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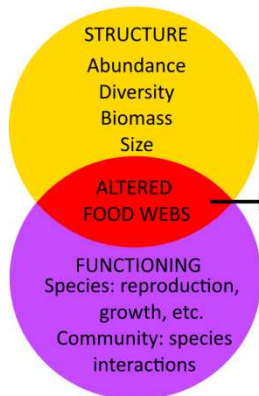
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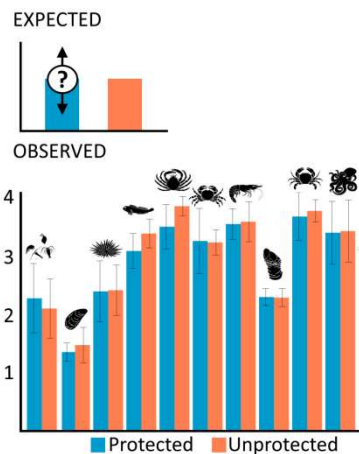
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## 24 GRAPHICAL ABSTRACT

Marine Protected Areas  
ecosystem changes

Benthic bottom-up  
effects on  
TROPHIC LEVELS



## WHY?

## POOR MANAGEMENT

Fishing pressure { ↑ Licences  
↓ No-take

## Management priority imbalance



## OTHER FACTORS

Environmental conditions  
Species-specific characteristics  
Sampling bias

25

26

## 27 HIGHLIGHTS

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

## 33 ABSTRACT:

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

56

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

## 59 1. INTRODUCTION

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

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

87 MPA characteristics (age, level of protection, and size of no-take area) also have significant  
88 effects on the conservation outcomes of MPAs, including ecosystem functioning variables  
89 (Edgar *et al.* 2014, Claudet 2018). Although the complexity of ecological processes in  
90 marine ecosystems is well known, most MPAs omit ecosystem functioning criteria in their  
91 design, which may jeopardize the outcomes of the management actions (Micheli *et al.*  
92 2004, Frid *et al.* 2008).

93 Cascading effects derived from shifts in prey-predator dynamics as a result of human  
94 intervention, such as habitat destruction, overfishing and species protection, may alter  
95 species interactions within marine ecosystems (Pauly *et al.* 1998, Soler *et al.* 2015, Yabsley  
96 *et al.* 2016). Both bottom-up and top-down processes are associated with trophic cascades  
97 within MPAs, regulating species interactions from respectively lower to upper trophic levels  
98 and vice versa (Pauly *et al.* 1998, Pinnegar *et al.* 2000, Soler *et al.* 2015, Di Lorenzo *et al.*  
99 2016). The trophic level defines the position of an organism within a food web (which can  
100 be based on primary producers or detritus) and is commonly used as an ecosystem  
101 indicator in fisheries management (Babcock *et al.* 2010, Bourdaud *et al.* 2016, Reed *et al.*  
102 2017) and thus in MPA ecosystem functioning (Davis *et al.* 2019). A higher mean trophic  
103 level (estimated from mass-balanced models) occurs as a result of protection of species in  
104 mature well established MPAs, mainly due to the increase in abundance of top-predators  
105 (Coll  ter *et al.* 2014). Nevertheless, despite the close correlation between mass-balanced  
106 models and stable isotope analysis for estimating trophic levels (Du *et al.* 2020), the effect  
107 of MPA implementation on the stable isotopic composition of different organisms remains  
108 largely unknown.

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

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

119 Stable isotope analysis is an indirect approach that is commonly used in trophodynamic  
120 studies as it provides information on assimilated food sources over a longer time scale than  
121 obtained by gut content analysis (Box *et al.* 2010, Deudero *et al.* 2011, Layman *et al.* 2012).  
122 Such timescales are closely related to the sampled tissue turnover rate, so that the lower  
123 the turnover rate the longer the timescale (Blanco *et al.* 2009, Cabanellas-Reboredo *et al.*  
124 2009, Deudero *et al.* 2009). This approach relies on the relationship between the lighter  
125 isotopes ( $^{12}\text{C}$  and  $^{14}\text{N}$ ) and the heavier isotopes ( $^{13}\text{C}$  and  $^{15}\text{N}$ ), which may help decipher the  
126 origin and pathways of organic matter of primary food sources ( $^{13}\text{C}/^{12}\text{C}$ ) and clarify the  
127 environmental conditions and trophic position of consumers ( $^{15}\text{N}/^{14}\text{N}$ ) (Post 2002,  
128 McCutchan *et al.* 2003, Caut *et al.* 2009, among others). Stable isotope analysis is a  
129 powerful tool in trophodynamic ecological studies (Post 2002), and it can even indicate the  
130 effectiveness of management by providing relevant information on foraging grounds,  
131 biogeographic distribution and anthropogenic impacts (Pearson *et al.* 2017, Pethybridge *et al.*  
132 *et al.* 2018, Raoux *et al.* 2020, Signa *et al.* 2020).

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

143

## 144 **2. MATERIALS AND METHODS**

### 145 *2.1. Study area*

146 The Illas Atlánticas Marine-Terrestrial National Park (Galicia, NW Spain) is a terrestrial and  
147 marine reserve formed by four main archipelagos that extend over a distance of 40 km in a  
148 north-south direction and cover a sea marine area of up to 728.52 km<sup>2</sup> (Figure 1). In the  
149 present study, only the three archipelagos facing open sea were studied (from north to  
150 south): Sálvora island, the Ons and Onza islands, and the Cíes islands, each located in  
151 front of a ria *sensu lato* (Cotton 1956). The Cíes islands archipelago was split into two  
152 different locations for sampling: the northern Monteagudo-Faro islands (two islands  
153 connected by a permanent sandbar) and the southern San Martiño island (separated from  
154 the northern islands by a channel of width ~500 m). In addition, management regulations  
155 are different in the northern and southern Cíes islands, with more restrictive management in  
156 terms of permitted uses in the latter (mainly due to the fact that only the northern islands  
157 are accessible to the public through tourist cruises and hold tourist services such as  
158 accommodation and restaurants).

