

Research Article

A worrying arrival: the first record of brown macroalga *Rugulopteryx okamuræ* in Madeira Island and its invasive risk

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Abstract

The brown macroalgae *Rugulopteryx okamuræ* is described as one of the most severe and threatening invasive marine macroalgae in European waters. This study reports the first record of *R. okamuræ* in the Madeira archipelago, which represents a new southern distribution limit of this species in NE Atlantic European waters. Morphological and molecular characters were used to confirm the species' identity, and its potential invasion risk in Madeiran waters was screened using the standard risk assessment tool AS-ISK. Results show that *R. okamuræ* has a medium-high risk of becoming invasive in Madeira Island under present and future climate scenarios. The greater risk of impact involves suppressing local species growth and the modification and degradation of local habitats, including trophic cascade effects. However, environmental and commercial impacts could also occur in case of an explosion of the invasive populations. This new introduction in Madeira coastal waters emphasises the need for regular monitoring of *R. okamuræ*, particularly to assess population dynamics to avoid establishing and further expansions. Finally, we recommend the evaluation of the possible derived impacts affecting rocky coastal communities and adopting the necessary mitigation measures and policies.

Key words: invasive species, marine bioinvasions, intertidal systems, ecological risk, Macaronesia

Introduction

The brown macroalgae *Rugulopteryx okamuræ* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim 2009 is originally from the Pacific Ocean but is currently widely distributed in subtropical and temperate western areas

(Lee 1986; Silva et al. 1987; Yoshida 1998). Individuals are characterised by a dichotomously branched thallus (10–20 cm high) with rhizoids in the basal parts of the thallus. This species was introduced into European waters, first recorded on Thau coastal lagoon (French Mediterranean coast) in the spring of 2002 (Verlaque et al. 2009). However, *R. okamurae* did not present an invasive behaviour until 2015 and 2016, when it was abundantly detected on both shores of the strait of Gibraltar (Afonso-Carrillo and Ocaña 2016; Altamirano et al. 2017) and more recently, in Provence (France) in 2018 (Ruitton et al. 2021) and in the Azores (Portugal) in 2019 (Faria et al. 2022a). In fact, this species has colonised hundreds of kilometres of coastline triggering several ecological and socio-economic impacts (Faria et al. 2022b; García-Gómez et al. 2020; Sempere-Valverde et al. 2021). In some locations, *R. okamurae* became the most abundant species in less than one year, covering almost 100% of the rocky bottoms between 5 and 20 m depth (García-Gómez et al. 2020; Ruitton et al. 2021; Faria et al. 2022b). In these regions, the accumulation of algal wrack on the coast has become a problem with implications for tourism, public health and the commercial sector, as the abundant floating biomass has caused a drastic reduction in fishing captures and an increase in net cleaning costs with significant repercussions to the local economy (García-Gómez et al. 2020, 2021a).

The success of *R. okamurae* is due to different aspects, including its high reproductive capacity, producing hundreds of individuals from a single specimen by vegetative propagules and asexual monospores, together with its colonization ability, leading to the massive occupation of rocky bottoms (Altamirano et al. 2017, 2019; García-Gómez et al. 2021a). Furthermore, *R. okamurae* has a highly competitive capacity against local biota (García-Gómez et al. 2021b) and the ability of this seaweed to store nitrogen, which would increase its occurrence in areas with occasional peaks in nutrient concentration, such as those created by local upwelling events and seasonal coastal eutrophication (Mercado et al. 2022). The high rates of the detachment of *R. okamurae* and the ability of its floating fragments to maintain high photosynthetic rates and reattach to hard substrata allow this species to rapidly expand after colonising suitable geographical areas (Figuerola et al. 2020).

