and

post-spawning octopuses were enriched in ^{15}N (Table 1; Fig. 2).

In sandy, $\delta^{13}\text{C}_{\text{norm}}$ of the organisms ranged between -19.6 and -5.3 ‰, and $\delta^{15}\text{N}$ ranged between 7.2 and 16.1 ‰. In rocky, $\delta^{13}\text{C}_{\text{norm}}$ ranged between -20.2 and -3.9 ‰, and $\delta^{15}\text{N}$ ranged between 7.5 and 14.8 ‰. The isotopic values of the organisms inhabiting rocky and sandy were significantly different (Table 2), but the crab *Cyrtograpsus* sp. did not show differences in $\delta^{15}\text{N}$ composition, and post-spawning octopuses did not differ in $\delta^{13}\text{C}$ between sites. Organisms in sandy were depleted in ^{13}C and enriched in ^{15}N with respect to rocky (Table 2; Fig. 3a, b). Although not compared statistically (due to lack of replicates in rocky), the isopod *Exosphaeroma* sp. also showed depleted ^{13}C values and enriched ^{15}N values in sandy.

An iso-space plot for the data (where the sources have been corrected by the trophic enrichment factors) is shown in Fig. 4. The model with the lowest DIC score was the one that considered hierarchically both factors, site and maturity condition, and no individual effect. The posterior density plot showed that site ($\sigma_{\text{Site}} = 1.55$) and condition ($\sigma_{\text{Condition}} = 0.94$) contribute about equally to the variation in diet of *O. tehuetchus*.

In both sites, the main prey was *B. rodriguezii* contributing more than 35 % to the diet. The remaining sources showed a lower proportion, but *C. isabellei* showed a higher contribution in rocky, and *Cyrtograpsus* sp. and *T. patagonica* showed a higher contribution in sandy when compared with rocky (Table 3).

The proportional contribution of the sources varied with the maturity condition of octopuses. The contribution of the chiton *C. isabellei* in the diet of mature octopuses was higher than the contribution in juvenile and post-spawning octopuses, while the isopod *Exosphaeroma* sp. showed a higher proportion in the diet of juvenile octopuses. Post-spawning octopuses fed mainly on the small mussel *B. rodriguezii* (Table 3).

Discussion

The results show that both the maturity condition of individuals and the habitat characteristics influence the food resources utilization by *O. tehuetchus* in Patagonian coasts. The isotopic composition of *O. tehuetchus* in both sites is within the range of values obtained by previous authors in the Patagonian coast (Forero et al. 2002; 2004), and to other coastal cephalopod species (Takai et al. 2000; Reñones et al. 2002). Diet shifts and increased trophic position related to cephalopods size are mentioned for different species (Ré and Gómez Simes 1992; Rodhouse and Nigmatullin 1996; Cherel and Hobson 2005; Cherel et al. 2009; Ruiz-Cooley et al. 2010). *O. tehuetchus* is a small octopus

Table 1 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean \pm SD) by maturity condition in rocky and sandy

			<i>df</i>	MS	<i>F</i>	<i>p</i> value
<i>Rocky</i>						
	$\delta^{13}\text{C}$					
Post	$-16.4 \pm 0.84\text{a}$	Sex	1	2.08	3.16	0.0879
Juvenile	$-15.4 \pm 0.76\text{b}$	Condition	2	6.07	9.23	0.0011
Mature	$-14.8 \pm 0.86\text{b}$	Interaction	2	0.23	0.35	0.7075
		Error	24	0.66		
	$\delta^{15}\text{N}$					
Mature	$13.9 \pm 0.39\text{a}$	Sex	1	0.28	2.72	0.1124
Post	$14.3 \pm 0.36\text{b}$	Condition	2	0.53	5.16	0.0137
Juvenile	$14.4 \pm 0.23\text{b}$	Interaction	2	0.14	1.37	0.2743
		Error	24	0.1		
	C:N					
Post	$3.4 \pm 0.05\text{a}$	Sex	1	0.00026	0.17	0.6851
Mature	$3.4 \pm 0.03\text{ab}$	Condition	2	0.01	4.93	0.0160
Juvenile	$3.5 \pm 0.04\text{b}$	Interaction	2	0.0019	1.22	0.3139
		Error	24	0.0015		
<i>Sandy</i>						
	$\delta^{13}\text{C}$					
Mature	-16.8 ± 1.51	Sex	1	0.12	1.13	0.2978
Post	-16.3 ± 0.90	Condition	2	0.93	8.62	0.0015
Juvenile	-16.2 ± 0.21	Interaction	2	8.46	78.76	<0.0001
		Error	24	0.11		
	$\delta^{15}\text{N}$					
Juvenile	15.2 ± 0.25	Sex	1	0.07	1.42	0.2455
Mature	15.4 ± 0.38	Condition	2	1.24	24.16	0.0001
Post	15.9 ± 0.19	Interaction	2	0.43	8.35	0.0018
		Error	24	0.05		
	C:N					
Mature	$3.4 \pm 0.03\text{a}$	Sex	1	0.0003	0.51	0.4836
Juvenile	$3.4 \pm 0.02\text{a}$	Condition	2	0.01	18.45	<0.0001
Post	$3.5 \pm 0.03\text{b}$	Interaction	2	0.0018	3.05	0.0661
		Error	24	0000.6		

