Stable isotopes reveal different dependencies on benthic and pelagic pathways between *Munida gregaria* ecotypes

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A B S T R A C T

Forage species are key nodes in the energy fluxes between benthic and pelagic zones. The squat lobster, *Munida gregaria*, is a common forage species of the Southern Hemisphere and its abundance is increasing. This species presents two ecotypes: one (*subrugosa*) that settles on the sea bottom immediately after the metamorphosis and another (*gregaria*) that remains in the water column for a variable period of time, before adopting a benthic habit. Hypotheses on its settlement are conflicting; one proposes that it is a density-dependent process of the sea bottom and the other that it is regulated by the availability of pelagic food. To rule out whether feeding factors could be significant in the decision-making process, the first step is to identify whether the ecotypes have different diets or not. This study compared the isotopic niche location, the trophic levels and the reliance on benthic or pelagic resources between ecotypes. Among all sampling events, the isotopic niches of the *gregaria* and the *subrugosa* ecotypes were significantly distant from each other. In addition, the difference in $\delta^{13}C$ values between ecotypes covered an average of 75% of the total difference between pelagic and benthic pathways. Benthic individuals relied mostly on benthic animals, while pelagic individuals relied on pelagic primary producers. Overall, results show that each ecotype feeds mainly on different energy pathways. Considering its feeding ecology and movements during life history, *M. gregaria* is a key component of bentho-pelagic coupling.

1. Introduction

Subtidal marine habitats are vertically divided into pelagic and benthic domains. These zones are connected via vertical processes, such as deposition of organic matter, upwelling of nutrients, and daily migration of organisms (Graf, 1992). Bentho-pelagic coupling is the umbrella term applied to describe all causal relationships (distinct to simple correlations) between these two domains (Smith et al., 2006). Forage species that form dense aggregations, feed on plankton, and are consumed by demersal predators are key actors of the bentho-pelagic coupling. Therefore, these species play an important role in marine food webs by transferring the energy of primary production events to top predators (Smith et al., 2011) all along the vertical distribution of benthic and pelagic zones. Likewise, population dynamics of forage species affect processes that go from the regulation of commercially important species (Pikitch et al., 2014) to massive oceanographic events (Carscadden et al., 2001).

The squat lobster *Munida gregaria* (Fabricius, 1793) is an important forage species of the Southern Hemisphere, commonly found in New Zealand, Chile, and Argentina. In Argentina, it is a major component of the marine animal biomass, with average densities of 4 tons mn^{-2} reported for San Jorge Gulf (Ravalli et al., 2013), and is observed from Buenos Aires (34° S) to Cabo De Hornos (56° S) (Boschi et al., 1992). Over the last decade the abundance of this species has grown on the Argentinian shelf, where acoustic studies detected an increase of densities in the water column (Diez et al., 2016a), and its biomass increased in benthic shrimp (*Pleoticus muelleri*) catches (Ravalli et al., 2013). This increment in abundance impacted on operational issues in the shrimp-fishery sector, hindering the access to the stocks (De la Garza et al., 2011), displacing shrimps' stock location (Roux et al., 2012), and changing catch composition (Ravalli et al., 2013). In terms of trophic dynamics, it had a major brunt on the diet of the Argentine hake *Merluccius hubbsi*, the most important finfish fishery resource of the Patagonian shelf. The hake has increased the consumption of *M. gregaria* up to 100% in several areas (Belleggia et al., 2017). Therefore, a better understanding of the main sources subsidizing the development of the '*M. gregaria*' population is crucial in this system.

This squat lobster presents two different morphotypes, "*gregaria*" and "*subrugosa*", with morphological differences in several features of the carapace, the third maxilliped, the eyestalk, and the mandible (Chilton, 1909; Rayner, 1935; Tapella and Lovrich, 2006; Williams, 1973). The differentiation between morphotypes occurs during larval

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development and is accompanied by a shift in their life history (Rayner, 1935). After the megalopa stage, while the *subrugosa* morphotype settles on the benthos directly and remains there for its entire life, the *gregaria* morphotype aggregates in swarms on the water column and settles later in the juvenile stage or first adult stages. The duration of the pelagic period for *gregaria* is supposed to be variable, described to be as long as the summer season (Zeldis, 1985). However, recent observations suggest the pelagic phase is longer in Argentina, since one- or two-year-old adults were found in the water column (Diez et al., 2012, 2016a) and pelagic swarms are detected throughout the year in Patagonia. Both *subrugosa* and *gregaria* morphotypes of this squat lobster show no genetic differentiation despite their pronounced bimodal morphologies and different life styles (Pérez-Barros et al., 2008; Wang et al., 2016) and represent two different ecotypes of this species (see Engelhard et al., 2011; Turesson, 1922 for ecotype definition).

Factors controlling the duration of the pelagic period are under discussion, but based on the available information, two hypotheses are the most widely supported: (1) As the species presents agonistic behavior, which is intensified at higher densities (Thiel and Lovrich, 2011), settlement is a density dependent process and higher abundance of benthic individuals triggers the development of the pelagic ones (Zeldis, 1985); and (2) the permanence of individuals in the water column is regulated by the availability of food (Varisco and Vinuesa, 2010; Williams, 1973), and hence, more successful phytoplankton production events are expected to generate bigger swarms, independently of the benthic densities.

