



This document is a postprint version of an article published in Marine Ecology Progress Series © Inter-Research after peer review. To access the final edited and published work see <https://doi.org/10.3354/meps13235>

Document downloaded from:



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18

Trophic plasticity in the sea urchin *Paracentrotus*  
*lividus*: herbivory, detritivory and omnivory as a  
function of resource availability and habitat features

Judith Camps-Castellà<sup>1,2\*</sup>, Javier Romero<sup>2</sup>, and Patricia Prado<sup>1</sup>

<sup>1</sup>IRTA-Aquatic Ecosystems. Ctra. Poble Nou Km 5.5, 43540 Sant Carles de la Ràpita,  
Tarragona, Spain

<sup>2</sup>Department of Evolutionary Biology, Ecology and Environmental Sciences, Section of  
Ecology, University of Barcelona, Av. Diagonal, 645, 08028 Barcelona, Spain

\*Correspondence author: [judithcamps@outlook.es](mailto:judithcamps@outlook.es)

1           **ABSTRACT**

2           Factors controlling herbivory pressure have a central importance in shaping the  
3 seascape. In the Mediterranean, the sea urchin *Paracentrotus lividus* is considered as a  
4 keystone herbivore in seagrass meadows and macroalgal communities. Here we  
5 explore the trophic behavior of this sea urchin in a shallow seagrass habitat of  
6 *Cymodocea nodosa* mixed with *Caulerpa prolifera* and interspersed with sandy areas in  
7 the Alfacs Bay, Ebro Delta (NW Mediterranean). The seasonal pseudo-indigenous  
8 bryozoan *Amathia verticillata* is locally very abundant, and there is also an important  
9 population of *Pinna nobilis*, providing hard substrate and hides, thus being a unique  
10 environment for assessing the sea urchin trophic behavior. To this end, an ensemble of  
11 food preference and foraging experiments, stomach contents and stable isotope  
12 analyses were conducted. Our results showed that sea urchins strongly prefer *A.*  
13 *verticillata* over other local resources, and there was also an important presence of the  
14 bryozoan in stomach contents (ca. 44 %), coupled with green and decayed seagrass  
15 leaves. Stable isotope analyses, evidenced that in the long-term, ca. 65 % of the diet of  
16 *P. lividus* appears to be based on decayed seagrass leaves, followed by the bryozoan  
17 and green seagrass leaves (21.7 and 13.3 %, respectively). The local availability of *P.*  
18 *nobilis* provides a preferred substrate for sea urchins which showed limited foraging  
19 movements into the surrounding seagrass beds, particularly when *A. verticillata* was  
20 attached to the pen shells. The apparently high contribution of animal and detrital  
21 food to *P. lividus* diet is unprecedented, and suggests an opportunistic feeding  
22 behavior in sea urchins in those habitats.

23   **Key words:** Trophic behavior, *Amathia verticillata*, seagrass beds, *Pinna nobilis*, stable  
24 isotopes

1        **1. INTRODUCTION**

2        Sea urchins, often regarded as keystone herbivores in rocky macroalgal  
3 communities and seagrass meadows, are capable of causing shifts from vegetation-  
4 dominated to unvegetated barrens in shallow marine waters during population  
5 outbreaks (see reviews by Eklöf et al. 2008, Ling et al. 2015). Factors triggering such  
6 major herbivory events have been primarily attributed to alterations in the strength of  
7 bottom-up regulation following nutrient enrichment, reduced control by top predators  
8 due to hunting or overfishing (Shepherd 1987, Estes et al. 1998, Ruiz et al. 2001,  
9 Tewfik et al. 2007), and to changes in ocean temperature due to global warming  
10 (Vergés et al. 2016). However, our ability to accurately predict herbivory impacts may  
11 also depend on a multiplicity of other variables related to foraging and feeding  
12 behavior, resource availability and accessibility (Prado & Heck 2011, Marco-Mendez et  
13 al. 2012, 2015), the effect of habitat (Boada et al. 2018), and the interplay between  
14 these variables.

15        Sea urchins are a widely diversified group, and some of the most emblematic  
16 species are voracious herbivores. The high C:N ratio found in macroalgae and,  
17 especially, in seagrasses, suggests a low nutritional value (Duarte 1990) and dietary  
18 deficit of nitrogen. Moreover, the abundance of refractory carbon such as lignin  
19 decreases digestibility of seagrasses by complexing with cellulose and hemicellulose  
20 (Bjorndal 1980). Sea urchins' tube feet are thought to be equipped with sensory  
21 receptors that help the process of food detection and selection (Pisut 2004),  
22 potentially related, at least in part, to the search of N sources. Foraging activity relies  
23 on a chemosensory ability that aids them in moving towards the source of the  
24 chemical cue and provides the capacity to discriminate among a variety of food items

1 (Lawrence et al. 2013). Chemical attractants indicative of palatability are believed to  
2 be primarily amino acids, although there also may be effects of quaternary ammonium  
3 bases, nucleotides and organic acids (Carr et al. 1996), which may account for  
4 observed patterns of enhanced consumption of nutrient enriched seagrasses or  
5 benthic macroalgae (Ruiz et al. 2001, Valentine & Heck 2001).

6 Despite most species of sea urchins are herbivores, it is not rare to find facultative  
7 omnivory in sea urchins (Lawrence et al. 2013), mostly due to the consumption of  
8 animal epiphytic communities associated with macrophytes, though some urchin  
9 species have been also shown to prey on larger benthic animals such as mussels and  
10 crustaceans (Watts et al. 2007, Wangensteen et al. 2011). Ingesting animals can  
11 compensate for the low nutritional value of seagrasses (Boudouresque & Verlaque  
12 2001, Prado et al. 2010) and, some urchin species (e.g. *Paracentrotus lividus*) use these  
13 food items as the in main source of dietary nitrogen (Tomas et al. 2005a, Marco-  
14 Mendez et al. 2012). Since patterns of leaf growth and senescence are strongly  
15 connected to those of epiphyte accrual (Cebrián et al. 1999), epiphyte biomass and  
16 distribution can also determine grazing preferences at the plant scale (see for instance  
17 Ott 1980, Prado et al. 2011). Hence, sea urchins can be regarded as generalist  
18 consumers with plastic feeding behavior (Lawrence 1975). In effect, new primary  
19 producers including both seagrasses and macroalgae may produce an assortment of  
20 secondary metabolites with potential deterrent activity that may also determine  
21 preferences, consumption rates, absorption efficiencies and ultimately the fitness of  
22 herbivorous invertebrates (Vergés et al. 2007a, Duarte et al. 2011). In addition, the  
23 shape and toughness of food resources may constrict the degree of manipulability by  
24 herbivores and further influence their trophic ecology (Vergés et al. 2007b).

1        Aside from the availability (in terms of chemical and structural aspects) of suitable  
2 food items, structural features of the habitat and the presence of predators may also  
3 determine patterns of sea urchin movement and feeding strategy. For instance, the  
4 sea urchin *P. lividus* (Lamarck) does not usually move across unvegetated areas and, in  
5 patchy mosaics, individuals tend to cluster on isolated seagrass patches or rocks or  
6 shells, and not venture beyond their refuges (Zavodnik 1980, Boudouresque &  
7 Verlaque 2013). Also, under intense predation pressure, sea urchins may hide within  
8 rocky or rhizome crevices or beneath boulders (Sala & Zabala 1996, Farina et al. 2009,  
9 Pessarrodona et al. 2019), thus restricting foraging ability.

10        To further explore the trophic plasticity of sea urchin, and how this plasticity  
11 interacts with habitat features, we took advantage of an uncommon species  
12 assemblage occurring in the Alfacs Bay, Ebro Delta (NW Mediterranean). There,  
13 seagrass meadows of *Cymodocea nodosa* (Ucria) Ascherson, sometimes mixed with  
14 the green alga *Caulerpa prolifera* (Forsskal) Lamouroux and sandy areas, constitute the  
15 dominant habitats in the shallower (0-1.5 m) margins of the bay (Mascaró et al. 2014).  
16 The bay also features one of the largest populations of the endangered pen shell,  
17 *Pinna nobilis* (Linnaeus) in the Mediterranean (Prado et al. 2014), which constitutes  
18 the only natural hard substrate in the area. The abundance of the urchin *P. lividus*  
19 within the seagrass is very low, as also reported for other meadows of *C. nodosa*  
20 (Boudouresque & Verlaque 2001, 2013), and individuals tend to aggregate on pen  
21 shells, which are located either in seagrass or isolated sand areas. The soft body  
22 branching bryozoan, *Amathia verticillata* (delle Chiaje) is locally very abundant during  
23 the summer period and this pseudo-indigenous species (Marchini et al. 2015) has likely

1 been possibly present in the Alfacs Bay for decades (see Zabala 1986) and could be a  
2 potential feeding resource for sea urchins.

3 In this context, the objective of this study was to investigate the trophic plasticity of  
4 *P. lividus* in relation to the seasonal availability of *A. verticillata* and its habitat use. To  
5 this end, an ensemble of food preference experiments, analyses of stomach contents  
6 and stable isotope determinations coupled with mixing models were combined with an  
7 assessment of habitat use and mobility, aimed at clarifying the trophic behaviour of  
8 the sea urchin in the presence of *A. verticillata*.

9

## 10 **2. MATERIALS AND METHODS**

### 11 **2.1. Study site**

12 The study was conducted in the Alfacs Bay (Ebro Delta, NW Mediterranean). The  
13 Alfacs Bay is a semi-confined estuarine area of 50 km<sup>2</sup> with an average depth of  
14 approx. 3 m and a maximum depth of 6 m. The northern shore of the bay receives  
15 nutrient and organic matter-rich freshwater discharges from rice agriculture between  
16 April and October (Garcés et al. 1999), while the southern shore (the Banya Sandspit)  
17 is more influenced by marine waters due to the proximity to the bay mouth (Sanmartí  
18 et al. 2018). The Banya Sandspit was included in the Ebro Delta Natural Park in 1986  
19 and is also a part of the Natura 2000 network of the European Union because of the  
20 importance of local seagrass beds of *C. nodosa* and, the presence of a large population  
21 of *P. nobilis* (Prado et al. 2014).

22 Sampling was conducted at two sites in summer 2018 along the Banya Sandspit  
23 (Site A: 40°35'N, 0°39'E, 0.6 m depth, and site B: 40°35'N, 0°39'E, 0.8 m depth; see Fig.  
24 1). Temperature in the area was  $28.3 \pm 0.49$  °C (mean  $\pm$  SE), salinity  $36.6 \pm 0.35$ , pH 8.1

1  $\pm 0.05$ , and dissolved oxygen  $6.1 \pm 0.29$  mg per liter. The Alfacs bay is subjected to  
2 minor tidal fluctuations typical from the Mediterranean Sea (Llebot et al. 2014), and  
3 storms are rare during the summer period, which is characterized by very calm waters.  
4 Both zones feature large meadows of *Cymodocea nodosa* (monospecific in site A and  
5 mixed with the green alga *Caulerpa prolifera* in site B) and are adjacent to the seagrass  
6 limit and sandy habitats. Other macroalgae (living or drift) were relatively scarce in the  
7 area and were not considered as major potential diet items. Sea urchins are frequently  
8 observed attached to the shells of *Pinna nobilis* which occurs both in seagrass and  
9 sandy areas. The soft-bodied, branching bryozoan *Amathia verticillata* is widely  
10 distributed within the Alfacs Bay during the summer period, growing at shallow depths  
11 (0-1 m) either attached to wooden pylons, seagrass and pen shells or as large detached  
12 floating mats similar in aspect and consistency to floating algae. The occurrence of *A.*  
13 *verticillata* in seagrass beds of *C. nodosa* in the presence of sea urchins is, to our  
14 experience, very rear, since the bryozoan usually appears in brackish sites with some  
15 freshwater influence which are close to the limit of tolerance for sea urchins (e.g.,  
16 Farrapeira 2011, Beiras et al. 2012).

