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Changes in the diet of the native sea urchin *Arbacia dufresnii* at different scenarios of the *Undaria pinnatifida* invasion (Patagonia, Argentina)

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ABSTRACT

Undaria pinnatifida is one of the most successful marine invasive species worldwide. Since its first detection in Nuevo Gulf (Argentina), changes in the species richness and diversity of native algae and macrofauna have been reported. Knowledge of the invasive alga-native grazer interaction is key to better understand ecosystem dynamics and the potential effects of *Undaria* on local food webs. The aim of this study was to compare the diet of the sea urchin *Arbacia dufresnii*, among different scenarios of invasion of *Undaria* in two northern Patagonian gulfs. To this end, we determined the relative contribution of kelp to the diet of the sea urchin by comparing the gut content and stable isotopes in different tissues of *A. dufresnii* from sites where the invasion of *Undaria* was advanced, relatively recent, or not recorded. Because *A. dufresnii* has a plastic feeding habit and *Undaria* represents a high input of algal biomass, our hypothesis was that *A. dufresnii* feeds on *Undaria* and that the contribution of the invasive kelp to the diet of the sea urchin will be greater according to the time elapsed since invasion. Our results confirmed that *A. dufresnii* fed on *Undaria* and assimilated in gonads and muscle. The contribution of *Undaria* to the sea urchin diet was related to the stage of invasion. In the site with an advanced stage of invasion, *A. dufresnii* consumed the kelp both in spring and summer, when sporophytes were mature and senescent, respectively. However, in the recently invaded site, *A. dufresnii* consumed *Undaria* only in summer, when the kelp was senescent. *Arbacia dufresnii* is an omnivore species with the plasticity to adapt its diet to a variety of resources depending on the availability in the environment, prioritizing faunal items. This work reinforces the importance of complementing gut analysis with stable isotope analysis to determine the contribution of items in the diet. Although the impacts of an invasive alga on food webs and ecosystem structure are difficult to evaluate, performing these analyses in different scenarios may allow better understanding the local food web structure and assessing the possible ecological consequences over the invaded habitat.

1. Introduction

The study of interspecific interactions allows us to understand ecosystem dynamics (Bertness, 1999), and, among these interactions, predation is a fundamental driver of the community structure (Duffy and Hay, 2001). On the other hand, introduction of species in marine environments represents a threat to native ecosystems, causing impacts on biological diversity, productivity, and economic resources (Carlton, 1999; Williams and Smith, 2007). Particularly, invasive algae species may dominate local benthic algal assemblages, causing changes in the community structure and modifying faunal assemblages through the provision of refuge or food (Deudero et al., 2011).

The kelp *Undaria pinnatifida* (hereafter referred to as *Undaria*) is one of the most successful marine invasive species worldwide and is recognized as one of the worst species in terms of ecological impact (Kulhanek et al., 2011; South et al., 2017). *Undaria* has successfully invaded many coasts of the world and rapidly expanded its distribution, covering extensive areas, mainly in subtidal coastal habitats (e.g. Perez et al., 1981; Hay and Luckens, 1987; Casas and Piriz, 1996; Silva et al., 2002; Thornber et al., 2004; Valentine and Johnson, 2005; Dellatorre et al.,

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2014; Suárez Jiménez, 2015; Epstein and Smale, 2017). The multiple ecological effects caused by this invasive alga on the invaded communities include a decrease in species richness and diversity of native algae, an increase in species richness and diversity of benthic macrofauna, an additional carbon subsidy to the coastal ecosystem and the possibility of becoming a new alternative food source able to modify the food web (e.g. Casas et al., 2004; Johnson et al., 2004; Thornber et al., 2004; Irigoyen et al., 2011; Suárez Jiménez et al., 2015; Tait et al., 2015). However, other studies have described its impacts as transient or inconspicuous (South et al., 2016; Epstein et al., 2019). In addition, *Undaria* is an annual species with a heteromorphic life cycle. The seasonal occurrence of some invasive algae species in the benthic ecosystem can cause changes in the available food sources, altering the consumer populations and, thus, shifting its trophic interactions (e.g. Deudero et al., 2011; Salvaterra et al., 2013; Suárez Jiménez et al., 2015; Suárez-Jiménez et al., 2017; Cabanillas-Terán et al., 2019). Thus, research performed on the ecological effects of this species is usually focused on the period when the macroscopic phase predominates. Knowledge regarding the incorporation of *Undaria* as a food source for native grazers is key to understand its potential effects on local food webs.

In their native area, *Undaria* is consumed by different herbivores, including some species of sea urchins (Tamaki et al., 2009; Ogasawara et al., 2011; Yang et al., 2021). Sea urchins play an important role in shallow communities such as coral reefs, seagrasses, and kelp forests, since they can structure them through grazing pressure (Steneck, 2013) and some species may even reduce invasive algae abundance (Valentine and Johnson, 2005; Cebrian et al., 2011). In the northern Patagonian gulfs of Argentina, the most abundant sea urchin is *Arbacia dufresnii* (Zaixso and Lizarralde, 2000; Brogger et al., 2013; Epherra, 2016), which has a plastic feeding habit, and may be considered as herbivorous, carnivorous or omnivorous depending on the availability of food in the environment (Vásquez et al., 1984; Penchaszadeh and Lawrence, 1999; Galván et al., 2009; Newcombe et al., 2012; Castro, 2014; Zárate, 2014). Some studies have found that the abundance of *A. dufresnii* is higher when *Undaria* is present (Irigoyen et al., 2011; Epherra et al., 2017). The local benthic macroalgal assemblages of these gulfs are characterized by small to medium size species that do not surpass more than 50 cm in height (Boraso de Zaixso et al., 1999; Boraso and Zaixso, 2008). As a consequence, the introduction of *Undaria* in Nuevo Gulf (Casas and Piriz, 1996) represents, after 20 years of invasion, not only the appearance of a new species in the community but also an increase of 800% in the availability of biomass for herbivores, as well as a new algal layer, which could grow up to 2 m high (Casas et al., 2008; Raffo et al., 2015). Similar conditions occur in San José Gulf, where the invasion of *Undaria* is much more recent (Irigoyen, 2009) and therefore, the kelp is less abundant (Epherra, 2016).