### 159 *2.2. Field sampling*

160 Sampling was conducted seasonally between March 2019 and September 2020, to prevent  
161 any potential bias towards a favourable sampling season. Nevertheless, seasonal analysis  
162 was not feasible for various reasons: weather conditions did not allow field sampling,  
163 absence of some species during certain seasons and/or pandemic restrictions. Thus, in  
164 order to deal with any seasonal bias in isotopic signatures and in accordance with the low  
165 turnover rate of the sampled tissues (MacAvoy *et al.* 2001, Blanco *et al.* 2009, Deudero *et*  
166 *al.* 2009), species-specific samples at each location were pooled (Michener and Kaufman  
167 2008) (Supplementary Material Table 1).

168 Four locations were sampled inside the MPA, and another four locations were sampled  
169 outside the MPA as reference locations (N total locations = 8, Figure 1). The island nature

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

197 length) and *P. lividus* (test diameter) were measured to the nearest 0.01 mm. All samples  
 198 were frozen immediately after sampling and stored at -20 °C until further processing.

### 199 2.3. Stable isotope analyses

200 Shell-free and bone-free muscle samples were taken from all macroinvertebrates and fish,  
 201 respectively. Individual fronds of macroalgae were sampled when possible (*C. tomentosum*,  
 202 *D. dichotoma*, Kelp, *Ploclanium* sp., and *Ulva* sp.) or otherwise were pooled (*A. armata*).

203 All samples were rinsed thoroughly with tap water, dried at 60 °C for 24 h and then ground  
 204 to a fine powder in a mortar and pestle. According to the study objectives and due to the  
 205 lack of consensus on the pre-treatment acidification of non-highly carbonated species  
 206 (Pomerleau *et al.* 2014, Pires-Teixeira *et al.* 2020), the samples were not acid-washed. A  
 207 homogeneous dried powdered extract (1.7mg ± 0.2) of each sample was placed in a  
 208 cadmium tin cup for analysis of the δ<sup>13</sup>C and δ<sup>15</sup>N stable isotope compositions by  
 209 continuous flow isotope ratio mass spectrometry (CF-IRMS) in a THERMO Finnigan MAT  
 210 253 mass spectrometer. Reference standards were included for C, IAEA-C6 sucrose, and  
 211 N, IAEA-N-2 ammonium sulphate. One sample of an internal reference was analysed every  
 212 10 samples in order to calibrate the system and compensate for the drift over time. The  
 213 analytical precision of the stable isotope analyses was based on the standard deviation of  
 214 the IAEA-C6 and IAEA-N-2 samples: 0.09‰ for δ<sup>13</sup>C and 0.11‰ for δ<sup>15</sup>N.

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

$$219 \quad \delta X = [(R_{\text{sample}} / R_{\text{reference}}) - 1] \times 10^3, \quad \text{Equation 1}$$

220 where *X* is <sup>13</sup>C or <sup>15</sup>N and *R* is the corresponding <sup>13</sup>C/<sup>12</sup>C or <sup>15</sup>N/<sup>14</sup>N ratio. The trophic level  
 221 of the organisms was calculated using the following formula (Minagawa and Wada 1984):

$$222 \quad \text{Trophic level} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}}) / \text{NEF} + \lambda, \quad \text{Equation 2}$$

223 where δ<sup>15</sup>N<sub>base</sub> is the δ<sup>15</sup>N isotopic signature of the base of the food web, λ is the trophic  
 224 level of δ<sup>15</sup>N<sub>base</sub> and NEF is the assumed δ<sup>15</sup>N trophic enrichment factor. For δ<sup>15</sup>N<sub>base</sub>,

225 we used the mean  $\delta^{15}\text{N}$  for primary producers (macroalgae and POM) at each location and  
226 assumed a  $\lambda$  value of 1. Species-specific *NEF* were considered when available (Hobson  
227 and Cherel 2006, Vanderklift *et al.* 2006, Dubois *et al.* 2007, Henschke *et al.* 2015),  
228 otherwise a general *NEF* (specific for the trophic guild) was applied (McCutchan *et al.* 2003,  
229 Caut *et al.* 2009, Henschke *et al.* 2015), in order to improve the accuracy of trophic  
230 estimates to apply proper ecosystem-based management (Du *et al.* 2020, Raoux *et al.*  
231 2020).

#### 232 2.4. Statistical analyses

233 Protective effects of the MPA on whole community isotopic signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$   
234 were analysed by fitting linear mixed models with Protection as a fixed factor (Protected vs  
235 Unprotected) and Location (South Cies, North Cies, Ons, Sálvora, Cape Silleiro, Cape  
236 Couso, O Grove, Cape Corrubedo) and Species (*A. armata*, *C. tomentosum*, *D. dichotoma*,  
237 Kelp, *Ploclanium* sp., *Ulva* sp., POM, zooplankton, *M. galloprovincialis*, *P. lividus*,  
238 *Lipophrys pholis*, *Maja* sp., *P. marmoratus*, *Palaemon* sp., *P. pollicipes*, *N. puber*, and *O.*  
239 *vulgaris*) as random effects. The effects of Protection (fixed factor, two levels) on the stable  
240 isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and trophic level of each species were analysed with  
241 linear mixed-effect models including size (when available) as a covariate and Location  
242 (eight levels) as a random effect. All models were run using the *lmer* function (Bates *et al.*  
243 2015), and the significance was evaluated by ANOVA with Satterthwaite approximation for  
244 degrees of freedom (Kuznetsova *et al.* 2017). Despite the robustness of linear mixed-  
245 effects models, which allow for violations of model assumptions (Schielzeth *et al.* 2020),  
246 models violating the normality of fixed-effects residuals were checked for outliers and re-run  
247 after elimination of these, to ensure that no severe violations of the assumptions occurred.  
248 However, the models that severely violated the assumption of normality ( $\delta^{13}\text{C}$  of *O. vulgaris*  
249 and  $\delta^{13}\text{C}$  of *D. dichotoma*) and those with singular boundary fit ( $\delta^{15}\text{N}$  of *O. vulgaris*,  $\delta^{13}\text{C}$  of  
250 Kelp and  $\delta^{13}\text{C}$  of *Ulva* sp.) were averaged by Location and re-run as linear models in order  
251 to assess the effects of protection (fixed factor) on the stable isotopic signatures. In  
252 addition, the *O. vulgaris* trophic level model was bound at zero for the random effect