17

## 18 **2.2. Distribution and abundance of *P. lividus* and *P. nobilis***

19 The abundance of sea urchins was estimated: (i) in seagrass (*C. nodosa*) meadows;  
20 (ii) pen shells (*P. nobilis*) dwelling in seagrass meadows and (iii) pen shells dwelling in  
21 unvegetated areas (sand), simultaneously with pen shell abundances in each habitat.  
22 At each site, randomly placed 50 x 50 cm quadrats (N=60) were used to assess  
23 abundances within the seagrass meadows (unattached to shells), whereas the number  
24 of pen shells and the number of sea urchins on pen shells was determined by using



1 randomly placed 10 m x 1 m transects across seagrass and sand habitats (N=10 for  
2 each habitat). The diameter of the test (excluding spines) of all sea urchins found in the  
3 quadrats or in the transects was measured to the nearest mm with Vernier plastic  
4 callipers (Prado et al. 2009).

5

### 6 **2.3. Abundance of food items**

7 The biomass (g dry weight [DW] m<sup>-2</sup>) of the two main macrophytes, the seagrass *C.*  
8 *nodosa* and the green alga *C. prolifera*, as well as of the bryozoan *A. verticillata*, were  
9 estimated in August 2018.

10 For *C. nodosa*, five 30 x 30 cm quadrats were haphazardly placed at each study site,  
11 and all the shoots within the quadrat were collected using a sharp knife, placed into a  
12 plastic bag and transported to the laboratory for shoot counts. Fifty shoots were also  
13 collected haphazardly from each site and dry-weighed (60°C until constant weight)  
14 order to convert the number of shoots per m<sup>2</sup> into g DW m<sup>-2</sup>. For *C. prolifera*, biomass  
15 was estimated by direct sampling, collecting all blades within 5 haphazardly-placed 30  
16 x 30 cm quadrats in each site. Each sample was dry-weighed in the laboratory. For *A.*  
17 *verticillata*, 5 x 5 m quadrats (N = 5) were haphazardly placed in both *C. nodosa* and  
18 sand habitats of each study site. The biomass within each quadrat was collected and  
19 transported to the laboratory where was determined as previously for dry weighting in  
20 plastic bags.

21

### 22 **2.4. Food preference experiments**

23 Three food choice experiments were conducted at different times (due to time and  
24 space restrictions) during August 2018, with sea urchins of different sizes: sub-adults

1 (1.5-2 cm), young adults (2-3 cm) and adults (> 3 cm). We use this modified  
2 terminology from Grosjean et al. (1998), because in their study authors refer to sub-  
3 adult categories as already strict herbivorous, whereas smaller sizes considered as  
4 juveniles (< 0.5-1 cm) still regularly ingest some animal material such as amphipods. All  
5 individuals (N = 18 of each size) were collected by snorkelling on a subtidal rocky area  
6 in the locality of Les Cases d'Alcanar (40°33'N, 0°31'E) located immediately outside the  
7 Alfacs Bay, and where abundant individuals of the different sizes were readily  
8 available, and transported to the laboratory in an aerated ice-box. Food items for the  
9 experiment (*C. nodosa*, *C. prolifera* and *A. verticillata*) were not available at the site of  
10 sea urchin collection and were gathered in the Alfacs Bay (site B) and transported to  
11 the laboratory in aerated containers. For all experiments, environmental conditions  
12 (salinity, temperature, pH, and oxygen) in the laboratory were kept as in the field,  
13 since seawater within the facilities is pumped from the Alfacs Bay. The photoperiod  
14 during the experiment was also maintained under natural light conditions.

15 For each food choice experiment (one per sea urchin size-class), individuals were  
16 kept randomly in six different tanks (40 L) with aeration (N = 3 individuals per tank)  
17 and allowed adjustment to laboratory conditions for 24 h with no food prior to each  
18 feeding trial. Individuals within each tank were separated by a mesh net and offered a  
19 simultaneous combination of the seagrass *C. nodosa*, the green alga *C. prolifera*, and  
20 the bryozoan *A. verticillata*. We randomly deployed 3 food bunches of a similar weight  
21 (5 to 6 g) of each species attached to a picket. A fourth group of 3 food bunches, which  
22 was separated from grazers by a mesh net, was used as a control to correct for  
23 autogenic changes in the bryozoan over the 24 h period. All bryozoan bunches were  
24 weighed before deployment (0.001 g accuracy). However, in the case of the seagrass

1 and alga, photographs and measures of each leaf (length and width) and frond  
2 (maximum length and width) included in each ramet were taken to determine  
3 consumption and avoid the larger methodological error associated to biomass loss  
4 over time and the need to use procedural controls, particularly in *C. prolifera*. Then,  
5 conversion to initial biomass (g wet weight [WW]) was conducted through the mean  
6 weight-area relationship in 1 cm<sup>2</sup> pieces (6 replicates for each species,  $0.0150 \pm 0.0008$   
7 g WW for *C. nodosa* and  $0.0194 \pm 0.0016$  g WW for *C. prolifera*). In *C. nodosa*, the bite  
8 marks already present at the beginning of the experiment were removed by cutting  
9 the leaf tips. In all cases, feeding trials were conducted over a 24 h period (usually,  
10 12:00 to 12:00 h).

11 After this period, the remaining food items were removed from each tank, the  
12 numbers of bite marks counted (seagrass and alga), and then blot-dried, and weighed  
13 (bryozoan) or photographed (macrophytes) for estimating weight and area losses. In  
14 the case of the bryozoan, control bunches were subjected to considerable weight loss  
15 (ca. 10 % over 24 h), and weight corrections were applied to the results (not applicable  
16 to macrophytes since conversions from area losses were based on initial WW). Losses  
17 by consumption in *C. nodosa* and *C. prolifera* were calculated as area differences  
18 between initial and final photographs using ImageJ v.1.52a. All results were expressed  
19 as the biomass consumed per individual per day.

20 For all individuals, the consumption of food items over the 24 h period was verified by  
21 sacrificing the animals and examination of gut contents.

1 **Dietary analyses of sea urchins**

2 *Stomach contents analyses.* Sea urchin were collected in August 2018 from site B  
3 (30 individuals from pen shells in the mixed seagrass meadow and 30 individuals from  
4 pen shells in sand at 2-4 m distance) and used for the analysis of stomach contents.  
5 The test of each individual was severed around the Aristotle's lantern with the aid of  
6 scissors and the stomach content preserved within sterile 50 mL glass vials in absolute  
7 ethanol for later examination under the stereomicroscope. A pseudo-quantitative  
8 approach (modified from Jones 1968) was adopted for determining the relative  
9 abundance of *C. nodosa* (green and detrital leaves), *C. prolifera*, and *A. verticillata*. To  
10 this end, the surface of a Petri dish (90 mm diameter) was divided into 36 cells of 1 x 1  
11 cm and we noted the number of times that each target item was present in each cell.

12 *Stable isotopes ( $\delta^{13}C$  and  $\delta^{15}N$ ) and nutrient content.* Five individuals from each  
13 habitat (mixed seagrass beds and sandy areas) among those sacrificed for stomach  
14 contents analyses were used for stable isotope analyses. The tissue for isotopic  
15 analyses was obtained by carefully removing buccal muscles from the Aristotle's  
16 lantern (Prado et al. 2010). For food items, green (N = 5) and detrital leaves (N = 5) of  
17 *C. nodosa* were collected, as well as the bryozoan (N = 5) in site A whereas *C. prolifera*  
18 was not analyzed due to its absence from stomach contents and zero consumption  
19 during food preference assays. We decided to analyze seagrass samples combined  
20 with their epiphytes because the extent of the importance of epiphyte communities in  
21 the diet of *P. lividus* has already been investigated in previous studies (Tomas et al.  
22 2006). Hence, we wanted to maximize the observation of differences between  
23 contributions of overall seagrass material coated with epiphytes (in green and decayed  
24 leaves) and that of *A. verticillata*.

1 All samples were dried separately at 60°C during 24 h and reduced to fine  
2 homogeneous powder in a ceramic mortar. Inorganic, non-dietary carbon from  
3 carbonate in encrusting epiphytic algae, bryozoans or other calcareous epiphytes on  
4 seagrass leaves was removed by acid wash with HCl 1M until no bubbling was  
5 observed (Prado et al. 2010). Since this procedure may alter  $\delta^{15}\text{N}$  values (Bunn et al.  
6 1995), samples containing carbonate traces were divided into two aliquots, one acid  
7 washed for  $\delta^{13}\text{C}$  and the other one (untreated) for  $\delta^{15}\text{N}$ .

8 Samples were analyzed with a Flash 1112 IRMS delta C series EA, Thermo Finnigan  
9 mass spectrometer connected to an elemental analyzer at the isotopic ratio mass  
10 spectrometry facility in the Serveis Científico-Tècnics of the University of Barcelona  
11 (Spain). Isotope ratios in samples were calculated from linear calibration curves  
12 constructed with standard reference materials of known composition and a blank  
13 correction. The difference in isotopic composition between the sample and reference  
14 materials is determined by:

$$15 \quad \delta_{\text{sample-standard}} = [(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}] \times 1000$$

16 where  $R_{\text{sample}}$  is the  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  in the sample;  $R_{\text{standard}}$  is the  $^{13}\text{C}/^{12}\text{C}$  or  
17  $^{15}\text{N}/^{14}\text{N}$  in the calibration material and  $\delta_{\text{sample-standard}}$  is the difference in isotopic  
18 composition of the sample relative to that of the reference (Vienna Peedee Belemnite  
19 and atmospheric nitrogen for carbon and nitrogen, respectively).

20 *Isotope mixing models.* The MixSiar Bayesian mixing model (Semmens et al. 2009)  
21 was used to identify the long-term biomass contributions of food items to sea urchin  
22 diet. The model was first outlined by Moore & Semmens (2008) and modified later to  
23 improve the robustness of results. MixSiar v.1.0.4 uses stable isotope signatures with  
24 their standard error (SE), and tissue-diet fractionation factors to estimate the

1 probability distributions (5<sup>th</sup>, 25<sup>th</sup>, 50<sup>th</sup>, 75<sup>th</sup>, and 95<sup>th</sup> percentiles) of each food item  
2 contribution to a mixture and accounts for uncertainty associated with multiple  
3 sources. The estimated median contribution (i.e., the 50 % percentile) represents the  
4 median source contribution value for each source and is usually given for comparative  
5 purposes. For  $\delta^{15}\text{N}$  we fed the model with the  $3.18 \pm 0.08$  fractionation value indicated  
6 for sea urchins feeding on seagrass habitat (Prado et al. 2012), whereas for  $\delta^{13}\text{C}$  we  
7 considered no fractionation (Michener & Schell 1994, Post 2002).

