Impact of the invasive brown alga *Rugulopteryx okamurae* on the benthic communities in the Northwestern Mediterranean Sea

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A B S T R A C T

Over the centuries, the Mediterranean Sea has been significantly impacted by biological invasions, hosting more than 1000 non-indigenous species. In the early 2000s, the brown alga *Rugulopteryx okamurae*, native to the northwestern Pacific Ocean, was introduced to the Thau Lagoon (Occitania, France) due to extensive shellfish farming activities. Since 2016, this species has proliferated, completely covering the rocky habitats along the Marseille coasts (Provence, France) and becoming the dominant alga. The impact of *R. okamurae* on flora and fauna was investigated by comparing rocky benthic assemblages of invaded and non-invaded sites. Results showed a change in the communities, with a significant decrease in species diversity and a homogenization of algal and invertebrate populations in invaded sites. *Rugulopteryx okamurae* is a habitat transformer species inducing a significant habitat shift. *Rugulopteryx okamurae* also seems to affect the different algal strata of the benthic community, thus impacting the entire canopy. However, a weak effect of *R. okamurae* was observed on the functional structure of native algal communities, comparing invaded and non-invaded areas. This lack of structural variation between these zones could be explained by the degradation of habitats within the Calanques National Park, due to decades of intense overgrazing by *Sarpa salpa* and sea urchins.

But an impact on the ecosystem functioning should not be excluded. It is noteworthy that *R. okamurae*, a species presumed to prefer colder waters, has shown resistance to successive marine heat waves, allowing it to continue spreading and dominating without impairment.

Keywords: Biological invasion Mediterranean sea Introduced algae Ecological impact *Rugulopteryx okamurae*

1. Introduction

Despite its relatively modest surface area (0.8% of the global ocean), the Mediterranean Sea harbors a high endemism, reaching more than one quarter of the whole of the Mediterranean biota (Bianchi and Morri, 2000; Coll et al., 2010). However, the it has undergone significant changes over time due to modifications of biotic and abiotic factors resulting from anthropogenic pressures (Claudet and Fraschetti, 2010; Lejeusne et al., 2010), such as fisheries, aquaculture, increased sedimentation, water degradation, urbanization, species invasion and heatwaves (Smith et al., 2023a). These perturbations act synergistically and have contributed to the degradation of marine habitats and associated species assemblages (Micheli et al., 2013).

Invasive species refer to organisms, which have been transported by humans, either deliberately or accidently, in a new environment where they have not evolved and cause ecological, economical and/or human health problems (Occhipinti-Ambrogi and Galil, 2010; Boudouresque and Verlaque, 2012). The Mediterranean Sea is one of the areas most affected by the occurrence of non-indigenous species (NIS), with over 1000 species recorded. Concerning the flora, 124 macroalgal species are considering likely introduced and among which 8 are invasive (Zenetos et al., 2010; Van Der Loos et al., 2023). For comparison, only one Magnoliophyta, *Halophila stipulacea,* is introduced and invasive in the Mediterranean Sea (Thibaut et al., 2022).

Invasive species are recognized as major contributors to global biodiversity loss, which can reduce the resilience of ecosystems (Maxwell et al., 2016; IPBES et al., 2023), leading to irreversible damage to ecosystems and profound alterations in the composition of native

communities, with associated impacts on the economy and human health (Carlton and Schwindt, 2024). Over the past few decades, the rate of introduction has increased, with an annual recording of 200 newly established alien species worldwide. The increase of transport and introduction of the invasive species is mainly influenced by economic drivers (IPBES et al., 2023). The principal vector of introduction of marine species is shipping, opening of new channel, aquaculture, fisheries, live seafood, tourism activities, research and habitat restoration (Minchin et al., 2009).

Invasive macroalgae are recognized for their detrimental effects on the local benthic biota, causing a change in species richness and diversity (South et al., 2017). In the worst-case scenario, "transformer" species can lead to the homogenization of habitats and thus changes in the composition of the community, affecting the overall structure of the food web (e.g. Deudero et al., 2011; Salvaterra et al., 2013). This phenomenon results in heightened competition for essential resources such as light, nutrients and space, ultimately impacting the growth and recruitment of the local community (e.g. Schaffelke and Hewitt, 2007; Cebrian et al., 2012). Striking examples of the impact of invasive species on local ecosystems in the Mediterranean Sea include *Asparagopsis taxiformis* (Navarro-Barranco et al., 2019a), *Caulerpa cylindracea* (Piazzi et al., 2007; Antolić et al., 2008), *Caulerpa taxifolia* (Boudouresque et al., 1995; Molenaar et al., 2009), *Lophocladia trichoclados* (as. *L. lallemandii*) (Ballesteros et al., 2007; Marbà et al., 2014) and *Worsmeleyella setacea* (Piazzi and Cinelli, 2000; De Caralt and Cebrian, 2013).