Prevention, early detection and eradication of non-indigenous species (NIS) are essential to avoid the socio-ecological impacts of biological invasions (Pearce et al. 2012; Castro et al. 2022), but it is a challenging task when dealing with species favoured by ocean warming and tropicalisation processes (Canning-Clode and Carlton 2017). Nonetheless, the requirement to produce an early warning system for eradication, mitigation and management policy decisions, makes an early evaluation of risks screening from NIS proliferation necessary. There are few examples in marine environments of a rapid response allowing the eradication of NIS in the early stages of the invasion, becoming cases of management success (Anderson 2005; Willan

et al. 2000). Risk evaluation protocols allow the implementation of the best procedures to reduce the risk of NIS introduction (Venette et al. 2021). In this context, the Aquatic Species Invasiveness Screening Kit (AS-ISK) is a method to identify potentially invasive aquatic species based on a questionnaire that the assessors answer based on updated peer-reviewed literature. This quantifies the risk associated with NIS invasiveness in a given area based on its biogeography, biological and ecological characteristics and previous records of invasions (Copp et al. 2016). As a result, AS-ISK allows an estimation of the potential of a NIS to present ecological, environmental and economic impacts under present and future climatic conditions.

Macaronesia is a vast oceanic region, located in the NE Atlantic Ocean, conformed by five volcanic archipelagos (Azores, Madeira, Canary Islands and Cabo Verde) influenced by a latitudinal gradient of temperatures from tropic areas in the south to more temperate ambients in the north. An increase of NIS has been reported in coastal systems of these remote islands over the last 30 years, including macroalgae, arthropods, echinoderms and fishes, among others (Castro et al. 2022 and references within), mainly transferred in ballast water and hull fouling of commercial shipping. More recently, these archipelagos have been also threatened by a tropicalisation process as a consequence of climate change, favouring the arrival of numerous NIS (Ribeiro et al. 2019; Schäfer et al. 2019; Castro et al. 2022). This study is the first report of *R. okamurae* in the Madeira archipelago (NE Atlantic), representing a new southern limit for its distribution in European waters and evidence of its expansion in the oceanic waters of the Macaronesian region. We confirm the species identification, using morphological and molecular characters. In addition, we assess and discuss the invasiveness potential of *R. okamurae* in the Archipelago of Madeira using the AS-ISK risk assessment tool.

Materials and methods

Rugulopteryx okamurae was observed in December 2021 in a rocky intertidal platform on the north coast of Madeira Island (32°48'41.0"N; 17°02'25.1"W) (Figure 1). The species was sharing habitat with other macroalgae species such as *Asparagopsis taxiformis* (Delile) Trevisan de Saint-León, 1845; *Halopteris scoparia* (Linnaeus) Sauvageau, 1904; *Padina pavonica* (Linnaeus) Thivy, 1960; articulated and encrusting corallines and other Dictyotales. Individuals of *R. okamurae* were also observed as epiphytes on other macroalgae species, specifically on *H. scoparia* and *A. taxiformis*. Ten collected individuals were identified by their morphological and anatomical characteristics under dissecting and compound microscopes. Eight samples from four different individuals were dried in silica for molecular analyses and vouchers were deposited in the AZB Herbarium Ruy Telles Palhinha at the University of Azores under deposit numbers