8

## 9 **2.5. Mobility experiment**

10 The movement of sea urchins dwelling on *P. nobilis* shells in seagrass and sandy  
11 habitats was assessed in the presence and in the absence of the bryozoan *A.*  
12 *verticillata*. To this end, locally available empty shells of *P. nobilis* were collected and  
13 placed within the seagrass meadow and in sand habitats within site A. Each pen shell  
14 was oriented in the same direction and separated from other replicates by ca. 3 m.  
15 Half of the pen shells in each habitat were attached a considerable amount of *A.*  
16 *verticillata* with a large cable tie (N = 5 per habitat and bryozoan treatment, except for  
17 the seagrass habitat without *A. verticillata* in which N = 10), and two individuals of *P.*  
18 *lividus* were carefully placed on each of them, considering always the same valve  
19 according to shell orientation.

20 After 24 h, the number of individuals that were either on the same or opposite  
21 valve, of the pen shell at a close distance (10-30 cm) or at a far distance (in a radius of  
22 2 m), or not found, was recorded for each habitat and bryozoan (presence/absence)  
23 treatment. Simultaneously, feeding activity and the food item being used were also

1 noted. Care was taken to avoid floating mats of *A. verticillata* around replicates  
2 without bryozoan addition.

3

#### 4 **2.6. Data analyses**

5 *Distribution and abundance of sea urchins.* Patterns in the abundance of sea urchins  
6 between seagrass and pen shells (fixed factor, two levels) and between sites (A and B,  
7 random factor, two levels) were investigated with a 2-way factorial ANOVA. Similarly,  
8 differences in the number of sea urchins on pen shells dwelling on seagrass and sandy  
9 habitats (fixed factor, two levels) and between sites (random factor, two levels) were  
10 also investigated with a 2-way factorial ANOVA. The association between pen shell and  
11 sea urchin abundances was assessed with regression analysis. The effects of the  
12 habitat where the pen shell was found (fixed factor, seagrass vs. sand) and site  
13 (random factor) on size frequencies of sea urchins (1 cm size classes) were assessed  
14 with a 2-way MANOVA.

15 *Abundance of food items.* Patterns in the biomass abundance of the main local food  
16 resources of *P. lividus* (fixed factor with two levels, the seagrass *C. nodosa* and the  
17 bryozoan *A. verticillata*) at the two study sites were investigated with a 2-way factorial  
18 ANOVA.

19 *Stomach contents analyses.* Differences in the abundance of food items (*C. nodosa*,  
20 *C. prolifera*, and *A. verticillata*) in stomach contents of individuals from seagrass and  
21 sand habitats was investigated with a one-way repeated measures ANOVA, using food  
22 item as the within-subject variable (see Martínez-Crego et al. 2015 for a similar  
23 approach) and the habitat (sand vs. seagrass) as a between-subject variable.

1        *Food preference experiments.* For each sea urchin size class (i.e., sub-adults, young  
2 adults, and adults), the Friedman ANOVA by ranks (Obremski & Conover 1981) and the  
3 Kendall's concordance coefficient (Kendall 1957) were used to assess differences in  
4 consumption rates among food items (*C. nodosa*, *C. prolifera* and *A. verticillata*), and  
5 the degree of agreement among the rankings (see Prado & Heck 2011 for a similar  
6 approach). Food items consistently showed evidences of grazing on at least one of the  
7 3 offered species; therefore, all replicates were included in the analyses. For each size  
8 class group, the entire set of observed consumption values (g WW) was ranked from  
9 the smallest to the largest, and then, non-parametric post hoc comparisons (Wilcoxon  
10 matched pairs test) were used to assess significant differences between diets.

11        *Stable isotopes and nutrient contents.* Differences in the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  composition  
12 of sea urchins between habitats (seagrass vs. sand) and in the stable isotope signatures  
13 and nutrient contents of food items used in food preference experiments were  
14 investigated with a one-way ANOVA.

15        *Mobility experiment.* Patterns of movement in and out pen shells within seagrass  
16 beds and sand habitats in the presence and in the absence of the bryozoan *A.*  
17 *verticillata* were investigated with a  $\chi^2$  goodness of fit test. Individuals were assigned  
18 two categories of movement: within the pen shell and outside the pen shell, and  
19 differences between observed and expected frequencies used to built a chi-square  
20 statistic for testing the overall significance of the patterns. Given the low power  
21 available for this type of analysis, patterns of movement were tested separately for  
22 habitat and bryozoan availability factors.

23        For all parametric analyses, homogeneity of variances and normality assumptions  
24 were tested by Cochran's test and Kolmogorov-Smirnov distribution-fitting test of the



1 residuals, respectively and transformed when necessary to meet ANOVA assumptions.  
2 The critical level of significance was fixed at  $p= 0.05$ . Student-Newman-Keuls (SNK)  
3 post hoc comparisons were used when necessary to identify significant differences in  
4 the interaction between habitats and zones. All ANOVA analyses were conducted using  
5 the Statistica v12.0 software.

6

### 7 **3. RESULTS**

#### 8 **3.1. Distribution and abundance of *P. lividus* and *P. nobilis***

9 There were not significant differences in the number of pen shells between habitats  
10 (seagrass vs. sand), sites, and their interaction (Table. 1a). However, the number of  
11 pen shells in site A ( $5.6 \pm 1.4$  and  $4 \pm 1.4$  individuals per  $10 \text{ m}^2$ ; in seagrass and sand,  
12 respectively) tended to be higher than in site B ( $5.4 \pm 0.6$  and  $1.2 \pm 0.6$  individuals per  
13  $10 \text{ m}^2$ ), also respectively in seagrass and sand). Sea urchins were clumped on pen  
14 shells and were seldom on the seagrass meadow. Consequently, densities were much  
15 higher on pen shells than in the seagrass beds ( $0.47 \pm 0.09$  and  $0.1 \pm 0.1$  individuals per  
16  $\text{m}^2$ , respectively), with no effects of site (Table 1b; Fig. 2a). A significant and positive  
17 association between the abundances of sea urchins and pen shells was therefore  
18 detected ( $r^2 = 0.602$ ,  $p < 0.001$ ,  $N = 40$ ).

19 The number of sea urchins per pen shell was between 4 and 6, with no influence of  
20 the habitat (sand or seagrass) where the pen shells occurred (Table. 1c, Fig. 2b).

21 The analysis of size distribution of sea urchins on pen shells showed no significant  
22 differences among habitats or sites (Table. 1d, Fig. 2c). The dominant size class was,  
23 consistently, that of large individuals of 5 to 6 cm test diameter or larger (6 to 7 cm),  
24 with no urchins smaller than 4 cm.

1        **3.2.        Abundance of food items**

2        The green alga *Caulerpa prolifera* was only found at site B, with an average biomass  
3        of  $54.44 \pm 10.79$  g DW m<sup>-2</sup>, and always mixed with *Cymodocea nodosa*. In contrast, the  
4        bryozoan *Amathia verticillata* was ubiquitous and the biomass did not differ by  
5        habitats, sites, or their interaction (Table 2a). The average biomass of seagrass was  
6        much higher than that of *A. verticillata* ( $255.62 \pm 28.71$  and  $3.86 \pm 1.21$  g DW m<sup>-2</sup>,  
7        respectively), particularly at site B (i.e., significant Site and Food item x Site effects)  
8        (Table. 2b, Fig. 3).

9  
10       **3.3.        Food preference experiments**

11       The preferred food for *P. lividus* was the bryozoan *A. verticillata*, as indicated by  
12       Friedman's tests for all sea urchin size classes including subadult, young adult and  
13       adult individuals (Table. 3, Fig. 4). There was no detectable consumption of the green  
14       alga *C. prolifera*, and seagrass was barely consumed. Although the same pattern was  
15       observed for the three size classes, the amount of bryozoan consumed increased  
16       proportionally to the size of the sea urchin (Table. 3, Fig. 4).

17  
18       **3.4.        Dietary analyses of sea urchins**

19       *Stomach contents analyses.* The stomach contents of sea urchins from pen shells in  
20       seagrass habitats did not differ significantly from those inhabiting pen shells in sand, as  
21       indicated by one-way repeated measures ANOVA (Table. 4). The abundance of *A.*  
22       *verticillata* ( $43.9 \pm 1.00$  %) in stomach contents was significantly higher than that of  
23       green ( $29.41 \pm 0.97$  %) and decayed seagrass ( $26.59 \pm 0.81$ ). There was also significant

1 Diet x Habitat interaction, apparently due to slightly higher contribution of *A.*  
2 *verticillata* to sea urchin diet in sand habitats (Table. 4).

3 *Stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and nutrient content.* There were no significant  
4 differences in the stable isotopes signatures ( $\delta^{13}\text{C}$ :  $-13.02 \pm 0.12$  and  $\delta^{15}\text{N}$ :  $12.72 \pm 0.10$ )  
5 and nutrient content (% C:  $38.35 \pm 0.36$  and % N:  $12.61 \pm 0.17$ ) between sea urchins in  
6 pen shells from seagrass and sandy habitats (two-tailed *t* test,  $p > 0.05$ ).

7 In contrast, there were significant differences in all stable isotopes signatures and  
8 nutrient content among the three potential food items (Table. 5a,d, Fig. 5a). The  $\delta^{13}\text{C}$   
9 signature was highest in green seagrass ( $-10.41 \pm 0.33$ ) followed by decayed seagrass ( $-$   
10  $12.88 \pm 0.31$ ) and lowest in *A. verticillata* ( $-15.12 \pm 0.09$ ). The  $\delta^{15}\text{N}$  signature showed  
11 highest values in decayed ( $10.27 \pm 0.28$ ) and green seagrass ( $9.26 \pm 0.36$ ), and lowest in  
12 *A. verticillata* ( $7.89 \pm 0.04$ ). For elemental content, C was highest in green seagrass  
13 ( $28.41 \pm 0.33$  %) and lower in decayed seagrass and *A. verticillata* ( $15.63 \pm 0.31$  and  $13.25$   
14  $\pm 0.09$  %, respectively), whereas N was much higher in *A. verticillata* and green seagrass  
15 ( $2.72 \pm 0.07$  and  $2.57 \pm 0.04$  %, respectively) than in decayed seagrass ( $1.71 \pm 0.09$  %).

16 *Isotope mixing models.* Given that no significant differences were observed in  
17 isotopic signatures of sea urchins from seagrass and sandy habitats, all data were pooled  
18 for use in the MixSiar Bayesian mixing model. Results for the 50% percentile using a  
19 tissue-diet fractionation factor of  $3.18 \pm 0.08$  for  $\delta^{15}\text{N}$  (Prado et al. 2012), and no  
20 fractionation for  $\delta^{13}\text{C}$  (Michener & Schell 1994, Post 2002) showed that the main local  
21 component of the sea urchin diet was decayed seagrass (65 %), followed by the  
22 bryozoan *A. verticillata* (21.7 %) and to a lesser extent green seagrass (13.3 %) (Fig. 5b).

23

1        **3.5.        Mobility experiment**

2        The presence of *A. verticillata*, as indicated by a  $\chi^2$  goodness of fit test had a  
3        significant influence on the mobility patterns of sea urchins ( $\chi^2 = 4.54$ ,  $df = 1$ ,  $p < 0.05$ )  
4        with higher numbers of individuals found outside the pen shells when the bryozoan was  
5        absent (Fig. 6). In contrast, the effect of type of habitat on the mobility is uncertain  
6        because two individuals from pen shells on exposed sandy habitats disappeared after  
7        24 h ( $\chi^2 = 1.98$ ,  $df = 1$ ,  $p > 0.05$ ). Yet, in the likely case that they were lost to predators  
8        differences between habitats would have been significant ( $\chi^2 = 2.89$ ,  $df = 1$ ,  $p < 0.05$ ).

