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Short communication

An outbreak of the invasive macroalgae *Rugulopteryx okamurae* in Alicante Bay and its colonization on dead *Posidonia oceanica* matte

Marc Terradas-Fernández[a,*,1], Carolina Pena-Martín[b,2], Miguel Valverde-Urrea[a,3], Alicia Gran[c,4], Fabio Blanco-Murillo[a,5], Laura Leyva[d,6], Eleuterio Abellán-Gallardo[a], Esther Beresaluze[a], Andrés Izquierdo[e,7], Yoana del Pilar-Ruso[a,8], Javier Aguilar[a,9], Yolanda Fernández-Torquemada[a,10]

[a] *Departamento de Ciencias del Mar y Biología Aplicada, Facultad de Ciencias, Universidad de Alicante, Aptdo. Correos 99, 03080 Alicante, Spain*
[b] *Dpto. Ciencias Ambientales y Recursos Naturales, Facultad de Ciencias, Universidad de Alicante, University of Alicante, 03690 Alicante, Spain*
[c] *Centro Oceanográfico de Baleares, Instituto Español de Oceanografía (IEO-CSIC), 07015 Palma, Spain*
[d] *Instituto Multidisciplinar para el Estudio del Medio, Universidad de Alicante, Aptdo. Correos 99, 03080 Alicante, Spain*
[e] *CIMAR - Centro de Investigación Marina de Santa Pola - Universidad de Alicante, Cuartel Torre D'en Mig s/n, Cabo de Santa Pola, Alicante 03130, Spain*

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ABSTRACT

We report the first occurrence of *Rugulopteryx okamurae* in Alicante Bay, the northernmost location in the Iberian Mediterranean where this species was detected. Collected specimens were studied using a morphological and molecular approach. A rapid assessment survey was conducted to assess its presence and abundance in the nearest beach-cast and infralittoral areas. The highest abundance was found over dead *P. oceanica* matte, occupying an important extension in the bay. We found detached fragments of *R. okamurae* dispersed by local hydrodynamics, accumulating on some tourist beaches in the region. The potential suitability of the Mediterranean for its spread necessitates monitoring programs to study its variation over time and potential impacts on native biota.

1. Introduction

Non-indigenous species (NIS) are on the rise worldwide (Katsanevakis et al., 2013), and this trend is evident in the Mediterranean, a semi-enclosed sea considered a significant hotspot for exotic species. Non-indigenous macrophytes (NIM) comprise 14.1% of non-indigenous species in this sea (Galil et al., 2014). NIM species are often accidentally introduced through maritime traffic (ballast water or fouling), aquaculture, aquariology, or via the Suez Canal (Lessepsian immigrants) or the Strait of Gibraltar (Galil et al., 2014). Some NIM can become invasive, causing ecological and economic impacts by altering habitats, replacing native species, and modifying trophic webs, community structure, and diversity (Tsirintanis et al., 2022). Notable examples of NIM with significant impacts in the Mediterranean include *Caulerpa taxifolia, C. cylindracea*, and *Asparagopsis taxiformis* (Tsirintanis et al., 2022). Recently, *Rugulopteryx okamurae* has been added to this list (Sempere-Valverde et al., 2021).

Rugulopteryx okamurae (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S.

* Corresponding author.
E-mail address: marc.terradas@ua.es (M. Terradas-Fernández).
[1] ORCID: 0000-0002-1916-5337
[2] ORCID: 0000-0001-5261-4838
[3] ORCID: 0000-0002-5708-5258
[4] ORCID: 0000-0002-7963-2558
[5] ORCID: 0000-0003-1759-9075
[6] ORCID: 0009-0002-1743-1010
[7] ORCID: 0000-0002-6887-5036
[8] ORCID: 0000-0003-2824-6136
[9] ORCID: 0000-0001-8668-5765
[10] ORCID: 0000-0003-0533-4109

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Kim (Dictyotales, Ochrophyta) is a brown alga native to the Asia-Pacific, ranging from tropical to tempered regions (Ruitton et al., 2021 and references therein). It was initially detected on the Mediterranean coast in 2002 at Thau Lagoon, possibly introduced through oyster aquaculture. Subsequent introductions of *R. okamurae* on the coasts of Marseille, Alboran Sea, and the Macaronesian region have exhibited invasive behavior (Ruitton et al., 2021; Sempere-Valverde et al., 2021; Faria et al., 2022). *Rugulopteryx okamurae* impacts the structure of benthic communities, outcompeting other NIM species and native communities (Ruitton et al., 2021). Additionally, the seaweed's ability to spread vegetatively and survive after detaching from the substrate leads to drifted thalli accumulating on the seafloor or washing up on beaches, impacting tourism, public health, and artisanal fishing (Faria et al., 2022; Mateo Ramírez et al., 2023).