Results of the two-way ANOVA for each of the variables with sex and maturity condition as main factors
Different letters indicate significant differences

with no larval stage, and after hatching the small benthic octopuses have habits similar to medium size and large octopuses, in accordance our analysis using stable isotopes did not show an increase in $\delta^{13}\text{C}$ nor $\delta^{15}\text{N}$ with body size. In addition, the isotopic composition of males and females was similar; this was recorded also in other cephalopod species (Takai et al. 2000) indicating similar diets between males and females.

Our results show that the maturity condition of octopuses has a high influence in their isotopic composition. Cephalopods intrinsic characteristics (i.e., short life span, semelparity, high growth rates and parental care) determine that the maturity condition of individuals affects the entire organism, influencing its physiology and several

biological features. Among other factors, octopus behavior (e.g., brooding females become less mobile and stay near the shelter) and feeding (e.g., individuals have the highest feeding rates before the reproductive season) change due to maturity condition, and these changes are reflected in their isotopic composition. Food deprivation may lead to enrichment of ^{15}N in body tissues (Michener and Lajtha 2007), which may explain the increased $\delta^{15}\text{N}$ of post-spawning octopuses. After reproducing, both males and females undergo senescence, which is a normal stage of an octopus life cycle, characterized by reduced feeding, loss of weight and uncoordinated movement, among others. During this stage of life, to provide energy to keep the eggs clean and oxygenated, and safe from predators, brooding

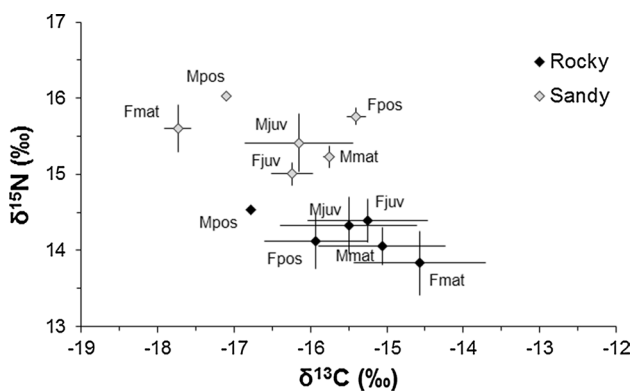


Fig. 2 Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (\pm SD) of *O. tehuilchus*. Fjuv, female juvenile; Fmat, female mature; Fpos, female post-spawning; Mjuv, male juvenile; Mmat, male mature; Mpos, male post-spawning

Table 2 Mean (\pm SD) and results of the *t* test ($\alpha = 0.05$) comparing the isotopic composition of organisms between sites (sandy vs. rocky)

