EU NON-NATIVE ORGANISM RISK ASSESSMENT SCHEME

Name of organism: ***Rugulopteryx okamurae*** (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim 2009

Author: Ministry for Ecological Transition and Demographic Challenge – Spain (MITECO)

Risk Assessment Area: The risk assessment area is the territory of the European Union (EU 27), excluding the outermost regions

Peer review 1: María Altamirano Jeschke, Departamento de Botánica y Fisiología Vegetal, Universidad de Málaga, Málaga, Spain
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Version	Date	File Name	Author(s)	Status and description
0.1	##/01/2020	*Rugulopteryx okamurae* RA	MITECO	First draft for internal review
1.0	##/02/2020	*Rugulopteryx okamurae* RA	MITECO	First draft for peer-review by the Scientific Forum
2.0	##/09/2020	*R. okamurae* RA 4 Sept. 2020	MITECO	Final RA following peer-review by the Scientific Forum: discussed at the meeting of the Scientific Forum of 17/11/2021
2.1	##/03/2021	RA R. okamurae March 2021 clean	MITECO	Updated version after comments from Scientific Forum on 17/11/2021 discussed at the meeting of the Scientific Forum of 13/04/2021: positive opinion
3.0	13/04/2021	Rugulopteryx_okamurae_final_20210413	MITECO	Final RA for submission to the IAS Committee

<table>
<tr><th colspan="2">EU CHAPEAU</th></tr>
<tr><td>QUESTION</td><td>RESPONSE</td></tr>
<tr><td>1. In how many EU member states has this species been recorded? List them.</td><td>It has been recorded in 2 member states: France (Verlaque et al. 2009) and Spain (Altamirano et al. 2016, García-Gómez et al. 2018).

Other relevant neighbouring countries where the species has been recorded:
Gibraltar (British Overseas Territory) (García-Gómez et al. 2018, 2020)
Morocco (El Aamri et al. 2018)</td></tr>
<tr><td>2. In how many EU member states has this species currently established populations? List them.</td><td>It has established populations in 2 member states: France (Verlaque et al. 2009), and Spain (Altamirano et al. 2016, Ocaña et al. 2016, García-Gómez et al. 2018, Navarro-Barranco et al. 2019)

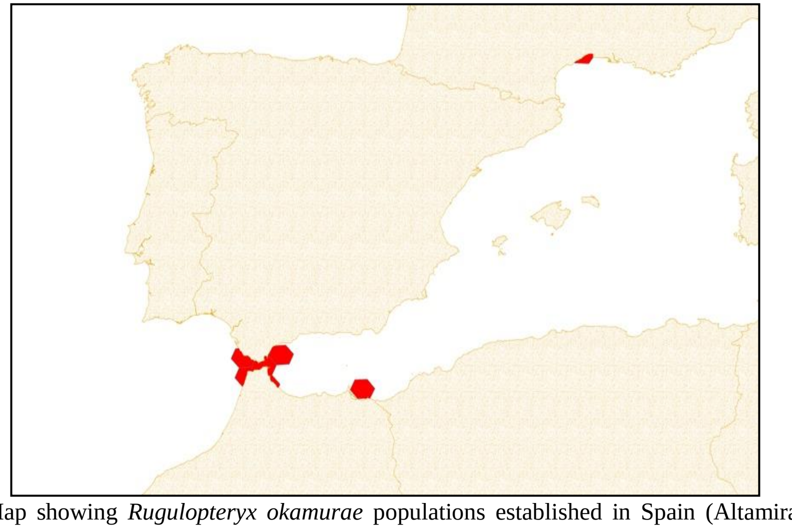
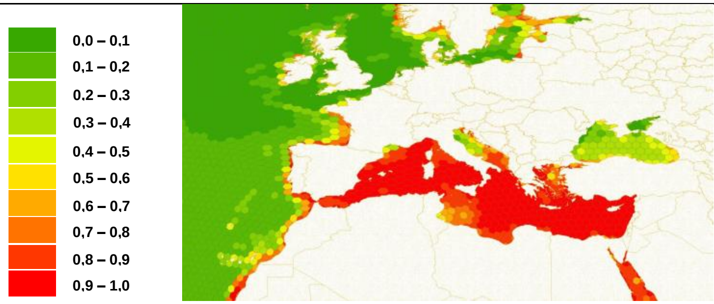


Figure.1: Map showing Rugulopteryx okamurae populations established in Spain (Altamirano et al. 2019a).

Other relevant neighbouring countries where the species has established:</td></tr>
</table>

	Gibraltar (British Overseas Territory) (García-Gómez *et al*., 2018, 2020), Morocco (El Aamri *et al*. 2018; García-Gómez *et al*. 2018, 2020).
3. In how many EU member states has this species **shown signs of invasiveness**? List them.	Within the risk assessment area, this species has only showed invasive behaviour in Spain (Altamirano *et al.* 2016, 2019a, b). Other relevant neighbouring countries where the species has shown signs of invasiveness: Gibraltar (British Overseas Territory) (García-Gómez *et al*. 2018, 2020) and Morocco (El Aamri *et al*. 2018, García-Gómez *et al*. 2018, 2020).
4. In which EU Biogeographic areas could this species establish?	*Rugulopteryx okamurae* could proliferate within the ecological environment of the Atlantic coast of Andalusia, the Mediterranean area and the Black Sea (Altamirano *et al.* 2016, 2019a, b; Muñoz *et al.* 2019). This species could establish in the following marine regions: North-east Atlantic Ocean (Bay of Biscay and the Iberian Coast); Mediterranean Sea (incl. Western and Central Mediterranean Sea, Aegean-Levantine Sea, Adriatic Sea, Ionian Sea); Black Sea area (the Sea of Marmara, Sea of Azov and Black Sea) (Muñoz *et al.* 2019).
5. In how many EU Member States could this species establish in the future [given current climate] (including those where it is already established)? List them.	In the future the organism could establish at least along the coasts of France, Spain, Portugal, Italy, Greece, Croatia, Slovenia, Cyprus and Malta where the temperature is suitable for its establishment as shown in south of Spain, where monthly mean temperature almost never fall below 15ºC all along the year and the maintained temperature peaks in summer may be a key factor for the blooming of the species (García-Gómez *et al*. 2020, Muñoz *et al*. 2019).

	 Figure 2: Environmental favourability model for *Rugulopteryx okamurae* in the Mediterranean and Atlantic coasts, based on the native and introduced distribution. The areas that present favourable conditions to accommodate the species are shown in warm colours (Altamirano *et al.* 2019a; Muñoz *et al.* 2019). The variables that were used to create this model are obtained from the platform Bio-ORACLE (Assis *et al*. 2017), and these variables were water temperature, salinity, nutrients, chlorophyll, speed of the ocean currents, phytoplankton, primary production, iron, light and surface light. These variables were chosen based on their predictive potential and are expected to characterize the environmental conditions that favor the presence of *R. okamurae* in its native range. Moreover, it is intended to identify other areas out of its native range that would favor the establishment of this species if it is introduced (Muñoz *et al*. 2019).
6. In how many EU member states could this species become invasive in the future [given current climate] (where it is not already established)?	It could become invasive in all countries mentioned previously: France, Portugal, Italy, Greece, Croatia, Slovenia, Cyprus, and Malta.

Distribution Summary: Member States and other relevant neighbouring countries

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Belgium				NA	
Bulgaria				NA	
Croatia			Yes	Yes	
Cyprus			Yes	Yes	
Denmark				NA	
Estonia				NA	
Finland				NA	
France	Yes	Yes	Yes	Yes	
Germany				NA	
Greece			Yes	Yes	
Ireland				NA	
Italy			Yes	Yes	
Latvia				NA	
Lithuania				NA	
Malta			Yes	Yes	
Netherlands				NA	
Poland				NA	
Portugal				NA	
Romania				NA	
Slovakia				NA	
Slovenia			Yes	Yes	
Spain	Yes	Yes	Yes	Yes	Yes
Sweden				NA	
United Kingdom				NA	
Gibraltar (British Overseas Territory)	Yes	Yes	Yes	Yes	Yes

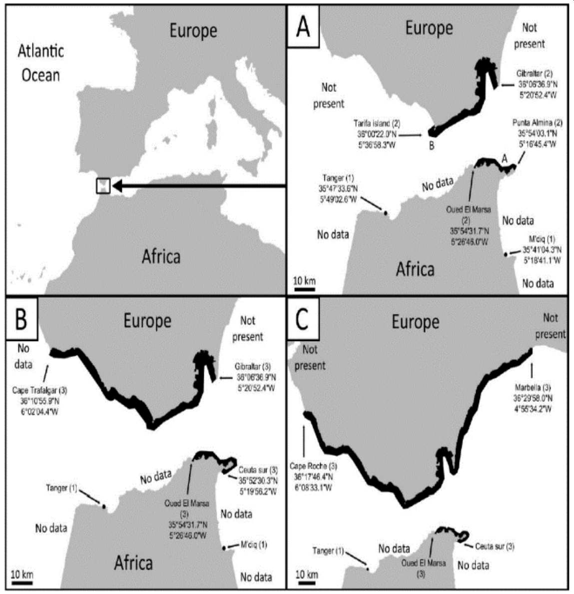
Distribution Summary: Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea				Yes	
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast			Yes	Yes	
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea			Yes	Yes	
Aegean-Levantine Sea			Yes	Yes	
Ionian Sea and the Central Mediterranean Sea			Yes	Yes	
Western Mediterranean Sea	Yes	Yes	Yes	Yes	Yes