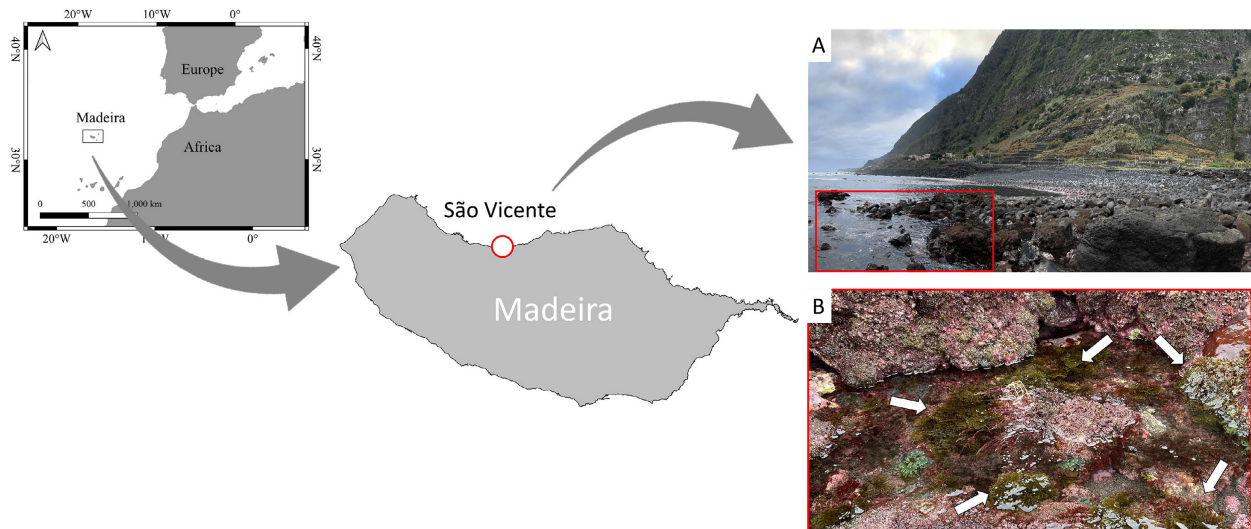


Figure 1. Location of the Madeira archipelago in NE Atlantic Ocean. Panels A and B show the rocky platform in São Vicente (north coast of Madeira) where *R. okamurae* was found. White arrows indicate some individuals in the rocky intertidal. Photo by Alejandro Bernal-Ibáñez.

MAD-22-01 and MAD-22-02. Genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen) and the two chloroplast protein-encoded genes *rbcL* and *psbA* were amplified with primers listed in Draisma et al. (2001) and Saunders and Moore (2013), respectively (see Faria et al. 2022a for additional details). Amplified PCR products were directly sequenced in both forward and reverse directions by STABVIDA sequencing services. The obtained DNA sequences were checked, edited and aligned using BIOEDIT v7.2 (Hall 1999) and verified by comparison through BLASTN search in the NCBI database. Retrieved sequences were deposited in GenBank.

We assessed the invasiveness potential of *R. okamurae* in Madeira using the free software AS-ISK v2.3 (Copp et al. 2016; Vilizzi et al. 2021). This screening protocol has 55 questions. The Basic Risk Assessment (BRA, 49 questions) include 13 questions on the biogeography and history of the species, such as commercial interest and trade, climatic affinities and previous records of impacts; and 36 questions on the Biology and Ecology of the species, including topics such as reproductive and life-span strategies, competitive traits against local species and trophic interactions (Copp et al. 2016). It also includes a Climate Change Assessment (CCA), with six questions that evaluate how predicted climate conditions are expected to affect the risks of NIS introduction, establishment, dispersal and impacts in the assessed area. The AS-ISK output includes a BRA score and BRA + CCA (composite) score for each assessor, ranging from –20 to 68 and from –32 to 80, respectively. Scores lower than 1 suggest that the species is unlikely to become invasive in the risk assessment area. Higher scores classify the species as having either a “medium risk” or a “high risk” of becoming invasive. The distinction between medium and high invasion risk depends upon a threshold value. We used the threshold of 32 for BRA and 27.25 for BRA + CCA, as provided by Vilizzi et al. (2021) for marine plants. The overall confidence factor (CF) of the assessment is computed

