Article

Rapid Spread of the Invasive Brown Alga Rugulopteryx okamurae in a National Park in Provence (France, Mediterranean Sea)

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Abstract: The temperate Northwest Pacific brown alga Rugulopteryx okamurae (Dictyotales, Phaeophyceae) was first discovered in 2002 in the Mediterranean Sea in the Thau coastal lagoon (Occitania, France) and then again in 2015 along the southern side of the Strait of Gibraltar, where it was assigned with invasive status. We report here on the first occurrence of the species in the Northwest Mediterranean Sea in Calanques National Park (Marseille, France) in 2018. By 2020, a large population had developed, extending over 9.5 km of coastline, including highly protected no-take zones. The seafood trade, with R. okamurae used as packing material for sea urchin Paracentrotus lividus shipments from Thau Lagoon, could be the vector of its introduction into the Marseille area. As observed in the Strait of Gibraltar, R. okamurae is spreading rapidly along the Marseille coasts, suggesting an invasive pathway. The subtidal reefs are densely carpeted with R. okamurae, which overgrows most native algal species. Fragments of the alga are continuously detached by wave actions and currents, sedimenting on the seabed and potentially clogging fishing nets, and thus, impacting artisanal fishing or washing up on the beaches, where they rot and raise concern among local populations.

Keywords: invasive species; NIS; Mediterranean Sea; Rugulopteryx okamurae; global change

1. Introduction

Non-indigenous species (NIS) are a part of global change. The Mediterranean Sea is considered the worldwide hotspot of biological invasions; it harbours almost 1000 NIS, more than 600 of which are definitively established (introduced or invasive) [1–4]. They originate from different source regions, both warm waters (e.g., the Red Sea) and cold waters (e.g., the Northwest Pacific) [5,6]. The Mediterranean can also function as a hub, exporting NIS; for example, the seagrass Halophila stipulacea (Forsskål) Ascherson, native to the Red Sea, transited through the Mediterranean Sea before being exported towards the Caribbean [6–9]. This highlights the fact that each new locality where an NIS is found can constitute a possible source for future introductions, even in distant localities and between oceans.

Originating from the temperate Northwest Pacific Ocean, in particular Japan and Korea, Rugulopteryx okamurae (E.Y. Dawson) I.K. Hwang, W.J. Lee and H.S. Kim (Dictyotales, Ochrophyta, kingdom Stramenopile) was reported for the first time outside its native area in European waters in 2002, in the coastal lagoon of Thau (Occitania, France, Mediterranean, [10]). In view of the extent of the colonized area when it was discovered, totalling a dozen kilometres of shoreline, its introduction probably preceded 2002. This first
Introduction is probably linked to the oyster farming facilities of *Magallana gigas* (Thunberg, 1793) regularly importing spat from Japan. Since then, *R. okamurae* has established a large population within this lagoon, mixed with many other algae introduced from the Northwest Pacific [11]. In 2015, *R. okamurae* was, for the first time, recorded in the Mediterranean open sea in the southern (Ceuta, Spain, and Morocco) and northern (Spain) sides of the Strait of Gibraltar, where it quickly became invasive [12–15]. From 2015 to 2020, *R. okamurae* exhibited extensive northerly and southerly geographical expansion from these two initial locations, along both the Atlantic and the Mediterranean coasts, ranking among the most invasive NIS in the Mediterranean Sea [14,16].

In its native area, the alga dwells on sublittoral rocky substrata from 0.5 to 35.0 m deep [17], while it can occur down to 40.0 m deep in the northern Bay of Ceuta (southern sector of the Strait of Gibraltar) [14]. Here, we report on the first sighting of *R. okamurae* in the Northwest Mediterranean open sea, in the marine protected area (MPA) of Calanques National Park (hereafter—CNP, Provence, France), and its rapid spread, with findings on its distribution and life cycle and hypotheses regarding its origin.

2. Materials and Methods

The CNP was established in 2012. It includes both terrestrial and marine areas and stretches from La Ciotat (east) to Marseille (west) (Figure 1). The land area covers 85 km² (land core area) and 26 km² (surrounding area). The marine area covers 435 km² (marine core area, including 58 km² of no-take zones (NTZ) and a small reinforced protection zone) and 977 km² (adjacent marine area—AMA) [18,19]. It is important to emphasize that the regulations of the CNP are effectively implemented and are well respected by users.