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

263

### 264 **3. RESULTS**

265 The overall range of  $\delta^{13}\text{C}$  at the base of the food web ranged from  $-32.82\text{‰} \pm 1.27$  to -  
266  $14.40\text{‰} \pm 1.32$  in protected locations and from  $-32.86\text{‰} \pm 0.81$  to  $-13.18\text{‰} \pm 1.88$  in  
267 unprotected locations (Table 1). The  $\delta^{15}\text{N}$  signatures ranged from  $5.43\text{‰} \pm 0.71$  to  $11.35\text{‰}$   
268  $\pm 0.61$  inside the MPA and from  $5.10\text{‰} \pm 0.53$  to  $12.02\text{‰} \pm 0.78$  outside the MPA (Table 1).  
269 More precisely, the  $\delta^{13}\text{C}$  signatures of the primary producers (including macroalgae and  
270 POM) ranged widely from  $-32.82\text{‰} \pm 1.27$  to  $-16.84\text{‰} \pm 1.44$  within protected locations  
271 areas and from  $-32.86\text{‰} \pm 0.81$  to  $-16.51\text{‰} \pm 1.06$  in unprotected locations. Conversely, the  
272 ranges of the  $\delta^{15}\text{N}$  signatures of primary producers were narrower, varying from  $5.43\text{‰} \pm$   
273  $0.71$  to  $6.15\text{‰} \pm 0.78$  in protected locations and from  $2.79\text{‰} \pm 1.53$  to  $5.10\text{‰} \pm 0.53$  in  
274 unprotected locations. The  $\delta^{13}\text{C}$  signatures of zooplankton, herbivores and filter feeders  
275 were respectively  $-20\text{‰} \pm 0.77$ ,  $-16.94\text{‰} \pm 1.28$  and  $-17.30\text{‰} \pm 0.25$ , in protected locations,  
276 and  $-20.59\text{‰} \pm 1.49$ ,  $-17.78\text{‰} \pm 1.88$  and  $-17.30\text{‰} \pm 1.05$ , in unprotected locations. Finally,  
277  $\delta^{13}\text{C}$  isotopic values for primary consumers ranged from  $-17\text{‰} \pm 0.22$  to  $-14.40\text{‰} \pm 1.32$  in  
278 protected locations and from  $-18.45\text{‰} \pm 0.65$  to  $-13.18\text{‰} \pm 1.88$  in unprotected locations,  
279 while for  $\delta^{15}\text{N}$ , the values ranged from  $10.47\text{‰} \pm 0.94$  to  $11.35\text{‰} \pm 0.61$  in protected areas  
280 and from  $9.80\text{‰} \pm 1.54$  to  $12.02\text{‰} \pm 0.78$  in unprotected locations. At the community level,

281 stable isotopic signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Figure 2) were not significantly affected by  
282 protection ( $p = 0.55$  and  $p = 0.35$ , respectively), although random effects of location proved  
283 significant for both  $\delta^{13}\text{C}$  ( $p < 0.001$ ) and  $\delta^{15}\text{N}$  ( $p = 0.003$ ), when controlled by species (Table  
284 2A; Supplementary Material Figure 1). Protection did not have significant effects on  $\delta^{13}\text{C}$   
285 and  $\delta^{15}\text{N}$  for any of the species studied (except *Ulva* sp. for  $\delta^{13}\text{C}$ , and the protection x size  
286 interaction for  $\delta^{13}\text{C}$  of *M. galloprovincialis*), while the effects of location at both  $\delta^{13}\text{C}$  and  
287  $\delta^{15}\text{N}$  were species-specific (Table 2B). Size had significant effects on  $\delta^{13}\text{C}$  of *P. pollicipes*  
288 and *P. lividus* and on  $\delta^{15}\text{N}$  of *L. pholis* and *P. lividus*).

289 The mean trophic levels (TLs) of organisms in the food web under study (excluding primary  
290 producers) ranged from  $1.34 \pm 0.16$  to  $3.66 \pm 0.41$  in protected locations and from  $1.46 \pm$   
291  $0.30$  to  $3.83 \pm 0.16$  in unprotected locations (Table 1). The mean TL values for locations in  
292 the MPA were not significantly different from those in unprotected locations (Table 3).  
293 However, the mean TL differed significantly between locations, although only between  
294 South Cíes and Cape Silleiro and Ons (Table 3). Species-specific mean TL ( $\pm$  SD) ranged  
295 from  $1.34 \pm 0.16$  (for *M. galloprovincialis*) to  $3.66 \pm 0.41$  (for *N. puber*) in protected locations  
296 and from  $1.46 \pm 0.30$  (for *M. galloprovincialis*) to  $3.83 \pm 0.16$  (for *Maja* sp.) in unprotected  
297 locations (Figure 3, Table 1). Species-specific models showed non-significant effects of  
298 protection on the TL for all of the studied species, and ontogenetic differences (size) in TL  
299 were only significant for *P. lividus* (Table 3). Location mainly explained the variation in TL  
300 for most of the species, regardless of the type of protection, except for *O. vulgaris*, *P. lividus*  
301 and zooplankton (Table 3). The main effects of Location on trophic level were species-  
302 specific: the trophic levels of *O. vulgaris*, *Palaemon* sp. and *P. lividus* were not affected by  
303 location, while those of *L. pholis*, *M. galloprovincialis*, *Maja* sp., *N. puber*, *P. marmoratus*, *P.*  
304 *pollicipes* and zooplankton varied significantly between locations (Figure 4, Table 3). The  
305 trophic level of *L. pholis* was higher in O Grove and Sálvora than in South and North Cíes,  
306 while it was also higher in Cape Couso than in South Cíes. For *M. galloprovincialis*, the  
307 trophic level was higher in Cape Corrubedo than in any other location, except North Cíes  
308 and Ons. The trophic level of *Maja* sp. was lower in South Cíes than in Cape Silleiro, Ons

