

Colonization on *Pinna nobilis* at a marine protected area: extent of the spread of two invasive seaweeds

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*Introduction of species is a major driver of global change and loss of biodiversity in ecosystems. The Mediterranean Sea is the most heavily invaded region in the world by introduced seaweeds. The fan mussel *Pinna nobilis* is the largest Mediterranean bivalve, and a protected species, present at depths from 0.5 m to 60 m. In Cabrera National Park marine protected area (MPA) (Balearic Islands, north-western Mediterranean) the population of *P. nobilis* is affected by the invasive seaweeds *Lophocladia lallemandii* and *Caulerpa racemosa*; however the extent of this colonization on the bivalve is unknown. Therefore, the main aims of the present study were to: (1) determine the extent of invasion of *L. lallemandii* and *C. racemosa* on the *P. nobilis* population; and (2) test if this invasion showed differences with depth. This is the first study assessing invasion by seaweeds on a large bivalve in a high density *P. nobilis* population. *Pinna nobilis* was distributed in all seagrass meadows surveyed within the MPA; we found a total of 872 live *P. nobilis* individuals, 449 and 423 of which were found at 10 and 20 m depth, respectively. High cover of *L. lallemandii* and low presence of *C. racemosa* were detected in the *P. nobilis* population, being quantified in 49.37% and 1.38% of the population of *P. nobilis*, respectively. Bathymetric differences on *Lophocladia* epizoisism over *P. nobilis* has been recorded in this study; with increasing values of *L. lallemandii* cover index, biomass and volume at deeper depths. *Pinna nobilis* could be a preferential substratum for *L. lallemandii* in *Posidonia oceanica* meadows in deeper waters, acting as a stepping-stone for invasive seaweeds and facilitating the invasion throughout seagrass meadows.*

Keywords: biological invasions, *Lophocladia lallemandii*, *Caulerpa racemosa*, *Pinna nobilis*, Mediterranean, alien species, introduced seaweeds, epizoisism, species interactions

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INTRODUCTION

The introduction of species is a major driver of global change and loss of biodiversity in ecosystems (Sakai *et al.*, 2001). The number of species introduced outside their natural ranges is rapidly increasing, although a relatively small proportion of transported and introduced species become invasive (Williamson & Fitter, 1996). However, the ability of ecosystem-engineering species to restructure and, hence, radically change the functioning of the recipient habitat and ecosystem process, is high (Crooks, 2002). Invasive species reduce the species diversity and alter the structure and functioning of the invaded ecosystems (Mack *et al.*, 2000; Boudouresque & Verlaque, 2002; MacDougall & Turkington, 2005).

The endemic fan mussel *Pinna nobilis* Linnaeus, 1758 is the largest Mediterranean bivalve and one of the biggest bivalves worldwide (Zavodnik *et al.*, 1991), living up to 28 yr (García-March & Márquez-Aliaga, 2007) and found at depths ranging from 0.5 to 60 m (Butler *et al.*, 1993). Its main habitats are soft sediments overgrown by seagrass meadows of *Posidonia oceanica* or *Cymodocea nodosa* (Zavodnik, 1967;

Zavodnik *et al.*, 1991). The anterior part of the fan mussel is buried in the seabed and usually anchored to the substratum or among rhizomes and shoots of *P. oceanica* by byssus threads (Richardson *et al.*, 1999; Templado, 2004). *Pinna nobilis* is a filter feeder with a significant ecological role responding to habitat alteration, and can, therefore, be used as an indicator of environmental impact on seagrass meadows. The population of *P. nobilis* has been greatly reduced over the Mediterranean during the last decades (Vicente & Moreteau, 1991) as a result of recreational and commercial fishing, extraction for decorative purposes and incidental killing by anchoring, bottom nets and trawling (Katsanevakis, 2006). Nowadays, *P. nobilis* is protected under ‘Habitats Directive’ to EU Member States, and it is also protected by the Protocol of the Barcelona Convention, to which most Mediterranean countries are signatories, and all forms of deliberate capturing or killing of them are prohibited (EEC, 1992).