9  
10        **DISCUSSION**

11        The sea urchin *Paracentrotus lividus* is not only a keystone seagrass herbivore but  
12        also a facultative omnivore (Prado et al. 2007, 2010, Wangensteen et al. 2011).  
13        Although animal epiphytes can be an important component of the sea urchin diet  
14        (Prado et al. 2010), to our knowledge, this is the first time the species has been shown  
15        to ingest such large quantities of animal material (22 to 44 % of the bryozoan *Amathia*  
16        *verticillata*, according to stable isotope and stomach contents analyses, respectively).  
17        Moreover, experimental results demonstrated a strong preference for this food item  
18        across size classes (sub-adults, young adults and adults), under the same  
19        environmental parameters than in the field. In the Mediterranean, the bryozoan *A.*  
20        *verticillata* is considered a pseudo-indigenous species, mostly associated to man-  
21        modified environments such as harbors and marinas (Ferrario et al. 2014) and its  
22        contribution to the diet of *P. lividus* might have passed unnoticed due to the  
23        infrequent occurrence of the two species in the same habitat. In some natural  
24        ecosystems, however, such as seagrass beds of the Alfacs and Fangar Bays in the Ebro

1 Delta, *A. verticillata* has been present for decades (see Zabala 1986, Camps & Prado  
2 2018), and seems to constitute an alternative food item for sea urchins during summer  
3 period. In other ecologically similar species such as the variegated sea urchin,  
4 *Lytechinus variegatus*, which is also reported as a major grazer in seagrass ecosystems  
5 of the Central American region (Rose et al. 1999), predation on a variety of benthic  
6 animals including mussels, crustaceans and epibionts is also common (McClintock et al.  
7 1982, Hammer et al. 2006, Watts et al. 2001, 2007). Besides, mixed formulated diets  
8 have been shown to boost growth compared to diets based only on vegetal material  
9 (Taylor et al. 2009, Prado et al. 2012). Despite the preference for the bryozoan, and its  
10 prevalence in stomach content during summer months, its long-term (ca. yearly, as  
11 indicated by isotopic analysis) contribution to sea urchin diet showed to be moderate  
12 (ca. 22 %), probably due to its patchy distribution in drifting mats and its seasonal  
13 (mostly summer) occurrence. According to isotopic analysis, detrital seagrass leaves  
14 appear to be the main diet source for sea urchins (ca. 65 %), particularly for C ( $\delta^{13}\text{C}$  of  
15 sea urchins and detrital material, -13.02 and -12.88, respectively), although this can be  
16 potentially explained by the preference of *P. lividus* for brown and epiphytised leaf  
17 tips. In effect, Ott (1981) defined *P. lividus* as a “pseudograzer” due to the large  
18 consumption of epiphytized brown tips which can be considered as a sort of standing  
19 debris. Further evidence comes from Ott & Maurer (1977) who showed enhanced  
20 consumption of decayed seagrass leaves compared to green leaves of *Posidonia*  
21 *oceanica* during food preference experiments, although they do not report whether  
22 the study was conducted in the presence or absence of epiphytes. However, the  
23 observations of a feeding behavior targeting leaf tips (brown and heavily epiphytized)  
24 are ubiquitous (Shepherd 1987, Cebrián et al. 1996, Pinna et al. 2009, Prado et al.

1 2011). In our study sites, detrital leaves are readily available to sea urchins dwelling on  
2 pen shells as detached debris, but results could be also partly due to the preference  
3 for leaf tips mentioned above. While the latter is a reasonable assumption for sea  
4 urchins found in pen shells within seagrass meadows, the former is, apparently, the  
5 only mechanism accounting for the observations (isotopic signals and stomach  
6 contents) in pen shells standing in bare sand. Similarly, the 13 % contribution of green  
7 seagrass leaves to sea urchin diet is possibly the resulting of recently detached  
8 material (storms, intense boating activity) from adjacent beds located only 2-4 m away  
9 from sandy areas. Yet, the species has been indicated to account for a 17 % removal of  
10 the leaf production of *P. oceanica* (Prado et al. 2007) and other authors such as  
11 Nedelec & Verlaque (1984) have found that green fragments of seagrass leaves were  
12 dominant over decayed leaves within stomach contents. In contrast, the avoidance of  
13 *Caulerpa prolifera* during food-choice experiments and its absence from stomach  
14 contents despite large local availability confirms the undergoing of chemical  
15 deterrence (see also Boudouresque & Verlaque 2001, 2013). Overall, this variability of  
16 results supports the trophic plasticity of *P. lividus* (Mazella et al. 1992, Prado et al.  
17 2010) and a certain opportunistic feeding strategy, in which the type of available  
18 drifting material seems to play a determinant role.

19 The availability of food items, palatability, and mechanisms for herbivore choice  
20 have been shown to have a major role in the foraging ecology of seagrass consumers  
21 (e.g. Prado et al. 2010, Prado & Heck 2011, Marco-Méndez et al. 2012, 2015). The  
22 nitrogen content of *A. verticillata* ( $2.7 \pm 0.07$  %) was similar to that of green leaves  
23 (2.6%), but in terms of nutritional value, the bryozoan showed ca. two times lower C: N  
24 ratios than seagrass resources, suggesting a more favorable assimilation of dietary

1 elements. In fact, the lower C: N ratio of epiphytes is regarded as a central factor  
2 mediating plant consumption, rich in refractory materials (i.e., structural  
3 carbohydrates) and have low assimilation (Tomas et al. 2005a, Marco-Mendez et al.  
4 2012). Hence, epiphytes may contribute to up to ca. 50 % of the diet of *P. lividus*, and  
5 to up to ca. 90% of the dietary N (Tomas et al. 2006). A similar mechanism has been  
6 indicated for determining the preference of *C. nodosa* over *P. oceanica* (higher C:N  
7 ratios) in *P. lividus* (Marco-Mendez et al. 2015). A plausible explanation could be that  
8 the animal component of seagrass leaves may constitute an enhanced source of N,  
9 often a limiting element for grazers which increases palatability, as evidenced by  
10 enhanced consumption of epiphytes and plant material during nutrient-enrichment  
11 experiments (Lapointe et al. 2004, Heck et al. 2000, 2006, Prado et al. 2010).

12       According to our mobility experiment and patterns of sea urchin distribution in the  
13 field *P. lividus* has a great pen shell fidelity, which can indirectly affect its foraging  
14 behavior. Most of the sea urchins (88 % of individuals) remained in the same pen shell  
15 after 24h. Although a low N value was used and further study is needed to confirm  
16 observed patterns, sea urchins appeared to be less prone to move away from their site  
17 and seek for food when *A. verticillata* was available. For connectivity patterns, the  
18 results of the experiment are inconclusive due to the disappearance of two individuals  
19 from pen shells in the sandy habitat but the total absence of individuals in this habitat  
20 (Zavodnik 1980, this study) suggest they could have been lost to predation (e.g., large  
21 sea breams; see Sala & Zabala 1996, Farina et al. 2014) since pen shells were ca. 4 m  
22 away from the seagrass bed. Also, relatively reduced foraging trips (only 3 individuals)  
23 were observed moving into the seagrass bed in the absence of *A. verticillata*. Recent  
24 research in the same study area has suggested that sea urchins can create barren sand

1 flats or seagrass “halos” (ca. 40-60 cm) around the pen shells where they dwell  
2 (Sanmartí et al. unpubl), which further support the long-term persistence of individuals  
3 within the same exact site. Individuals located on sandy areas or in seagrass halos have  
4 granted a large supply of detached plant material from the adjacent beds (2-4 m  
5 distance) as well as drifting mats of *A. verticillata* during the summer period. In a  
6 similar but more extreme instance, shallow individuals of *P. lividus* living under very  
7 exposed conditions may resist dislodgement by waves by burrowing cup-shaped  
8 cavities in the rocky substrate where they may remain for a long period of time or  
9 even permanently and such behavior completely determines their feeding on arriving  
10 algal propagules (Boudouresque & Verlaque 2001). In contrast, significant mobility  
11 may occur in connected seascapes when densities of individuals are high and food  
12 resources become scarce in the original habitat. For instance, Boada et al. (2018)  
13 suggested that migration of individuals from rocky macroalgal substrates into adjacent  
14 seagrass beds was responsible for the abundance and demographic structure of  
15 populations across sublittoral habitat mosaics. Since post-settlement mortality can be  
16 a considerable bottleneck in seagrass habitats (Prado et al. 2009), this process can  
17 explain the presence of similar adult densities across connected seascapes (Boada et  
18 al. 2018) and account for the large proportion of the plant primary production  
19 reported to be consumed by sea urchins (ca. 17 %; Prado et al. 2007).

20 The preferred qualities of hard substrate habitats may be related to enhanced  
21 recruitment success (Tomas et al. 2004) due to facilitation of anchorage and/or  
22 locomotion (Boudouresque & Verlaque 2001) and to greater structural complexity  
23 favoring the survival of individuals (Farina et al. 2014). In patchy seagrass beds of *C.*  
24 *nodosa*, attributes of habitat complexity affecting refuge availability and modulating



1 sea urchin predation such as shoot density and depth of the root-rhizome layer (Farina  
2 et al. 2009) are comparatively lower than in more robust *P. oceanica* meadows. Hence,  
3 pen shells appear to constitute the most similar and readily available alternative to the  
4 substrate provided by rocky habitats (Zavodnik 1980), and habitat preference may  
5 constrict foraging trips into less suitable environments more exposed to predators  
6 and/ or local hydrodynamics. For instance, the bottom predatory snail *Hexaplex*  
7 *trunculus* (Farina et al. 2014) is very abundant within local seagrass beds of *C. nodosa*  
8 and might significantly increase predatory risk within this habitat. Besides, the  
9 relatively rapid migration of subaqueous dunes over the seagrass beds (average speed  
10 of 13 m yr<sup>-1</sup>) is a natural process (see Marbà & Duarte 1995, Marbà et al. 1994) that  
11 may negatively affect sea urchin mobility and from which pen shells can provide a  
12 long-term protection.

13 To conclude, although the importance of *P. lividus* as a keystone herbivore in  
14 shallow seagrass ecosystems is irrefutable (e.g. Tomas et al. 2005b, Prado et al. 2007,  
15 2008), the results of this study document the great trophic plasticity and opportunistic  
16 feeding behavior of the species. This shift to a strongly preferred animal species such  
17 as *A. verticillata* differs from the traditional herbivorous role of this sea urchin.  
18 Individuals appear to be largely constricted in mobility within pen shell habitats, and to  
19 feed on drifting *A. verticillata* during the summer period, and on detached seagrass  
20 material when the bryozoan is not available. Foraging trips of *P. lividus* are also  
21 reduced when it dwells in pen shells within sandy areas where sea urchins are not  
22 usually found. Overall, our study shows that the feeding plasticity of *P. lividus* is  
23 strongly related to both nutritional factors and connectivity features of the habitat  
24 which determine its trophic role. Given that *A. verticillata* is often regarded as

1 unwanted fouling organism (Marchini et al. 2015), our findings might also have an  
2 application in pest control.

3

4 **Acknowledgements:** Authors are grateful to professor Kenneth Heck for his  
5 extensive review that greatly improved the manuscript. Also, we thank Dr. Jordi  
6 Sorribas, the UTM-CSIC technician Xavier Rayo and the Dr. David Amblàs for help on  
7 creation Ebro Delta map with QGis. We thank Pep Cabanes and Josep Maria Costas for  
8 technical assistance during fieldwork and David Mateu for this help with sample  
9 processing in the laboratory. We are also grateful to Neus Sanmartí for help during  
10 stable isotope processing and to Joan Camps for help during food preference  
11 experiments.

