

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹

Name of organism: *Asterias amurensis* Lutken, 1871

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outmost regions.

Peer review 1: Jack Sewell, The Marine Biological Association, UK

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¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: This risk assessment covers one species: *Asterias amurensis* Lutken, 1871

Subspecies/varieties: *Asterias amurensis f. amurensis* Lutken, 1871
Asterias amurensis f. robusta Djakonov, 1950

Both subspecies are covered in the Risk Assessment.

Phylum: Echinodermata, **Class:** Stelleroidea, **Order:** Forcipulatida, **Family:** Asteriidae

Synonyms: *Allasterias migrata* Sladen, 1879
Allasterias rathbuni var. nortonensis Verrill, 1909 †
Asteracanthion rubens var. migratum Doderlein, 1879
Asterias acervispinis Djakonov, 1950
Asterias amurensis f. acervispinis Djakonov, 1950
Asterias amurensis f. flabellifera Djakonov, 1950
Asterias amurensis f. gracilispinis Djakonov, 1950
Asterias amurensis f. latissima Djakonov, 1950
Asterias flabellifera Djakonov, 1950
Asterias gracilispinis Djakonov, 1950
Asterias latissima Djakonov, 1950
Asterias pectinata Brandt, 1835 (Synonym according to Fisher (1930))
Asterias rubens var. migratum Sladen, 1879
Parasterias albertensis Verrill, 1914 (Synonym according to Fisher (1930))

Common names: North Pacific sea star (EN); Japanese sea star (EN); Japanese starfish (EN); purple-orange sea star (EN); northern Pacific sea star (EN); flatbottom sea star (EN); Nordpazifischer Seestern (DE)

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Asterias amurensis is a starfish that can grow up to 50cm in diameter. It is yellow with red and purple pigmentation on its five arms, and a small central disk. The undersides are completely yellow and arms are unevenly covered with small, jagged-edged spines. It shows a wide range of colours on its dorsal side: orange to yellow, sometimes red and purple. These spines line the groove in which the tube feet lie, and join up at the mouth in a fan-like shape (MPSC, 2015).

Similar species: According to Verrill (1914) it most resembles the species *Asterias forbesi* (North-west Atlantic) and *A. rubens* from the north-east Atlantic, which is native to the RA area. It is distinguished by its lack of interradial plates and the evenly reticulated arrangement of the dorsal plates.

Fisher (1930) notes the following: “Differing from *A. rubens* in having, when adult, conspicuously broader rays, more numerous, usual shorter abactinal spinelets; 5 to 10 channeled or scoop-shaped superomarginal spines instead of two to five more or less terete, tapered, cylindrical, or clavate ones; in having a more nearly plane actinal surface (sharply defined by the rather thin edge of the ray, bearing a chevaux-de-frise of superomarginal spines), and by having a broader, flatter actinal interradial region; intermarginal spines frequently present.

In more simple terms, a number of ‘photo-ID features’ could be used in combination to differentiate between *A. rubens* and *A. amurensis*.

- *Asterias rubens* appears to consistently display a straight row of spines along the centre of the dorsal surface of each arm. In *A. amurensis* this central, upper row is almost always irregularly arranged, waved or ‘zig-zagging’.
- In orange specimens of *A. amurensis* arm tips often appear to have been ‘dipped in purple paint’. *Asterias rubens* do not appear to display this colour pattern.
- In purple specimens of *A. Amurensis* the tip region of the arms is more densely purple than rest of arm and usually with white, yellow or pale patches/ blotches around the spines towards the central disk. *Asterias rubens* is more uniformly coloured along the length of the arms and is lacking pale blotches around the spines.

Asterias amurensis is very variable. While large specimens all have the same general appearance and are easily recognized, there is considerable diversity in the number, robustness and form of the spines.

The breadth of the ray increases with age in such a way as to alter very materially the appearance of the animals. Quite immature specimens are therefore unlike the adults in general appearance.”

Besides the congeneric *A. rubens*, two other, common to the RA area, starfish species present some similarities with *A. amurensis* but should not be difficult to differentiate. These are:

- *Leptasterias mueleri*: this species has a ‘rosette of spines on the dorsal surface rather than single spines. The central row of dorsal spines is arranged in a straight line, while the arm tips are white/ pale in purple specimens. This species is sometimes confused with young *A. rubens* but the common features are different in *A. amurensis*.
- *Marthasterias glacialis* often has purple tips to arms but has conspicuous knobs and large thorn-like spines arranged in straight lines and should not be mistaken for *A. amurensis*. It is also a larger species (up to 80 cm across but usually smaller) and usually has a pale blue colouration, although it can also be grey, brown or white.

A number of starfish species exist in the aquarium trade (e.g. *Echinaster* (*Echinaster*) *sepositus* (Retzius, 1783), which is also native to the RA area) but they are distinctly different from *A. amurensis* and *Asterias* species in general.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: Extensive work has been carried out in Australia, where the species is considered invasive since the 1990s (see McEnnulty et al., 2001). It is widely viewed as one of the most serious invasive marine pests in Australia (Hayes et al., 2005) and considerable scientific effort has been devoted to the prevention of its spread and its management (e.g. Goggin, 1998; Joint SCC/SCFA, 1999; Hayes et al., 2004; Bax et al., 2006; Aquenal, 2008; MPSC, 2015).

Asterias amurensis is on New Zealand’s register of unwanted and notifiable marine organisms (<http://www.environmentguide.org.nz/issues/marine/marine-biosecurity/invasive-marine-species/>).

The species was assessed with the CMIST screening-level risk assessment tool for NIS for Pacific North America in relation to the Japanese Tsunami Marine Debris JTMD (Therriault et al., 2018), and was one of the highest scoring species. Higher scores for the species may be expected in Pacific N. America, due to the higher proximity to donor regions and an already confirmed introduction event with JTMD.

It is included in the MSFD UK priority surveillance species list (version 1 – Stebbing et al., 2015) and was assessed with the MI-ISK protocol by Townhill et al. (2017) for the UK, where it was one of the highest scoring species (5th highest score out of 20, driven by high likelihood of spread and impact). The last two studies are directly relevant to the RA area (particularly Atlantic Europe), in terms of climatic conditions but also risk of introduction and spread.

Included in the marine species Horizon Scanning exercise carried out under the auspices of JRC (Tsiamis et al., 2020), and scored medium (weighed score of 31), identified as posing a threat to the NE Atlantic.

Considered as a “door knocker species” in the Nordic region (covering Sweden, Norway, Denmark, Finland, Estonia, Lithuania, Latvia, Iceland and the Faroe Islands) but was not risk assessed by the NOBANIS Horizon Scanning exercise (NOBANIS, 2015).

Assessed as a potential invader of medium impact by the Irish Horizon Scanning exercise (O’Flynn et al., 2014).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

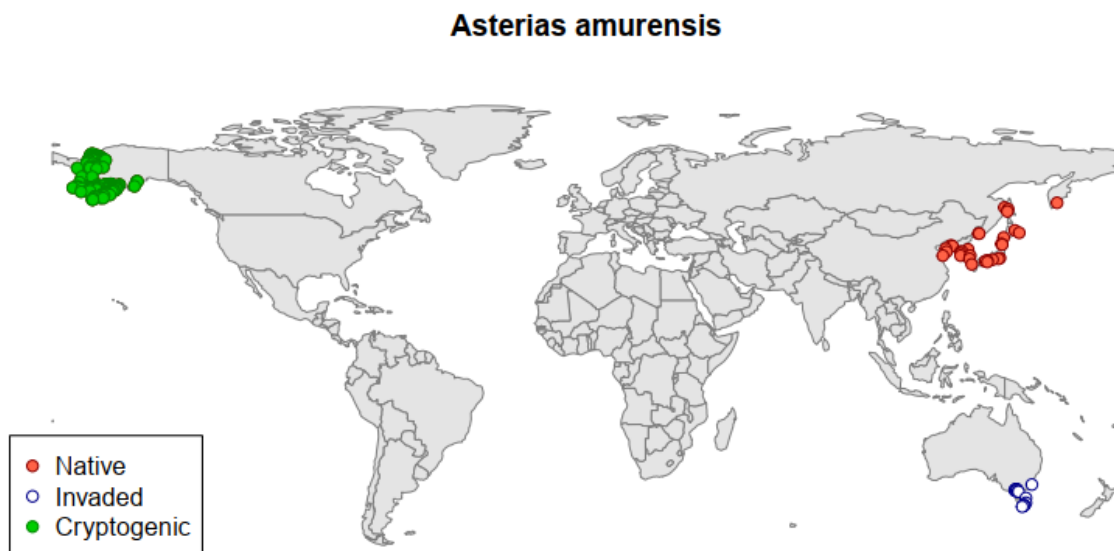


Figure 1: Occurrence records of *A. amurensis* in the native (red) and invaded (blue) range. Data points retrieved from the literature, biodiversity databases and screened as explained in A4-A6 and the modelling Annex (Annex IX). Map created by Bjorn Beckmann.

Response: *Asterias amurensis* is one of the most common northern Pacific starfish species, native to the cold-temperate, coastal waters of Korea, Russia, Japan (Lee et al., 2004) and Northern China (Li et al., 2018). It is common in the Eastern Bering Sea from the eastern Chukchi Sea to the Gulf of Alaska and British Columbia (Smith & Armistead, 2014 and references therein); in Alaska it is considered cryptogenic (McLoughlin & Bax, 1993, cited in Talman et al., 1999; McClory & Gotthardt, 2008; MPSC, 2015). *Asterias amurensis* inhabits a variety of coastal habitats, from muddy, sandy, to coarser and more consolidated bottoms (Smith & Armistead, 2014), to kelp beds (Won et al., 2013) and rocky sheltered areas of intertidal zones (Aquenal, 2008). In its native range it is typically found at depths down to approximately 100m (Gabaev, 2018) but more commonly below 40-50m (Hatanaka & Kosaka, 1959; D'yakonov, 1968).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Outside its native distribution, the species has been introduced to Tasmania and Victoria, in temperate Australia. In Tasmania it is believed to have been introduced as early as 1982 but was only correctly identified in 1992 (see Dunstan & Bax, 2007). The population in the Derwent estuary (Tasmania) was estimated in 1994 to be more than 30 million individuals (Goggin 1998). Today the species can be found all along the south and east coast of Tasmania (MPSC, 2015). In Port Phillip Bay, Victoria, it first appeared in 1995 with a few adult individuals, while spawning was confirmed in 1997 (Parry & Cohen, 2001). Within 3 years of confirmed establishment the population had reached ≈ 165 million individuals (Parry et al., 2004).

In Australia, it is predominantly found on shallow (<25m) soft sediment habitats and reefs, mostly limited to protected embayments and estuaries (Richardson et al., 2016; NIMPIS, 2020), as well as artificial structures, such as wharves and piers (Aguenal, 2008). It is not clear whether the narrower range of colonized habitats in the invaded range vs the native range (see previous question) is due to different habitat availability/distribution, time since colonization or other selection factors.

In Alaskan waters, where the species is considered cryptogenic, it occurs at depths down to 200m (Smith & Armistead, 2014 and references therein).

Note: Global Biodiversity Information Facility (<http://www.gbif.org>, occurrence data: https://www.gbif.org/occurrence/search?taxon_key=5187508) contains a number of records based on non-validated citizen science observations from South-East Asia (Indonesia, Thailand, Philippines), southern USA (Gulf of Mexico, California) as well as various European locations (see question A6 for details). These are considered questionable (see also Byrne et al., 2016), are not included in the peer reviewed literature on the non-native range of the species and were ignored for the purposes of this Risk Assessment.

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

² Convention on Biological Diversity, Decision VI/23

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): None

Response (6b): None

Note: as above (A5), GBIF and OBIS records based on non-validated citizen science observations (besides, no photograph was supplied) were not taken into account in the answer to A6. For the RA area, these refer to records from Greece, Portugal, Spain, France, the UK, the Netherlands, corresponding to the marine regions North-East Atlantic and Mediterranean Sea. The presence of the species is not confirmed in the peer-reviewed literature (e.g. Tsiamis et al., 2020), in the European Alien Species Information Network (EASIN) or in national biodiversity databases, e.g. the NBN Atlas for the UK (<https://nbnatlas.org/>), ELNAIS for Greece (<https://elnais.hcmr.gr/>), Netherlands Soortenregister for the Netherlands (<https://www.nederlandsesoorten.nl/>), thus it is considered extremely unlikely that these observations belong to *A. amurensis*.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu 2.1, Qu 2.9 and Annex VIII & IX for details). For purposes of mapping and assessing the risk of establishment, the following tolerance limits were defined:

- Average surface temperature of the warmest month < 20°C
- Average surface temperature of the coldest month > 5°C
- Minimum surface salinity > 20psu

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: moderately likely, low confidence

Black Sea: unlikely, high confidence (BUT moderately likely with medium confidence ONLY for the Sea of Marmara, which is outside the RA Area)

In the Black Sea and the Baltic Sea, establishment of *A. amurensis* will be prevented by low salinities (Black Sea range of 14-19 psu for surface salinity – Baltic Sea beyond the Kattegat <15 psu). The species requires salinities above 22 psu for spawning, fertilization, and embryonic development (i.e. salinity near the seabed) and above 20 psu for complete larval development, i.e. surface salinity (Kashenko, 2005). The only exception here is the Sea of Marmara (part of the Black Sea), which exhibits surface salinities > 20 psu and fully marine bottom salinities (Beşiktepe et al., 1994) and can potentially support establishment of *A. amurensis*. Establishment in the Mediterranean Sea is expected to be limited by high temperatures to the northern, cooler basins.

Response (7b): The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu 2.1, Qu 2.9 and Annex VIII & IX for details). Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the developed models is described in Annex IX and considers scenarios RCP 2.6 and RCP 4.5 by 2070. See also Qu. 2.10.

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, medium confidence

Mediterranean Sea: unlikely, low confidence

Black Sea: unlikely, high confidence (Sea of Marmara: moderately likely, medium confidence)

The species distribution model predicted a very small reduction in projected suitability for *A. amurensis* for all EU marine subregions under future climate change and a small northward shift of the overall suitable area for the species. Suitable conditions in the Mediterranean are likely to become even more restricted spatially and temporally (Annex VIII – Figure 2), rendering establishment in this marine subregion unlikely. The possibility of physiological adaptations and/or introductions from southern Japan, where the sea surface temperature of the warmest month can exceed 27 C, drives the low confidence of this assessment. In the Bay of Biscay and the Iberian coast suitable conditions for establishment are expected to last for shorter time periods in the future, hence the lower likelihood score. In the Greater North Sea, an increase in sea temperature will offer suitable conditions for spawning and larval development for prolonged periods in the south and east of the region, extending into the winter months, which under current conditions are too cold for the survival of larvae (see Annex VIII – Figure 1).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): None

Response (8b): None

See also comment in Qu. A6

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)

- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Belgium, Croatia, Denmark, France, Germany, Greece, Ireland, Italy, Netherlands, Portugal, Slovenia, Spain, Sweden; and the United Kingdom

Response (9b): Belgium, Denmark, France, Germany, Ireland, Netherlands, Portugal, Spain, Sweden; and the United Kingdom

The response to 9b is based on the RCP4.5 scenario for the period 2050/2070. The aspect of climate change most likely to affect the organism's ability to establish is an increase in winter sea surface temperatures. Higher winter temperatures in the Mediterranean Sea will exceed the species' upper limit for larval survival/development, particularly throughout the southern basin, while the same change will favour larvae for longer periods in the eastern North Sea. (see Annexes VIII & IX for details on modelling and future climate conditions in the RA area).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: for details and references see Qu. 4.1.

In Australia, *Asterias amurensis* reached very high densities (up to 24 individuals/m²) and developed massive populations within a relatively short time of its establishment (Byrne et al., 1997b; Grannum et al., 1996) in the two main invaded areas, where it has become the dominant benthic invertebrate predator. By the late 1990s, the adult population in Derwent was estimated at 30 million sea stars, and in Port Phillip Bay at 165 million individuals by 2003 (Parry et al., 2004).

With its voracious appetite and preference for bivalve prey, it has caused drastic population declines of (commercially important) native bivalve species and severely reduced the recruitment of juvenile bivalves to the benthos (Ross et al., 2002; 2004). It has been implicated in population declines of fish species, with which it competes for food (Parry & Hirst, 2016) and was considered responsible for the precipitous decline of the endemic fish species *Brachionichthys hirsutus* (Barrett et al., 1996, Ross et al., 1999), which was subsequently considered critically endangered. The mechanism was hypothesized to involve predation by *A. amurensis* on stalked ascidians, which form the primary spawning substrate of *B. hirsutus*, and subsequent reduction of key habitat availability (Bruce & Green, 1998; Green et al., 2012), although direct evidence for this is lacking.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: None. *Asterias amurensis* is not present in the risk assessment area.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: None. *Asterias amurensis* is not present in the risk assessment area.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response:

Species of the genus *Asterias* have been used in the past in their native range (e.g. Japan, Canada and the USA), albeit to a limited extent, for the production of fish meal, compost or fertiliser (McEnnulty et al., 2001; Barkhouse et al., 2007 and references therein). For example, in Japan, where the large

amounts of *A. amurensis* by-catch from fisheries and aquaculture activities constitute a waste disposal problem, the species has been used to produce plant growth promoting compost (Line, 1994; Ishii et al., 2007). In Denmark, a starfish meal processing plant was inaugurated in 2019, utilising starfish *A. rubens* caught in an important mussel cultivation area, intended for animal feed. Similar uses can be envisaged for *A. amurensis*, in case the species is introduced to the RA area.

Preliminary research work has been conducted with *A. amurensis* in China, in order to obtain saponin (Hwang et al., 2011) and collagen (Hao and Li, 1999) from viscera or body wall for biotechnological purposes. In another area, the gametes of *A. amurensis* have been used in the assessment of marine environmental quality in Japan (Yu, 1998; Yu et al., 1998; Lee and Choi, 2003).

It is listed as a medicinal species in Asia (Goya & Youngsung, 2017), primarily in relation to its use in homeopathy.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name:

a) TRANSPORT-STOWAWAY (ship/boat ballast water)

Asterias amurensis is believed to have been introduced to Tasmanian waters from central Japan via ballast water discharge (Ward & Andrew, 1995). Based on a thorough evaluation of alternative pathways (Hayes et al., 2004), ballast water transfer is still considered the most plausible pathway of introduction of this species to Tasmania, as well as its subsequent spread to Victoria, mainland Australia (Dunstan & Bax, 2008).

b) TRANSPORT-STOWAWAY Hitchhikers on ship/boat (excluding ship/boat hull fouling)

This pathway covers specifically cases where species are transported with boat/ship in locations other than ballast water and hull fouling (namely where water is held or collected within the hull, such as sea chests, bilge water and within the hull itself – IUCN, 2017). While it is unlikely that starfish would remain attached to vessels when underway for any length of time, their translocation is possible in sheltered niche areas (Dommissie & Hough, 2004; MAF, 2011). For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissie & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from internal surfaces of fishing/recreational vessels offering further support that the species can be translocated via this pathway (Hayes et al., 2004).

c) TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector)

Asterias amurensis is a notorious pest in shellfish aquaculture, both in the native and the invaded range, and is known to settle on and infest oyster trays, mussel lines and scallop spat collectors, as well as salmon cages, on-shore and offshore abalone facilities and other fish-farming equipment (e.g. Gabaev, 2018; Dommissie & Hough, 2004). Even though introduction via this pathway is not documented for the species anywhere in the invaded range, it is considered possible that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, into the RA area.

d) TRANSPORT-STOWAWAY (ship/boat hull fouling)

Asterias amurensis DNA was found in biofouling samples collected from the external surfaces of fishing and recreational vessel hulls examined in Australia, in the absence of *A. amurensis* larvae in the surrounding water, offering support that the species can be translocated via vessel fouling (Hayes et al., 2004). However, it is unlikely that starfish would remain attached to vessels when underway for any length of time (Dommissie & Hough, 2004; MAF, 2011), such that this pathway is not considered a risk for primary introductions from existing native and invasive populations and is instead discussed in conjunction with the pathway “Hitchhikers on ship/boat” only in the Risk of Spread section.

a) TRANSPORT-STOWAWAY (ship/boat ballast water)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This will depend on the larval densities in the donor location, the time of the year (i.e. if uptake of ballast water coincides with the peak spawning period), as well as the densities of the adult population, which will affect the reproductive output. The highest larval densities ever recorded in the Derwent estuary, Tasmania were 1000/m³ (Sutton & Greene, 2002), other studies however reported much lower densities during spawning season, in the range of 50/m³ in areas with smaller adult populations (Dommissse & Hough, 2004). By the late 1990s, the adult population in Derwent was estimated at 30 million sea stars, and in Port Phillip Bay at 165 million individuals by 2003 (Parry et al., 2004). During outbreak events in China, adult *A. amurensis* densities can reach 300ind./m² (Li et

al., 2018), although much lower densities have been reported for outbreaks in Japan (i.e. 6 ind/m² – Nojima et al., 1986) and similar densities of 7 ind/m² are maintained throughout the year in Australia (MPSC, 2015); with a reproductive output of up to 19 million eggs per female (Buttermore et al., 1994), there is a very high potential for extremely high larval densities in the donor region.