Recently, *Rugulopteryx okamurae*, an invasive brown alga that originated from the temperate waters of the northwestern Pacific Ocean has been spreading in European waters. It was initially introduced in 2002 in the Thau Lagoon (Occitania, France) likely due to the importation of Japanese oyster (*Magallana gigas*) for oyster cultivation (Verlaque et al., 2009). Subsequently, the species was spotted in the open sea in 2013 at Agde (France) (Lauret and Verlaque, 2013). Currently, the species has successfully colonized extensive areas, including the Gulf of Lion, the southern regions of Spain on both sides of the Strait of Gibraltar (Altamarino-Jeschke et al., 2016; Ocaña et al., 2016; García-Gómez et al., 2018; Sempere-Valverde et al., 2020; Roca et al., 2022), the Alicante Bay (eastern Spain) (Terradas-Fernández et al., 2023), various locations along the Atlantic Ocean, such as the coasts of Portugal, Madeira (Bernal-Ibáñez et al., 2022; Herrero et al., 2023), Azores (Faria et al., 2022a), and the Canary Islands (RJ Haroun, personal communication). More recently, it has also been identified in the northern part of Sicily (Bellissimo et al., 2023). In the introduced area, the species is present all year round with sporophyte dominance, and so far, no form of sexual reproduction has been reported, indicating the existence of other modes of propagation such as fragmentation and/or apospory (Navarro-Barranco et al., 2019b). Once *R. okamurae* colonizes the rocky substrates, they can cover up to 100% of the area, leading to significant alterations in both flora and fauna (Sempere-Valverde et al., 2020; García-Gómez et al., 2021a; Faria et al., 2022b). This species demonstrates an ability to attach to any solid substrate (García-Gómez et al., 2021b) and can also persist on the deep seafloor for extended periods, even in low-light conditions (Estévez et al., 2022; Mateo-Ramírez et al., 2023). Its proliferation can produce huge amount of biomass, leading to accumulation on beaches (Rueda et al., 2023; Terradas-Fernández et al., 2023). In Spain 5000 tons of this algal deposit were removed from the Ceuta coastline in 2015 (Ocaña et al., 2016), similar events occurred also in every invaded location along the Atlantic seaboard due to tides. In contrast, in the Mediterranean Sea, massive beaching is highly localized to narrow coves that are exposed to wave action (Ruitton et al., 2021). Drift specimens over the sea floor have been observed down to 450 m depth (Rueda et al., 2023). This species is also a threat to the local economy, particularly to fishermen who suffer from clogged nets and reduced catches (Ruitton et al., 2021; Altamirano-Jeschke et al., 2023; Báez et al., 2023).

Rugulopteryx okamurae was initially discovered in 2016 at Riou Island, which is situated within the No Take Zone of the Calanques National Park (CNP) in Marseille (Provence, France). Since then, it has spread westward throughout the CNP, carpeting the rocky substrate in some locations. The aim of this study was to assess the impact of *R. okamurae* on the macroalgae and benthic invertebrate communities on rocky substrates in shallow waters in the northwestern Mediterranean Sea. To monitor the spatial and temporal evolution of benthic communities, *in situ* sampling of fauna and flora in the CNP was carried out.

2. Materials & methods

2.1. Studied area

The studied area is located within the Calanques National Park (CNP) (Marseille, Provence, France, Mediterranean Sea). Two study sites, Tiboulen de Maïre Island (named here Tiboulen) and Cap Croisette (named here Croisette), were selected. These areas have seen an expansion of *Rugulopteryx okamurae* since 2020 and 2018, respectively. Both sites are located 675 m apart within the core area of the CNP, ensuring comparable environmental and conservation conditions. The core area is subject to regulated fishing with authorized species and quotas available online: https://www.calanques-parcnational.fr/fr (accessed on December 20, 2024) (Fig. 1).

2.2. Sampling

The fauna and flora communities of rocky substrates were sampled seasonally at Tiboulen and Croisette on photophilic sub-vertical rocks (~20–30° slope) at a depth of 5 m. Four sampling periods were chosen: September and December 2021, April and June 2022. We assessed the community spatial variability at different spatial scale (from 10 m between sub-site to hundreds of meters between sites) in invaded and non-invaded locations. A total of 95 samples were collected by sampling three quadrats of 400 cm^2 in each sub-site on each date (Supplementary Fig. S1). This surface area is commonly used as a minimal area for studying Mediterranean rocky benthic communities (Boudouresque and Belsher, 1979). Within each quadrat, the entire community was scrapped using a chisel hammer by Scuba divers and collected in an identified ziplock bag. At each site, two invaded sub-sites and two non-invaded sub-sites were selected: inv1 - inv2 and no1 - no2 for Tiboulen and inv3 - inv4 and no3 - no4 for Croisette. One sample has been lost for the sub-sites inv2 in June.

In the laboratory, the contents of each quadrat were analyzed and identified down to species level wherever possible. For the algal community, only the dominant species greater than 1 cm were identified (Supplementary Table S1). The algal community was characterized based on cover, wet weight (ww) and dry weight (dw). Cover represents total surface of the thallus of each macro-algae using graph paper; it can of course exceed 100% of the covering as species have a 3D shape. For macro-invertebrates the number of individuals, wet weight (ww) and the dry weight (dw) were measured. The identification of different taxa was carried out using available literature, with species names verified through WORMS for invertebrates (https://www.marinespecies.org/) and AlgaeBase (https://www.algaebase.org/) for macroalgae. Each macro-algae and macro-invertebrate were weighed with a scale (Mettler Toledo AE100 Analytical Balance; Precision ± 0.1 mg) then placed in a drying oven at 60 °C for 24 h and re-weighed. Data were then converted taking into account the surface of the quadrat ($g.dw.m^{-2}$, $ind.m^{-2}$).

2.3. Data analysis

All statistical analyses and graphical representation were performed with R software 4.2.2. To investigate community structure, PERmutational Multivariate Analysis of VAriance (PERMANOVA) and non-Metric multidimensional scaling (nMDS) the package *vegan* version 2.6.4 (Okasen, 2013) were used and figures done with *ggplot2* (*version 3.4.1.*).