Based on the above, the aim of this study was to compare the contribution of *Undaria* to the diet of *A. dufresnii* in different scenarios of invasion of *Undaria*. The diet of *A. dufresnii* was studied through gut content and stable isotope analyses. Gut content analysis provides information of the recent food ingested while the stable isotope analysis provides complementary information on the assimilated food integrated over a period of time (Yatsuya and Nakahara, 2004; Phillips et al., 2014) and, therefore, allows establishing trophic interactions (Vander Zanden et al., 1997). Furthermore, since the isotopic enrichment varies between tissue types by turnover rates, the study of the isotopic composition of multiple tissues simultaneously provides more complete information about the feeding habit of a target species (Martínez del Rio et al., 2009). For this purpose, the diet of *A. dufresnii* was analyzed comparing the gut content and the $\delta^{15}N$ and $\delta^{13}C$ isotope values among sites with different invasion times of *Undaria*, to identify the relative contribution of the invasive kelp in the diet of the sea urchin, and the variability in $\delta^{15}N$ and $\delta^{13}C$ among tissues. Because *A. dufresnii* has a plastic feeding habit and *Undaria* represents a large input of edible algal biomass, our hypothesis was that *A. dufresnii* feeds on *Undaria* and that the contribution of the invasive kelp will be greater in an advanced stage of invasion.

2. Materials and methods

2.1. Study area

San José Gulf (SJG) and Nuevo Gulf (NG) are located in the north of Argentine Patagonia (Fig. 1). These gulfs are semi-enclosed basins where surface temperature does not exceed 20 °C (Amoroso and Gagliardini, 2010; Dellatorre et al., 2012). The sites selected for the present study are shallow rocky reefs of limestone platforms with similar characteristics prior to the invasion of *Undaria* (Zaixso et al., 1998; Boraso de Zaixso et al., 1998). Samplings were carried out during 2012, when the sites presented differences in the timing and stage of *Undaria* invasion. In SJG, two sampling sites were selected: Punta Tehuelche (SJG-PT; 42°23′S, 64°17′W), where the invasion was relatively recent (less than 8 years; Irigoyen, 2009) and Zona 39 (SJG-Z39; 42°23′S, 64°04′W), where the kelp had not been recorded. In NG, one site was selected: Punta Cuevas (NG-PC, 42°46′44"S, 64°59′52"W), where the invasion was in advanced stage (over 20 years, Casas and Piriz, 1996; Raffo et al., 2012). Sites presented similar species composition but different abundances of macro-invertebrates and cover of algal species, for instance, the bivalves *Aulacomya atra atra* and *Mytilus* sp. were dominant benthic species in SJG-PT (Epherra, 2016). Particularly, the density of *A. dufresnii* was higher in invaded than in non-invaded sites (Table 1; Epherra et al., 2017). The percentage of biomass of *Undaria* at each site and season is presented in Table 1.

2.2. Sampling

According to the annual life cycle of *Undaria* reported by Casas et al. (2008) for the study area, the sea urchins were collected in four periods: i) autumn (May), during the gametophyte phase, when the hard bottoms were free of sporophytes; ii) winter (August), when the sporophytes

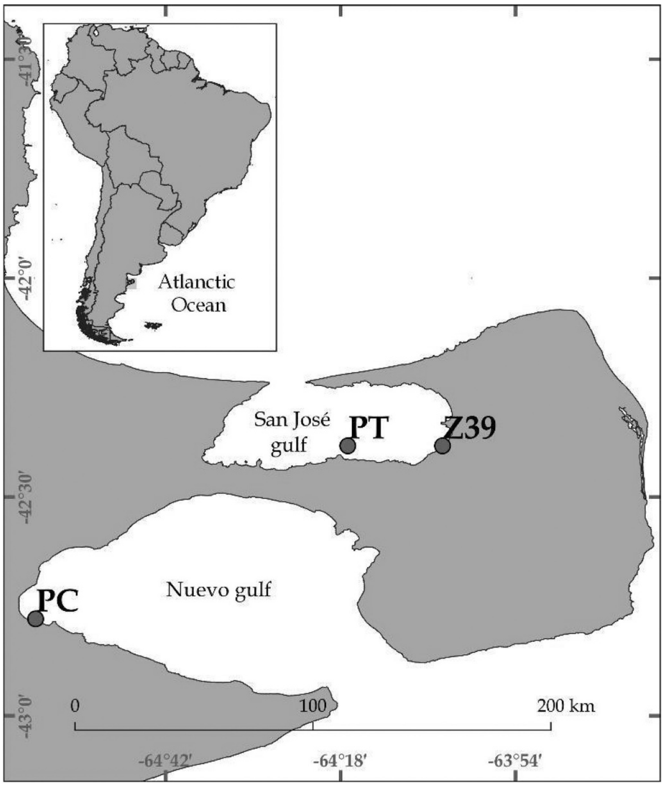

Fig. 1. Map showing the location of the study sites in San José Gulf (northern Argentine Patagonia): Punta Tehuelches (PT), where the invasion of *Undaria pinnatifida* was recent and Zona 39 (Z39), where the kelp was absent, and in Nuevo gulf: Punta Cuevas (PC), where kelp invasion was advanced.

Table 1

Percent of biomass of *Undaria pinnatifida* with respect to total algal biomass and density *of Arbacia dufresnii* by season and site (Nuevo Gulf-Punta Cuevas (NG-PC), San José Gulf-Punta Tehuelches (SJG-PT), and San José Gulf-Zona 39 (SJG-Z39)). Data extracted and adapted from Epherra (2016).

	% *Undaria pinnatifida*				*Arbacia density*			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
NG-PC	74.08 ± 18.94	0.73 ± 2.01	30.4 ± 18.83	78.80 ± 12.44	9.90 ± 6.91	3.46 ± 1.35	8.31 ± 2.74	*
SJG-PT	40.11 ± 32.18	5.15 ± 6.60	0.04 ± 0.10	43.94 ± 21.27	2.06 ± 1.48	8.88 ± 4.50	8.95 ± 2.63	18.13 ± 12.37
SJG-Z39					2.99 ± 1.59	3.71 ± 3.91	5.26 ± 6.86	3.80 ± 4.38

* No density data.

were starting to grow, *Undaria* formed dense stands and the mean densities were maximum; iii) spring (November), when sporophytes were mature, showing the highest average of biomass, and iv) summer (January), when the kelp was in senescence stage.