According to Kaluza et al. (2010), the ports of Japan, Korea and northern China are well connected in terms of trading with the ports of the Mediterranean and Atlantic Europe (especially the Le Havre – Hamburg range) with thousands of journeys per year through the Suez Canal. With increasing sea surface temperatures (SST) and extended ice melting periods in the Arctic, some of this shipping traffic is expected to be diverted to the Northern Sea Route (Arctic passage), which is currently being used by a relatively small number of commercial vessels on a “trial” basis (Fernandez et al., 2014). However, an annual increase in traffic volume of 20% is expected over the next 25 years (Miller & Ruiz, 2014; Rosenhaim et al., 2019). Less maritime traffic takes place between temperate Australasia and the RA area (see Annex VIII for a more detailed analysis).

The lowest estimates of the volumes of ballast water taken-up, transferred and discharged into world oceans each year are around 10 billion tonnes (Interwies & Khuchua, 2017), whereas just one cubic metre of ballast water may contain from 21 up to 50,000 zooplankton specimens (Locke et al., 1991, 1993; Gollasch, 1997) and a heavy bulk carrier can carry up to more than 130,000 tonnes of ballast water (GloBallast, 2009). It is thus evident that high numbers of planktonic propagules of *A. amurensis* are likely to enter the RA area via ballast water and this remains true in the event of a possible eradication.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Pelagic larval duration (PLD) of *A. amurensis* larvae can range between 50 and 120 days at temperatures between 19°C and 10°C respectively (Bruce et al., 1995 and references therein), while a journey from the north-west Pacific to the RA area lasts approximately 6 weeks (information retrieved from shipping companies’ itinerary pages). Despite the high PLD of the species, there is high uncertainty regarding the likelihood of survival of its larvae in ballast water when travelling through tropical regions. Ballast water temperature follows the temperature of the surrounding water with 1-2 days delay (Gollasch et al., 2000; Lenz et al., 2018). In tropical seas sea surface temperature can reach values of >30 °C in the summer and as high as 27-28 °C in the winter, far above the optimal temperature for survival of *A. amurensis* larvae as surmised by the literature (see e.g. Qu. 2.7). Nevertheless, under laboratory conditions, larvae have survived temperatures of 27.9 °C for up to 6 days but died within hours at 31.3 °C (Sagara & Ino, 1954). The dominant hypothesis about the introduction pathway to Australia is ballast water, presumably due to the sheer volume transported with international shipping and the number of larvae that could be carried in it. Even if this is indeed

the case and *A. amurensis* larvae have already survived one such trip, the time travelled through warm waters between the native region and Australia is presumably much shorter than the respective time spent in the tropics while traveling from the west Pacific to the RA area.

For vessels navigating the Northern Sea Route in the future, the transit time can be reduced by e.g. approximately 10 days for a journey between Japan and Rotterdam (ABS, 2013). Lower temperatures in the Arctic will extend larval development duration, while shorter trips will potentially increase likelihood of survival for the larvae. Reproduction is clearly not possible during ballast transport of larvae, since this would require mature adults to be present in ballast sediments and furthermore to be diverting resources to gamete production and maturation in a stressful and food poor environment.

The overall likelihood of survival with current shipping routes is assessed as moderate with low confidence.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) entered into force in September 2017. It requires ships in international traffic to apply ballast water management measures, in particular:

- ballast water exchange in open seas, away from coastal areas (D-1 standard for an interim period)
- fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule phased in up to 8 September 2024). D-2 standard requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfers of ballast water mediated species.

Ballast Water Exchange (BWE) is currently practiced and requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at least 200 metres in depth (David et al., 2007 and BWMC Guideline 6). Even though BWE can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid 2007), its application has severe limitations, primarily dependant on shipping patterns of a port (e.g., shipping routes, length of voyages) and local specifics i.e., distance from nearest shore, water depth (David et al., 2007), particularly for EU Seas where these conditions are often not possible to meet. Also, organisms may still remain in the volume of ballast not exchanged and in ballast sediment, or BWE may not be possible due to weather conditions or other safety restrictions. The survival of zooplanktonic organisms (including *A. amurensis*) is thus not unlikely when only BWE measure are implemented.

As a result, ballast water treatment has been deemed necessary, such that ships shall discharge (in relation to the organism size range of interest for *A. amurensis*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension (IMO D-2 standard).

Ballast water treatment options include mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2010) and can vary with treatment method but the application of many combined methods (e.g. Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. As such, the survival of *A. amurensis* larvae in ballast water with full implementation of the D-2 standard (i.e. after 2024) is considered unlikely.

With some of the global fleet already compliant with the D2 standard, the chances of larval survival are currently assessed as moderate. Full implementation of the BWMC by 2024, despite its current operational challenges, is expected to reduce the likelihood of survival before the Arctic route becomes relevant for the species.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: After September 2017, with the BWMC coming into effect and gradually being implemented, detection of larval stages in ballast water during Port State Control inspections may be possible. According to Resolution MEPC.252(67), if initial inspections of ballast water samples indicate non-compliance with the D-2 standard, detailed inspections will be carried out. eDNA methodologies are rapidly becoming one of the fastest and most cost-efficient tools for the detection of NIS⁵ in introduction water samples (Darling & Frederick, 2018; Borrell et al., 2017; Koziol et al., 2019) and have proven suitable for the detection of *A. amurensis* larvae specifically in port water samples as well as water samples from the internal spaces of small vessels (Hayes et al., 2004). In fact, due to their similarities with closely related species and the lack of taxonomic expertise, genetic methods are the only way to unequivocally identify *A. amurensis* larvae (Bruce et al., 1995). However, full implementation of the BWMC is not anticipated until 2024. Until then, the likelihood that *A. amurensis* will enter the RA area undetected in ballast waters remains high, particularly since eDNA methodologies are not yet widely used. On the other hand, if the species is targeted through a national monitoring programme with molecular methods, larvae in water samples from ports/harbours can be currently detected.

⁵ NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of “alien species” as used in the framework of Regulation (EU) 1143/2014

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: In the Mediterranean Sea, potential recipient ports are relatively evenly spaced but slightly more concentrated along the central and western Mediterranean, with a small number in Malta, southern Italy and Spain acting as the main transshipment hubs (Rodrigue, 2020). Conversely, in Atlantic Europe, the main ports of entry are located within a more confined region, i.e. both coasts of the English Channel, but principally the Le Havre-Hamburg range along the north coast of continental Europe.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* is assumed to have been transported from central Japan to Tasmania as larvae in ballast water. The species has a very high reproductive output and can reach high densities during outbreak events, both at the seabed but also in surface waters during peak spawning periods. Despite the long PLD of the species, a number of conditions need to be met for the successful introduction of viable propagules from possible donor regions into the RA area, most importantly uptake of a sufficient number of larvae (i.e. during spawning) and transportation during the winter months, which would offer the best chances of survival during passage from tropical waters.

Out of the two main donor regions, north-western Pacific ports in Japan, Korea and China have the highest maritime connectivity with potential recipient ports in the RA area although some shipping traffic exists with southern Australia as well. Overall, the likelihood of introduction in the RA area as larvae in ballast water is judged as moderate but this applies to Atlantic Europe and primarily the Greater North Sea region. Concerning the Mediterranean Sea, considering that the primary shipping route from Asia, as well as the main transshipment hubs are located along the southern basins (Rodrigue, 2020), successful introduction and entry are considered far less likely in the Mediterranean parts of the RA area. The same applies for the Black Sea and the Baltic Sea. Concerning Arctic shipping routes, these are currently only used on a “trial” and seasonal basis with significant associated challenges and are expected to become more widely available around mid-century (Melia et al., 2017). While the increased use of the Arctic passage in the future is expected to increase the potential for introduction of *A. amurensis* (and other NIS) via ballast water, this will be of concern

further into the future, by which time it will be largely ameliorated by full implementation of the BWMC.

b) TRANSPORT-STOWAWAY Hitchhikers on ship/boat (excluding ship/boat hull fouling)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The translocation of starfish is documented in sheltered niche areas of both large commercial and small recreational vessels (Dommissé & Hough, 2004; MAF, 2011). For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissé & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from the internal surfaces of fishing and recreational vessel hulls, offering further support that the species can be translocated via this pathway (Hayes et al., 2004).

At this point it should be considered that DNA detected with meta-barcoding of a fouling sample may come from non-viable propagules or parts of organisms present in the stomachs of filter feeders or entrained within the fouling community. In this particular case however, fouling samples tested positive for *A. amurensis* DNA in the absence of *A. amurensis* larvae in the surrounding water, which was concurrently tested, as they were collected outside the species spawning season (Hayes et al., 2004). This does not necessarily mean that the detected DNA corresponds to viable propagules and indeed recent advances in environmental monitoring of marine pests are suggesting the combined use of eDNA and eRNA for a more accurate representation of viable communities (Pochon et al., 2017).

While it is certainly possible for the species to settle on the internal surfaces of ship/boat hulls and be transported over long distances as part of a fouling community, the likelihood of large number being transported via this pathway is not very high. Only a few such incidents are reported in the literature (see also Frey et al., 2014 for other asteroid species), and the number of individuals detected is small. On the other hand, the amount of maritime traffic between possible donor and recipient regions is very high (see Qu. 1.3a), considering both large merchant vessels as well as leisure craft. Based on the above, reinvasion after eradication is possible. Entry into the environment would require dislodgment of fouling material or active movement by the adults/juveniles. Alternatively, propagules could enter the environment in the form of gametes by at least a pair of *A. amurensis*, an event which is not considered very likely.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: If entrained in areas of the vessel with low food availability, *Asterias* species are capable of surviving prolonged periods of starvation, up to several weeks (St-Pierre & Gagnon, 2015) or even months (Vevers, 1949). If, on the other hand, starfish are transported in sea chests, where heavy fouling can accumulate (i.e. providing food resources, primarily in the form of bivalves), not only survival but also growth and maturation may be possible. For *A. amurensis*, this is attested by the survival and translocation of live individuals on debris from the Tohoku tsunami of 2011 from Japan to the west coast of North America almost a year later (Carlton et al., 2017; 2018). Reproduction would require the presence of at least a pair of adults and would be unlikely to happen under starvation conditions, when the species would mobilise its energy reserves for survival (Aguera & Byrne, 2018).

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Fouling organisms such as *A. amurensis* can be transported for weeks or months in mature fouling communities or sea chests. Implementing practices to control and manage biofouling can greatly assist in reducing the risk of the transfer of invasive marine species.

While the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001 (AFS Convention) addresses anti-fouling systems on ships, its focus is on the prevention of adverse impacts from the use of anti-fouling systems and the biocides they may contain, rather than preventing the transfer of invasive aquatic species.

Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species were adopted on 15 July 2011 [RESOLUTION MEPC.207(62)] The Biofouling Guidelines represent a decisive step towards reducing the transfer of invasive aquatic species by ships. Especially vessel cleaning during dry-docking in a shipyard generates a very low biosecurity risk because the debris is sent to local deposit and residue water from cleaning is collected (Bohn et al., 2016). Moreover, maintenance during dry-docking involves the re-application of anti-fouling paint, which seems to be efficient for up to 1-1.5 years – thereafter heavy fouling can start occurring (Sylvester et al., 2011; Frey et al., 2014). Nevertheless, dry-docking frequency is determined by performance (fuel consumption below a certain threshold) and can range from 0.5-5 years (Bohn et al., 2016).

On the other hand, in-water cleaning (IWC) of hulls (used as an additional tool, in-between dry-dock cleaning), especially without capturing the biofouling debris, might represent a higher risk of introducing NIS relative to land based cleaning in dry-docks with land based waste disposal because physical disturbance of the fouling communities may trigger the release of propagules or viable gametes (Hopkins & Forrest, 2008). This is especially significant with *A. amurensis* as regeneration following fragmentation may result in the proliferation of individuals. High migratory distance (reported as at least 10-15 m/day in Japan – Miyoshi et al., 2018) and survival at depth mean that even offshore activity may pose a threat.

There has been a proliferation of new IWC technologies in the past decade (e.g. <https://www.ecosubsea.com/>, <http://econetsaustralia.com/>) that capture debris and render it non-viable through e.g. UV treatment, although such systems sometimes fail to contain all of the removed debris (for reviews see Zabin et al., 2016; Scianni & Georgiades, 2019).

The suite of measures described above can prove effective against *A. amurensis* and other fouling organisms, if fully implemented, although sea-chests would still remain higher risk areas and may require more frequent in-water treatment. However, anti-fouling practices are not legally required, and can be financially costly, making it likely that a number of vessels traveling between contaminated and uncontaminated marinas and ports will not have been treated, motivating a likely score for this question. Even though there does not appear to be any comprehensive analysis of the compliance levels to the MEPC 2011 biofouling guidelines, a considerable reduction in the arrival of high-risk vessels was observed in New Zealand, after biofouling management measures became mandatory (Hayes et al., 2019).

Although (as with physical hull cleaning), antifouling is not currently a legal requirement, there is potential that treatments with biocidal compounds may prove an effective method of controlling fouling and reduce the likelihood of introduction and spread.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is unlikely to be detected upon introduction, unless thorough inspections of fouling communities in sea chests and internal hull surfaces are carried out, which is currently not a routine practice. Even then, because of the small size of newly settled individuals, the likelihood of detection via visual inspections remains low.

In order to reach GES targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes. Monitoring should focus on introduction hotspots (e.g. ports, marinas, aquaculture plots) and this will increase the detectability of *A. amurensis* entering the RA area through hull fouling, particularly if molecular methods are employed (Hayes et al., 2004).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Besides the big ports, which are relevant for large commercial vessels and are described in Qu. 1.7a, marinas for recreational vessels also constitute possible points of introduction and these are much more widespread throughout the RA area. There are over 4500 marinas in Europe, of which the largest percentage is small facilities (EC, 2017), but there are some that receive leisure craft from ports outside the EU (e.g. Gittenberger et al., 2017). Additionally, there are numerous open mooring and anchoring areas for recreational vessels, particularly in touristic hotspots, attracting high numbers of vessels often during the summer months.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* are capable of settling on ships/boat internal surfaces and sea chests and surviving over the time period it takes for a journey from the possible donor regions to the RA area, especially considering that management measures for this pathway are not mandatory. Maritime traffic between the west Pacific and EU ports and harbours is generally high but the number of individuals transported via this pathway and entering the environment is unlikely to be high. Thus, introduction with ships/boats fouling is considered to be moderately likely.

c) TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Even though introduction via this pathway is not documented for the species anywhere in the invaded range, it is considered possible that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, into the RA area.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *A. amurensis* on cultured molluscs can display high densities, e.g. in the order of 10 ind/m² on suspended scallop spat collectors (Gabaev, 2018 - for more details see Qu 4.9). The main commercial bivalve species, with which *A. amurensis* could be accidentally imported into the RA area are the oyster *Magallana gigas* and the clam *Ruditapes phillipinarum*, both of which constitute preferred prey for the starfish in its native range, as well as settlement habitat in the case of *M. gigas* (see also Qu 1.5c). However, shellfish imports from countries outside the EU are not very common and are prohibited in many cases, if not requiring strict quarantine procedures.

Available information in the peer reviewed literature suggests that bivalve culture in Europe is largely dependent on harvesting/collecting wild seed from nearby locations to aquaculture plots, bivalve seed from hatcheries to a smaller extent and, when necessary, imports of seed from other EU countries (Muehlbauer et al., 2014, Robert et al., 2013; Occhipinti-Ambrogi et al., 2016; Marchini et al., 2016). Small quantities of bivalves and other cultured molluscs may still be imported from non-EU countries (e.g. small quantities of oysters *C. gigas*, up to 3-4 tonnes per year, were directly imported from Japan and Korea into the Netherlands between 2004 and 2008 – Haydar & Wolff, 2011), but more extensive information on bivalve imports from countries outside the EU could not be found. The non-native abalone species *Haliotis discus hannai*, originally imported from Japan, is cultivated in Ireland (Hannon et al., 2013) and Spain (<http://abalonbygma.com/en/abalone-exclusive-seafood/>, see also Cook, 2014), where current production takes place in closed systems and relies on local hatcheries for seed. If more stock was imported from the native range, it would be subjected to the stringent controls of COUNCIL REGULATION (EC) No 708/2007 (see Qu.1.5c), such that this species is not considered to pose additional risks for new introductions of *A. amurensis* into the RA area.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Reproduction would not be possible considering the short duration of bivalve transfers. However, survival is very likely, given the abundance of suitable prey species and the conditions under which shellfish consignments are transported, which may actually enhance the likelihood of survival of contaminant species, as well by providing moisture and protection from harsher conditions (Minchin, 2007; Curtis et al., 2015).

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock.

The bivalves *Crassostrea gigas* (now *Magallana gigas*) and *Ruditapes philippinarum*, listed in Annex IV, constitute exceptions and can be moved without any risk assessment or quarantine; however local/national legislation exists that can limit the translocation possibilities of species like *M. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. Additionally, if the import region is a Natura2000 area, regulations can be much stricter as they aim to protect the conservation objectives of the protected area first.

Other initiatives have produced codes of conduct for the transfer of bivalve seed/stock at the national/regional level, such as the ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005 (ICES, 2005).

The implementation of EC regulation 708/2007 (EC 2007) introduces a high biosecurity level for most bivalve transfers from areas outside the EU, that has already proven to be effective in preventing new introductions of marine alien species (Katsanevakis et al., 2013; Zenetos, 2019). However, the exemption of these two species, which both constitute common prey items of *A. amurensis* in its native (Kim, 1969) and invaded range (Dommissse & Hough, 2004), means that *M. gigas* and *R. philippinarum* consignments potentially infested with *A. amurensis* would not be subjected to mandatory control measures before being released into the wild, unless stricter national/regional regulations apply, thus increasing the risk of introduction of the species. On the other hand, shellfish growers are aware of the risks posed to bivalve stock by the introduction of *Asterias* species and would be expected to adhere to codes of conduct for thorough inspections and other additional measures if necessary.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In situations where regular inspection of stock is a normal part of shellfish farm operations, *Asterias amurensis* can and has been detected on bivalve stock as early as recently settled juveniles of 10-15mm diameter, on e.g. mussel lines (Garnham, 1998; Martin & Proctor, 2000). Thus, early detection is not impossible, as long as there is awareness of the risk of introduction of the species and the ability to discriminate it from native species. However, *A. amurensis* is very variable in appearance, closely resembles the native common starfish *A. rubens* and even more so at the juvenile stage (See Qu. A2). Thus, inspections during aquaculture operations by unsuspecting/untrained staff could easily misidentify it.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Concerning the two species that are exempted from the EC regulation 708/2007, i.e. *M. gigas* and *R. philippinarum*, their cultivation is widespread throughout the RA area, particularly that of *M. gigas*. *Magallana gigas* is extensively cultivated, particularly in Atlantic Europe (Muehlbauer et al., 2014) but also in some of the Mediterranean countries (Greece, Italy, Mediterranean France and Spain) to a smaller extent (Rodrigues et al., 2015). *Ruditapes philippinarum* is cultivated mainly in Italy, Spain, Portugal, France, Ireland and the UK, where the species has naturalized and developed substantial wild populations, which provide the seed for culture operations, alongside hatcheries (Moura et al., 2017). Other non-native bivalve species (potential vectors of *A. amurensis*) intended for aquaculture would be subjected to the Regulation stipulations for strict inspections, quarantine, etc, making introduction highly unlikely – This follows from Qu. 1.5c. Besides, these two are the main species that fulfil both conditions, i.e. have been/are still occasionally imported for aquaculture into the RA area and can act as vectors of *A. amurensis* as they constitute prey species and/or settlement habitat.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species may be introduced as pest, primarily on imported oysters *M. gigas* and clams *R. philippinarum* from the North West Pacific. Available literature suggests that shellfish imports from countries outside the EU are generally limited in the past couple of decades and well regulated. The risk of introduction is associated with a few species listed in Annex IV of Council Regulation (EC) No 708/2007 if stricter local/regional regulations are not in place (see Qu. 1.5c) and with illegal/unreported transfers. Even for the exempted species of Annex IV however, the amount imported from outside the EU is at levels low enough to render this pathway unlikely.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Baltic Sea: unlikely, high confidence

Greater North Sea: moderately likely, medium confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, low confidence

Mediterranean Sea: moderately likely, medium confidence

Black Sea: unlikely, high confidence

The most likely pathways of introduction of *A. amurensis* in the RA area are through shipping vectors. While ballast water can potentially transport a large number of larvae, a number of conditions need to be met for the successful introduction of viable propagules from possible donor regions into the RA area, primarily uptake and transportation during thermally suitable times of the year. Moreover, the increasing implementation of the BWMC D2 standard will gradually reduce even further the survival probability of *A. amurensis* larvae entrained in ballast water.