309 and Cape Corrubedo. The trophic levels of *N. puber* was higher in Sálvora than in Ons and  
310 the trophic level of *P. marmoratus* in Sálvora was higher than in the South Cíes, Cape  
311 Couso, Ons, O Grove and Cape Corrubedo. The trophic level of *P. marmoratus* was higher  
312 in Cape Silleiro than in South Cíes. The trophic level of *P. pollicipes* was lower in Cape  
313 Silleiro and South Cíes than in North Cíes (where it was also higher than in O Gove and  
314 Sálvora), Cape Couso, Ons and Cape Corrubedo. Finally, the trophic level of zooplankton  
315 was higher in Ons than in Cape Silleiro.

316

#### 317 **4. DISCUSSION**

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

328 Studies comparing multi-trophic level stable isotope signatures at the base of the food webs  
329 inside and outside MPAs are scarce and mostly focused on local single MPAs (Guest *et al.*  
330 2009, Dannheim *et al.* 2014, Hofmeister 2018). Dannheim *et al.* (2014) reported no  
331 significant effects of protection on community trophic level after cessation of trawling  
332 (despite minor species-specific changes). However, the time scale of the study (20 months)  
333 was shorter than the mean time (5.13 years) required to detect direct effects on fisheries  
334 target species and even much shorter than that required (13.1 years) at a community level  
335 through indirect trait-mediated effects (Babcock *et al.* 2010). This constraint may be even  
336 more important when considering trawl fisheries, which are known to have strong impacts

337 on benthic organisms, sediment dynamics and trophic interactions that may take longer  
338 than 20 months to recover (Dannheim *et al.* 2014). The MPA under study was designed in  
339 2002 to provide ecosystem resilience while allowing for artisanal professional fisheries  
340 (BOE, July 1<sup>st</sup> 15/2002). Although the threshold time to detect indirect effects at a  
341 community level (13.1 years) has been reached, the management plan for the MPA has  
342 only recently been published (PRUX PNMTIA, 2019). In addition, despite removing fishing  
343 pressure by establishing no-take areas has resulted in substantial species-specific shifts in  
344 stable isotope signatures (Guest *et al.* 2009, Hofmeister 2018), the current MPA design  
345 includes very small no-take and restricted areas (0.44% and 10.02%, respectively) and an  
346 overall fishing ground of over 89%. Hence, the known benefits of MPA zonation (partly  
347 protected areas surrounding no-take areas) through spillover effect (Di Lorenzo *et al.* 2020),  
348 may be jeopardized by the large impacts of small-scale artisanal fisheries across the food  
349 webs (Coleman *et al.* 2013, Corrales *et al.* 2020).

350 In the present study, no differences between protected and unprotected marine areas in  
351 the stable isotope composition were found at either the community or at species-specific  
352 level (Table 2); not even in species targeted by artisanal fisheries both inside and outside  
353 the MPA, namely *P. lividus*, *M. galloprovincialis*, *Maja* sp., *P. pollicipes*, *N. puber* and *O.*  
354 *vulgaris* (Freire and García-Allut 2000, Ouréns *et al.* 2015). Most consumer species studied  
355 here are targeted by the Galician small-scale artisanal fisheries (Cambiè *et al.* 2012), both  
356 inside and outside the MPA, which may lead, through cascading, to the overall equivalent  
357 trophic levels. In addition, the small scale of the studied MPA may also contribute to the  
358 homogenized stable isotopic composition as both prey and predators could move inside  
359 and outside the MPA. However, this is unlikely since most mobile species considered (*L.*  
360 *pholis*, *Maja* sp., *N. puber*, *O. vulgaris*, *P. lividus*, *P. marmoratus*, and *Palaemon* sp.) are  
361 sedentary or have relatively small home ranges (Cannicci *et al.* 1999, Bernárdez *et al.*  
362 2003, Hereu 2005, Silva *et al.* 2014, Roma *et al.* 2018, Arechavala-Lopez *et al.* 2019). A  
363 homogenization of TL has been also associated with a spillover effect towards unprotected  
364 locations or to a lack of fishery limitations within protected locations (Branch *et al.* 2010).

365 Accordingly, the overall high levels of artisanal fishing activity (3235 12-m vessels in 2004)  
366 in the study region (from Cape Corrubedo to Cape Silleiro) is reflected by the high fishing  
367 pressure (with over 19 vessels per square km) described in only one of the three  
368 archipelagos (Cíes Islands) that form the MPA (Freire and García-Allut 2000, Cambiè *et al.*  
369 2012, Ouréns *et al.* 2015), which may limit the potential spillover effects.

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

380 Differences in TL and the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  content were location-specific, which suggests a  
381 stronger influence of local environmental factors or population characteristics than that of  
382 protection. Changes in the main trophic level within MPAs are commonly related to the  
383 inclusion of top predators looking for shelter and foraging grounds (Pinnegar and Polunin  
384 2000). However, when considering shellfish fisheries, the overall outcome of protection on  
385 food web dynamics remains unclear, as most studies focus on the effects of protection on  
386 community composition and richness, and species biomass, abundance and size  
387 (Lemasson *et al.* 2019). Local disturbances (i.e. species-specific fisheries) in species-  
388 specific trophodynamics (Supplementary Material Table 2) may also be offset by a myriad  
389 of trophic interactions in marine ecosystems (Albouy *et al.* 2019), especially in generalist  
390 feeders (Lawton *et al.* 2012) targeted by fisheries as is the case of species included in this  
391 study (Dunne 1977, Guerra 1978, Milton 1983, Bernárdez *et al.* 2000, Cannicci *et al.* 2002,  
392 Aguzzi *et al.* 2005, Janas and Baranska 2008, Silva *et al.* 2010). Generalist feeders are