Adult *A. dufresnii* individuals were randomly collected by scuba diving at each site and season. At the laboratory, all individuals were narcotized by immersion for 15 min in a 5% $MgCl_2$ solution. The gut, Aristotle's lantern muscles, and gonads were dissected. The gut was fixed in 5% formaldehyde with boric acid and preserved in 70% ethanol for further content analysis. Muscles and gonads were rinsed in distilled water, dried at 60 °C for 24 h and preserved for stable isotope analysis.

To ensure that the samples of sea urchin reflected the consumed food sources and the period of time during which the tissues of interest were synthesized (Phillips et al., 2014), at the beginning of the study (summer 2012), potential food sources ($n = 3$ individuals at each selected item) were collected for stable isotope analysis. The algae collected included *Undaria* and the most representative and abundant benthic algae species (*Ulva* spp., *Codium* spp., *Dictyota dichotoma*, *Ceramium* sp., *Lomentaria clavellosa* and *Polysiphonia* spp.) (Epherra, 2016), while bivalves included common mussel (*Mytilus edulis platensis*), ribbed mussel (*Aulacomya atra atra*) and scallop (*Aequipecten tehuelchus*). Samples were rinsed in distilled water, and dried at 60 °C for 48 h.

2.3. Gut content analysis

The gut of the individuals collected from each site during the different seasons in which the *Undaria* sporophytes occurred (winter, spring and summer) ($n = 6$, $N = 54$) were removed and pellets separated from the tissue, using a stereomicroscope. Then, all pellets from each individual were diluted to standardize the volume of the contents to 3 mL. Six subsamples of 150 μL were taken from each sample and observed under a microscope. The abundance of each animal item was calculated as the average in the six subsamples, standardized relative to total gut contents. Instead, because algae were observed as fragments the percentage of cover of each algal item was estimated as the number of cells or fraction of them occupied in a grid. The percentages obtained for each subsample were averaged to calculate the percentage of each item in each gut content. The percentage cover was relative because 100% of the coverage was calculated by adding the total number of cells occupied by the different algal items. All food items were identified to the lowest taxonomic level possible. Shell fragments and completely digested material were not considered.

2.4. Stable Isotope analysis

Aristotle's lantern muscles from the sea urchins collected in all seasons from each site ($n = 5$, $N = 60$) were used for stable isotope analysis. This is the most recommended tissue for this analysis in sea urchins because it integrates assimilated sources over long terms (Cabanillas-Terán et al., 2019). However, in omnivorous species the gonads can provide complementary information because they are dual organs, producing gametes and storing nutrients (Hughes et al., 2006), and because they are metabolically active tissues (Prado et al., 2012). For this reason, gonadal tissue from sea urchins collected in summer and winter from each site ($n = 3$, $N = 18$) was additionally used.

Untreated samples were homogenized, weighed (animal items: ~ 1 ± 0.2 mg; algal items: between 1 and 3 mg), and placed in tin combustion capsules for stable isotope analysis. C and N percentages and isotopic values were determined at the Stable Isotope Facility of the University of California, Davis (USA). The stable isotope value was expressed using the standard δ notation relative to carbonate Vienna PeeDee Belemnite, and atmospheric nitrogen was calculated using the following equation: δX (‰) = [(R sample/ R standard) -1] x 1000, where X: ^{13}C or ^{15}N and $R = {}^{13}C{:}{}^{12}C$ or $^{15}N{:}^{14}N$. To avoid a biased value due to the lipid content in gonads, a correction on $\delta^{13}C$ values ($\delta^{13}C$ normalized $= \delta^{13}C$ untreated-3:32 + 0:99C: N) was applied to samples with a C: N ratio greater than 3.5 (Post et al., 2007).

2.5. Data analysis

Differences in the gut content of sea urchins between sites and seasons were tested by multivariate two-way PERMANOVA, performed separately for animal abundance and algal cover. Abundance and cover data were square-root transformed and a Bray Curtis similarity matrix was used in all the analyses. The factor "season" was nested in the factor "site". When appropriate, pair-wise tests ($\alpha = 0.05$) were used as a posteriori check of significant differences. Similarity percentage (SIMPER) routines were used to evaluate which items contributed the most to the differences between sites and seasons.

Isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of sea urchins were compared by three-way PERMANOVA to examine differences between tissues, sites and seasons (nested in the factor "Site"). Untransformed data were used to perform similarity matrices using Euclidean distances. Pair-wise tests were used, where appropriate, for a posteriori comparisons of the means. Differences in isotope values among sources were tested by one-way PERMANOVA. These results allowed combining food sources to be used in the mixing models (more details below). All statistical analyses were carried out with Primer v6.1.12 (Clarke and Gorley, 2006) with the permutational multivariate PERMANOVA+ v1.0.1 add-on (Anderson, 2003).

Independent analyses of muscles and gonads of sea urchins were performed. Seasons were not considered as factor because there were no significant differences for $\delta^{13}C$ or $\delta^{15}N$ values (see Results). The food source contribution to the diet of *A. dufresnii* at each site was estimated using the Bayesian isotopic mixing model SIAR (Stable Isotope Analysis in R, Parnell and Jackson, 2013), following the guidelines suggested by Phillips et al. (2014). Although it was not possible to analyze all the food sources at the three sites, isotopic values of each alga species in the two sites of SJG were pooled to perform the mixing models. On the other hand, isotopic values of food sources statistically similar or related in some way (such as those belonging to the same taxa or functional group) were combined to reduce the number of sources (below six or seven) and thus avoid reducing the discriminatory power of mixing models. Taxa considered for the mixing model analyses were: brown algae *U. pinnatifida*, *D. dichotoma*, green algae *Codium* spp., *Ulva* spp., and red filamentous algae (*Ceramium* sp., *Polysiphonia* spp.), and L. *clavellosa*, and the bivalves *(M. edulis platensis*, *Aulacomya atra atra,* and *A. tehuelchus*).