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**Figure 1.** Calanque National Park (CNP), with boundaries and zoning (a) and monitored sites (b).
Field surveys on the benthic macroalgae and organisms as well as fish communities have been carried out by SCUBA diving within the framework of several research programmes aimed at monitoring the ecological status of the marine ecosystems of Calanques National Park. A compilation of all the macroalgae found within CNP is available [20].

Since the early 2000s, yearly routines on the benthic ecosystems and fish surveys have been conducted in several sites from the Frioul Islands (Site 13) to the Riou Island (Site 10) (Figure 1). Sites 11 through 13 are located within the AMA of CNP, 1 through 5 in the marine core area and 6 through 10 in the NTZs of CNP. In 2018 (first sighting of *Ruguloptyrx okamurae*), 2019 and 2020, the 13 sites were thoroughly monitored (Figures 1 and 2), enabling the mapping of occurrence of *R. okamurae* and the building of a photographic database. In addition to these 13 sites, a few occasional observations were made at additional sites. The monitoring consisted of visiting each site on a yearly basis between April and September (a period of widespread development of *R. okamurae*). At each site, an area of about 150 m long, and between 0 and 20 m deep, was covered by 3 scientific divers where *R. okamurae* was searched for over the rocky substratum. About twenty photographs were taken at each site to document the whole algal community’s coverage and composition with depth. Where *R. okamurae* was present, 5 photos were taken at 50 cm from the substrate to estimate the alga coverage using an image analysis tool. At Sites 2, 3 and 4, where *R. okamurae* was strongly established, wider explorations of the area were conducted to determine the limits of its range, and GPS positions were noted. All the results were implemented on a geographical information system (ArcGIS pro 2.7 ESRI®) to estimate the distance of coastline impacted by the alga.

In February 2019, at Site 3 (43° 12.680’ N, 05° 20.385’ E; rocky substrate, Figure 2), the first site where *Ruguloptyrx okamurae* was discovered, the surface covered by this alga was estimated (%) by the visual examination of twenty 1 m² quadrats, at 5 and 12 m deep. In September 2020, the wet and dry biomass of *R. okamurae* was assessed by collecting attached specimens within 5 quadrats of 400 cm² at 6 m deep, corresponding to the maximum development of *R. okamurae*. The wet biomass was measured by weighing the algae on an Ohaus Navigator XT scale to the nearest 0.1 g after placing them on absorbent paper for 10 min. Algae were then placed in a drying oven at 60 °C until a constant weight was reached, and the dry weight was then measured.

Morphological identification of *R. okamurae* was done according to the methods of [10,21]. It is worth emphasizing that the authors, who participated in the discovery of *R. okamurae* in the Thau Lagoon [10,11] and were very familiar with this species and all the macroalgae in the region, were easily alerted at the first sight of this species in Marseille.

### 3. Results

#### 3.1. Observation and Species Expansion

Analysis of the photographic database between the early 2000s and 2017 did not reveal the presence of *R. okamurae* at any site; the first observation of clumps of this species was dated July 2018 (Site 3, Figure 2). Dense populations (covering up to 85%) were reported, in February 2019 only, all around Maïre Island (Site 3) and along the adjacent mainland coast (Site 4), between 3 m and 15 m deep, on rocky reefs (Figures 3 and 4). The impacted coastline was then estimated to be 5978 m long. By the end of 2020, newly invaded sites were detected (Sites 1, 2, 5 and 6; Table 1) and the affected coastline reached 9569 m. Only sublittoral reefs have been colonized so far; *R. okamurae* was not observed either on sandy habitats or in the seagrass *Posidonia oceanica* (Linnaeus) Delile meadows.
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Figure 3. A rocky reef carpeted with \textit{Rugulopteryx okamurae} in February 2019. Red seaweeds are \textit{Sphaerococcus coronopifolius} Stackhouse, a native species. Photo © Sandrine Ruitton.

Figure 4. \textit{Rugulopteryx okamurae} in February 2019. Photo © Sandrine Ruitton.

Since 2018, the expansion of \textit{R. okamurae} has been rapid and overwhelming (Figure 2) and the species seems to have settled durably in the Marseille area. The NTZ (Site 6) does not constitute a barrier to its spread.