Diez et al. (2012) described the swarm's vertical movements along the water column using hydroacoustic surveys and identified aggregations of *gregaria* both next to the bottom and at the surface. They suggested that vertical movements respond to feeding purposes, where individuals could be swimming down to eat on the sea bottom but would remain at the water column later due to the density of benthic individuals. A prediction derived from the first hypothesis and Diez et al. (2012) observation is that individuals from both origins share benthic food sources, and their diets are mainly overlapped. In contrast, the second hypothesis predicts that pelagic and benthic individuals should have distinct diets feeding in different pathways. In this scenario, the growth of *gregaria* ecotype population would be mostly subsidized by the pelagic pathways. Therefore, it would be important to rule out if the diet of the *gregaria* ecotype overlaps with the diet of the *subrugosa* ecotype.

The diet of *M. gregaria* was studied using gut content analysis on the Beagle Channel and San Jorge Gulf, locations that are 1000 km apart. Benthic individuals from the Beagle Channel fed on crustaceans, worms, macroalgae, sediment, and particulate organic matter (Romero et al., 2004). There, pelagic individuals presented similar prey items, but they also contained unicellular algae (Diez et al., 2012). Analogous results were found in the stomach contents of benthic individuals from the San Jorge Gulf (Vinuesa and Varisco, 2007), but for this area, there are records of consumption of fishery discards (Varisco and Vinuesa, 2007; Vinuesa and Varisco, 2007). On the other hand, pelagic individuals from San Jorge Gulf had planktonic components as dominant items in their stomach contents, such as copepods and dinoflagellates (Varisco and Vinuesa, 2010). This matches what is described for pelagic individuals of New Zealand, which graze on harmful dinoflagellates in significant amounts (MacKenzie and Harwood, 2014). However, until now, diet comparison between ecotypes based on stomach contents have not been done at the same location and time.

Stomach content analysis strongly depends on time and place of capture, reflecting only undigested items at the moment of capture. Not only does it detect a fraction of the diet, but it also overestimates food items that are more difficult to digest. In addition, not everything in the stomach is incorporated into the animal. Stable Isotope Analysis (SIA) is broadly applied to describe food habits in marine ecology and integrates digested items over longer amounts of time. The diet between morphotypes was compared at the Beagle Channel using SIA

(Pérez-Barros et al., 2010). However, all samples came from the benthos since individuals were captured using trawl nets. Then, feeding ecology of the *gregaria* morphotype at its pelagic phase remains unstudied.

The northern Patagonian shelf is a highly productive area where industrial and artisanal fisheries overlap with tourist and recreational activities. Coasts of the Patagonian gulfs are breeding sites for several species of marine mammals and seabirds (Yorio, 2009), which also includes both nursery and feeding grounds for fish and invertebrates of commercial importance like hakes, anchovies; *Engraulis anchoita*, and shrimps. Particularly at San Jorge Gulf, two important bottom-trawling fisheries take place; the industrial fishery of the shrimp, the most important crustacean fishery of Western South America, and the industrial fishery of the hake, which is the principal finfish fishery of the country (Góngora et al., 2012). Both fisheries discard thousands of tons of by-catch every year, including *M. gregaria*. In contrast, there is no fishing activity at Nuevo Gulf.

At this system, the squat lobster represents a major component of the pelagic and benthic biomass, it is important to correctly identify the nutritional pathways of each ecotype, which would contribute to pinpointing factors controlling the pelagic phase. The main goal of the present study was to test the isotopic niche location of co-occurring pelagic and benthic individuals of *M. gregaria* and estimate the contribution of pelagic and benthic energetic pathways to their diets at several locations of the Patagonian shelf.

2. Materials and methods

The study was conducted in the northern sea of Patagonia, Argentina. Four sites were located at San Jorge Gulf and one at Nuevo Gulf (Fig. 1 & Table 1). Sampling events occurred in three different seasons throughout 2016–2017. Sampling event 1 took place in summer of 2016 in the north western coast of San Jorge Gulf; events 2, 3 and 4 took place the following spring, sampling south western coast, the center and north eastern coast of San Jorge Gulf; and event 5 occurred the following autumn 2017 in Nuevo Gulf (Fig. 1).