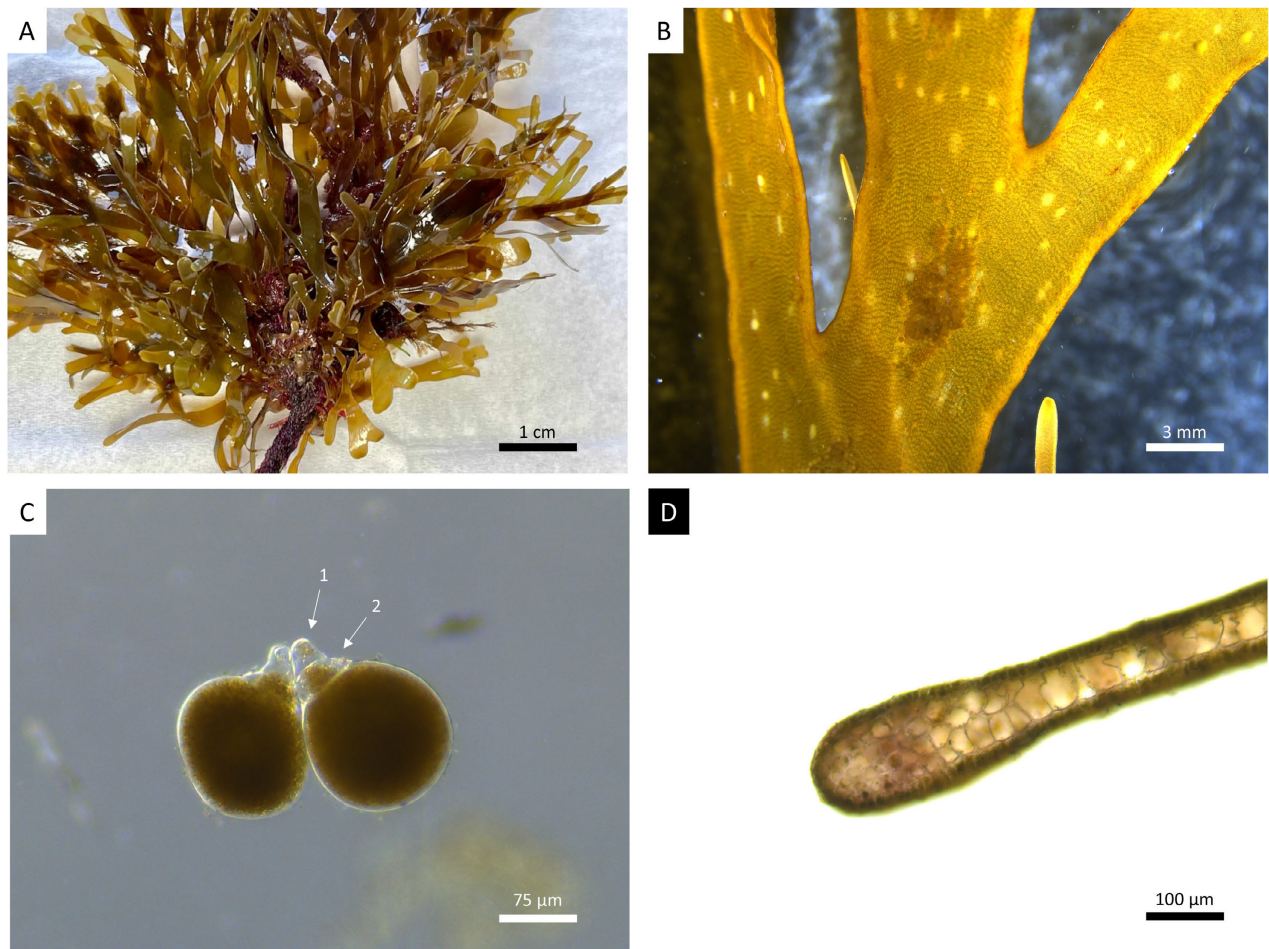


Figure 2. (A) *Rugulopteryx okamurae* thallus growing over *A. taxiformis*. (B) Thallus surface with asexual monosporangia, (C) borne on two stalked cells (white arrows) and (D) transverse section of the thallus with multiple layers of medullary cells in the margin. Photographs by Alejandro Bernal-Ibáñez (A) and Andrea Albert-Fonseca (B,C,D).

from a confidence level assigned to each question (low, medium, high and very high) and ranges from $CF = 0.25$ to a maximum confidence of $CF = 1$.