In studies of trophic ecology, it is suggested to estimate mean diet–tissue discrimination factors (DTDF) (Newsome et al., 2010) because fractionation rates can vary among species, tissues, diet quality and trophic level (McCutchan Jr et al., 2003; Vanderklift and Ponsard, 2003). Therefore, an a priori test was performed to estimate the feasible values of DTDF in this study because of the considerable variation in DTDF documented in sea urchins and the omnivorous potential of *A. dufresnii*. The routine proposed by Smith et al. (2013) was conducted before running the isotopic mixing models, to determine whether the consumers were inside the convex mixing polygon and bound to all potential sources through an iterative procedure using the distributions of the DTDF. Usual DTDF values of 1‰ for δ^{13}C and 3.4‰ for δ^{15}N were tested (DeNiro and Epstein, 1981; Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001). Since mixing polygon sensitivity analyses for the two sea urchin tissues analyzed showed that many consumers are located outside the 95% mixing polygon, other bibliographic values were tested.

Considering the data from the three sites, the best fit to the models (1500 iterations) was the one with the following values: 1 ± 0.5 for C and 3.8 ± 0.7 for N for muscle, and 1.3 ± 0.3 for C and 1.81 ± 0.3 for N15 for gonads (Fredriksen, 2003; Prado et al., 2012). All estimations of stable isotope analysis were written and performed in the R language version 4.0.0 (R Core Team, 2020).

3. Results

3.1. Gut content

Gut contents revealed many small pellets and pieces of algae. These pellets were composed of a high percentage of sediment, as well as digested material. Algal items were dominant over animal items, although these quantities were not statistically compared. All urchins had algae in their guts, whereas animal items occurred in fewer individuals (Table 2). The most frequent items were ostracods. Recruits of bivalves were abundant at invaded sites (Table 2).

Undaria was present in the gut of *A. dufresnii* individuals from both invaded sites (SJG-PT and NG-PC), and absent in those of individuals from the non-invaded site (SJG-Z39). In addition, it was recorded in samples collected from NG-PC (site whit an advanced stage of invasion) in spring and summer, and those collected from SJG-PT (recently invaded site) in summer. In general, a wide diversity of ingested algae was observed and gut contents varied between sites and seasons (PERMANOVA, Table 3). Percentage cover of algae was different between invaded sites (SJG-PT and NG-PC: $t = 2.0531$, P (perm) = 0.0448), and that was also reflected in the highest average dissimilarity value. *Undaria* contributed to the dissimilarity between the site with an advanced stage (NG-PC) of invasion and both sites from SJG (Contribution %, NG-PC/SJG-PT = 7.83; NG-PC/SJG-Z39 = 6.09) (SIMPER, Supplementary Material, Table 1). Regarding the factor “season”, at SJG-Z39, the cover of algae in gut contents of individuals collected in spring differed from those collected in summer ($t = 1.415$, P (perm) = 0.0273), whereas, at the invaded sites, differences were observed between winter and summer (SJG-PT: $t = 1.4667$, P (perm) = 0.034; and NG-PC: $t = 1.4447$, P (perm) = 0.0106) (Fig. 2a). At NG-PC, *Undaria* contributed to the dissimilarity among seasons, whereas at SJG-PT, it only contributed to the dissimilarity in summer (SIMPER, Supplementary Material, Table 2). The abundance of animal items did not vary among sites or seasons (Table 3, Fig. 2 b).

3.2. Stable isotopes

The δ^{13}C and δ^{15}N values of *A. dufresnii* significantly varied among sites and between tissues, but the isotopic signatures were similar during the year. In addition, since the differences found among sites and between tissues did not depend on other factors (Table 4a), the comparisons were performed for each tissue. For Aristotle's lantern muscles, significant differences among sites were found for both isotopes (Table 4b). The δ^{13}C values varied from −18.90 to −13.11‰ at NG-PC, from −17.60 to −14.14‰ at SJG-PT, and from −19.73 to −16.07‰ at SJG-Z39, and showed no significant differences between invaded sites (t: 1.76, P (perm) = 0.1517). However, invaded sites were enriched with ^{13}C compared with the non-invaded site (SJG-PT/SJG-Z39: $t = 7.23$, P (perm) = 0.0292; NG-PC/SJG-Z39: $t = 6.97$, P (perm) = 0.0053). Instead, δ^{15}N values showed significant differences only between invaded sites (NG-PC/SJG-PT: $t = 5.31$, P (perm) = 0.0074; SJG-PT/SJG-Z39: $t = 1.53$, P (perm) = 0.2262; NG-PC/SJG-Z39: $t = 1.32$, P (perm) = 0.2513). The δ^{15}N values varied from 15.04 to 17.63‰ at NG-PC, from 16.50 to 18.67‰ at SJG-PT, and from 15.49 to 18.27‰ at SJG-Z39. SJG-PT was the site with the highest average values of δ^{15}N (17.29, sd: 0.86), while NG-PC showed the lowest average value of δ^{15}N (16.38, sd: 0.81) (Fig. 3). No significant differences were found for δ^{13}C and δ^{15}N values in gonadal tissue either between sites or seasons (Table 4c). The δ^{13}C values varied between −22.22 and − 14.7‰ while δ^{15}N varied between 11.27 and 15.17‰ (Fig. 3).

The δ^{13}C and δ^{15}N values of food sources significantly varied among food items and sites, and their interactions were significant (Table 4d). At each site, *a posteriori* comparisons of $\delta\ ^{13}$C and $\delta\ ^{15}$N values allowed combining food sources to be used in the mixing model (Fig. 3). The mixing models analysis showed that both algal and animal items were assimilated by *A. dufresnii*. *Undaria* was assimilated in both muscle and gonad, but its contribution was different between them (Fig. 4). At SJG-PT, the contribution of *Undaria* was higher in gonads, whereas at NG-PC it was higher in muscle. The sources were assimilated in different proportions and a greater diversity of sources contributed to the gonads (Fig. 4).

At NG-PC, the main assimilated item was *Codium* spp., contributing more than 50% to the diet in both tissues. In gonads, all remaining sources showed similar and poor contribution, while *Undaria* and bivalves showed a higher contribution in muscle (Fig. 4a). In contrast, sea urchins from SJG-PT fed mainly on bivalves, without any other relevant source contribution, while in gonads, they fed mainly on *Undaria* and *Codium* spp. (Fig. 4b). Sea urchins of SJG-Z39 consumed all food sources analyzed. In muscle, bivalves were the most important assimilated source, followed by the exotic red alga L. *clavellosa.* In gonads, there was a higher contribution of filamentous red algae, while the proportional contributions of the remaining sources were considerably overlapped (Fig. 4c).