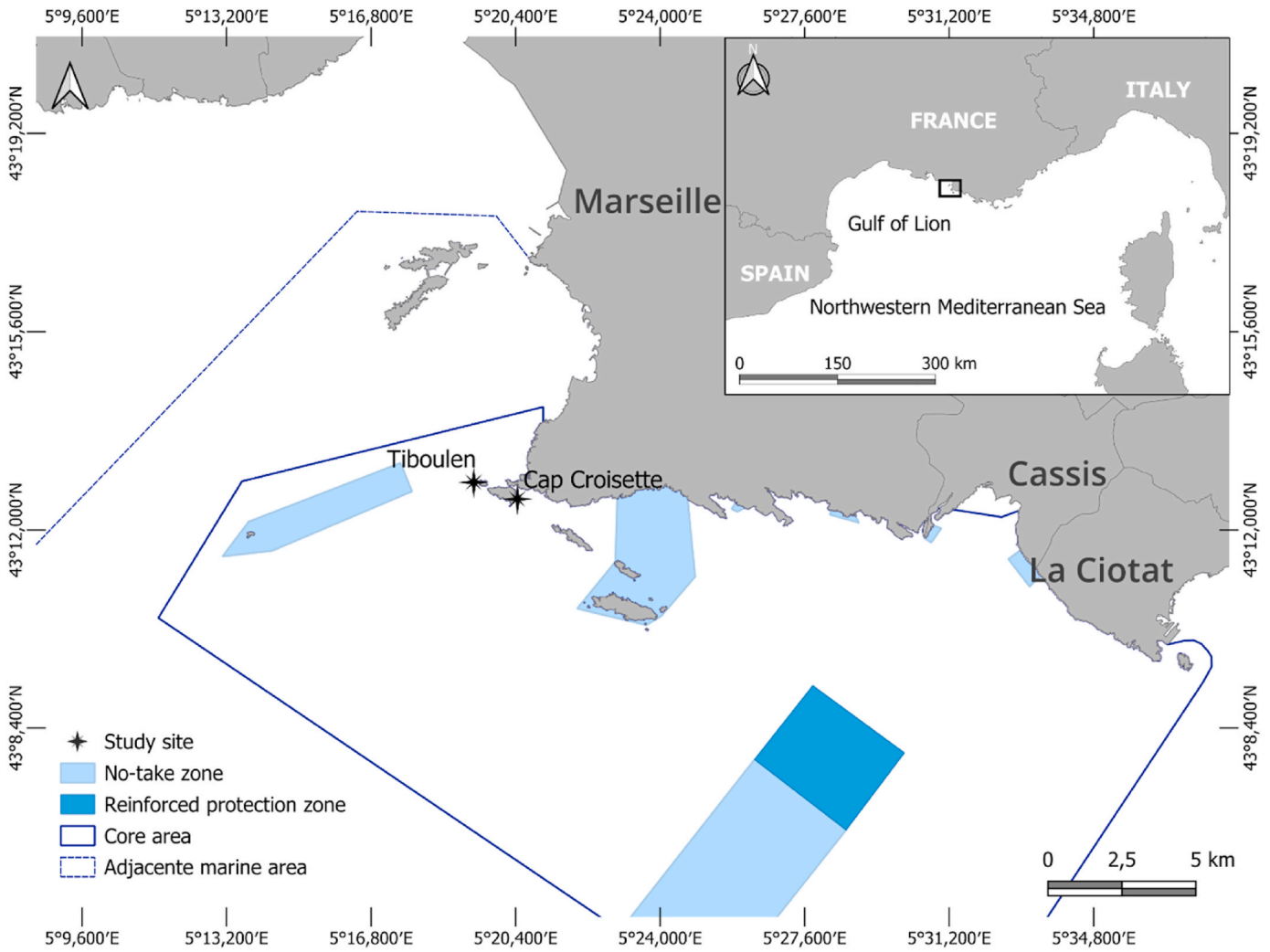

Fig. 1. Calanques National Park (CNP) boundaries with regulations and study sites.

Data (algae dw, invertebrate dw and invertebrate numbers) were first square root transformed. The data on the abundance of the algal community is dominated by *Rugulopteryx okamurae*, which is only found in the invaded sites. As *R. okamurae* was considered a disturbance to the benthic community to examine its impact on the diversity and structure of benthic communities, it was excluded from the analysis, except for the Community Structure Index (Ics), to understand the structure of the algal community at invaded and non-invaded locations.

As *Lithophyllum incrustans* is an encrusting alga attached to the rocky substrate, it was removed from the dataset to avoid altering the dry mass results. Distance matrix using Bray-Curtis dissimilarities was then calculated and an nMDS analysis performed on the entire community as well as only on the algae community (k = 2, trymax = 100). On the same distance matrix, we performed a 3-way mixed model PERMANOVA on invaded and non-invaded locations, sites and month, followed by a pairwise PERMANOVA analysis on the factors site (sub-site).

To investigate the impact of *R. okamurae* on morpho-functional groups of algae sampled at both sites, as conducted in Faria et al. (2022b), different groups were formed following the description provided by Steneck and Dethier (1994): (i) encrusting (*Peyssonnelia* spp., *Lithophyllum incrustans*), (ii) articulated (*Corallina berteroi, Jania rubens*), (iii) corticated (*Sphaerococcus coronopifolius, Codium vermilara, Codium bursa, Caulerpa cylindracea, Colpomenia peregrina, Asparagopsis armata, Halopteris scoparia*) and (iv) foliose corticated (*Padina pavonica, Zanardinia typus*). The dataset was tested for normality using the Shapiro test, and a Wilcoxon -Mann Whitney-test was performed to compare the invasion factor.

To evaluate the number of taxa (species, genera, families or higher taxa) in each condition, taxon richness was used (point diversity; for diversity as a function of scale, see Sala and Knowlton, 2006). The Pielou index (Pielou, 1966) was used to assess the equitability of the taxa present. The Shannon-Wiener Index was used as a standard estimate of taxon richness per quadrat and abundance in the samples (quadrats), considering rare species (Shannon and Weaver, 1948), while the Simpson Index (Simpson, 1949) was used to evaluate the probability of encountering the same taxon in different samples, considering the more abundant taxa. Normality was tested, and non-parametric Wilcoxon-Mann Whitney tests were conducted on the diversity indices.

To evaluate the impact of *R. okamurae* on the structural composition of algal assemblages in both invaded and non-invaded locations, the functional index Ics was computed on each quadrat using the dry mass of algae, following the methodology outlined in Ar Gall and Le Duff (2014). This index is derived from three primary parameters: the Functional Organization Index (Io), Stratification Index (Is), and Taxonomic Index (It). The calculation is expressed as Ics = Io + Is + It, as detailed in Ar Gall and Le Duff (2014). The Ics values range from 0.3 to 1.8, low Ics score correspond to a community dominated by annual low structural species, and higher score correspond to perennial species forming a high canopy.