393 less susceptible to changes in abundance and density of their prey, which will therefore  
394 mask potential changes in the trophic web (Dannheim *et al.* 2014) as may have occurred in  
395 the present study. Nevertheless, shifts in the stable isotope composition of keystone  
396 species have been reported for the lobster *Jasus edwardsii* and the pen-shell *Pinna nobilis*  
397 with depleted  $\delta^{15}\text{N}$  values within no-take areas in Tasmania (Guest *et al.* 2009) and the  
398 Mediterranean (Alomar *et al.* 2015), respectively. Such depletion was the result of targeting  
399 easier and more available prey of lower trophic level when feeding inside MPAs (Guest *et*  
400 *al.* 2009) and the influence of anthropogenic nutrient inputs outside MPAs (Alomar *et al.*  
401 2015). Conversely, no significant differences were found between protected and  
402 unprotected areas in the  $\delta^{15}\text{N}$  of *Crassotrea gigas* and *Perna canaliculus* in New Zealand  
403 (Salomon *et al.* 2008), or in *Octopus bimaculatus* in South California that shifted prey  
404 sources (evidenced by changes in  $\delta^{13}\text{C}$ ) of the same trophic level (similar overall  $\delta^{15}\text{N}$ )  
405 (Hofmeister 2018). Species not targeted by fisheries (including macroalgae, sea urchins,  
406 common snails, bivalves and crabs) showed negligible effects of protection on the  $\delta^{13}\text{C}$  and  
407  $\delta^{15}\text{N}$  signatures even in long-term no-take areas (Guest *et al.* 2009, Dannheim *et al.* 2014,  
408 Hofmeister 2018).

409 Banning fishing activity often results in low social acceptance and in trades-off between  
410 conservation and socioeconomic objectives (Voyer *et al.* 2014, Basurto *et al.* 2017).  
411 However, a global assessment reported increasing abundance (385%) and biomass  
412 (820%) of highly targeted invertebrate species within no-take areas relative to fished areas  
413 (Lester *et al.* 2009), but also relative to partially protected areas (Sciberras *et al.* 2015).  
414 Hence, in order to better balance ecological and socioeconomic benefits and conservation  
415 goals, a common practice in ecosystem-based management is to combine partly protected  
416 areas and no-take areas within single or multiple MPAs (Halpern *et al.* 2010, Sala and  
417 Giakoumi 2018, Zupan *et al.* 2018, Corrales *et al.* 2020, Di Lorenzo *et al.* 2020). This is  
418 particularly relevant in regions such as the study area, in which up to 60% of the total  
419 employment is related to fisheries (Villasante *et al.* 2016). Coupling marine spatial planning  
420 and ecosystem-based management is especially challenging in such highly dynamic

421 ecosystems and, therefore, requires dynamic management actions (Game *et al.* 2009,  
422 D'Aloia *et al.* 2019). The MPA under study is, however, spatially fixed by nature. Integration  
423 of regional conservation measures in a dynamic decision framework of zonation  
424 prioritization will enhance MPA network goals (Beger *et al.* 2015), mainly in regions with  
425 highly complex fishery activity such as the studied MPA and surrounding areas (Ouréns *et*  
426 *al.* 2015).

427 The bottom-up trophodynamic processes presented here provide a new perspective for  
428 marine management, which traditionally focuses on top-down regulations from fisheries and  
429 which has recently shifted towards more holistic ecosystem-based management (Tam *et al.*  
430 2017). Marine protected areas are not ecologically isolated and cannot mitigate the effects  
431 of anthropogenic disturbances, including climate change (Bates *et al.* 2019) or invasive  
432 species (Blanco *et al.* 2020). Integrating ecological and human-related processes in  
433 conservation management actions is extremely challenging (Frid *et al.* 2008, Beger *et al.*  
434 2010, Balbar and Metaxas 2019), especially in small MPAs in which species home ranges  
435 go beyond MPA boundaries (Green *et al.* 2015). In the last decade, conservation targets  
436 have moved from single species/habitat towards preservation of ecosystem functioning in  
437 seascapes considering long-term changes due to climate change, emerging new uses,  
438 policy changes and technological advances (Beger *et al.* 2010, Engelhard *et al.* 2017, Gissi  
439 *et al.* 2019). However, species not only interact horizontally in space, but also vertically in  
440 the trophic web, and the incorporation of trophodynamics in marine management will  
441 hopefully be the next step in conservation research.

442

## 443 **5. CONCLUSION**

444 This research, in which the Illas Atlánticas National Park was considered a case study,  
445 provides insights into how baseline sources and the lower trophic community respond to  
446 MPA designation and management. The observed limited effects of protection and the  
447 highly location-dependant variability indicate that although other factors (mainly particular  
448 environmental conditions) may influence location-specific effects, the fishing regulations

449 within the MPA do not have effects in the trophic level at the base of the food web when  
450 compared to unprotected (fished) areas. Despite study limitations described, the overall  
451 high human use of the MPA and the limited effects of protection on marine food webs  
452 reported suggest that the zonation design and the MPA management should be revised  
453 taking into account trophic data (in order to ensure functioning resilience within the MPA).  
454 More restrictive actions may not be well accepted in a highly fishing-dependant society.  
455 However, the resulting benefits of larger no-take areas should provide both short-term  
456 resources and long-term ecological outcomes, particularly if the design of the MPA links  
457 natural and socio-economic variables in order to endorse management policies by using  
458 decision-support software tools. Moreover, including ecosystem functioning (particularly  
459 prey-predator interactions and trophic indicators) in conservation plans would greatly  
460 improve ecological outcomes and the services provided.