4. Discussion

Arbacia dufresnii is an omnivorous sea urchin that inhabits Patagonian gulfs, with plasticity to adapt its diet to a variety of resources depending on the availability in the environment. At the invaded studied sites, this sea urchin fed on *Undaria*, which was even assimilated in different types of tissues, suggesting a complete incorporation of the invasive kelp to the *A. dufresnii* diet.

In most trophic studies on invasive algae, the role of omnivorous species has been generally ignored (Santamaría et al., 2021). Integrating both types of diet analyses (gut content and stable isotope analysis) as we did, provided a complementary view of the feeding habit of *A. dufresnii*. According to the gut content, this sea urchin is an omnivore with a tendency to herbivory, instead, however, stable isotopes showed a strong tendency to carnivory. The gut content analysis showed small pellets with a high percentage of sand as well as pieces of algae, while the stable isotope analysis showed a consistently higher contribution of assimilated animal food items. Since animal tissue is more rapidly digested than the algae (Fernández and Boudouresque, 2000; Agnetta et al., 2013), the gut content analysis may be biased towards less digestible material (Cobb and Lawrence, 2005; Wangensteen et al., 2011; Gil et al., 2021), and the results may be interpreted as a false tendency to herbivory. For example, in the gut content of *Arbacia lixula*, fragments of filamentous algae were intact, while animal items showed complete digestion (Oliveira, 1991; Wangensteen et al., 2011). Similarly, in the guts of *A. dufresnii,* filamentous algae were often observed,

Table 2
Relative frequency (RF), average percentage cover of algae (%) and average abundance of animal items in gut contents of *Arbacia dufresnii* individuals from San José Gulf and Nuevo Gulf by season.

	San José Gulf- Zona 39						*San José Gulf- Punta Tehuelche*						Nuevo Gulf – Punta Cuevas					
	Summer		Winter		Spring		Summer		Winter		Spring		Summer		Winter		Spring	
	RF	%	RF	%	RF	%	RF	%	RF	%	RF	%	RF	%	RF	%	RF	%
Algae																		
Chlorophyta																		
Chaetomorpha sp.													0.33	0.17	0.50	0.26	0.33	5.25
Cladophora sp.															0.67	2.35	0.83	5.39
Codium spp.[1]	0.83	20.66	0.33	22.36	0.57	1.49	0.33	1.31	0.33	33.43	0.17	6.67	0.50	0.97	0.17	0.16	0.67	8.75
Ulva sp.[2]															0.50	1.43	0.17	0.40
Ulva spp.	0.17	14.96	0.17	0.16	0.14	38.81	0.17	79.44	0.50	5.38	0.50	23.53	0.33	4.69	0.33	7.90	0.67	6.13
Phaeophyta																		
Dictyota dichotoma	0.50	1.82	0.67	32.63	0.86	9.14	0.33	4.18	0.67	26.66	0.67	9.92	0.83	15.94	0.67	2.87	0.83	8.67
Ectocarpaceae	0.50	1.01	0.83	1.42	1.00	2.85	0.17	4.90	0.50	0.39	0.67	11.15	0.50	7.88	0.83	1.31	0.67	0.56
Ectocarpus siliculosus	0.67	3.42	0.67	0.84	0.86	3.50	1.00	13.57			0.67	9.16	0.67	10.46	0.33	1.17	0.33	2.68
Sphacellaria sp.													0.67	3.65	0.83	0.90	0.83	0.37
Undaria pinnatifida							**0.83**	**27.13**					**0.33**	**51.45**			**0.33**	**45.92**
Rhodophyta																		
Acrochaetium sp.													0.33	1.61			0.33	1.90
Anotrichium sp.	0.83	9.81	1.00	11.18	1.00	2.15					0.17	0.34	0.67	14.52	0.67	2.33	0.17	2.08
Antithamnion sp.	0.33	9.99	0.67	2.38	0.29	2.02					0.17	3.51	0.17	0.18	0.17	0.50		
Callithamnion sp.	0.50	1.03	0.17	0.41	0.86	1.08	0.33	5.02					0.17	1.17	0.33	0.41	0.67	1.30
Ceramium sp.	1.00	7.86	0.50	2.00	0.86	10.48							0.67	5.87	1.00	13.89	1.00	18.47
Gelidium sp.	0.67	23.24	0.17	0.29	0.43	1.51	0.17	0.98	0.17	0.11			0.17	6.67	0.17	0.06		
Gracilaria gracilis													0.33	3.05	0.17	5.17		
Heterosiphonia sp.	0.33	0.70	0.33	5.71	0.14	14.29			0.17	0.92	0.17	6.14			0.17	0.73	0.17	2.22
Lomentaria clavellosa			0.17	12.22	0.57	44.61			0.17	0.18	0.33	8.66			0.50	44.33	0.50	17.24
Medeiothamnion sp.					0.14	0.39									0.17	1.23		
Polysiphonia spp.[3]	1.00	27.93	1.00	21.84	0.86	12.24	0.33	3.91	0.17	11.92	0.50	7.64	0.83	32.12	1.00	21.54	1.00	21.53
Stylonema sp.	0.50	0.44	1.00	1.43	1.00	1.81	0.17	0.74	0.17	0.01	0.50	2.72	0.67	0.59	0.67	1.64	0.33	4.70
Delesseriaceae	0.17	15.82	0.17	5.93	0.29	11.61	0.50	22.66	0.83	25.53	0.33	9.23						
Animals																		
	RF	Ab	RF	Ab	RF	Ab	RF	Ab	RF	Ab	RF	Ab	RF	Ab	RF	Ab	RF	Ab
Porifera																		
Octocorallia							0.17	2.67										
Foraminifera	0.17	6.50	0.33	7.50	0.57	2.79	0.17	3.00	0.17	3.33	0.33	4.67					0.83	3.33
Bryozoa																	0.17	
Hydrozoa																		
Leptothecata	0.5		0.83		0.43		0.33						0.17		0.33			
Arthropoda																		
Copepoda	0.17	8.83			0.14	9					0.33	4.67	0.33	2.67	0.33	2.83	0.17	2.67
Cladocera					0.14	3.17									0.17	3		
Ostracoda	0.50	4.11	0.67	3.71	0.71	4.30	0.50	3.78			0.33	2.92	0.17	2.67			0.17	2.5
Isopoda													0.17	3				
Halacaridae	0.17	5.83	0.33	2.92	0.14	3.17									0.17			
Crustacean articles	0.5		0.5		1						0.17				0.17		0.17	
Nematoda	0.33	2.83	0.17	3.33	0.14	3.33	0.17	2.67										
Mollusca (recruits)																		
Pelecipoda			0.50	6.56	0.43	5.72	0.50	5.50			0.33	5.83	0.17	2.67			0.33	1.5
Gastropoda					0.14	5.33	0.17	7			0.17	3.33						

[1] *Codium* spp. (*Codium fragile*, *Codium decorticatum*, *Codium vermilara*).