	Rocky	Sandy	<i>t</i>	<i>df</i>	<i>p</i> value
<i>B. rodriguezii</i>					
$\delta^{13}\text{C}_{\text{norm}}$	-18.2 ± 0.05	-19.5 ± 0.07	32.83	8	<0.0001
$\delta^{15}\text{N}$	11.9 ± 0.08	11.4 ± 0.12	7.58	8	0.0001
C:N	4.1 ± 0.14	4.2 ± 0.9	-1.54	8	0.1613
<i>T. patagónica</i>					
$\delta^{13}\text{C}_{\text{norm}}$	-15.1 ± 0.25	-14.7 ± 0.25	-2.79	8	0.0234
$\delta^{15}\text{N}$	11.9 ± 0.09	13.9 ± 0.24	-17.67	8	<0.0001
C:N	3.5 ± 0.03	3.5 ± 0.04	0.03	8	0.9765
<i>C. isabellei</i>					
$\delta^{13}\text{C}$	-10.7 ± 0.89	-12.4 ± 0.83	3.15	8	0.0135
$\delta^{15}\text{N}$	9.6 ± 0.37	13.6 ± 0.40	-16.41	8	<0.0001
C:N	3.5 ± 0.08	3.5 ± 0.20	-0.01	8	0.9942
<i>Cyrtograpsus sp.</i>					
$\delta^{13}\text{C}_{\text{norm}}$	-10.6 ± 1.87	-15.6 ± 1.02	5.21	8	0.0008
$\delta^{15}\text{N}$	9.5 ± 0.94	10.5 ± 0.65	-1.86	8	0.1002
C:N	5.7 ± 1.01	3.9 ± 0.10	4.1	4	0.0149
<i>O. tehuilchus</i> Juvenile					
$\delta^{13}\text{C}$	-15.4 ± 0.76	-16.2 ± 0.21	3.28	10	0.0083
$\delta^{15}\text{N}$	14.4 ± 0.23	15.2 ± 0.25	-7.88	18	<0.0001
C:N	3.5 ± 0.04	3.4 ± 0.02	2.58	13	0.0228
<i>O. tehuilchus</i> Mature					
$\delta^{13}\text{C}$	-14.8 ± 0.86	-16.8 ± 1.51	4.25	18	0.0005
$\delta^{15}\text{N}$	13.9 ± 0.39	15.4 ± 0.38	-8.52	18	<0.0001
C:N	3.4 ± 0.03	3.4 ± 0.03	1.15	18	0.2647
<i>O. tehuilchus</i> Post-spawning					
$\delta^{13}\text{C}$	-16.4 ± 0.84	-16.3 ± 0.90	-0.27	18	0.7925
$\delta^{15}\text{N}$	14.3 ± 0.36	15.9 ± 0.19	-12.13	18	<0.0001
C:N	3.4 ± 0.05	3.5 ± 0.03	-3.29	18	0.0041

Significant differences ($P < 0.05$) are shown in bold

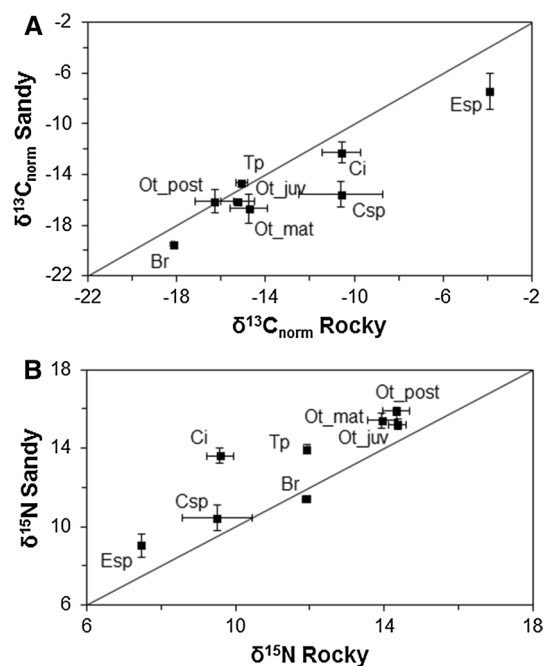


Fig. 3 a $\delta^{13}\text{C}_{\text{norm}}$ (mean \pm SD) of species in sandy versus rocky habitats. b $\delta^{15}\text{N}$ (mean \pm SD) of species in sandy versus rocky habitats. Ot_juv, *Octopus tehuilchus* juvenile; Ot_mat, *O. tehuilchus* mature; Ot_post, *O. tehuilchus* post-spawning; Tp, *Tegula patagónica*; Ci, *Chaetopleura isabellei*; Br, *Brachidontes rodriguezii*; Csp, *Cyrtograpsus sp.*; Esp, *Exosphaeroma sp.*

females metabolize muscle tissue, which keeps the metabolic rate at a high level (O’Dor and Wells 1978). Males also undergo senescence, which cause inactivation of the posterior salivary and digestive glands, and diminution of appetite, which normally causes death after reproduction (Anderson et al. 2002). Although we did not evaluate this issue directly, our results suggest that these processes and physiological changes in the life cycle of cephalopods are reflected in their isotopic composition and need to be considered in studies of their predators.