3. Results

3.1. Impact of Rugulopteryx okamurae on the whole community

A total of 48 and 52 taxa were identified in invaded and non-invaded sites, respectively with 38 were invertebrates in both locations and 10 algae for invaded sites and 14 algae for non-invaded sites. A clear distinction appears between the invaded and non-invaded communities across all sites and months (Fig. 2, Table 1). Multivariate analyses reveal significant differences in the structure and composition of benthic assemblages based on invasion, site, and month factors for the dry mass of the entire community. Additionally, the interactions between the different factors were found to be significantly different (p-value <0.05) (Table 1A). PERMANOVA pairwise tests on sub-sites indicates that non-invaded sub-sites differed significantly between sites (p-value <0.05) (Tiboulen and Croisette) but not within site (p-value >0,05) (no1 = no2; no3 = no4) (Table 1B). Conversely, no differences were detected between invaded sub-sites at Tiboulen and Croisette (inv1 = inv2 = inv3 = inv4) (Table 1C).

All diversity indices show significantly higher values when *R. okamurae* is absent (Wilcoxon -Mann Whitney-test, p-value <0.05) (Fig. 3). The species richness index indicates a significantly greater number of species in non-invaded locations compared to invaded locations, with 18.1 ± 3.4 (mean ± sd) species compared to 12.9 ± 3.8, respectively. Similarly, the Shannon and Simpson indices are higher in non-invaded locations, suggesting a more diversified and complex community when *R. okamurae* is absent. The Pielou index is also higher in non-invaded locations, indicating a more uniform community in the absence of the invasive species.

3.2. Impact of Rugulopteryx okamurae on algal community

Throughout the study period, 3 species with relative abundances lower than 1% were recorded in invaded locations compared to 9 species in non-invaded locations (Fig. 4). In both conditions, *Lithophyllum incrustans* was the species the most abundant in term of biomass whatever the month. This species represents the encrusting stratum of the algal communities on photophilic rocky reefs. *Corallina berteroi* is the second most abundant species in the invaded and non-invaded locations. Its relative abundance ranges from 5.9% to 23.8% in the invaded sites. In the non-invaded locations, *C. berteroi* is slightly more abundant than in the invaded locations, with values between 8.6% in April to 30% in June. In the invaded locations, *Rugulopteryx okamurae* is consistently present throughout the year with a maximum development in June (61.5% of the algal biomass) and a minimum in December (21.03% of the algal biomass). The species diversity is higher in the non-invaded locations, the turf forming strata (a mixture of small and filamentous algae such as *Chondracanthus acicularis, Ceramium ciliatum,* and *Sphacelaria cirrosa*) being more abundant in the non-invaded locations with

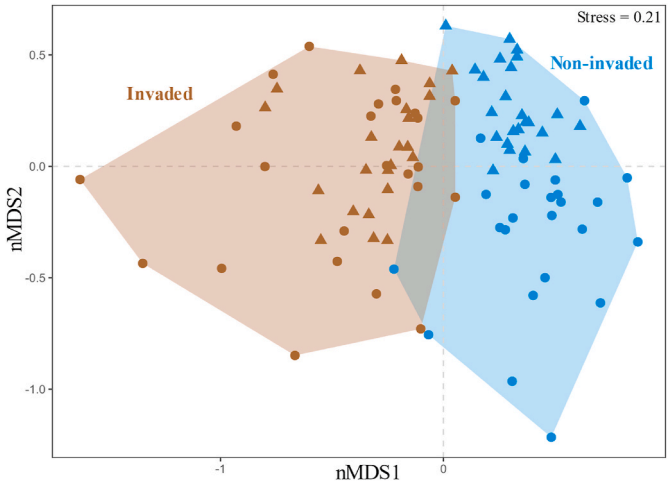

Fig. 2. Non-metric multidimensional scaling (nMDS) based of Bray-Curtis dissimilarity indices between quadrats showing the structure of the shallow water benthic assemblages in The non-invaded (in blue) and invaded sites (in brown) by Rugulopteryx okamurae at Croisette (circles) and Tiboulen (triangles). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Multivariate analyses on the whole community structuration. A) Three-way PERMANOVA for fauna and flora dry biomass with month, invasion and sites as factors. B) Pairwise PERMANOVA test results on sub sites with non-invaded locations at Tiboulen (no1 and no2) and Croisette (no3 and no4). C) Pairwise PERMANOVA test results on sub sites with invaded locations at Tiboulen (env1 and env2) and Croisette (env3 and env4), Significance level: ***p < 0.001; **p < 0.01; *p < 0.05.

A	Df	SumOfSqs	R^2	F	Pr
Sites	1	1.299	0.069	11.130	**0.001** ***
Invasion	1	3.050	0.162	26.131	**0.001** ***
Month	3	1.820	0.096	5.198	**0.001** ***
Sites x Invasion	1	0.436	0.023	3.737	**0.002** **
Sites x Month	3	0.839	0.044	2.396	**0.001** ***
Invasion x Month	3	1.249	0.066	3.568	**0.001** ***
Sites x Invasion x Month	3	0.968	0.051	2.764	**0.001** ***
Residual	79	9.220	0.488		

B. WITHIN NON-INVADED SITES			
Group 1	Group2		
no1	no2	0.038	0.423
no1	no3	0.232	**0.001** ***
no1	no4	0.251	**0.001** ***
no2	no3	0.133	**0.004** **
no2	no4	0.166	**0.001** ***
no3	no4	0.060	0.158

C. WITHIN INVADED SITES			
Group 1	Group 2		
inv1	inv2	0.037	0.524
inv1	inv3	0.053	0.287
inv1	inv4	0.059	0.192
inv2	inv3	0.042	0.478
inv2	inv4	0.057	0.242
inv3	inv4	0.036	0.644

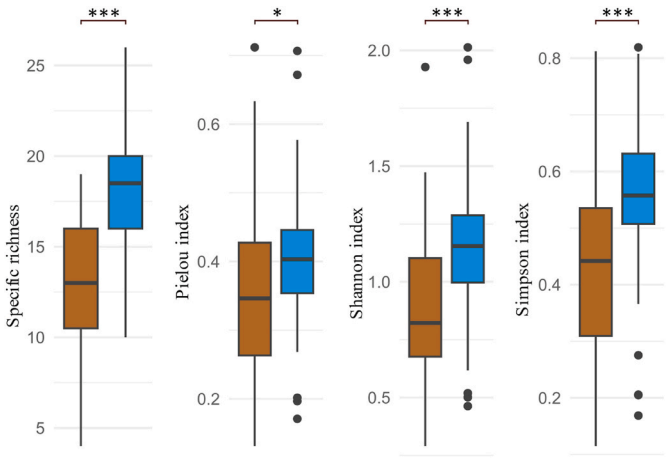

Fig. 3. Diversity index of fauna and flora community on invaded locations (brown) and non-invaded locations (blue) (Species richness, Pielou index, Shannon index, Simpson index), Significance level: ***p < 0.001; **p < 0.01; *p < 0.05 (Wilcoxon -Mann Whitney-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