12

1        **4. LITERATURE CITED**

- 2        Beiras R, Durán I, Bellas J, Sánchez-Marín P (2012) Biological effects of contaminants:  
3        *Paracentrotus lividus* sea urchin embryo test with marine sediment elutriates. ICES  
4        Tech Mar Environ Sci 51
- 5        Bjorndal KA (1980) Nutrition and grazing behavior of the green turtle *Chelonia mydas*.  
6        Mar Biol 56:147-154
- 7        Boada J, Farina S, Arthur R, Romero J, Prado P, Alcoverro T (2018) Herbivore control in  
8        connected seascapes: habitat determines when population regulation occurs in the  
9        life history of a key herbivore. Oikos 127:1195-1204
- 10       Boudouresque CF, Verlaque M (2013) *Paracentrotus lividus*. Dev Aquacult Fish Sci  
11       38:297-327
- 12       Boudouresque CF, Verlaque M (2001) Ecology of *Paracentrotus lividus*. Dev Aquacult  
13       Fish Sci 32:177-216
- 14       Bunn SE, Loneragan NR, Kempster MA (1995) Effects of acid washing on stable isotope  
15       ratios of C and N in penaeid shrimp and seagrass: Implications for food-web studies  
16       using multiple stable isotopes. Limnol Oceanogr 40:622-625
- 17       Camps J, Prado P (2018) *Polycerella emertoni* associated to *Amathia verticillata* in the  
18       Ebro Delta, NE Spain (Western Mediterranean). P.675. In: Yokes M, Andreou V,  
19       Bakiu R, Bonanomi S and others (2018) New Mediterranean Biodiversity Records  
20       (November 2018). Mediterr Mar Sci 19:673-689
- 21       Carr WE, Netherton III JC, Gleeson R A, Derby CD (1996) Stimulants of feeding behavior  
22       in fish: analyses of tissues of diverse marine organisms. Biol Bull 190:149-160

- 1 Cebrián J, Enriquez S, Fortes M, Agawin N, Vermaat JE, Duarte CM (1999) Epiphyte  
2 accrual on *Posidonia oceanica* (L.) Delile leaves: implications for light absorption.  
3 Bot Mar 42:123-128
- 4 Cebrián J, Duarte, CM, Marbà N, Enríquez S, Gallegos M, Olesen B (1996) Herbivory on  
5 *Posidonia oceanica*: magnitude and variability in the Spanish Mediterranean. Mar  
6 Ecol Prog Ser 130:147-155
- 7 Duarte C, Acuña K, Navarro JM, Gómez I (2011). Intra-plant differences in seaweed  
8 nutritional quality and chemical defenses: importance for the feeding behavior of  
9 the intertidal amphipod *Orchestoidea tuberculata*. J Sea Res 66:215-221
- 10 Duarte CM (1990) Seagrass nutrient content. Mar Ecol Prog Ser 6:201-207
- 11 Eklöf JS, De la Torre-Castro M, Gullström M, Uku J, Muthiga N, Lyimo T and others  
12 (2008) Sea urchin overgrazing of seagrasses: a review of current knowledge on  
13 causes, consequences, and management. Estuar Coast Shelf Sci 79:569-580
- 14 Estes JA, Tinker MT, Williams TM, Doak DF (1998) Killer whale predation on sea otters  
15 linking oceanic and nearshore ecosystems. Science 282:473-476
- 16 Farina S, Arthur R, Pagès JF, Prado P, Romero J, Vergés A and others (2014) Differences  
17 in predator composition alter the direction of structure-mediated predation risk in  
18 macrophyte communities. Oikos, 123: 311-1322
- 19 Farina S, Tomas F, Prado P, Romero J, Alcoverro T (2009) Seagrass meadow structure  
20 alters interactions between the sea urchin *Paracentrotus lividus* and its predators.  
21 Mar Ecol Prog Ser 377:131-137
- 22 Farrapeira, CMR (2011) The introduction of the bryozoan *Zoobotryon verticillatum*  
23 (delle Chiaje, 1822) in the northeast of Brazil: a cause for concern. Biol Invasions  
24 13(1):16-16

- 1 Ferrario J, Marchini A, Lodola A, Ambrogi AO (2014) The pseudoindigenous bryozoan  
2 *Zoobotryon verticillatum* along the Mediterranean and European Atlantic Coasts.  
3 Biol Mar Medit 21:117-118
- 4 Garcés E, Delgado M, Masó M, Camp J (1999) In situ growth rate and distribution of  
5 the ichthyotoxic dinoflagellate *Gyrodinium corsicum* Paulmier in an estuarine  
6 embayment (Alfacs Bay, NW Mediterranean Sea). J Plankton Res 21:1977-1991
- 7 Grosjean P, Spirlet C, Gosselin P, Vaitilingon D, Jangoux M (1998) Land-based, closed-  
8 cycle echiniculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata):  
9 A long- term experiment at a pilot scale. J Shellfish Res 17:1523-1531
- 10 Hammer H, Watss S, Lawrence A, Lawrence J, Desmond R (2006) The effect of dietary  
11 protein on consumption, survival, growth and production of the sea urchins  
12 *Lytechinus variegatus*. Aquaculture 254:483-495
- 13 Heck KL Jr, Valentine JF, Pennock JR, Chaplin G, Spitzer PM (2006) Effects of nutrient  
14 enrichment and grazing on shoalgrass *Halodule wrightii* and its epiphytes: results of  
15 a field experiment. Mar Ecol Prog Ser 326:145-156
- 16 Heck KL Jr, Pennock JR, Valentine JF, Coen LD, Sklenar SA (2000) Effects of nutrient  
17 enrichment and small predator density on seagrass ecosystems: an experimental  
18 assessment. Limnol Oceanogr 45:1041-1057
- 19 Holmer M, Duarte CM, Boschker HTS, Barrón C (2004) Carbon cycling and bacterial  
20 carbon sources in pristine and impacted Mediterranean seagrass sediments. Aquat  
21 Microb Ecol 36:227-237
- 22 Jones RS (1968) Micronesica. Guam 4:269-371
- 23 Kendall MG (1957) Rank Correlation Methods. Biometrika 44:298

- 1 Lapointe BE, Barile PJ, Yentsch CS, Littler MM, Littler DS, Kakuk B (2004) The relative  
2 importante of nutrient enrichment and herbivory on macroalgal communities near  
3 Norman's Ponds Cay, Exumas Cays, Bahamas: a "natural" enrichment experiment. J  
4 Exp Biol Ecol 298:275-301
- 5 Lawrence JM, Lawrence AL, Watts SA (2013) Feeding, digestion and digestibility of sea  
6 urchins. Dev Aquacult Fish Sci 38:135-154
- 7 Lawrence JM (1975) The relationship between echinoids and marine plants. Oceanogr  
8 Mar Biol Annu Rev 13:213-286
- 9 Ling SD, Scheibling RE, Rassweiler A, Johnson CR, Shears N, Connell SD and others  
10 (2015) Global regime shift dynamics of catastrophic sea urchin overgrazing. Philos  
11 Trans R Soc Lond B Biol Sci 370:20130269
- 12 Llebot C, Rueda FJ, Solé J, Artigas ML, Estrada M (2014) Hydrodynamic states in a wind-  
13 driven microtidal estuary (Alfacs Bay). J Sea Res 85:263-276
- 14 Marbà N, Duarte CM (1995) Coupling of seagrass (*Cymodocea nodosa*) patch dynamics  
15 to subaqueous dune migration. J Ecol 83:381-389
- 16 Marbà N, Cebrián J, Enríquez S, Duarte CM (1994) Migration of large-scale subaqueous  
17 bedforms measured with seagrasses (*Cymodocea nodosa*) as tracers. Limnol  
18 Oceanogr 39:126-133
- 19 Marchini A, Ferrario J, Minchin D (2015) Marinas may act as hubs for the spread of the  
20 pseudo-indigenous bryozoan *Amathia verticillata* (Delle Chiaje, 1822) and its  
21 associates. Sci Mar 79:355-365
- 22 Marco-Méndez C, Ferrero-Vicente LM, Prado P, Heck KL, Cebrián J, Sánchez-Lizaso JL  
23 (2015) Epiphyte presence and seagrass species identity influence rates of herbivory  
24 in Mediterranean seagrass meadows. Estuar Coast Shelf Sci 154:94-101

- 1 Marco-Méndez C, Prado P, Heck KL, Cebrián J, Sánchez-Lizaso JL (2012) Epiphytes  
2 mediate the trophic role of sea urchins in *Thalassia testudinum* seagrass beds. *Mar*  
3 *Ecol Prog Ser* 460:91-100
- 4 Martínez-Crego B, Arteaga P, Ueber A, Engelen AH, Santos R, Molis M. (2015).  
5 Specificity in mesograzers-induced defences in seagrasses. *PloS one* 10:e0141219
- 6 Mascaró O, Romero J, Pérez M (2014) Seasonal uncoupling of demographic processes  
7 in a marine clonal plant. *Estuar Coast Shelf Sci* 142:23-31
- 8 Mattson Jr WJ (1980) Herbivory in relation to plant nitrogen content. *Annu Rev Ecol*  
9 *Syst* 11:119-161
- 10 Mazzella L, Buia MC, Gambi MC, Lorenti M, Russo GF, Scipione MB and others (1992)  
11 Plant-animal trophic relationships in the *Posidonia oceanica* ecosystem of the  
12 Mediterranean Sea: a review. In: *Plant-Animal Interactions in the Marine Benthos*.  
13 Clarendon Press, Oxford, p 165-187
- 14 McClintock JB, Klinger TS, Lawrence JM (1982) Feeding preferences of echinoids for  
15 plant and animal food models. *Bull Mar Sci* 32:365-369
- 16 Michener RH, Shell DM (1994) Stable isotope ratios as tracers in marine aquatic food  
17 webs. In: Lajtha K, Michener RH (eds) *Stable isotopes in ecology and environmental*  
18 *Science*, Blackwell Scientific, London, p 138-157
- 19 Moore JW, Semmens BX (2008) Incorporating uncertainty and prior information into  
20 stable isotope mixing models. *Ecol Lett* 11:470-480
- 21 Nedelec H, Verlaque M (1984) Alimentation de l'oursin *Paracentrotus lividus* (Lamarck)  
22 dans un herbier à *Posidonia oceanica* (L.) Delile en Corse (Mediterranee-France). In:  
23 *International workshop on Posidonia oceanica beds*, Vol 1, GIS Posidonie Publishing,  
24 Marseilles, France, p 349-364