Six independent AS-ISK screenings were carried out, assessing the risk of *R. okamurae* invasion in Madeiran waters and accounting for possible variations in the assessors' opinion. The AS-ISK questions were answered based on bibliographic information extracted from peer-reviewed literature (indexed journals and books) whenever possible. When this information was not available, the answers were selected using non-peer-reviewed literature and professional judgement (i.e., best guess).

Results

At a macroscopic level, collected individuals reached 9–18 cm in height and 89–134 mm in width. Individuals showed an apical dichotomy with two lobes of different sizes and numerous proliferous branchlets on both surfaces (Figure 2A). Transverse sections showed mature sporangia with two stalk cells on the basis (Figure 2B, C). Cross-sections of the thallus showed a medulla with at least two layers of medullary cells or a set of cells grouped at the margin (Figure 2D). The BLASTN searches for *rcbL* sequences

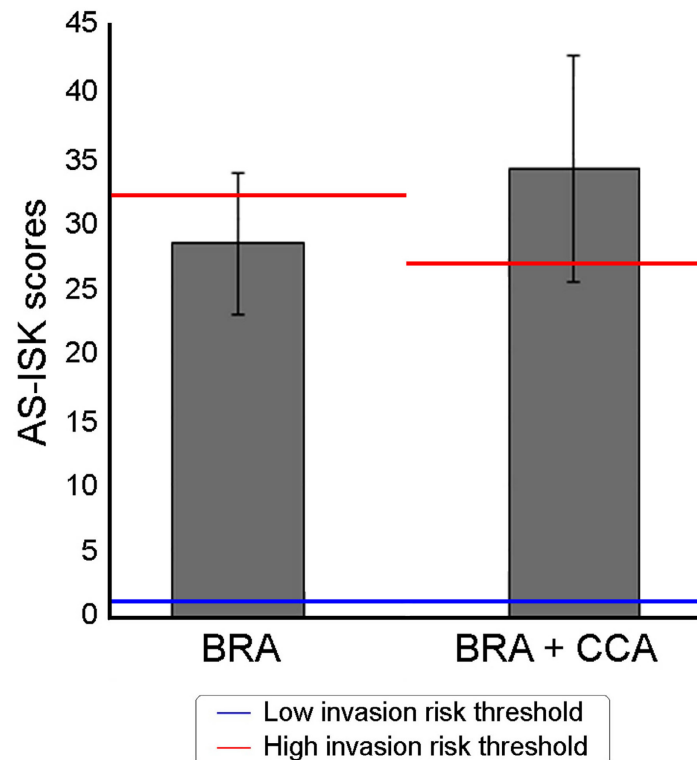


Figure 3. Average Risk Assessment (AS-ISK) scores and standard deviation for the Basic Risk Assessment (BRA, 49 questions) and the BRA + Climate Change Assessment (BRA + CCA, 55 questions), including the threshold values between low, medium and high invasion risk for BRA and BRA + CCA.

(as of 02.07.2022) were 99.2% to 100% identical to sequences from individuals identified as *R. okamurae* from Azores and also from its native origin, Japan and Korea. Likewise, psbA sequences were closely associated with *R. okamurae* individuals (98,9–100% identical). The rbcL and psbA sequences representing the Madeira specimens were deposited under GenBank accession numbers ON393999–ON394000 for rbcL and ON677528–ON677529 for psbA, respectively. The molecular analysis corroborates the morphological identification of the species, representing the first documented occurrence of this macroalgae in Madeira.

A total of 51 bibliographical sources were used to answer the AS-ISK questions. After carrying out the assessment, the six assessors converged to a document with all AS-ISK answers (see Supplementary material Appendix I). The average risk of invasion was medium for the Basic Risk Assessment (BRA), with an average confidence factor of $CF = 0.73 (\pm 0.04)$, although two of the six assessments resulted in a high risk of invasion (Figure 3). For the BRA + Climate Change Assessment (BRA + CCA), five of six assessments classified the risk of *R. okamurae* becoming invasive in Madeira Island as high, with a $CF = 0.51 (\pm 0.02)$.