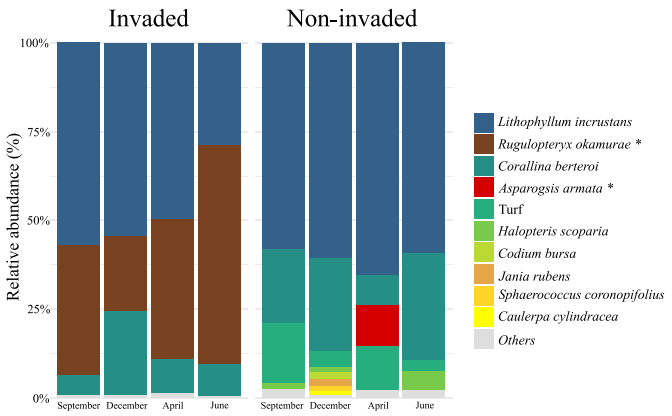

Fig. 4. Seasonal variation of abundance of the algal dry mass of the species in function of the invasion whatever the sites. Taxa are listed in descending order of abundance, except for Others. Others include species with an abundance lower than 1%. (3 species for invaded locations and 9 species for non-invaded locations). Stars indicate invasive species.

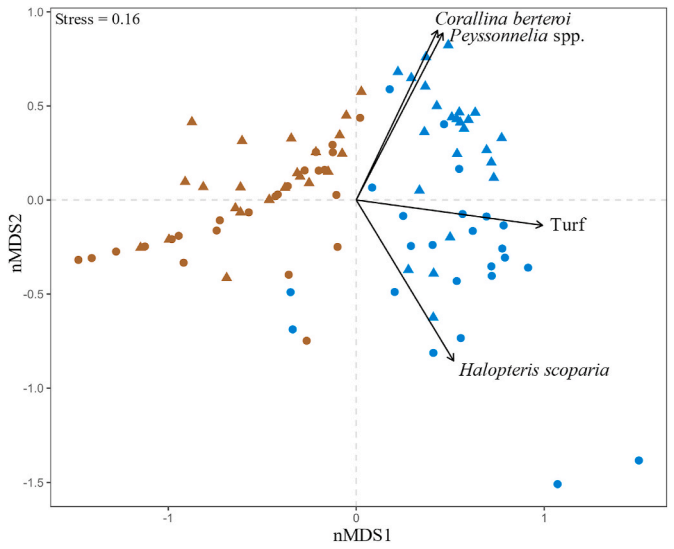

Fig. 5. Non-metric multidimensional scaling (nMDS) based on Bray-Curtis dissimilarity indices on algal assemblages in invaded (in brown) and non-invaded (in blue) locations by Rugulopteryx okamurae at Croisette (circles) and Tiboulen (triangles).
Only the species with a correlation coefficient greater than r > 0.25 were represented as arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

an algal mass ranging from 3.1% in June and 16.8% in September, while a maximum value of 0.2% is reported in September in the invaded locations (Supplementary Fig. S2). *Codium bursa*, *Codium vermilara*, and *Colpomenia peregrina* are exclusively found in non-invaded locations. Additionally, the biomass of other invasive species *Asparagopsis armata, Caulerpa cylindracea* and *Womersleyella setacea,* show significantly lower abundance in the presence of *R. okamurae* with 11.6% for *A. armata* in April and 1.2% for *C. cylindracea* in December in the non-invaded locations (Fig. 4, Supplementary Table S2).

A. armata, another invasive species, historically known to colonize the Tiboulen site in dense population, is strongly impacted by *R. okamurae*, its biomass being reduced by order of magnitude between the non-invaded sites (343.85 ± 88.50 g.dw.m^{-2}) and the invaded ones (0.18 ± 0.18 g.dw.m^{-2}) (Wilcoxon-Mann Whitney-test 0.004, p-value <0.05) (Fig. 4).

The nMDS analysis revealed two distinct groups, with invaded locations forming one cluster, while non-invaded locations exhibiting more heterogeneity. Communities in invaded locations differed significantly from non-invaded locations (Fig. 5). Samples from non-invaded locations appear to be positively correlated with high algal abundances, particularly with *C. berteroi*, *Peyssonnelia* spp., *H. scoparia* and the turf, all of which belong to various strata. In contrast, samples from invaded locations are negatively correlated with the abundance of the algal community (Fig. 5). The invasion by *R. okamurae* is the main factor influencing the algal composition whatever the season or site ($p < 0.001$***) (Table S3).

Diversity indices such as species richness, Shannon index, and Simpson index are significantly higher in non-invaded location (Wilcoxon-test; p-value <0.05) (Fig. S2). That indicates that there are more species, with greater Shannon and Simpson indices when *R. okamurae* is absent. Only the variation of Pielou index is not significant, which indicates no difference for the equitability of the algal composition.

There is a weak difference in the algal structure measure by the Ics index in the invaded and non-invaded locations but significative (Wilcoxon-Mann Whitney-test p-value = 5.37e^{-4}, p-value <0.05; Table 2). The Ics scores are around 0.6 and indicate an absence of structuring species at both sites (Table 2).

Abundance (dw or cover) of each stratum (articulated, corticated, encrusting, and corticated foliose) is significantly lower in invaded locations (Fig. 6), regardless of the morpho-functional type of algae.