Values of δ^{13}C and δ^{15}N were used to describe and compare the diet of the two ecotypes of *M. gregaria* in terms of their trophic position, isotopic niche location, and the degree of association with benthic or pelagic pathways. In comparative studies using SIA, isotopic baselines are a necessary reference of each isotopic value to the main trophic pathways of its local environment. Achieving the correct estimation and differentiation of pelagic and benthic baselines is logistically and analytically challenging. Primary consumers are the best choice to build isotopic baselines because they integrate longer and more important temporal variations in a marine ecosystem, such as phytoplankton blooms (Jennings and Warr, 2003). Bivalves were used to estimate the benthic baseline, since these filter feeders use a combination of phytoplankton and associated material that supports benthic production (Jennings and Warr, 2003). Zooplankton smaller than 300 μm were used to estimate the pelagic baseline, as in Badalamenti et al. (2008), since this fraction is composed mainly of small herbivore copepods (Pérez Seijas et al., 1987; Giménez, 2018). The combination of pelagic grazers (zooplankton) and benthic filter feeders (bivalves) was successfully used to estimate the reliance on benthic/pelagic affinity prey by Le Loc'h et al. (2008). Then, at each sampling location, a sample set was built with individuals of both ecotypes and samples of primary consumers from both pelagic and benthic pathways. To minimize temporal variation within each sample set, all the components were collected the same day. Plankton nets (mesh size 160 μm) were used to capture pelagic individuals of *M. gregaria* and zooplankton. Fresh samples of zooplankton were split by size into two subsamples (<300 μm and >300 μm). Benthic organisms were collected with a small beam trawl (~0.6 m horizontal opening) and sorted fresh. Species composition of bivalves was different at each sampling location, but consensus was possible by selecting species with the same epifaunal habit and size range (Table 1). All squat lobsters of the study were adults (cephalothorax
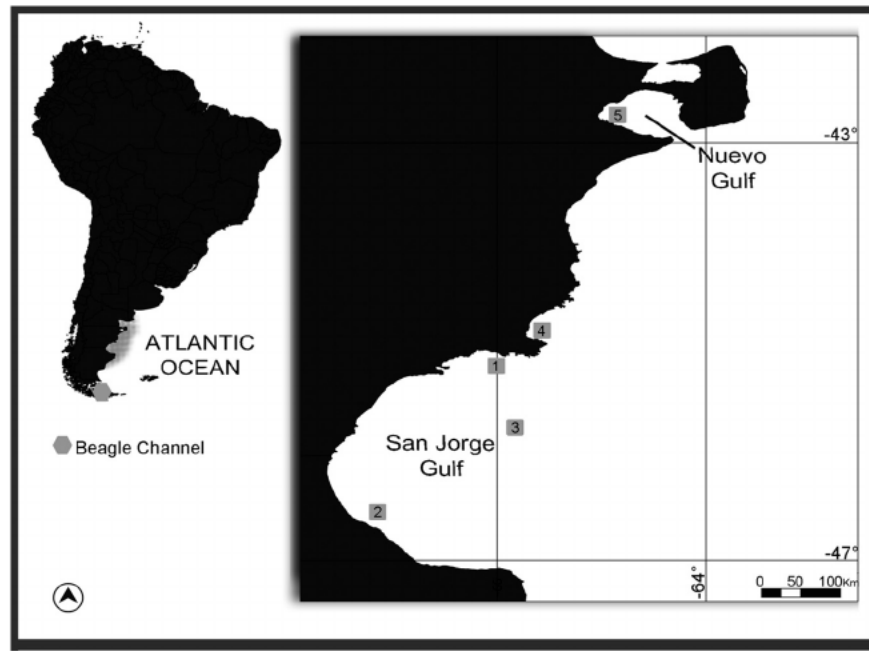
Fig. 1. Study site location. Sampling events are noted by numbers in order of occurrence = N° 1: January 2015; N° 2, 3, 4: November 2016; N° 5: March 2017. The Beagle Channel (frequently mentioned as an antecedent) is noted by a hexagon.

length > 13 mm) and samples were frozen immediately after collection. Back at the laboratory, samples were assigned to each morph, ensuring all benthic samples used were only *subrugosa* morph, and the pelagic samples were only *gregaria* morph. Individuals were dissected selecting muscle tissue. Muscle samples and zooplankton samples were dried for 72 h at 60 °C, converted into a fine powder and ≈1.25 μg per sample were sent for stable isotope determination, without applying any treatment. Sampling event n° 1 was analyzed at the Isotope Facility at California Davis University, and later sampling events, n° 2, 3, 4, 5 were analyzed at the Stable Isotopes Center of the University of New Mexico. Internal standards used in Davies were G-11 nylon, G-13 bovine liver, G-17 USGS-41 glutamic acid, and G-9 glutamic acid. Mean SDs of reference materials were 0.11‰ and 0.20‰ for δ^{13}C and δ^{15}N values respectively. For samples analyzed at the Stable Isotopes Center of the University of New Mexico, internal standards were soy, tuna and whey protein, casein, IAEA N1, IAEA N2, USGS 42 and USGS 43. Mean SDs of reference materials were 0.03‰ and 0.08‰ for δ^{13}C and δ^{15}N values respectively.

Isotope niche location were compared following the hypothesis-testing framework proposed by Turner et al. (2010) in which centroid locations of both ecotypes are studied using nested linear models and residual permutation procedures (see Turner et al., 2010 for statistical details). As centroids are the mean values of a group, their location represents the core of the aggrupation. Therefore, if distances between groups are statistically significant, a difference between the diets of each group could be assumed. Secondly, samples of all sites were grouped, and pelagic and benthonic niches were compared using centroid distances and testing the overlap between their standard ellipses (Jackson et al., 2011). Standard ellipses describe the bivariate variability in the isotopic space and the overlap between the ellipses of both groups was interpreted as the overlap between their isotopic niches (Jackson et al., 2011).

The contribution of the pelagic and the benthic energetic pathways to the nutrition of each ecotype (reliance on benthic/pelagic affinity prey) was estimated by solving a mixing model with two sources (e.g. Melville and Connolly, 2005).

$$\delta^{x}X_{consumer} - \Delta^{x}X = \sum f_{source\ i}\delta^{x}X_{source\ i} \qquad (1)$$

and

$$\sum f_{source\ i} = 1 \qquad (2)$$

The trophic enrichment factors (TEF) should be the trophic discrimination values (Δ^{15}N and Δ^{13}C) multiplied by the difference between the trophic level (TL) of the consumer and the TL of the organisms used to estimate the isotopic baselines (λ).

$$TEF = \Delta^{x}X \left(TL_{consumer} - TL_{baseline}\right) \qquad (3)$$

Table 1
General information of sample collection events. Sampling events are arranged in columns in consecutive order. Rows show sampling date, location name, coordinates (lat/long), depth and the species used to estimate the benthic baseline.