SECTION A – Organism Information and Screening

Stage 1. Organism Information	**RESPONSE** **[chose one entry, delete all others]**	**COMMENT**
1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Yes, it is clearly a single taxonomic entity. It has been described in 2009 as *Rugulopteryx okamurae* (E.Y. Dawson) I.K. Hwang, W.J. Lee & H.S. Kim 2009. Taxonomic reappraisal of *Dilophus okamurae* (Dictyotales, Phaeophyta) from the western Pacific Ocean. *Phycologia* 48 (1): 1-12. It can be confused with other species of the genus *Dictyota*, such as *Dictyota pinnatifida*, *D. dichotoma, D. spiralis, D. cyanoloma or D. fasciola.*	Kingdom: Chromista; Phylum: Ochrophyta; Class: Phaeophyceae; Subclass: Dictyotophycidae; Order: Dictyotales; Family: Dictyotaceae, Tribe: Dictyoteae Synonyms: *Dictyota marginata*, *Dictyota okamurae*, *Dilophus marginatus* and *Dilophus okamurae.*
2. If not a single taxonomic entity, can it be redefined? (if necessary, use the response box to re-define the organism and carry on)	N/A	
3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment)	Yes. A rapid risk assessment for *Rugulopteryx okamurae* was produced by the University of Málaga (Spain) commissioned by the Ministry of Ecological Transition of Spain in October 2019 (Altamirano *et al*. 2019a).	The mentioned risk assessment for *Rugulopteryx okamurae* describes the species as an effective invasive species, because since its detection in Ceuta (Spain, Northern Africa) in 2016, its expansion along the Andalusian coast has been exponential. The analysis is based on an evaluation that answers ten questions designed to determine the level of risk and assign the species a category (high, medium or low risk) according to the scores obtained. Scores are justified and argued with a comment or reference to published scientific and technical evidence. The values between 0-12 points are

		considered low risk, values between is considered 13-19 is medium risk and high risk values greater than 19 with a maximum of 21 points. *R. okamurae* obtained a punctuation of 17.
4. If there is an earlier risk assessment is it still entirely valid, or only partly valid?	The previous mentioned risk assessment (Altamirano *et al*. 2019a) can be considered valid, although species distribution may have changed locally.	
5. Where is the organism native?	*Rugulopteryx okamurae* is a common native organism of the warm and temperate North-western Pacific Ocean, of Korea (Lee & Kang 1986), Japan (Yoshida *et al*. 1990, China (Tseng 1984), Taiwan (Huang 2000) and Philippines (Silva *et al*. 1987).	 Figure 3: Native distribution of *R. okamurae* (Altamirano *et al.* 2019a; Muñoz *et al.* 2019).
6. What is the global distribution of the organism (excluding the risk assessment area)?	North-western Pacific Ocean coasts (Korea, Japan, China, Taiwan and Philippines where is native. Although records are available in Mexico, as *Dilophus okamurai*, and in the Gulf of California (Dawson 1950, Norris 2010), in both works it is stated that identifications require additional information to be confirmed.	

7. What is the distribution of the organism in the risk assessment area?	*Rugulopteryx okamurae* is now established in the Thau Lagoon of France near Montpellier (Verlaque *et al*. 2009), and in the Atlantic and Mediterranean coasts of southern Spain: Ceuta, Cádiz (Altamirano *et al*. 2016), Málaga (CAGPYDS 2019), the Chafarinas Islands (Altamirano, pers. obs), and Gibraltar (Ocaña *et al*. 2016). Drifted material of the species has also been found in Huelva, Granada and Almería (Altamirano, pers. obsv.). Latest records of the species show an exponential expansion along the Andalusian coast: in 2017–2018 *R. okamurae* has advanced from Tarifa to Trafalgar-Caños de Meca (36°10055.900N, 6°02004.400W – southern Atlantic coast of Andalusia). By July 2019, it had advanced from Trafalgar to Cape Roche (36°17046.400N, 6°08033.100W– southern Atlantic coast of Andalusia) and from Gibraltar to Marbella (36°29058.000N, 4°55034.200W– southern Mediterranean coast of Andalusia) (Figure 4). The progress towards the Mediterranean coast has been very fast reaching the nearby coast of Granada and Almería (García-Gómez *et al*. 2020).	 **Figure 4.** Distribution of *R. okamurae* in the Strait of Gibraltar and nearby area Map A from El Aamri et al., (2018) and García-Gómez et al., (2018), bo describing results from summer 2017; Map B and C from García-Gómez et a (2018): B describing results from 2017-2018 and C from 2018-July 2019.
8. Is the organism known to be invasive (i.e. to threaten organisms, habitats or ecosystems) anywhere in the world?	Since its detection in 2015 in Ceuta, Spain (Altamirano *et al*. 2016), the species produces important ecological impacts, such as alterations of the marine habitat (including protected areas in the Natura 2000 Network) and loss of biodiversity. No invasive behaviour has been reported anywhere else.	Ecological impacts in Southern Spain: - Habitat modification due to rapid colonization and monopolization of marine ecosystems, and accumulations of detached biomass. - Loss of marine biodiversity and alteration of the structure of the communities, causing

		the physical displacement of the rest of the species due to the occupation of the substrate and preventing the fixation of larvae or propagules of other species. - Impact to habitats and species of community interest in spaces of the Natura 2000 Network. - Affected communities: Kelp forests, *Carpodesmia/Treptacantha spp* communities, *Posidonia oceanica* meadows, eulittoral and infralittoral communities of seaweeds, maeërl communities, epiphytic fauna of invertebrates. - Affected species: *Laminaria ochroleuca*, *Saccorhiza polyschides*, *Carpodesmia/Treptacantha* species (eg *C. usneoides*), *Lithophyllum byssoides*, *Gymnogongrus crenulatus*, *Sphaerechinus granularis*, *Leptogorgia sarmentosa*, *Eunicella* spp., *Paramuricea clavata*, *Charonia lampas*, *Astroides calycularis*, *Corallium rubrum*, *Patella ferruginea*. <u>Ecological impacts in Gibraltar and Morocco:</u> A preliminary distribution map of *R. okamurae* in the Strait of Gibraltar has been constructed by García-Gómez et al. (2018). Within the limits indicated in this map, it was the dominant seaweed in the macrophytobenthos. As mentioned by García-Gómez *et al*. (2018) the species presented a high invasive character where it was established in this area.

		Particularly concerning are its effects on the El Estrecho Natural Park, which is integrated into the Intercontinental Reserve of the Biosphere of Andalusia (Spain) and Morocco (El Aamri *et al*. 2018; García-Gómez *et al*. 2020).
9. Describe any known socio-economic benefits of the organism in the risk assessment area.	There are no socio-economic benefits of *R. okamurae* although the species could in the future become interesting for the industry because of secondary metabolites.	The taxonomic family to which *R. okamurae* belongs, presents a considerable diversity of secondary metabolites with possible commercial interest, with terpenes being one of the best represented groups, but there is no evidence of practical application (Yamase *et al*. 1999; De Paula *et al*. 2011, Suzuki *et al*. 2002). Terpenoids have been shown to have various biological activities that make them very interesting as a target for exploitation by the industry. On the one hand, its compounds have been shown to possess α- inhibitory activity glucosidase, enzyme that regulates processes of synthesis and quantity of glycoproteins and glycolipids. These last two are involved in diseases such as diabetes, certain forms of hyperlipoproteinemia and obesity. Therefore, it has been proposed that glucosidase inhibitors may be useful for the treatment of this type of diseases. Other biological activities of *R. okamurae* compounds are antifungal, antibiotic, anti-inflammatory, insecticide and antiviral (De Paula et al. 2011). High efficacy against cancers such as leukemia has been demonstrated (Harada and Kamei 1997). Finally, a very interesting use of terpenes is their antifouling ability, so they are good candidates to be included as a component in the

		paints used for coating the ships, taking into account that they are also less toxic than other synthetic compounds (Fusetani 2004).