The main contributor to the risk of invasion by *R. okamurae* in Madeira is the biology and ecology of the species (Figure 4). In this group of questions, the high generation of propagules by *R. okamurae*, its environmental tolerance and its ability to suppress the growth of local species

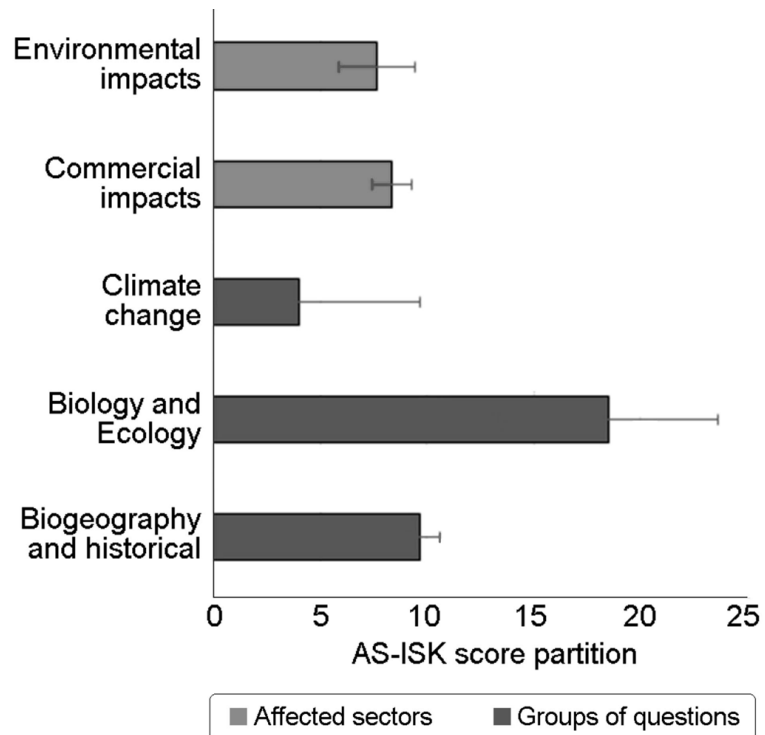


Figure 4. Average Risk Assessment (AS-ISK) scores and standard deviation for the socio-economic risks: affected sectors; based on a group of questions: Biogeographic and bioclimatic affinities and Historical invasiveness records (13 questions), Biological and Ecological traits (36 questions) and invasiveness under projected Climate change (6 questions).

and promote changes in local habitats and ecosystems were the main contributors to the risk of invasion (questions 14 to 49 in Appendix I). The second contributor to the risk of invasion is the biogeography and historical group of questions (questions 1 to 13). In this group, the questions with higher scores were focused on the history of impacts caused by *R. okamurae* in areas with a similar climate to Madeira and the existing risk of spread in the assessed area. The risks of commercial and environmental impacts by *R. okamurae* in Madeira were similar (Figure 4). These include the possible accumulation of wrack deposits and floating biomass on beaches, strongly affecting tourism as the main economic driver in Madeira (Fortuna and Vieira 2007), and shallow waters directly impacting recreational and artisanal fisheries, with the entanglement of the algae in fishing nets and aquaculture facilities (see Appendix I). Finally, the risk of invasion in future climate conditions (CCA, questions 45 to 50) had the higher variability among assessors (Figure 4). These differences in the assessments could be related to the paradox that the projected climate in Madeira is warmer, which would favour *R. okamurae*, but also more oligotrophic, which might reduce the success of *R. okamurae* in the area.