3.2. Life Cycle and Impact

Dense populations of \textit{R. okamurae} have been observed year-round since mid-2018, with maximums occurring during spring to late summer. In September 2020, off the south coast of the Maïre Island, the mean wet and dry biomass weights at 6 m deep were 1131.5 g.m$^{-2}$ (SD = 201.7) and 149.0 g.m$^{-2}$ (SD = 31.1), respectively. From spring to autumn, fragments break off from the substrate even without strong hydrodynamism and form large accumulations of drifting litter over the bottom (layers of up to 60 cm thick have been reported), while other fragments remain floating in the water column. Some of these drifting algae are swept away by currents and can pile up in protected areas, such as in the Callelongue Cove (between Sites 2 and 4), where they wash ashore (Figure 5). In winter, \textit{R. okamurae} is still far spread. At the most colonized site, it covered up to 85% (SD = 13) of the substrate at 5 m deep and 69% (SD = 21) at 12 m deep in February 2019 (Table 1).

In Marseille, sublittoral reef canopy-forming \textit{Cystoseira} (sensu lato) forests have been almost eradicated within the last century, as in many other Mediterranean areas, because of pollution, overfishing, and the resulting overgrazing [22,23]. The native reef communities are now bush-like stands dominated by \textit{Corallina ferreyrae} E.Y. Dawson, Acleto, and Foldvik (syn. \textit{C. caespitosa} R.H. Walker, J. Brodie, and L.M. Irvine) and \textit{Halopteris scoparia} (Linnaeus) Sauvageau. In areas invaded by \textit{R. okamurae}, the main native algal species that still remain are, in order of decreasing abundance, \textit{Corallina ferreyrae}, \textit{Sphaerococcus coronopifolius} Stackhouse (Figure 3), and \textit{Codium bursa} (Linnaeus) C. Agardh.
Table 1. *Rugulopteryx okamurae* presence and the main characteristics of distribution (% cover, depth) at the study sites. See Figure 1 for the locations of the sites with their number (No.).

| No. | Sites                                      | 2018                  | 2019                  | 2020                          |
|-----|--------------------------------------------|-----------------------|-----------------------|--------------------------------|
| 1   | Saména                                     | No sighting           | No sighting           | Spot sighting, from 3 to 4 m deep. Percentage cover < 1% from 1.4 to 3 m deep, reaching 20% at 3–6 m deep. |
| 2   | Cap Croisette                              | No sighting           | No sighting           | Continuous sighting all around the island. Percentage cover reaching 85% at 5 m deep and 69% at 12 m deep. Maximum sighted depth: 20 m. |
| 3   | Maïre Island                               | No in situ sighting but spot sighting in the south of the island (a posteriori photo analysis). | Continuous sighting all around the island. Percentage cover reaching 85% at 5 m deep and 69% at 12 m deep. Maximum sighted depth: 20 m. | Continuous sighting all around the island. Maximum sighted depth: 25 m. |
| 4   | Coast from Callelongue Cove to Marseilleveyre Cove | No sighting           | Continuous sighting, from 3 to 12 m deep. Percentage cover reaching 60% at this depth. | Continuous sighting. Percentage cover reaching 80% in some areas, around 6 m deep. |
| 5   | Jarre Island                               | No sighting           | No sighting           | Spot sighting only on the north side of the island, at 3 to 5 m deep. Percentage cover < 1%. |
| 6   | Plane Island                               | No sighting           | No sighting           | Spot sighting only on the northwest side of the island, at 3 to 5 m deep. Percentage cover around 2%. |
| 7   | Cortiou                                    | No sighting           | No sighting           | No sighting |
| 8   | Planier Island                             | No sighting           | No sighting           | No sighting |
| 9   | West of Riou Island                        | No sighting           | No sighting           | No sighting |
| 10  | East of Riou Island                        | No sighting           | No sighting           | No sighting |
| 11  | Cap Caveau, Frioul Island                  | No sighting           | No sighting           | No sighting |
| 12  | Gros Estéou, Frioul Island                 | No sighting           | No sighting           | No sighting |
| 13  | Tiboulen du Frioul, Frioul Island          | No sighting           | No sighting           | No sighting |

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All individuals of *R. okamurae* attached to the substrate were intact without epibionts or any evidence of grazing, which could be an indication of chemical defence. In autumn, during the senescence period, that is, when fragments of individuals break away from the substrate, a few epibionts were observed colonizing the alga.
4.3. Impact