SECTION B – Detailed assessment

PROBABILITY OF ENTRY

Important instructions:

- Entry is the introduction of an organism into the risk assessment area. Not to be confused with spread, the movement of an organism within the risk assessment area.
- For organisms which are already present in the risk assessment area, only complete the entry section for current active pathways of entry or if relevant potential future pathways. The entry section need not be completed for organisms which have entered in the past and have no current pathways of entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential entry of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	**moderate number**	**high**	The known pathways are unintentional. There is no evidence of intentional pathways. The species presents a considerable diversity of secondary metabolites with possible interest for the industry, but there is no evidence of commercial use (see also question 9 in section EU chapeau).
1.2. List relevant pathways through which the organism could enter. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 1.3 to 1.10 (copy and paste additional rows at the end of this section as necessary).	**-Maritime transportation:** **1.Ballast Waters BW** **2. Hull Fouling HF** **-Marine aquaculture**	**high**	As indicated by Katsanevakis *et al.* (2013), more than half of aliens in Europe were probably introduced via shipping. Maritime traffic, through ballast water or fouling communities (biofouling) of the ships, is the main vector of introduction of alien species in the marine environment (Ruiz *et al*. 2000).
Pathway name:	**Maritime transportation: Ballast waters**		

1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	**accidental**	**high**	Along with fouling (inlays in boat hulls), ballast waters represents a principal pathway of entry for algae (Ruíz *et al.* 1997; Ribera 2003; Katsanevakis *et al.* 2013). Both pathways could explain the introduction of *R. okamurae* in the Strait of Gibraltar from its native areas in the Pacific, taking into account the intense maritime traffic in this region.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	**very likely**	**high**	Ballast waters has also been responsible for the introduction of many exotic marine species (Ruiz *et al.* 1997; Gollasch *et al.* 2000), including macroalgae (Carlton & Geller 1993; Smith *et al.* 1999; Gollasch *et al.* 2002; David *et al.* 2007). Up to 15 different species were identified from ballast water samples from 12 vessels that made routes through the Mediterranean, with different origins, both from within the Mediterranean and from other waters (Flagella *et al.* 2007). The importance of this vector in the transport of macroalgae propagules is not well known yet, but it is known that conditions during transport in ballast waters can induce the formation of spores and propagules (Kolwalkar *et al.* 2007), and that microscopic stages of macroalgae can withstand long periods of darkness and subsequently develop when conditions improve (Leukart & Lüning 1994; Worm *et al.* 2001; Santelices *et al.* 2002; Carney & Edwards 2006).
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**very likely**	**very high**	Given the high capacity of *R. okamurae* to produce vegetative propagules and asexual monospores and the presence of two large commercial ports in the Strait of Gibraltar (Algeciras and Tangier Med in Morocco), it is suspected that ballast waters may be an important vector of introduction of the species, especially in this

			region. In fact, Rosas-Guerrero *et al.* (2018) observed that portions of adult thallus of the species had survival rates between 80-100% after being grown in dark conditions for three weeks, depending on the temperature during cultivation, and that these thalli even increased their biomass during this time. Moreover, after the dark period the thallus maintained the same survival rates when they passed to lighting conditions, which simulated the release phase after transport in ballast water.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	**moderately likely**	**medium**	Conditions during transport in ballast waters can induce the formation of spores and propagules (Kolwalkar *et al*. 2007), and microscopic stages of macroalgae can withstand long periods of darkness and subsequently develop when conditions improve (Leukart & Lüning 1994; Worm *et al*. 2001; Santelices *et al*. 2002; Carney & Edwards 2006). As *R. okamurae* produces vegetative propagules and asexual monospores and two large commercial ports are in the Strait of Gibraltar (Algeciras and Tangier Med in Morocco), it is suspected that ballast waters may be an important vector of introduction of the species, especially in this region. However, if adequate systems for ballast water control were implemented the probability of entry of the species would be greatly reduced. It is necessary to make effective the International Convention for the control and management of ballast water and sediments of ships, made in London on February 13, 2004, that entered into force in Spain on September 8, 2017 (BOE 282, of November 22, 2016). This is especially important in regions such as the Strait

			of Gibraltar, which withstand heavy commercial maritime traffic in the form of large merchants and tourist cruises, which maintain their maneuverability thanks to ballast waters.
1.7. How likely is the organism to enter the risk assessment area undetected?	**very likely**	**very high**	Through the ballast waters, spores and propagules can be introduced (Kolwalkar *et al.* 2007) undetected and microscopic stages can withstand long periods of darkness (Leukart & Lüning 1994; Worm *et al.* 2001; Santelices *et al.* 2002; Carney & Edwards 2006; Rosas-Guerrero *et al.* 2018). Furthermore, due the morphological similarity with native *Dictyota* species, *R. okamurae* may be producing cryptic invasion.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**very likely**	**high**	In a study about the reproductive phenology of *R. okamurae* on the coast of Cádiz carried out between January and August 2017, monospores and vegetative propagules were observed in the thallus that were collected all months of the study, although its abundance increased from May and show a maximum in August. In addition, during the months of April and August some thallus also presented tetrasporangia, but less abundant and frequent than the monosporangia (Pulido 2017; Pulido & Altamirano 2017). The bloom of this species in the Strait of Gibraltar could have been associated with the temperature peak in July 2015 and was possibly linked to global warming (García-Gómez et al., 2020). With no control on introduction vectors, new inoculums may be arriving along the whole year, increasing the probabilities of success of the species.

1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**very likely**	**very high**	The Spanish marine habitats are suitable for the establishment of *R. okamurae*, as evidenced by the presence of the species fixed to the substrate on the coasts of Malaga and Cádiz, and as revealed by the distribution models in other areas of the Spanish coast, mainly in its Mediterranean side. These models were developed for the previous risk analysis and have shown that there are other areas with potential to accommodate the species, in addition to its native area. These include the Mediterranean Sea as a whole, including the Spanish coasts and the Balearic archipelago, and the Atlantic coast of Andalusia, which have very high favourable values. Additionally, as indicated previously in this Risk Assessement, the following marine regions: North-east Atlantic Ocean, Mediterranean Sea and the Black Sea could present optimal conditions for the organism.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**very likely**	**high**	As indicated previously, this is probably the principal pathway of entry. Katsanevakis *et al.* (2013) describes that more than half of aliens in Europe were probably introduced via shipping. Maritime traffic, through ballast water or fouling communities (biofouling) of the ships, is the main vector of introduction of alien species in the marine environment (Ruiz *et al*. 2000). If no management measures are taken, it is very likely that propagules continue to arrive from the area of origin through ballast waters (Rosas-Guerrero *et al.* 2018).
End of pathway assessment, repeat as necessary.			
Pathway name:	**Maritime transportation: Hull fouling**		

1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	**accidental**	**high**	Along with ballast waters, fouling (inlays on boat hulls), represents a principal pathway of entry for algae (Ruíz *et al*. 1997; Ribera 2003; Katsanevakis *et al*. 2013). Both vectors could explain the introduction of *R. okamurae* in the Strait of Gibraltar from its native areas in the Pacific, taking into account the intense maritime traffic in this region.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	**very likely**	**high**	Most macroalgae species are capable of adhering to ship hulls (Schaffelke *et al*. 2006; Hewitt *et al*. 2007). Ribera (2003) mentions 39 species of exotic macroalgae that have been introduced in new regions in this way. Recruitment can occur through macroscopic thallus, such as juvenile sporophytes of the invading brown seaweed *Undaria pinnatifida* (Hay 1990); but mainly, it is microscopic stages of the life cycle that travel by fouling (Lewis *et al*. 2004), as was the case of gametophytes of *U. pinnatifida* (Wotton *et al*. 2004) and other species of brown algae such as *Phloiocaulon* or *Punctaria* (Coutts 1999). Monospores or propagules of *R. okamurae* may have arrived to introduced areas by hull fouling although up to now no scientific evidence support this hypothesis.
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**likely**	**medium**	Although there is no evidence that *R. okamurae* has reached the shores of the western Mediterranean adhered to ship hulls, it has been observed that the species is capable of adhering to surfaces of very diverse nature and composition, such as glass, iron, rubber or ceramic (García-Gómez *et al*. 2018). However, the changing conditions associated with this kind of transport, especially those related to the temperature and salinity, as well as the effect of friction caused by the crossing speed and the swell, and the presence of antifouling substances, make the transport by this vector less likely (Hewitt *et al*. 2007).

1.6. How likely is the organism to survive existing management practices during passage along the pathway?	**moderately likely**	**medium**	The presence of antifouling substances makes the transport by this vector less likely (Hewitt *et al*. 2007).
1.7. How likely is the organism to enter in the risk assessment area undetected?	**very likely**	**very high**	Due the morphological similarity with native *Dictyota* species, *R. okamurae* may be producing cryptic invasion.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**very likely**	**high**	In a study about the reproductive phenology of *R. okamurae* on the coast of Cádiz carried out between January and August 2017, monospores and vegetative propagules were observed in the thallus that were collected all months of the study, although its abundance increased from May and show a maximum in August. In addition, during the months of April and August some thallus also presented tetrasporangia, but less abundant and frequent than the monosporangia (Pulido 2017; Pulido & Altamirano 2017). Also, the bloom of this species in the Strait of Gibraltar has been associated with the temperature peak in July 2015 and possibly linked to global warming (García-Gómez *et al*. 2020). With no control on introduction vectors, new inoculums may be arriving along the whole year, increasing the probabilities of success of the organism.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**very likely**	**high**	The Spanish marine habitats are suitable for the establishment of *R. okamurae*, as evidenced by the presence of the species on the coasts of Malaga and Cádiz, and as revealed by the distribution models (see fig 2) in other areas of the Spanish coast, mainly in its Mediterranean side. These models were developed for the previous risk analysis and have shown that there are other areas with potential to accommodate the species.