461

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

	PROTECTED								UNPROTECTED							
	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		TL				$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)		TL			
	N	Mean	SD	Mean	SD	Mean	SD	N	Mean	SD	Mean	SD	Mean	SD		
Primary producers																
<i>Asparagopsis armata</i>	22	-30.92	1.46	6.15	0.78	-	-	19	-30.93	1.16	5.36	0.33	-	-		
<i>Codium tomentosum</i>	29	-19.00	2.41	5.64	1.12	-	-	29	-18.92	2.13	5.33	0.80	-	-		
<i>Dyctiota dichotoma</i>	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	-	-		
<i>Plocclanium</i> sp.	27	-32.83	1.27	5.56	0.80	-	-	28	-32.86	0.81	5.50	0.80	-	-		
<i>Ulva</i> 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																
<i>Paracentrotus lividus</i>	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																
<i>Mytilus galloprovincialis</i>	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																
<i>Lipophrys pholis</i>	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		
<i>Maja</i> 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		
<i>Pachygrapsus marmoratus</i>	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		
<i>Palaemon</i> 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		
<i>Pollicipes pollicipes</i>	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																
<i>Necora puber</i>	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		
<i>Octopus vulgaris</i>	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		

798  
 799 **Table 2.** Summary of the significance and effect size of (A) Protection as a fixed factor, Location and Species as random factors on  $\delta^{13}\text{C}$  and  
 800  $\delta^{15}\text{N}$  stable isotope signatures for each species of the whole community, and (B) Protection (fixed factor), Location (random factor) and Species  
 801 (random factor) on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures for each species. F and p values, and LRT are derived from linear mixed effects  
 802 models, except for those in which the normality assumption was not met (Protection F and p values derived from a linear models averaged by  
 803 Location). Significant differences ( $p < 0.05$ ) are shown in bold.

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A)			Protection		Species		Location	
			F-value	p-value	F-value	p-value	LRT	p-value
Overall	$\delta^{13}\text{C}$	0.405	0.548	380.23	<b>&lt;0.0001</b>	13.53	<b>&lt;0.001</b>	
	$\delta^{15}\text{N}$	0.999	0.351	325.06	<b>&lt;0.0001</b>	9.03	<b>0.003</b>	
B)			Protection		Size		Location	
			F-value	p-value	F-value	p-value	LRT	p-value
Primary producers								
<i>Asparagopsis armata</i>	$\delta^{13}\text{C}$	0.005	0.945	-	-	3.216	0.073	
	$\delta^{15}\text{N}$	4.389	0.084	-	-	9.338	<b>0.002</b>	
<i>Codium tomentosum</i>	$\delta^{13}\text{C}$	0.002	0.969	-	-	2.312	0.128	
	$\delta^{15}\text{N}$	0.388	0.291	-	-	2.895	0.089	
<i>Dyctiota dichotoma</i>	$\delta^{13}\text{C}$	0.010	0.923	-	-	-	-	
	$\delta^{15}\text{N}$	1.382	0.291	-	-	0.454	0.501	

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

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

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808 **Table 3.** Summary of the significance of Protection and effect size, of Location and size  
 809 on species trophic level. Protection and size F and p values are derived from linear  
 810 mixed effect models including Location as a random factor (with the exception of  
 811 *Octopus vulgaris* which had no random effect). Location F and p values are derived

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

812 from a linear model. Significant differences ( $P < 0.05$ ) are shown in bold.

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815 **Figures captions**

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

821

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

826

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

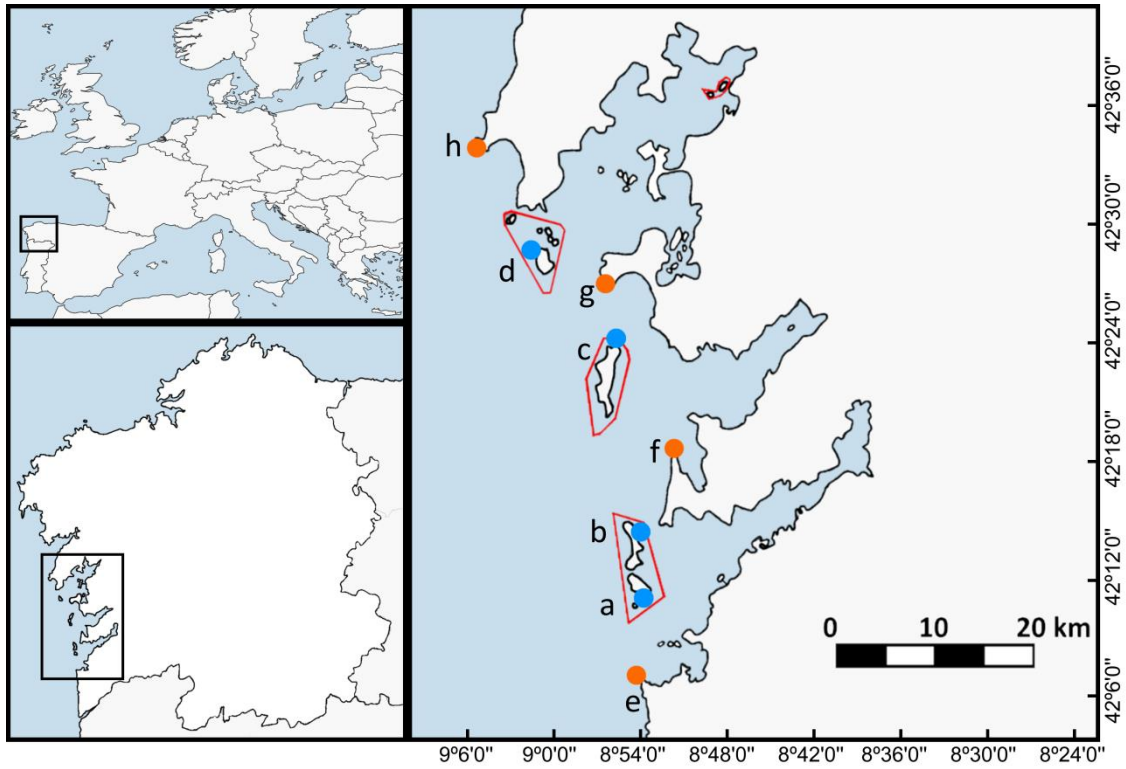
832

833 Figure 4. Species-specific mean ( $\pm$  SD) trophic level (TL) in the locations studied (from  
 834 south to north) in protected (bluish bars) areas (South Cíes, North Cíes, Ons, and  
 835 Sálvora) and unprotected (reddish bars) areas (Cape Silleiro, Cape Couso, O Grove,  
 836 and Cape Corrubedo). Zp: Zooplankton. Mg: *Mytilus galloprovincialis*. Pl: *Paracentrotus*  
 837 *lividus*. Lp: *Lipophrys pholis*. Ms: *Maja* sp. Pm: *Pachygrapsus marmoratus*. Ps:  
 838 *Palaemon* sp. Pp: *Pollicipes pollicipes*. Np: *Necora puber*. Ov: *Octopus vulgaris*. Lower  
 839 case letters represent Tukey post-hoc significant differences between locations ( $P <$   
 840 0.05).