Sampling event	1		2		3		4		5	
Date	January 2016		November 2016		November 2016		November 2016		March 2017	
Location (lat/long)	North West GSJ		South West GSJ		Center East GSJ		North East GSJ		Golfo Nuevo	
	45.04	65.78	46.90	65.71	46.62	65.71	45.91	65.46	42.76	65.01
Depth (m)	27		72		96		74		25	
Benthic baseline species	*Aequipecten tehuelchus*		*Neilonella sulculata*		*Malletia cumingii*		*Pitar rostratus*		*Diplodonta patagonica*	

The TL of each consumer can be estimated following the equations by Post (2002a):

$$TL_{consumer} = \lambda$$

$$+ \left[\delta^{15}N_{consumer} - \left(\alpha \; \delta^{15}N_{benthic\;baseline} + (1-\alpha) \; \delta^{15}N_{pelagic\;baseline}\right)\right] / \Delta^{15}N$$

$$(4)$$

where α is the proportion ultimately derived from the base of the benthic pathway (Post, 2002a). Given the proportional input α is in the equation for TL, and TL is in the equation of the mixing model, there is circular reasoning without mathematical solution. Therefore, we built an iterative routine to calculate feasible values of TL and α (Post, 2002a). To estimate the feasible values of $TL_{consumer}$, we performed an iterative procedure slightly modifying the routine developed to evaluate the model outright for mixing models (Smith et al., 2013). In Eq. (3) $TL_{consumer}$ varied from 2 to 3.5 (by 0.1 TL increments) and consumers were tested to satisfy the point in the polygon assumption (Smith et al., 2013, example of outcome in Fig. 2). To identify the feasible values of TL of each ecotype, we estimated the probable contributions of each pathway using the Bayesian model SIAR (Parnell et al., 2010). In the absence of specific $\Delta^{15}N$ and $\Delta^{13}C$ values for *M. gregaria*, we used an approximation derived from values of other marine decapods. Literature informed $\Delta^{15}N$ values ranging from 2.4‰ (*Litopenaeus vannamei* Parker et al., 1989) to 3.6‰ (*Nihonotrypaea japonica* and *N. harmandi* Yokoyama et al., 2005). Thus, we used the general value of $\Delta^{15}N = 3$‰. On the other hand, informed $\Delta^{13}C$ values were higher than the general value $\Delta^{13}C = 1$‰, ranging from 1.7‰ (*Litopenaeus vannamei* Parker et al., 1989) to 2.2‰ (*Nihonotrypaea japonica* and *N. harmandi* Yokoyama et al., 2005); then, we ran the iterative procedure for three possible values: 1, 1.5, and 2‰. Uncertainty around eTEF was set at

0.5‰ and around sources at 0.3‰. All calculations and iterative procedures were written and performed in the R language (version 3.4.3, R Development Core Team, 2017).

3. Results

A total of 58 samples of squat lobster were analyzed, with 4 or 5 individuals per ecotype per station. Meanwhile, a minimum of three samples per of zooplankton and bivalves were used to construct pelagic and isotopic baseline. Zooplankton and bivalves $\delta^{13}C$ values showed enough distance to allow possible differentiations between pelagic and benthic pathways (mean difference of 3.5‰) (Table 2). Similarly, pelagic and benthic squat lobsters presented a mean difference of 2.5‰ in their $\delta^{13}C$ values. *Gregaria* ecotype (pelagic) varied between −17.4 and −19.2‰, while zooplankton signature did so between −19.2 and −21.8‰. On the other hand, *subrugosa* ecotype (benthic) presented higher $\delta^{13}C$ values, a more restricted range (from −15 to −16‰) and higher proximity to the benthic baseline values (−15 to −17.9‰; Table 2; See scheme in Fig. 3). Considering both C and N isotopic composition, results showed that in both gulf all along the sampling events, benthic and pelagic individuals of *M. gregaria* occupied different locations at the bivariate isotopic space (Fig. 4a, b, c, d, e; Table 2). Centroids between ecotypes were located significantly distant from each other (all p values < 0.005). In addition, data grouped by ecotype also presented similar values among groups, which could be split into two main domains of pelagic and benthic individuals, ellipses of which did not overlap, and centroids were again located significantly distant from each other ($p = 0.001$, Fig. 4f; Table 2). Then, the described pattern was constant throughout gulfs, deeps and seasons.