			These include the Mediterranean Sea as a whole, which have very high favourable values. Additionally, as indicated previously in this Risk Assessement, the following marine regions North-east Atlantic Ocean, Mediterranean Sea and the Black Sea could present optimal conditions for the organism. Release of propagules from ship hulls to suitable habitat may occur in harbour areas in the introduced region, as well as along the coast.
1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**likely**	**medium**	As indicated by Katsanevakis *et al*. (2013), more than half of aliens in Europe were probably introduced via shipping. Maritime traffic, through ballast water or fouling communities (biofouling) of the ships, is the main vector of introduction of alien species in the marine environment (Ruiz *et al*. 2000).
End of pathway assessment, repeat as necessary.			
Pathway name:	**Marine aquaculture**		
1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	**accidental**	**high**	As in the case of some other non-native species, the introduction of *R. okamurae* in Thau Lagoon is associated with oyster imports (*Crassostrea gigas*) carried out later than 1977, probably in 1994, from Korea (Verlaque *et al*. 2009). It is an accidental entry.
1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	**moderately likely**	**low**	In the Alboran Sea, although it is not considered an important commercial activity, several companies are engaged in oyster farming (Robles 2010), so the presence of *R. okamurae* may be associated with this introduction vector. However, due to the high capacity of the species to produce vegetative propagules and asexual monospores, few individuals may carry big amounts of inoculum through this pathway.

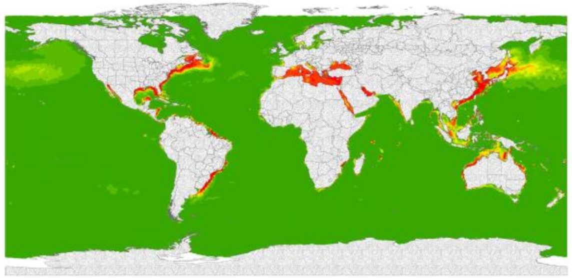
1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	**likely**	**medium**	It is likely that microscopic stages of *R. okamurae* can survive the passage through this pathway, especially propagules and monospores.
1.6. How likely is the organism to survive existing management practices during passage along the pathway?	**unlikely**	**medium**	It will depend on the management treatment. Taking into account the high propagation capacity of *R. okamurae*, mainly due to asexual and vegetative mechanisms, it is essential to avoid the dispersion of thallus by vectors linked to human activities, since it is not possible to stop dispersal by sea currents, especially in some spaces or habitats of community interest.
1.7. How likely is the organism to enter in the risk assessment area undetected?	**very likely**	**high**	*Rugulopteryx okamurae* can enter Europe via marine aquaculture unintentionally without being detected, as suggested by Verlaque (2009). Besides, due to morphological similarity with native *Dictyota* species, *R. okamurae* may be producing cryptic invasion.
1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	**very likely**	**high**	Aquaculture is an activity that takes place along the year. Due to suitable temperature most part of the year, as demonstrated with its establishment in south of Spain, the arrival could be successful at least in the Mediterranean. While there is no control on introduction vectors, new inoculums may be arriving along the whole year, increasing the probabilities of success.
1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	**likely**	**high**	The Spanish marine habitats are suitable for the establishment of *R. okamurae*, as evidenced by the presence of the species on the coasts of Malaga and Cádiz. These areas include the Mediterranean Sea biogeographical region, which has favourable factors for its establishment as shown by the model (Fig. 6).

1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	**very likely**	**high**	The presence of the species in France is associated to this pathway (Verlaque *et al.* 2009). Due to the commercial success of oyster farming, the Thau lagoon acts as a donor of oyster populations for its cultivation in many parts of the Mediterranean and Atlantic (Mineur *et al.* 2007; Verlaque *et al.* 2007). In the Alboran Sea, although it is not considered an important commercial activity, several companies are engaged in oyster farming (Robles 2010), so the presence of *R. okamurae* may be associated with this activity.
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways (comment on the key issues that lead to this conclusion).	**very likely**	**high**	The species is already established in Southern Spain coasts, spreading mainly to the Mediterranean Sea. New introductions may be still happening through maritime transport at the Strait of Gibraltar or at other ports in the Mediterranean. Ballast water may be the main vector for introduction of the species, as pieces of thalli can survive long periods of darkness (Rosas-Guerrero *et al.* 2018). Marine aquaculture on the other hand could be a potential entry pathway and the cause of unintentional or accidental dispersal of invasive macroalgae such as *R. okamurae* in the Mediterranean. In the Alboran Sea, although it is not considered an important commercial activity, several companies are engaged in oyster farming (Robles 2010), so the presence of *R. okamurae* may be associated with this activity.

PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already well established in the risk assessment area, only complete questions 1.15 and 1.21 then move onto the spread section. If uncertain, check with the Non-native Species Secretariat.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.12. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions in Europe and the organism's current distribution?	**very likely**	**very high**	Though the distribution of this species was originally concentrated in the northwestern end of the Pacific, the model detects climatically suitable areas for *R. okamurae* in other sites of the Earth, like the northern and western coast of Australia, Persian Gulf, Red Sea, most of the American coast and the Mediterranean Sea.  Figure 5: Environmental favourability model for *R. okamurae* on a global scale, showing (in red) the areas that present the most similar conditions to the native distribution area of this species (Altamirano *et al.* 2019a; Muñoz *et al.* 2019).
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions in the risk assessment area and the organism's current distribution?	**very likely**	**very high**	Based on the global model that considers the known distribution of this species (the native distribution plus the areas where it was introduced), the obtained results show the potential distribution of the species according to the environmental conditions to which *R.*

			okamurae is exposed in all its distribution range. Therefore, the map shows the ecologically suitable places for this species. Moreover, it identifies other areas out of its native range where the establishment of this species would be favoured if it is introduced as shown in fig. 2.
1.14. How likely is it that the organism will become established in protected conditions (in which the environment is artificially maintained, such as wildlife parks, glasshouses, aquaculture facilities, terraria, zoological gardens) in the risk assessment area? Subnote: gardens are not considered protected conditions	**very likely**	**medium**	There is no evidence for this species to become established in protected conditions but it is known to be easily cultivated under controlled laboratory conditions (Rosas-Guerrero *et al*. 2018).
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	**widespread**	**high**	On the basis of the environmental characteristics of the native areas of distribution, it can be concluded that the Andalusian Atlantic coast, the Strait of Gibraltar region and the entire Mediterranean coast are environmentally very similar and therefore these areas could host the species. The Black Sea and north Africa are also shown as suitable. Furthermore, it has to be considered that the host communities of *R. okamurae* in the new invaded areas (mainly *Carpodesmia/Treptacantha* species communities and photophilic ones), are well represented in the Mediterranean Sea.
1.16. If the organism requires another species for critical stages in its life cycle, then how likely is the organism to become associated with such species in the risk assessment area?	**NA**		

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<td>1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?</td>
<td>very likely</td>
<td>high</td>
<td>
In the Strait of Gibraltar, R. okamurae is responsible of community homogenization, just a few species remain in the ecosystem, mainly invasive macro-algae like Asparagopsis armata, Asparagopsis taxiformis and Caulerpa cylindracea. The host ecosystems in the early stages of invasion are Fucaidean algae bottoms with species of the genus Carpodesmia/Treptacantha or Sargassum, kelp forests and photophilic bottoms where coralline algae prevail. The invasive species competes successfully with all these species and homogenizes the community at the sea bottom.

It has been found that R. okamurae has driven a large change in a short time in community composition one year after the first recordings of the species in 2016, reaching exponential growth of up to 65% average cover, much to the detriment of the coverage of native macrophytes as Mesophyllum sp. and coral threatened species as Astroides calycularis, clearly reflecting the impact within partially shaded areas (García-Gómez et al., 2020) (Figure 6).