In this study, we first report the establishment of *R. okamurae* in Alicante Bay (SE Spain) using molecular and morphological approaches. We propose a rapid assessment method (RAS) involving scuba diving and analysing beach-cast macrophyte wrack patches to detect and quantify this alga. We also discuss ecological considerations to aid in developing strategies to prevent its further expansion.

2. Material and methods

First samples were collected for teaching purposes from Alicante Bay (38°18.81′ N - 000°30.567′ W, 38°18.472′ N - 000°30.278′ W, 38°18.345′ N - 000°29.869′ W) at depths ranging from 6.5 to 15.8 m after using Van Veen grabs and Naturalist's and skid dredges. These samples were collected from a highly degraded area near Alicante Port, characterized by a bottom predominantly composed of dead *P. oceanica* matte covered with a layer of silty sediments, and the presence of the macroalgae *Caulerpa prolifera* (Blanco-Murillo et al., 2022).

2.1. Morphological and molecular identification

To determine morphology, specific identification keys were followed (Cormaci et al., 2012). Specimens used for morpho-anatomical observations were preserved on sheets and in a 4% formaldehyde solution. They were deposited in the ABH Herbarium (University of Alicante) under the numbers ABH-Algae 828–830. Subsamples from ABH-Algae 830 were utilized for molecular studies and anatomical images.

For molecular analysis, DNA was extracted according to CTAB protocol with slight modifications in the extraction buffer composition (0.1 M Tris HCl, 0.05 M EDTA, 2 M NaCl, 0.05 M DTT, PVP 1% and 3% CTAB) (Sambrook and Russell, 2007). The *rbc*L gene was amplified using the pair of primers rbcL68F and S3R (Draisma et al., 2001). On the other hand, *psb*A gene was amplified using the pair of primers psbAF1 and *psb*AR2 (Saunders and Moore, 2013). PCR products were sequenced by an external laboratory (STAB vida, Caparica, Portugal). The consensus sequences were deposited in the NCBI database (Accession Number OQ851594 and OQ851595) and compared to the NCBI database by the Blastn tool (accessed on 15 April 2023).

2.2. Rugulopteryx distribution in Alicante Bay

A rapid assessment method was used to cover extensive areas to rapidly measure its abundance and dispersion during March 2023. Firstly, we conducted a comprehensive field survey to quantify *R. okamurae* in detritus belts washed ashore at Alicante Bay, including the adjacent Santa Pola (South) and San Juan (North) beaches. The entire coastline was covered on foot due to its accessibility. The degree of continuity of the detrital belt was classified into six categories (Braun-Blanquet, 1979; Ballesteros et al., 2007; Terradas-Fernández et al., 2022) (0.1: detected, 1: scarce clumps, 2: regular but small patches, 3: regular and almost continuous patches, 4: continuous belts with small, interspersed gaps, 5: continuous belts). Based on the results of the previous sampling, a total of 12 dives were conducted at depths ranging from 5 to 15 m to assess the abundance of *R. okamurae* in the infralittoral area of Alicante Bay (Supplementary material, Table A1). In each dive, a transect of 50 m long and 2 m wide was performed to assess the presence and the degree of continuity of *R. okamurae* canopy using the same aforementioned procedure. Data on the type of substrate or habitat (seagrass meadows, sand, rock, dead *P. oceanica* matte, etc.) were also collected, along with information on the presence of other species.

