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Multiple effects of a *Gracilaria vermiculophylla* invasion on estuarine mudflat functioning and diversity

D. Davoult[a,*], G. Surget[b,c], V. Stiger-Pouvreau[b,c], F. Noisette[a], P. Riera[a], D. Stagnol[a], T. Androuin[a], N. Poupart[b,c]

[a] Sorbonne Universités, UPMC Univ Paris 6, CNRS, UMR 7144, Station Biologique de Roscoff, F. 29680 Roscoff, France
[b] Université de Bretagne Occidentale, Institut Universitaire Européen de la Mer, 29280 Plouzané, France
[c] CNRS, UMR 6539 LEMAR-IUEM-UBO, Institut Universitaire Européen de la Mer, 29280 Plouzané, France

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ABSTRACT

The invasive Japanese seaweed *Gracilaria vermiculophylla* has become established over the past several years in numerous European estuaries, from Portugal to Norway. In the Faou estuary (48.295°N-4.179°W, Brittany, France), it forms a dense population at the mud's surface. The effects of *G. vermiculophylla* on metabolism, diversity, and the food web were studied. Community gross primary production (GPP) and respiration (CR) during emersion, chlorophyll-*a* content, macrofaunal and meiofaunal diversity and abundance, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of representative macrofaunal species and main food sources were measured at low tide in winter, spring, summer 2014, and winter 2015. Results show significant seasonal variation in GPP and CR. Moreover, GPP was significantly higher in areas where *G. vermiculophylla* was present than in the control area (bare mud). However, this high GPP appeared to be linked to the increase in biomass in primary producers, with their efficiency (primary productivity, i.e. assimilation number) remaining relatively stable compared with the control area. Significant variation in abundance of meiofauna and macrofauna was also detected and new epifaunal species were collected, mainly in *Gracilaria*-colonized areas. Isotopic food-web Bayesian mixing models strongly suggested that *G. vermiculophylla* plays a major role in the diet of some dominant species. Mechanisms interacting with the functioning and diversity of the mudflat are discussed. Finally, the invasive seaweed *G. vermiculophylla* affected the mudflat ecosystem in three ways: as a new primary producer (increase in metabolism), as a habitat-forming species (changes in diversity and abundance of macrofauna and meiofauna), and as a new abundant food source, likely through the detrital pathway.

1. Introduction

Among reported marine non-indigenous species (NIS) in Europe, between 20 and 40% are macroalgae (Schaffelke et al., 2006; Stiger-Pouvreau and Thouzeau, 2015). Owing that some of them can act as foundation species (Dayton, 1972; Ellison et al., 2005) or ecosystem engineers (Jones et al., 1994, 1997), they may deeply alter the structure and functioning of local communities by changing abiotic conditions (Jones et al., 1997), local diversity (Wallentinus and Nyberg, 2007 and references therein), and food webs (Hastings et al., 2007). Reports on these changes generally indicate negative effects on indigenous species (Levine et al., 2003), although there may also be some positive effects (Crooks, 2002). Studies on macroalgal introductions generally focus on rocky shores and explore potential competition with native seaweeds for space (Schaffelke and Hewitt, 2007 and references therein). Less frequently, non-indigenous seaweeds can also colonize biotopes that originally have no significant macroalgal populations. For example, the perennial red seaweed *Gracilaria vermiculophylla* (Ohmi) Papenfuss is now reported on the Pacific coast of USA, on the west and east coasts of the North Atlantic (Freshwater et al., 2006; Thomsen et al., 2007, 2009; Krueger-Hadfield et al., 2017) and especially along the French Atlantic coasts (Stiger-Pouvreau and Thouzeau, 2015).

This species, originating from East Asia, can be found in Europe from Norway to Portugal (Rueness, 2005; Hammann et al., 2013 and references therein), as well as along the Atlantic coast of Morocco (Guillemin et al., 2008; Krueger-Hadfield et al., 2017). Its introduction in France likely occurred in the vicinity of oyster farms (Mollet et al., 1998): a recent study on this species using population genetics (Krueger-Hadfield et al., 2017) indicates that the probable main source of the invasion is northeastern Japan, the area from which the majority of *Crassostrea gigas* oysters were imported during the 20th century. In its native range, the species is characterized by a haplo-diplontic life cycle

* Corresponding author.
E-mail address: davoult@sb-roscoff.fr (D. Davoult).

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and is attached by holdfasts to hard substrata. Along west and east coasts of the North Atlantic, its populations are dominated by diploid thalli without holdfasts that colonize estuarine mudflats through vegetative fragmentation (Krueger-Hadfield et al., 2016). Similarly, in French mudflats, *G. vermiculophylla* occurs without attaching to a (however small) hard substratum. The presence of *G. vermiculophylla* on mudflats on the northeastern coasts of the United States seems to be linked to, or at least facilitated by, the presence of the tube-dwelling worm *Diopatra cuprea* (Thomsen and McGlathery, 2005; Wright et al., 2014). Nevertheless, it has been reported in various types of substrata in Europe, including immersed soft sediments and stones, e.g. in the Baltic Sea (Weinberger et al., 2008), where it may constitute a competitor of the native brown alga *Fucus vesiculosus*.

Invasive *G. vermiculophylla* may be less palatable than *G. vermiculophylla* in its native range both for grazers from its native range and from its invaded range (Hammann et al. (2013). Invasive populations of *G. vermiculophylla* defend themselves better against bacterial epibionts isolated from their respective introduced ranges than from the ones from their native range, suggesting a rapid adaptation of its chemical defense mechanism to new bacterial epibionts in the invaded range (Saha et al., 2016).