[2] *Ulva* sp. (previously named as genus *Enteromorpha*).

[3] *Polysiphonia* spp. (species of gender *Polysiphonia* and *Melanothamnus harveyi* (previously named as *Neosiphonia harveyi* or *Polysiphonia argentinica*).

Table 3
Results of the two-way PERMANOVA for *Arbacia dufresnii* gut contents among sites (Nuevo Gulf-Punta Cuevas, San José Gulf-Punta Tehuelches, and San José Gulf-Zona 39) and seasons (winter, spring and summer). (a) Percentage cover of algae. (b) Abundance of animal items. Significant p- values are in bold.

Source of variation	a) Algal items					b) Animal items				
	df	SS	MS	Pseudo-F	P (perm)	df	SS	MS	Pseudo-F	P (perm)
Site	2	27,614	13,807	3.6073	**0.0054**	2	11,242	5,620.8	1.3433	0.2755
Season (Site)	6	22,997	3,832.8	1.7474	**0.0009**	6	26,471	4,411.8	1.504	0.0693

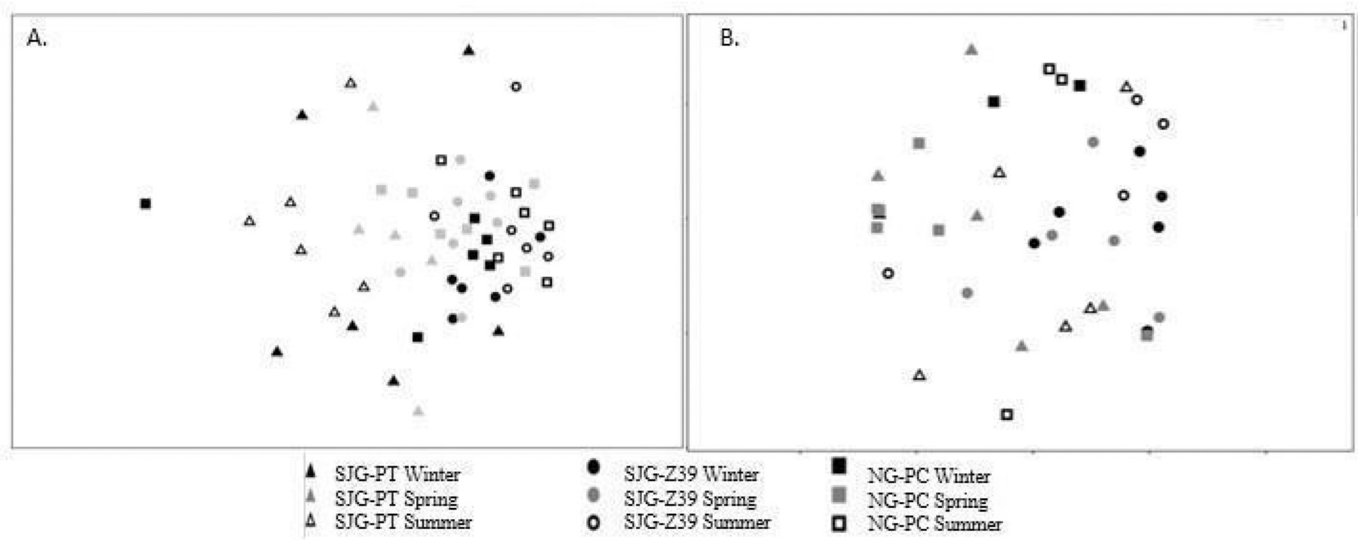

Fig. 2. Multidimensional scaling (MDS) plot of Bray Curtis similarities (square root transformed) of *Arbacia dufresnii* gut contents among sites (SJG-PT: San José Gulf-Punta Tehuelches, SJG- Z39: San José Gulf,-Zona 39, and NG-PC: Nuevo Gulf-Punta Cuevas,) and seasons (winter, spring and summer) for a) cover of algae (Stress: 0.21), and b) abundance of animal items (Stress: 0.24).

Table 4
Results of the PERMANOVA for isotopic values (δ^{13}C and δ^{15}N) of (a) *Arbacia dufresnii*, (b) muscles of Aristotle's lanterns, (c) gonads, and (d) food sources. In the respective models the factors included are: Ti: tissues (muscle and gonads of *Arbacia dufresnii*), Si: sites (Nuevo Gulf-Punta Cuevas, San José Gulf-Punta Tehuelches, and San José Gulf-Zona 39) and Se: seasons (nested in "site"; winter, spring, and summer). Significant p- values are in bold.

	$^{13}\delta$C					$^{15}\delta$N				
Source	df	SS	MS	Pseudo-F	P(perm)	df	SS	MS	Pseudo-F	P(perm)
a) Sea urchin										
Tissue (Ti)	1	5.94	5.94	9.96	**0.0491**	1	179.99	179.99	346.96	**0.0007**
Site (Si)	2	38.84	19.42	13.56	**0.0002**	2	11.78	5.88	7.46	**0.0198**
Season (Site)	9	11.45	1.27	0.73	0.6794	9	7.14	0.79	1.02	0.4481
Tissue x Site	2	0.54	0.27	0.45	0.6796	2	7.73	0.87	1.67	0.3198
Tissue x Season (Site)	3	1.79	0,59	0.34	0.7901	3	1.56	0.52	0.66	0.5726
b) Muscle										
Site	2	50.57	25.28	30.681	**0.0005**	2	7.56	3.78	5.33	**0.0418**
Season (Site)	9	7.33	0.81	0.56	0.8274	9	6.39	0,71	1.09	0.3923
c) Gonads										
Site	2	13.94	6.97	3.70	0.2624	2	6.22	3.11	3.47	0.1823
Season (Site)	3	5.65	1.88	1.06	0.4133	3	2.68	0.89	0.73	0.5587
d) Food Source										
Site	2	160.90	80.45	89.24	0.0001	2	21.19	10.59	36.85	0.033
Food	13	1213.30	93.33	103.54	0.0001	13	48.54	3.73	12.98	0.0001
Site x Food	8	28.94	3.62	40.13	0.0024	8	36.51	45.64	15.87	0.0001