Results showed that the specific value for decapods, $\Delta^{13}C = 2$‰ was the best choice to satisfy the point in polygon assumption of the mixing
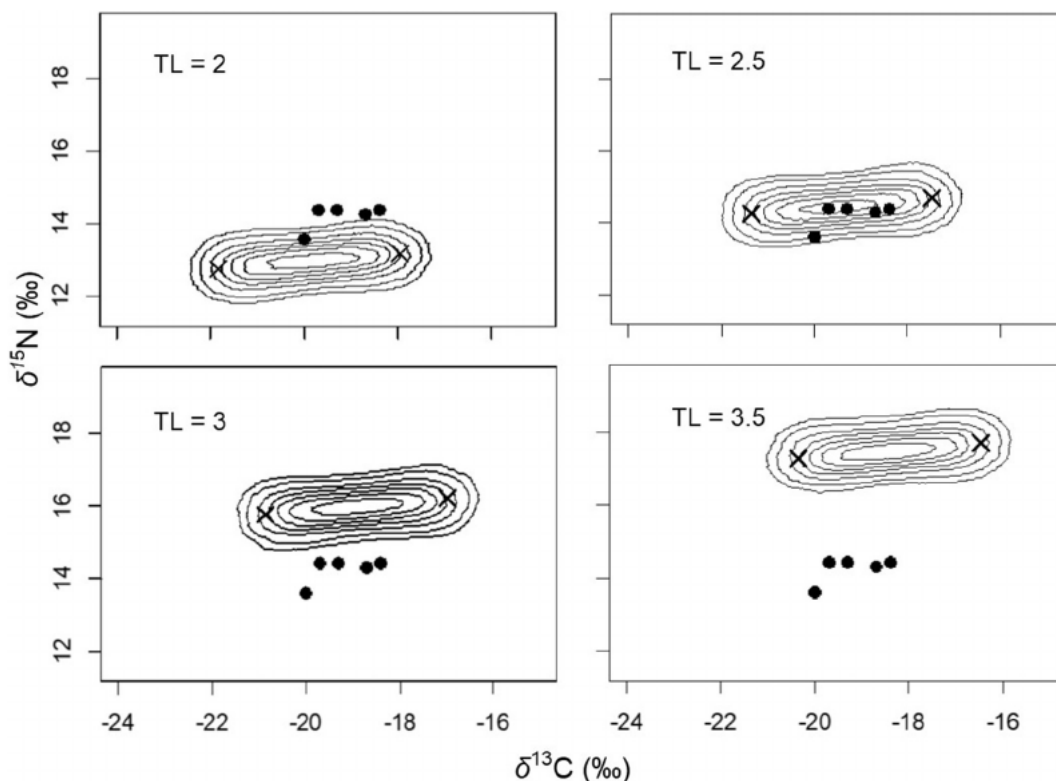

Fig. 2. Example of the iterative routine used to estimate feasible trophic levels (TL). Black dots show *Munida gregaria* isotopic composition; crosses show pelagic and benthic mean baselines corrected by the effective TEF, and lines are the probability contours which indicate how often a mixing polygon enclosed an area. Models with any consumer outside the outermost contour (the 5% contour), do not satisfy the "point in polygon" assumption (see Smith et al., 2013). Panels show four of the iterations performed for *gregaria* ecotype in site 5 (Nuevo Gulf). Model with TL = 2.5 satisfied "point in polygon" assumption while TL = 2, 3 and 3.5 do not.

Table 2
Stable isotopes composition per group. Columns contain sampling size (n), $\delta^{15}N$ & $\delta^{13}C$ mean values, standard deviation (SD) and the ratio of total Carbon/Nitrogen composition (C:N) per group of zooplankton, bivalves and *Munida gregaria* ecotypes. Total difference between pelagic and benthic pathways, and between *gregaria* (pelagic) and *subrugosa* (benthic) ecotypes of *M. gregaria* are expressed in absolute values.

Sampling event	1						2						3						4						5					
	n	$\delta^{15}N$ (‰)	SD	$\delta^{13}C$ (‰)	SD	C:N	n	$\delta^{15}N$ (‰)	SD	$\delta^{13}C$ (‰)	SD	C:N	n	$\delta^{15}N$ (‰)	SD	$\delta^{13}C$ (‰)	SD	C:N	n	$\delta^{15}N$ (‰)	SD	$\delta^{13}C$ (‰)	SD	C:N	n	$\delta^{15}N$ (‰)	SD	$\delta^{13}C$ (‰)	SD	C:N
Zooplankton	6	12.0	0.2	−21.7	0.7	4.0	3	10.8	0.7	−18.2	0.1	3.9	3	12.8	0.1	−19.8	0.1	3.8	3	11.6	0.1	−19.5	0.1	4.0	3	12.7	0.7	−21.8	0.7	3.9
Bivalves	3	13.4	0.1	−17.8	0.9	3.3	3	14.2	0.9	−16.1	0.6	3.4	3	13.7	0.6	−15.4	0.2	3.7	3	13.1	0.1	−16.3	0.4	4.0	3	13.1	0.3	−17.9	0.4	3.7
Gregaria Ecotype	6	13.6	0.2	−18.8	0.3	4.0	4	11.9	0.4	−17.8	0.3	3.5	5	12.9	0.3	−17.4	0.2	3.6	4	12.7	0.5	−17.6	0.3	3.4	5	14.2	0.3	−19.2	0.6	3.6
Subrugosa Ecotype	15	15.6	0.3	−16	0.6	3.7	5	14.1	1.3	−15.9	0.6	3.2	4	15.6	0.6	−15.4	0.2	3.1	5	14.9	0.3	−15	0.2	3.2	5	15.7	0.5	−15.8	0.8	3.3
Difference of pathways		1.4		3.8				3.4		2.1				0.9		4.4				1.5		3.2				0.4		3.9		
Difference of ecotypes		2.0		2.8				2.2		1.9				2.7		2.0				2.2		2.6				1.5		3.4		

model. The TL of the *gregaria* ecotype was lower than the TL of the *subrugosa* ecotype (Table 3). The TL of the *gregaria* ecotype varied between TL = 2 and TL = 2.6, while the TL of the *subrugosa* ecotype varied from TL = 2.4 to TL = 3. Contributions of the pelagic and benthic pathways to the nutrition of the sampled individuals were different for both ecotypes. The *gregaria* ecotype (pelagic) consumed resources of both energetic pathways (Table 2), although the pelagic pathways contributed more (from 40% to 87%) than the benthic (from 13% to 60%). The nutrition of the *subrugosa* ecotype (benthic) relied mostly on benthic resources (Table 3), and the pelagic pathway was important (higher than 40%) only in two extreme cases of the range of feasible contributions (Site 2 with TL = 2.7 and Site 3 with TL = 3).