For more than 10 years, *G. vermiculophylla* has been considered as an occasional species on French coasts, without any effect on ecosystems (Martínez-Lüscher and Holmer, 2010). Currently, it is now broadly distributed in estuarine ecosystems where it visibly constitutes a habitat-forming species at the surface of the mud. *G. vermiculophylla* can be now considered as invasive because it tends to occupy a large part of the mudflats it has colonized. It also represents a new benthic primary producer on the mudflat, the microphytobenthos being the only primary producers until this invasion.

The expansion of the species has been monitored in three estuaries in the Bay of Brest since 2013 (Surget et al., 2017). Among them, the Faou estuary appears to be the most heavily colonized field site (Surget et al., 2017). Therefore, this estuary was chosen for a study on the impact of *G. vermiculophylla* that was carried out from February 2014 to January 2015. The aim was to determine whether this alga present in mudflats previously colonized only by microphytobenthos (1) is significantly modifying mudflat metabolism (primary production and respiration) as a new primary producer, (2) is significantly modifying the diversity and abundance of the benthic community (macrofauna and meiofauna) as a habitat-forming species, and (3) is significantly modifying the macrobenthic food web by providing a new food source.

2. Materials and methods

All sampling and measurements were performed simultaneously during low tide, about 4.10 m above chart datum, in February, May, September 2014, and January 2015 in the Faou estuary (48.295°N-4.179°W, Brittany, France) (Fig. 1).

2.1. Ecosystem metabolism

Ecosystem metabolism was measured during low tide with three 0.071 m^2 benthic chambers to estimate CO_2 fluxes at the air-sediment interface using the method described in Migné et al. (2002). Sediment (including *G. vermiculophylla* when present) was enclosed down to 10 cm depth. Changes in air CO_2 concentration (ppm) in the benthic chamber (10 L) were measured with an infrared gas analyzer (LiCor Li-820) for 10–15 min. CO_2 concentrations were recorded in a data logger (LiCor Li-1400) at a 5 s frequency. CO_2 flux was calculated as the slope of the linear regression of CO_2 concentration (μmol mol^{-1}) against time (min) and expressed in mg C m^{-2} h^{-1} assuming a molar volume of 22.4 L at standard temperature and pressure. Transparent chambers were used to estimate the net benthic community production (NCP), the difference between community gross primary production (GPP) and community respiration (CR). Opaque chambers were used to estimate CR. During light incubations, incident photosynthetically available radiation (PAR, 400–700 nm) was monitored with a LiCor SA-190 quantum sensor. On each sampling date, stratified sampling was performed, with three replicates on the *Gracilaria*-colonized area (% cover *G.* vermiculophylla > 50%) and three replicates on the bare-mud area, considered as the control. Benthic chambers were deployed within a few meters of each other to limit any spatial variation.

Considering the low number of replicates and the absence of homoscedasticity even after metric transformation, we used the non-parametric Scheirer-Ray-Hare test (Sokal and Rohlf, 1995), the non-parametric equivalent of a two-way ANOVA, on sampling date (n = 4) and colonization status (area) (bare-mud and *G. vermiculophylla*-colonized areas, n = 2).

2.2. Chlorophyll a

Four replicates of 1.96 cm^2 and 1 cm depth (including *G. vermiculophylla* when present) were sampled within each benthic chamber at each sampling date during low tide. Samples were kept cool, in the dark, and brought back to the laboratory where they were stored at −24 °C until analysis. Fresh samples were ground 30 s in pure acetone (5 mL), placed in the dark at 4 °C for at least 4 h, and centrifuged (4 °C, 3500 rpm, Eppendorf Centrifuge 5810R) to extract chlorophyll *a*. Chlorophyll *a* contents (Chl a) were determined on homogenized supernatants using spectrophotometry according to the trichromatic method described in Jeffrey and Humphrey (1975). In microplates (UVStar F-Bottom, Greiner Bio-one), optical density (OD) of 200 μL samples was read at 630, 647, 664, and 750 nm with a POLARstar Omega spectrophotometer (BMG Labtech). Chlorophyll *a* contents were calculated using the following equation and expressed in $mg.m^{-2}$:

$$\text{Chl a } (mg.m^{-2}) = 50 \times [11.85 \times (OD_{664} - OD_{750}) - 1.54 \times (OD_{647} - OD_{750}) - 0.08 \times (OD_{630} - OD_{750})]/1.96$$

Data were also analyzed using the Scheirer-Ray-Hare test.

2.3. Macro- and meiobenthos

On each occasion, three 0.1 m^2 quadrats were sampled on the *Gracilaria*-colonized area and three other quadrats on the bare-mud area for macrofauna identification. Samples were isolated in the field on sieves of 1 mm mesh size, and stored in 4% salted and buffered formalin in the lab. Individuals were identified at the species level and counted.

Three replicates (1.77 cm^2, 2 cm deep) were also collected on the *Gracilaria*-colonized area along with three other replicates from the bare-mud area, and sieved with a mesh size of 40 μm. Individuals of the meiofauna were identified, belonging to the 10 following taxonomic categories: nematodes, platyhelminths, interstitial polychaetes, oligochaetes, harpacticoid copepods, ostracods, halacarid mites, foraminifers, gastropods, and bivalves.

Given the low number of replicates and the absence of homoscedasticity even after metric transformation, we used the non-parametric Scheirer-Ray-Hare test.

2.4. Food web

2.4.1. Sample collection and preparation

Invertebrates and the main potential organic matter sources from the sampling area were collected on the four sampling dates. Suspended particulate organic matter (POM) from the site was sampled by collecting 2 L of seawater. POM was obtained by filtration on pre-combusted Whatman GF/F glass fiber membranes within 2 h after collection. Membranes were then acidified (10% HCl) to remove carbonates, briefly rinsed with Milli-Q water, dried (60 °C), and kept at −32 °C until analysis. Sediment samples were taken by scraping the upper 1 cm