Two recognized invasive seaweeds have spread rapidly through the Mediterranean: *Lophocladia lallemandii* (Montagne) F. Schmitz (hereafter *L. lallemandii*) and *Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque (Verlaque *et al.*, 2003; hereafter *C. racemosa*). The red algae *L. lallemandii* is widespread throughout the tropics and subtropics and was probably introduced in the Mediterranean via the Suez Canal (Boudouresque & Verlaque, 2002). *Lophocladia*

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lallemandii is a filamentous algae showing defence metabolites and sexual reproduction, which give it a high invasive potential, growing on a wide range of substrata (bare bedrock, rocky seaweed bottoms, *Posidonia oceanica* seagrass meadows and over coralligenous communities; Ballesteros, 2006). There are few studies concerning invasion of *L. lallemandii* in the Mediterranean Sea, most in the western Mediterranean (Patzner, 1998; Cebrian & Ballesteros, 2010; Bedini et al., 2011). *Lophocladia lallemandii* induces an increase in mortality in *P. oceanica* meadows (Ballesteros et al., 2007), grows over different benthic invertebrates (Patzner, 1998; Deudero et al., 2010) and generates a stress response in *P. nobilis* individuals (Box et al., 2009). The green algae *C. racemosa* has spread extensively throughout the Mediterranean during the last few decades, invading all kind of habitats (Verlaque et al., 2004; Piazzini et al., 2005). The rapid expansion relies on very efficient reproductive strategies, both sexual and asexual (Panayotidis & Züljevič, 2001). Numerous studies have dealt with its taxonomy, biology and ecology, distribution and spread, seasonality and dynamics and impacts on macrophyte assemblages (see review in Klein & Verlaque, 2008). However, few studies have been undertaken to quantify the effects of *C. racemosa* on the native fauna communities (Vázquez-Luis et al., 2008, 2009, 2010; Box et al., 2010). Previous studies dealing with *Caulerpa taxifolia* detected lower densities of the bivalve *Andara trapezia*; moreover, the bivalves presented lower dry tissue weight when living in *C. taxifolia* meadows, which suggests effects on bivalve body condition (Wright et al., 2007).

In the marine protected area (MPA) of Cabrera National Park (Balearic Islands, north-western Mediterranean) the population of *P. nobilis* is colonized by the invasive seaweeds *L. lallemandii* and *C. racemosa*; however, the extent of this colonization over *P. nobilis* shells is unknown. Knowledge about colonization of invasive algae at different habitats and depths can provide information for further management of the endemic species, knowledge about the colonization degree of the algae on the bivalve and contribute to knowledge about the links among invasiveness and native and alien species interaction. There are no clear examples of MPAs providing refuge against invasions. Thus, the main objectives of the present study were: (1) to find out the extent of the invasion of *L. lallemandii* and *Caulerpa racemosa* on the *P. nobilis* population; and (2) to test if this invasion differed between depths.

MATERIALS AND METHODS

Study area

The study was carried out at the MPA of Cabrera National Park in the Balearic Islands, north-western Mediterranean (Figure 1). The MPA was established in 1991 with an area of 100.21 km², of which 87.03 km² are maritime. *Caulerpa racemosa* was recorded in the MPA in 2003 growing at 30–35 m depth, and rapidly spread to almost all benthic communities between 0 and 65 m depth. *Lophocladia lallemandii* was also recorded for the first time in 2003 and is currently present in nearly all habitats between 5 and 45 m depth (Cebrian et al., 2011). The fieldwork was carried out within the *P. oceanica* seagrass meadows in the MPA, since it is the main habitat for the bivalve *P. nobilis*.

Sampling design

Pinna nobilis individuals were sampled at two different depths (10 and 20 m). Visual censuses along line transects of 30 m length and 2.5 m width (total area per transect of 75 m²) were conducted by SCUBA diving in order to survey the *P. nobilis* population density, size (as shell maximum width) and the presence of the two alien algae on the shells. The field survey was carried out at the end of July 2011 (average seawater temperature of 25°C), ensuring high abundances and presence of *C. racemosa* and *L. lallemandii* (Cebrian & Ballesteros, 2009, 2010). A total of 122 line transects allocated at 15 sites were established at 10 m depth, whereas 81 distributed among 12 sites were established at 20 m depth. Surveys were conducted by two divers. An index (cover index) was designed to measure the coverage of the invasive species (*L. lallemandii* and *C. racemosa*) on the two valves of each *Pinna* individual, by giving each invasive species a score ranking from 0 to 4: CI₀ (0%), CI₁ (≤25%), CI₂ (25–50%), CI₃ (50–75%) and CI₄ (75–100%).