but these were not assimilated in the tissues analyzed, except where *Undaria* was absent. Blades of the green *Ulva* spp. and brown *D. dichotoma* were often recorded in gut contents but not assimilated in either tissue at any of the three sites, despite being the two most abundant algal resources in these gulfs (Epherra, 2016). *Ulva* spp. are frequently consumed and preferred by several species of sea urchins (Navarrete et al., 2008), whereas *D. dichotoma* is avoided presumably due to the presence of deterrent secondary metabolites (Cobb and Lawrence, 2005; Gil et al., 2021). The fact that these algae were ingested but not assimilated may indicate that *A. dufresnii* is an opportunistic omnivore, like many other regular sea urchins (Emson and Moore, 1998), or an omnivore with a tendency to carnivory when animal items are available. Previous studies analyzing fatty acids in gonads found that *A. dufresnii* fed on brown algae species in Nuevo Gulf, although the author also indicated an omnivorous diet (Zárate, 2014). To our knowledge, there are no similar studies for San José Gulf, but our results

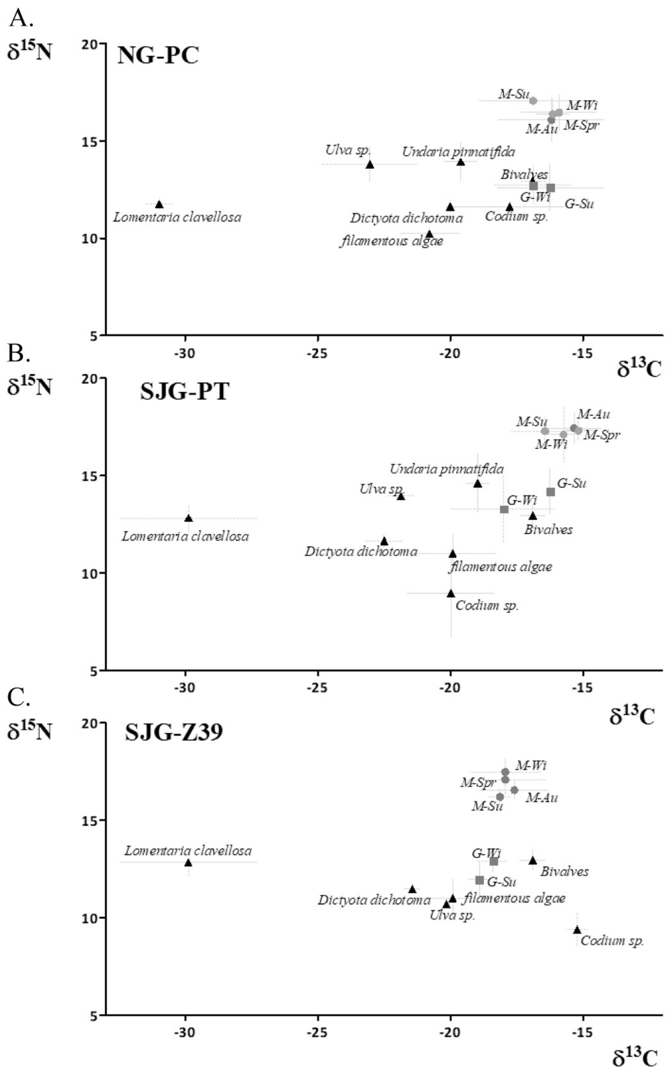

Fig. 3. Mean values (±SD) of stable isotope values of carbon and nitrogen of the potential food sources and tissues in different seasons of the sea urchin *Arbacia dufresnii* from: a) Nuevo Gulf-Punta Cuevas (NG-PC), b) San José Gulf-Punta Tehuelches (SJG-PT), and c) San José Gulf-Zona 39 (SJG-Z39). G: gonadal tissue, M: muscle tissue, Au: autumn, Su: summer; Wi: winter.

suggest that these populations could assimilate more animals than algae. In contrast, *Codium* spp. were frequently observed in gut content and were assimilated in both tissues. The siphonous structure of *Codium* spp. could be easier to eat and assimilate rather than the parenchymatous and double layered cell arrangement of *Ulva* blades. Likewise, *Undaria* was assimilated in tissues with high or low metabolic rates. These results support the suggestion made by Suárez Jiménez et al. (2017) that this invasive kelp can play a similar role to that of native algae as a food resource.

The isotopic values here found in both tissues of *A. dufresnii* studied did not vary between seasons, suggesting that only one season sampling would provide representative information of the diet. The gonads showed a higher proportion of assimilated algae than the muscle. Although gonads are lipid-rich tissues and, therefore, the depletion in δ^{13}C can reduce the accuracy in identifying the kinds of source material (Polunin et al., 2001; Post, 2002), gonads have also fast turnover rates and short-term dietary histories (Polunin et al., 2001). While the Aristotle's lantern muscle is the most recommended tissue for isotopes analysis in sea urchins (Cabanillas-Terán et al., 2019), our results showed that gonads can provide information regarding assimilated algae species, helping to understand omnivorous feeding habits. In addition, the phenomenon of isotopic routing must be considered when analyzing the diet of *A. dufresnii* because it accounts for the differential allocation of isotopically diverse sources to specific tissues (Gannes et al., 1998). Although in the present study the mean values of both isotopes in the gonads were not different between sites, the mixture models showed different contributions of source items in the diet. Instead, the muscular tissue analysis of *A. dufresnii* showed different assimilated sources among sites. Both the highest δ^{15}N value observed in sea urchins from SJG-PT and the fact that the contribution of bivalves was greater than 80% support carnivorous habits in this site. This result is consistent with the fact that both recruits of bivalves are an important item of the diet of *A. dufresnii* (Penchaszadeh and Lawrence, 1999; Zaixso, 2004) and a higher abundance of bivalves in the field in this site (Epherra, 2016). Since the δ^{13}C isotope provides information of the ultimate carbon source for an organism (Post, 2002), the ^{13}C enrichment found in sea urchins from invaded sites supports the hypothesis that the incorporation of *Undaria* could modify the use of resources and,