3.3. Impact of Rugulopteryx okamurae on the fauna

Among the 41 taxa recorded, Polychaeta and *Sipunculus (Sipunculus) nudus* dominate the community in terms of numbers of individuals particularly in the non-invaded locations (Figs. 7 and 8). Only a few taxa were specific at the invaded or non-invaded locations. The gastropod *Calliostoma laugieri* and the bivalves *Cerastoderma glaucum* and *Modiulus barbatus* were only found in the invaded locations (Supplementary Table S4). The gastropods *Clanculus jussieui*, *Mangelia scabrida* and *Jujubinus striatus* were only found in the non-invaded locations but all these species are quite rare with a very low relative abundance (<3%) (Supplementary Table S4). Among the 41 taxa, a few have a relative abundance> 3 % (Supplementary Table S4, Fig. 7) and the relative abundance of four taxa significatively differs between invaded and non-invaded locations. Polychaeta, *S. (Sipunculus) nudus* and *Anomia ephippium* are more abundant in the non-invaded locations whereas Copepoda are significantly more abundant in the invaded locations than in non-invaded locations (16.5 ± 28.2 and 1.0 ± 5.0 respectively) (Fig. 8).

The difference in the faunal community can be explained mainly by the level of invasion, the month, the sites and all the interactions between these factors (Supplementary Table S5).

Taxon richness was higher in non-invaded locations. Pielou index was significantly higher in invaded locations, suggesting greater evenness among species when *R. okamurae* is present. However, the Simpson and Shannon indices did not show statistical significance for the invertebrate biota (Supplementary Fig. S3).

4. Discussion

In this study, we demonstrated the significant impact of *Rugulopteryx okamurae* on the algal and faunal communities of rocky reefs around Marseille. Its overwhelming presence has transformed local habitats, as previously reported in the Azores (Faria et al., 2022b) and the Strait of Gibraltar (García Gómez et al., 2021a). *Rugulopteryx okamurae* can therefore be classified as a habitat-transforming species (Richardson et al., 2000; Boudouresque and Verlaque, 2012), capable of colonizing entire rocky substrates. It forms a dense canopy 15–20 cm high with a substantial biomass, ranging from 87 g.dw.m^{-2}–949 g.dw.m^{-2} in dry weight and from 525 g.ww.m^{-2}–5054 g.ww.m^{-2} in wet weight throughout the study year (Supplementary Table S6).

Table 2
Mean ± sd of Io (organization index), Is (stratification index), It (taxonomic index) and Ics (index of community structure) in locations invaded and not invaded by *Rugulopteryx okamurae* (Wilcoxon -Mann Whitney-test).

Invasion	Organization index (Io)	Stratification index (Is)	Taxonomic index (It)	Index of community structure (Ics)
Invaded	0.25 ± 0.02	0.23 ± 0.01	0.16 ± 0.01	0.64 ± 0.03
Non - invaded	0.25 ± 0.02	0.22 ± 0.01	0.15 ± 0.04	0.62 ± 0.05
p-value	0.195 ns	**<0.001** ***	**<0.05** **	**<0.001****

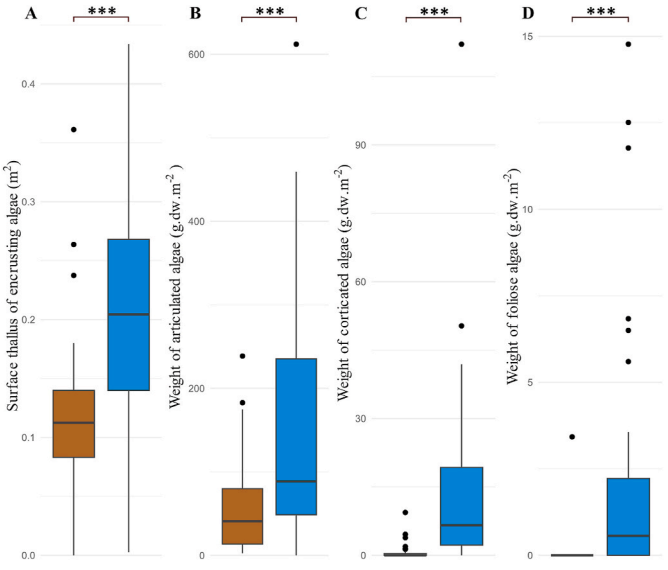

Fig. 6. Influence of the invasion by Rugulopteryx okamurae of (A) the cover of encrusting macroalgae and dry biomass of (B) articulated, (C) corticated, and (D) corticated foliose macroalgae (brown = invaded, blue = non - invaded), Significance level: ***p < 0.001; **p < 0.01; *p < 0.05 (Wilcoxon -Mann Whitney-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The presence of this standing species clearly modifies the light conditions, the nutrient availability for other benthic algae and the sedimentation for invertebrates. The rocky substrate colonization by *R. okamurae* appears to homogenize local diversity. Habitat homogenization simplifies the diversity, structure, and function of native species (O'Connor and Crowe, 2005) as well as the diversity and resilience of the ecosystems (Downing et al., 2012). This emerges as an important underestimated consequence of biological invasions (Olden and Rooney, 2006; Occhipinti-Ambrogi and Galil, 2010).

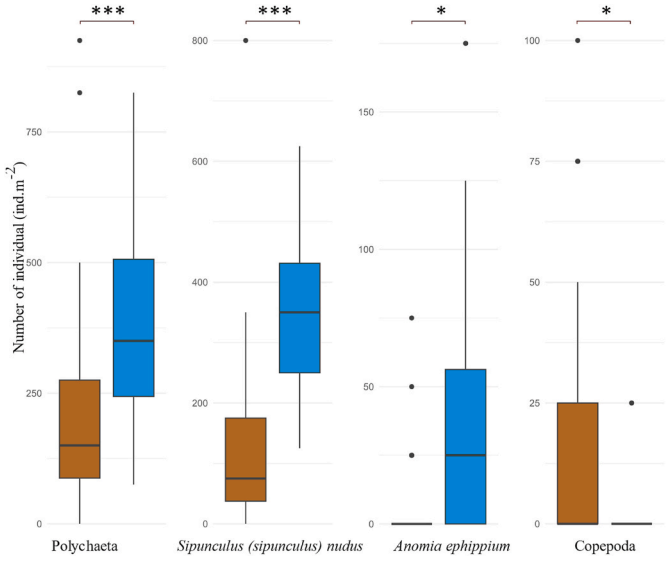

Fig. 8. Variation of abundance of invertebrate organisms by m^2 (ind. m^{-2}) in invaded and non-invaded locations (**brown = invaded site, blue = non-–invaded site**), Significance level: ***p < 0.001; **p < 0.01; *p < 0.05 (Wilcoxon -Mann Whitney-test). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