In Ceuta (south sector of the Strait of Gibraltar), large biomasses of \textit{R. okamurae} have resulted in recurrent massive strandings on the beaches since 2017, localised downstream of the prevailing current [15]. More than 5000 metric tonnes of stranded \textit{R. okamurae} has been removed from the beaches, and fishermen have reported the clogging of fishing nets resulting in a decline in fish catches [15]. In Marseille, the same has been occurring, with artisanal fishermen complaining that drifting \textit{R. okamurae} clog their nets (September/October 2020), making them less efficient and heavier to lift back onboard the boat (Sandrine Ruitton, personal communication). Invaded areas are becoming unsuitable for fishing, down to 10 m deep. In semi-enclosed, very shallow and small coves, such as the Callelongue Cove, \textit{R. okamurae} accumulated over the shore in long wide mats, which rotted and produced H2S, bothering local inhabitants and sea-goers (Figure 5).

Figure 5. A heap of dry fragments of \textit{Rugulopteryx okamurae} cast ashore at the head of the Callelongue Cove (between Sites 2 and 4, see Figure 2), early June 2021. The girl (Emma Thibaut, 140 cm tall) gives the scale. Photo © Thierry Thibaut.

4. Discussion

4.1. Spread

Since its first observation in the Marseille area in July 2018, \textit{Rugulopteryx okamurae} has rapidly established an extensive population, from Cap Croisette to Marseilleveyre Cove and around Maïre Island, showing a steady increase in its spatial distribution. Despite occurring for 20 years or more in the Thau Lagoon, \textit{R. okamurae} has not displayed such invasive behaviour there as in the Strait of Gibraltar and in Marseille [10]. This might be due to its drastic regression during winter, which is probably linked to the low surface temperature of the lagoon during this period (down to 4–5 °C in January; [24]). Conversely, in Marseille, with the winter sea surface temperature usually above 13 °C, this alga persists throughout the winter, and therefore, rapidly spreading when conditions are favourable. A similarly rapid spread and invasive behaviour have also been reported in the Strait of Gibraltar [14].

The question of how \textit{Rugulopteryx okamurae} spreads remains unresolved. In Thau Lagoon, Marseille (France) and Spain, no gametophytes have ever been observed so far [10,13,16], as they were in its source area (Japan [25]). Asexual reproduction with vegetative propagation by forming propagules (proliferous branchlets arising on the thallus surface) that grow into new plants appeared to be the main mode of reproduction in sink areas [17]. The absence of sexual reproduction is a common feature of invasive macroalgae in the Mediterranean [26]. This has been observed, in particular, for the green algae \textit{Codium fragile} (Suringar) Hariot subsp. \textit{tomentosoides} (Van Goor) P.C. Silva [27] and \textit{Caulerpa...}
taxifolia (M. Vahl) C. Agardh [28,29] and the red alga Womersleyella setacea (Hollenberg) R.E. Norris [30–32].

In Marseille, the discovery of Rugulopteryx okamurae was made through routine and long-term monitoring of the marine environment. Field surveys and environmental monitoring by scientists allow for the early detection of NIS as well as the ability to carry out studies to identify the drivers of biological invasions and their consequences.

4.2. Origin

In the Thau Lagoon, the introduction of R. okamurae was linked to the import of spat of the Japanese oyster Magallana gigas from Japan and Korea for aquaculture [10,11]. In the Strait of Gibraltar (Spain), it is presumed that the vector of introduction could be maritime transportation by ballast waters from Asian vessels that transit through the Strait of Gibraltar [14,33]. According to [14], the large bloom in the south of the Iberian Peninsula is probably linked to a temperature peak in July 2015 (23.9 °C) and, more generally, with global climate change, while the species may have already been present but unnoticed in the region for several years due to possible confusion with Dictyota pinnatifida Kützing. Under these conditions, this invasion could be described as cryptic in its early stages.

