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ESTIMATING BENTHIC TROPHIC LEVELS TO ASSESS THE EFFECTIVENESS OF MARINE PROTECTED AREA MANAGEMENT

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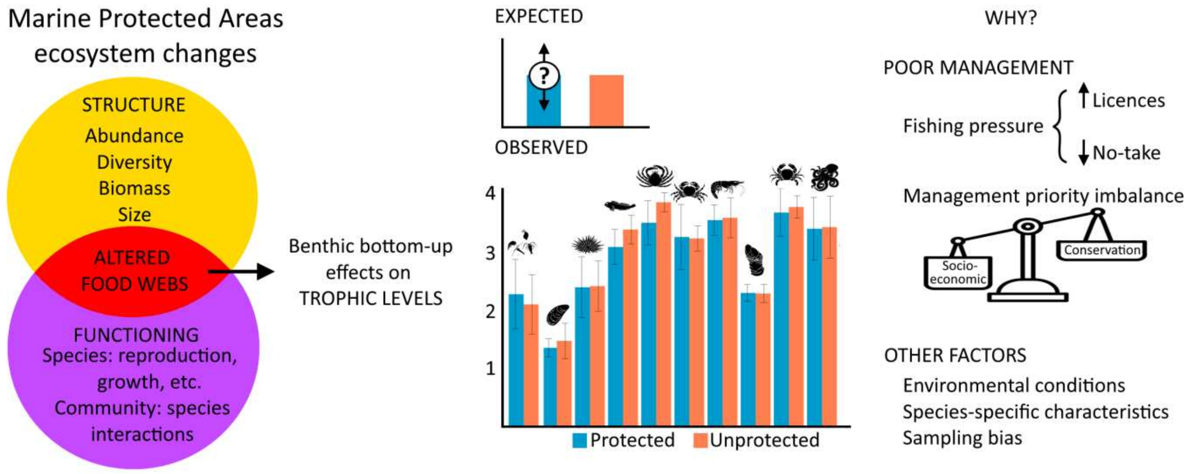
GRAPHICAL ABSTRACT



HIGHLIGHTS

- Bottom up effects of MPAs on trophic level are often ignored.
- Trophodynamics inside/outside MPAs can be assessed by stable isotopes analysis.
- Protection did not significantly affect trophic level in Illas Atlánticas MPA.
- Trophic levels in MPAs vary across locations but not across management regimes.
- Poorly managed MPAs do not appear to have protective effects on food web dynamics.

ABSTRACT:

Designating and managing marine protected areas (MPAs) can mitigate many ocean threats. Banning fishing activities within MPAs enhances the robustness of food-web dynamics and thus increases trophic resilience. Ecosystem function indicators, such as mean trophic level, are increasingly applied in conservation management. Stable isotope analysis is a common tool in trophodynamic studies as it provides information about food sources and trophic level within food webs. In contrast to the traditional top-down approaches in conservation management (mainly for fisheries), this study focuses on bottom-up responses to protection according to the target species in regional small-scale fisheries. The present study aimed to examine how MPA status affects trophodynamics in the rocky reefs of the Illas Atlánticas Marine-Terrestrial National Park (Galicia, NW Spain). Results showed no differences between inside and outside the MPA in species stable isotopic signatures or trophic level. However, these results should be considered with caution due to some limitations in the study design (small number of sites per location, biogeographic differences associated with the island nature of the MPA, or seasonal variability). Nevertheless, the lax fishing management, the lack of proper implementation (the MPA was established in 2002 without a management plan until 2019), and the small size of the studied MPA may result in ineffective conservation outcomes that could have been reflected in the stable isotopic content of the food web. The large number of "paper park" MPAs existing worldwide are not only detrimental to the perception of marine protection, but also provide poor protection of marine ecosystems. Subject to further studies accounting for both environmental and management factors on stable isotope signatures, trophic interactions can form a cost-effective tool for monitoring MPA effectiveness.

KEYWORDS: trophic interactions, stable isotopes, Illas Atlánticas Marine-Terrestrial National Park, ecosystem functioning, conservation

1. INTRODUCTION

Human activities have many deleterious effects on ecosystem structure and functioning in the marine environment, with subsequent degradation of associated ecosystem services (Crain *et al.* 2009, Halpern *et al.* 2015, Bishop *et al.* 2017). Such activities may affect ecosystem functioning by altering trophodynamic interactions within the seascape (Gascuel and Pauly 2009). The effects of local perturbations and coastal disturbances (e.g. habitat destruction and fishing) may quickly spread to different patches of the seascape through the food web (Albouy *et al.* 2019). Conservation actions must consider both biological features (e.g. ecosystems, species) and ecological processes (e.g. dispersal, migration, foraging) to ensure environmental persistence (Frid *et al.* 2008, Balbar and Metaxas 2019). Management and conservation measures such as banning/limiting exploitation and/or habitat protection are essential to address the increasing and accelerating tendency for ocean use (Bishop *et al.* 2017). As one of the most efficient conservation actions, the number of marine protected areas (MPAs) designated worldwide has increased in the last few decades (www.mpatlas.orgwww).

As a result of banning fishing activities within MPAs, both the abundance and size of predators increase (i.e. their overall biomass), thereby reducing the prey population (Micheli *et al.* 2004). However, when fisheries outside the MPA boundaries target prey species, their biomass may also increase (Guest *et al.* 2009), and thus the robustness of food-web dynamics and trophic resilience may also increase (Cheng *et al.* 2019). Fish biomass has been estimated to be 670% higher in no-take (fully protected) areas and 183% higher in partially protected areas (where artisanal fishing is allowed) than in unprotected areas (Sala and Giakoumi 2018). However, the increase in biomass within MPA boundaries can only be ensured in effectively managed and well-enforced MPAs (Soler *et al.* 2015). Changes in species biomass are directly related to the respective trophic level within the community (Pauly *et al.* 2001, Pinnegar *et al.* 2002). Hence, the aforementioned overall increase in biomass within MPAs is expected to lead to an increase in the overall trophic level of such ecosystems. However, other factors including environmental context, biotic interactions and

MPA characteristics (age, level of protection, and size of no-take area) also have significant effects on the conservation outcomes of MPAs, including ecosystem functioning variables (Edgar *et al.* 2014, Claudet 2018). Although the complexity of ecological processes in marine ecosystems is well known, most MPAs omit ecosystem functioning criteria in their design, which may jeopardize the outcomes of the management actions (Micheli *et al.* 2004, Frid *et al.* 2008).

Cascading effects derived from shifts in prey-predator dynamics as a result of human intervention, such as habitat destruction, overfishing and species protection, may alter species interactions within marine ecosystems (Pauly *et al.* 1998, Soler *et al.* 2015, Yabsley *et al.* 2016). Both bottom-up and top-down processes are associated with trophic cascades within MPAs, regulating species interactions from respectively lower to upper trophic levels and vice versa (Pauly *et al.* 1998, Pinnegar *et al.* 2000, Soler *et al.* 2015, Di Lorenzo *et al.* 2016). The trophic level defines the position of an organism within a food web (which can be based on primary producers or detritus) and is commonly used as an ecosystem indicator in fisheries management (Babcock *et al.* 2010, Bourdaud *et al.* 2016, Reed *et al.* 2017) and thus in MPA ecosystem functioning (Davis *et al.* 2019). A higher mean trophic level (estimated from mass-balanced models) occurs as a result of protection of species in mature well established MPAs, mainly due to the increase in abundance of top-predators (Colléter *et al.* 2014). Nevertheless, despite the close correlation between mass-balanced models and stable isotope analysis for estimating trophic levels (Du *et al.* 2020), the effect of MPA implementation on the stable isotopic composition of different organisms remains largely unknown.

The effects of altered food webs at multiple trophic levels are still poorly understood (Griffith *et al.* 2018). At the base of food webs, changes in community structure encompass species-specific shifts in stable isotope signatures and trophic level in long-term no-take areas (Guest *et al.* 2009, Hofmeister 2018) and 20 months after trawling cessation (Dannheim *et al.* 2014). Similarly, a recent meta-analysis of predator trophic levels, determined by stable isotope analysis, revealed a higher mean trophic level outside MPAs

(De Lope Arias *et al.* 2016), even though predator biomass, abundance and size are known to increase within MPA boundaries (Cheng *et al.* 2019). However, the meta-analysis mainly considered areas that were only partly protected, which tends to jeopardize conservation goals relative to full protection (no-take MPAs) (Zupan *et al.* 2018).