841

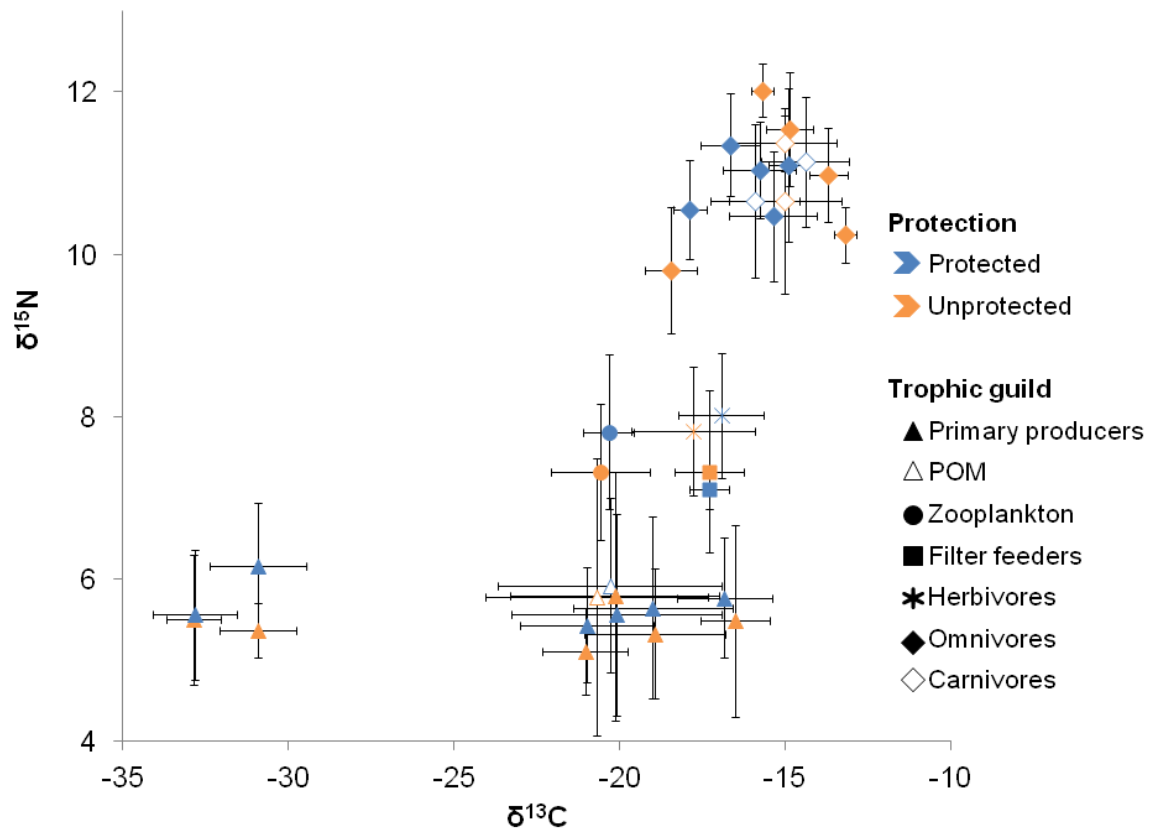
842 **Figure 1**

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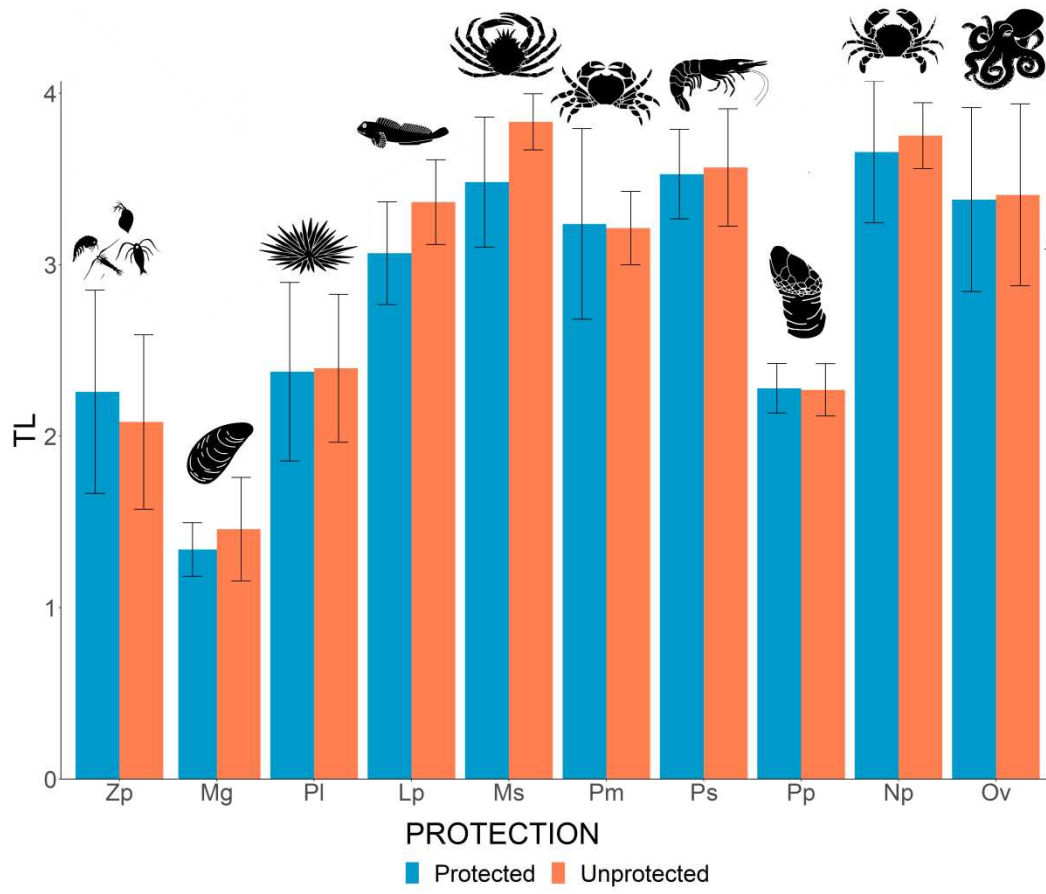
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846 **Figure 2**