Although both coastal areas have environmental differences, mussels were the main prey of octopuses with smaller contributions of the remaining prey. This is interesting because this could indicate a preference of *O. tehuilchus*. In the sandy intertidal, mussels are less abundant and have a patchy distribution (pers. obs.) but still constituted a high proportion of the diet of this octopus. Thus, although described as a generalist predator with a wide trophic spectrum (Ré and Gómez Simes 1992), this octopus may have preferred items. The Bayesian mixing model indicated that contributions of the remaining prey are different in sandy and rocky habitats. This may be related to the availability of prey in each site, but also to the presence

Fig. 4 $\delta^{13}\text{C}_{\text{norm}}$ and $\delta^{15}\text{N}$ values for *O. tehuelchus* and sources (mean \pm SD) in the food web of sandy and rocky habitats. Tp, *Tegula patagónica*; Ci, *Chaetopleura isabellei*; Br, *Brachidontes rodriguezii*; Csp, *Cyrtograpsus* sp.; Esp, *Exosphaeroma* sp. Sources corrected by the trophic enrichment factors

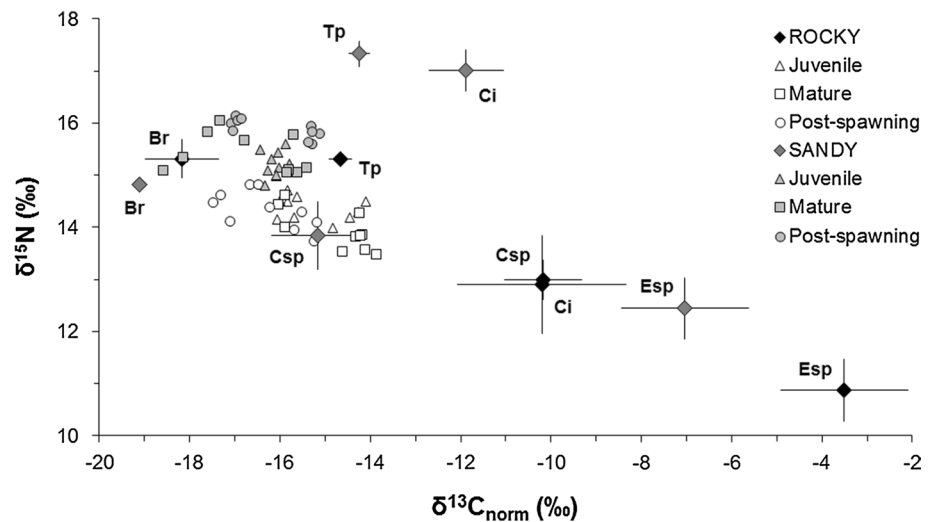


Table 3 Proportional contribution (50 % quantiles) of the potential sources to *O. tehuelchus* diet in two sites

	<i>B. rodriguezii</i>	<i>Cyrtograpsus</i> sp.	<i>C. isabellei</i>	<i>Exosphaeroma</i> sp.	<i>T. patagónica</i>
Rocky	0.495 (0.139–0.830)	0.072 (0.001–0.617)	0.275 (0.004–0.675)	0.027 (0.001–0.265)	0.010 (0.001–0.154)
Sandy	0.377 (0.095–0.764)	0.162 (0.001–0.625)	0.079 (0.001–0.469)	0.087 (0.001–0.390)	0.146 (0.006–0.445)
Juvenile	0.539 (0.376–0.647)	0.089 (0.001–0.546)	0.232 (0.003–0.427)	0.062 (0.001–0.288)	0.005 (0.001–0.062)
Mature	0.460 (0.24–0.563)	0.055 (0.001–0.711)	0.406 (0.001–0.541)	0.007 (0.001–0.092)	0.003 (0.001–0.060)
Post-spawning	0.617 (0.413–0.724)	0.031 (0.001–0.530)	0.267 (0.003–0.373)	0.013 (0.001–0.112)	0.016 (0.001–0.127)

95 % Credible intervals (Bayesian credible intervals) in parenthesis

and abundance of shelters and the behavior of octopuses. In the sandy-pebbly bottoms of San Antonio Bay shelters for *O. tehuelchus* are scarce, and individuals remain buried or hidden under empty shells of mollusks, rocks or artificial refuges. In consequence, octopuses may display an opportunistic behavior, consuming the available prey in their surrounds. In the rocky intertidal, shelters are highly abundant and usually nearby large mussel beds, which are the main prey of this octopus. Since the limitations of the sampling procedure can affect isotope signatures (i.e., acid treatment for carbonate extraction, Mateo et al. 2008; Schlacher and Connolly 2014; and ^{13}C -depleted lipid contents, Post et al. 2007; Phillips et al. 2014) proportions of the different items to the diet of octopuses must be considered for comparative purposes and not as exact estimates of diet.