Stable isotope analysis is an indirect approach that is commonly used in trophodynamic studies as it provides information on assimilated food sources over a longer time scale than obtained by gut content analysis (Box *et al.* 2010, Deudero *et al.* 2011, Layman *et al.* 2012). Such timescales are closely related to the sampled tissue turnover rate, so that the lower the turnover rate the longer the timescale (Blanco *et al.* 2009, Cabanellas-Reboredo *et al.* 2009, Deudero *et al.* 2009). This approach relies on the relationship between the lighter isotopes (^{12}C and ^{14}N) and the heavier isotopes (^{13}C and ^{15}N), which may help decipher the origin and pathways of organic matter of primary food sources ($^{13}C/^{12}C$) and clarify the environmental conditions and trophic position of consumers ($^{15}N/^{14}N$) (Post 2002, McCutchan *et al.* 2003, Caut *et al.* 2009, among others). Stable isotope analysis is a powerful tool in trophodynamic ecological studies (Post 2002), and it can even indicate the effectiveness of management by providing relevant information on foraging grounds, biogeographic distribution and anthropogenic impacts (Pearson *et al.* 2017, Pethybridge *et al.* 2018, Raoux *et al.* 2020, Signa *et al.* 2020).

Understanding the function and structure of food webs is essential to improve predictive approaches and ecological indicators for effective application of management actions. Traditionally, efforts have mainly been directed to higher trophic guilds (predators), and information on lower trophic positions in the food web is scarce (but see Guest *et al.* 2009, Dannheim *et al.* 2014, Hofmeister 2018). The present study aims to tackle this challenge by deciphering the effects of bottom-up protection by studying the stable isotopic signatures of the food web in an MPA off the west coast of the Iberian Peninsula (the Illas Atlánticas Marine-Terrestrial National Park) as an indicator of how conservation measures affect the underlying trophic interactions. Understanding how the lower fractions of the food web are modified in MPAs should improve the methods available for evaluating MPA performance.

2. MATERIALS AND METHODS

2.1. Study area

The Illas Atlánticas Marine-Terrestrial National Park (Galicia, NW Spain) is a terrestrial and marine reserve formed by four main archipelagos that extend over a distance of 40 km in a north-south direction and cover a sea marine area of up to 728.52 km2 (Figure 1). In the present study, only the three archipelagos facing open sea were studied (from north to south): Sálvora island, the Ons and Onza islands, and the Cíes islands, each located in front of a ria *sensu lato* (Cotton 1956). The Cíes islands archipelago was split into two different locations for sampling: the northern Monteagudo-Faro islands (two islands connected by a permanent sandbar) and the southern San Martiño island (separated from the northern islands by a channel of width ~500 m). In addition, management regulations are different in the northern and southern Cíes islands, with more restrictive management in terms of permitted uses in the latter (mainly due to the fact that only the northern islands are accessible to the public through tourist cruises and hold tourist services such as accommodation and restaurants).

2.2. Field sampling

Sampling was conducted seasonally between March 2019 and September 2020, to prevent any potential bias towards a favourable sampling season. Nevertheless, seasonal analysis was not feasible for various reasons: weather conditions did not allow field sampling, absence of some species during certain seasons and/or pandemic restrictions. Thus, in order to deal with any seasonal bias in isotopic signatures and in accordance with the low turnover rate of the sampled tissues (MacAvoy *et al.* 2001, Blanco *et al.* 2009, Deudero *et al.* 2009), species-specific samples at each location were pooled (Michener and Kaufman 2008) (Supplementary Material Table 1).

Four locations were sampled inside the MPA, and another four locations were sampled outside the MPA as reference locations (N total locations = 8, Figure 1). The island nature of the MPA does not allow for similar reference locations. Therefore, in order to minimize habitat-related differences, sampling locations (both inside and outside MPA) were selected according to similar benthic assemblages on hard bottom habitat (i.e. rocky reefs) dominated by kelp forests, which also determined the wave-facing orientation of the sampling sites. At each location, two 50 m underwater (8 m depth) transects separated by a distance of more than 50 m were sampled, with the aim of assessing the main common trophic guilds potentially representative of the base of the food web: particulate organic matter (POM), zooplankton, primary producers (*Asparagopsis armata, Codium tomentosum*, *Dyctiota dichotoma*, *Saccorhiza polyschides*/*Laminaria ochroleuca* referred to as kelp hereafter, *Ploclamium* sp., and *Ulva* sp.), filter feeders (*Mytilus galloprovincialis*), herbivores (*Paracentrotus lividus*), omnivores (*Lipophrys pholis*, *Maja* sp., *Pachygrapsus marmoratus*, *Palaemon sp.,* and *Pollicipes pollicipes*) and carnivores (*Necora puber* and *Octopus vulgaris*). More specifically, the sampling included the POM of the upper (50 cm) sea surface layer (N = 63), the plankton community in the subsurface (2 m) layer (N = 57), six different macroalgal species (N = 312), up to eight different species of macroinvertebrates (N = 292) and one fish species (N = 27) (Table 1). The POM was sampled by vacuum filtering 3 L of seawater through a pre-combusted (450 ºC, 4 hours) Whatman GF/F glass microfibre microfiber filter. Plankton samples were collected using a WP2 plankton net (250 µm mesh size, 45 cm diameter), which was towed through the subsurface of the water for 15 minutes at a velocity of 2 knots. Samples of macroalgae, sea urchins, shrimps, stalked barnacles, mussels, intertidal crabs and fish, as well as other macroinvertebrate fauna (when available), were collected by hand or by scuba diving in the intertidal and deeper zones respectively. Samples not harvested by hand were provided by local fishers and fishing guilds. In order to avoid unnecessary death of the animals, when possible, one leg of each *Maja* sp., *N. puber* and *P. marmoratus* specimen and a piece of arm (of length about 4-5 cm) from each octopus (*O. vulgaris*) were sampled. Individual size of *L. pholis* (total length), *M. galloprovincialis* (shell length), *P. pollicipes* (rostro-carinal length) and *P. lividus* (test diameter) were measured to the nearest 0.01 mm. All samples were frozen immediately after sampling and stored at -20 ºC until further processing.

2.3. Stable isotope analyses

Shell-free and bone-free muscle samples were taken from all macroinvertebrates and fish, respectively. Individual fronds of macroalgae were sampled when possible (*C. tomentosum*, *D. dichotoma*, Kelp, *Ploclamium* sp., and *Ulva* sp.) or otherwise were pooled (*A. armata*). All samples were rinsed thoroughly with tap water, dried at 60 ºC for 24 h and then ground to a fine powder in a mortar and pestle. According to the study objectives and due to the lack of consensus on the pre-treatment acidification of non-highly carbonated species (Pomerleau *et al.* 2014, Pires-Teixeira *et al.* 2020), the samples were not acid-washed. A homogeneous dried powdered extract (1.7mg ± 0.2) of each sample was placed in a cadmium tin cup for analysis of the $\delta^{13}C$ and $\delta^{15}N$ stable isotope compositions by continuous flow isotope ratio mass spectrometry (CF-IRMS) in a THERMO Finnigan MAT 253 mass spectrometer. Reference standards were included for C, IAEA-C6 sucrose, and N, IAEA-N-2 ammonium sulphate. One sample of an internal reference was analysed every 10 samples in order to calibrate the system and compensate for the drift over time. The analytical precision of the stable isotope analyses was based on the standard deviation of the IAEA-C6 and IAEA-N-2 samples: 0.09‰ for $\delta^{13}C$ and 0.11‰ for $\delta^{15}N$.

Stable isotope signatures were measured by comparison of the ratio of the most abundant isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) in the sample and the international isotopic standards. Stable isotopic ratios of carbon and nitrogen were expressed in δ notation, in parts per thousand (‰) deviations from the standards according to the following equation:

$$\delta X = [(Rsample / Rreference) - 1] \times 10^3, \qquad \text{Equation 1}$$

where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The trophic level of the organisms was calculated using the following formula (Minagawa and Wada 1984):

$$Trophic\ level = (\delta^{15}Nconsumer - \delta^{15}Nbase)/ NEF + \lambda, \qquad \text{Equation 2}$$

where $\delta^{15}Nbase$ is the $\delta^{15}N$ isotopic signature of the base of the food web, λ is the trophic level of $\delta^{15}Nbase$ and NEF is the assumed $\delta^{15}N$ trophic enrichment factor. For $\delta^{15}Nbase$, we used the mean $\delta^{15}N$ for primary producers (macroalgae and POM) at each location and assumed a λ value of 1. Species-specific *NEF* were considered when available (Hobson and Cherel 2006, Vanderklift *et al.* 2006, Dubois *et al.* 2007, Henschke *et al.* 2015), otherwise a general *NEF* (specific for the trophic guild) was applied (McCutchan *et al.* 2003, Caut *et al.* 2009, Henschke *et al.* 2015), in order to improve the accuracy of trophic estimates to apply proper ecosystem-based management (Du *et al.* 2020, Raoux *et al.* 2020).