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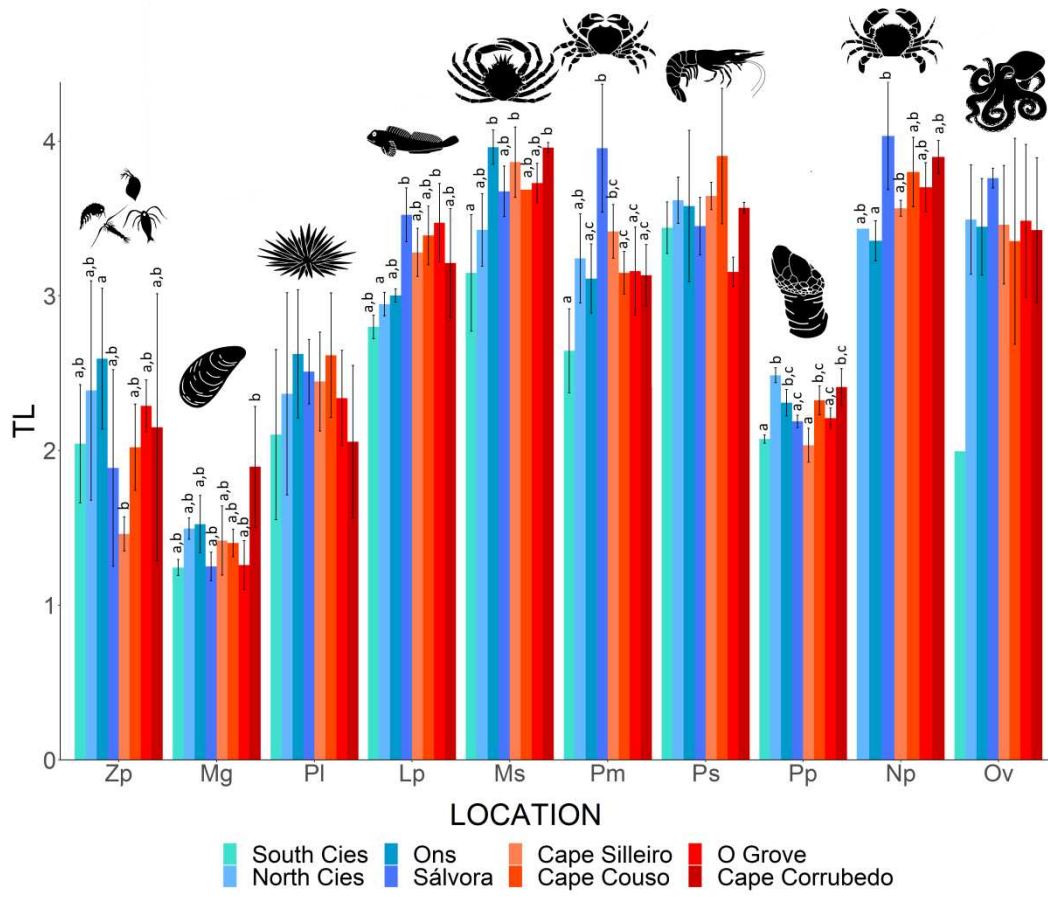
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849 **Figure 3**

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852 **Figure 4**



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## 855 SUPPLEMENTARY MATERIAL

856

857 Table 1. Mean ( $\pm$  SD) values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures (‰) and number of samples (in brackets) of the studied species in  
 858 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	
		$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Primary Sources	<i>Asparagopsis armata</i>	-31.23 $\pm 1.45$ (7)	6.39 $\pm 0.71$	-31.45 $\pm 1.75$ (3)	6.94 $\pm 1.51$	-29.93 $\pm 1.50$ (6)	6.09 $\pm 0.36$	-31.29 $\pm 1.12$ (6)	5.55 $\pm 0.17$	-32.04 $\pm 0.59$ (6)	5.25 $\pm 0.20$	-30.46 $\pm 0.79$ (3)	5.83 $\pm 0.32$	-30.00 $\pm 1.09$ (6)	5.40 $\pm 0.21$	-31.02 $\pm 0.83$ (4)	5.13 $\pm 0.37$
	<i>Codium tomentosum</i>	-19.88 $\pm 2.37$ (9)	6.30 $\pm 1.29$	-20.05 $\pm 1.69$ (9)	5.62 $\pm 0.93$	-17.08 $\pm 2.39$ (8)	5.37 $\pm 0.92$	-18.37 $\pm 2.00$ (3)	4.45 $\pm 0.31$	-19.22 $\pm 2.47$ (9)	5.72 $\pm 0.84$	-18.52 $\pm 2.01$ (7)	5.55 $\pm 0.63$	-18.23 $\pm 2.33$ (8)	5.11 $\pm 0.86$	-20.06 $\pm 0.98$ (5)	4.65 $\pm 0.32$

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

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

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

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

