



Under the canopy: Community-wide effects of invasive algae in Marine Protected Areas revealed by metabarcoding



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ABSTRACT

We analysed with multigene (18S and COI) metabarcoding the effects of the proliferation of invasive seaweeds on rocky littoral communities in two Spanish Marine Protected Areas. The invasive algae studied were *Caulerpa cylindracea*, *Lophocladia lallemandii* and *Asparagopsis armata*. They are canopy-forming, landscape-dominant seaweeds, and we were interested in their effects on the underlying communities of meiobenthos and macrobenthos, separated in two size fractions through sieving. A new semiquantitative treatment of metabarcoding data is introduced. The results for both markers showed that the presence of the invasive seaweed had a significant effect on the understory communities for *Lophocladia lallemandii* and *Asparagopsis armata* but not for *Caulerpa cylindracea*. Likewise, changes in MOTU richness and diversity with invasion status varied in magnitude and direction depending on the alga considered. Our results showed that metabarcoding allows monitoring of the less conspicuous, but not least important, effects of the presence of dominant invasive seaweeds.

1. Introduction

Metabarcoding of DNA is emerging as a powerful tool for biodiversity assessment and monitoring (Taberlet et al., 2012; Baird and Hajibabaei, 2012; Bohmann et al., 2014; Thomsen and Willerslev, 2015; Aylagas et al., 2016). This technique, albeit still subject to some limitations, will likely become a cornerstone in decision making of management bodies in the near future (Kelly et al., 2014a; Danovaro et al., 2016). In the marine realm, eukaryotic diversity has been analysed using metabarcoding in plankton and sediment communities (reviewed in Carugati et al., 2015; Bucklin et al., 2016; Sinniger et al., 2016). These studies aimed at diverse applications, including community description, beta-diversity patterns, impact assessment, or study of ecological interactions, among others (e.g., Bik et al., 2012; Fonseca et al., 2014; Pawlowski et al., 2016; Brannock et al., 2016; Guardiola et al., 2015, 2016). Less work has been performed on hard-substrate natural communities (e.g., Pearman et al., 2016), which are among the most affected by human activities. The complex nature of these communities, composed of a tri-dimensional array of superimposed strata (from canopy-forming organisms to cryptic microhabitats), poses methodological challenges for the application of metabarcoding techniques (Wangensteen and Turon, 2017).

Metabarcoding has also been used for the study of introduced and invasive marine species. Research in this field followed two different approaches, one focusing on the early detection of particular pest species (targeted or active surveillance; e.g., Ardura et al., 2015; Simpson et al., 2016), and the other involving monitoring of communities for signs of appearance of alien species (passive surveillance; e.g., Comtet et al., 2015; Zaiko et al., 2015; Abad et al., 2016; Brown et al., 2016; Xiong et al., 2016). Another side of invasion biology is the assessment of the impact of alien species on native assemblages. This is usually performed by traditional community analysis methods, involving sampling, sorting, identification, and preparation of qualitative and/or quantitative inventories (e.g., Piazzini et al., 2001; Balata et al., 2004; Box et al., 2010). These tasks are time-consuming, strongly dependent on available taxonomic expertise, and in practice applied only to the analysis of the larger elements of the fauna and flora which constitute only a minor fraction of the diversity present (Blaxter, 2016). The use of metabarcoding can greatly improve the sensitivity and breadth of the assessment of biodiversity shifts linked to the proliferation of invasive species, but the potential of this approach remains largely unexplored. Metabarcoding allows analysing not only the larger organisms, but also the smaller components of the eukaryotic diversity, likely the first to respond to perturbations and to suffer from cascading

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events (Schwindt et al., 2001; Gallucci et al., 2012). At the same time, the definition of taxonomic units based on sequence tags allows comparison across spatially and temporally distant studies, which is hardly possible with traditional inventory lists where many taxa are not identified at the species level and are thus in practice unavailable for comparison with other studies.

Among invasive species, seaweeds profoundly alter hard-substrate sublittoral communities, resulting in economic and ecological impacts worldwide (Schaffelke et al., 2006; Williams and Smith, 2007). The effects of the invasive algae are particularly important when they affect benthic habitats harbouring endangered species and long-lived, slow-growing organisms, which are very sensitive to disturbances (Ballesteros, 2006; Casas-Guell et al., 2016). However, although there is increasing concern about the effects of invasive algae on these habitats, they are commonly assessed by measuring changes in the most apparent or emblematic species (often their disappearance). The focus is therefore on measuring lethal effects of the invasion, which are likely irreversible considering the slow dynamics of these habitats (e.g., Cebrian et al., 2012). The integral community-wide study of the habitats undergoing invasion by alien seaweeds afforded by metabarcoding can allow a fine-scale assessment of their effects, both lethal and sub-lethal. At the same time, it provides a tool for monitoring these effects over time, for early detection of alterations, and for follow-up of restoration efforts.

The goal of the present study is to analyse with multigene (18S and COI) metabarcoding the effects of the proliferation of three invasive seaweeds on rocky littoral communities in two Spanish National Parks. Marine reserves have a pivotal role in the conservation of biodiversity, but their performance in the face of non-native species is not well understood (Byers, 2005; Kellner and Hastings, 2009). Evidence to date suggests that reserves are highly vulnerable to invasive species (reviewed in Burfeind et al., 2013) and thus management plans for reserves should include measures to prevent or counteract their impact.