2.4. Statistical analyses

Protective effects of the MPA on whole community isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ were analysed by fitting linear mixed models with Protection as a fixed factor (Protected vs Unprotected) and Location (South Cies, North Cies, Ons, Sálvora, Cape Silleiro, Cape Couso, O Grove, Cape Corrubedo) and Species (*A. armata, C. tomentosum*, *D. dichotoma*, Kelp, *Ploclamium* sp., *Ulva* sp., POM, zooplankton, *M. galloprovincialis, P. lividus*, *Lipophrys pholis*, *Maja* sp., *P. marmoratus*, *Palaemon sp., P. pollicipes N. puber*, and *O. vulgaris*) as random effects. The effects of Protection (fixed factor, two levels) on the stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) and trophic level of each species were analysed with linear mixed-effect models including size (when available) as a covariate and Location (eight levels) as a random effect. All models were run using the *lmer* function (Bates *et al.* 2015), and the significance was evaluated by ANOVA with Satterthwaite approximation for degrees of freedom (Kuznetsova *et al.* 2017). Despite the robustness of linear mixed-effects models, which allow for violations of model assumptions (Schielzeth *et al.* 2020), models violating the normality of fixed-effects residuals were checked for outliers and re-run after elimination of these, to ensure that no severe violations of the assumptions occurred. However, the models that severely violated the assumption of normality ($\delta^{13}C$ of *O. vulgaris* and $\delta^{13}C$ of *D. dichotoma*) and those with singular boundary fit ($\delta^{15}N$ of *O. vulgaris*, $\delta^{13}C$ of Kelp and $\delta^{13}C$ of *Ulva* sp.) were averaged by Location and re-run as linear models in order to assess the effects of protection (fixed factor) on the stable isotopic signatures. In addition, the *O. vulgaris* trophic level model was bound at zero for the random effect

(Location), which led to a singular model that required more parsimonious parameterization (Bates *et al.* 2015) and was therefore simplified into a linear model with Protection as a fixed factor. Species for which trophic level models had significant random effects of Location were refitted in linear models with Location as a fixed factor in order to assess the effects of Location on species trophic level to evaluate potential trends related to location. Tukey's post-hoc tests were implemented using the *multcomp* package. Trophic level analysis was not used for primary consumers as these were considered the basal food sources in the food web, and therefore TL = 1 was assumed for all of them. All statistical computations were conducted in R version 4.0.3 (R Core Team 2020) with R studio interface (R Studio Team 2020).

3. RESULTS

The overall range of $\delta^{13}C$ at the base of the food web ranged from -32.82‰ ± 1.27 to -14.40‰ ± 1.32 in protected locations and from -32.86‰ ± 0.81 to -13.18‰ ± 1.88 in unprotected locations (Table 1). The $\delta^{15}N$ signatures ranged from 5.43‰ ± 0.71 to 11.35‰ ± 0.61 inside the MPA and from 5.10‰ ± 0.53 to 12.02‰ ± 0.78 outside the MPA (Table 1). More precisely, the $\delta^{13}C$ signatures of the primary producers (including macroalgae and POM) ranged widely from -32.82‰ ± 1.27 to -16.84‰ ± 1.44 within protected locations areas and from -32.86‰ ± 0.81 to -16.51‰ ± 1.06 in unprotected locations. Conversely, the ranges of the $\delta^{15}N$ signatures of primary producers were narrower, varying from 5.43‰ ± 0.71 to 6.15‰ ± 0.78 in protected locations and from 2.79‰ ± 1.53 to 5.10‰ ± 0.53 in unprotected locations. The $\delta^{13}C$ signatures of zooplankton, herbivores and filter feeders were respectively -20‰ ± 0.77, -16.94‰ ± 1.28 and -17.30‰ ± 0.25, in protected locations, and -20.59‰ ± 1.49, -17.78‰ ± 1.88 and -17.30‰ ± 1.05, in unprotected locations. Finally, $\delta^{13}C$ isotopic values for primary consumers ranged from -17‰ ± 0.22 to -14.40‰ ± 1.32 in protected locations and from -18.45‰ ± 0.65 to -13.18‰ ± 1.88 in unprotected locations, while for $\delta^{15}N$, the values ranged from 10.47‰ ± 0.94 to 11.35‰ ± 0.61 in protected areas and from 9.80‰ ± 1.54 to 12.02‰ ± 0.78 in unprotected locations. At the community level, stable isotopic signatures of δ^{13}C and δ^{15}N (Figure 2) were not significantly affected by protection ($p = 0.55$ and $p = 0.35$, respectively), although random effects of location proved significant for both δ^{13}C ($p < 0.001$) and δ^{15}N ($p = 0.003$), when controlled by species (Table 2A; Supplementary Material Figure 1). Protection did not have significant effects on δ^{13}C and δ^{15}N for any of the species studied (except *Ulva* sp. for δ^{13}C, and the protection x size interaction for δ^{13}C of *M. galloprovincialis*), while the effects of location at both δ^{13}C and δ^{15}N were species-specific (Table 2B). Size had significant effects on δ^{13}C of *P. pollicipes* and *P. lividus* and on δ^{15}N of *L. pholis* and *P. lividus*).

The mean trophic levels (TLs) of organisms in the food web under study (excluding primary producers) ranged from 1.34 ± 0.16 to 3.66 ± 0.41 in protected locations and from 1.46 ‰ 0.30 to 3.83 ± 0.16 in unprotected locations (Table 1). The mean TL values for locations in the MPA were not significantly different from those in unprotected locations (Table 3). However, the mean TL differed significantly between locations, although only between South Cíes and Cape Silleiro and Ons (Table 3). Species-specific mean TL (± SD) ranged from 1.34 ± 0.16 (for *M. galloprovincialis*) to 3.66 ± 0.41 (for *N. puber*) in protected locations and from 1.46 ± 0.30 (for *M. galloprovincialis*) to 3.83 ± 0.16 (for *Maja* sp.) in unprotected locations (Figure 3, Table 1). Species-specific models showed non-significant effects of protection on the TL for all of the studied species, and ontogenetic differences (size) in TL were only significant for *P. lividus* (Table 3). Location mainly explained the variation in TL for most of the species, regardless of the type of protection, except for *O. vulgaris*, *P. lividus* and zooplankton (Table 3). The main effects of Location on trophic level were species-specific: the trophic levels of *O. vulgaris*, *Palaemon* sp. and *P. lividus* were not affected by location, while those of *L. pholis*, *M. galloprovincialis*, *Maja* sp., *N. puber*, *P. marmoratus*, *P. pollicipes* and zooplankton varied significantly between locations (Figure 4, Table 3). The trophic level of *L. pholis* was higher in O Grove and Sálvora than in South and North Cíes, while it was also higher in Cape Couso than in South Cíes. For *M. galloprovincialis*, the trophic level was higher in Cape Corrubedo than in any other location, except North Cíes and Ons. The trophic level of *Maja* sp. was lower in South Cíes than in Cape Silleiro, Ons and Cape Corrubedo. The trophic levels of *N. puber* was higher in Sálvora than in Ons and the trophic level of *P. marmoratus* in Sálvora was higher than in the South Cíes, Cape Couso, Ons, O Grove and Cape Corrubedo. The trophic level of *P. marmoratus* was higher in Cape Silleiro than in South Cíes. The trophic level of *P. pollicipes* was lower in Cape Silleiro and South Cíes than in North Cíes (where it was also higher than in O Gove and Sálvora), Cape Couso, Ons and Cape Corrubedo. Finally, the trophic level of zooplankton was higher in Ons than in Cape Silleiro.

4. DISCUSSION

The present study provides the first relevant multi-species information on stable isotope signatures throughout the boundaries of the Illas Atlánticas Marine-Terrestrial National Park and surrounding unprotected areas. The study findings indicated that, despite certain limitations discussed later on, protection has no effects at the lower levels of the trophic web in the MPA under study (Figure 2 and 3). The findings also revealed location-specific differences at the species-specific level (Figure 4). The incorporation of this type of information in spatial planning is essential to improve ecosystem-based management actions (Bourdaud *et al.* 2016, Reed *et al.* 2017, Tam *et al.* 2017), and, therefore, the data presented here could help understanding the ecosystem functioning within and around the studied MPA to further enhance its efficiency.