The variation in food source utilization in the two coastal areas furthers our understanding of trophic relationships of *O. tehuelchus*. In coastal ecosystems, local stressors may have a strong influence in the population characteristics of octopuses, and phenotypic plasticity in response to environmental variability is one of the main characteristics of cephalopod's life history (Forsythe et al. 2001;

Jackson et al. 2007). Thus, the environmental characteristics jointly with differences in the proportions of alternative prey in the diet may explain the variations in the structure and dynamics of *O. tehuelchus* populations in San Matías Gulf (Storero et al. 2010, 2012, 2013a). In this sense, differential food source utilization could be related to differential contribution of energy from prey, and it could explain the differences in the growth pattern and life span, as well as in the reproductive traits and the recruitment success of *O. tehuelchus* in the sandy and rocky intertidals.

In both sites, trophic relationships are correlated to the maturity condition of octopuses. Mature octopuses include in their diet a larger proportion of chitons, and juveniles feed on a higher proportion of small isopods but they also include crabs and chitons in their diet. Probably due to juvenile's high growth rates and active feeding, the small and highly abundant isopods that live beneath rocks and crevices, constituted their preferred prey (considering prey handling time and maximizing energy). Previous dietary studies mentioned *Exosphaeroma* sp. as the main item prey for juveniles of *O. tehuelchus* (Ré and Gómez Simes 1992). On the contrary, Post-spawning octopuses,

which are known to have reduced feeding, feed mainly on the sessile and abundant mussels and the remaining prey do not contribute substantially. Within- and among populations trophic variation are generally little understood due to the limited ability of gut content analysis to elucidate feeding patterns (Vander Zanden et al. 2000). Using SIA, we showed here that within-population trophic variation in cephalopods (i.e., due to maturity condition) need to be considered when evaluating and comparing variation among sites. Differences in octopus diet (due to maturity condition or habitat characteristics) may in turn affect not only the dynamics of populations, as mentioned before, but also the species trophic niches and its role in communities. In San Matías Gulf, *O. tehuelchus* is found in high densities in the intertidal and shallow subtidal areas (Iribarne 1991; Storero et al. 2013b). Octopuses are active predators able to consume a large number of prey, affecting the structure of communities (Ambrose 1986). Thus, the observed variation in food sources utilization jointly with the high densities of octopuses suggests that the species may be playing an important role as predator in the intertidal ecosystems of San Matías Gulf.

The isotopic values determined in this study are, in some way, close to those found in previous studies for organisms in the rocky intertidal of North Patagonia (Forero et al. 2002, 2004; Martinetto et al. 2011). However, our results also highlight the importance of site-specific effects as we discuss hereafter. In sandy, the species were depleted in ^{13}C and enriched in ^{15}N with respect to rocky. These results suggest that several processes may be playing major roles structuring the ecological communities in the San Antonio Bay ecosystem, and different hypotheses may be proposed to explain this pattern.

1. Land-derived nutrients from the coastal city of San Antonio Oeste have been suggested as drivers of the eutrophication in San Antonio Bay. Primary producers assimilate these nutrients (Teichberg et al. 2010; Martinetto et al. 2010; 2011), which ultimately results in consumers having higher $\delta^{15}\text{N}$ values. We were not able to obtain ^{15}N values of primary producers in the study areas. However, previous experiments in San Antonio Bay reported high $\delta^{15}\text{N}$ values for macroalgae located near the city (Martinetto et al. 2011). Other studies have also shown that cephalopods may reflect in their isotopic composition the enriched ^{15}N from the wastewater inflow (Takai et al. 2002).
2. The presence of the bioturbator crab *N. granulata* may affect the direction and magnitude of nutrient benthic fluxes (Botto et al. 2005, 2006; Fanjul et al. 2011). Crab burrows may stimulate denitrification, which increases baseline $\delta^{15}\text{N}$ (Botto et al. 2005), and con-

sequently species inhabiting San Antonio Bay could show ^{15}N enriched values.

3. Benthic invertebrates may rely on a larger variety of organic matter sources (e.g., marsh grasses, plankton, benthic algae, chemosynthetic and photosynthetic bacteria, and detritus). Thus, if a variety of primary producers influences the baseline signal, it is transmitted along the food chain, and may explain the different isotopic composition of species between sites.

In summary, there are differences between sites and our evidences suggest that the described hypotheses contribute to produce the observed pattern, potentially affecting the San Antonio Bay food web at different trophic levels.

Acknowledgments LPS thanks “Suncho” Fidel and “Cacho” Montenegro for collecting octopuses, Sandro Acosta and Nestor Dieu for technical assistance, and Matías Ocampo Reinaldo for comments on the manuscript. Authors acknowledge the helpful suggestions of reviewers. This work was funded by CONICET (PIP 2012-424 to OI) and ANPCyT (PICT 2013-1926 to LPS and PICT 2012-042 to OOI). LPS, FB, MAN, OOI acknowledge financial support from CONICET (Argentina).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Human and animal rights All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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