Individuals of *P. nobilis* were randomly sampled (36 individuals at 10 m depth and 48 at 20 m depth), at each depth, by scraping all seaweed epizoics on the shell surface, in order to calculate the biomass and volume of invasive species. From each sample, *L. lallemandii* was separated and processed to calculate volume (ml), then dried for 48 h at 60°C, and weighed to calculate biomass (g dw). Colonizable surface area of the shells (as the shell part not buried in the sediment and exposed to be colonized) was calculated from images of 35 empty shells collected during fieldwork through ImageJ software (Abramoff et al., 2004). A correlation between maximum width of *P. nobilis* shell and colonizable surface was conducted. The calculation of colonizable surface was carried out in order to standardize the results of biomass and volume. Therefore, values of volume and biomass of invasive seaweeds were expressed in ml cm⁻² for volume, and g dw cm⁻² for biomass.

Data analysis

Cover index of invasive seaweeds on *P. nobilis* individuals gathered from the visual census were analysed according to a two-factor model, where the main factors were 'Depth' fixed at two levels (10 m and 20 m) and 'Site' random (with 15 levels at 10 m and 12 levels at 20 m). Volume and biomass data from *P. nobilis* shells were analysed using a two-factor model with main factors 'Depth' fixed at two levels (10 m and 20 m) and '*L. lallemandii*' fixed at five levels (CI₀, CI₁, CI₂, CI₃ and CI₄). To investigate whether the cover index differed across depths and sites and to test differences of biomass and volume across depths and levels of *L. lallemandii* (CI), a permutational multivariate analysis of variance (PERMANOVA) was applied, based on the Bray–Curtis dissimilarities of untransformed data (Anderson, 2001; McArdle & Anderson, 2001). After the permutational test, a pair-wise test was carried out to detect differences between groups. Multivariate statistical analyses were performed using PRIMER-E software (Clarke & Gorley, 2006) with the add-on package PERMANOVA+ (Anderson et al., 2008).

RESULTS

A total of 872 living *Pinna nobilis* individuals were studied, of which 449 and 423 were found at 10 m and 20 m depths,