Studies comparing multi-trophic level stable isotope signatures at the base of the food webs inside and outside MPAs are scarce and mostly focused on local single MPAs (Guest *et al.* 2009, Dannheim *et al.* 2014, Hofmeister 2018). Dannheim *et al.* (2014) reported no significant effects of protection on community trophic level after cessation of trawling (despite minor species-specific changes). However, the time scale of the study (20 months) was shorter than the mean time (5.13 years) required to detect direct effects on fisheries target species and even much shorter than that required (13.1 years) at a community level through indirect trait-mediated effects (Babcock *et al.* 2010). This constraint may be even more important when considering trawl fisheries, which are known to have strong impacts on benthic organisms, sediment dynamics and trophic interactions that may take longer than 20 months to recover (Dannheim *et al.* 2014). The MPA under study was designed in 2002 to provide ecosystem resilience while allowing for artisanal professional fisheries (BOE, July 1st 15/2002). Although the threshold time to detect indirect effects at a community level (13.1 years) has been reached, the management plan for the MPA has only recently been published (PRUX PNMTIA, 2019). In addition, despite removing fishing pressure by establishing no-take areas has resulted in substantial species-specific shifts in stable isotope signatures (Guest *et al.* 2009, Hofmeister 2018), the current MPA design includes very small no-take and restricted areas (0.44% and 10.02%, respectively) and an overall fishing ground of over 89%. Hence, the known benefits of MPA zonation (partly protected areas surrounding no-take areas) through spillover effect (Di Lorenzo *et al.* 2020), may be jeopardized by the large impacts of small-scale artisanal fisheries across the food webs (Coleman *et al.* 2013, Corrales *et al.* 2020).

In the present study, no differences between protected and unprotected marine areas in the stable isotope composition were found at either the community or at species-specific level (Table 2); not even in species targeted by artisanal fisheries both inside and outside the MPA, namely *P. lividus*, *M. galloprovincialis*, *Maja* sp., *P. pollicipes*, *N. puber* and *O. vulgaris* (Freire and García-Allut 2000, Ouréns *et al.* 2015). Most consumer species studied here are targeted by the Galician small-scale artisanal fisheries (Cambiè *et al.* 2012), both inside and outside the MPA, which may lead, through cascading, to the overall equivalent trophic levels. In addition, the small scale of the studied MPA may also contribute to the homogenized stable isotopic composition as both prey and predators could move inside and outside the MPA. However, this is unlikely since most mobile species considered (*L. pholis*, *Maja* sp., *N. puber*, *O. vulgaris*, *P. lividus*, *P. marmoratus*, and *Palaemon* sp.) are sedentary or have relatively small home ranges (Cannicci *et al.* 1999, Bernárdez *et al.* 2003, Hereu 2005, Silva *et al.* 2014, Roma *et al.* 2018, Arechavala-Lopez *et al.* 2019). A homogenization of TL has been also associated with a spillover effect towards unprotected locations or to a lack of fishery limitations within protected locations (Branch *et al.* 2010).

Accordingly, the overall high levels of artisanal fishing activity (3235 12-m vessels in 2004) in the study region (from Cape Corrubedo to Cape Silleiro) is reflected by the high fishing pressure (with over 19 vessels per square km) described in only one of the three archipelagos (Cíes Islands) that form the MPA (Freire and García-Allut 2000, Cambiè *et al.* 2012, Ouréns *et al.* 2015), which may limit the potential spillover effects.

Some limitations of the present study should be considered for a conclusive interpretation of the results. Other than fisheries (Pinnegar and Polunin 2000), further factors such as species ontogeny, environmental changes in space and time and prey availability strongly influence stable isotope composition (De Lope Arias *et al.* 2016, Reed *et al.* 2017) and, subsequently, species trophic levels. Moreover, one of the main constraints of the present study was the location of reference sites (mainland), which were geographically different from protected sites (islands) by nature. While recognizing such limitations, deciphering the effects of local characteristics (both environmental and community-related) on tropho-dynamic relationships is beyond the scope of the present study and should be further investigated

Differences in TL and the $\delta^{13}C$ and $\delta^{15}N$ content were location-specific, which suggests a stronger influence of local environmental factors or population characteristics than that of protection. Changes in the main trophic level within MPAs are commonly related to the inclusion of top predators looking for shelter and foraging grounds (Pinnegar and Polunin 2000). However, when considering shellfish fisheries, the overall outcome of protection on food web dynamics remains unclear, as most studies focus on the effects of protection on community composition and richness, and species biomass, abundance and size (Lemasson *et al.* 2019). Local disturbances (i.e. species-specific fisheries) in species-specific trophodynamics (Supplementary Material Table 2) may also be offset by a myriad of trophic interactions in marine ecosystems (Albouy *et al.* 2019), especially in generalist feeders (Lawton *et al.* 2012) targeted by fisheries as is the case of species included in this study (Dunne 1977, Guerra 1978, Milton 1983, Bernárdez *et al.* 2000, Cannicci *et al.* 2002, Aguzzi *et al.* 2005, Janas and Baranska 2008, Silva *et al.* 2010). Generalist feeders are less susceptible to changes in abundance and density of their prey, which will therefore mask potential changes in the trophic web (Dannheim *et al.* 2014) as may have occurred in the present study. Nevertheless, shifts in the stable isotope composition of keystone species have been reported for the lobster *Jasus edwardsii* and the pen-shell *Pinna nobilis* with depleted $\delta^{15}N$ values within no-take areas in Tasmania (Guest *et al.* 2009) and the Mediterranean (Alomar *et al.* 2015), respectively. Such depletion was the result of targeting easier and more available prey of lower trophic level when feeding inside MPAs (Guest *et al.* 2009) and the influence of anthropogenic nutrient inputs outside MPAs (Alomar *et al.* 2015). Conversely, no significant differences were found between protected and unprotected areas in the $\delta^{15}N$ of *Crassotrea gigas* and *Perna canaliculus* in New Zealand (Salomon *et al.* 2008), or in *Octopus bimaculatus* in South California that shifted prey sources (evidenced by changes in $\delta^{13}C$) of the same trophic level (similar overall $\delta^{15}N$) (Hofmeister 2018). Species not targeted by fisheries (including macroalgae, sea urchins, common snails, bivalves and crabs) showed negligible effects of protection on the $\delta^{13}C$ and $\delta^{15}N$ signatures even in long-term no-take areas (Guest *et al.* 2009, Dannheim *et al.* 2014, Hofmeister 2018).

Banning fishing activity often results in low social acceptance and in trades-off between conservation and socioeconomic objectives (Voyer *et al.* 2014, Basurto *et al.* 2017). However, a global assessment reported increasing abundance (385%) and biomass (820%) of highly targeted invertebrate species within no-take areas relative to fished areas (Lester *et al.* 2009), but also relative to partially protected areas (Sciberras *et al.* 2015). Hence, in order to better balance ecological and socioeconomic benefits and conservation goals, a common practice in ecosystem-based management is to combine partly protected areas and no-take areas within single or multiple MPAs (Halpern *et al.* 2010, Sala and Giakoumi 2018, Zupan *et al.* 2018, Corrales *et al.* 2020, Di Lorenzo *et al.* 2020). This is particularly relevant in regions such as the study area, in which up to 60% of the total employment is related to fisheries (Villasante *et al.* 2016). Coupling marine spatial planning and ecosystem-based management is especially challenging in such highly dynamic ecosystems and, therefore, requires dynamic management actions (Game *et al.* 2009, D'Aloia *et al.* 2019). The MPA under study is, however, spatially fixed by nature. Integration of regional conservation measures in a dynamic decision framework of zonation prioritization will enhance MPA network goals (Beger *et al.* 2015), mainly in regions with highly complex fishery activity such as the studied MPA and surrounding areas (Ouréns *et al.* 2015).

The bottom-up trophodynamic processes presented here provide a new perspective for marine management, which traditionally focuses on top-down regulations from fisheries and which has recently shifted towards more holistic ecosystem-based management (Tam *et al.* 2017). Marine protected areas are not ecologically isolated and cannot mitigate the effects of anthropogenic disturbances, including climate change (Bates *et al.* 2019) or invasive species (Blanco *et al.* 2020). Integrating ecological and human-related processes in conservation management actions is extremely challenging (Frid *et al.* 2008, Beger *et al.* 2010, Balbar and Metaxas 2019), especially in small MPAs in which species home ranges go beyond MPA boundaries (Green *et al.* 2015). In the last decade, conservation targets have moved from single species/habitat towards preservation of ecosystem functioning in seascapes considering long-term changes due to climate change, emerging new uses, policy changes and technological advances (Beger *et al.* 2010, Engelhard *et al.* 2017, Gissi *et al.* 2019). However, species not only interact horizontally in space, but also vertically in the trophic web, and the incorporation of trophodynamics in marine management will hopefully be the next step in conservation research.