- 1 Obremski T, Conover WJ (1980) Practical nonparametric statistics. Technometrics  
2 23:415
- 3 Ott JA (1981). Adaptive strategies at the ecosystem level: examples from two benthic  
4 marine systems. Mar Ecol 2:113-158
- 5 Ott J, Maurer L (1977) Strategies of energy transfer from marine macrophytes to  
6 consumer levels: the *Posidonia oceanica* example. In: Biology of benthic organisms,  
7 Pergamon, p 493-502
- 8 Pessarrodona A, Boada J, Pagès JF, Arthur R, Alcoverro T (2019) Consumptive and non-  
9 consumptive effects of predators vary with the ontogeny of their prey. Ecology  
10 100:e02649
- 11 Pinna S, Pais A, Chessa L, Sechi N, Ceccherelli G (2009) Spatial variation of  
12 *Paracentrotus lividus* and *Sarpa salpa* herbivory on *Posidonia oceanica* seagrass  
13 meadows. Estuar Coast Shelf Sci 84:21-27
- 14 Pisut DP (2004) The distance chemosensory behavior of the sea urchin *Lytechinus*  
15 *variegatus*. PhD dissertation, Georgia Institute of Technology, USA
- 16 Post DM (2002) Using stable isotopes to estimate trophic position: models, methods,  
17 and assumptions. Ecology 83:703-718
- 18 Prado P (2018) Seagrass epiphytic assemblages are strong indicators of agricultural  
19 discharge but weak indicators of host features. Estuar Coast Shelf Sci 204:140-148
- 20 Prado P, Caiola N, Ibáñez C (2014) Habitat use by a large population of *Pinna nobilis* L.  
21 in shallow waters. Sci Mar 78:555-565
- 22 Prado P, Carmichael RH, Watts SA, Cebrián J, Heck Jr KL (2012) Diet-dependent  $\delta^{13}\text{C}$   
23 and  $\delta^{15}\text{N}$  fractionation among sea urchin *Lytechinus variegatus* tissues: implications  
24 for food web models. Mar Ecol Prog Ser 462:175-190



- 1 Prado P, Heck Jr KL (2011) Seagrass selection by omnivorous and herbivorous  
2 consumers: determining factors. *Mar Ecol Prog Ser* 429:45-55
- 3 Prado P, Collier CJ, Romero J, Alcoverro T (2011) Distinctive types of leaf tissue damage  
4 influence nutrient supply to growing tissues within seagrass shoots. *Mar Biol*  
5 158:1473-1482
- 6 Prado P, Romero J, Alcoverro T (2010) Nutrient status, plant availability and seasonal  
7 forcing mediate fish herbivory in temperate seagrass beds. *Mar Ecol Prog Ser*  
8 409:229-239
- 9 Prado P, Romero J, Alcoverro T (2009) Welcome mats? The role of seagrass meadow  
10 structure in controlling post-settlement survival in a keystone sea-urchin species.  
11 *Estuar Coast Shelf Sci* 85:472-478
- 12 Prado P, Farina S, Tomas F, Romero J, Alcoverro T (2008) Marine protection and  
13 meadow size alter fish herbivory in seagrass ecosystems. *Mar Ecol Prog Ser* 371:1-  
14 21
- 15 Prado P, Tomas F, Alcoverro T, Romero J (2007) Extensive direct measurements of  
16 *Posidonia oceanica* defoliation confirm the importance of herbivory in temperate  
17 seagrass meadows. *Mar Ecol Prog Ser* 340:63-71
- 18 Rose CD, Sharp WC, Kenworthy WJ, Hunt JH and others (1999) Overgrazing of a large  
19 seagrass bed by the sea urchin *Lytechinus variegatus* in Outer Florida Bay. *Mar Ecol*  
20 *Prog Ser* 190:211-222
- 21 Ruiz JM, Pérez M, Romero J (2001) Effects of fish farm loadings on seagrass (*Posidonia*  
22 *oceanica*) distribution, growth and photosynthesis. *Mar Pollut Bull* 42:749-760

- 1 Sala E, Zabala M (1996) Fish predation and the structure of the sea urchin  
2 *Paracentrotus lividus* populations in the NW Mediterranean. Mar Ecol Prog Ser  
3 140:71-81
- 4 Sanmartí N, Solé L, Romero J, Pérez M (2018) Seagrass-bivalve facilitative interactions:  
5 Trait-mediated effects along an environmental gradient. Mar Environ Res 133:99-  
6 104
- 7 Semmens BX, Ward EJ, Moore JW, Darimont CT (2009) Quantifying inter-and intra-  
8 population niche variability using hierarchical Bayesian stable isotope mixing  
9 models. PloS one 4:e6187
- 10 Shepherd SA (1987) Grazing by the sea urchin *Paracentrotus lividus* in *Posidonia* beds  
11 at Banyuls, France. In: Colloque International sur *Paracentrotus lividus* et les oursins  
12 comestibles. GIS Posidonie, Marseille, p 83-86
- 13 Taylor AM, Powell ML, Watts SA, Lawrence AL (2009) Formulated feed supports weight  
14 gain and survivorship in juvenile sea urchins *Lytechinus variegatus*. J World Aquacult  
15 Soc 40(6):780-787
- 16 Tewfik A, Rasmussen JB, McCann KS (2007) Simplification of seagrass food webs across  
17 a gradient of nutrient enrichment. Can J Fish Aquat Sci 64:956-967
- 18 Tomas F, Alvarez-Cascos D, Turon X, Romero J (2006) Differential element assimilation  
19 by sea urchins *Paracentrotus lividus* in seagrass beds: implications for trophic  
20 interactions. Mar Ecol Prog Ser 306:125-131
- 21 Tomas F, Turon X, Romero J (2005a) Effects of herbivores on a *Posidonia oceanica*  
22 seagrass meadow: importance of epiphytes. Mar Ecol Prog Ser 287:115-125

- 1 Tomas F, Turon X, Romero J (2005b) Seasonal and small-scale variability of herbivory  
2 pressure on the temperate seagrass *Posidonia oceanica* (L.). Mar Ecol Prog Ser  
3 301:95-107
- 4 Tomas F, Romero J, Turon X (2004) Settlement and recruitment of the sea urchins  
5 *Paracentrotus lividus* in two constrating habitats in the Mediterranean. Mar Ecol  
6 Prog Ser 282:173-184
- 7 Valentine JF, Heck Jr KL (2001) The role of leaf nitrogen content in determining  
8 turtlegrass (*Thalassia testudinum*) grazing by a generalized herbivore in the  
9 northeastern Gulf of Mexico. J Exp Mar Biol Ecol 258:65-86
- 10 Vergés A, Doropoulos C, Malcolm HA, Skye M, Garcia-Pizá M and others (2016) Long-  
11 term empirical evidence of ocean warming leading to tropicalization of fish  
12 communities, increased herbivory, and loss of kelp. Proc Natl Acad Sci USA  
13 113(48):13791-13796
- 14 Vergés A, Becerro MA, Alcoverro T, Romero J (2007a) Experimental evidence of  
15 chemical deterrence against multiple herbivores in the seagrass *Posidonia oceanica*.  
16 Mar Ecol Prog Ser 343:107-114
- 17 Vergés A, Becerro MA, Alcoverro T, Romero J. (2007b) Variation in multiple traits of  
18 vegetative and reproductive seagrass tissues influences plant–herbivore  
19 interactions. Oecologia 151:675-686
- 20 Wangensteen OS, Turon X, García-Cisneros A, Recasens M, Romero J, Palacín C (2011)  
21 A wolf in sheep’s clothing: carnivory in dominant sea urchins in the Mediterranean.  
22 Mar Ecol Prog Ser 441:117-128

- 1 Watts SA, McClintock JB, Lawrence JM (2007) Ecology of *Lytechinus variegatus*. In:
- 2 Lawrence JM (ed) Edible sea urchins: biology and ecology, 2<sup>nd</sup> edn, Developments in
- 3 Aquaculture and Fisheries Science, Vol 37. Elsevier Press, NY, p 473–497
- 4 Watts SA, McClintock JB, Lawrence JM (2001) The ecology of *Lytechinus variegatus*. In:
- 5 J.M. Lawrence (Ed.), Edible Sea Urchins: Biology and Ecology. Elsevier Science Press,
- 6 Amsterdam: 375-393
- 7 Zabala M (1986) Fauna del Briozous dels Països Catalans. PhD Thesis. University of
- 8 Barcelona, Catalonia, 825 pp
- 9 Zavodnik D (1980) Distribution of Echinodermata in the north Adriatic insular region.
- 10 Acta Adriat 21:437-468

1 **Table 1.** Two-way ANOVA for: a) density of *P. nobilis* (habitats: seagrass and sand); b)  
 2 density of sea urchins (substrates: seagrass and pen shells); and c) density of sea urchins  
 3 on pen shells (habitats: seagrass and sand). Densities of sea urchins in seagrass and pen  
 4 shells were square root transformed to meet ANOVA assumptions. d) Two-way  
 5 MANOVA for sea urchin size classes observed on pen shells across habitats (*C. nodosa*  
 6 and sand) at the two study sites. PN= pen shells, CN= *C. nodosa*. Statistically significant  
 7 results are indicated in **bold**.  
 8

<b>ANOVAs</b>				
a) <i>P. nobilis</i> abundances	df	MS	<i>F</i>	<i>p</i>
Habitat= H	1	8.07	0.22	0.64
Site= Si	1	0.004	0.0001	0.99
H x Si	1	8.86	0.24	0.62
Error	36	10.63		
<hr/>				
b) Sea urchin abundance	df	MS	<i>F</i>	<i>p</i>
Habitat= H	1	2.77	7.23	<b>0.009</b>
Site= Si	1	0.17	0.44	0.50
H x Si	1	0.23	0.60	0.44
Error	76	0.38		
SNK	PN> CN			
<hr/>				
c) Sea urchin abundance on pen shells	df	MS	<i>F</i>	<i>p</i>
Habitat= H	1	8.07	0.22	0.63
Site= Si	1	0.004	0.0001	0.99
H x Si	1	8.85	0.24	0.62
Error	36	36.21		
<hr/>				
<b>MANOVA</b>				
d) Abundances of sea urchin size classes		Wilk's $\lambda$	$F_{4, 33}$	<i>p</i>
Habitat= H		0.978	0.179	0.947
Site= Si		0.861	1.326	0.280
H x Si		0.884	1.074	0.384

1 **Table 2.** Two-way ANOVA results testing for: a) differences in the biomass of the  
 2 bryozoan *A. verticillata* between habitats and sites; and b) differences in the biomass of  
 3 the two major food items (*A. verticillata* and *C. nodosa*) at the two study sites.  
 4 Abundances of bryozoan (AV) and *C. nodosa* (CN) were square root transformed to meet  
 5 ANOVA assumptions. Statistically significant results are indicated in **bold**.

6

7

8

<b>ANOVAs</b>				
a) Biomass <i>A. verticillata</i>	df	MS	<i>F</i>	<i>p</i>
Habitat= H	1	112.68	7.35	0.22
Site= Si	1	640.89	0.08	<b>0.000</b>
H x Si	1	15.33	0.53	0.47
Error	15	28.81		
b) Biomass of food items	df	MS	<i>F</i>	<i>p</i>
Food item= F	1	50.10	447.28	<b>0.000</b>
Site= Si	1	0.59	5.28	<b>0.029</b>
F x Si	1	0.81	7.22	<b>0.012</b>
Error	27	0.11		
SNK (F)	CN> AV			
SNK (Si)	Site B> Site A			
SNK (R x Si)	CN-Site B> CN-Site A> AV-Site A= AV-Site B			

1 **Table 3.** Friedman’s ANOVA  $\chi^2$  and Kendall’s coefficient of concordance ( $W$ ) for ranked  
 2 consumption rates on offered food items including leaves of *C. nodosa* (CN), the  
 3 bryozoan *A. verticillata* (AV) and the green alga *C. prolifera* (CP). In Wilcoxon matched  
 4 pairs (WMP) post hoc comparisons, significant differences in consumption rates  
 5 between pairs of diet items are indicated in **bold**.