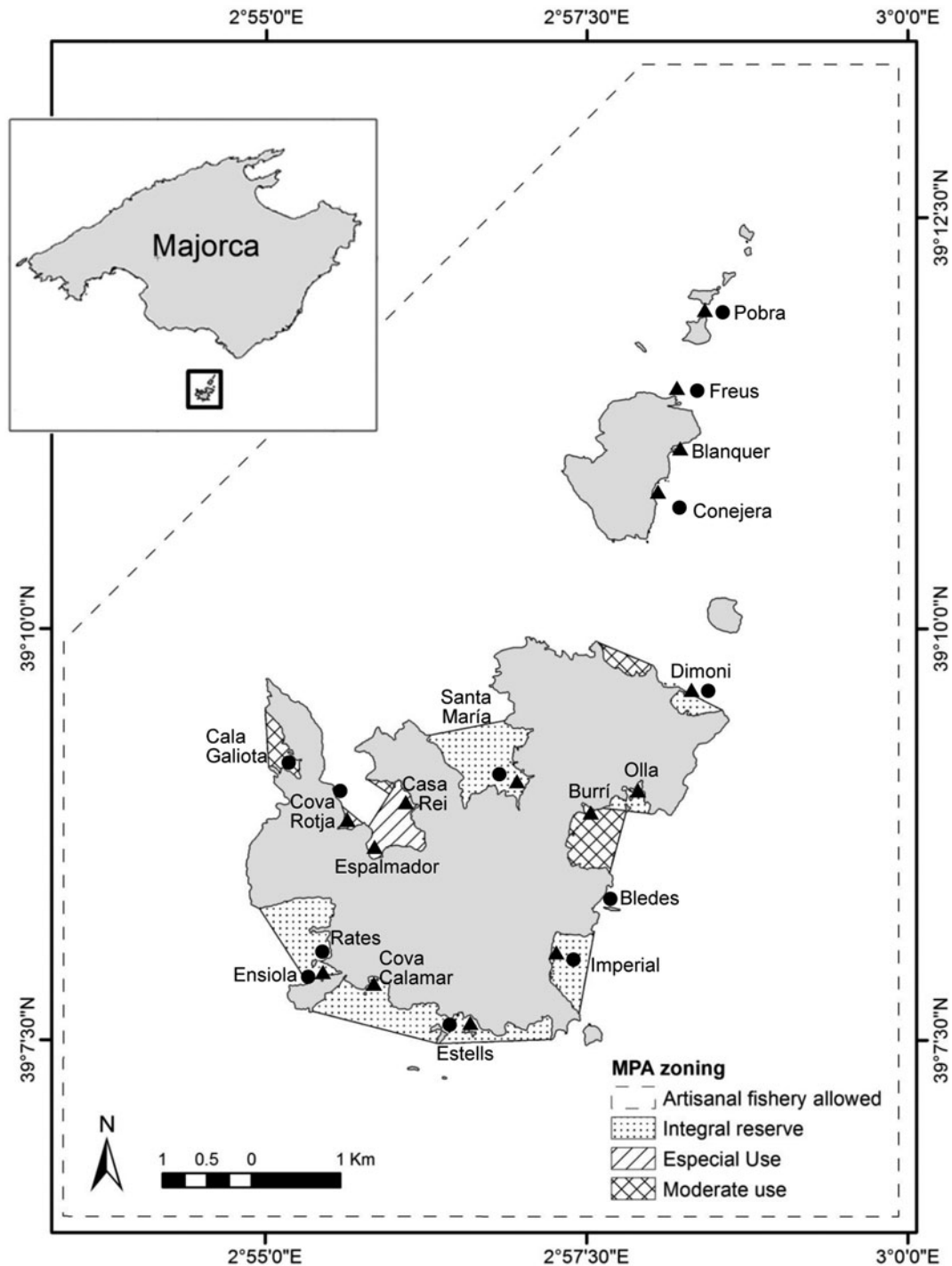


Fig. 1. Study area (ACNP) and different localities (N = 18) sampled for epiphytism of invasive seaweed *Lophocladia lallemandii* over endemic bivalve *Pinna nobilis* shells. Triangles, 10 m depth; circles, 20 m depth.

respectively. *Pinna nobilis* is distributed in all seagrass meadows censused within the Cabrera MPA. The size–frequency distribution was unimodal with individuals ranging from 2.2 to 30 cm in maximum width. Densities of *P. nobilis* individuals at 10 m depth varied from 0 to 37.3 ind 100m^{-2} , while at 20 m depth ranged from 0 to 29.3 ind 100m^{-2} . In terms of mean values, densities (\pm standard error) of *P. nobilis* populations at 10 m and 20 m depth presented 4.9 ± 0.5 ind 100m^{-2} and 6.9 ± 0.7 ind 100m^{-2} , respectively, including data from transects with values of zero.

The cover index showed a high presence of *Lophocladia lallemandii* and a low presence of *Caulerpa racemosa*, being quantified in 49.37% and 1.38% of *P. nobilis* population, respectively. Given the low incidence of *C. racemosa* on *P. nobilis* shells, results are focused on the colonization by the invasive seaweed *L. lallemandii*. Most shells were non-epiphyted by invasive seaweeds at shallow waters with percentages of 63.3% at 10 m and 35.7% at 20 m depth (Figure 2). The pattern of epizoism was similar at both depths (Figure 2). In contrast, the cover index of *L.*