The algae chosen have a big impact in terms of landscape changes (i.e., they are canopy-forming, engineer species). However, changes in the dominant algal species likely imply changes in the understory compartment, and little is known about effects on the smaller components of the community. In other words, does the presence of invasive algae affect the communities “under the canopy”? The small organisms in benthic communities are the most diverse, and likely the first to respond to environmental alteration. We want to showcase the potential of metabarcoding for this kind of studies, detect changes in eukaryotic biodiversity (meio- and macro-organisms), and set the baseline for future monitoring efforts on these communities. To our knowledge, this is the first time that such questions are addressed by metabarcoding DNA in the context of marine invasion biology.

2. Material and methods

2.1. Algal communities selected

We have studied macroalgal forest communities invaded by three alien seaweeds: *Lophocladia lallemandii* (Montagne) F. Schmitz and *Caulerpa cylindracea* Sonder in the Mediterranean, and *Asparagopsis armata* Harvey in the Atlantic.

L. lallemandii is probably coming from the Red Sea via the Suez Canal (Verlaque, 1994; Streftaris and Zenetos, 2006). It is currently distributed throughout most of the Mediterranean Sea, covering several types of substrates and homogenizing the appearance of benthic seascares (Patzner, 1998; Ballesteros et al., 2007; Cebrian and Ballesteros, 2010). *C. cylindracea* is an endemic species from south-western Australia. The mode of introduction in the Mediterranean remains speculative; however, maritime traffic (ballast water and ship hull fouling) and the aquarium trade are the most likely vectors for this high-impact alga. *A. armata* is native to western Australia, this species was probably introduced into European waters through oyster aquaculture.

Nowadays it is distributed throughout Europe in both the Atlantic and the Mediterranean shores. All species have a high invasive potential and all of them are included in the black list of invasive species from IUCN (Otero et al., 2013). Both *C. cylindracea* and *L. lallemandii* were recorded for the first time in the study area in 2003 and rapidly spread to almost all benthic communities present between 0 and 45 m depth (Cebrian et al., 2011), while *A. armata* invasion goes back to the late 90's in the area studied (Guiry and Guiry, 2017).

2.2. Sampling

Samples were taken by scuba diving at two Spanish National Parks: the Cabrera Archipelago (Balearic Islands, Northwestern Mediterranean) and the Atlantic Islands of Galicia (Galicia, Northeastern Atlantic) (Fig. S1). Samples of *Lophocladia lallemandii* were collected in Cabrera Island (October 2015) at 10–12 m depth in a vertical wall facing SE, located in the “Imperial” islet (39°07'30.32"N 2°57'37.14"E). *Caulerpa cylindracea* samples were collected at 30–32 m depth in the same wall and dates as the *L. lallemandii* samples. For these algae, replicate samples were collected in areas visually dominated by the invasive seaweed, while the control samples were taken in zones (interspersed with the former) visually free from them. *Asparagopsis armata* was sampled in the Cies Islands (Galicia) in May 2015 in a shallow community (4–6 m depth) facing E in the “Penela dos Viños” islet (42°12'52.59"N 8°53'50.34"W). This community was completely dominated by *A. armata*, mostly in the sporophyte phase (also known as *Falckenbergia rufolanosa*). It was impossible to sample clearly uninvaded zones at this same spot, so the control samples were taken at the same habitat, depth and orientation in a site in the “Illa do Monteagudo” about 1 km from the first place (42°13'32.93"N 8°53'51.29"W), in a community dominated by *Cystoseira nodicaulis* (Whitening) M. Roberts. Hereafter, we will refer to these datasets (comprising both the invaded and the non-invaded samples) as the Ll (*Lophocladia lallemandii*), Cc (*Caulerpa cylindracea*) and Aa (*Asparagopsis armata*) datasets. Representative images of the communities sampled are shown in Fig. S2.

Sampling followed the protocol described in Wangenstein and Turon (2017). In short, triplicate samples for each condition were obtained by scraping with hammer and chisel quadrats of 25 × 25 cm to bare rock while SCUBA diving. The samples were placed underwater in plastic bags. Water was eliminated by filtering (63 µm mesh sieve) and replaced with absolute ethanol. Three size fractions (A: > 10 mm; B: 1–10 mm; C: 63 µm–1 mm) were obtained from each replicate sample using a column of sieves. Fraction A (megabenthos sensu Rex and Etter, 2010) was dominated by the canopy-forming algal species and was not used in this study as the objective was to assess changes in the smaller components of the community. The retained fractions B and C (macro-benthos and meiobenthos, respectively, Rex and Etter, 2010) were then homogenized with a blender and stored at –20 °C until DNA extraction. All equipment was thoroughly washed and cleaned with sodium hypochlorite between successive samples.

2.3. DNA processing

DNA was extracted from 10 g of each homogenized sample using PowerMax Soil DNA Isolation Kit (www.mobio.com). Two genes were amplified: a 100–110 bp fragment in the v7 region of the 18S rRNA gene, using the 18S_allshorts primers (Guardiola et al., 2015; forward: 5'-TTTGTCTGTTAATTSCG-3' and reverse: 5'-TCACAGACCTGTTAT TGC-3), and a fragment of the COI gene, amplified with a modification of the forward mlCOIintF primer (Leray et al., 2013): 5'-GGWACWRGWTGRACWITITAYCCYCC-3' and the reverse jgHCO2198 primer (Geller et al., 2013): 5'-TAIACYTCIGGRTGIC-CRAARAAYCA-3'. The forward primer incorporated two more wobble bases and two inosine nucleotides in the most degenerate positions, relative to the original mlCOIintF. This was done for increased

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