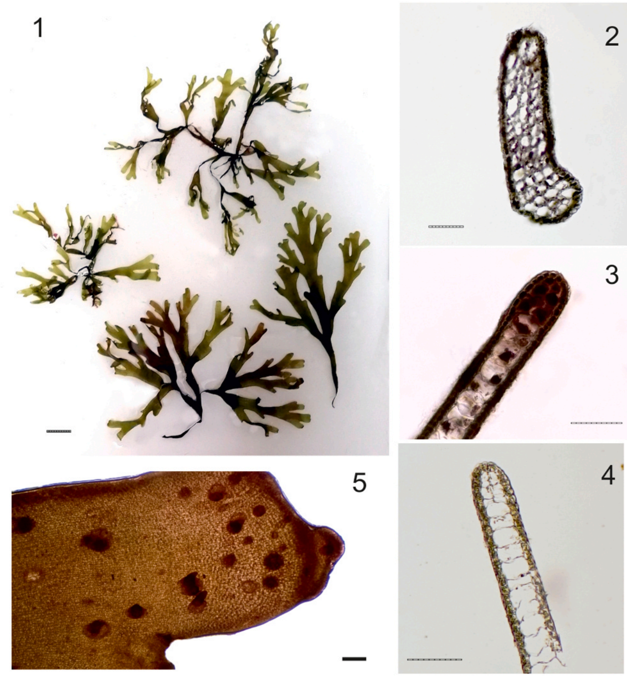

Fig. 1. 1–5. Specimens collected by dredging in Aguamarga beach (ABH-Algae 828). 1. Habit showing different morphotypes (Scale-bar: 1 cm). 2. Basal section of the thallus. 3. Section in the middle zone. 4. Section near the apex. 5. Propagules on a specimen from Santa Pola beach (ABH-Algae 830). Scale-bar 2–5: 100 μm.

3. Results and discussion

3.1. Morphological and molecular identification

Thalli were from 3 to 10 cm high, and 3–10 cm wide. The colour was yellowish brown without any iridescence. Neither sporangia nor gametangia were detected, although propagules were abundant as proliferous branches (Fig. 1.5). Thalli were dichotomously to anisotomously branched (Fig. 1.1 and Supplementary material Fig A.1), with obtuse apices showing a distinctly apical cell (Supplementary material Fig A.5), and rhizoids in the basal parts (Supplementary material Fig A.2). Cross-section of the base (first 2–4 mm) shows a multi-layered medulla (3–5 layers) (Fig. 1.2), which then changes to centrally monostromatic, with 3–4 layers in the margin along most of the thallus (Fig. 1.3 and Supplementary material Fig A.3). In the last 5–10 mm towards the apex, the margin decreases to 2 strata (Fig. 1.4 and Supplementary material Fig A.4).

Resulting sequences matched with *R. okamurae* individuals from the western Pacific Ocean and with recently detected ones in the NE Atlantic Ocean (Faria et al., 2022) (rbcL: GenBank accession No. KJ000694; psbA: GenBank accession No. ON677529.1) (Supplementary material, Table A2). All matches showed an E. value < 0.01.Fig. 1.