Entrainment in ships' sea chest and other hull internal surfaces is a plausible mode of transport but less likely to be responsible for a large number of propagules entering the RA area. Based on the maritime traffic between potential source regions and EU ports, introduction and entry of *A. amurensis* larvae is considered most likely in the Greater North Sea region (with important transshipment hubs) and less likely in the Mediterranean Sea, where most shipping traffic nowadays is concentrated along its southern parts.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The response is based on the RCP 4.5 scenario for 2070.

Baltic Sea: unlikely, medium confidence

Greater North Sea: moderately likely, medium confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, low confidence

Mediterranean Sea: moderately likely, low confidence

Black Sea: unlikely, high confidence

A reduction in the extent and duration of ice-cover at northern latitudes under future climate conditions is likely to make the Northern Sea Route (via the Arctic Ocean) more attractive to global shipping and possibly add new ports to the potential entry points for *A. amurensis*, although big operational challenges are still associated with this (Hansen et al., 2016). Even if this happens

however, the main recipient area in the RA area where introduction is likely will remain the Greater North Sea. Increased traffic in Siberian harbours however may facilitate a stepping stone movement of *A. amurensis* larvae throughout Arctic waters towards the North-East Atlantic.

At the same time, heat waves, can cause mass mortality of aquaculture bivalves, leading to increased shellfish transfers to replete the stocks (Rodrigues et al., 2015), and this is more likely to happen in the Mediterranean Sea. More shellfish movements may be associated with a higher risk of introduction if the stocks/seed originate from areas outside the EU with a high risk of contamination with *A. amurensis* and the necessary precautions are not taken.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: *Asterias amurensis* is an arctic-boreal species that can survive a wide range of temperatures. In the northwestern Sea of Japan (Russia), the species overwinters under negative temperatures of -1.6 to -1.9°C, and in shallow-water bays in summer it lives at temperatures of up to 28-30°C (Gabaev, 2018 and references therein). In Alaskan waters *A. amurensis* can withstand sub-zero temperatures for weeks and it reproduces in the summer months (see also Qu. 2.7). *Asterias amurensis*, in its native range, spawns primarily in late winter-early spring, at optimal temperatures between 5°C and 17°C (Li et al., 2018) but is reported to spawn at temperatures of up to 23°C (Novikova, 1978). This upper temperature limit to spawning comes in contrast with all other published literature derived both from field studies as well as laboratory experiments (e.g. Kashenko, 2005; Lee et al., 2004; Sutton & Bruce, 1996; Byrne et al., 2016), which indicates that normal larval development can occur at a maximum temperature of 20°C.

These thermal tolerances are depicted in the global distribution of the species, combined with temperature data, retrieved from BIO-ORACLE (Assis et al., 2018, URL: <http://www.bio-oracle.org/>) and MARSPEC (Sbrocco & Barber 2013), URL: <http://www.esapubs.org/archive/ecol/E094/086/metadata.php>).

Concerning its salinity requirements, Kashenko (2003) suggests a minimum value of 22 psu for the survival of adult *A. amurensis*, based on laboratory experiments. Kashenko (2005) reports a similar tolerance to salinity during embryonic development, which is arrested at 22 psu. Other, pelagic, larval stages are able to survive salinities at an absolute minimum of 18 psu but exhibit higher survivorship above 20 psu (Kashenko, 2005). Thus, salinity limitations are expected to prevent establishment of the species in the Black Sea (surface salinities in the range of 14-19 psu), except for the Sea of Marmara with salinities > 20 psu and the Baltic Sea, except at the very entrance of the western Baltic.

For purposes of mapping and assessing risk of establishment, the following tolerance limits were defined:

- Average surface temperature of the warmest month < 20°C
- Average surface temperature of the coldest month > 5°C
- Minimum surface salinity > 20psu

Considering the above, the species is very likely to find suitable abiotic conditions for establishment throughout most of Atlantic Europe, with the exception of the Bay of Biscay, where sea surface temperatures are generally higher than 20°C in the summer months but cooler between October and June. In the Mediterranean Sea however, there are two short time windows for larval development, in April-May and November-December, depending on the donor region of larvae being Japan/Korea or Australia. Propagules arriving to the Mediterranean from China in the winter months are still likely to find suitable temperatures for development and settlement upon release. Thus, the likelihood of establishment in the Mediterranean Sea is considered as moderate (See Annexes VIII & IX for more details).

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE		CONFIDENCE	
	very isolated		low
	isolated		medium
	moderately widespread		high
	widespread		
	ubiquitous		

Response: *Asterias amurensis* inhabits a variety of coastal habitats, from muddy, sandy, to coarser and more consolidated bottoms (Smith & Armistead, 2014), to kelp beds (Won et al., 2013) and rocky sheltered areas of intertidal zones (Aguenal, 2008). In its native range it is typically found at depths down to approximately 100m (Gabaev, 2018) but more commonly below 40-50m (Hatanaka & Kosaka, 1959; D'yakonov, 1968), while in Alaskan waters it occurs at depths down to 200m (Smith & Armistead, 2014 and references therein). Some of these habitats, such as kelp beds and the rocky intertidal, are more widespread in Atlantic Europe, but the sublittoral soft and mixed sediment habitats are practically ubiquitous throughout the shallow waters (down to 200m) of all EU Seas.

The species is well known to aggregate over shellfish grounds (mostly bivalve molluscs), natural or cultivated (e.g. Paik et al., 2005; Gabaev, 2018), aquaculture equipment, such as suspended bivalve spat collectors, mussel lines and oyster trays (Dommissse & Hough, 2004; Gabaev et al., 2005) and piers/wharves (Aguenal, 2008). Such habitats are also widespread in the RA area, where extensive bivalve cultivation (and harvesting) takes place (Muehlbauer et al., 2014).

The organism does not require another species to complete its life cycle.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In the RA area, *A. amurensis* is likely to face competition by asteroid species with a similar trophic position and functional role in the ecosystem, i.e. *A. rubens*, *Marthasterias glacialis*, as well as crab species with a similar dietary preference for bivalves, e.g. *Carcinus maenas*, *C. aestuarii*. The outcome of such interactions is difficult to predict, it is known however that subtidal starfish aggregations can consist of more than one species of asteroids, e.g. *A. rubens* and *M. glacialis* occurring together in Brittany (France) and sharing bivalve resources (Guillou, 1996) or *Leptasterias polaris* and *A. rubens* (referred to with the synonym *A. vulgaris*) in the Gulf of Saint Lawrence (North America), preying on *Mytilus edulis* (Gaymer & Himmelman, 2002). Often the outcome of such interactions is resource partitioning by prey size or depth zone, rather than competitive exclusion (Gaymer et al., 2002). As an example, in Japan, *A. amurensis* dominates the shallow waters of Sendai Bay to be gradually replaced beyond the depth of 50 m by the congeneric *A. nippon* (now *Distolasterias nippon*) (Hatanaka & Kosaka, 1959). Conversely, in Port Phillip Bay, Australia, *A. amurensis* is mostly found in water deeper than 15 m and *Coscinasterias muricata* in the shallow parts of the bay (Parry, 2015).

Even more informative is the case of co-existence of *A. rubens* with *A. forbesi* (the third boreal, closely related species of the genus *Asterias*) in parts of their range in the north-west Atlantic, where the two species display high overlap in space (geographic and bathymetric), time, mean body size, diet composition and mean prey size (Menge, 1979). Menge (1979) suggested that the two species in this region compete only infrequently and their population dynamics were controlled by storms, disease, competition and prey patchiness, in order of importance.

As regards competition with other taxonomic groups, the interaction between *C. maenas* and *A. amurensis* in Australia, where they are both introduced, appears to be one of resource competition, resulting in partitioning of bivalves according to size between predators, with *A. amurensis* consuming the large bivalves and *C. maenas* the small ones (Ross et al., 2004). On the other hand, competitive interactions with predatory crabs for bivalve prey can result in sub-lethal injuries to arm tips of *A. amurensis* (Ling & Johnson, 2013), which are more pronounced in juvenile starfish.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: *Asterias amurensis*, like many asteroid species, is generally considered to be a terminal consumer in food webs; it does however have some documented predators. In its native range the species is consumed by the starfish *Solaster paxillatus* and the red king crab *Paralithodes camtschaticus* (Gabaev, 2018). In Australia, predation by the native starfish *Coscinasterias muricata* is suspected to be responsible for high post-settlement mortality of *A. amurensis* juveniles in Port Phillip Bay (Parry & Cohen 2001), while in laboratory experiments *C. muricata* was identified as the most efficient predator of *A. amurensis* among a suite of species examined (Botsios 2001, in Parry 2015). Nevertheless, further research is required to determine whether such native predators have the potential to influence *A. amurensis* establishment and densities in the field (Aguenel, 2008). On the other hand, in Australia, Ling & Johnson (2013) observed a high-incidence of sub-lethal injuries to *A. amurensis* by the native spider crab *Leptomithrax gaimardi*, occurring in large aggregations, and suggested that seasonal aggregations of the large spider crab can have strong but localized effects on *A. amurensis*.

Regarding the RA area, native starfish predators of the congeneric *A. rubens* may be able to exert some predation pressure on *A. amurensis*, particularly at the post-settlement, juvenile stage. Such predators include the species *Crossaster paposus* (Castilla, 1972), the specialized echinoderm predators *Luidia ciliaris* and *L. sarsi*, which are known to control *A. rubens* populations (Guillou, 1996), possibly native species of the genus *Coscinasterias*, such as *C. tenuispina*, but also large predatory crab species. It is noteworthy that the native predator *P. camtschaticus* is introduced and invasive to Norway and currently spreading to the south and west, while smaller related stone crabs (e.g of the genus *Lithodes*, which is known to prey on asteroid species – Fuhrmann et al., 2017), may fulfil a similar role. The potential of predation by these species to limit *A. amurensis* populations is unknown, but judging from the establishment of the species in Australia in the presence of native predators, it is thought that establishment is unlikely to be prevented.

With respect to parasites and pathogens, male *A. amurensis* are liable to gonad parasitisation by the ciliate parasite *Orchitophrya stellarum* (Byrne et al., 1997a), which causes complete atrophy of the testes, leading to castration. In Japan, infestation rates were as high as 100% in some populations (Byrne et al., 1997a). This has implications for recruitment of the species, as it decreases the concentration of male gametes and, consequently, the chances of successful external fertilisation. *Orchitophrya stellarum* has a circumboreal distribution and is known to parasitize a number of asteroid species, including the native to the RA area *A. rubens*, albeit with a lower prevalence (Jangoux, 1987). It has also been found in asteroids from the Mediterranean Sea (Febvre et al., 1981 in Jangoux, 1987). Since this ciliate infests mature males, it is considered unlikely that it will affect the potential for establishment by larval propagules, it can however affect the following generations and adults entering the RA area by other vectors.

Sea star wasting disease (SSWD) can cause mass mortality events in asteroid species, accounting for strong population declines and shifts in the demography of the affected species. SSWD is an umbrella term used to describe a suit of clinical signs starting with the appearance of skin lesions which may enlarge over time to allow internal organs to protrude, leading to the animal's death (Hewson et al., 2019). While clinical signs are similar for different affected species, the etiologies vary and can range from abiotic factors to pathogens or combinations of both (Hewson et al., 2018). Such events have become more wide-spread and prevalent in the last decade but are unpredictable. Asteroid wasting

events have been documented for *A. amurensis* both in the native area (China) and in Australia (Hewson et al., 2019) but also for different species in European waters (Thorpe & Spencer, 2000; Staehli et al., 2009).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: While Ballast Water Exchange (BWE) and Ballast Water Treatment (BWT) can reduce propagule pressure and, consequently, the rate of establishment (see Q1.5a for details), these management practices are not always possible or yet in effect. On the other hand, bivalve transportations for aquaculture purposes (which constitute a pathway of spread) offer suitable habitats to *A. amurensis* in the form of the aquaculture plots themselves and, thus, facilitate establishment. Moreover, seed harvesting devices and seed relaying offer favourable substrates and conditions for settlement and growth, enhancing establishment potential. Management practices during aquaculture operations (such as the inspection of stock and removal of predators or the removal of fouling species from seed/spat before relaying to on-grow sites) can reduce establishment potential. On the other hand, management of the natural environment (e.g. MPAs with no-take zones), is likely to limit early detection and removal by stakeholders, affording some protection to early populations.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Eradication may be attempted at an early stage of invasion by manual removal with divers and/or snorkeling, i.e. targeting juveniles and adults. If the population has not yet reached sexual maturity the chances of local eradication are relatively high (see Millers, 2015; Richardson et al., 2016 and references therein for an overview of known eradication campaigns of *A. amurensis* in Australia). In the case of reproducing populations however, the very high fecundity and long pelagic larval duration of the species (see following question) would provide a high number of propagules able to disperse over long distances and negate eradication efforts. Poorly planned and executed removal

campaigns, involving chopping and fragmentation of adults may have the opposite effect, by increasing the reproducing population (see following question for details).

Size at maturity for *A. amurensis* (i.e. distance from the center of the disc to the arm tip) is reported to vary between 46-55 mm (Ino et al., 1955; Hatanaka and Kosaka 1959; Li et al., 2018) and is generally reached at one year of age. The fact that the species takes a year and grows relatively large before maturity increases the likelihood of detection before a reproductive population develops and can facilitate eradication campaigns (Crombie et al., 2007; Millers, 2015).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Asterias amurensis is a dioecious species (i.e. sexes are separate with the male and female reproductive organs being in different individuals) with external fertilization and an annual reproductive cycle (Novikova, 1978), although damaged individuals can reproduce asexually by the regeneration of arms attached to a portion of the central disc (Ward & Andrew, 1995). One-year old females produce approximately 0.4 – 2.8 million eggs and two-year old 5.3 – 15.5 million (Hatanaka & Kosaka, 1959).

Spawning is reported at different times in different locations, ranging from late January/February to July (e.g. Akkeshi Bay, Hokkaido, Japan) and at temperatures ranging from 5 °C to 23 °C (Li et al., 2018; Hatanaka & Kosaka, 1959 and references therein). Spawning takes place primarily in February-April, at temperatures between 5°C and 17°C (Lee et al., 2004; Yu et al., 1998; Kim, 1968; Hatanaka & Kosaka 1959; Kashenko, 2005), although in Alaska the species spawns in the summer (Smith & Armistead, 2014). In some locations however there are two spawning events; such is the case in China, where spawning takes place in October-November (coinciding well with the lower bottom

water temperature (14.29°C) and again in March-May (Li et al., 2018) and also in Peter the Great Bay, Russia, with spawning events in June-July and in September, at temperatures of 17°C and 23°C, respectively (Novikova, 1978). In the invaded range, *A. amurensis* spawns during the austral winter, at similar degrees of latitude, but in the southern hemisphere, indicating photoperiodic regulation of gametogenesis, as well as modulation by temperature (Smith & Armistead, 2014). The adaptability of the species with respect to spawning periods and temperatures is very likely to facilitate establishment in the RA area, where suitable temperatures for reproduction are encountered throughout much of its extent at different times of the year (see Annex VIII).

Fertilised eggs are demersal and develop into swimming and feeding larvae (termed bipinnariae) within 60h to 120h of fertilization depending on water temperature (20°C and 10°C respectively) (Lee et al., 2004). Larval duration in the plankton also varies with temperature, as well as with feeding conditions, and can range between 50 and 120 days at temperatures between 19°C and 10°C respectively (Bruce et al., 1995 and references therein). In the native range (Korea-Japan), settlement occurs during June-July, while early juveniles appear during August-September (Paik et al., 2005).

Early Tasmanian populations, sampled in 1994, showed a considerable loss of genetic diversity compared to native Russian and Japanese populations – in the order of 30-40% - indicating a small number of initial colonisers and a genetic bottleneck (Ward & Andrew, 1995). Despite the above, the species was a very successful invader in Tasmanian and later on in Victorian waters.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Considering that the most likely life stage of *A. amurensis* to enter the RA area is the planktonic larva, with the narrowest abiotic requirements, if the environmental conditions they encounter upon entry are unfavourable for survival, they are unlikely to establish or form casual populations. Casual populations may occur in the case of the arrival of adults/juveniles as hitchhikers on vessels, and this vector is unlikely to transport *A. amurensis* in large numbers or frequently (see Risk of Introduction section). Thus, the occurrence of casual populations is deemed unlikely.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu 2.1, Qu 2.9 and Annex VIII & IX for details). The reader is encouraged to consult Annex VIII in particular for a full explanation of the rationale.

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: moderately likely, low confidence

Black Sea: unlikely, high confidence (Sea of Marmara: moderately likely, medium confidence)

The likelihood of a successful establishment of *A. amurensis* in the RA area will be largely determined by a combination of source region, arrival time (i.e. time of the year) and abiotic conditions of the recipient region when the first larvae arrive. The survival and settlement of the first introduced larvae is likely to be of high importance for initial establishment, since, once this happens, the species has demonstrated its adaptability to local conditions by changing the seasonality of its reproductive cycle (see the Australian invasion, also spawning periods throughout its native range).

Considering spawning times in the donor regions, travel duration and arrival times (see Annex VIII), the larvae of the species are very likely to find suitable abiotic conditions for establishment during the whole year throughout most of Atlantic Europe, with the exception of the Bay of Biscay, where sea surface temperatures are generally higher than 20°C in the summer months but cooler between October and June. In the Mediterranean Sea however, there are two short time windows for larval development, in April-May and November-December, depending on the donor region of larvae being Japan/Korea or Australia. Propagules arriving to the Mediterranean from China in the winter months are still likely to find suitable temperatures for development and settlement upon release. Thus, the likelihood of establishment in the Mediterranean Sea is considered as moderate. Furthermore, if it is adults rather than larvae that first arrive, temperatures at the seabed are generally lower in the summer months due to stratification and this would favour establishment even in areas with thermally marginal surface waters, as long as there is a sufficient window for spawning and dispersal.

In the Black Sea and the Baltic Sea, establishment of *A. amurensis* will be prevented by low salinities (Black Sea range of 14-19 psu for surface salinity – Baltic Sea <15 psu). The species requires salinities above 22 psu for spawning, fertilization, and embryonic development (i.e. salinity near the seabed) and above 20 psu for complete larval development, i.e. surface salinity (Kashenko, 2005).

Biotic interactions with native species and in particular with the con-generic *A. rubens*, which is very similar ecologically and functionally, have the potential to alter the likelihood of establishment but this is very difficult to predict.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The response is based on the RCP4.5 scenario for the period 2050/2070. Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the isotherm approach is described in Annex VIII and for the developed models in Annex IX. See also Qu. A7.