5. CONCLUSION

This research, in which the Illas Atlánticas National Park was considered a case study, provides insights into how baseline sources and the lower trophic community respond to MPA designation and management. The observed limited effects of protection and the highly location-dependant variability indicate that although other factors (mainly particular environmental conditions) may influence location-specific effects, the fishing regulations within the MPA do not have effects in the trophic level at the base of the food web when compared to unprotected (fished) areas. Despite study limitations described, the overall high human use of the MPA and the limited effects of protection on marine food webs reported suggest that the zonation design and the MPA management should be revised taking into account trophic data (in order to ensure functioning resilience within the MPA). More restrictive actions may not be well accepted in a highly fishing-dependant society. However, the resulting benefits of larger no-take areas should provide both short-term resources and long-term ecological outcomes, particularly if the design of the MPA links natural and socio-economic variables in order to endorse management policies by using decision-support software tools. Moreover, including ecosystem functioning (particularly prey-predator interactions and trophic indicators) in conservation plans would greatly improve ecological outcomes and the services provided.

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Table 1. Mean (± SD) stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) and trophic level (TL) of the target species grouped by trophic guilds within the Illas Atlánticas Marine-Terrestrial National Park (protected) and reference (unprotected) locations.

	PROTECTED							**UNPROTECTED**						
		$\delta^{13}C$ (‰)		**$\delta^{15}N$ (‰)**		**TL**			**$\delta^{13}C$ (‰)**		**$\delta^{15}N$ (‰)**		**TL**	
	N	Mean	SD	Mean	SD	Mean	SD	N	Mean	SD	Mean	SD	Mean	SD
Primary producers														
Asparagopsis armata	22	-30.92	1.46	6.15	0.78	-	-	19	-30.93	1.16	5.36	0.33	-	-
Codium tomentosum	29	-19.00	2.41	5.64	1.12	-	-	29	-18.92	2.13	5.33	0.80	-	-
Dyctiota dichotoma	23	-20.99	2.01	5.43	0.71	-	-	22	-21.03	1.29	5.10	0.53	-	-
Kelp	31	-20.09	3.18	5.56	1.25	-	-	32	-20.15	3.17	5.79	1.53	-	-
Ploclamium sp.	27	-32.83	1.27	5.56	0.80	-	-	28	-32.86	0.81	5.50	0.80	-	-
Ulva sp.	25	-16.84	1.44	5.77	0.74	-	-	25	-16.51	1.06	5.48	1.19	-	-
Particulate organic matter						-	-						-	-
POM	33	-20.29	3.38	5.92	1.08	-	-	30	-20.68	3.36	5.78	1.71	-	-
Zooplankton														
Zooplankton	33	-20.33	0.77	7.81	0.96	2.26	0.59	24	-20.59	1.49	7.32	0.84	2.08	0.51
Herbivore														
Paracentrotus lividus	30	-16.94	1.28	8.01	0.78	2.38	0.52	35	-17.78	1.88	7.82	0.79	2.40	0.43
Filter feeders														
Mytilus galloprovincialis	17	-17.30	0.25	7.10	0.60	1.34	0.16	31	-17.30	1.05	7.32	1.00	1.46	0.30
Omnivores														
Lipophrys pholis	12	-16.66	0.51	11.35	0.61	3.07	0.30	15	-15.69	1.43	12.02	0.78	3.36	0.25
Maja sp.	17	-15.77	0.89	11.03	0.64	3.48	0.38	10	-14.87	1.83	11.54	0.33	3.83	0.16
Pachygrapsus marmoratus	12	-15.37	0.59	10.47	0.94	3.24	0.56	12	-13.18	1.88	10.24	0.58	3.21	0.21
Palaemon sp.	11	-14.92	1.11	11.10	0.60	3.53	0.26	12	-13.71	2.78	10.98	0.70	3.57	0.34
Pollicipes pollicipes	18	-17.88	0.22	10.55	0.49	2.28	0.14	21	-18.45	0.65	9.80	1.54	2.13	0.38
Carnivores														
Necora puber	7	-14.40	1.32	11.14	0.79	3.66	0.41	15	-15.01	1.58	11.37	0.34	3.75	0.19
Octopus vulgaris	11	-15.91	1.36	10.66	0.94	3.38	0.54	33	-15.00	1.71	10.66	1.14	3.41	0.53

Table 2. Summary of the significance and effect size of (A) Protection as a fixed factor, Location and Species as random factors on $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures for each species of the whole community, and (B) Protection (fixed factor), Location (random factor) and Species (random factor) on $\delta^{13}C$ and $\delta^{15}N$ stable isotope signatures for each species. F and p values, and LRT are derived from linear mixed effects models, except for those in which the normality assumption was not met (Protection F and p values derived from a linear models averaged by Location). Significant differences ($p < 0.05$) are shown in bold.

A)		**Protection**		**Species**		**Location**	
		F-value	**p-value**	**F-value**	**p-value**	**LRT**	**p-value**
Overall	$\delta^{13}C$	0.405	0.548	380.23	**<0.0001**	13.53	**<0.001**
	$\delta^{15}N$	0.999	0.351	325.06	**<0.0001**	9.03	**0.003**

B)		**Protection**		**Size**		**Location**	
		F-value	**p-value**	**F-value**	**p-value**	**LRT**	**p-value**
Primary producers							
Asparagopsis armata	$\delta^{13}C$	0.005	0.945	-	-	3.216	0.073
	$\delta^{15}N$	4.389	0.084	-	-	9.338	**0.002**
Codium tomentosum	$\delta^{13}C$	0.002	0.969	-	-	2.312	0.128
	$\delta^{15}N$	0.388	0.291	-	-	2.895	0.089
Dyctiota dichotoma	$\delta^{13}C$	0.010	0.923	-	-	-	-
	$\delta^{15}N$	1.382	0.291	-	-	0.454	0.501

Kelp	$\delta^{13}C$	0.046	0.838	-	-	-	-
	$\delta^{15}N$	0.082	0.786	-	-	4.582	**0.032**
Ploclamium sp.	$\delta^{13}C$	0.017	0.902	-	-	9.063	**0.003**
	$\delta^{15}N$	0.014	0.909	-	-	6.567	**0.010**
Ulva sp.	$\delta^{13}C$	9.272	**0.023**	-	-	-	-
	$\delta^{15}N$	0.329	0.587	-	-	9.619	**0.002**
Particulate organic matter							
POM	$\delta^{13}C$	0.264	0.625	-	-	0.758	0.384
	$\delta^{15}N$	1.666	0.243	-	-	0.354	0.552
Zooplankton							
Zooplankton	$\delta^{13}C$	0.741	0.440	-	-	0.046	0.830
	$\delta^{15}N$	1.920	0.219	-	-	3.434	0.064
Herbivore							
Paracentrotus lividus	$\delta^{13}C$	0.562	0.482	19.948	**<0.0001**	17.201	**<0.0001**
	$\delta^{15}N$	0.467	0.519	17.786	**<0.0001**	3.359	0.067
Filter feeder							
Mytilus galloprovincialis	$\delta^{13}C$	Interaction effects: F-value: 9.765; P-value: **0.005**				9.79	**0.002**
	$\delta^{15}N$	0.215	0.659	1.129	0.294	13.172	**<0.001**
Omnivore							
Lipophrys pholis	$\delta^{13}C$	0.402	0.549	0.007	0.935	9.359	**0.002**
	$\delta^{15}N$	2.506	0.163	4.923	**0.038**	10.289	**0.001**
Maja sp.	$\delta^{13}C$	1.513	0.266	-	-	1.291	0.256
	$\delta^{15}N$	2.599	0.158	-	-	1.185	0.276

Pachygrapsus marmoratus	δ^{13}C	4.619	0.075	-	-	20.519	**<0.0001**
	δ^{15}N	0.216	0.659	-	-	6.224	**0.013**
Palaemon sp.	δ^{13}C	0.532	0.494	-	-	7.331	**0.007**
	δ^{15}N	0.048	0.835	-	-	9.163	**0.002**
Pollicipes pollicipes	δ^{13}C	4.076	0.093	4.331	**0.046**	11.855	**<0.001**
	δ^{15}N	0.528	0.496	2.625	0.116	15.626	**<0.0001**
Carnivore							
Necora puber	δ^{13}C	0.515	0.510	-	-	0.426	0.514
	δ^{15}N	0.502	0.509	-	-	2.905	0.088
Octopus vulgaris	δ^{13}C	1.394	0.282	-	-	-	-
	δ^{15}N	0.277	0.617	-	-	-	-

Table 3. Summary of the significance of Protection and effect size,of Location and size on species trophic level. Protection and size F and p values are derived from linear mixed effect models including Location as a random factor (with the exception of *Octopus vulgaris* which had no random effect). Location F and p values are derived from a linear model. Significant differences (P < 0.05) are shown in bold.