3.2. Rugulopteryx distribution in Alicante Bay

Rugulopteryx okamurae dominated the infralittoral zone near

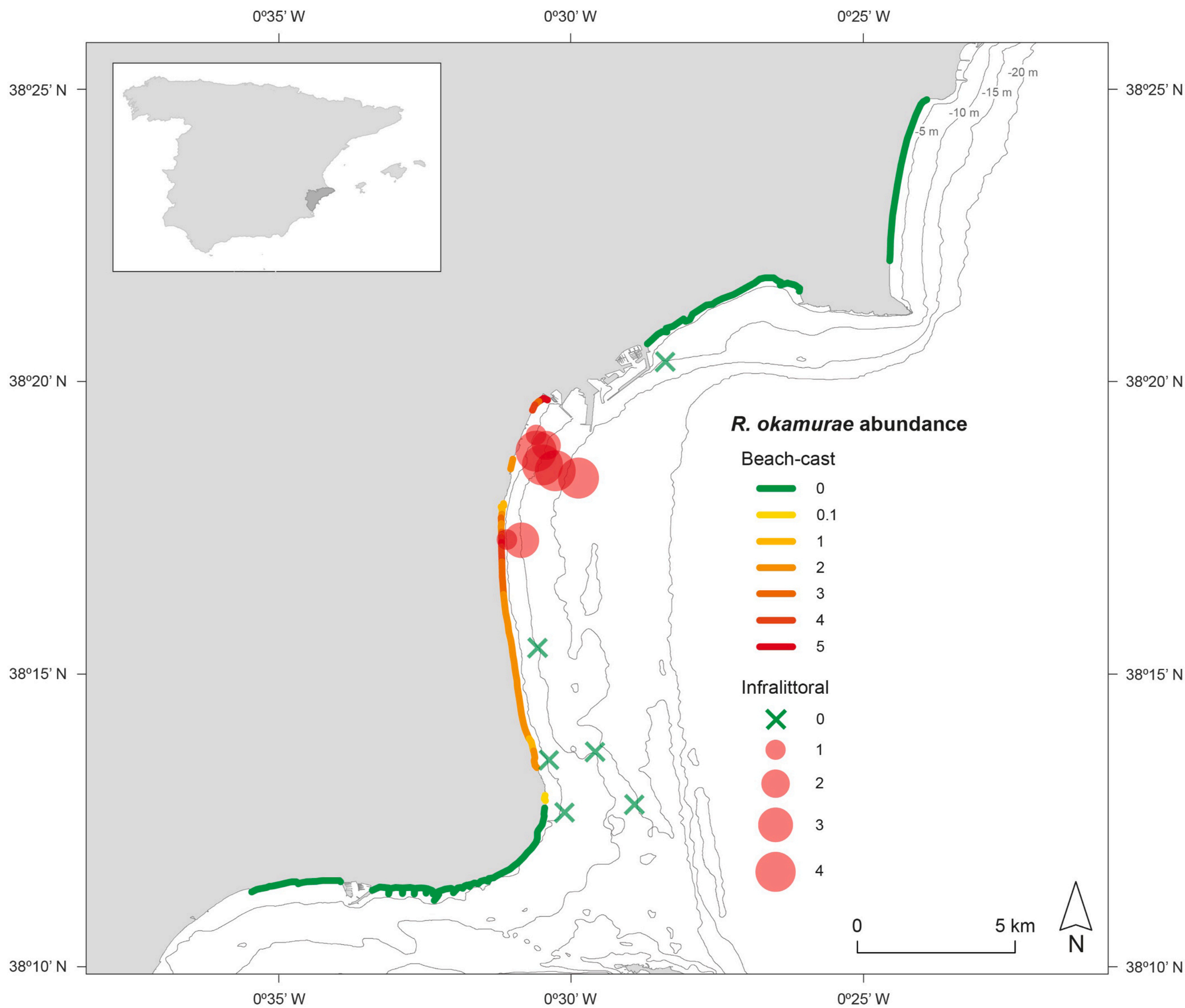

Fig. 2. Map of Alicante Bay and sites sampled for the presence of *R. okamurae* on beach-cast or at the infralittoral zone. Red circles mark sites where *R. okamurae* was detected in the infralittoral (size indicates abundance of this species, see Material and Methods); green crosses mark studied sites where this species was not found. The same colour scale was applied for beach-cast by using lines as symbols (showing a more continuous sampling).

Alicante Port (Fig. 2, Supplementary material Fig. B). The species showed a higher cover in the southern vicinity of Alicante Port, where we also detected another exotic species (Supplementary material, Table A1), such as *Asparagopsis taxiformis* and *Codium fragile* (with lower cover). Other species found in the area were *Caulerpa prolifera, Flabellia petiolata, Padina pavonica, Dictyopteris polypodioides, Dictyota* spp and *Cymodocea nodosa*. Maximum cover and size of *R. okamurae* was found over dead *P. oceanica* matte. This species was also found as a drift over sediment mainly composed of sand and spots of *C. nodosa*. We did not detect this species through diving at southern locations (Arenales and Cape of Santa Pola) or north of Alicante Port, where healthy *P. oceanica* meadows are present. *Rugulopteryx* was also dominant in the detritic belts along the shore, extending from the southern dock of Alicante Bay to the beaches located at the centre of the bay (Urbanova beach), forming continuous belts and established detritic reefs or "banquettes" (Fig. 2, Supplementary material Fig. B). Other frequent species at these beach-cast patches were *P. oceanica, C. nodosa, Phyllophora* sp., *Asparagopsis taxiformis, C. prolifera, Ulva* spp., *Colpomenia sinuosa, Jania adhaerens, Treptacantha sauvageauana* and *Dictyota* spp. We also detected *R. okamurae* in the beach-cast of sites south of Alicante Bay. At some of these beach-cast wrack patches, the thalli of *R. okamurae* were more fragmented, and detached algae were spreading in the water. However, this species has not been detected in other nearby locations north of Alicante Port, nor south of Cape of Santa Pola.

Rugulopteryx okamurae has an invasive behaviour in most of the locations where it has been recently detected as exotic species. In many cases first observations coincide with an outbreak of this species showing a wide ecological valence (Ruitton et al., 2021). In our study, *R. okamurae* covered most of the infralittoral zone near Alicante Port, becoming the primary detritic component washed ashore along the coast. The non-detection in other peripheral stations of Alicante Bay could indicate that the invasion has its origin in Alicante Port, and it is still located or there are some environmental constrains that could slow down the expansion. We hypothesized that *R. okamurae* entered the bay through the activity of Alicante Port, as ports and marinas are significant sources of alien species, introduced via fouling or ballast water (Katsanevakis et al., 2013). In fact, in Alicante Port some other non-indigenous species have been detected, such as *Bostrycapulus aculeatus* and *Oculina patagonica* (Izquierdo et al., 2007). Moreover, ballast water has been proposed as the main entrance way for *R. okamurae* at the Strait of

Gibraltar (Rosas-Guerrero et al., 2018).