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, medium confidence

Mediterranean Sea: unlikely, low confidence

Black Sea: unlikely, high confidence (Sea of Marmara: moderately likely, medium confidence)

The species distribution model predicted a very small reduction in projected suitability for *A. amurensis* for all EU marine subregions under future climate change and a small northward shift of the overall suitable area for the species. This largely agrees with previous modeling work, carried out by Townhill et al. (2017), which predicted a slight poleward shift in the projected suitability for *A. amurensis* along the continental shelf of Atlantic Europe in the order of 40-50 km. According to the isotherm approach, the warming of the Mediterranean Sea but also the Bay of Biscay and the Iberian coast, is likely to further restrict the potential for establishment of *A. amurensis* in these regions.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response:

Natural larval dispersal: *Asterias amurensis* is a highly fecund species (up to 19 million eggs per female) with pelagic larvae that can stay in the water column for up to 120 days, depending on the temperature (Bruce et al., 1995 – see also Qu. 2.7). It has the potential to develop dense populations, which increases the fertilization success, since the species is a broadcast spawner, and the ability to reproduce at different times of the year between temperatures of 5°C and 20°C. In Atlantic Europe, the thermal requirements for successful larval development are met throughout most of the year, except for the winter months in the western North Sea and the summer months in the Bay of Biscay (see Annex VIII and Qu. 2.1). This can lead to a significant potential for natural dispersal, depending of course on local/regional hydrodynamic regimes. In the Mediterranean Sea, higher sea surface temperatures will limit both the period with conditions suitable for development (November to May – See Annex VIII), as well as the duration of larval development, which can be as low as 50-60 days at 19°C (Bruce et al., 1995). Even at these temperatures, 2 months of natural dispersal with oceanic currents would be sufficient for a considerable spread.

Rafting: Drifting macroalgae are known to support entire faunal assemblages and transport them over long distances, in the range of 100s of kilometers, with starfish species having been sampled on numerous occasions from such natural rafting objects (Thiel & Gutow, 2005; Fraser et al., 2011).

Besides translocated adults or juveniles, competent larvae of *A. amurensis* could also settle on already drifting macroalgae or other natural objects (Byrne et al., 2016), enhancing the potential for spread through this mechanism. Additionally, the species has the potential for 'ballooning' (filling body cavity with air and using currents to migrate or settle on floating objects) which has led to 'swarming' in Tokyo Bay (Kurata et al., 1954, cited in Nelson et al., 2016) and may enhance the potential for spread via rafting or natural dispersal.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 4.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway names:

- a) **TRANSPORT-STOWAWAY (ship/boat ballast water)**
The evidence base in the Australian invaded range suggests that *Asterias amurensis* was introduced to Tasmanian waters from central Japan via ballast water discharge (Ward & Andrew, 1995). Based on a thorough evaluation of alternative pathways (Hayes et al., 2004), ballast water transfer is still considered the most plausible pathway of introduction of this species to Tasmania, as well as its subsequent spread to Victoria, mainland Australia (Dunstan & Bax, 2008). With respect to the RA area, movement of vessels between ports within its boundaries is less restricted and ballast water regulations do not apply to short journeys within states. This means that the potential of ballast waters transport of larvae to act as a vector of spread is very significant.
- b) **TRANSPORT-STOWAWAY (Hitchhikers on ship/boat & ship/boat hull fouling)**
While it is unlikely that starfish would remain attached to vessels when underway for any length of time, their translocation is possible in sheltered niche areas (Dommissse & Hough, 2004; MAF, 2011). For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissse & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from 3 fishing and recreational vessel hulls, both from internal and external surfaces, offering further evidence that the species can be translocated via vessel fouling (Hayes et al., 2004).
- c) **TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector)**

Asterias amurensis is a notorious pest in shellfish aquaculture, both in the native and the invaded range, and is known to settle on and infest oyster trays, mussel lines and scallop spat collectors, as well as salmon cages, on-shore and offshore abalone facilities and other fish-farming equipment (e.g. Gabaev, 2018; Dommissse & Hough, 2004). Spread via this pathway is not documented for the species in the invaded range, it is considered however that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, throughout the RA area.

d) **TRANSPORT-STOWAWAY (angling/fishing equipment)**

In both the native and invaded range, *A. amurensis* is caught, often in high quantities, as by-catch of demersal and bivalve fisheries (e.g. Hatanaka & Kosaka, 1959, Aquenal, 2008, Smith & Armistead, 2014), both by bottom towed gear as well as by longlines and hooks by professional and recreational fishermen (Parry et al., 2003). Since the species is not targeted as a commercial catch, it is discarded by the fishermen (but see potential waste disposal solutions in A13 and later in this section). Depending on discard practices and disposal of by-catch, entrainment in fishing/angling equipment poses a high risk of unintentionally translocating the species.

e) **TRANSPORT-STOWAWAY (machinery/equipment & floating debris)**

This pathway includes a diverse array of vectors of different sizes and mobility, such as mobile drilling rigs, energy extraction devices, moorings and buoys, as well as equipment dislodged by storms or damaged structures and debris lost at sea and drifting (e.g. aquaculture equipment, pontoons, pier debris, etc.). Such structures are often heavily fouled with mature fouling communities (Nall, 2015; Astudillo et al., 2009), which can provide both settlement space and abundant food resources for *A. amurensis*. Some of them enter and stay in ports for extended periods of time (e.g. tidal and wave energy devices – Loxton et al., 2017b), or are in frequent contact with offshore support vessels and can thus enhance the connectivity network for propagules of the species.

a) **TRANSPORT-STOWAWAY (ship/boat ballast water)**

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: It can be stated with high certainty that this pathway is unintentional. There is no doubt that uptake of larvae in ballast water is accidental. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: For reproductive output and ship ballast volume & potential larval concentration, see Qu. 1.3a. With respect to spread of the organism within the EU, transshipment operations constitute the main maritime traffic that will act as the vector for spread. Important transshipment hubs are situated along the southern Mediterranean (serving the rest of the Mediterranean and the Black Sea) and the Le Havre-Hamburg range, serving the UK, the Baltic and Scandinavia (Notteboom et al., 2013). Considering that *A. amurensis* larvae are favoured by water temperatures encountered in many parts of the RA area, it is considered likely that sufficient numbers can be transferred with ballast water along this pathway. Finally, in many parts of the European Seas, ballast in passenger ferries is also a potential vector mainly due to high traffic volume potential. Due to the routes taken, measures described in the ballast water convention cannot easily be followed (exchange at depth and distance from shore) so the risk of depositing propagules at suitable locations is higher.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Short Sea shipping routes/trips within EU ports in particular are shorter than international shipping trips from Asia and Australia (where introduction events may originate from), such that survival in ballast water during passage along this pathway is much more likely. Most importantly, sea surface temperatures (which determine ballast water temperature) are generally within the optimal range

for *A. amurensis* larval survival (i.e. 5-20 °C), particularly in Atlantic Europe. In the Mediterranean favourable thermal conditions are restricted to the autumn and winter months (see Annex VIII). This is also the case for ferry routes between many European locations.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) entered into force in September 2017. It requires ships in international traffic to apply ballast water management measures, in particular:

- ballast water exchange in open seas, away from coastal areas (D-1 standard for an interim period)
- fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule phased in up to 8 September 2024). D-2 standard requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfers of ballast water mediated species.

Ballast Water Exchange (BWE) is currently practiced and requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at least 200 metres in depth (David et al., 2007 and BWMC Guideline 6). Even though BWE can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid 2007), its application has severe limitations, primarily dependant on shipping patterns of a port (e.g., shipping routes, length of voyages) and local specifics i.e., distance from nearest shore, water depth (David et al., 2007), particularly for EU Seas where these conditions are often not met. Also, organisms may still remain in the volume of ballast not exchanged, or BWE may not be possible due to weather conditions or other safety restrictions. The survival of zooplanktonic organisms (including *A. amurensis*) is thus not unlikely when only BWE measure are implemented.

BWE for EU short sea shipping routes is usually restricted to the second criterion of at least 50 nm from the nearest land and in waters at least 200 metres in depth in the Mediterranean Sea and is often not even feasible within these limits in northern European Seas (David et al., 2007), such that ballast water exchange is not likely to be effective in preventing the spread of *A. amurensis* (and other organisms potentially transferred in ballast water) within European Seas.

As a result, ballast water treatment has been deemed necessary, such that ships shall discharge (in relation to the organism size range of interest for *A. amurensis*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension (IMO D-2 standard).

Ballast water treatment options include mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2010) and can vary with treatment method but the application of many combined methods (e.g. Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. As such, the survival of *A. amurensis* larvae in ballast water with full implementation of the D-2 standard (i.e. after 2024) is considered unlikely. Until then (i.e. currently), planktonic propagules of the species are likely to survive in ballast water.

It is worth noting that, even after full implementation of D2, exemptions can be granted by member states on ‘low risk’ routes (Stuer-Lauridsen et al., 2018) and it is possible that e.g. internal routes by passenger ferries etc may fall into this. If *A. amurensis* arrives undetected at a port included in this exemption it may have chance to spread to others before detection and interception is possible.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: After September 2017, with the BWMC coming into effect and gradually being implemented, detection of larval stages in ballast water during Port State Control inspections may be possible. According to Resolution MEPC.252(67), if initial inspections of ballast water samples indicate non-compliance with the D-2 standard, detailed inspections will be carried out. eDNA methodologies are rapidly becoming one of the fastest and most cost-efficient tools for the detection of NIS⁶ in introduction water samples (Darling & Frederick, 2018; Borrell et al., 2017; Koziol et al., 2019) and have proven suitable for the detection of *A. amurensis* larvae specifically in port water samples as well as water samples from the internal spaces of small vessels (Hayes et al., 2004). However, full implementation of the BWMC is not anticipated until 2024. Until then, the likelihood that *A. amurensis* will enter the RA area undetected in ballast waters remains high. On the other hand, if the species is targeted through a national monitoring programme with molecular methods, larvae in water samples from ports/harbours can be currently detected.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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⁶ NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of “alien species” as used in the framework of Regulation (EU) 1143/2014

	moderately likely likely very likely		high
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Response: If ballast water exchange occurs in open seas rather than in coastal areas, transfer of planktonic larvae to suitable substrate will be hampered. If, however, untreated ballast water is released in ports, estuaries or other coastal areas, then establishment will be dependent on availability of suitable habitat. Considering a) the breadth of habitat that characterizes the species; b) the wide distribution of such habitats in the RA area and c) the invasion history of the species in Australia, which is closely connected with ports and estuaries, there is a high likelihood that *A. amurensis* can transfer to a suitable habitat after release with ballast water.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Given the amount of maritime traffic between ports and harbours of the RA area (thousands of journeys per year), the ballast volume of commercial vessels (10^4 - 10^5 tonnes) and the potentially high densities of both adult *A. amurensis* on the seabed as well as larvae in surface waters, the potential rate of spread of the species via ballast water is considerable but mainly in Atlantic Europe (see also Risk of Establishment). Due to the more limited extent and duration of favourable environmental conditions in other marine regions/subregions of the RA area, the likelihood that dense populations will develop there (and hence the corresponding larval density in the seawater in ports and harbours) is lower.

b) TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: There is direct evidence that *A. amurensis* can travel in the sea chests of large commercial vessels and that it can settle on external and internal surfaces of small recreational (and professional) craft (Hayes et al., 2004 – see also Qu 1.3b). Moreover, it is possible that larvae may be transported in sea water systems, live wells or other deck basins of recreational craft or other small commercial craft (MPSC, 2015). In these cases, the entrainment of the species on ships/boats hulls and niche areas is accidental.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: While the maritime traffic by large commercial vessels as well as smaller professional and recreational craft is very high, only a few incidents of entrainment on hulls have been reported in the literature, and the number of individuals detected was small. For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissie & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from 3 fishing and recreational vessel hulls, both from internal and external surfaces (Hayes et al., 2004). This does not necessarily mean the presence of viable propagules (as discussed in Qu. 1.3b) but is nevertheless an indication that passive or active settlement may be taking place. Considering that travel durations within the RA area are much shorter compared to journeys from the native region, particularly for small craft, it is conceivable that larvae or juveniles sheltering among protected spaces in a fouling community (e.g. barnacle or bivalve shells) can remain entrained and be translocated.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: If entrained in areas of the vessel with low food availability, *Asterias* species are capable of surviving prolonged periods of starvation, up to several weeks (St-Pierre & Gagnon, 2015) or even months (Vevers, 1949). If, on the other hand, starfish are transported in sea chests, where heavy fouling can accumulate (i.e. providing food resources, primarily in the form of bivalves), not only survival but also growth and maturation may be possible. For *A. amurensis*, this is attested by the survival and translocation of live individuals on debris from the Tohoku tsunami of 2011 from Japan to the west coast of North America almost a year later (Carlton et al., 2017; 2018). Reproduction would require the presence of at least a pair of adults and would be unlikely to happen if starvation conditions are reached (e.g. depletion of resources in sea chests), when the species would mobilise its energy reserves for survival (Aguera & Byrne, 2018). On the other hand, poorly maintained vessels (largely recreational) are a particular risk if moored for long periods of time and moved between marinas occasionally because they have the potential to harbour reproducing populations in stable, sheltered conditions for suitable periods of time to enable reproduction to occur.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Even though there are methods to prevent and minimize fouling on vessels hulls, these management measures are not mandatory in the RA area and can be expensive and time consuming, which may create a barrier to implementation. Thus, they are not carried out at a frequency and prevalence sufficient to prevent the entrainment and translocation of species like *A. amurensis* via ships hulls and niche areas (see also Qu 1.5b).

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is unlikely to be detected upon introduction, unless thorough inspections of hull fouling communities are carried out, which is currently not a routine practice. Even then, because the species can also settle on internal surfaces of vessels (Hayes et al., 2004), the likelihood of detection via visual inspections remains low.

In order to reach GES targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes. Monitoring should focus on introduction hotspots (e.g. ports, marinas, aquaculture plots) and this will increase the detectability of *A. amurensis* entering the RA area through hull fouling, particularly if molecular methods are employed (Hayes et al., 2004).

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Entry into the environment would require dislodgment of fouling material or active movement by the adults/juveniles. Alternatively, propagules could enter the environment in the form of gametes by at least a pair of *A. amurensis*, an event which is not considered very likely. In any case, ports and harbours provide abundant suitable habitats for colonization for *A. amurensis*, which a) is a habitat generalist and b) develops high densities on wharves and piers, due to their high food availability (Aguenal, 2008).

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Despite direct evidence that *A. amurensis* can settle on external and internal surfaces of vessels and can be transported via this pathway, the translocation risk is likely to be smaller compared to that associated with the ballast water of large commercial vessels (Hayes et al., 2004), primarily due to the smaller number of individuals potentially transported via hull fouling at one time. Additionally, the environmental limitations for establishment that apply in the different marine subregions of the RA

area (see Annex VIII) will also make entrainment and transport more likely in the North-East Atlantic (as in 3.9a).

- c) **TRANSPORT-CONTAMINANT Contaminant on animals** (except parasites, species transported by host/vector)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Spread via this pathway is not documented for the species in the invaded range, it is considered however that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, throughout the RA area.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis*, like many of its congeners, is a notorious pest of shellfish aquaculture both in its native and the invaded range. It is known to infest and predate on scallops, oysters, mussels, clams and abalone (see Qu. 4.9 for details and references) and could easily spread within the RA area with routine bivalve transfer operations. All countries along the European Atlantic and the

Mediterranean coasts involved in the cultivation of bivalves are currently conducting transfer activities (Muehlbauer et al., 2014; Occhipinti-Ambrogi et al., 2016; Marchini et al., 2014; Rodrigues et al., 2015). These activities include transfers at all life stages, from field sites to wild fishery sites or from field to culture sites, from nearshore to onshore facilities or from nearshore wild bottom beds to offshore hanging cultivation devices (Muehlbauer et al., 2014).

Asterias amurensis can achieve very high densities when food resources are abundant, from 300 ind/m² during outbreaks in China (Li et al., 2018) to a more modest but still considerable 6 ind/m² in Japan (Nojima et al., 1986) and 7 ind/m² around shellfish beds and aquaculture facilities in Australia (MPSC, 2015). It also settles on spat collecting devices (see Qu 4.9 for details), which poses an even higher risk of spread, due to the high volume of spat transfers and the small size of the starfish at this stage.

Additionally, *A. amurensis* can reproduce asexually by regeneration of its arms, as long as part of the central disc is attached (see Qu. 2.7), thus, even a very small number of adult individuals could potentially lead to successful spread via this pathway if e.g. damaged during dredging operations to harvest the transferred material.

An additional means of transport in the invaded range was considered to be salmon cages, which can be heavily fouled with bivalves and act as settlement surfaces for starfish (Dommissé & Hough, 2004). Nevertheless, in a survey of aquaculture operators, there were no *A. amurensis* (or any native starfish species) reported from salmon cages and it was thought that application of anti-fouling on fish cages reduces the attachment of bivalves and hence settling starfish (Dommissé & Hough, 2004).

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* is a broadcast spawner so it will not reproduce during transport, neither will it increase within the small duration of such operations (within the RA area). It is very likely to survive though, as the shellfish themselves and the methods used to contain them during transport may actually enhance the likelihood of survival of contaminant species by providing moisture and protection from harsher conditions (Minchin, 2007).

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: Management practices relevant to this pathway fall into two categories.

The first one is legislation regarding aquaculture transfers. At the EU level, COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock. Importantly, in relation to spread within the RA area, the regulation does not apply to movements of locally absent species within the Member States (i.e. in this case cultivated native species of bivalves) “except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2.” Additionally, the bivalves *M. gigas* and *R. philippinarum*, listed in Annex IV, which are common prey species for *A. amurensis*, constitute exceptions and can be moved without any risk assessment or quarantine. However local/national legislation exists that can limit the translocation possibilities of species like *M. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. Moreover, if the import region is a Natura2000 area, regulations can be much stricter as they aim to protect the conservation objectives of the protected area first. In general, restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk NIS are available.

The second category of measures are standard industry practices to minimize predator infestations, in this case commonly employed in the RA area to reduce predation by the native starfish *A. rubens*. There is a variety of methods, depending on the cultivated bivalve species and type of cultivation (bottom or suspended culture) that include manual removal (e.g. handpicking from suspended lines), immersion in chemical solutions or freshwater, removal by traps or specialized towed gear (see McEnnulty et al., 2001 and Barkhouse et al., 2007). The efficiency of these methods is variable but would allow opportunities for spread.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: If the species transfers at the juvenile stage, recently settled on bivalve spat, it is likely to go undetected due to its small size (<10mm ray length, Davenport & McLoughlin, 1993). Perfunctory visual inspections during bivalve aquaculture operations are likely to miss small size individuals. Moreover, juvenile/young *A. amurensis* could easily be misidentified for the native *A. rubens*, which is also a common shellfish culture pest and frequently encountered during transfer operations. See Qu. A2 for a comparison between *A. rubens* and *A. amurensis*.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If *A. amurensis* infested bivalve seed/stock is relayed on cultivation plots without any prior management measure, the likelihood of transfer to other suitable habitats (the cultivation plots themselves are suitable habitats) is very high. These plots are often situated in coastal areas in close proximity to suitable natural habitat to which individuals may spread. The high mobility of the adults (Miyoshi et al., 2018) augments this mechanism of spread.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Bivalve transfers are a likely mechanism of entraining and translocating *A. amurensis* in the RA area, particularly considering that suitable environmental conditions for the establishment of new populations are widespread in areas where a high volume of transfer operations takes place (i.e. throughout shallow coastal areas of Atlantic Europe, as well as the northern Adriatic, northern Aegean and the western Mediterranean).