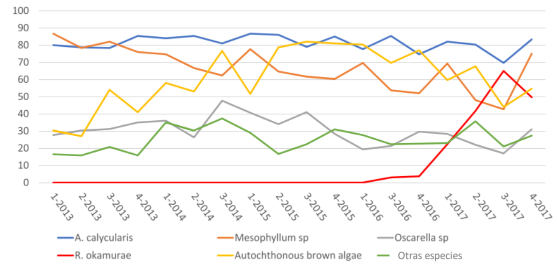

Figure 6. Temporal variation of benthic mean percent species coverage at Tarifa Island (Cádiz) monitoring station fixed quadrats from years 2013 to 2017 (1, 2, 3, 4 refer to random sampling times within each year). Species with less than 10% coverage (Alcyonium sp., Aplidium sp., Asparagopsis armata, Crambe sp, Ircinia sp. and Polycitor adriaticum) were grouped under “Other species” (García-Gómez et al., 2020).
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1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	**NA**		It is not known if there is any species that could prevent the establishment of *R. okanurae* if the conditions are suitable.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	**very likely**	**very high**	Effective control methods for *R. okamurae* that can be applied in the current stage of invasion are not known. The most important action and with more probability of success is, without any doubt, to prevent the introduction and spread of this species. The survival and reproduction capacities of the species once introduced into a new area, make difficult any management practice, although eradication may be effective at the early moments of the introduction (no evidence yet).
1.20. How likely are management practices in the risk assessment area to facilitate establishment?	**moderately likely**	**medium**	Considering that no management practice has been developed for this species yet, general practice should avoid the release and spread of propagules into the open sea. Management practices to avoid the introduction and dispersal of the species are not likely to facilitate its establishment. If the algae are collected, it is important to prevent its dispersion, having in mind the high propagation capacity of *R. okamurae*. Moreover, if it is located in the sea bottom and is taken above the photic zone, it can survive and colonize new shallow areas (this species can survive up to three weeks in the darkness).
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	**very likely**	**very high**	*Rugulopteryx okamurae* shows an extraordinary competition and colonization ability, with a disproportionate and unprecedented biomass increment that is not comparable to previous invasions in the Spanish coasts by any other macroalgae like

			Asparagopsis taxiformis (Altamirano *et al.* 2008; Zanolla *et al.* 2018a, b, c), *Lophocladia lallemandii* (Patzner 1998; Cabanelles *et al.* 2010; Deudero *et al.* 2010), *Caulerpa taxifolia* (Verlaque *et al.* 2015) or *Caulerpa cylindracea* that was considered the most severe invasive macroalgae in the Mediterranean in history, until this moment (Klein & Verlaque 2008). Part of the successful invasion performance of the species is due to its high propagation capacity through vegetative and asexual structures that can easily escape from management strategies such as eradications campaigns. Thalli can grow directly over the hard substrata but also on other seaweeds as epiphyte or even on animals such as sea urchins and sea cucumbers. The consequence could be eradication campaigns with low success rates when the population is too big and not followed by monitoring.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment?	**very likely**	**medium**	Reproduction takes place mainly by asexual and vegetative mechanisms. *R. okamurae* is a marine species that occurs from eulittoral basins to depths of more than 30 m. The invasive potential of a single thallus is substantial, taking into account that more than 100 spores and 25 propagules have been counted in 1 cm^2 of thallus (Altamirano *et al.* pers. obs.) and each one can generate a new clone individual, regardless of whether the thallus is fixed to the substrate or free in the water column. However, there is still a big lack of knowledge on the biological performance of the species in the introduction areas.
1.23. How likely is the capacity to spread of the organism to facilitate its establishment?	**likely**	**medium**	Taking into account the high propagation ability of *R. okamurae* that was observed in the Strait of Gibraltar, it is essential to avoid its dispersal by vectors linked to human activities. However, secondary introductions

			must indeed be happening, mainly due to fisheries activities (thalli entangled in the nests and released for cleaning in the sea again) and sea currents. Although floating material can sink to depths out of the photic zone, due to its survival capacity to darkness (Rosas-Guerrero *et al*. 2018), these thalli can get again to the photic area thanks to sea currents and establish in new distant places, with high proliferation capacity due to monospores and propagules. Up to now, however, there is no scientific evidence on the attachment capacity of these propagules to the new substrata, although it is suspected to be high.
1.24. How likely is the adaptability of the organism to facilitate its establishment?	**very likely**	**medium**	Ecological performance of the species at the new invaded areas at the Strait of Gibraltar (perennial status, wide bathymetric range from intertidal rock-pools to depth below -30m, and host communities, presence of allelopathic substances like terpenes) leads to suggest a high adaptability of the species, although ecological performance along the year is still unknown.
1.25. How likely is it that the organism could establish despite low genetic diversity in the founder population?	**very likely**	**very high**	Up to now the presence of the gametophyte of *R. okamurae* in the invaded area has not been confirmed, as only thalli with vegetative propagules, asexual monospores or in scarce cases, tetraspores, have been found (Altamirano *et al.* 2016, 2019a, b). This leads to the conclusion that invasive populations are formed and increased by clonal mechanisms with low or absent genetic variability, although sexual reproduction maybe occurring as well.
1.26. Based on the history of invasion by this organism elsewhere in the world, how likely is to establish in the	**very likely**	**very high**	The species has not exhibited invasive performance elsewhere in the world besides Southern Spain and Northern coast of Morocco, up to now.

risk assessment area? (If possible, specify the instances in the comments box.)			As supported also by modelling (Muñoz et al. 2019) the species could find favourable areas not only on the Spanish coasts, but also on other European coasts, mainly Mediterranean.
1.27. If the organism does not establish, then how likely is it that transient populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is established because of continual release, is an example of a transient species.	**likely**	**medium**	As long as maritime vectors are not under control, new inoculum of the species may continue to enter in the risk assessment area.
1.28. Estimate the overall likelihood of establishment (mention any key issues in the comment box).	**very likely**	**very high**	The species is already established and widespread within the risk assessment area in Southern Spain. Considering that within three years since its identification in 2016 the species has spread along the whole southern Spain coast and part of the northern coast of Morocco, and that new local populations are being reported in the last months expanding its distribution, it is expected that the species may confirm the distribution model proposed.

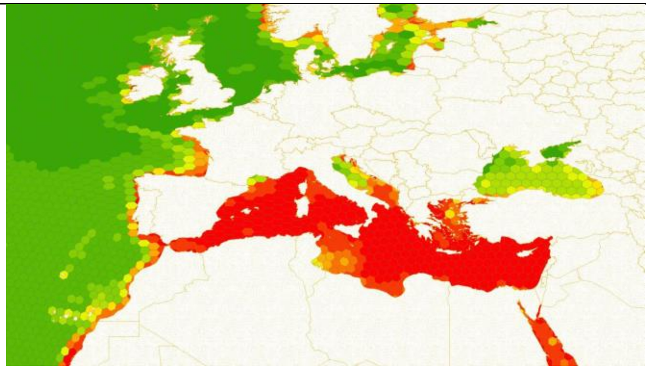
PROBABILITY OF SPREAD

Important notes:

- Spread is defined as the expansion of the geographical distribution of a pest within an area.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
2.1. How important is the expected spread of this organism in the risk assessment area by natural means? (Please list and comment on the mechanisms for natural spread.)	**massive**	**high**	Short to medium dispersal through marine currents is already happening, as it was observed in the movement of the organism in the Straits of Gibraltar. The progress towards the Mediterranean coast has been very fast and it can be expected that it will soon reach the nearby coast of Granada and Almería (García-Gómez *et al*. 2020). Drifting material is observed in the water column which can move tens of kilometres and cause the colonization of new areas mediated by sea currents or local vectors. Since the first draft of the RA (in February 2020) new easterly records of *R. okamurae* have been observed in Granada and Almería provinces, probably facilitated by sea currents.
2.2. How important is the expected spread of this organism in the risk assessment area by human assistance? (Please list and comment on the mechanisms for human-assisted spread.)	**major**	**high**	Ballast waters can be an important vector of introduction, but also of dispersion to nearby areas, especially in the Mediterranean Sea where big tourism cruises move between harbours as it would be the case with green algae forming massive proliferations (also known as “blooms”) *Ulva ohnoi* and *Ulva pertusa* (Zanolla *et al*. 2019). Probably more important for local spread of the species are vectors related with fisheries activities, responsible of secondary introductions. In addition, other potential vectors responsible for the dispersion of the species and secondary introductions must be added. Fishermen in the areas affected by the

			species, report that they suffer decreases in their catches and deterioration of their nets because thalli of the species are trapped in them in massive quantities. These nets in many cases are dragged and / or cleaned within the sea, which favours the dispersion of the species, especially taking into account the reproductive thalli with the presence of monospores and vegetative propagules. Similarly, other fishing gear may be favouring the dispersal of the species unintentionally. Other possible dispersion vectors of the species may be: - anchors or boat anchoring systems, both fishing and recreational; - diving equipment - marine litter; - trawlers and uprights that collect the biomass from the sea. Marine aquaculture could be a potential entry pathway and the cause of unintentional or accidental dispersal of invasive macroalgae such as *R. okamurae* in the Mediterranean and in general in the risk assessment area.
2.3. Within the risk assessment area, how difficult would it be to contain the organism?	**very difficult**	**high**	Effective control methods for *R. okamurae* that can be applied in the current state of the invasion are unknown. Only prevention of new introductions is suggested as a potential way to contain the species.