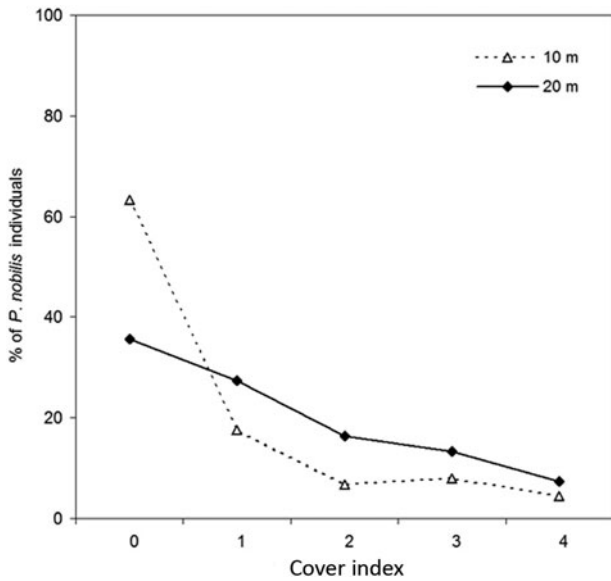


Fig. 2. Percentage of *Pinna nobilis* individuals epiphytized at each *Lophocladia lallemandii* cover index at 10 and 20 m depth. See Materials and Methods section for details on codes.

lallemandii was higher in deeper areas (Figure 2). Incidence of *L. lallemandii* cover on *P. nobilis* population was significantly higher at 20 m depth (depth, PERMANOVA, $P < 0.05$; Table 1 and Figure 3).

An exponential relationship between maximum shell width and colonizable surface was found ($P < 0.001$, Figure 4). Biomass and volume of *L. lallemandii* at different depths showed a similar pattern, but with different values, being both higher at 20 m depth; the R^2 values of the linear regression were similar ($P < 0.001$ for both depths), but the line slope differed between depths (Table 2; Figure 5). However, significant differences between depths were only found for volume at CI2, being higher at 20 m (depth \times *Lophocladia*, PERMANOVA, $P < 0.01$; Table 3). Significant differences between sites were detected, with Santa Maria, Casa Rei, Cova Rotja and Espalmador being the least invaded areas (site, PERMANOVA, $P < 0.01$; Table 1 and Figure 6). Moreover, the variability of colonization cover index differed between sites; the least invaded areas showed low variability, whereas locations with higher values of cover index showed higher variability of the colonization level.

Table 1. Results of two-factor multivariate PERMANOVA for *Lophocladia lallemandii* coverage on *Pinna nobilis* shells. Post-hoc test: depth: 20 m > 10 m ($P = 0.012$); sites: S. Maria = Casa Rei = Cova Rotja = Espalmador < other sites ($P < 0.05$).

Source of variation	<i>L. lallemandii</i> coverage		
	df	MS	P (perm)
Depth	1	31.796	0.016*
Sites (depth)	25	9.4011	0.001**
Residual	845	1.251	
Total	871		

MS, mean square; p, level of significance; df, degrees of freedom; *significant at $P < 0.05$; **significant at $P < 0.01$.

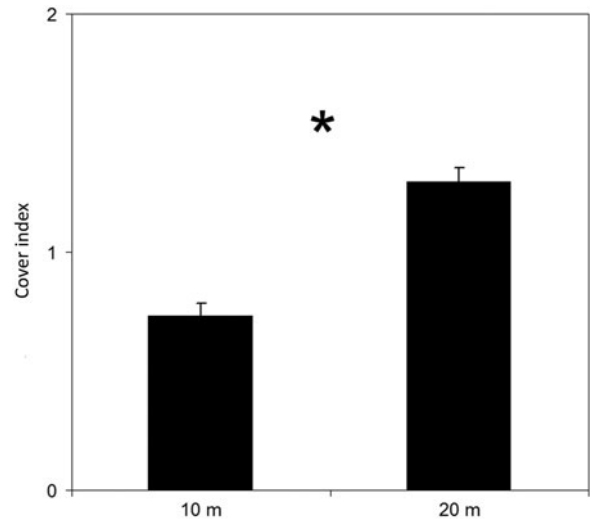


Fig. 3. Mean values (\pm standard error) of *Lophocladia lallemandii* cover index on *Pinna nobilis* living shells at 10 and 20 m depth. * $P < 0.05$

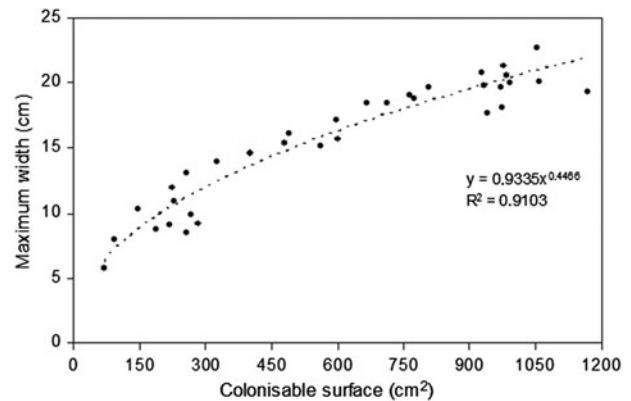


Fig. 4. Exponential function of the regression line of maximum shell width (cm) and colonizable surface (cm²) of *Pinna nobilis* (N = 35).