Taking into account the degree of regulation and management in the industry and the fact that in many cases transfers are predominantly conducted within Member States, spread to distant locations through this pathway may be less important than spread through ship-mediated pathways.

d) TRANSPORT-STOWAWAY (fishing/angling equipment)

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: In both the native and invaded range, *A. amurensis* is caught, often in high quantities, as by-catch of demersal and bivalve fisheries (e.g. Hatanaka & Kosaka, 1959, Aquenal, 2008, Smith & Armistead, 2014), both by bottom towed gear as well as by longlines and hooks by professional and recreational fishermen (Parry et al., 2003). Since the species is not targeted as a commercial catch, it is discarded by the fishermen (but see potential waste disposal solutions in A13 and later in this section). Depending on discard practices and disposal of by-catch, entrainment in fishing/angling equipment poses a high risk of unintentionally translocating the species. Additionally, starfish may be entrained in wet wells or damp crevices on the deck (Kinloch et al., 2003).

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This will depend on the densities the species has achieved where fishing operations take place, the type of fishing gear used and the discard practices. Gear that is submerged for a long time, comes into contact with the seabed and is unselective (gill and trawl nets, dredges, traps and pots) is ranked the most likely to entrain and spread marine pests and particularly *A. amurensis* (Hayes 2002; Kinloch et al., 2003). Considering that *A. amurensis* can dominate the by-catch of demersal fisheries (see Qu 4.9 for details) and how widespread these operations are in the RA area, there is a high risk that a sufficient number of individuals to establish a new population can spread via this pathway, especially if the species is discarded at locations far away from the point of capture. Since this is a continuously active pathway of spread, reinvasion after eradication is also possible, unless preventative measures are taken.

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias* species generally display relatively high survival rates when discarded, unless heavily damaged during fishing operations (e.g. for *A. rubens* see Bergmann & Moore, 2001; Ramsay et al., 2001; Revill, 2012 for review). If it remains entangled in wet fishing equipment or damp crevices on the deck, it also has high chances of survival until the fishing gear is submerged again or the vessel returns to harbour. Furthermore, even damaged individuals can regenerate and have the capability to reproduce after translocation, even though they cannot reproduce along the pathway, being broadcast spawners. Some fishing practices fragment individuals and this will increase the number of viable propagules.

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are currently no management practices addressing starfish by-catch during fishing operations and their discarding at sea. Handling of by-catch can be variable and depends on fishing gear and practices, such that survival of the species is generally considered likely during fishing operations. The Common Fisheries Policy (CFP) of 2013, which aims at gradually eliminating the practice of discarding through the introduction of the landing obligation by 2019, only applies to regulated commercial species.

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If *A. amurensis* is caught together with native species in high quantities, as is often the case during fishing operations, it would be easy to miss on the deck, as by-catch is not given much attention. Additionally, juveniles and even adults may be overlooked or mistaken as “unusual looking” native *A. rubens* by untrained individuals (see Qu. 3.7c).

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: This will depend again on discard practices, fishing operations (e.g. if the gear is used multiple times during the same fishing trip before being cleaned and stored) and where the species has been entrained. If discarded at sea, it is very likely that individuals will survive and be released over suitable depths and substrates.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: Fishing/angling equipment is considered a high risk vector for the translocation and spread of *A. amurensis*, mainly due to the high numbers of individuals that can potentially be entrained and survive transport but also due to the fact that fishing operations are so widespread throughout the RA area. Nevertheless, the geographic spread of the species via this route would be relatively limited, hence a moderately rapid rate of spread may be expected.

e) TRANSPORT-STOWAWAY (machinery/equipment & floating debris)

Qu. 3.3e. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

This pathway includes a diverse array of vectors of different sizes and mobility, such as mobile drilling rigs, energy extraction devices, moorings and buoys, as well as equipment dislodged by storms or damaged structures and debris lost at sea and drifting (e.g. aquaculture equipment, pontoons, pier debris, etc.). Such structures are often heavily fouled with mature fouling communities (Nall, 2015; Astudillo et al., 2009), which provide both settlement space and abundant food resources for *A. amurensis*. Some of them enter and stay in ports for extended periods of time (e.g. tidal and wave energy devices – Loxton et al., 2017b), or are in frequent contact with offshore support vessels and can thus enhance the connectivity network for propagules of the species.

Qu. 3.4e. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This is difficult to predict as it will depend on the density of nearby populations, the larval density and hydrodynamic patterns in the vicinity of the different structures, the biofouling communities that develop on them and their frequency of movement between locations. As corroborating information, it is worth noting that the native starfish *A. rubens* is frequent and abundant in the subtidal zone of both fixed windmill pilings (de Mesel et al., 2015), floating, mobile wave energy devices (Nall, 2015), as well as navigational buoys (Orton & Fraser, 1930). Thus, even though

it is possible that significant numbers of *A. amurensis* may be found on mobile structures (should the species invade the RA area), particularly juveniles and young individuals, it is less likely that extensive spread will occur during the course of one year.

As far as smaller, dislodged equipment and debris are concerned, these may be the carriers of denser aggregations of *A. amurensis*. It is well documented that the starfish heavily settles and fouls e.g. aquaculture cages and piers (see previous sections for details), that are often damaged due to poor maintenance or extreme weather and lost at sea. This may increase spread sporadically and unpredictably over longer distances as this lighter material can drift for considerable distances depending on local hydrodynamic conditions.

Qu. 3.5e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: If *A. amurensis* find sufficient food resources on the submerged part of these structures (which is often the case, due to extensive fouling), they are very likely to survive, grow and even reproduce, if they remain undisturbed and attached for long enough (maturation in *A. amurensis* occurs at approximately 1 year – see Qu. 2.7), especially under the favourable environmental conditions of Atlantic Europe. Moreover, when such equipment/machinery is transferred between locations it is usually towed at low speeds (e.g. typically around 2 knots for a mobile drilling rig – Kinloch et al., 2003), which reduces the likelihood of dislodgement of the starfish.

Moreover, *A. amurensis* as part of fouling communities on floating debris also has high chances of survival. The most characteristic and extreme example is that of the translocation of live individuals on pier debris from the Tohoku tsunami of 2011 from Japan to the west coast of North America almost a year later (Carlton et al., 2017; 2018).

Qu. 3.6e. How likely is the organism to survive existing management practices during spread?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Floating/mobile marine devices have different challenges to ships/boats when it comes to fouling. Because this category covers a variety of objects with different purposes and operational characteristics, there are no overall standards for anti-fouling, like in the shipping sector. Nevertheless, anti-fouling coatings/paints can be used on moorings, buoys and sensors, and mechanical removal of

fouling communities is part of the maintenance procedure for marine renewable energy (MRE) devices (Loxton et al., 2017a; Venkatesan et al., 2017). With anti-fouling measures, the same limitations apply as for vessels, paints are effective for a period of 1.5-2 years, after which heavy fouling can start developing. As such, they would not affect the survival of *A. amurensis* on objects remaining at sea for long periods. In the case of mechanical removal (e.g. in-water cleaning), survival during spread would depend on the method employed, the disposal of the generated waste and whether cleaning takes place before a device is moved to another location.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This will depend on the level of inspection of the fouling communities during maintenance or before transportation of the machinery/equipment. Considering that taxonomic characterization of the attached biota is not a priority in these cases and given the similarity of *A. amurensis* with the native common starfish *A. rubens*, the likelihood that the introduced species will remain undetected seems high. See also Qu. 1.6b, 1.6c, 3.7c.

With regards to floating debris, all natural sink areas can receive floating debris (Rech et al., 2016) such that rafting *A. amurensis* could potentially get stranded all along the risk assessment area, lowering the chances of detection away from monitoring hotspots.

Qu. 3.8e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* utilizes a wide range of habitats in relatively shallow coastal areas (see Qu. 2.2 for details), where machinery/equipment is most commonly deployed. Moreover, ports and harbours, where such equipment is also transferred and stored, provide additional artificial habitats, highly suitable for colonization due to the high food availability they present. For example, in the Derwent river estuary, Tasmania, *A. amurensis* near wharf structures were present in higher densities and had larger gonads compared to *Asterias* occurring on natural substrates (Ling, 2000; Aquenal, 2008). It is also important to note that many of the structures in question are anchored or connected by other means to the seabed, providing a bridge or direct physical link over which developed starfish can

move between the structure and the seabed to forage or search for suitable habitat and conspecifics. Finally, spawning of individuals living as part of these fouling communities would release planktonic propagules to the water column at locations with conditions suitable for development.

Asterias amurensis attached to floating debris is also likely to transfer to suitable habitats as the drifting material approaches the shore; moreover, its wide depth range and relatively high adult mobility can potentially expand the spatial scale of suitable habitats around rafting communities.

Qu. 3.9e. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: Mobile machinery and equipment has a high likelihood to act as a settlement surface for the larvae of *A. amurensis*, as well as adult individuals if it comes in contact with the seabed or port/harbour structures that can host high densities of the species. The rate of spread however is not expected to be high, as these objects generally spend long periods at sea and are moved only infrequently.

On the other hand, a moderate rate of spread may be expected for *A. amurensis* rafting on dislodged and drifting objects, such as aquaculture gear and damaged structures/pieces, given the affinity of the species for such objects. Increased storminess resulting from climate change has the potential to increase this risk even further.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE		CONFIDENCE	
	very easy		low
	easy		medium
	with some difficulty		high
	difficult		
	very difficult		

Response: Naturally dispersing organisms are very difficult to contain, especially species such as *A. amurensis*, with high fecundity, long pelagic larval duration and the capability to develop extremely dense populations in a very short time (See Qu. 2.7 for the biological characteristics of the species and A5 for its invasion history in Australia). This was exemplified in Australia, where despite removal

efforts, the species spread from Tasmania to Port Phillip Bay in Victoria and from there to adjacent bays within a relatively short period of time (Millers, 2015; MPSC, 2015)

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: Unaided dispersal and multiple pathways of human-aided spread create a considerable potential for spread of *A. amurensis* in the north-east Atlantic. Mature females are highly fecund, producing between 0.5 and 15.5 million eggs per year (see Qu. 2.7 for details). The species has long-lived planktonic larvae, whose physiological requirements are met during prolonged periods in this region; additionally, it can spread rapidly via ballast water and can also be translocated with hull fouling, bivalve consignments, and fishing equipment, although at a more moderate rate with the latter pathways. In the Mediterranean Sea, if the species is introduced and establishes, its distribution is likely to be more disjunct in the cooler, northern basins of this marine region, due to less favourable environmental conditions for larval development. Natural dispersal is expected to be more limited and human-aided spread will likely proceed at a more moderate rate due to the smaller number of potential nodes.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: The rate of spread is not expected to change significantly in Atlantic Europe under foreseeable climate change conditions, therefore a rapid rate of spread may still be expected.

Higher frequency and severity of storms can increase the amount of time vessels have to spend in port, increasing the likelihood of entrainment (Galil et al., 2019); it may also increase the likelihood that equipment with mature fouling communities (e.g. buoys, cages) can be detached from docks, marinas and aquaculture facilities.

In the Mediterranean Sea, an overall increase in sea surface temperatures will limit even more the potential for natural dispersal. At the same time, heat waves, can cause mass mortality of aquaculture bivalves, leading to increased shellfish transfers to replete the stocks (Rodrigues et al., 2015). More shellfish movements may be associated with a higher risk of introduction if the stocks/seed originate from areas with a high risk of contamination with *A. amurensis* and the necessary precautions are not taken.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

NOTE: Some information is given for the functional role of the organism in its native range as a point of reference for impacts in the invaded range. It is acknowledged that these effects are not directly transferable to the invaded range and they are not taken into account when assessing the impact score for this question.

Asterias amurensis is an opportunistic generalist predator with a preference for bivalves (Lockhart & Ritz, 2001). It feeds on a variety of infaunal and epifaunal species including mollusks, ascidians,

bryozoans, sponges, crustaceans, polychaetes, fish and echinoderms (Hatanaka & Kosaka, 1959; Fukuyama and Oliver, 1985), as well as scavenging on carrion (Smith & Armistead, 2014 and references therein). In common with other *Asterias* species, *A. amurensis* in its native range undergoes periodic population density fluctuations (Gabaev, 2018) or “outbreak-like” population increases (Uthicke et al., 2009). Outbreak events are associated with heavy predation effects on wild and cultured bivalve populations (see Qu. 4.9)

They also compete for food with native (commercial) flatfish species (main food items are small crustaceans, lamellibranchs and small fish) Hatanaka & Kosaka (1959).

In Australia, *Asterias amurensis* reached densities up to 24 individuals/m² over a relatively short time (10 yrs) after its introduction (Byrne et al., 1997a, Grannum et al., 1996). These are densities far higher than those reported during outbreak events in its native range (e.g. 6 individuals/m² during an outbreak in Japan – Nojima et al., 1986). The starfish also has the ability to maintain high densities when food availability is high (MPSC, 2015). It has thus become the dominant benthic invertebrate predator in the two main invaded areas (Port Phillip Bay, Victoria and Derwent Estuary, Tasmania) with massive populations (See also Qu. A5, Qu. 1.3a for quantitative information).

The species was demonstrated to dramatically reduce the recruitment of native bivalves. In a manipulative experiment in Tasmania, densities of juveniles of the bivalve *Fulvia tenuicostata* were reduced by ca. 15 fold (from 580 to 35/m²) in the presence of sea stars at background densities relative to the treatment without sea stars, effectively arresting a massive settlement event (Ross et al., 2002). Predation by the starfish was also responsible for drastic population declines of adult *F. tenuicostata*, as well as another bivalve species *Katelysia rhytiphora* (Ross et al., 2004).

Asterias amurensis has been implicated in the dangerous decline in the 1980s of populations of the Tasmanian endemic spotted handfish, *Brachionichthys hirsutus* (Barrett et al., 1996, Ross et al., 1999), which was subsequently considered critically endangered. The mechanism was hypothesized to involve predation by *A. amurensis* on stalked ascidians, which form the primary spawning substrate of *B. hirsutus*, and subsequent reduction of key habitat availability (Bruce & Green, 1998; Green et al., 2012), although direct evidence of this is lacking (Green et al., 2012). Spotted handfish had already suffered historical habitat degradation due to scallop dredging, mooring disturbances and coastal urbanization (Wong et al., 2018). It is a species strictly restricted to the Derwent estuary, with low dispersal capability due to the lack of a planktonic larval stage, and genetic isolation between local populations. The species is vulnerable to stochastic processes that can lead to serial local extinctions such that continued conservation action (i.e. deployment of artificial spawning habitats and captive breeding program) is considered essential for its survival. (Stuart-Smith et al., 2020). In this context, the cumulative effect of *A. amurensis* on its habitat and subsequent survival can be considered a major impact.

Asterias amurensis has also been suggested to cause large changes to fish populations through competition for food (Parry & Hirst, 2016). During its peak abundance in Port Phillip Bay, *A. amurensis* biomass was equivalent to 56% of total fish biomass in the deep region of the bay, where sharp declines of 3 demersal fish species were observed. These species have a strong dietary overlap with *A. amurensis*, with bivalves and polychaetes being their preferred prey items (Parry & Hirst, 2016). In Alaskan waters (cryptogenic status – see Qu A4), *A. amurensis* is one of the main bivalve predators with the potential to affect the production of commercial crabs and fishes competing for the same resources, such as starry flounders (*Platichthys stellatus*) and king (*Paralithodes* spp.) crabs (Fukuyama & Oliver, 1985; Smith & Armistead, 2014). Nevertheless such impacts have not actually been documented.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the risk assessment area, the functional and ecological role that *A. amurensis* plays in benthic communities is fulfilled by the native, con-specific and closely related *A. rubens*. This is the case in Atlantic Europe, where *A. amurensis* is more likely to be introduced, establish and spread. Like *A. amurensis* in the north-west Pacific, *A. rubens* is also a keystone benthic predator (Casties et al., 2015), that undergoes periodic population outbreaks (Uthicke et al., 2009), during which the species can develop extensive aggregations with high densities (“feeding fronts”) and decimate bivalve beds (Hancock, 1955; Gallagher et al., 2008). Thus, the potential biodiversity impacts of *A. amurensis* will depend to a large extent on its interaction with *A. rubens* and the density/distribution it achieves in relation to its sister species. The literature suggests that asteroid species can often co-exist, either sharing or partitioning prey resources (e.g. in terms of prey size) or by partitioning the habitat (for an extensive discussion see Qu. 2.3). *Asterias rubens* in particular has been shown to only infrequently compete with *A. forbesi* in parts of their range in the north-west Atlantic (Menge, 1979), where it is suggested that their dynamics are controlled by environmental stochasticity and prey patchiness. A higher tolerance of *A. rubens* larvae to slightly lower temperatures (down to 2 °C - Benitez Villalobos et al., 2006) and salinities (locally as low as 14-15 psu - Casties et al., 2015) may give it a competitive advantage at higher latitudes. While difficult to predict, it is hypothesised that, as long as there is sufficient prey availability and favourable conditions for successful recruitment, biotic interactions with *A. rubens* will not significantly affect the potential of *A. amurensis* to cause population declines

of native species (mainly bivalves) in the RA area, such that moderate impacts may be expected, similar to elsewhere in the invaded range (Qu. 4.1). On the other hand, impacts of *A. rubens* population outbreaks on native species may be exacerbated by simultaneous or staggered outbreaks of *A. amurensis* and this may limit system recovery from natural events.

With regards to hybridisation, within the genus *Asterias*, hybridization has been documented between *A. rubens* and *A. forbesi* in the laboratory, producing viable, fertile adults (Harper & Hart, 2005) but is limited in wild populations of the north-west Atlantic (Gulf of Maine to Nova Scotia), where the two species are sympatric (Harper & Hart, 2007). Considering the close phylogenetic relationships between all three species (Wares, 2001), hybridization between *A. rubens* and *A. amurensis* may be possible.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: A list of habitats of conservation concern that may be threatened by *A. amurensis* through predation is as follows:

Mussel beds on infralittoral rock are part of the wider Reef NATURA-1170 habitat type (Annex I of the Habitats Directive). The habitat is also part of the sublittoral rocky seabeds and kelp forests (code 11.24), listed as endangered in the Resolution no. 4 of the Council of Bern Convention (1996)

(Salomidi et al., 2012). They are under severe threat from *A. amurensis* which can obliterate mussel bed populations (Gallagher et al., 2008).

Mussel beds on circalittoral rock (EUNIS A4.24).

Sublittoral mussel beds on sediment (EUNIS A5.62). Within the Habitats Directive, this biotope can be protected as Reefs (habitat type 1170).

Modiolus modiolus (horse mussel) beds/reefs are considered a type of Annex I biogenic reef habitat, an OSPAR listed habitat and a Habitat of Principal Importance across the UK (Morris, 2015). They significantly modify the underlying habitat and provide substratum and refuge for a wide variety of species, including commercially important bivalves (Baxter et al., 2011). Despite their sparse and patchy distribution and the somewhat intermittent recruitment, dense reefs are generally thought to be very stable in the long term (Holt et al., 1998; Rees, 2009). They are subject to predation by *A. rubens* (Tillin & Tyler-Walters, 2015) and could come under threat by *A. amurensis* as well. Predation by starfish (and crabs) is thought to be particularly important for the survival of juveniles, however it is suspected that survival is greatly enhanced by juveniles living within the mass of adult's byssus threads where predators cannot easily get them (Holt et al., 1998; Rees, 2009). Thus, irreversible impacts by *A. amurensis* are considered unlikely.

Sabellaria spp. reefs are listed as protected habitats under Annex I of the Habitats Directive and as a threatened and/or declining priority habitat by OSPAR (OSPAR, 2008). They provide topographically complex structures with varied microhabitats which can support high levels of biodiversity (NRW, 2019). Native starfish *A. rubens* are commonly found predating on these habitats but due to the lack of knowledge on prey-predator interactions with the reef building worms and the associated fauna, the level of risk cannot be assessed (Gibb et al., 2014). *Asterias amurensis* is known to feed on tube-building polychaetes (e.g. *Lagis koreni*, in Smith & Armistead, 2014) such that it could constitute a threat to *Sabellaria* reefs as well, however there is large uncertainty associated with this assessment. Nevertheless, the starfish has the potential to impoverish the diversity of these reef habitats by predating on the erect epifauna they support.