Discussion

The arrival and establishment of *R. okamurae* in Madeira reflect its high capacity for dispersal and invasion in subtropical oceanic areas, such as the four archipelagos composing Macaronesia. This new record represents the

new southern limit distribution of the species in European waters, previously delimited to the African coast of the Strait of Gibraltar. After its first record in the Azores region in 2019, it was observed in Madeira in less than two years. Although we do not know the introduction vector of *R. okamurae* in Madeira, nor where it came from, the fact that it was found on the north coast of Madeira, less urbanised, may suggest that ocean currents have favoured its arrival instead of human-assisted transport. The north of Madeira is an area with high hydrodynamics with a dominance of winds and currents from the north (Johnson and Stevens 2000). Taking into account the abundant biomass produced by *R. okamurae* in the Strait of Gibraltar and Azores, and the ability of the detached fragments to maintain high photosynthetic rates while floating, one cannot exclude the possibility of individuals of *R. okamurae* (or its reproductive structures), arriving in Madeira by floating through ocean currents from the Azores or the African coast (García-Gómez et al. 2018, 2021b; Figueroa et al. 2020; Faria et al. 2022a).

Moreover, other floating macroalgae have reached the coasts of the Macaronesian archipelagos, as is the case of the invasive *Sargassum muticum* (Yendo) Fensholt, 1995, where individuals floating were found on the northern island of Lanzarote, Canary Islands (Álvarez-Canali et al. 2021). Furthermore, *R. okamurae* can attach to floating objects and withstand long periods of darkness, so it could travel in ballast waters or be attached to marine debris (Rosas-Guerrero et al. 2018; García-Gómez et al. 2021a, b). Finally, the longline fishing fleet of Madeira, which approaches the Strait of Gibraltar and Azores (Delgado et al. 2018), could have discarded tangled biomass near Madeira, so it could also be considered a possible vector of introduction. However, better knowledge of the resistance of *R. okamurae* to air exposure and desiccation would help to acknowledge the likeliness of this vector, therefore contributing to the implementation of management measures.

The rapid invasion process of *R. okamurae* in the Strait of Gibraltar (García-Gómez et al. 2020) and in Azores (Faria et al. 2022b), where it became the dominant algae in a short period, should be an alert for its control in Madeira. The ecological consequences of the invasion in these locations have involved a restructuring of shallow-water benthic communities, producing changes in the diversity and species richness and impacting seagrasses, coralligenous and maerl habitats (Faria et al. 2022b; García-Gómez et al. 2020, 2021a; MITECO 2021; Sempere-Valverde et al. 2021; Appendix 1). Specifically, other authors have observed drastic reductions in the abundance of other native macroalgal species due to competition for the substrate and other resources favoured by the high invasiveness of *R. okamurae* (Faria et al. 2022; García-Gómez et al. 2021b). However, it is unknown to which extent *R. okamurae* beds can act as a surrogate for these impacted habitats (e.g., see Navarro-Barranco et al. 2021). Overall, the commercial and environmental impacts have been high in these areas,

where the extraction of at least 13 commercially important species has been seriously reduced (MITECO 2021). The combination of impacts on fishing extraction and beach management in Andalusia had an estimated cost of 140000 euros per month (Altamirano et al. 2019, as cited in MITECO 2021). Furthermore, aquaculture facilities' maintenance costs associated with net cleaning are likely to increase. Although it is not likely that the impacts will be similar in Madeira than in continental Europe, as the shallow platform available for the production of biomass is much smaller in oceanic islands than on continental coasts (Johnson and Stevens 2000).