2.4. Based on the answers to questions on the potential for establishment and spread in the risk assessment area, define the area endangered by the organism.	Mediterranean Sea (incl. Western and Central Mediterranean Sea, Aegean-Levantine Sea, Adriatic Sea, Ionian Sea), as well as part of the North-east Atlantic Ocean and the Black Sea.	**high**	 Figure 7: Environmental favourability model for *Rugulopteryx okamurae* in the Mediterranean and western European coasts, based on the native and introduced distribution. The areas with favourable conditions to accommodate the species are shown in warm colours (Altamirano *et al.* 2019a; Muñoz *et al.* 2019).
2.5. What proportion (%) of the area/habitat suitable for establishment (i.e. those parts of the risk assessment area were the species could establish), if any, has already been colonised by the organism?	**0-10**	**medium**	In Alboran Sea where the species is established, the coverage ranged from 100% to 40% of the sea bottom depending on substrata and depth, occupying areas of hectares (Altamirano *et al*. 2019a). Considering the predicted distribution of the species in the Mediterranean, it is suspected that the species is at the beginning of its expansion phase. In the Strait of Gibraltar, *R. okamurae* covers most of the illuminated rocky bottoms, with a range from zero to 40 m in depth. However, the species highest coverages percentages (70-100%) are registered from 5 to 30 m deep, were associated with well-lit horizontal surfaces. More than 90% coverage is achieved between 10 and 20 m, where the exotic species displace the native biota. Moreover, besides its overwhelming growth at the subtidal levels, it was also observed in the shadowed areas of intertidal pools (García-Gómez *et al.*, 2018, 2020).

2.6. What proportion (%) of the area/habitat suitable for establishment, if any, do you expect to have been invaded by the organism five years from now (including any current presence)?	**33-67**	**low**	New records of *R. okamurae* are available in remote areas from where it is known that the species is currently established. The new records come from Adra, 150 km to the east, and Punta Umbría, 110 km to the west of the nearest places, from material collected by trawlers and uprights, respectively. Both localities are within the areas that the model recognizes of high favourability for the species, with values greater than 0.9, and it would be important to know if this presence of material deposited on the beaches affects subsequent establishment of the species on the coast.
2.7. What other timeframe (in years) would be appropriate to estimate any significant further spread of the organism in the risk assessment area? (Please comment on why this timeframe is chosen.)	**10**	**medium**	Considering that since its identification in 2016 and first record in Ceuta, the species has spread along Southern coast of Spain in Alboran Sea in three - four years, and taking into account its propagation capacity by propagules and monospores together with secondary vectors and natural sea currents, it is expected that *R. okamurae* will further spread along its predicted suitable areas on European coasts (Muñoz *et al*. 2019).
2.8. In this timeframe what proportion (%) of the endangered area/habitat (including any currently occupied areas/habitats) is likely to have been invaded by this organism?	**10-33**	**medium**	The models have not provided an answer to this specific question. However, 10-33% would be a reasonable estimate in view of the models and taking into account the knowledge about the biology of this species.
2.9. Estimate the overall potential for future spread for this organism in the risk assessment area (using the comment box to indicate any key issues).	**rapidly**	**high**	Marine aquatic ecosystems are difficult to manage to prevent the dispersion of invasive species. Marine currents along with human activities can cause a difficult to predict dispersion. However, taking into consideration that the species has spread along Southern coast of Spain in Alboran Sea in three years, and on the other hand its propagation capacity by propagules and monospores

			together with secondary vectors and natural sea currents, it is expected that *R. okamurae* will further spread along its predicted suitable areas on European coasts. Furthermore, both Atlantic and Mediterranean European coasts exhibit high favourability for the species in the present climatic scenario (Muñoz *et al.* 2019). García-Gómez *et al.* (2020) consider that coincidences between maximum Sea Surface Temperature (SST) and explosive peaks and colonizing capacity of the species registered in section 2.7 must be considered to estimate the overall potential for future spread of *R. okamurae.*

PROBABILITY OF IMPACT

Important instructions:

- When assessing potential future impacts, climate change should not be taken into account. This is done in later questions at the end of the assessment.
- Where one type of impact may affect another (e.g. disease may also cause economic impact) the assessor should try to separate the effects (e.g. in this case note the economic impact of disease in the response and comments of the disease question, but do not include them in the economic section).
- Note questions 2.10-2.14 relate to economic impact and 2.15-2.21 to environmental impact. Each set of questions starts with the impact elsewhere in the world, then considers impacts in Europe separating known impacts to date (i.e. past and current impacts) from potential future impacts. Key words are in bold for emphasis.

QUESTION	RESPONSE	CONFIDENCE	COMMENTS
2.10. How great is the economic loss caused by the organism within its existing geographic range, including the cost of any current management?	**major**	**very high**	The species produced no economic loss in its native geographic range, nor in Thau Lagoon in France. However, economic impact associated to fisheries activities and beach management in Southern Spain, has been estimated major in short time, rising to nearly one million and three hundred thousand euros in nine months (Altamirano *et al*. 2019a).
2.11. How great is the economic cost of the organism currently in the risk assessment area excluding management costs (include any past costs in your response)?	**major**	**very high**	*Rugulopteryx okamurae* produces significant detrimental economic impacts since its detection in Spanish waters in 2016, with significant direct and indirect economic losses. The costs are mainly related to the fishing sector as well as to the management of massive accumulation of biomass on the beaches. Losses in ecosystems services has not been estimated. As an indicator of the economic impact on the fishing sector, it has been estimated the economic losses in captures by fisheries associations from Huelva, Cádiz and Málaga provinces. The economic loss in captures in nine months was nearly nine hundred thousand euros,

			and this amount is underestimated due to lacks information provided by fisheries associations (Altamirano *et al*. 2019a). Thirteen different fish species captures have been affected by *R. okamurae* with decreases in captures ranging from 20 to 48%. Damage to fishery gear and equipment (mainly nets) should be considered as well (Altamirano *et al.* 2019a).
2.12. How great is the economic cost of the organism likely to be in the future in the risk assessment area excluding management costs?	**massive**	**high**	The expansion of the organism along the Mediterranean Sea, will entail significant economic losses because of its effects on numerous species, on which the fishing sector depends. Losses in the tourism sector as well as the loss in ecosystems services are also important. The total economic cost of impacts on fisheries and local administration for beaches managements rises to nearly one million and three hundred thousand euros in nine months. If these values are put in time and geographic context in the predicted expansion of the species in the Mediterranean Sea, a bigger economic impact can be expected, besides the economic losses in ecosystem services.
2.13. How great are the economic costs associated with managing this organism currently in the risk assessment area (include any past costs in your response)?	**major**	**high**	An economic impact in Southern Spain due to *R. okamurae* is derived from the removal of drifted material on the beaches. In a nine months period, a total of 11 thousand tons of drifted material have been removed from beaches in affected localities with expenses rising to nearly four hundred thousand euros (Altamirano *et al.* 2019a). Management of this biomass means a big problem for local administrations, and a big public impact.

			The beaches are covered by this seaweed until it can be removed, with major consequences for the tourism sector. Furthermore, cleaning of the fishing nets produces important economic impacts in terms of salaries and affected goods. Currently the regional administration in Andalusia is allocating economic and human resources in monitoring the species but none is done for preventing, containing or controlling the species. The total economic cost of impacts on fisheries and local administration for beaches management rises to nearly one million and three hundred thousand euros in nine months.
2.14. How great are the economic costs associated with managing this organism likely to be in the future in the risk assessment area?	**major**	**high**	As the established area of the species is currently big and predicted to increase, it can be suggested that costs in managing the species may increase exponentially together with its expansion.
2.15. How important is environmental harm caused by the organism within its existing geographic range excluding the risk assessment area?	**minimal**	**high**	In its native geographic range, the species behaves as an accompanying species of kelp forest for example, with no invasive performance (Sano *et al.* 2001).
2.16. How important is the impact of the organism on biodiversity (e.g. decline in native species, changes in native species communities, hybridisation) currently in the risk assessment area (include any past impact in your response)?	**major**	**high**	In Spain, *R. okamurae* has shown a very fast spread, colonizing most of the hard seabed substrates in those areas where the species is present. The expansion of *R. okamurae* is causing a significant environmental impact on native benthic communities, immediately reflected in a loss of biodiversity in the short term, in a change in the structure and composition of species communities in the long-term (Altamirano *et al.* 2017, 2019a; Ocaña *et al.*

			2016; García-Gómez *et al.* 2018; CAGPYDS 2018, 2019). In Morocco the situation is similar as described by El Aamri *et al.* (2018) and Sempere-Valverde *et al.* (2021). Present coverage of the species has not been estimated, but the value will be over hundreds of hectares including marine protected areas. *Rugulopteryx okamurae* exhibits an extraordinary competitive and colonization capacity with an , unprecedented increase in biomass that exceeds previous invasions in the Spanish coast by other macroalgae such as *Asparagopsis taxiformis*, *Lophocladia lallemandii Caulerpa taxifolia* or more recently *Caulerpa cylindracea*. In relation to the affected habitat, this species is affecting important habitats of high ecological value included in the Natura 2000 Network, such as the ZEC Natural Park of the Strait (ES0000337), the Natural Park Cabo de Gata, the ZEC Seabed of the Bay from Estepona (ES6170036), the ZEC El Saladillo-Punta de Baños (ES6170037), and the ZEC from Calahonda (ES6170030) on the Andalusian coast and the ZEC from Monte Hacho (ES0000197), unique species in Europe where marine biodiversity shows the transition zone between Atlantic and Mediterranean waters, and where several protected and endemic species such as *Posidonia oceanica, Astroides calycularis, Leptogorgia sarmentosa, Treptacantha ballesterosii, Cladocora caespitosa*, *Corallium rubrum* etc. inhabit.