	Protection		**Size**		**Location**	
Species	**F-value**	**P-value**	**F-value**	**P-value**	**F-value**	**P-value**
Overall (mean trophic level)	0.157	0.707	-	-	2.089	**0.044**
Zooplankton	0.595	0.473	-	-	2.339	**0.038**
Paracentrotus lividus	0.017	0.899	17.637	**<0.001**	1.967	0.075
Mytilus galloprovincialis	0.551	0.485	1.059	0.309	8.491	**<0.001**
Lipophrys pholis	2.733	0.150	1.562	0.231	5.764	**0.001**
Maja sp.	2.120	0.192	-	-	5.777	**0.001**
Pachygrapsus marmoratus	0.008	0.934	-	-	5.859	**0.002**
Palaemon sp.	0.136	0.724	-	-	1.960	0.130
Pollicipes pollicipes	0.028	0.872	0.122	0.729	11.106	**<0.001**
Necora puber	0.338	0.586	-	-	4.059	**0.013**
Octopus vulgaris	0.395	0.533	-	-	1.273	0.291

Figures captions

Figure 1. Map of the study area, with red lines defining the boundaries of the Illas Atlánticas Marine-Terrestrial National Park, NW Spain. Blue dots denote protected sampling locations (a: South Cies island, b: North Cies islands , c: Ons island, d: Sálvora jsland). Orange dots denote unprotected sampling locations (e: Cape Silleiro, f: Cape Couso, g: O Grove, h: Cape Corrubedo).

Figure 2. Biplot of $\delta^{13}C$ and $\delta^{15}N$ values (mean ± SD) of the main trophic guilds (primary producers, POM, zooplankton, herbivores, filter feeders, and primary consumers) inside (blue) and outside (orange) of the Illas Atlánticas Marine-Terrestrial National Park.

Figure 3. Mean (± SD) trophic level (TL) of consumers in protected (blue) and unprotected (orange) locations. Zp: Zooplankton. Mg*: Mytilus galloprovincialis.* Pl: *Paracentrotus lividus.* Lp: *Lipophrys pholis.* Ms*: Maja* sp*.* Pm: *Pachygrapsus marmoratus.* Ps*: Palaemon* sp. Pp*: Pollicipes pollicipes.* Np*: Necora puber.* Ov: *Octopus vulgaris.*.

Figure 4. Species-specific mean (± SD) trophic level (TL) in the locations studied (from south to north) in protected (bluish bars) areas (South Cíes, North Cíes, Ons, and Sálvora) and unprotected (reddish bars) areas (Cape Silleiro, Cape Couso, O Grove, and Cape Corrubedo). Zp: Zooplankton. Mg*: Mytilus galloprovincialis.* Pl: *Paracentrotus lividus.* Lp: *Lipophrys pholis.* Ms*: Maja* sp. Pm: *Pachygrapsus marmoratus.* Ps*: Palaemon* sp. Pp*: Pollicipes pollicipes.* Np*: Necora puber.* Ov: *Octopus vulgaris.*Lower case letters represent Tukey post-hoc significant differences between locations ($P < 0.05$).

Figure 1

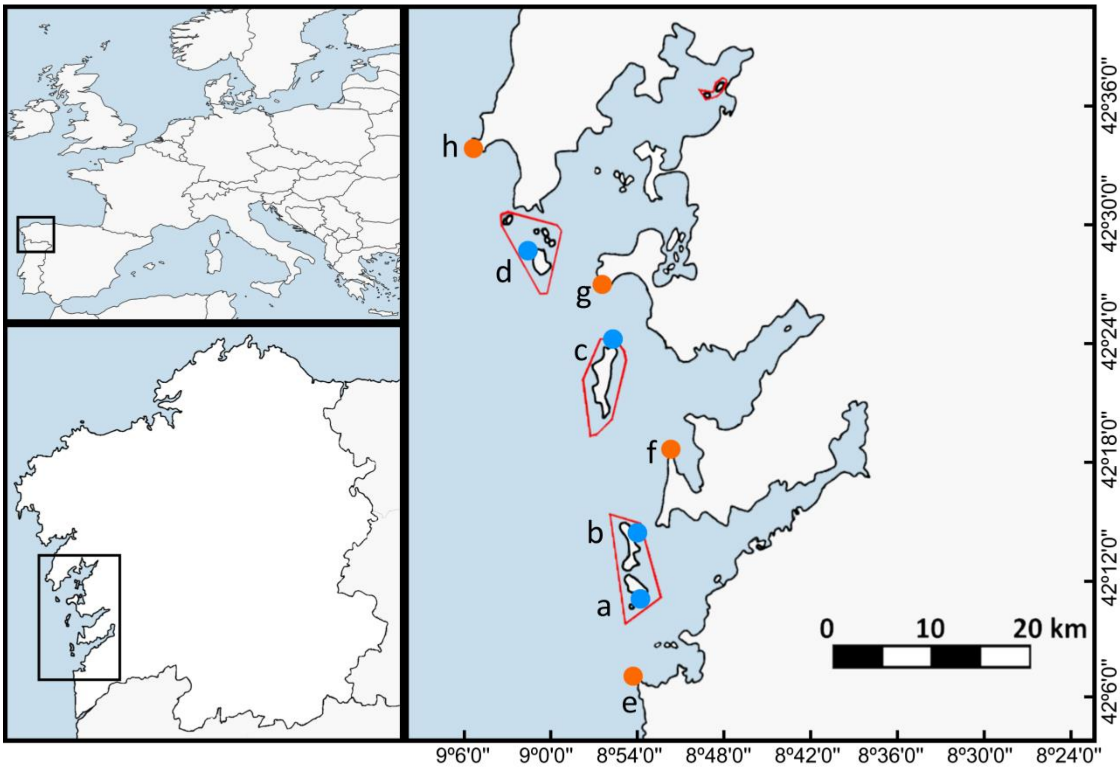
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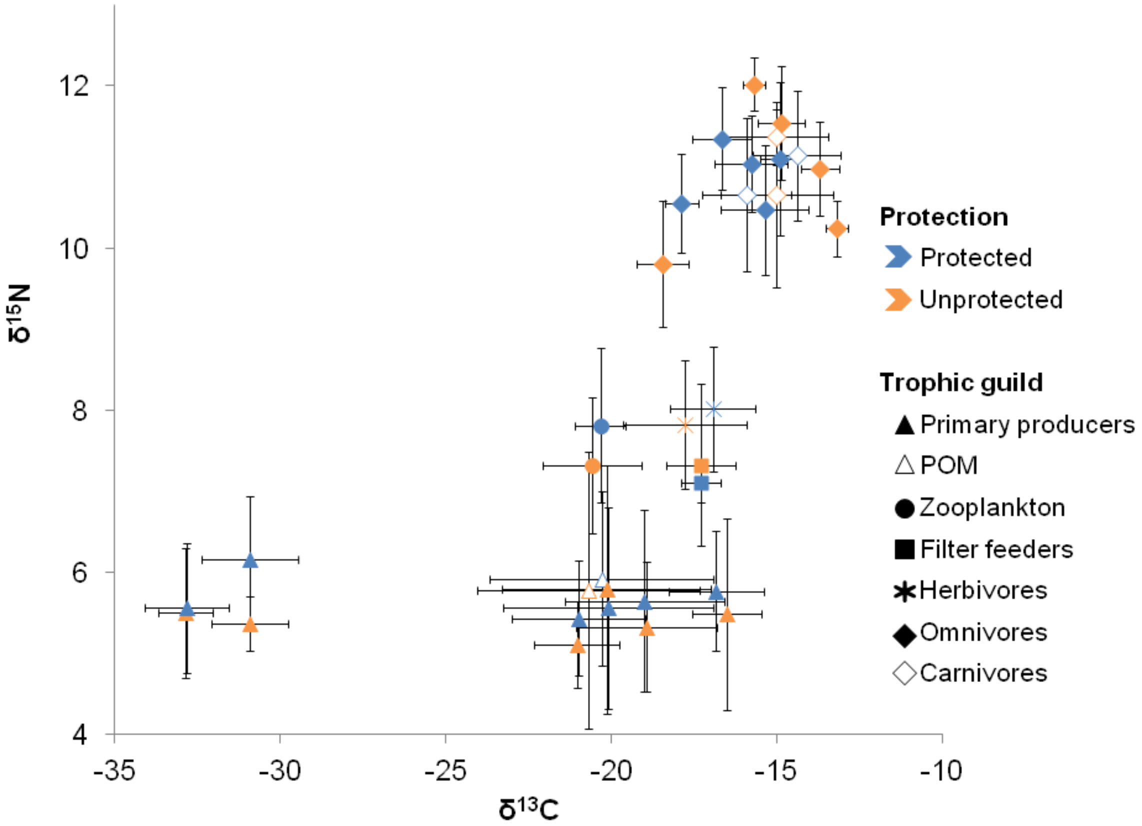
Figure 2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Primary producers
POM
Zooplankton
Filter feeders
Herbivores
Omnivores
Carnivores