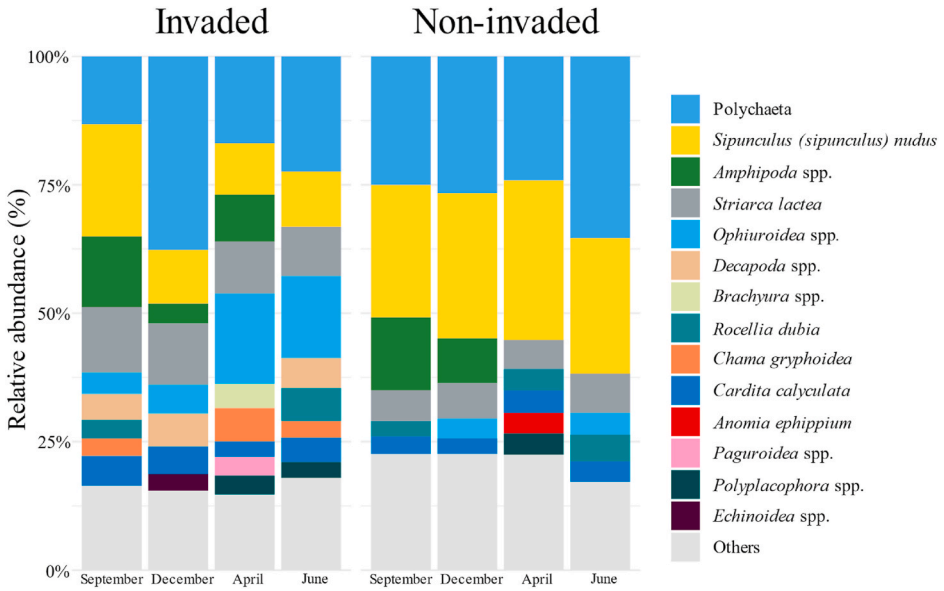

Fig. 7. Seasonal variation of abundance of the invertebrate species in function of the invasion whatever the sites. *Taxa are listed in descending order of abundance, except for Others represent taxa with an abundance lower than 3%.*

Homogenization has previously been documented in Mediterranean assemblages invaded by *Caulerpa cylindracea* (Klein and Verlaque, 2009a). For instance, while the presence of *C. cylindracea* led to an increase in alpha diversity, it resulted in a loss of beta diversity within the coastal ecosystem (Pacciardi et al., 2011). Similar findings were observed with *Caulerpa taxifolia*, which also caused a significant decrease in species richness and overall diversity (Verlaque and Fritayre, 1994).

The PERMANOVA results showed that the factors Sites, Invasion, and Month, along with their interactions, were all significant. Among these, Invasion was the primary factor explaining most of the variation between samples, as indicated by consistently higher R^2 values for both the overall community and the algal assemblage. (Table 1; Table S3). However, for invertebrates, all three factors appear to contribute equally to the variability of the data, with relatively low R^2 values for each, but highly significant p-values ($p < 0.001$***) (Table S5). Thus, it appears that spatial and temporal heterogeneity, particularly for mobile epifaunal invertebrates, is predominantly shaped by environmental conditions. For example, the epifaunal community associated with the invasive alga *Asparagopsis armata*, compared to the epifaunal community of *Halopteris scoparia*, depends more on site-specific factors rather than the influence of the host alga itself (Navarro-Barranco et al., 2019a). This pattern of spatial heterogeneity was also observed in certain invertebrate species, particularly the Peracarida, Mollusca and Polychaeta, which exhibited a greater influence from the site than from the host alga (*Dictyota dichotoma* or *R. okamurae*) (Navarro-Barranco et al., 2019b). This phenomenon could be attributed to the relatively short life cycle of many epifaunal species, which enhances their capacity for rapid colonization of specific habitats and increases their sensitivity to environmental fluctuations, thus amplifying the variability in species composition across the studied sites or sampling periods. (Navarro-Barranco et al., 2021).

Decline in the diversity and species richness of the fauna and flora community when the invasive species *R. okamurae* is present, particularly noticeable in algal assemblages, was revealed by the low Shannon and Simpson indices (Fig. S2). Similar findings have already been observed for *A. taxiformis*, an invasive Rhodophyta on Favignana Island (Sicily, Italy), where the diversity index decreased in correlation with the progression of the invasion (Mancuso et al., 2022). However, no significant differences in Pielou's index were found between sites invaded and non-invaded by *R. okamurae* (Fig. S2). This suggests that *R. okamurae* does not affect the evenness of the remaining species in benthic communities within the invaded sites, but rather leads to a reduction in the overall abundance of the present algal species (Fig. 6). Studies comparing communities invaded by *C. cylindracea* and non-invaded communities in Marseille showed similar results to those for *R. okamurae*, with Shannon and Simpson indices being lower in invaded areas. However, when the biomass of *C. cylindracea* was taken into account, the Pielou index was significantly lower, probably due to its dominance, which could explain the lack of variation in this index between the invaded and non-invaded communities of *R. okamurae* in this study (Klein and Verlaque, 2009b).