This alga has been introduced in eutrophic regions of the Mediterranean Sea, such as Thau lagoon, Gulf of Lyon or Alboran Sea (Ruitton et al., 2021), suggesting local nutrient enrichment may encourage its expansion (Mercado et al., 2022). The area of Alicante Bay, where *R. okamurae* was detected, is highly degraded due to various anthropogenic activities, including the presence of a desalination plant and sewage discharges from Alicante city (Blanco-Murillo et al., 2022). The degradation of this area has led to the loss of *P. oceanica* meadows at depths less than 15 m, where it has remained as a bottom of dead matte covered with muddy sediments, and in some sites, opportunistic algae, such as *Caulerpa prolifera* or *C. cylindracea* (Blanco-Murillo et al., 2022). Thus, the nutrient enrichment from sewage discharge and available substrate from dead *P. oceanica* matte may provide an optimal environment for *R. okamurae* colonization, displaying opportunistic behaviour like other algae invasions in the area (Terradas-Fernández et al., 2020).

However, the broad native range of this species, from tempered to tropical waters in the Asian-Pacific region, along with the broad ecological valence showed in those invaded locations, may indicate that the expansion across the rest of the southeast and east of Spain is an ongoing process. Moreover, some proposed models of expansion of this alga agree with an acute expansion through most of the Mediterranean waters (Muñoz et al., 2019). Part of the dispersal potential of this species is due to its high vegetative reproduction and its survivability in adverse environmental conditions (Rosas-Guerrero et al., 2018), but also due to its ability to remain photosynthetically active once separated and uprooted from the substrate as detached thalli (Mateo-Ramírez et al., 2023). In our study, we have detected some of these detached thalli on beach shores where we did not find living submerged individuals, which were probably dispersed by the action of currents and waves. The easy dispersion of fragments may lead to the species appearing in new areas. Detached thalli of this alga have been found retained by *Cymodocea nodosa* shoots, which could act as collector traps for this species. The frequent proliferative branches observed accord with the high capacity of vegetative reproduction of this species, while the non-detection of sporangia could be attributed to the winter sampling period. Some studies suggest that this species may have a greater invasive capacity in warm months when the frequency of thallus with propagation structures increases (Salido and Altamirano, 2021).

Considering the current distribution and invasive potential of this species, the implementation of broad and coordinated long-monitoring strategies to detect new introductions and evaluate their impacts along with some measures to slow down its expansion, becomes crucial. For these purposes, a manual has been drafted involving Spanish public administration (Ministry for the Ecological Transition and the Demographic Challenge BOE-A-2022–13838), which highlights the need for preventing new introductions, management and control of existing populations, and the coordination between the different agents responsible for such management. In this sense, the implementation of a rapid assessment method to detect populations in other locations is of utmost importance. Rapid assessment surveys (RAS) have proved to be an effective tool in early detection of exotic species (Bishop et al., 2015). We recommend applying RAS in the vicinity and in urbanized areas with ports and marinas, as they are the source of new putative introductions, along with fishing ports where fishing activities can promote the invasion (Katsanevakis et al., 2013). The rapid visual sampling of detritic belts can also be an effective tool for detecting and locating other established populations, as it has already been proven to other exotic species, such as *Pinctada radiata* or *Cerithium scabridum* (Martínez-Ortí and Escutia, 2021). This sampling method is easy and cost-effective for monitoring large areas, making it suitable for citizen science projects. However, expert validation is necessary to avoid misidentifications (Faria et al., 2022).

Our study reinforces the prediction of *R. okamurae*'s spread along the south-eastern Spanish coast (Muñoz et al., 2019), underscoring the importance of monitoring programs. We also recommend getting in contact with fishermen, diving clubs, and other stakeholders to raise awareness about this species on our coast and implement preventive measures.

CRediT authorship contribution statement

Conceptualization (**MTF**, **CPM**, **YFT**), Methodology (**MTF**, **CPM**, **FBM**, **AI**, **YFT**), Formal analysis (**MVU**), Investigation (**MTF**, **CPM**, **MVU**, **AG**, **FBM**, **LL**, **EAG**, **EB**, **AI**, **YPR**, **JA**, **YFT**), Writing – original draft (**MTF**, **CPM**, **MVU**, **YFT**), Writing – review & editing (**MTF**, **CPM**, **FBM**, **YFT**), Visualization (**AG**, **LL**).

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aquabot.2023.103706.

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