4. Discussion

Across all samples, pelagic and benthic pathways showed important differences in $\delta^{13}C$ values, which allowed an easy discrimination of their contributions to the diet of both ecotypes. Overall, the isotopic niches of pelagic and benthic individuals of *M. gregaria* resulted different. The present results are the first evidence of a trophic difference in the isotope ratios of co-occurring individuals of each ecotype. In all sampling events, the *gregaria* ecotype showed lower $\delta^{13}C$ values; moreover, the difference in the mean isotope ratios of the ecotypes followed the differences between the pelagic and benthic pathways (Fig. 4). In addition, the $\delta^{15}N$ values of the *gregaria* ecotype were always lower than their benthic conspecifics, and closer to TL = 2. Thus, pelagic microalgae are likely to be the main food item of the *gregaria* ecotype, while the benthic group seems to be feeding mostly on detritus and benthic secondary consumers.

The results presented here do not support the hypothesis of pelagic individuals mainly eating on the benthic floor (Diez et al., 2012) and using the water column to escape from the agonistic behavior of conspecifics (Hypothesis 1; Zeldis, 1985). Based on the previously postulated hypotheses, availability of food in the water column could be playing an important role in the duration of the pelagic phase (Hypothesis 2; Varisco and Vinuesa, 2010; Williams, 1973). The main process controlling the duration of the pelagic phase for the *gregaria* ecotype remains unknown, but it is still possible that conspecific benthic densities could be shaping their permanence on the water column (Zeldis, 1985). However, even if the agonistic behavior is the key factor triggering the pelagic population, at least they do use different food resources at that stage, and phytoplankton availability is important. Considering the wide distribution of the species in the Southern Hemisphere, the extent of the patterns described here could be local and it would be interesting to test such niche differentiation at other location of *M. gregaria* distribution.

Our results are in agreement with previous findings by Varisco and Vinuesa (2010) about pelagic individuals consuming phytoplankton in the San Jorge Gulf, and it is important to consider several consequences of this behavior: In one hand, interactions between primary consumers, such as competition for resources, could be taking place. In Peru, for example, the squat lobster, *Pleuroncodes monodon*, and the Peruvian anchoveta, *Engraulis ringens*, were found to share their ecologic niche (Gutiérrez et al., 2008). Likewise, there is a niche overlap between the lobster *Nephrops norvegicus* and *Munida sarsi* in Bay Biscay, USA and it was suggested that the competition for resources could be compromising the stock recovery of the lobster (Lovrich and Thiel, 2011 and citations within). In the Beagle Channel, a spatial overlap between *Sprattus fueguensis* and *gregaria* ecotype was reported, and a niche overlap was suggested (Diez et al., 2012; Diez et al., 2018). Another possible interaction could be taking place in San Jorge Gulf with the shrimp *Peisos petrunkevitchi*, an abundant forage species that feed on phytoplankton and detritus (Mallo and Boschi, 1982). Between 2006 and 2014, the frequency of occurrence of *P. petrunkevitchi* in the diet of the hake changed from 45% to 4%. This happened in correspondence with an increase in the occurrence of *M. gregaria* on the hake's diet
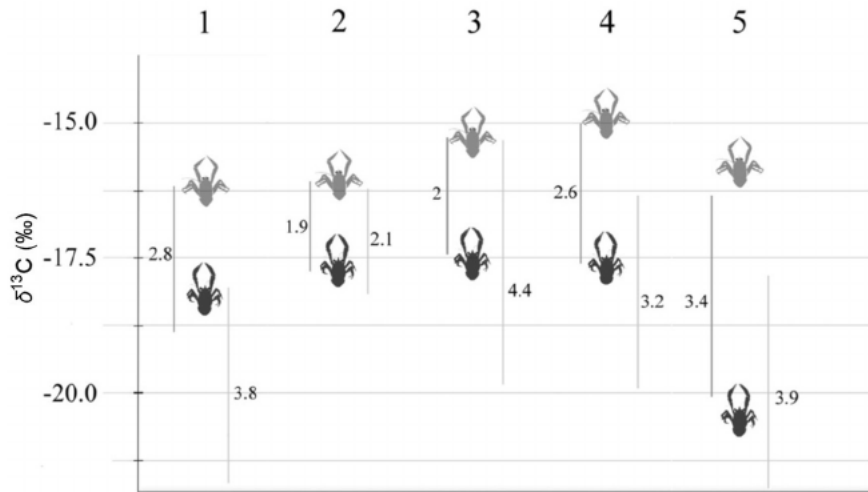
Fig. 3. Schematic comparison between mean δ^{13}C values of *Munida gregaria* ecotypes and isotopic baselines. Comparisons are arranged by sampling event order. *Gregaria* ecotype is drawn in black while *subrugosa* ecotype is drawn in grey. Bold lines show the differences between ecotype's values; whereas the lighter lines show the difference between the pelagic and the benthic pathways. Numbers next to each line indicate the absolute difference in δ^{13}C values.