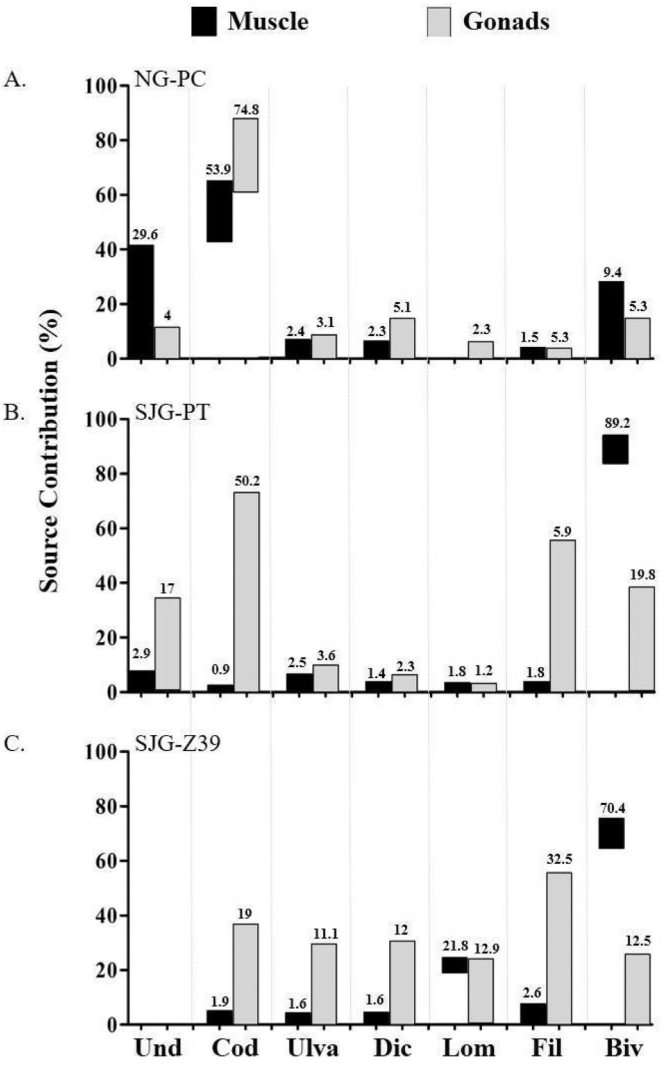

Fig. 4. Range of contribution percentages of the potential food sources to the diet of the sea urchin *Arbacia dufresnii* in a) Nuevo Gulf-Punta Cuevas (NG-PC), b) San José Gulf-Punta Tehuelches (SJG-PT), and c) San José Gulf-Zona 39 (SJG-Z39), according to the type of tissue used (muscle or gonad). Bars show the percentage values between 5 and 95% of possible contributions. The number above the bar means the average contribution. Und: *Undaria pinnatifida*, Cod: *Codium* spp., Ulva: *Ulva* spp., Dic: *Dictyota dichotoma*, Lom: *Lomentaria clavellosa*, Fil: filamentous algae (*Ceramium* sp., *Polysiphonia* spp.), and Biv: bivalves (*Mytilus edulis platensis*, *Aulacomya atra atra, Aequipecten tehuelchus*).

consequently, the trophic structure of the associated communities.

The consumption of an invasive alga species by a native grazer is a fundamental issue to understand its potential impact on the food web and ecosystem structure in invaded habitats (Deudero et al., 2011; Suárez Jiménez et al., 2015). For example, in New Zealand, laboratory feeding assays have shown that *Undaria* can contribute to the local food web because it is consumed by the amphipod *Aora typica* and the gastropods *Cookia sulcata* and *Haliotis iris* at consumption rates comparable to those of native algae (Suárez Jiménez et al., 2015). In addition, in sandy beaches of New Zealand, the amphipod *Bellorchestia quoyana* consumes *Undaria* at higher rates than native kelps (Suárez-Jiménez et al., 2017). Moreover, the native crab *Pugettia producta* consumes *Undaria* and the native kelp *Macrocystis pyrifera* in similar proportions, which was its preferred food prior to the invasion of the kelp *Undaria* in California, USA (Thornber et al., 2004). In these cases, the ecological similarity between kelps could favor the consumption of *Undaria*. In contrast, native algal communities in the study area do not include species that are morphologically or functionally similar to *Undaria*. Despite the differences, *A. dufresnii* fed on *Undaria* and our results clearly show changes in the diet of the sea urchin between invaded and non-invaded sites. In addition, the time since *Undaria* became established on the invaded habitat could influence the grazing and assimilation by *A. dufresnii*. When algae are introduced to a new habitat, native grazers may not immediately recognize invasive algae as food or be unable to avoid biochemical compounds at the early stages of invasion (Trowbridge, 1995; Shea and Chesson, 2002; Callaway and Ridenour, 2004; Crooks, 2011). At the site that had been invaded for more than 20 years, *A. dufresnii* fed on *Undaria* both when sporophytes were mature and during the senescence stage. In contrast, at the site where the *Undaria* invasion had less than 8 years, *A. dufresnii* consumed only senescent *Undaria*. Although these differences may be due to particular characteristics of each gulf, at the early stage of invasion sea urchins may not be able to tolerate biochemical deterrents accumulated on reproductive and young parts (Hay and Fenical, 1988; Teso et al., 2009; Lawrence et al., 2013). Thus, in a recent invasion scenario, *A. dufresnii* fed and assimilated *Undaria* in its tissues only when blades and sporophylls were in a degradation process. Whilst further studies are needed to explore this hypothesis, the consumption and assimilation of *Undaria* by *A. dufresnii* could increase over the course of the invasion process.

In summary, the native sea urchin *A. dufresnii* fed on the invasive kelp *Undaria* and was able to assimilate it in its tissues. The contribution of *Undaria* to the diet of *A. dufresnii* varied between invaded sites, being consumed and assimilated in greater proportion in the site with longer invasion history. The results of this work highlight the importance of complementing techniques to determine the contribution of food sources to the diet of omnivores. Although the impacts of invasive algae on food webs and ecosystem structure are difficult to evaluate, and more studies are needed to explore the effect of invasion time, this baseline study in northern Patagonian gulfs may allow better understanding of the local food web structure before and after the invasion of *Undaria*.

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Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fooweb.2022.e00221.

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