			The observations carried out over the last three years (Altamirano et al. 2017, 2019; Ocaña et al. 2016; Morocco: El Aamri et al. 2018; García-Gómez et al. 2018; CAGPYDS 2018, 2019) show a colonization of most hard substrates, with a wide bathymetric range in which *R. okamurae* develops showing large variation of the coverage with the depth. Between the surface and 20m depth there is complete coverage of the hard substrates by *R. okamurae*, reaching out 90-100%. It produces a full domination of the substrata, being the only single species of macroalgae happening, with few spots of another invasive species, *Asparagopsis armata*. Towards deeper levels the coverage decreases but remains at high levels, around 30-40%. In depths around 50m, the presence of the species has also been observed, but less abundant. Due to the high proliferation of this species, the benthic ecosystem is being deeply transformed affecting all marine communities. In the area of the ZEC of the Strait of Gibraltar, impacts on kelp forests have been detected, significantly affecting the most representative species of these formations such as *Laminaria ochroleuca* and *Saccorhiza polyschides*. These kelp forests have disappeared from the zone due to *R. okamurae*. Forests of *Treptacantha usneoides* and other species of the genus have also been affected, included in the List of Threatened and Endangered Species of the Protocol on Specially Protected Areas and Biological Diversity in the Mediterranean (BOE April 23, 2014); these fucales forest have also disappeared in many places at the Strait of Gibraltar with the losses in important ecosystem services, like refuge and breed habitat for fishes and invertebrates, among others. Development of *R. okamurae* beds are so fast that the above mentioned

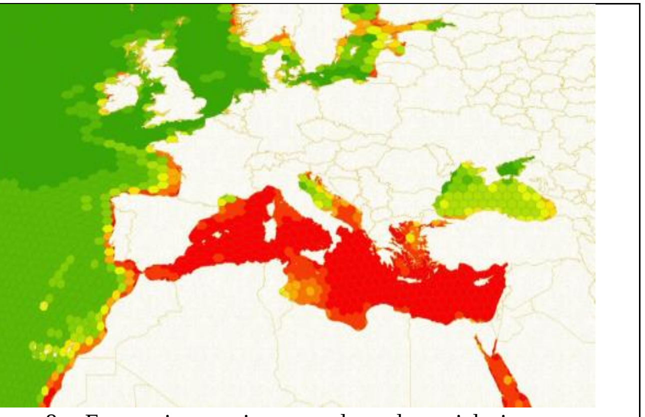
			native species cannot compete and new recruits seem not to be able to grow under the invader canopy. Only previous invasive species like *Asparagopsis armata* or *Caulerpa cylindracea* are able to coexist with few thalli with *R. okamurae*. Other species included in the Protocol mentioned above, such as *Lithophyllum byssoides* or *Gymnogongrus crenulatus*, are also being affected by the presence of *R. okamurae*. In Malaga coast, in the El Saladillo-Punta de Baños ZEC, *Posidonia oceanica* meadows, endemic species of the Mediterranean, and also included in the Paris Protocol have also been affected (CAGPYDS, 2019). *Rugulopteryx okamurae* widely compete with this plant. Regarding wildlife, numerous invertebrates, especially those of sessile life, are being affected by the development of *R. okamurae*, such as tubes of tubular polychaetes, soft surfaces of holothuria, shellfish crustaceans, sponges, *Sphaerechinus granularis* urchin (García-Gómez *et al.* 2018). The coralligene communities have also been affected by the development of *R. okamurae* (Ocaña *et al.* 2016, García-Gómez *et al.* 2018; CAGPYDS 2018). Various types of gorgonians such as *Leptogorgia sarmentosa*, *Eunicella* spp and *Paramuricea clavata* are being particularly affected, to which the algae adheres and covers them almost completely. Furthermore, endangered species such as the *Charonia lampas* horn, the star coral *Astroides calycularis*, the red coral *Corallium rubrum*, or even intertidal species such as the *Patella ferruginea* are also affected (García-Gómez *et al.*, 2018; El Aamri *et al.*, 2018). Invertebrate fauna associated to *R. okamurae* differs significantly from that of the native *Dictyota dichotoma*, with unpredictable consequences (Navarro-Barranco *et al*. 2019).

			Additionally, the accumulation and decomposition of the large biomass generated by this species both in the intertidal region and on the beaches as well as on the seabed could be causing indirect impacts on coastal ecosystems.
2.17. How important is the impact of the organism on biodiversity likely to be in the future in the risk assessment area?	**major**	**medium**	Given the major impact on ecosystems and marine species, which is occurring in the area where it has been detected in Spain, it is foreseeable that the future impact in the area of possible colonization, such as the Mediterranean and Black Sea, will be of the same level.
2.18. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services, caused by the organism currently in the risk assessment area (include any past impact in your response)?	**major**	**medium**	The significant deterioration of different marine communities in invaded areas reflects the important alteration of ecosystems' functions, so far not studied, given the recent invasion of the species *R. okamura*e. A large amount of material has been observed both deposited in the bottom, occupying platforms with little slope, or occupying clearings of sand, whose thickness has reached 45-50 cm in height. Important ecosystem services like refuge and breed zones for commercial fisheries like spider crabs and sole fish, have been affected, as economic impact data shows. Recreational and social services linked to touristic activities in a touristic hot spot in Europe have also been affected, like in worldwide well-known beaches of Marbella and Tarifa (Altamirano *et al.* 2019b; García-Gómez *et al*. 2020). The alteration of ecosystem services has caused social alarm and loss of income as reported in local press:

			https://www.diariodealmeria.es/agriculturadealmeria/tardanza-Gobierno-facilita-propagacion-invasora_0_1504049881.html (Diario de Almería 2020) https://www.preferente.com/noticias-de-turismo/sargazo-espanol-un-alga-asiatica-invade-las-playas-andaluzas-292213.html (preferente.com 2019).
2.19. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services, caused by the organism likely to be in the risk assessment area in the future?	**major**	**medium**	Taking into account the effects of *R. okamurae* on the biocenosis invaded so far, an important alteration of the ecological function of the ecosystems is foreseen, also considering its explosive colonization in most of the hard seabed substrates where the species is present until now in Spain. Considering this in the context of predicted favourable areas in the Mediterranean, an important alteration of ecosystems function in the future is expected.
2.20. How important is decline in conservation status (e.g. sites of nature conservation value, WFD classification) caused by the organism currently in the risk assessment area?	**major**	**medium**	The decline in conservation status caused by the organism currently in the risk assessment area is major because of the damage to species and habitats in several protected areas of the Natura 2000 Network. This however has not been fully evaluated yet. Altamirano *et al.* 2019a,b and García-Gómez *et al.* 2020 mentioned some examples of localities where this species affected sites of nature conservation value.This invasion is affecting habitats in the Natura 2000 Network, such as ZEC Natural Park of the Strait (ES0000337), ZEC Seabed of the Bay from Estepona (ES6170036), ZEC El Saladillo-Punta de Baños (ES6170037), and ZEC from Calahonda (ES6170030) on the Andalusian coast and ZECMonte Hacho (ES0000197) for the Spanish African coast.

2.21. How important is decline in conservation status (e.g. sites of nature conservation value, WFD classification) caused by the organism likely to be in the future in the risk assessment area?	**major**	**medium**	An important decline in the conservation status of protected areas and of special ecological value is expected where this seaweed settles. In the long term, a change in the structure and composition of species is expected, as observed with other macroalgae invasions in the Mediterranean. The sites of nature conservation value are generally more sensitive to biological invasions. As the models show, the organism is likely to establish and invade other habitats of great value in the risk assessment area. The species is already present in places where one year ago the model predicted it would be, and which are protected marine areas, such as Cabo de Gata (https://www.diariodealmeria.es/agriculturadealmeria/tardanza-Gobierno-facilita-propagacion-invasora_0_1504049881.html (Diario de Almería 2020)).
2.22. How important is it that genetic traits of the organism could be carried to other species, modifying their genetic nature and making their economic, environmental or social effects more serious?	**unknown**	**low**	No information on this has been found.
2.23. How important is social, human health or other harm (not directly included in economic and environmental categories) caused by the organism within its existing geographic range?	**minimal**	**medium**	There is no evidence on risks to human health, and / or animal or plant health due to parasites or pathogens of *R. okamurae*. However, a study of the impact of the substances released into the environment by decomposing biomass is recommended.

			The effect of the released substances that may be produced as a result of the decomposition of the biomass of *R. okamurae*, must be studied in both the water column, and the air in gaseous form, and that could produce some kind of sanitary impact to both the native flora and fauna and the people.
2.24. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	**unknown**	**low**	No information on this has been found.
2.25. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	**unknown**	**low**	No other effects are known. It may produce impact on marine infrastructures, like waste water pipes or energy plants pipes.
2.26. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?	**major**	**high**	Considering that there are no known predatory organisms, parasites or pathogens that may affect the species in the invaded area, the impact of *R. okamurae* are of major importance.
2.27. Indicate any parts of the risk assessment area where economic, environmental and social impacts are particularly likely to occur (provide as much detail as possible).	[insert text + attach map if possible]	**medium**	Given the invasive potential of the species and its environmental characteristics detected in the invaded area, it is expected that both the Mediterranean Sea, the Atlantic coasts and the Black Sea will be affected.