DISCUSSION

This is the first study assessing seaweed colonization on a large bivalve, in a high density *Pinna nobilis* population. Results showed a high level of epizoism of the alien *Lophocladia lallemandii* on *P. nobilis* shells (approximately half the population was affected). Different *L. lallemandii* epizoism over *P. nobilis* has been recorded in this study, with increasing values of *L. lallemandii* cover index, biomass and volume at deeper depths.

Previous studies carried out on the seaweed community in the Cabrera MPA did not find significant differences in *L. lallemandii*'s biomass as dry weight at 10 m and 30 m depth (Cebrian & Ballesteros, 2010). On the contrary, Bedini *et al.* (2011) found that in the Tuscan Archipelago the percentage cover and biomass of *L. lallemandii* increased with depth throughout the investigated bathymetric range. However, this study was carried out only at 3, 6 and 9 m depth. In our case all variables (cover index, biomass and volume) were higher at 20 m depth. It is likely that *P. nobilis* individuals living at 20 m are more exposed to invasion. One factor that could be affecting this is the lower protection offered by *P. oceanica* meadows at this depth. In fact, a study carried

Table 2. Mean values ± standard error of *Lophocladia lallemandii* biomass (g dw cm⁻²) and volume (ml cm⁻²) by different levels of epiphytism and depth.

Cover index	Shallow (10 m)			Deep (20 m)		
	N	Biomass (g dw cm ⁻²)	Volume (ml cm ⁻²)	N	Biomass (g dw cm ⁻²)	Volume (ml cm ⁻²)
0	10	0	0	10	0	0
1	12	0.0007 ± 0.0001	0.0137 ± 0.0049	7	0.0005 ± 0.0002	0.0085 ± 0.0034
2	8	0.0015 ± 0.0002	0.0264 ± 0.0069	17	0.0026 ± 0.0002	0.0307 ± 0.0034
3	3	0.0017 ± 0.0002	0.0376 ± 0.0076	14	0.0028 ± 0.0003	0.0465 ± 0.0029
4	3	0.0035 ± 0.0006	0.0589 ± 0.0077	0	–	–

N, number of samples.

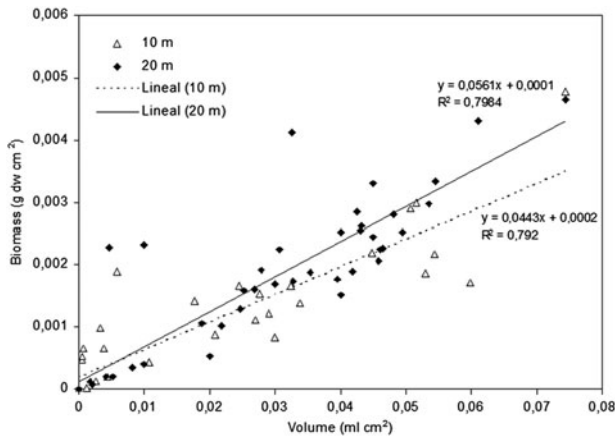


Fig. 5. Linear regression of volume and biomass of *Lophocladia lallemandii* at two depths (10 and 20 m).

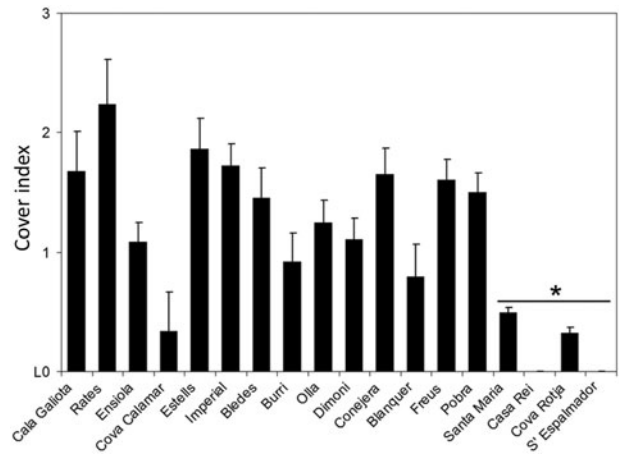


Fig. 6. Mean values (+standard error) of *Lophocladia lallemandii* coverage on *Pinna nobilis* living shells at sampled sites.