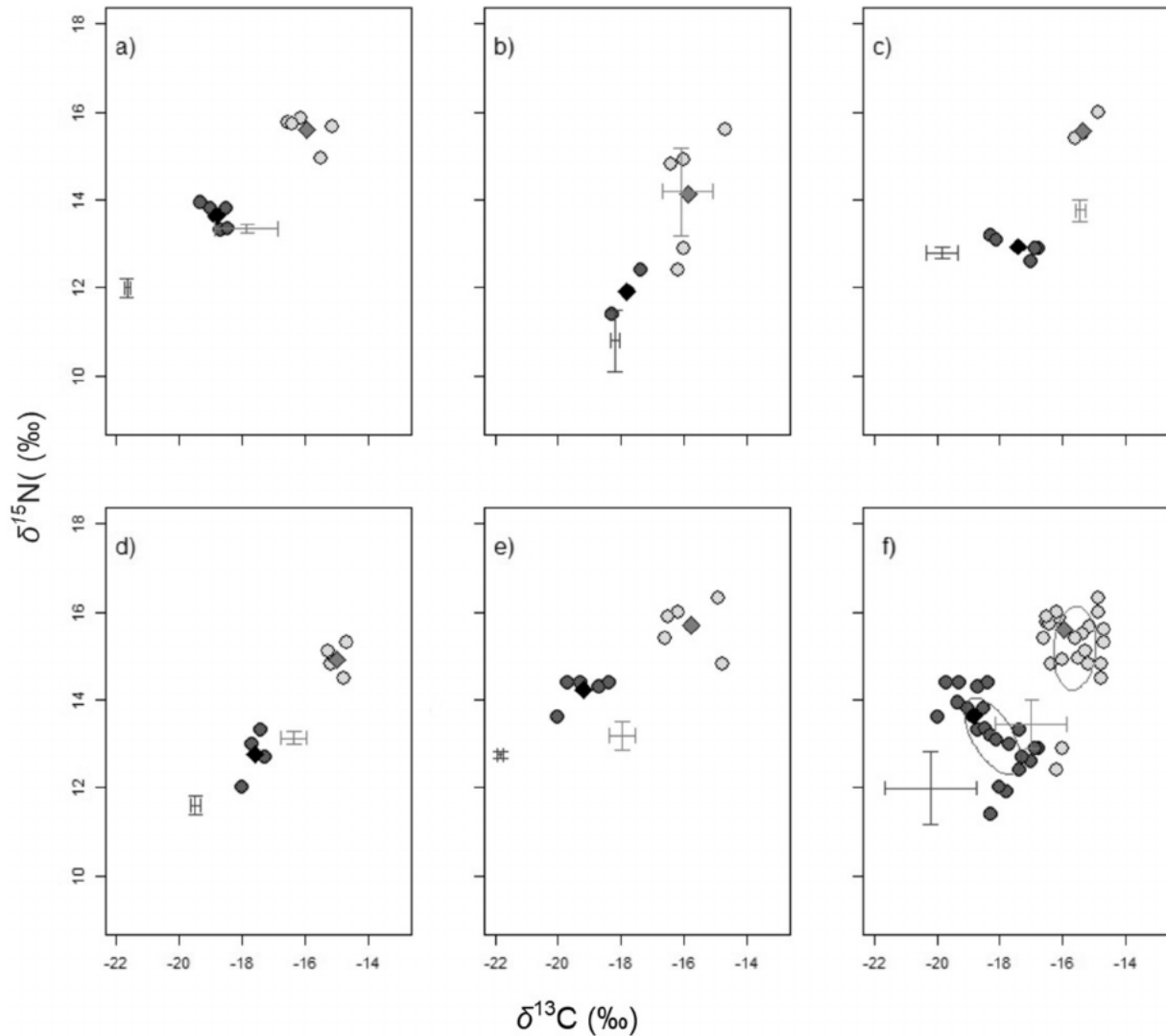**Fig. 4.** Biplots δ^{13}C vs δ^{15}N. Captions are organized by sampling event order a = 1, b = 2, c = 3, d = 4 & e = 5. Caption "f" shows values of all sampling events combined. Light grey dots represent benthic individuals while dark grey dots represent pelagic individuals. Centroid location of each group is indicated by a diamond while pelagic and benthic baselines and their standard deviation are represented by error bars, following the ecotype tone: light is benthic and dark is pelagic. In caption "f" each ecotype is bordered by its corresponding corrected standard ellipse SEAc.

Table 3

Trophic level (TL) and the Reliance on Bentic/Pelagic Affinity Prey (RBAP/RPAP) of each ecotype at each sampling event. All values are presented in ranges of most feasible values. RBAP and RPAP are expressed in percentages.