			 Figure 8: Economic, environmental and social impacts are particularly likely to occur in the areas that present favourable conditions to accommodate the species (shown in warm colors) (Altamirano *et al.* 2019a; Muñoz *et al.* 2019). Specially fishing and touristic areas may suffer important impacts, similar to that already happened in Southern Spain.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Entry	**very likely**	**high**	Regarding the distribution areas of *R. okamurae* in the European territory, since 2002 there is evidence of its presence in France, in the Thau lagoon, on the French Mediterranean coast near Montpellier (Verlaque et al. 2009), while in Spain the species was identified for the first time in Ceuta in 2015 (Altamirano *et al*. 2016). The entry pathways are priori unintentional, potentially associated with **ballast waters, hull fouling and marine**

			aquaculture, without ruling out others. It is unknown if there are intentional pathways of entry. The seaweed has shown survival rates between 80-100% after being grown in dark conditions for three weeks, depending on the temperature during cultivation. During this period thalli even increased their biomass (Rosas-Guerrero et al., 2018). This enhances the entry of organisms with ballast water, which is supposed to be the main pathway of entry. In addition to the entire coast of the Iberian Peninsula, including the Portuguese, Spanish and French Atlantic coasts and the Balearic archipelago, there are other areas with the potential to accommodate the species, highlighting the whole of southern Europe, which has very high probability to be colonized.
Summarise Establishment	**very likely**	**very high**	The species is already established in the risk assessment area. Species distribution models highlight the high and widespread favourability for the species in the Mediterranean Sea, but also in other areas of Europe, such as the Black Sea and the Atlantic coasts. Taking into account the high propagation capacity of *R. okamurae*, observed in the populations introduced in the Strait of Gibraltar, and the extraordinary competitive and colonization capacity with an increase in excessive biomass, it is very likely that the species will establish once entered in an area that presents favorable conditions.
Summarise Spread	**rapidly**	**high**	The potential pathways responsible for the spread of the species and secondary introductions are the following: - Marine currents

			- Fishing gear and equipment; - Recreational boats - Diving equipment - Funding systems - Marine litter Possibly the most important pathway at the moment in the spread of *R. okamurae* is linked to fishing activities. Gear accidentally collects fixed and / or suspended algae material, sometimes in large quantities. Taking into account the high propagation capacity of *R. okamurae*, mainly due to asexual and vegetative mechanisms, it is essential to avoid thallus´ spread by human activities, since it is not possible to stop spread by marine currents.
Summarise Impact	**major**	**high**	*Rugulopteryx okamurae* has shown a very rapid spread extending from the area of the Strait of Gibraltar to the Atlantic and Mediterranean coasts of Cadiz and Malaga, as well as to the Chafarinas Islands, Huelva and Almeria, in just over 3 years. The species exhibits an extraordinary competitive and colonization capacity with an excessive increase in biomass and subsequent impacts on marine species and ecosystems and their functions. The accumulation and decomposition of the excessive biomass generated by this species, both in the intertidal and beaches, as in the seabed, could be causing major impacts on marine ecosystems, as well as large economic losses. **Environmental impact:** *R. okamurae* shows large variation of the coverage with the depth. Between the surface and 20m depth there is a practically complete covering of the hard substrates by *R. okamurae* covering 90-100%. It produces a full

			domination of the substrata, being the only species of macroalgae, with few spots of another invasive species, *Asparagopsis armata*. More deeply, the level of covering decreases but remains at high levels. The benthic ecosystem is being deeply transformed affecting all marine communities with a very important loss of biodiversity as the first and most obvious consequence, and a change in the structure and composition of species in the long term (Altamirano *et al*. 2017, 2019a; Ocaña *et al*. 2016; El Aamri *et al*. 2018; García-Gómez *et al*. 2018; CAGPYDS 2018, 2019). Numerous invertebrates are affected by the development of *R. okamurae*: i.e. tubes of tubular polychaetes, soft surfaces of holothuria, crustaceans, sponges, *Sphaerechinus granularis* sea urchin (García-Gómez *et al*. 2018). The coralligene communities have also been affected by the development of *R. okamurae* (Ocaña *et al*. 2016, García-Gómez *et al*., 2018; CAGPYDS, 2018). Various types of gorgonians such as *Leptogorgia sarmentosa*, *Eunicella* spp and *Paramuricea clavata* are being particularly affected, in which the algae adheres and covers them almost completely. Furthermore, endangered species (*Charonia lampas* horn, the star coral *Astroides calycularis*, the red coral *Corallium rubrum*, or even intertidal species such as the *Patella ferruginea* are also affected (García-Gómez *et al*., 2018; El Aamri *et al*., 2018). *R. okamurae* affects very relevant spaces of great ecological value, included in the Natura 2000 Network, such as the ZEC Natural Park of the Strait (ES0000337), the Natural Park Cabo de Gata, the ZEC Seabed of the Bay from Estepona (ES6170036), the ZEC El Saladillo-

			Punta de Baños (ES6170037), and the ZEC from Calahonda (ES6170030) on the Andalusian coast and the ZEC from Monte Hacho (ES0000197) in the Spanish African coasts. **Economic impact:** Economic impact of the species is highly associated to losses in fisheries and beach management. Total amount estimated for nine months is higher than one million euros. Tourism sector also has experienced great losses the last summers. **Social impact:** Recreational and social services linked to touristic activities have also been affected. The large amounts of biomass accumulated on the beach create social alarm.
Conclusion of the risk assessment	**high**	**high**	*Rugulopteryx okamurae* exhibits an extraordinary competitive and colonization capacity with an excessive increase in biomass not even comparable with respect to previous invasions in the Spanish coast by other macroalgae such as: *Asparagopsis taxiformis* (Altamirano *et al*. 2008; Zanolla *et al*. 2018a, b, c), *Lophocladia lallemandii* (Patzner 1998; Cabanelles *et al*. 2010; Deudero *et al*. 2010), the so-called "killer algae" *Caulerpa taxifolia* (Verlaque *et al*. 2015), or the most recent *Caulerpa cylindracea* considered in its moment as the most serious in the history of invasive macroalgae species in the Mediterranean (Klein & Verlaque 2008). There are no previous control experiences that can serve as a reference.

			Prevention and control of pathways of entry and spread, especially those related to human activities, are necessary to avoid more impact in the affected area and in new areas where the species could be introduced and establish. Among the possible actions associated with the control of the species, the inclusion in the European list is considered very positive to help identify the pathways of introduction and dispersion of human activities (ballast water, marine crops, ship hulls, as well as fishing-related activities), prevent new introductions in order to prevent the spread of the species, especially in some space or habitat of community interest.
ADDITIONAL QUESTIONS - CLIMATE CHANGE			
3.1. What aspects of climate change, if any, are most likely to affect the risk assessment for this organism?	[Temperature rise]	**medium**	Changes in seawater temperature could affect its vegetative and reproductive performance. Probable the species could reproduce and spread faster during temperature peaks (García-Gómez *et al*. 2020) Coincidences between maximum Sea Surface Temperature (SST) and explosive peaks and colonizing capacity of the species (registered in section 2.7) should be considered to estimate the overall potential for future spread of *R. okamurae.*
3.2. What is the likely timeframe for such changes?	**10 years**	**medium**	The explosive growth and colonizing capacity of *R. okamurae* in a very short time coincided in the Strait of Gibraltar and nearby areas with the maximum Sea Surface Temperature (SST) of the period between 2000–2017, 23.9 °C in July 2015 (García-Gómez *et al*. 2020). In this regard, it is expected that global warming may facilitate a shift to dominance by *R. okamurae*, accelerating the scarcity of species in the global biota.

3.3. What aspects of the risk assessment are most likely to change as a result of climate change?	[EU CHAPPEAU]	**low**	Under foreseeable climate, the species could establish further to the north part of the risk assessment area but there is no model or evidence to affirm that.
ADDITIONAL QUESTIONS - RESEARCH			
4.1. If there is any research that would significantly strengthen confidence in the risk assessment, please summarise this here.	[molecular tools and genetic analysis]	**medium**	It would be necessary to identify other vectors and assess their importance, such as transport in ship hulls or marine cultures of various kinds, in order to act on them. It is also necessary to be able to know the sources of origin of the inoculums that are introduced in the Mediterranean, so that, together with the knowledge of the vectors, to be able to identify the main transport routes of *R. okamurae*, and to be able to develop management plans focused on an efficient prevention. In this sense, molecular tools and genetic analysis can be of great help, as already shown. It is also necessary to study the life cycle of the species in the new introduced areas, in order to understand its propagation systems and identify its weaknesses in biology. It is also interesting to study the ecological performance of the species in order to understand its competitive capacity with native communities. Assessing its genetic variability is important to predict its fate in the invaded area and resistance to changes in the environment. It becomes necessary to develop management strategies for the species and test eradication and content protocols.

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