Table 3. Results of two-factor multivariate PERMANOVA for *Lophocladia lallemandii* biomass and volume of shells of *Pinna nobilis*. Post-hoc test: depth × *L. lallemandii*: L2 volume: 20 m > 10 m (*P* = 0.006).

Source of variation	Biomass (g dw cm ⁻²)			Volume (ml cm ⁻²)		
	df	MS	<i>P</i> (perm)	df	MS	<i>P</i> (perm)
Depth	1	108.62	0.484	1	484.62	0.467
<i>L. lallemandii</i>	4	9687.6	0.001**	4	30144	0.001**
Depth × <i>L. lallemandii</i>	3	408.25	0.026**	3	2383.9	0.002**
Residual	76	160.24		76	603.06	
Total	84			84		

MS, mean square; *P*, level of significance; df, degrees of freedom; **significant at *P* < 0.01.

out by Marba *et al.* (2002) found that in Santa Maria mean shoot densities were: 1000 sh m⁻² at 7 m, 761.68 sh m⁻² at 13 m and 461.33 sh m⁻² at 20 m. Therefore, individuals living at 20 m depth are less protected by the seagrass. *P. oceanica* shoots are one of the preferred habitats for invasive species, thus, due to reduction of this habitat in deeper areas, *L. lallemandii* settles on new substratum such as *P. nobilis* individuals. Hydrodynamics could also affect the invasion process since *L. lallemandii* can be easily detached from the substratum by water movements (Cebrian & Ballesteros, 2010), *P. nobilis* being a new settlement substratum. Generally, the upper region of the exposed part of the *P. nobilis* shell is the first part to be colonized, and other authors suggested that vertical substrata seem to facilitate the spread of *L. lallemandii* when compared with horizontal substrata (Bedini *et al.*, 2011). Therefore, *P. nobilis* could be a preferential substratum

for *L. lallemandii* in *P. oceanica* meadows in deeper areas, acting as a stepping-stone and facilitating the invasion of the algae throughout seagrass meadows.

Regarding geographical variation, the sites with a lower level of *L. lallemandii* incidence are areas with extensive *P. oceanica* meadows, semi-enclosed and with little habitat heterogeneity. Healthy *P. oceanica* meadows have been suggested to act as a biological barrier against invasion (Ceccherelli & Cinelli, 1999; Ceccherelli *et al.*, 2000). Occhipinti-Ambrogi & Savini (2003) suggested that a robust native ecosystem can successfully fight competitor newcomers. Therefore, a healthy pristine community could represent a natural obstacle to bioinvasion. Moreover, sites with a lower level of epizoism could have less propagule pressure since they are less exposed to currents and hydrodynamics; thus, the most epizoited areas are places more exposed to storms. However, we must take into account in the interpretation of these results the fact that some sampled sites presented only one depth.

It is important to remark that a low percentage of the *P. nobilis* population was epizoited to a maximum *L. lallemandii* cover level (L4: 4.5% at 10 m and 7.3% at 20 m). It is possible that the invasive seaweed was not found at maximum spread level, or that some mechanisms control the invasion process. Species interactions are a major driver of ecosystem dynamics (Sergio *et al.*, 2006) and are important in invasion biology and ecology (Colautti *et al.*, 2004). Specialist herbivores control plants in their native range, whereas generalist herbivores provide resistance to invasion in recipient communities (Maron & Vilà, 2001; Parker *et al.*, 2006). For marine algae, vulnerability to native herbivores may be an important