Figure 3

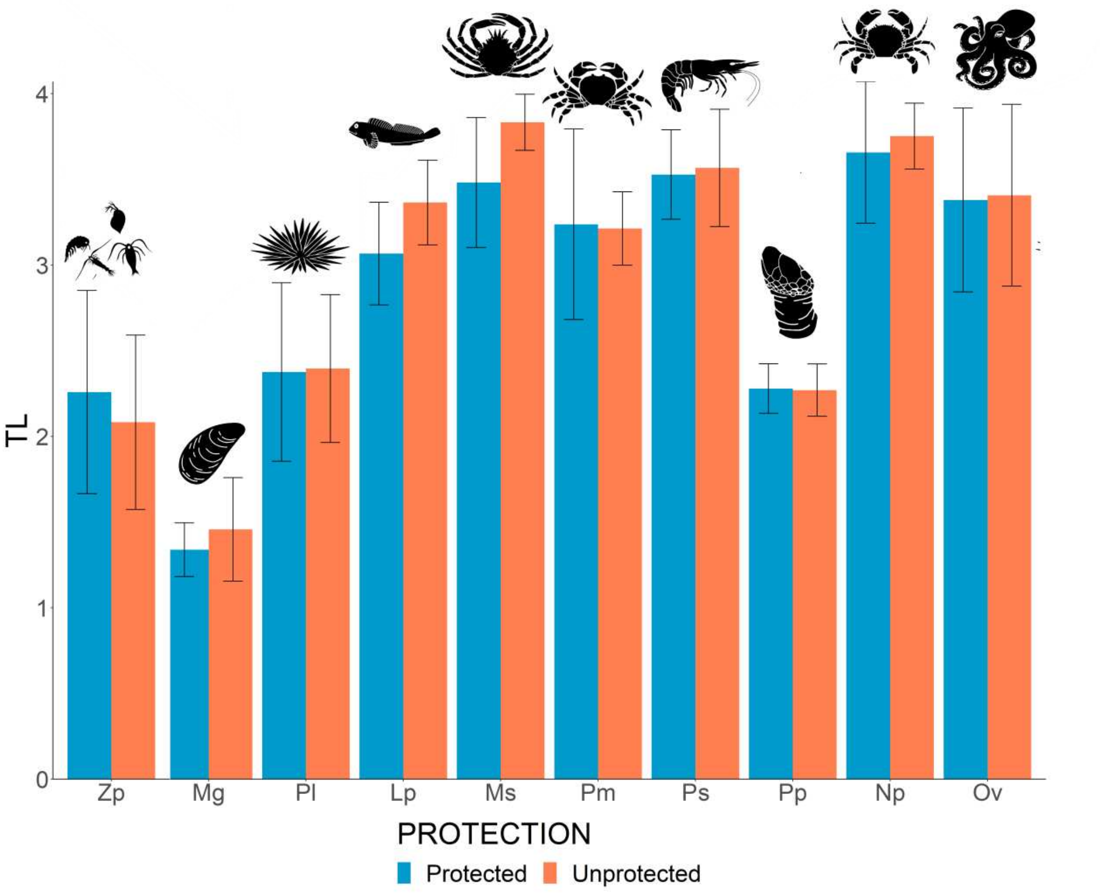
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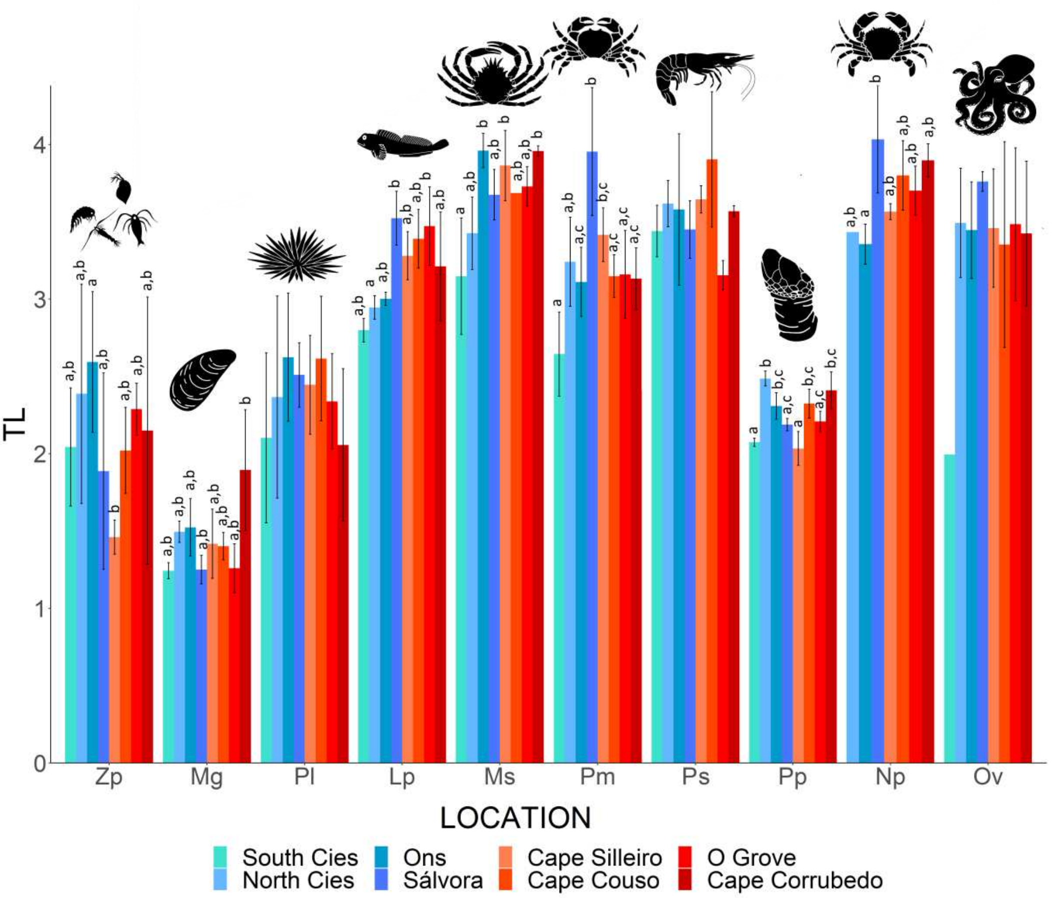
Figure 4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South Cies
North Cies
Ons
Sálvora
Cape Silleiro
Cape Couso
O Grove
Cape Corrubedo

SUPPLEMENTARY MATERIAL

Table 1. Mean (± SD) values of δ^{13}C and δ^{5}N stable isotope signatures (‰) and number of samples (in brackets) of the studied species in protected (South Cíes, North Cíes, Ons, and Sálvora) and unprotected (Cape Silleiro, Cape Couso, O Grove, and Cape Corrubedo) areas.

		Protected								**Unprotected**							
		South Cies		**North Cies**		**Ons**		**Sálvora**		**Silleiro**		**Couso**		**O Grove**		**Corrubedo**	
		δ^{13}C	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**	**δ^{13}C**	**δ^{15}N**
Primary Sources	*Asparagopsis armata*	-31.23	6.39	-31.45	6.94	-29.93	6.09	-31.29	5.55	-32.04	5.25	-30.46	5.83	-30.00	5.40	-31.02	5.13
		± 1.45	± 0.71	± 1.75	± 1.51	± 1.50	± 0.36	± 1.12	± 0.17	± 0.59	± 0.20	± 0.79	± 0.32	± 1.09	± 0.21	± 0.83	± 0.37
		(7)		(3)		(6)		(6)		(6)		(3)		(6)		(4)	
	Codium tomentosum	-19.88	6.30	-20.05	5.62	-17.08	5.37	-18.37	4.45	-19.22	5.72	-18.52	5.55	-18.23	5.11	-20.06	4.65
		± 2.37	± 1.29	± 1.69	± 0.93	± 2.39	± 0.92	± 2.00	± 0.31	± 2.47	± 0.84	± 2.01	± 0.63	± 2.33	± 0.86	± 0.98	± 0.32
		(9)		(9)		(8)		(3)		(9)		(7)		(8)		(5)	