6

7

Consumer (n=19, df= 2)	Friedman’s ANOVA $\chi^2$	Kendall’s $W$	$p$
Subadults WMP post hoc:	25.72 AV > CN = CP	0.714	<b>0.000</b>
Young adults WMP post hoc	27.25 AV > CN = CP	0.75	<b>0.000</b>
Adults WMP post hoc:	28.00 AV > CN = CP	0.77	<b>0.000</b>

1 **Table 4.** One-way repeated measures ANOVA results for differences in the composition  
 2 of food items within stomach contents (Diet within subjects' factor: *A. verticillata* (AV),  
 3 green *C. nodosa* (G-CN) and decayed *C. nodosa* (D-CN)) between sea urchins from pen  
 4 shells located in *C. nodosa* (CN) and sand (S) habitats. Statistically significant results are  
 5 indicated in **bold**.

6

7

<b>RM-ANOVA</b>				
	df	MS	<i>F</i>	<i>p</i>
Habitat= H	1	154.94	1.41	0.23
Error	58	109.83		
Diet= Di	2	925.52	51.38	<b>0.000</b>
Di x H	2	3313.87	14.43	<b>0.000</b>
Error	116	18.01		
SNK (Di)	AV > D-CN = G-CN			
SNK (Di x Ha)	AV(S) > AV(CN) = G-CN(CN) = D-CN(S) = G-CN(S) = D-CN(S)			

8



1 **Table 5.** One-way ANOVA for differences in isotopic signatures and nutrient content  
 2 among food items (*A. verticillata*: AV, green *C. nodosa*: G-CN, and decayed *C. nodosa*: D-  
 3 CN): a)  $\delta^{13}\text{C}$ ; b)  $\delta^{15}\text{N}$ ; c) % C; and d) % N. Statistically significant results are indicated in  
 4 **bold.**

5

6

7

<b>ANOVAs</b>				
a) $\delta^{13}\text{C}$	df	MS	<i>F</i>	<i>p</i>
Food item= F	2	27.682	24.73	<b>0.000</b>
Error	12	0.370		
SNK	AV < D-CN < G-CN			
b) $\delta^{15}\text{N}$	df	MS	<i>F</i>	<i>p</i>
Food item= F	2	7.164	20.36	<b>0.000</b>
Error	12	0.352		
SNK	AV < G-CN < D-CN			
c) %C	df	MS	<i>F</i>	<i>p</i>
Food item= F	2	332.07	24.60	<b>0.000</b>
Error	12	13.495		
SNK	AV = D-CN < G-CN			
d) %N	df	MS	<i>F</i>	<i>p</i>
Food item= F	2	1.489	60.03	<b>0.000</b>
Error	12	0.024		
SNK	D-CN < G-CN = AV			

1 **Fig. 1.** Map of the study zone (Alfacs bay, Ebro Delta), showing the sampling sites (A  
2 and B).

3

4 **Fig. 2.** Distribution and abundance of *P. lividus*. a) Number of sea urchins per 10 m<sup>2</sup> found  
5 directly on the seagrass *C. nodosa* (CN) and on *P. nobilis* (PN) substrates at the two study  
6 sites. b) Abundance of sea urchins per 10 m<sup>2</sup> on *P. nobilis* (PN) dwelling in seagrass (CN)  
7 or sand (S) habitats at the two study sites. c) Abundance of sea urchins (number per 10  
8 m<sup>2</sup>) of the different size classes on *P. nobilis* (PN) dwelling in *C. nodosa* (CN) and sand (S)  
9 habitats at the two study sites. Error bars are SE.

10

11 **Fig. 3.** Biomass of potential food items for the sea *P. lividus*, i.e. the macrophytes *C.*  
12 *nodosa* (CN) and *C. prolifera* (CP), and the bryozoan *A. verticillata* (AV) (g DW m<sup>-2</sup>), in  
13 both seagrass (CN) and sand (S) habitats at the two study sites (A and B). Error bars are  
14 SE.

15

16 **Fig. 4.** Biomass (g WW) consumed in 24h-lasting experiments of the three food items  
17 offered to *P. lividus* (*C. nodosa* (CN), *C. prolifera* (CP) and the bryozoan *A. verticillata*  
18 (AV)), categorized by sea urchins size classes (sub-adults, young adults and adults). Error  
19 bars are SE.

20

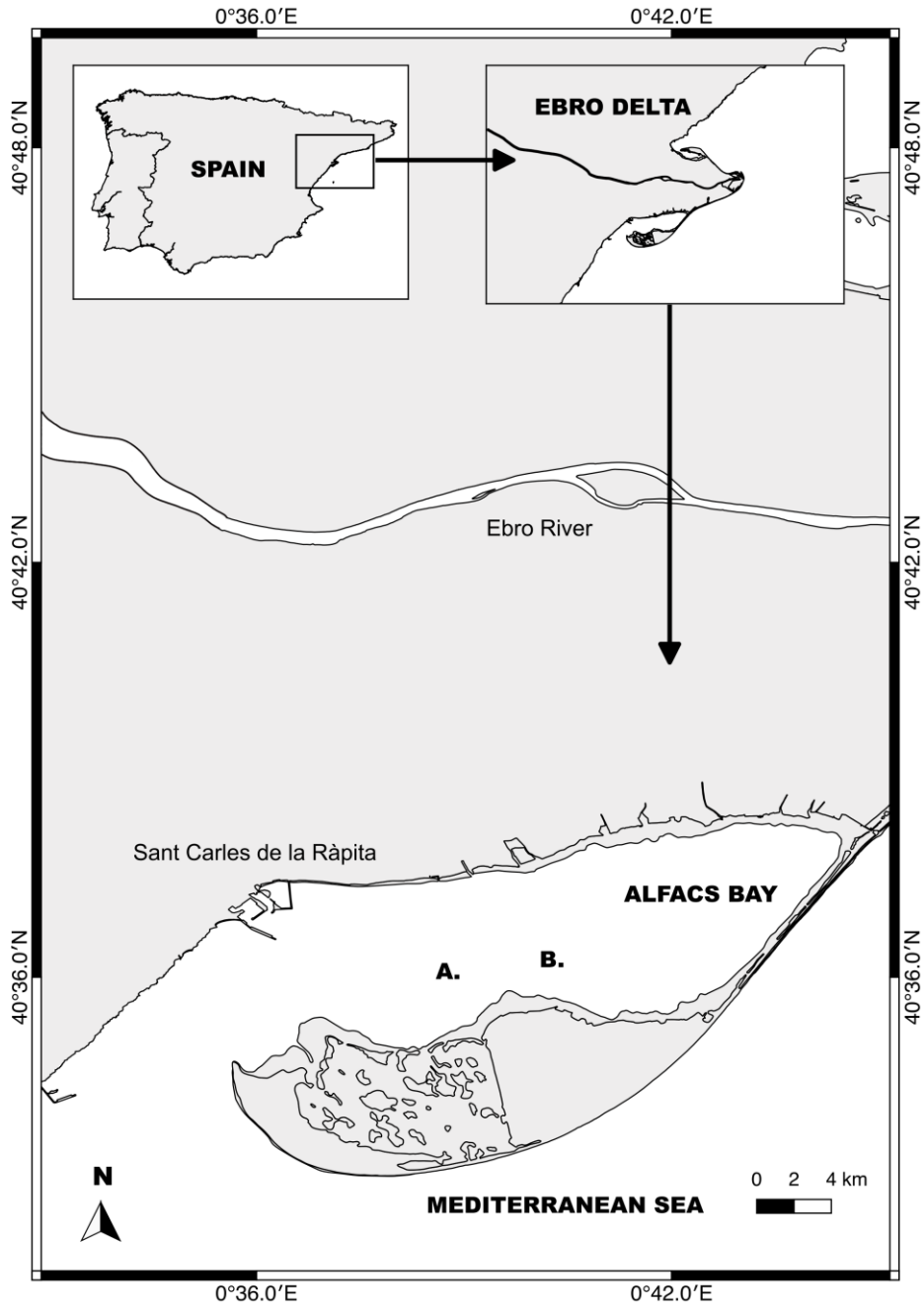
21 **Fig. 5.** Dietary analyses of *P. lividus* dwelling on pen shells from seagrass and sand  
22 habitats (pooled data, since there were no significant differences; for further details see  
23 text). a) Stable isotope signatures ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of *P. lividus* (PL) tissue and of food  
24 items including *A. verticillata* (AV), and green and detrital *C. nodosa* (G-CN and D-CN,

1 respectively). b) Results of the MixSiar Bayesian mixing model showing the contribution  
2 of each considered food items (*A. verticillata* (AV), green and detrital *C. nodosa*, (G-CN  
3 and D-CN, respectively)) to sea urchin diet. The boxes of boxplot indicate the median  
4 and the interquartile range. Error bars are SE.

5

6 **Fig. 6.** Number of sea urchins observed on and outside *P. nobilis* (PN) 24 h after their  
7 manipulative placement in treatments with and without the supply of *A. verticillata*  
8 (AV). The habitat factor (seagrass vs. sand) was pooled due to limitations in the  
9 necessary power for the analysis, for further details see the materials and methods  
10 section.