Ostrea edulis beds on shallow sublittoral muddy mixed sediment are part of the wider Reef NATURA-1170 habitat type (Annex I of the Habitats Directive). They are included in the European Red List of Habitats as Critically Endangered (EC 2016). These habitats provide important ecosystem functions, as described in Qu 4.7 and 4.9 (namely high biodiversity and productivity, nutrient cycling and benthic-pelagic coupling, increased water quality, etc.), which are under direct threat by *A. amurensis* predation. In the Mediterranean Sea, both mussel beds (*M. galloprovincialis*) and native oyster beds (*Ostrea edulis*) are included in the European Red List of Habitats as Vulnerable (EC 2016). Infralittoral mussel beds are of conservation concern (Near Threatened to Critically Endangered) across the regional seas. Nevertheless, *A. amurensis* is not expected to develop very high densities on a wide scale throughout the Mediterranean, such that habitats of conservation concern are not considered under very serious risk.

Loss of both infaunal and epifaunal bivalve populations has the potential to affect the overwintering success of protected diving seabirds such as common eider (*Somateria mollissima*) and common scoter (*Melanitta nigra*), which depend upon these prey resources for their survival (Bräger et al., 1995).

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Provisioning services: Biomass – Reared aquatic animals & Wild animals: *Asterias amurensis* affects ecosystem services primarily through its negative impacts on wild and cultivated bivalve populations, as well as on commercial fisheries. Loss of food provisioning services could constitute a major impact but at the local scale, since the species heavily affects many different cultured species, including scallops, oysters and mussels, wild harvested species of clams and cockles but also interferes with trawl and long-line fisheries as by-catch (see Qu 4.1 & 4.9).

Regulation & maintenance services: Regulation of physical, chemical, biological conditions: Additionally, it is thought likely that it affects other ecological processes, such as nutrient cycling, by heavily reducing infaunal populations (Joint SCC/SCFA, 1999), and regulating and maintenance services through water quality regulation (bivalve suspension feeders remove particulate organic matter, reduce nitrogen load and improve water quality (Salomidi et al., 2012).

Cultural services: Direct, in-situ and outdoor interactions – Physical and experiential: Finally, it may be affecting the amenity and recreational value of the infested areas by reducing recreational harvests (MPSC, 2015). However, such impacts have not been quantified or assessed in the invaded range.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A	CONFIDENCE	
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The establishment and development of dense population of *A. amurensis* in the RA area has the potential to severely impact food provisioning and regulating services through predation, as described in Qu. 4.6.

Provisioning services: Biomass – Reared aquatic animals & Wild animals: Mussel, oyster beds and reefs, cockle and clam beds are present throughout the regional seas (with similar structure and function albeit different species and community composition) and support both commercial and recreational harvests.

Regulation & maintenance services: Regulation of physical, chemical, biological conditions & lifecycle maintenance: Filter feeding bivalves and the biogenic structures they create offer valuable regulating and maintenance services in three ways. 1) Through water quality regulation (bivalve suspension feeders remove particulate organic matter, reduce nitrogen load and improve water quality (Salomidi et al., 2012), 2) through water flow regulation and coastal protection - three-dimensional structures dissipate wave energy (Boström et al., 2011; Scyphers et al., 2011) - and 3) through lifecycle maintenance processes by providing important feeding and nursery habitats (references in Scyphers et al., 2011 for oysters, for mussels see Salomidi et al., 2012; Gundersen et al., 2017). Predation on these organisms will impact on these ecosystem services.

Cultural services: Direct, in-situ and outdoor interactions – Physical and experiential: Cultural services in the form of recreation, tourism and the degradation of important/symbolic habitats may also be impacted (Katsanevakis et al., 2014; Gundersen et al., 2017).

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case,

please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Population outbreaks of *A. amurensis* in its native range are known to cause significant economic damages to fisheries and aquaculture, particularly of bivalve species, such as scallops, oysters, clams and mussels. For example, the damage on the marketable shellfishes caused by the starfish in Tokyo Bay in 1954 amounted to as much as 400 million yen (Kim, 1968), while the outbreak that occurred in the coastal waters of Qingdao, China in 2006-2007, caused economic losses up to 1.5 million US dollars in mariculture of scallop, abalone, and clams (Zhou and Wang, 2008 in Li et al., 2018). Besides predation on the seabed, the species also settles on spat collectors, where a high abundance of juvenile *A. amurensis* (with a mean value of about 10 ind./m²), can result in 95% mortality of juvenile scallops *Mizuhopecten yessoensis* (as *Patinopecten yessoensis*) (Gabaev, 1987, cited in Gabaev, 2018).

Additionally, it is a nuisance to trawl fisheries, increasing the by-catch and damaging nets (Nojima et al., 1986), while at the same time creating a waste disposal problem. In Hokkaido, Japan, the amount of starfish removed from aquaculture and fishing grounds and disposed as waste on land is estimated to be about 15,000 tons per year (Ishii et al., 2007). In the Gulf of Alaska and the Bering Sea (cryptogenic status – see Qu A4), *A. amurensis* also constitutes a large part of the by-catch and damages trawl nets (D. Urban, pers. comm in Smith & Armistead, 2014).

In the invaded range, large numbers of *Asterias amurensis* adults and juveniles have been found on scallop spat collector bags and suspended 'grow out' cages, mussel long-lines and oyster trays (Dommissse & Hough, 2004 and references therein). It is believed that, in all cases, settlement from plankton directly onto the different gears used is the most likely origin of the sea stars (Martin and Proctor 2000). In Tasmania, losses of commercial scallop spat over a settlement season due to *A. amurensis* predation were reported to be as high as 50% (S. Crawford pers. comm. in Hutson et al., 2005) or even 100%, according to one scallop farmer (Dommissse & Hough, 2004), resulting in an overall economic loss of 1 million AUD to the industry in 2000 (Aquenal, 2008). Additionally, in 2006, 25 tonnes of *A. amurensis* were caught as by-catch by commercial scallop fishermen on the east coast of Tasmania (Aquenal, 2008). *A. amurensis* heavily reduces the densities and potential for recruitment of wild populations of commercially important clams and cockles (Ross et al., 2002; 2004). Commercial long line fishermen are also affected in Victoria, Australia, where in areas of high infestation they report significant losses of bait and the majority of their hooks catching *A. amurensis* (Parry et al., 2003).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Throughout the world, predation by starfish is considered one of the primary causes of mortality of cultivated bivalves, such that prevention/mitigation measures are standard practice for shellfish growers (e.g. see Barkhouse et al., 2007; Calderwood et al., 2016; Kamermans & Capelle, 2019). During outbreak events, *Asterias* species can eliminate entire mussel beds (e.g. Gallagher et al., 2008; Paul-Burke & Burke, 2013) and threaten local resources and industries (Magnesen & Redmond, 2012). Considering the high abundances and predation mortality rates *A. amurensis* can achieve within cultivated bivalves, both on the seabed and on suspended culture means, such as lines, trays, spat collectors (see Qu. 4.9), it has the potential to cause major economic losses to the shellfish culture industry. For reference, predation mortality by *A. amurensis* on scallop spat collectors can result in 95% mortality of juvenile scallops in northern Russia and up to 50-100% in Tasmania (Qu. 4.9). Significant resources are at risk throughout the RA area, including mussel, oyster, scallops and other cultivated molluscs, as well as harvested wild populations of cockles and clams. Considering that such bivalve resources are already under predation pressure by the native *A. rubens*, additional and/or prolonged predation impacts by *A. amurensis* can exacerbate the economic losses.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Costs related to shipping (ballasts/fouling) will be borne by the shipping companies. Significant costs can be associated with the ratification of relevant legislation, e.g. the Ballast Water Management Convention, in ensuring its compliance, related to planning, monitoring, enforcement and capacity-building. These costs however are not specific to *A. amurensis* and will prevent/reduce the introduction of a wide range of species carried by ballast water.

Significant costs are anticipated if the shellfish aquaculture sector is heavily impacted but overall estimates will depend on the extent and intensity of infestation. Shellfish growers routinely employ a variety of measures to combat predation by *Asterias* species (for a review see Barkhouse et al., 2007). These range from removals with towed gear (e.g. starfish mops, dredges) or traps, manual removal by hand, chemical methods, with estimated costs in the range of 10^3 - 10^4 USD per application (Barkhouse et al., 2007). These are costs that will need to be applied on a yearly basis, where the species is established and has attained high population densities. Cost estimates for the subsequent disposal of the starfish waste could not be found but need to be considered. On the other hand, potential commercial exploitation of the harvested populations (as outlined in Qu. A13) could reduce the negative costs associated with disposal. A ban of imports or restrictions in the movement of shellfish seed/stock from infected areas could have potentially significant economic implications for shellfish producers (but the alternative of allowing the risk of introduction may be even more harmful, i.e. cost of inaction – see Qu. 4.9 & 4.11).

If eradication is attempted at an early stage of invasion, estimates from a successful eradication in Australia indicate costs in the range of 500.000 AUD for a small and shallow area (<1ha and shallower than 15m) over a period of one year (Crombie et al., 2007). Population removal from artificial structures in the Derwent estuary (Australia) by divers has been estimated to incur a cost of approximately 250K AUD per year (Aquenal, 2008), while monitoring costs for this species alone were calculated in the range of 10^4 - 10^5 AUD per year (Aquenal, 2008). These are examples of costs

related to different management measures, which may need to be used in combination or be repeated depending on the stage of the invasion.

For long-established and dense populations (e.g. in Port Phillip Bay, Australia), Bax et al. (2006) examined the possibility of genetic control methods and arrived at a rough cost estimate >20 million AUD for research, infrastructure and operational costs. This management option however has not materialised and was expected to be met with public concerns and legislative obstacles in Australia (Aguenal, 2008), such that the potential costs were not taken into account for the score of this question.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: *Asterias amurensis* can accumulate paralytic shellfish toxins (Asakawa et al., 1997), and considering its (potential) uses for the production of fish meal, compost or fertiliser (McEnnulty et al., 2001; Barkhouse et al., 2007) there may be concerns for indirect effects on human health. It is expected however that most of the toxin will be leached out or destroyed during the processing steps (e.g. cooking and sterilization are known to remove up to 90% of PSP toxins – EFSA, 2009 and references therein) or diluted by the other non-toxic components such that the danger to human health will be minimized. No information was found on human PSP intoxications via this indirect pathway and the metabolic routes of the toxins can vary considerably between different species (Vilarinho et al., 2018) such that there is high uncertainty associated with this assessment.

On the other hand, through its effects on recreational activities (i.e. fishing, harvest of bivalves – see Qu. 4.6 & 4.9) and the aesthetic values of the marine environment, it could potentially impact tourism and the amenity value of coastal areas (Aguenal, 2008).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As in Qu. 4.14. Minor social and health impacts may be anticipated in the RA area in the future.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Male *A. amurensis* are liable to gonad parasitisation by the ciliate parasite *Orchitophrya stellarum* (Byrne et al., 1997a), which causes complete atrophy of the testes, leading to castration. The same ciliate species infests native asteroids in the RA area, i.e. *A. rubens* in Atlantic Europe and *Sclerasterias richardi* in the Mediterranean Sea but with a lower prevalence (Jangoux, 1987). It was hypothesized that the high infestation rates in native Japan were due to the fact that *A. amurensis* was a new host for *O. stellarum* there (Byrne et al., 1997a). It is possible that the establishment of dense populations of *A. amurensis* can mediate an increase in *O. stellarum* infestations of other asteroids but high uncertainty is associated with this assessment.

Besides the risks posed for native species, *O. stellarum* is highly infectious and pathogenic to blue crabs *Callinectes sapidus* (Miller et al., 2013), another introduced species to European waters, which has already gained economic importance (Mancinelli et al., 2017).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No other impacts were found.

Qu. 4.18. 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?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Biotic interactions of *A. amurensis* with other species in its native and invaded range, as well as the RA area are discussed in Qu. 2.3, 2.4 & 4.3, 4.16. In Australia, the possibility for natural control has been suggested in relation to the parasite *O. stellarum* (see above) but also to the native starfish *Coscinasterias muricata* (Parry, 2015), which predares on *A. amurensis* juveniles and small adults. Possible predators of *A. amurensis* (e.g. native starfish and crab species) were also identified in Qu. 2.4, however the potential of predation by these species to limit *A. amurensis* populations is unknown. Judging from the establishment of extremely dense populations in Australia in the presence of native predators and competitors, it is thought that the impacts of the species are unlikely to be significantly moderated by natural control. On the other hand, *O. stellarum* is present and a parasite of native *A. rubens* in the RA area with the potential to infect *A. amurensis* with high prevalence rates (Byrne et al., 1997a), should it invade the RA area, hence the score of this question is accompanied by low confidence.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response: *Asterias amurensis*, like other asteroid species, is a benthic keystone predator with a propensity for population outbreaks that can have strong impacts on local (mainly) bivalve populations. In its invaded Australian range, it quickly developed massive populations and also maintained high densities after the first population boom. The ability to behave in a similar manner in the RA area will depend to some extent on its interaction with its sister species, the native *A. rubens*, which is very similar functionally and ecologically. Driven by examples in the literature and assuming that the two species can co-exist, *A. amurensis* has the potential to cause moderate environmental impacts in the RA area, and primarily the North East Atlantic region, through predation and, to a lesser extent, competition. Thus, population declines (locally very severe, and likely stochastic) of native species of mussels, oysters, scallops and clams may be expected, as well as declines in erect macrofauna and habitat forming polychaetes and demersal fish species that utilise the same prey resources as the starfish. Impacts on bivalve populations, although likely to be widespread, are not anticipated to be irreversible. Bivalves are broadcast spawners with high dispersal potential and relatively short generation times. Furthermore, native bivalve species are widespread and generally well connected, able to offset sudden population losses or bad recruitment years.

Mussel and oyster beds/reefs, which are listed as endangered habitats and fulfil an important ecological role are anticipated to come under threat. Of these, the most vulnerable to predation by *A. amurensis* are the *M. modiolus* reefs and the diverse fauna they support. Where infaunal bivalves are impacted, this may have implications for sediment processes and nutrient cycling, while declines in epifaunal, filtering bivalves will affect water quality regulating services but such impacts are likely to be transient. Food provisioning services through declines in commercial and recreational harvests will also be affected. The economic impacts of *A. amurensis* are likely to be even more serious (major) due to its demonstrated ability elsewhere in the invaded range to cause heavy mortalities of commercially harvested in the wild and cultivated bivalve species and, most importantly, their spat/seed. These impacts may be readily mitigated, as similar native starfish predators are routinely removed from bivalve culture plots and devices, but enhanced management practices for additional starfish populations (and possibly at different times of the year if recruitment seasons for native and alien starfish species do not exactly overlap) will entail additional significant costs. Other fisheries sectors, e.g. demersal trawling and long-lining may also suffer by increased by-catch and loss of catch.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response:

Due to the higher tolerance of *A. amurensis* for higher temperatures compared to *A. rubens*, both at the larval and the adult stage, a future warming of the seas is likely to change the dynamics of the two species in favour of the invader and push *A. rubens* northwards. In this case it may be expected that *A. amurensis* solely assumes the role of the keystone benthic predator in areas no longer suitable for *A. rubens* but the ecological and functional niche will not remain empty. The severity of the impacts is not likely to change in Atlantic Europe, the Mediterranean Sea however will present *A. amurensis* with even less favourable environmental conditions and for shorter periods of time, lowering the risk of strong recruitment, population outbreaks and associated predation impacts, such that native species, habitats and resources will be at lower risk.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	<p>The most likely pathways of introduction of <i>A. amurensis</i> in the RA area are through shipping vectors. While ballast water can potentially transport a large number of larvae, a number of conditions need to be met for the successful introduction of viable propagules from possible donor regions into the RA area, primarily uptake and transportation during thermally suitable times of the year. Moreover, the increasing implementation of the BWMC D2 standard will gradually reduce even further the survival probability of <i>A. amurensis</i> larvae entrained in ballast water.</p> <p>Entrainment in ships' sea chests and hull internal surfaces is a plausible mode of transport but less likely to be responsible for a large number of propagules entering the RA area. Based on the maritime traffic between potential source regions and EU ports, introduction and entry of <i>A. amurensis</i> larvae is considered more likely in the Greater North Sea region and less likely in the Mediterranean Sea. New Arctic Ocean shipping routes will not change the main recipient areas.</p>
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	<p>Taking into account spawning times in the donor regions, travel duration and arrival times at the different parts of the RA area, the species is very likely to find suitable abiotic conditions for establishment during most of the year throughout most of Atlantic Europe, with the exception of the Bay of Biscay. Likelihood of establishment in the Mediterranean Sea is considered as moderate. Under future climate change, a small northward shift of the overall suitable area for the species is predicted, while the Mediterranean is expected to become widely unsuitable for establishment due to high water temperatures.</p>
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	<p>Unaided dispersal and multiple pathways of human-aided spread create a considerable potential for spread of <i>A. amurensis</i> in the north-east Atlantic. The species has long-lived planktonic larvae,</p>

			whose physiological requirements are met during prolonged periods in this region; additionally, it can spread rapidly via ballast water and can also be translocated with hull fouling, in sea chests, bivalve consignments, and fishing equipment.
Summarise Impact*	minimal minor moderate major massive	low medium high	<i>Asterias amurensis</i> has the potential to cause moderate environmental impacts in the RA area, and primarily the North East Atlantic region, through predation and, to a lesser extent, competition. Population declines (locally very severe) of native species of mussels, oysters, scallops and clams may be expected, as well as declines in demersal fish species and protected bird populations that utilise the same prey resources as the starfish. Mussel and oyster beds/reefs, which are listed as endangered habitats and fulfil an important ecological role are anticipated to come under threat. Where infaunal bivalves are impacted, this may have implications for sediment processes and nutrient cycling, while declines in epifaunal, filtering bivalves will affect water quality regulating services. Food provisioning services through declines in commercial and recreational harvests will also be affected. The economic impacts of <i>A. amurensis</i> are likely to be major due to its demonstrated ability elsewhere in the invaded range to cause heavy mortalities of commercially used bivalve species.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	<i>Asterias amurensis</i> is a keystone predator of benthic invertebrates, mainly bivalve molluscs, with a well-studied invasion history in Australia, where it was most likely introduced via ballast water. The North-east Atlantic is the most likely recipient region and offers highly suitable conditions for establishment and possibilities for rapid spread. The species has the propensity for massive population outbreaks, which can be very persistent in the invaded range, and can cause severe population declines of benthic bivalve and fish species. It constitutes a serious threat to bivalve fisheries and the aquaculture industry.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
 – if not recorded, established or invasive
 ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

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

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	-	-	-	-	-
North-east Atlantic Ocean	-	-	YES	YES	-
Bay of Biscay and the Iberian Coast	-	-	YES	YES	-
Celtic Sea	-	-	YES	YES	-
Greater North Sea	-	-	YES	YES	-
Mediterranean Sea	-	-	YES	-	-
Adriatic Sea	-	-	YES	-	-
Aegean- Levantine Sea	-	-	YES	-	-
Ionian Sea and the Central Mediterranean Sea	-	-	YES	-	-
Western Mediterranean Sea	-	-	YES	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Modeately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁷	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁷ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water⁸	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
	Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes <u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
			Mediation of nuisances of anthropogenic origin <u>Smell reduction; noise attenuation; visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
		Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

⁸ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		Lifecycle maintenance , habitat and gene pool protection	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		Pest and disease control	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		Soil quality regulation	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