In France, the probability of a natural spread from Thau to Marseille (150 km in a straight line), is very low, considering the dominant westwards Northwest Mediterranean current systems [34] and the absence of known intermediate populations. A few clumps of the alga were seen at Cap d’Agde (Occitania, France), in 2013 (T. Thibaut and M. Verlaque, personal communication), a few kilometres west of the Thau Lagoon, a location coherent with the dominant current. In Marseille, the introduction of R. okamurae by ships coming from the Pacific Ocean is possible (due to a potential release of ballast water upon arrival at the port of Fos, west of Marseille, which is strictly forbidden) but unlikely; no R. okamurae has been observed near or inside the harbour. Its introduction is more probably related to the importing of live shellfish or sea urchins (Paracentrotus lividus (Lamarck, 1816)) for human consumption since fresh specimens of R. okamurae, used as packing material for P. lividus from Thau Lagoon, were found at a local fish market in Marseille (M. Verlaque, personal communication, 10 January 2021). Sea urchins are considered a delicacy and their consumption is very popular in Provence. Our hypothesis is that sea urchins were carried to sea by recreational boaters as food supply, and that tests and packing material were then thrown overboard. Introductions related to seafood packing material have already been reported (see e.g., [35]).

Overall, the Thau, Gibraltar and Marseille populations could result from distinct introduction events. The Marseille population corresponds either to a distinct introduction event or to a dispersal via a secondary vector from Thau (e.g., seafood trade) illustrated by the hopscotch jump model. Once in a region (Thau, Strait of Gibraltar and Marseille), the spread of R. okamurae follows the diffusion dispersal pattern (wave of the advance model) (see [36] for dispersal models).

4.3. Impact

In Ceuta (south sector of the Strait of Gibraltar), large biomasses of R. okamurae have resulted in recurrent massive strandings on the beaches since 2017, localised downstream of the prevailing current [15]. More than 5000 metric tonnes of stranded R. okamurae has been removed from the beaches, and fishermen have reported the clogging of fishing nets resulting in a decline in fish catches [15]. In Marseille, the same has been occurring, with artisanal fishermen complaining that drifting R. okamurae clog their nets (September/October 2020), making them less efficient and heavier to lift back onboard the boat (Sandrine Ruitton, personal communication). Invaded areas are becoming unsuitable for fishing, down to 10 m deep. In semi-enclosed, very shallow and small coves, such as the Callelongue Cove, R. okamurae accumulated over the shore in long wide mats, which rotted and produced H₂S, bothering local inhabitants and sea-goers (Figure 5).
In the Strait of Gibraltar (Spain and Morocco), *R. okamurae* has become the dominant alga on the coastal rocky bottoms and replaced the native species *Dictyota dichotoma* (Hudson) J.V. Lamouroux [37], leading to a major change in the macrofaunal assemblages with a higher number of species and abundance of individuals and profound alteration of the species composition of autochthonous macroalgal communities. When the other invasive algae *Asparagopsis armata* Harvey, *A. taxiformis* (Delile) Trevisan and *Caulerpa cylindracea* Sonder were also present, *R. okamurae* appeared to be the most competitive and displaced the three former species [13]. No herbivory has been reported on this alga, which is strongly linked to the presence of anti-herbivory diterpenes [38].

*Rugulopteryx okamurae* caused a shift in the coralligenous habitat of Jbel Moussa (southern Strait of Gibraltar, west of Ceuta). In only one year, *R. okamurae* became the most abundant species and carpeted the native community, leading to an increase in the number of dead colonies of the red gorgonian *Paramuricea clavata* (Risso, 1826) and a significant decline in the cover of the red alga key species *Mesophyllum expansum* (Philippi) Cabioch and M.L. Mendoza (Corallinaceae) [16,39,40].

Both in the Strait of Gibraltar and in Marseille, *R. okamurae* does not respect the boundaries of marine protected areas, and its impact is as strong within as outside them. This has been demonstrated in many introduced species in the Mediterranean and worldwide [41–45], and once again contradicts the old theory of Charles Elton [46] regarding the resistance of pristine ecosystems to biological invasions (ecological resistance theory) [47].

### 5. Conclusions

Clearly, *R. okamurae* can induce significant changes in benthic assemblages and the functioning of marine ecosystems. Therefore, it is important to monitor the fate of this invasion (spread, stabilization, or natural decline; see [47]), which is part of the current dynamics of global change. *Rugulopteryx okamurae* can also cause discomfort (unpleasant smells) for the inhabitants and impact the activity of artisanal fishing. The boundaries of a national park (MPA) do not restrict its expansion. Finally, seafood trade should be subject to precautionary measures regarding their capacity as a vector in the dispersal of introduced species, which is not currently the case.

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