A weak difference for Ics between invaded and non-invaded locations is unexpected. Typically, the rocky shallow water in the Mediterranean Sea should harbor marine forests, which create extensive canopies that serve as habitats for marine species (Thibaut et al., 2005; Orellana et al., 2019; Smith et al., 2023b). Despite being an area with extensive regulations and protection, the CNP has been previously degraded and exploited over centuries leading to decreased ecosystem diversity and changes in the marine littoral landscape. The decline of the marine algal forest has been reported in the CNP, mainly due to habitat destruction and overgrazing by herbivores (Blanfuné et al., 2022). No single individuals of marine forest forming species as *Cystoseira sensu lato* or *Sargassum* spp. were observed in all the sampled sites. The vegetation is dominated by low-structural species forming bushy or turf stratum. The currently dominant species were *Lithophyllum incrustans, Corallina berteroi*, and *H. scoparia*. The presence of these fewer structuring species could explain the weak discernible differences in the Ics between invaded and non-invaded locations, resulting in a low Ics in both cases. For example, in Ar Gall and Le Duff (2014) they found that the community dominated by *Pelvetia canaliculata*, a small fucales (15 cm high), had the lowest Ics score ($Ics = 0.64 \pm 0.14$), while the highest score was found for the *Laminaria digitata* community with a higher canopy ($Ics = 1.19 \pm 0.24$). In highly stressed marine environments, annual species are favored due to their rapid growth rates and active reproduction. In contrast, undisturbed marine environments usually feature perennial species with slower growth rates (i.e *Cystoseira s.l.*). Our results for invaded and non-invaded locations show a community dominated by annual species with small size. The highest Ics score in the invaded location can be explained by the massive density and abundance of *R. okamurae*, which has no equivalent in terms of algal dominance in the non-invaded sites. Therefore, the low-structured algal communities are sustained by an intense grazing by the herbivore teleost *Sarpa salpa* (Vergés et al.,2009) which represents half the total fish biomass of the CNP (Le Diréach et al., 2023). We did not observe grazing of *R. okamurae* by this fish probably due to a high concentration of dilkamural, a deterrent compound (Casal-Porras et al., 2021). The dominance of the unpalatable alga *R. okamurae* is a major change on the functional diversity in the studied area.

Same species of invertebrates were significantly less abundant in invaded sites (Polychaeta, *Sipunculus* (*sipunculus*) *nudus*, *Anomia ephippium*). The difference could be explained by the dense canopy formed by *R. okamurae*, which may lead to a reduction in dissolved oxygen due to the respiration of algae and decomposition of algal detritus beneath the canopy (D'Avanzo and Kremer, 1994). Additionally, changes in sedimentation and water column flow can contribute to alterations in environmental parameters as already observed with *C. taxifolia* invasion leading to a decrease in oxygen and an increase in sulfide levels, impacting the life traits of the invertebrate biota (Gribben et al., 2009). Conversely, Navarro-Barranco et al. (2019b) found that the abundance of polychaetes was currently higher when *R. okamurae* was present. However, the authors concentrated exclusively on the epifauna by meticulously collecting each individual alga in plastic bags in order to limit the loss of species. In the present study, the sampling protocol, which employed the use of a hammer and chisel, facilitated the comprehensive collection of the entire benthic community, extending to the bedrock. However, this method results in a significant loss of organisms belonging to the mobile epifauna. Therefore, integrating the methodology employed by Navarro-Barranco et al. (2019b) with the sampling protocol used in this study would offer a more thorough assessment of the impact of invasive species on the entire benthic community.

The abundance of Copepods increased in the presence of *R. okamurae*, which has never been reported previously with *R. okamurae*. However, earlier studies have shown that greater meiofaunal abundance is associated with increasing algal biomass (Ape et al., 2018). Therefore, the dense beds of *R. okamurae* create numerous microhabitats that provide refuge for copepods from predators. The difference in abundance between copepods and polychaetes in invaded sites can be attributed to their habitat. Copepods reside above the substrate, in the surrounding water of *R. okamurae*, while polychaetes inhabit the sediment under the canopy.

We observed a clear over-competition between *R. okamurae* and *A. armata.* That species was introduced in the area around Marseille in the late 19th century forming dense beds of 20 cm high gametophytes on all rocky substrates during the spring cold period. The sporophyte (*Falkenbergia* stage) is present throughout the year. *Asparagopsis armata* does not bloom in sites invaded by *R. okamurae*. Competition between invasive algae is common but interactions are few documented. Thus, the competition between two *Caulerpa* has been observed in Tuscany, with *C. cylindracea* out-competing *C. taxifolia* (Piazzi and Ceccherelli, 2002). Futhermore, the arrival of *C. cylindracea* in a habitat previously

dominated by another introduced species, *Womersleyella setacea*, unexpectedly conspicuously increased the species point diversity (Balata et al., 2000). In the Alboran Sea, *Asparagopsis taxiformis,* recently introduced, and outcompeted the earlier-introduced *A. armata* (Zanolla Balbuena, 2014).

5. Conclusion

This study demonstrates the significant impact of the invasive brown alga *Rugulopteryx okamurae* on the fauna and flora communities of the rocky substrate, following its introduction along the Marseille coast in 2016. This species has extensively colonized the entire rocky shoreline, resulting in a significant habitat shift and alterations in the local landscape. The observed impact affects the taxon richness and diversity of the local ecosystem, as well as the stratification of the algal assemblage, ultimately modifying ecosystem functioning. Currently, the invasion of *R. okamurae* is confined to the coldest regions of the Mediterranean Sea (Gulf of Lion, Alboran Sea and Sicily Channel). Despite frequent marine heat waves in the Mediterranean, the spread of *R. okamurae* has not been slowed in the CNP. Given their limited spatial and temporal scope, heat waves are unlikely to significantly promote the establishment of non-indigenous species (NIS). However, the overall rise in temperature in the Mediterranean could favour thermophilic species, including non-native ones such as *Halophila stipulacea*, which recently made a significant leap northward, covering several hundred kilometers to reach Cannes (Thibaut et al., 2022). Conversely, the rise in temperature may impede the spread of more cold-tolerant species and restrict the expansion of *R. okamurae* to the eastern Mediterranean (Ruitton et al., 2021). This raises questions about its potential future expansion into warmer parts of the Mediterranean Sea and its potential impact on a broader array of benthic assemblages.

CRediT authorship contribution statement

Marie Borriglione: Writing – original draft, Investigation, Formal analysis. **Sandrine Ruitton:** Writing – review & editing, Methodology, Conceptualization. **Florian Boyer:** Investigation. **Delphine Thibault:** Resources. **Aurélie Blanfuné:** Methodology. **Dorian Guillemain:** Resources. **Marc Verlaque:** Writing – review & editing. **Charles-François Boudouresque:** Writing – review & editing. **Thierry Thibaut:** Writing – original draft, Methodology, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2024.109010.

Data availability

Data will be made available on request.

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