determinant of invasion success. In the case of invasive seaweeds and herbivore control, recent studies through a combined examination of sea urchin *Paracentrotus lividus* diets and experimental manipulations to measure interaction strength, concluded that introduced *C. racemosa* is likely to be affected directly by consumption, whereas direct effects on introduced *L. lallemandii* are unlikely, because it is avoided (Cebrian *et al.*, 2011; Tejada *et al.*, 2013). Other studies showed that *L. lallemandii* is also avoided by the herbivorous fish *Sarpa salpa* (Tomas *et al.*, 2011). However, in the enclosed areas with lower levels of occurrence of *L. lallemandii* on *P. nobilis* shells, herbivorous control by mesograzers could occur, since we detected a facilitative interaction between the two species. Positive interactions are defined as non-trophic interspecific interactions that increase the average individual fitness of one species (Callaway, 2007). Probably, *P. nobilis* enhances the coexistence of the mesograzer mollusc *Haliotis tuberculata*, since it has been observed that a high percentage of pen shells present *H. tuberculata* on their valves (personal observation). Previous studies showed that *H. asinina* have 72.2% of red algae ingestion (Tahil & Juinio-Menez, 1999). Clavier & Chardy (1989) showed that *H. tuberculata* is absent in areas deeper than 8 m (northern Brittany, France), finding a correlation between abundance and biomass with reduction in water current. In our study, areas with lowest epizoisism levels by *L. lallemandii* correspond to possible optimal areas for *H. tuberculata* populations, since we have observed individuals of this species on *P. nobilis* shells, indicating that this association could be producing herbivore control through grazing of seaweeds on *P. nobilis* shells. However, further studies are necessary to test if ormers could be acting as biological control of *L. lallemandii* spread in these areas.

Several effects of *L. lallemandii* invasion on *P. nobilis* can be envisaged, both on the community structure of associated epizoisms, and on the fan mussel's physiological responses. Thus, at community level, studies carried out by Banach-Esteve (2011) demonstrated shifts in the native epizoisic community after seaweed colonization at the Cabrera MPA. Moreover, *P. nobilis* physiological status is modified, with enhanced malondialdehyde (MD) levels and thioredoxin reductase (TR) repair activity (Box *et al.*, 2009). In this regard, the increased antioxidant enzyme activity is not enough to protect *P. nobilis* from the negative effects induced by *L. lallemandii* colonization (Box *et al.*, 2009). At the same time, changes in *P. nobilis* feeding at invaded seagrass meadows occur with variable food source contributions of almost 20% of *Lophocladia* (Cabanellas-Reboredo *et al.*, 2010). Another possible effect is on the hydrodynamics of fluxes surrounding *Pinna*, as the three-dimensional structure of the invasive seaweed *L. lallemandii* can modify the particle flux and filtration rates of encrusting organisms (Deudero *et al.*, 2010). The high cover of *L. lallemandii* over *P. nobilis* could reduce the availability of water flow around the sessile mollusc and its filtering ability. Bivalves are in general suspension feeders; a high seaweed coverage reduces water current velocities and, therefore, its feeding capacity (Bartoli *et al.*, 2001; Tyler, 2007). Moreover, effects of *L. lallemandii* on other benthic invertebrates have been detected. It is known that *L. lallemandii* represents an alteration to *Reteporella grimaldii* natural conditions by evidence of physiological responses (Deudero *et al.*, 2010). These authors also found that distribution patterns of *Reteporella grimaldii* clearly differed in invaded and non-

invaded areas, finding reduced colony abundance in invaded areas (Deudero *et al.*, 2010). Similarly, all these changes could have unknown consequences for the biology, reproduction and survival of the *P. nobilis* population. However, *L. lallemandii* has a seasonal growth pattern, with high growth rates in summer and autumn and low biomass during winter and spring (Cebrian & Ballesteros, 2010). Therefore, the effect of *L. lallemandii* on *P. nobilis* is temporally constrained to the warmer period, and *P. nobilis* could recover to a healthier situation after colonization. Thus, manipulative experiments controlling the environmental conditions will be needed to show the exact effect of *L. lallemandii* on fan mussels.

In conclusion, high colonization rates of *L. lallemandii* over the endemic bivalve *P. nobilis* are encountered, mainly increasing with depth. This invasion might be promoting some indirect effects on the life history of the protected species, with further unknown consequences. The present results highlight the need to further address interaction across natural communities and alien-species-invaded systems before further cascade effects are driven.

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