11

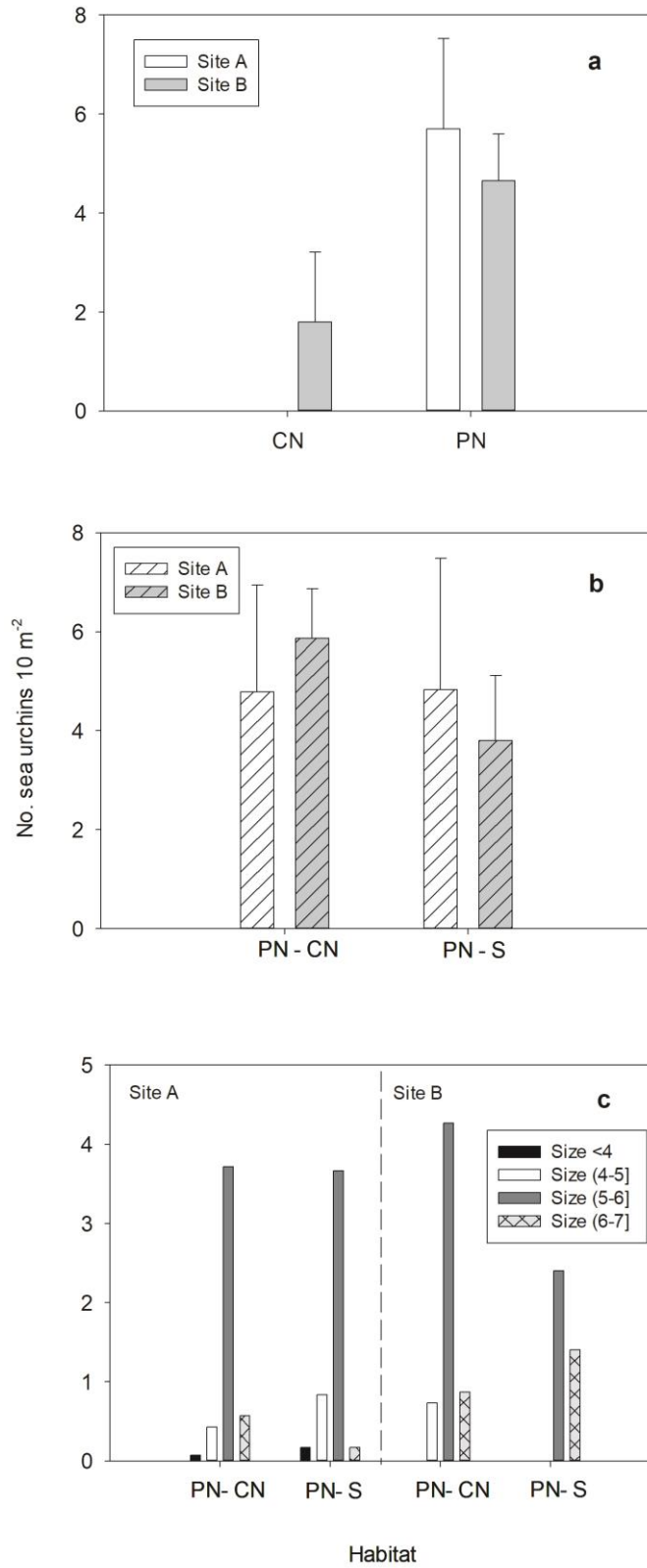


1

2 **Fig. 1.**

3

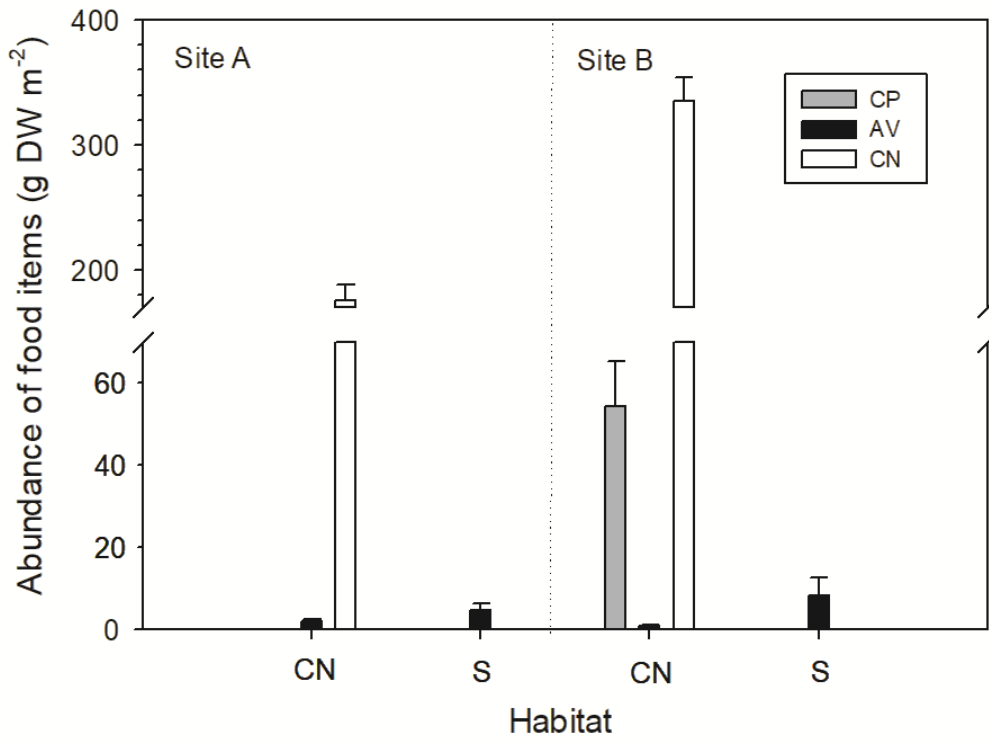
Running title: Trophic plasticity in *P. lividus*



1

2 **Fig. 2.**

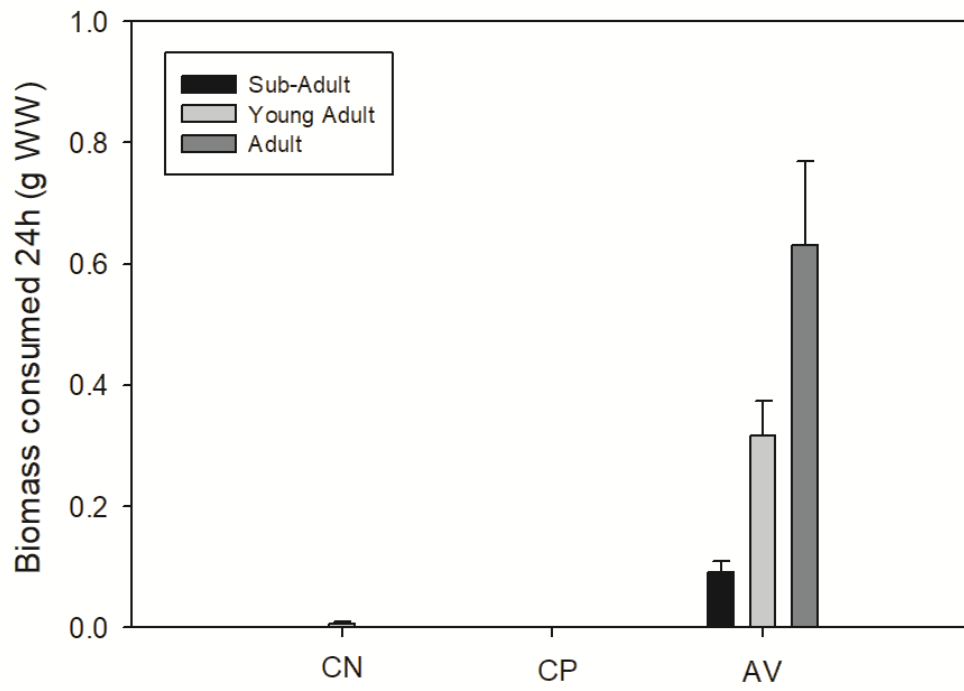
3



1

2 **Fig. 3.**

3

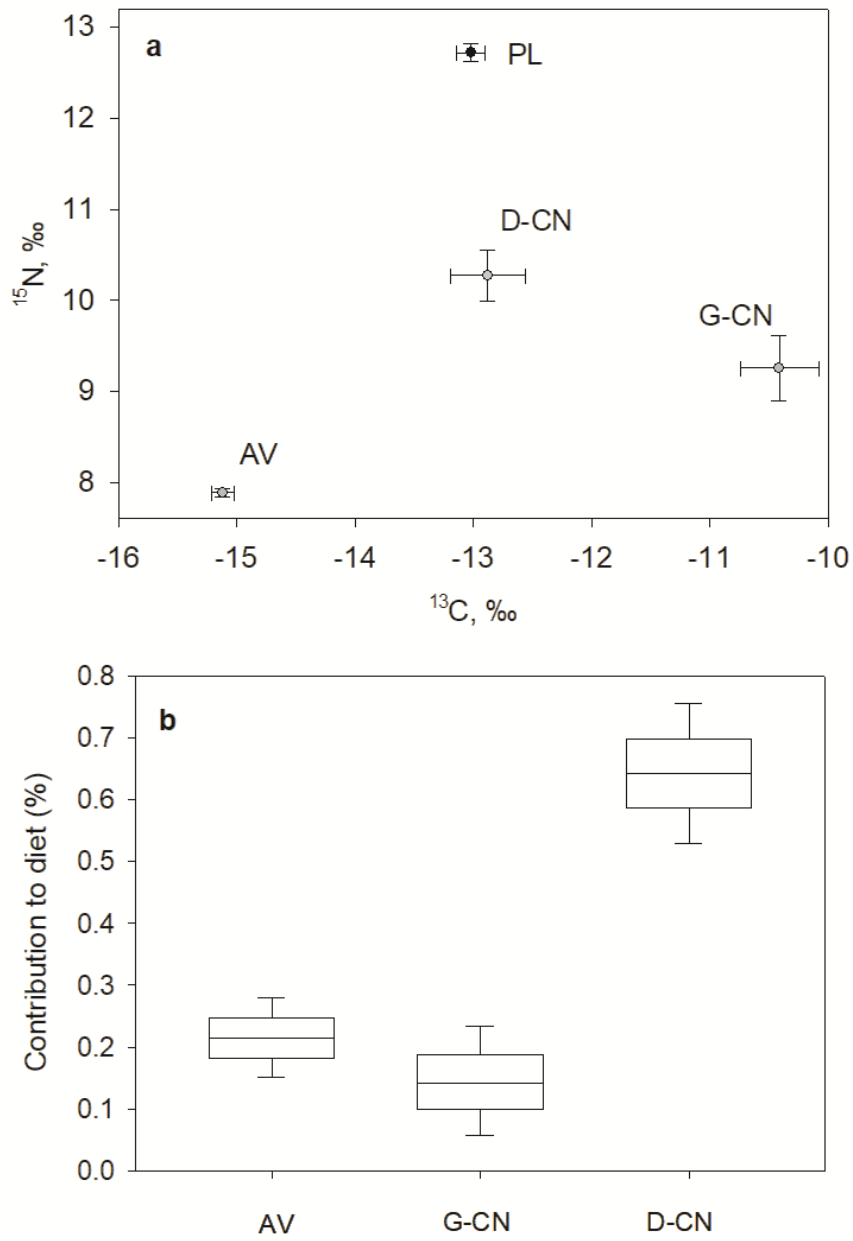


1

2 **Fig. 4.**

3

1



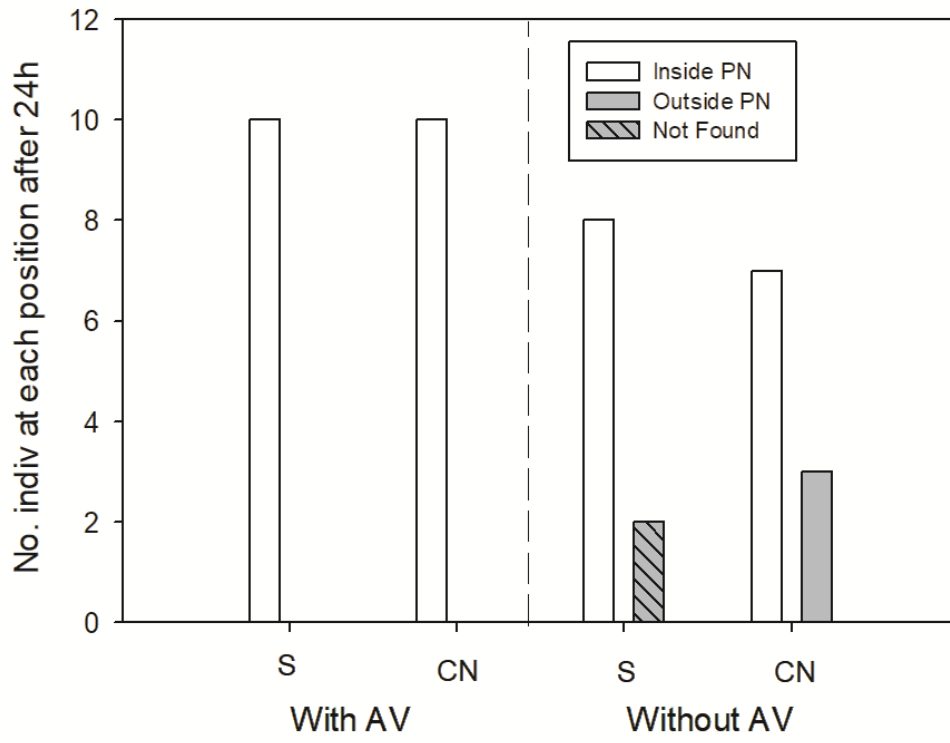
2

3 **Fig. 5.**

4



1



2

3 **Fig. 6.**