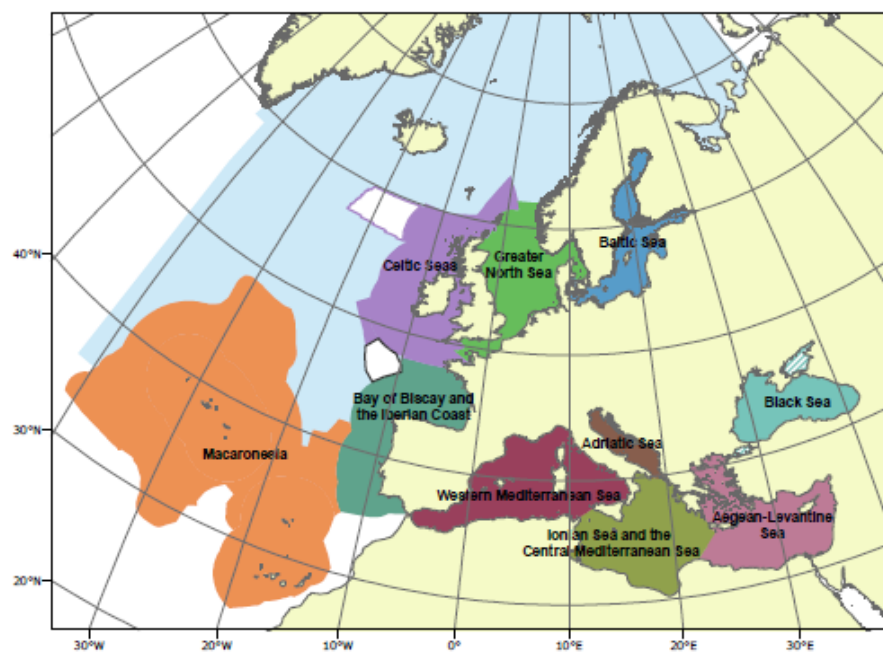
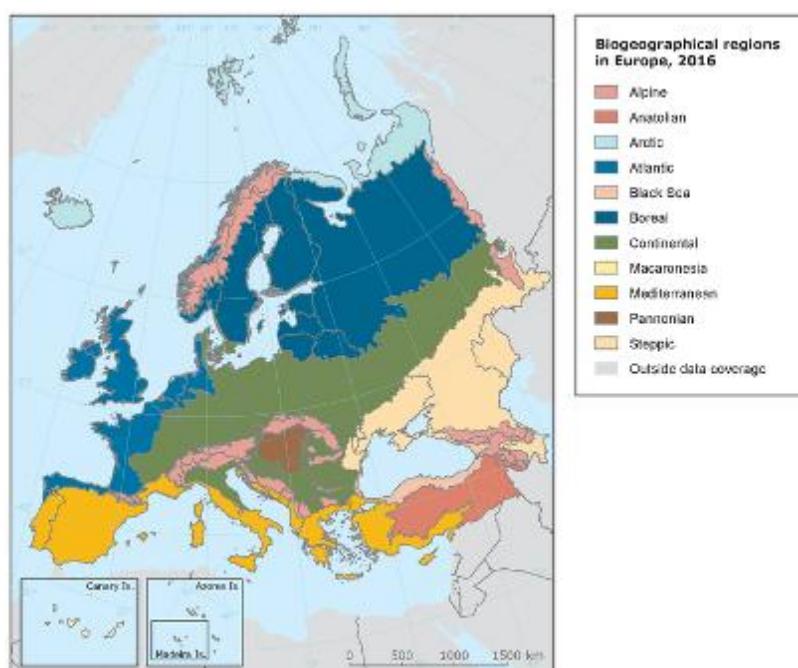
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Larval biology/ecology of *A. amurensis* and invasion risk through ballast water

Marika Galanidi (text and analysis specifically developed for the purposes of this risk assessment)

Spawning for *A. amurensis* is reported at different times in different locations, ranging from late January/February to July (e.g. Akkeshi Bay, Hokkaido, Japan) and at temperatures ranging from 5 °C to 23 °C. In Japan and Korea, spawning takes place primarily in February-April, at temperatures between 5°C and 20°C (Paik et al., 2005; Lee et al., 2004; Yu et al., 1998; Kim, 1968; Hatanaka & Kosaka, 1959; Kashenko, 2005). In Peter the Great Bay, Russia and Alaska the species has shifted its spawning period to the summer months (June-September) and in China it is reported to reproduce between October and January (Li et al., 2018; Zhang et al., 2014). In some locations however there are two spawning events; such is the case in Yantai, China, where spawning takes place in October-November (coinciding well with the lower bottom water temperature (14.29°C) and again in March-May (Li et al., 2018) and also in Peter the Great Bay, Russia, with spawning events in June-July and in September, at temperatures of 17°C and 23°C, respectively (Novikova, 1978). This upper temperature limit to spawning comes in contrast with all other published literature derived both from field studies as well as laboratory experiments (e.g. Kashenko, 2005; Lee et al., 2004; Sutton & Bruce, 1996; Byrne et al., 2016), which indicate that normal larval development can occur at a **maximum temperature of 20°C**. This was consequently regarded as the maximum temperature threshold for establishment, with the **minimum temperature threshold set at 5°C**. In the invaded range, *A. amurensis* spawns during the austral winter from July to October, at similar degrees of latitude and a similar temperature range (i.e. 8-14°C, Byrne et al., 2016), but in the southern hemisphere, indicating photoperiodic regulation of gametogenesis, as well as modulation by temperature (Smith & Armistead, 2014). Fertilised eggs are demersal and develop into swimming and feeding larvae (termed bipinnariae) within 60h to 120h of fertilization depending on water temperature (20°C and 10°C respectively) (Lee et al., 2004). Larval duration in the plankton also varies with temperature, as well as with feeding conditions, and can range between 50 and 120 days at temperatures between 19°C and 10°C respectively (Bruce et al., 1995 and references therein).

Larvae taken up in ballast water at possible source regions are estimated to arrive at release locations in the RA area after a travel period of approximately 6 weeks. It can be seen from the following table that larvae can, theoretically, arrive at the RA area at any time of the year (this without taking into account the frequency of connections between source area ports and arrival ports).

Source region	Spawning period	Temperature	Estimated arrival	Ecoregion
Japan-Korea	February-May	5-20°C	April-July	NW Pacific
Russia-Alaska	June-September	7-??	August-November	NW/NE Pacific
Australia	July-October	8-14°C	September-December	Temperate Australasia
China	October-January	5-14.3°C	December-March	NW Pacific

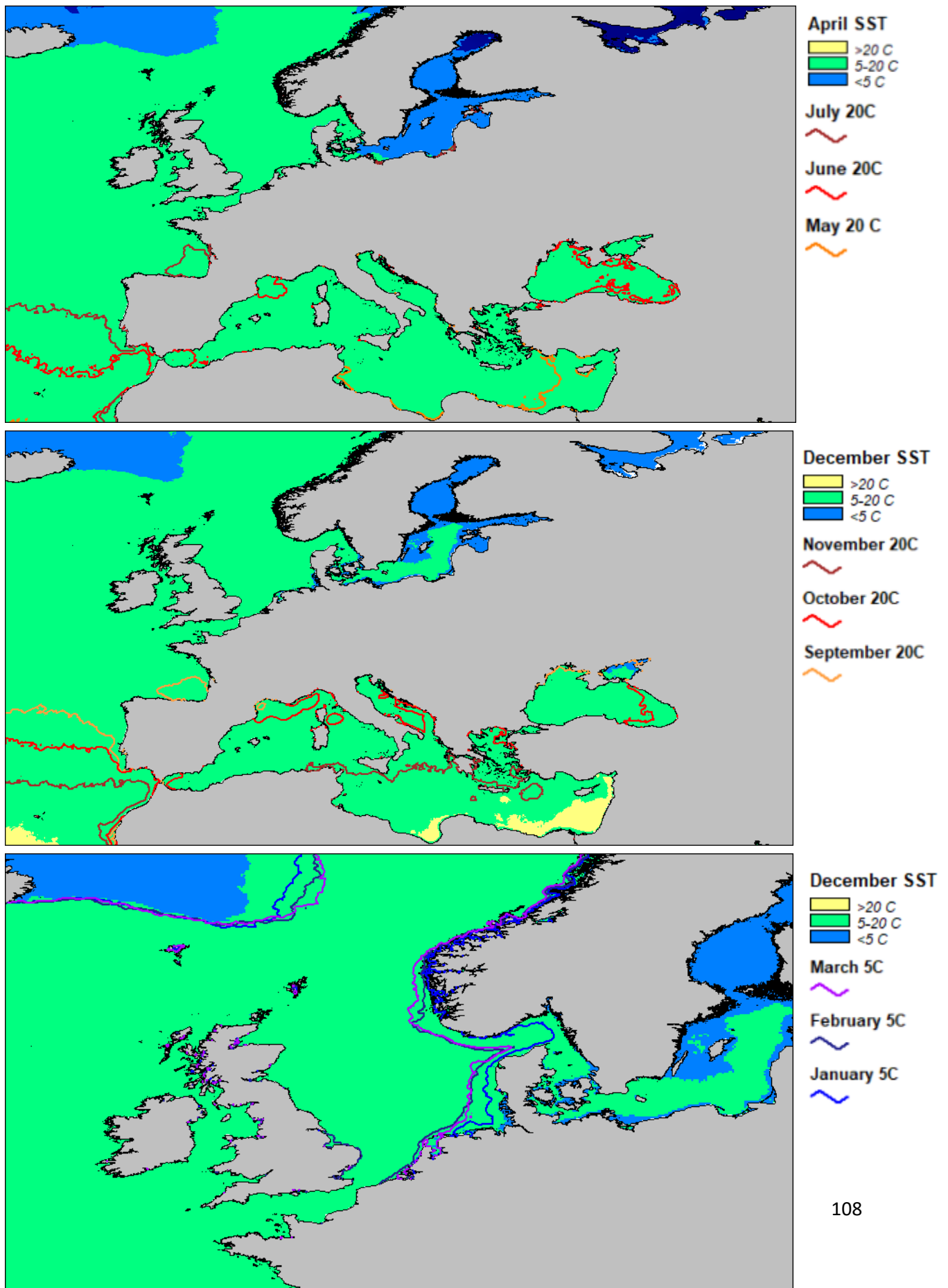
Based on the analysis of Kaluza et al. (2010), Alaska and north-eastern Russia are unlikely to act as donor regions of larvae via ballast water. Ports and coastal waters of Japan, Korea and China are well connected with Mediterranean and Eastern Atlantic ports via the Asia/Europe shipping route and receive a large amount of vessel traffic (Verny et al., 2009; Kaluza et al., 2010). Less intense maritime connections exist between Europe and temperate Australia.

In the Mediterranean Sea, propagules arriving from China in the winter months are likely to find suitable temperatures for development and settlement upon release (Temperature data layers retrieved from MARSPEC, Sbrocco et al., 2013, URL: <http://www.esapubs.org/archive/ecol/E094/086/metadata.php>). However, larvae in ballast arriving from Japan/Korea and from Australia will have a two-month window, in April-May and November-December respectively, to complete development and settle. Between June and October, sea surface temperatures will prevent normal development and survival of *A. amurensis* larvae in the Mediterranean (Figure 1). In Atlantic Europe, sea surface temperatures fall within the 5-20°C range throughout the period that *A. amurensis* larvae may be expected to arrive from Japan/Korea and for the most part from Australia, although larvae arriving from Australia later in the year will encounter increasingly colder temperatures in the Wadden Sea and around Denmark and south-eastern UK. Larvae arriving to Atlantic Europe from China in the winter months will find themselves at the lower limit of their thermal tolerance in the shallow, inshore areas of the western North Sea and along parts of the UK. The Celtic Seas, English Channel and Iberian coast/Bay of Biscay offer favourable establishment conditions throughout the year, with the exception of the Bay of Biscay (BoB), where temperatures are slightly above the 20°C threshold during the summer.

In summary, the likelihood of initial establishment of *A. amurensis* in the RA area will be determined by a combination of donor region, arrival time and abiotic conditions of the recipient region when the first larvae arrive. The survival and settlement of the first introduced larvae is likely to be of high importance for a successful invasion, since, once this happens, the species has demonstrated its adaptability to local conditions by changing the seasonality of its reproductive cycle (see the Australian invasion, also spawning periods throughout its native range). From that point on, natural dispersal and secondary spread can lead to further establishment within the RA area (see following Annex with the results of the species distribution model).

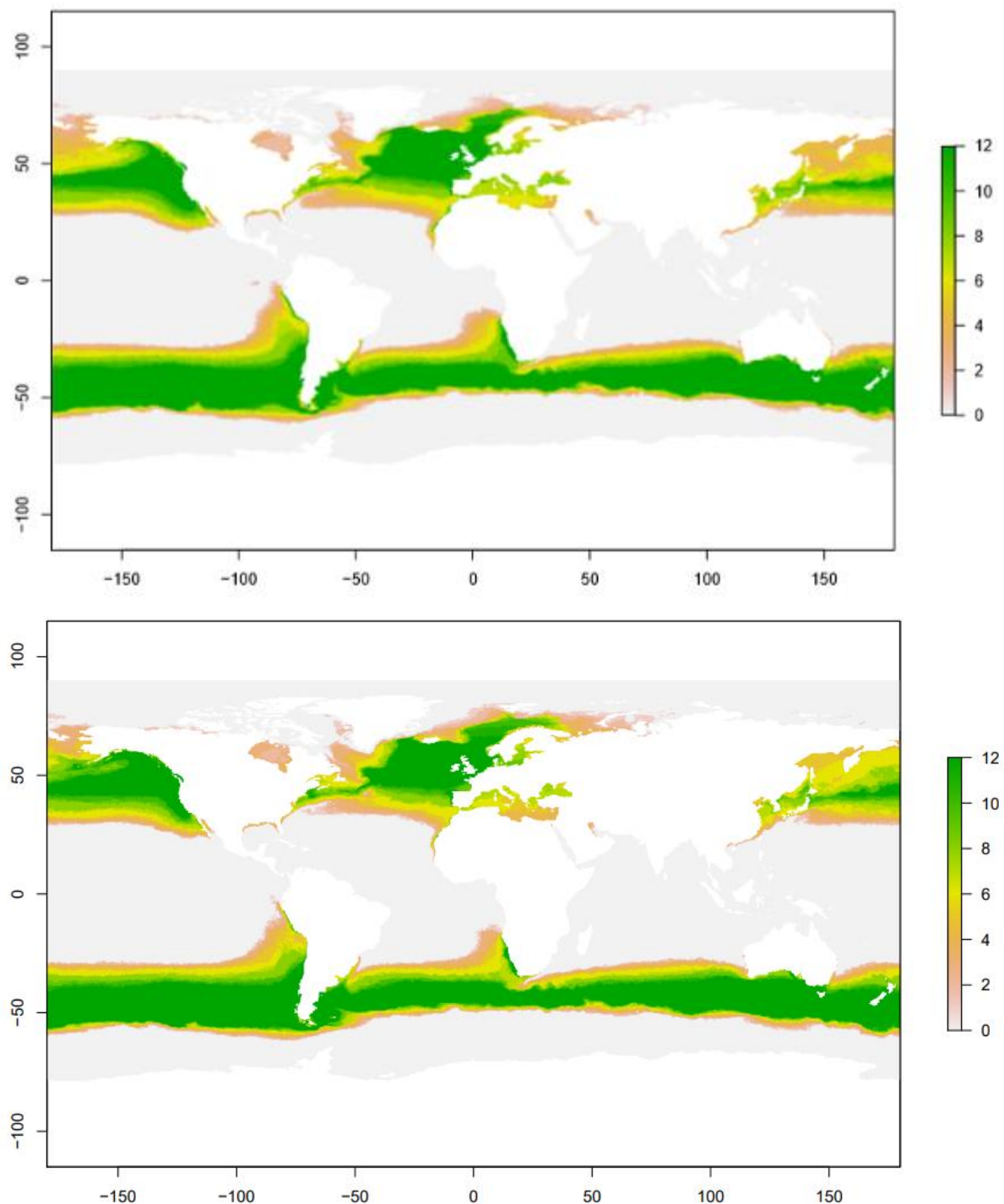
Likelihood of establishment by larvae of <i>A. amurensis</i> (by primary introduction)	
Mediterranean	1 main donor region – suitable in winter – high maritime connectivity only in the south – moderately likely
Iberian Coast	3 donor regions – suitable throughout the year – moderate to low maritime connectivity – moderately likely (not the Bay of Biscay)
Celtic Seas	3 donor regions – suitable throughout the year – moderate maritime connectivity – moderately likely
Greater North Sea	3 donor regions – suitable throughout the year – high maritime connectivity – likely
Black Sea	Restricted by salinity – UNLIKELY (except for the Sea of Marmara)
Baltic Sea	Restricted by salinity – UNLIKELY

Figure 2. Top: April Sea Surface Temperature (SST) and 20°C SST contours for the months of May-July. Middle: December SST and 20°C SST contours for October-November. Suitable conditions (SST<20°C) for *A. amurensis* larval development are encountered westwards and northwards of the contour lines. Bottom: December SST and 5°C SST contours for January-March.



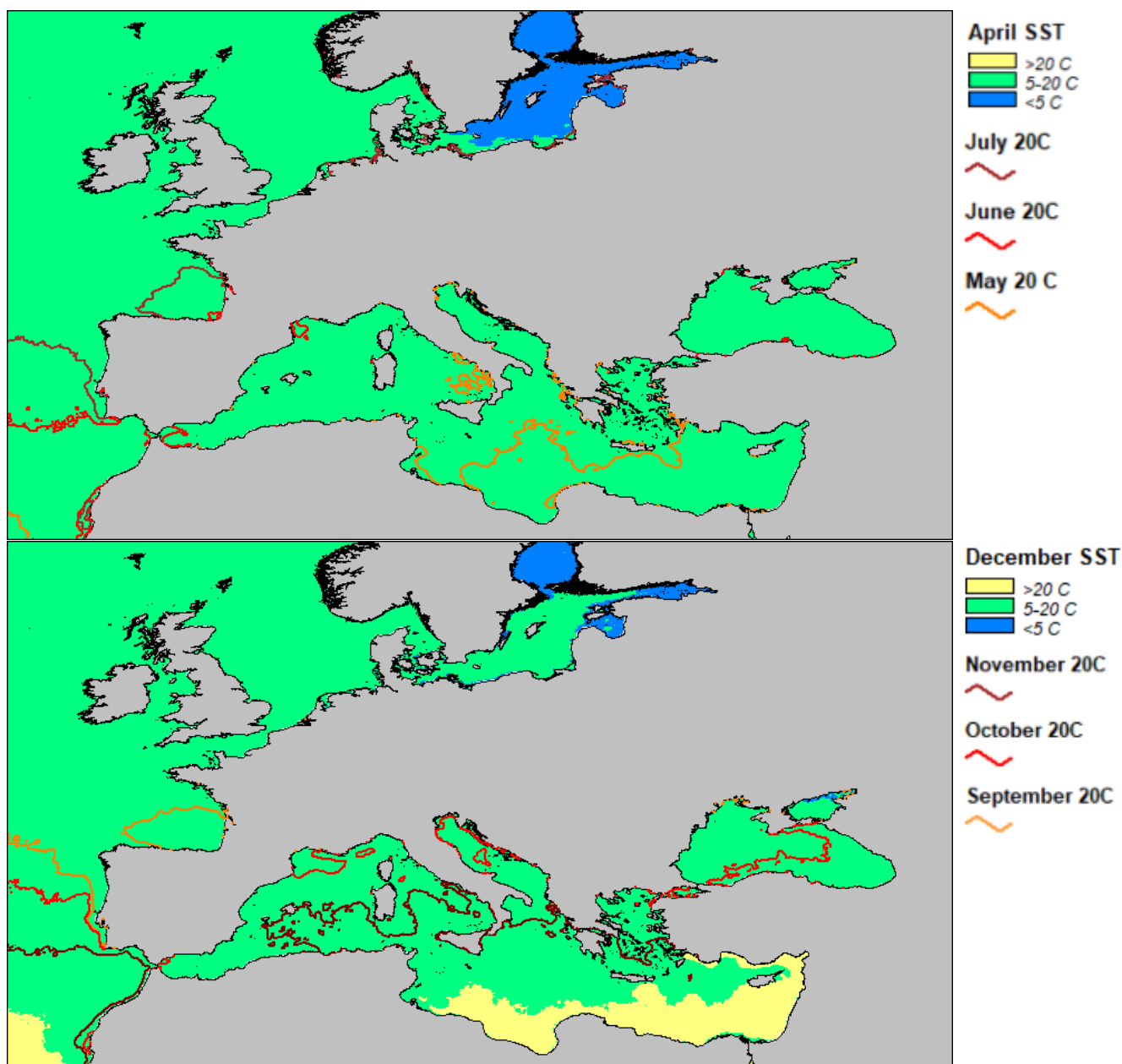
The same principles are expressed in the following map, as number of months with thermal conditions suitable for establishment (i.e. mean monthly temperatures between 5-20 °C).

Figure 3. Number of months with conditions suitable for establishment, i.e. mean monthly temperatures between 5-20 °C. Top: Under current climate conditions. Bottom: Under RCP4.5 in 2070. Maps created by Björn Beckmann.