Rugulopteryx okamurae has invaded different ecoregions with a similar climate but higher primary productivity than Madeira and local upwelling events (Tyberghein et al. 2012; Freitas et al. 2019; Faria et al. 2022a; Ruitton et al. 2021; Mercado et al. 2022). These differences in nutrient availability could restrict the production of biomass by *R. okamurae*, which would prevent it from becoming an explosive invader in Madeira. However, more research is needed on the factors (i.e. temperature and nutrients) that promote and/or limit the growth of this species in its exotic range of distribution (Figuerola et al. 2020; Mercado et al. 2022). It has been pointed out that *R. okamurae* has a high adaptability to a wide range of depths, light conditions and nitrate concentrations (Merchán et al. 2020; García-Gómez et al. 2020; Muñoz-Jiménez et al. 2020; Rosas-Guerrero et al. 2020). It can store nutrients, which would contribute to resisting oligotrophic conditions and take advantage of nutrient peaks, both natural, such as land runoff and upwelling events, and anthropogenic, such as that resulting from the increase in population during the tourist season (Campuzano et al. 2010; Mercado et al. 2022). Furthermore, the photosynthetic rates of *R. okamurae* are higher than other Dictyotales, which would grant a competitive advantage when colonising hard bottoms (Figuerola et al. 2020). Considering this, it is likely that *R. okamurae* will succeed in colonising hard substrates in Madeira, although it is not clear if it will be able to produce massive amounts of biomass in the oligotrophic conditions of the island. Nevertheless, *R. okamurae* produces terpenoids and other secondary metabolites that suppress the growth of native fauna and are a deterrent against herbivores and predators (García-Gómez et al. 2018 and references therein; Casal-Porras et al. 2021). These metabolites and the decomposition of large amounts of biomass near-shore and in-depth waters seriously degrade the local ecosystems of the strait of Gibraltar and the Alboran Sea, causing the mortality of fish and other organisms (MITECO 2021). Therefore, close monitoring of the development of *R. okamurae* should be implemented in Madeira, considering early eradication techniques in vulnerable habitats, such as tidepools, and protected areas, such as the MPA of Garajau (see question 36 in Appendix 1).

Particular attention should be paid to Madeira's southern coast, dominated by a barren state characterised by a high density of sea urchins where algal

cover, mainly marine forests, has declined in recent decades (Bernal-Ibáñez et al. 2021a). We hypothesise that the arrival of a species of algae with great invasive potential, such as *R. okamuræ*, in these highly resilient degraded systems may lead to a phase shift. This will allow us to understand if certain bioinvasions can act as a tipping point, reshaping the structure and functionality of barren systems where herbivory and tropicalization are the main drivers modulating rocky coastal ecosystems (Alves et al. 2001; Bernal-Ibáñez et al. 2021b). In addition, it will be necessary to determine the role that the invasion of *R. okamuræ* may have on marine forests. As other authors have observed (García-Gómez et al. 2018, 2021b), and as we have detected in Madeira, this species has the capacity to grow on many different natural substrates, including the thallus of other macroalgae. Therefore, this invasion may pose a further threat to Madeiran marine forests already in a critical condition in Madeira by the synergistic effects of herbivory and tropicalization, among other local impacts (Bernal-Ibáñez et al. 2021a, b).

Future efforts must monitor this established population on the north coast and try to avoid a possible expansion over the coasts of Madeira. Further studies exploring the physiological responses of *R. okamuræ* to the tropicalization process occurring in Macaronesia, and the biotic resistance to native herbivores through the invasion process (Santamaría et al. 2021) should also be considered. Taking into account the possible limits of a natural control of *R. okamuræ* by local herbivores at the initial stages of the invasion (Santamaría et al. 2021), we cannot exclude that the possible expansion of *R. okamuræ* in Madeira may lead to the monopolisation and homogenization of the coastal rocky bottoms producing several adverse ecological and socio-economic implications for society. Next, actions and policies for the control and early eradication of the species should be evaluated and adopted as soon as possible to mitigate or avoid undesirable consequences.

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Authors' contribution

Alejandro Bernal-Ibáñez: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, writing – original draft. Sahar Chebaane: investigation and data collection, data analysis and interpretation, writing – review and editing. Juan Sempere-Valverde: sample design and methodology, investigation and data collection, data analysis and interpretation, writing – review and editing. João Faria: sample design and methodology, data analysis and interpretation, writing – review and editing. Patrício Ramalhosa: writing – review and editing. Manfred Kaufmann: writing – review and editing. Marta Florido: investigation and data collection, writing – review and editing. Andrea Albert-Fonseca: investigation and data collection. João Canning-Clode: funding provision, writing – review and editing. Ignacio Gestoso: research conceptualization, writing – review and editing. Eva Cacabelos: research conceptualization, writing – review and editing.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Risk Assessment (RA) questionnaire – *Rugulopteryx okamurae*.

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