	Dyctiota dichotoma	-22.74	5.98	-22.37	5.45	-19.66	5.32	-19.48	5.08	-21.37	5.19	-20.83	5.56	-20.90	5.22	-20.79	4.66
		± 2.04	± 0.84	± 1.79	± 0.77	± 0.77	± 0.20	± 0.64	± 0.75	± 1.22	± 0.37	± 0.62	± 0.08	± 1.86	± 0.75	± 1.30	± 0.42
		(5)		(6)		(6)		(6)		(8)		(3)		(5)		(3)	
	Kelp	-19.80	5.90	-21.25	4.72	-19.62	6.25	-19.86	4.97	-20.97	6.21	-21.31	5.98	-19.00	6.21	-18.57	3.99
		± 2.33	± 1.09	± 3.93	± 1.19	± 4.09	± 1.01	± 1.99	± 1.26	± 2.73	± 1.71	± 3.51	± 1.13	± 3.15	± 1.55	± 2.55	± 0.71
		(9)		(7)		(9)		(6)		(8)		(10)		(9)		(5)	
	Ploclamium sp.	-32.85	6.16	-33.82	5.10	-32.86	5.69	-31.80	5.00	-32.92	4.98	-32.54	5.78	-33.59	5.88	-31.91	5.15
		± 1.03	± 0.54	± 1.23	± 1.16	± 0.94	± 0.39	± 1.34	± 0.19	± 0.47	± 0.33	± 0.42	± 0.50	± 0.76	± 1.12	± 0.36	± 0.45
		(9)		(6)		(6)		(6)		(7)		(7)		(9)		(5)	
	Ulva sp	-17.01	6.18	-16.85	6.19	-16.76	5.66	-16.78	5.26	-16.40	6.30	-16.77	6.27	-16.35	4.48	-16.55	5.05
		± 0.84	± 0.43	± 1.25	± 0.83	± 2.03	± 0.61	± 1.27	± 0.85	± 1.13	± 0.95	± 0.66	± 0.86	± 1.71	± 1.19	± 0.24	± 0.51
		(6)		(4)		(9)		(6)		(6)		(6)		(7)		(6)	
	POM	-22.02	6.41	-20.49	5.96	-18.15	5.46	-20.58	5.82	-22.02	5.97	-20.01	5.28	-18.99	6.16	-22.54	5.63
		± 1.38	± 0.88	± 1.65	± 0.88	± 5.69	± 1.48	± 0.40	± 0.80	± 2.90	± 1.82	± 1.67	± 0.71	± 4.30	± 1.63	± 3.04	± 2.69
		(9)		(9)		(9)		(6)		(7)		(8)		(9)		(6)	
Zooplankton	Zooplankton	-20.73	7.95	-20.40	7.95	-20.07	8.15	-20.00	6.88	-21.71	6.53	-19.83	7.20	-20.52	7.86	-20.90	7.04
		± 0.96	± 0.61	± 0.67	± 1.13	± 0.64	± 0.73	± 0.65	± 1.02	± 0.01	± 0.18	± 0.54	± 0.45	± 1.55	± 0.27	± 2.13	± 1.38

		(9)		(9)		(9)		(6)		(3)		(6)		(9)		(6)	
Filter feeders	*Mytilus galloprovincialis*	-17.42	7.20	-17.37	7.60	-17.00	7.58	-17.30	6.41	-16.01	7.37	-17.32	7.09	-17.51	6.78	-17.80	8.58
		± 0.15	± 0.20	± 0.11	± 0.26	± 0.44	± 0.70	± 0.11	± 0.35	± 2.38	± 0.84	± 0.33	± 0.34	± 0.66	± 0.60	± 0.72	± 1.47
		(6)		(3)		(3)		(5)		(4)		(12)		(9)		(6)	
Herbivores	*Paracentrotus lividus*	-17.66	8.04	-17.41	7.92	-16.36	8.20	-15.88	7.88	-19.46	8.10	-17.88	8.15	-16.20	7.94	-16.93	6.89
		± 0.82	± 0.88	± 1.19	± 1.05	± 0.86	± 0.66	± 1.56	± 0.33	± 1.97	± 0.51	± 1.25	± 0.64	± 2.04	± 0.49	± 0.83	± 0.79
		(9)		(8)		(7)		(6)		(9)		(12)		(6)		(8)	
Omnivores	*Lipophrys pholis*	-16.71	11.14	-17.27	10.98	-16.65	11.00	-16.01	12.27	-16.15	11.95	-17.18	12.02	-14.67	12.47	-15.80	11.17
		± 0.09	± 0.21	± 0.10	0.21	± 0.30	± 0.12	± 0.38	± 0.47	± 0.57	± 0.42	± 0.18	± 0.51	± 1.51	± 0.69	± 1.20	± 0.95
		(3)		(3)		(3)		(3)		(3)		(3)		(6)		(3)	
	Maja sp.	-16.31	10.79	-16.23	10.82	-15.58	11.82	-14.15	11.08	-14.01	11.80			-15.84	11.53	-15.01	11.41
		± 0.44	± 0.79	± 0.32	± 0.49	± 0.49	± 0.23	± 0.40	± 0.34	± 2.95	± 0.48	-14.08	11.21	± 1.60	± 0.27	± 0.92	± 0.07
		(5)		(6)		(3)		(3)		(3)		(1)		(3)		(3)	
	Pachygrapsus marmoratus	-15.89	9.73	-15.38	10.44	-15.25	10.03	-14.94	11.66	-10.21	10.86	-14.18	10.08	-14.44	10.33	-13.90	9.68
		± 0.36	± 0.57	± 0.33	± 0.60	± 0.09	± 0.47	± 0.97	± 0.87	± 0.60	± 0.37	± 0.44	± 0.29	± 0.31	± 0.60	± 0.98	± 0.42
		(3)		(3)		(3)		(3)		(3)		(3)		(3)		(3)	
	Palaemon sp.	-14.60	11.40	-15.17	11.23	-15.15	11.02	-14.66	10.61	-10.04	11.34	-13.25	11.67	-14.83	10.32	-16.70	10.59
		± 2.33	± 0.35	± 0.51	± 0.31	± 0.12	± 1.03	± 0.02	± 0.39	± 2.20	± 0.19	± 0.95	± 0.92	± 1.00	± 0.20	± 0.16	± 0.07

		(3)		(3)		(3)		(2)		(3)		(3)		(3)		(3)	
	Pollicipes pollicipes	-17.74 ± 0.09	10.34 ± 0.10	-17.71 ± 0.06	11.35 ± 0.18	-17.92 ± 0.25	10.55 ± 0.32	-18.05 ± 0.12	9.95 ± 0.15	-19.35 ± 1.00	9.70 ± 0.41	-18.53 ± 0.18	10.57 ± 0.35	-18.40 ± 0.13	10.37 ± 0.25	-17.90 ± 0.60	8.40 ± 2.35
		(3)		(3)		(9)		(3)		(3)		(9)		(3)		(6)	
Carnivores	*Necora puber*	-	-	-17.06	10.84	-14.34 ± 0.47	10.55 ± 0.27	-13.56 ± 0.64	11.83 ± 0.73	-16.09 ± 1.18	11.18 ± 0.11	-14.26 ± 1.89	11.45 ± 0.47	-15.66 ± 1.69	11.47 ± 0.33	-14.79 ± 0.25	11.28 ± 0.22
		-		(1)		(3)		(3)		(3)		(6)		(3)		(3)	
	Octopus vulgaris	-17.78	8.37	-16.74 ± 1.02	10.97 ± 0.74	-15.81 ± 1.29	10.74 ± 0.66	-14.47 ± 0.08	11.26 ± 0.13	-13.88 ± 1.12	10.95 ± 0.81	-15.30 ± 1.93	10.51 ± 1.40	-15.74 ± 1.29	11.02 ± 1.04	-15.90 ± 1.35	10.29 ± 0.98
		(1)		(2)		(6)		(2)		(10)		(15)		(3)		(5)	

Figure 1. Biplot of δ^{13}C and δ^{15}N values (mean ± SE) of the studied species by trophic guild (primary producers, POM, zooplankton, herbivores, filter feeders and primary consumers) inside (blue) and outside (orange) the Illas Atlánticas marine-terrestrial National Park. Aa: *Asparagopsis armata,* Ct: *Codium tomentosum,* Dd: *Dyctiota dichotoma,* K*:* Kelp, Pr: *Ploclamium* sp*,* Ur: *Ulva* sp.*,* POM: Particulate organic matter*,* Zp: Zooplankton*,* Mg: *Mytilus galloprovincialis,* Pl: *Paracentrotus lividus,* Lp: *Lipophrys pholis,* Ms: *Maja* sp.*,* Pm: *Pachygrapsus marmoratus,* Ps: *Palaemon* sp.*,* Pp: *Pollicipes pollicipes,* Np: *Necora puber,* Ov: *Octopus vulgaris.*