Sampling event		1	2	3	4	5
Gregaria ecotype	TL	2.1–2.5	2–2.2	2–2.1	2–2.3	2.2–2.6
	RPAP (%)	45–71	70–87	50–55	40–60	41–64
	RBAP (%)	29–55	30–13	45–50	40–60	36–59
Subrugosa ecotype	TL	2.8–3	2.4–2.7	2.5–3	2.7–2.8	2.8–3
	RPAP (%)	3.0–10	34–49	19–46	12.0–10	10.0–13
	RBAP (%)	90–97	66–51	54–81	80–88	87–90

(Belleggia et al., 2017). As previously mentioned, the squat lobster is a major component of pelagic and benthic biomass, and in Argentina, its abundance is increasing. In that context, it would be important to closely describe those potential interactions both at San Jorge and at the Beagle Channel.

The *gregaria* ecotype has been described as an important consumer of toxic microalgae in the waters of New Zealand (MacKenzie and Harwood, 2014). Studies showed it actively grazes on the massive blooms of dinoflagellates, significantly reducing the numbers of algal cells, and sequestering the paralytic shellfish toxin. As this ecotype is a common prey for many valuable populations in the Argentinian Sea (Belleggia et al., 2017; D'Agostino, 2017; Schiavini et al., 2005; Scioscia et al., 2014), and given that deaths by intoxication are strongly underreported (Shumway et al., 2003), the present results corroborate that the *gregaria* ecotype is a potential vector for toxicity in the Patagonian shelf. It would be interesting to develop specific studies on the toxicology of its predators.

In south Chile, SIA studies show that the benthic community (with the *Subrugosa* ecotype as an important component) is subsidized by the pelagic pathway, making phytoplankton an item of considerable importance to their diets (Zapata-Hernández et al., 2016). Our results did not demonstrate the pelagic pathway as an important source of nutrition for the *subrugosa* ecotype, but it was present as a minor percentage. Squat lobsters produce high nutritive feces in abundant amounts and with rapid gut clearance (Lovrich and Thiel, 2011). The feces of the pelagic ecotype constitute a dominant component of marine snow in some ecosystems (Lovrich and Thiel, 2011) and particularly in San Jorge Gulf (Massé-Beaulne, 2017). The consumption of the *gregaria* ecotype's feces by benthic individuals could be one possible mechanism for the inclusion of pelagic resources in the diet of the benthic individuals. The dominance of benthic resources in the *subrugosa* ecotype's diet was consistent with the two different and co-occurring feeding habits described for this squat lobster (Romero et al., 2004), which in shallow water was consuming small invertebrates and macroalgae, while in deep waters, POM dominated its diet.

Previous studies showed *M. gregaria* has a notable flexibility in its feeding habits and can act as predator, detritivore, deposit feeder, scavenger, cannibal, and suspense and bacterial feeder (Diez et al., 2016b; Romero et al., 2004; Varisco and Vinuesa, 2007; Varisco and Vinuesa, 2010; Vinuesa and Varisco, 2007). At the same time, it is an extended prey of several marine mammals (Alonso et al., 2000; D'Agostino, 2017), fishes (Sánchez and Prenski, 1996; Belleggia et al., 2017), and marine birds (Schiavini et al., 2005; Scioscia et al., 2014). Thus, when consuming phytoplankton and being consumed by top predators, they immediately shorten the food chain on one or even more levels (Longhurst et al., 2004; Lovrich and Thiel, 2011). Consequently, *M. gregaria* is a key trophic node, capturing energy from the primary production and injecting it into the food webs, shortening the food web, and making energy fluxes more efficient (Lovrich and Thiel, 2011; Diez et al., 2016b). Short food webs are theoretically more robust, with faster return times to the equilibrium (Pimm, 2002). Likewise, the length of the food webs is supposed to be determined by the ecosystem size (diversity of habitats and species richness) rather than by resource

availability (Post, 2002b), which is consistent with previous descriptions of the San Jorge gulf's trophodynamic (Milessi, 2008).

In summary, results presented here on the trophic ecology of this forage species, in the context of the literature and previous studies, show that the *gregaria* ecotype plays an important role contributing to the bentho-pelagic coupling in the Patagonian shelf with at least four possible mechanisms: (1) as plankton feeders that have been reported to be major prey of *M. hubbsi*, the most abundant demersal fish; during daily migration, *M. hubbsi* swims up on the water column and forages on the *gregaria* ecotype (Belleggia et al., 2017); (2) as dominant plankton feeders with rapid gut clearance contributing to the organic matter that support benthic production; (3) as pelagic swimmers with vertical migrations which include the utilization of resources from the benthic pathway (reliance on benthic resources from 13% to 60% Table 2); and (4) in the settlement process, pelagic individuals of *M. gregaria* change habitat, relocating their biomass, developed in and mainly by the pelagic zone, to the benthic zone.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property. We further confirm that any aspect of the work covered in this manuscript that has involved either experimental animals or human patients has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). She is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from: alejojoaquini@yahoo.com.ar, gastontrobbiani@hotmail.com, davidedgalvan@gmail.com and manuelafunes@yahoo.com.ar.

Declarations

Ethics approval and consent to participate

Non applicable.

Consent for publication

Non-applicable.

Competing interests

None of the authors of this study have any competing interests in the manuscript.

Authors' contributions

Manuela Funes contributed to fieldwork design, sample collection. Performed data analysis and manuscript writing.

Alejo Joaquin Irigoyen contributed to fieldwork design, sample collection and manuscript writing.

Gastón Andrés Trobbiani contributed to fieldwork design, sample collection and manuscript writing.

David Edgardo Galván provided the idea, designed the experiment and contributed to data analysis and in manuscript writing.

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