Similar isotherm maps were produced for a future scenario of climate change (rough estimate based on a maximum increase in seawater temperatures of 0.8 °C by 2070, according to the medium timeframe RCP 4.5 scenario). It appears that, under future climate change, the conditions suitable for establishment in the Mediterranean Sea will shrink even more both in space and in time. Thus, future establishment for the Mediterranean is considered unlikely.

Figure 3: As in Figure 1 but for under RCP4.5 in 2070. Top: April Sea Surface Temperature (SST) and 20°C SST contours for the months of May-July. Bottom: December SST and 20°C SST contours for October-November. Suitable conditions (SST<20°C) for *A. amurensis* larval development are encountered westwards and northwards of the contour lines.



ANNEX IX Species Distribution Model

Projection of environmental suitability for *Asterias amurensis* establishment in Europe

Björn Beckmann, Marika Galanidi and Dan Chapman

10 June 2020

Aim

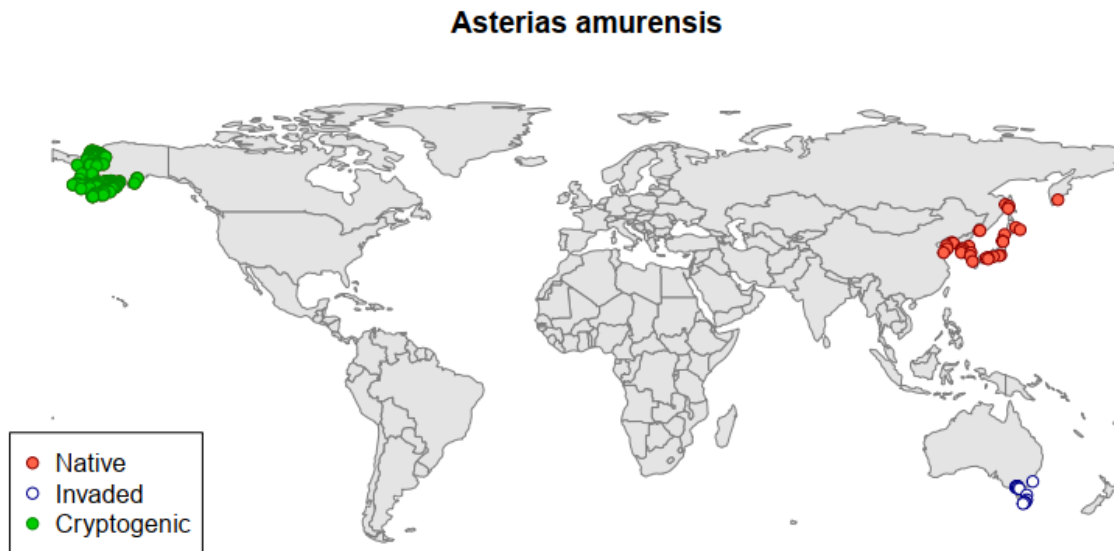
To project the suitability for potential establishment of *Asterias amurensis* in Europe, under current and predicted future climatic conditions.

Data for modelling

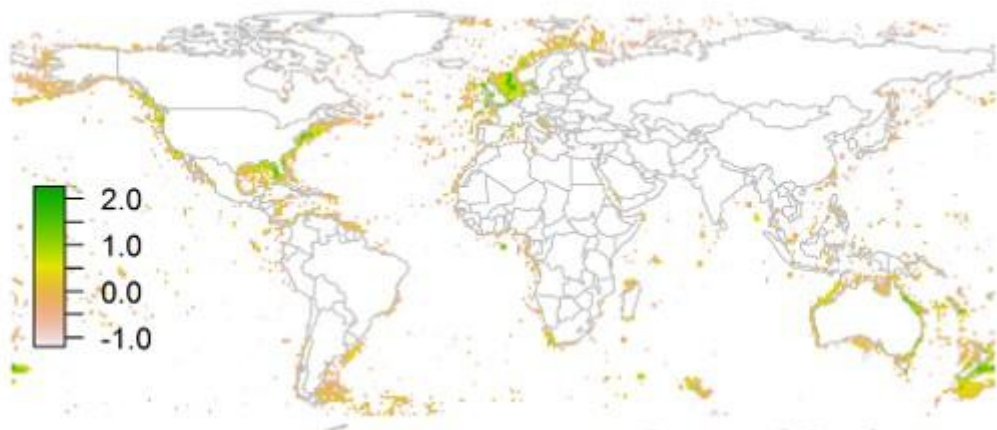
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (360 records), the Ocean Biogeographic Information System (OBIS) (285 records), the Biodiversity Information Serving Our Nation database (BISON) (219 records), the Integrated Digitized Biocollections (iDigBio) (170 records), the Atlas of Living Australia (99 records), iNaturalist (65 records), and some additional records from the risk assessment team. We scrutinised occurrence records and removed any dubious ones and records where the georeferencing was too imprecise or outside of the coverage of the predictor layers. The records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 205 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Asteroidea records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Asterias amurensis* and used in the modelling, showing native and invaded distributions. (b) The recording density of Asteroidea on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Predictors describing the marine environment were selected from the ‘Bio-ORACLE2’ set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein et al., 2012, Assis et al. 2017), supplemented by variables calculated from MARSPEC monthly sea surface temperature data (Sbrocco & Barber 2013). Both were originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Asterias amurensis*, the following variables were used in the modelling:

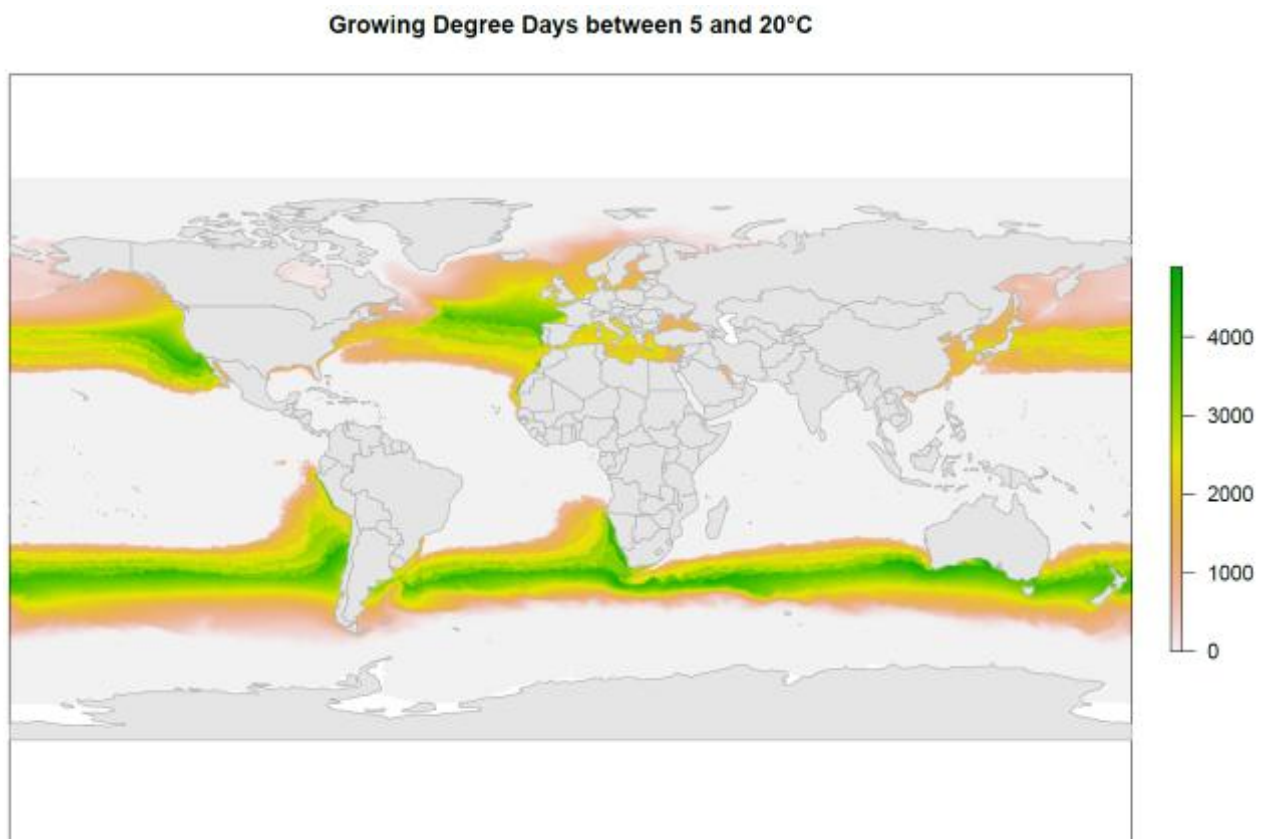
- Mean temperature (tempmean_ss)

- Maximum long-term temperature (templtmax_ss)
- Minimum long-term temperature (templtmin_ss)
- Minimum salinity (salinitymin_ss)
- Mean bathymetry (bathymean)
- Growing Degree Days between 5 and 20°C (gdd_5_20)

All parameters (except depth) are measured at the sea surface.

Growing Degree Days (GDD) have been widely used as a measure of the “biologically useful” sum of warmth between threshold temperatures where processes of interest occur (McMaster and Wilhelm 1997). For *Asterias amurensis*, larval development occurs between approximately 5 and 20°C (see Risk Assessment for details). GDD are usually calculated from daily temperature data, but since these are not available for the marine environment at a global scale, GDD were calculated from MARSPEC monthly mean temperature data: First, all grid cells with temperatures less than 5°C or more than 20°C were excluded for each month. Then, any positive difference from the lower temperature threshold was multiplied by the number of days in the month. For example, a grid cell with a temperature of 7°C in January had a value of $7^{\circ}\text{C} - 5^{\circ}\text{C} = 2^{\circ}\text{C} \times 31 \text{ days} = 62 \text{ GDD}$ for January. Finally, GDD values for all months were summed to produce an annual total for each grid cell (Fig. 2).

Figure 2. Annual Growing Degree Days (GDD) between 5 and 20°C for the current climate, calculated from MARSPEC monthly mean sea surface temperatures.



To estimate the effect of climate change on the potential distribution of *Asterias amurensis*, equivalent modelled future conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. Projections for the 2070s were calculated as averages of projections for the 2040s and 2090s (which are the time periods available on Bio-ORACLE2). Future monthly mean temperatures for calculating Growing Degree Days were approximated by adding the temperature difference between Bio-ORACLE2 current and future scenarios to the MARSPEC monthly temperature layers, because no future layers for MARSPEC are available.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Asterias amurensis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 500km buffer around the native range occurrences. This criterion was also applied to areas where the species is considered cryptogenic and has been present for more than 150 years (Fisher, 1930); AND
- A 200km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following information was taken into account in order to determine abiotic parameter thresholds.

Spawning of *A. amurensis* is reported at different times in different locations, ranging from late January/February to July (e.g. Akkeshi Bay, Hokkaido, Japan) and at temperatures ranging from 5°C to 23°C. In Japan and Korea, spawning takes place primarily in February-April, at temperatures between 5°C and 20°C (Paik et al., 2005; Lee et al., 2004; Yu et al., 1998; Kim, 1968; Hatanaka & Kosaka, 1959; Kashenko, 2005). In Peter the Great Bay, Russia and Alaska the species has shifted its spawning period to the summer months (June-September) and in China it is reported to reproduce between October and January (Li et al., 2018; Zhang et al., 2014). In some locations however there are two spawning events; such is the case in Yantai, China, where spawning takes place in October-November (coinciding well with the lower bottom water temperature (14.29°C) and again in March-May (Li et al., 2018) and also in Peter the Great Bay, Russia, with spawning events in June-July and in September, at temperatures of 17°C and 23°C, respectively (Novikova, 1978). This upper temperature limit to spawning comes in contrast with all other published literature derived both from field studies as well as laboratory experiments (e.g. Kashenko, 2005; Lee et al., 2004; Sutton & Bruce, 1996; Byrne et al., 2016), which indicate that normal larval development can occur at a maximum temperature of 20°C. This was consequently regarded as the maximum temperature threshold for establishment, with the minimum temperature threshold set at 5°C.

Concerning salinity requirements, Kashenko (2003) suggests a minimum value of 22 psu for the survival of adult *A. amurensis*, based on laboratory experiments. Kashenko (2005) reports a

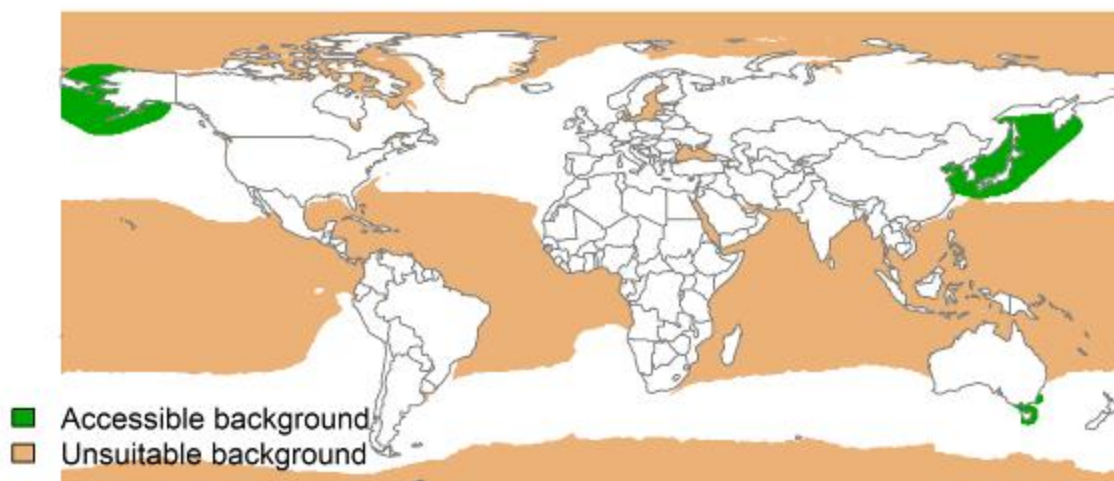
similar tolerance to salinity during embryonic development, which is arrested at 22 psu. Other, pelagic, larval stages are able to survive salinities at an absolute minimum of 18 psu but exhibit higher survivorship above 20 psu (Kashenko, 2005). Considering that small scale salinity variations of inshore areas are not likely to be reflected in the predictor data layer, which is modelled itself and not highly resolved, we opted for a salinity threshold a few units below the value for adult survival indicated in the literature but still allowing larval development.

- Consequently, the following rules were applied to define a region expected to be highly unsuitable for *Asterias amurensis* at the spatial scale of the model:
 - Minimum salinity (salinitymin_ss) < 20 psu
 - Maximum long-term temperature (templtmax_ss) < 5°C
 - Minimum long-term temperature (templtmin_ss) > 20°C

Altogether, 2.4% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (205), weighting the sampling by a proxy for recording effort (Figure 3).

Figure 3. The background from which pseudo-absence samples were taken in the modelling of *Asterias amurensis*. Samples were taken from a 500km buffer around the native range and a 200km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the

confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Asterias amurensis* was most strongly determined by Mean bathymetry (bathymean), accounting for 42.9% of variation explained, followed by Growing Degree Days between 5 and 20°C (gdd_5_20) (30.8%), Mean temperature (tempmean_ss) (9.9%), Minimum long-term temperature (tempmin_ss) (7.3%), Maximum long-term temperature (tempmax_ss) (6.1%) and Minimum salinity (salinitymin_ss) (3.1%) (Table 1, Figure 4).

Table 1. Summary of the cross-validation predictive performance (ROC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Mean bathymetry (bathymeanss)	Growing Degree Days between 5 and 20°C (gddss)	Mean temperature (tempmeanss)	Minimum long-term temperature (templtminss)	Maximum long-term temperature (templtmaxss)	Minimum salinity (salinityminss)
GLM	0.987	0.759	0.964	no	33	2	20	27	14	4
GAM	0.994	0.812	0.973	yes	32	1	28	18	16	4
GBM	0.992	0.818	0.966	yes	46	51	0	0	0	2
RF	0.994	0.819	0.976	yes	50	40	2	3	2	3
Maxent	0.884	0.692	0.758	no	56	22	4	6	2	10
Ensemble	0.995	0.820	0.978		43	31	10	7	6	3

Figure 4. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

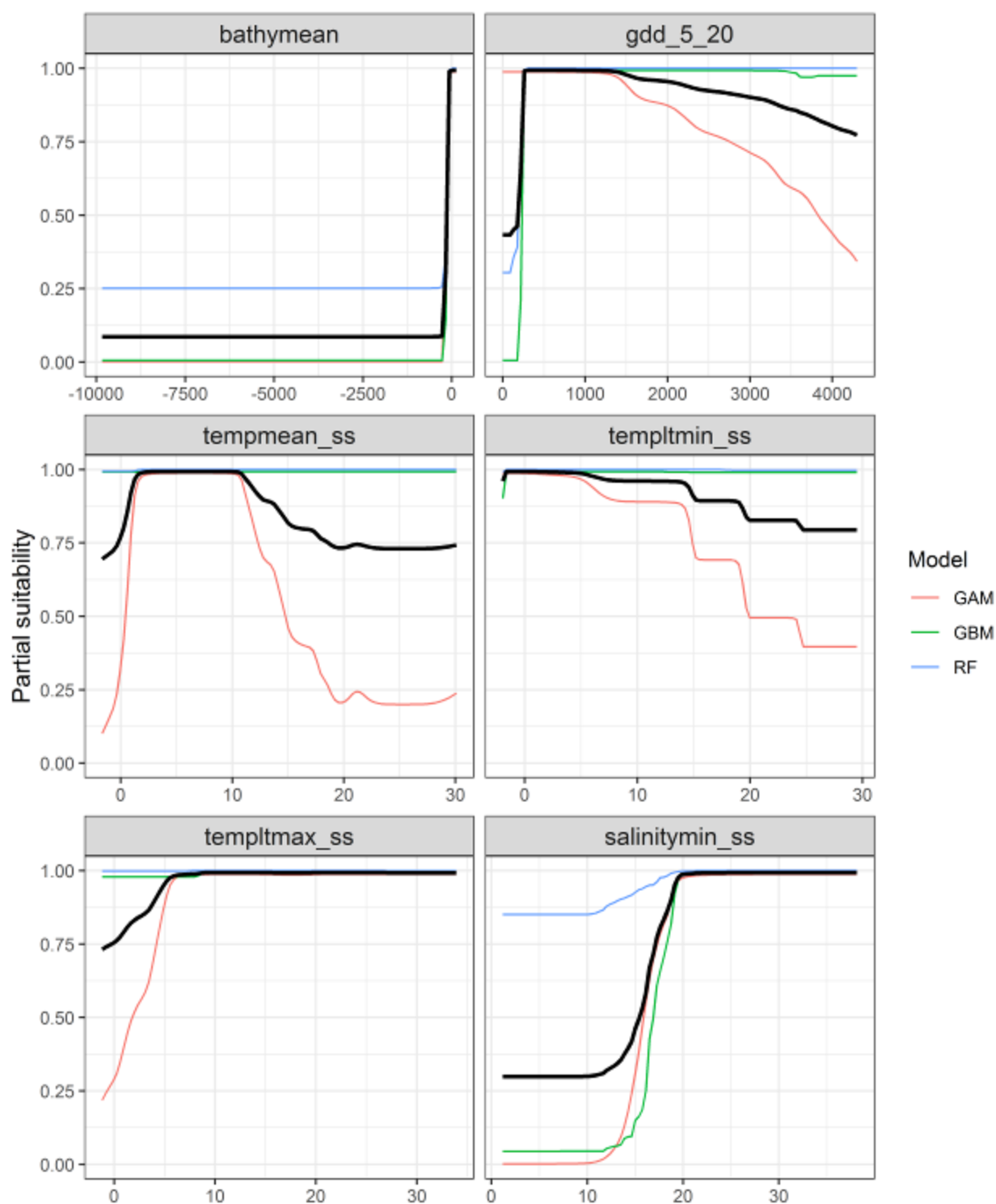


Figure 5. (a) Projected global suitability for *Asterias amurensis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.67 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

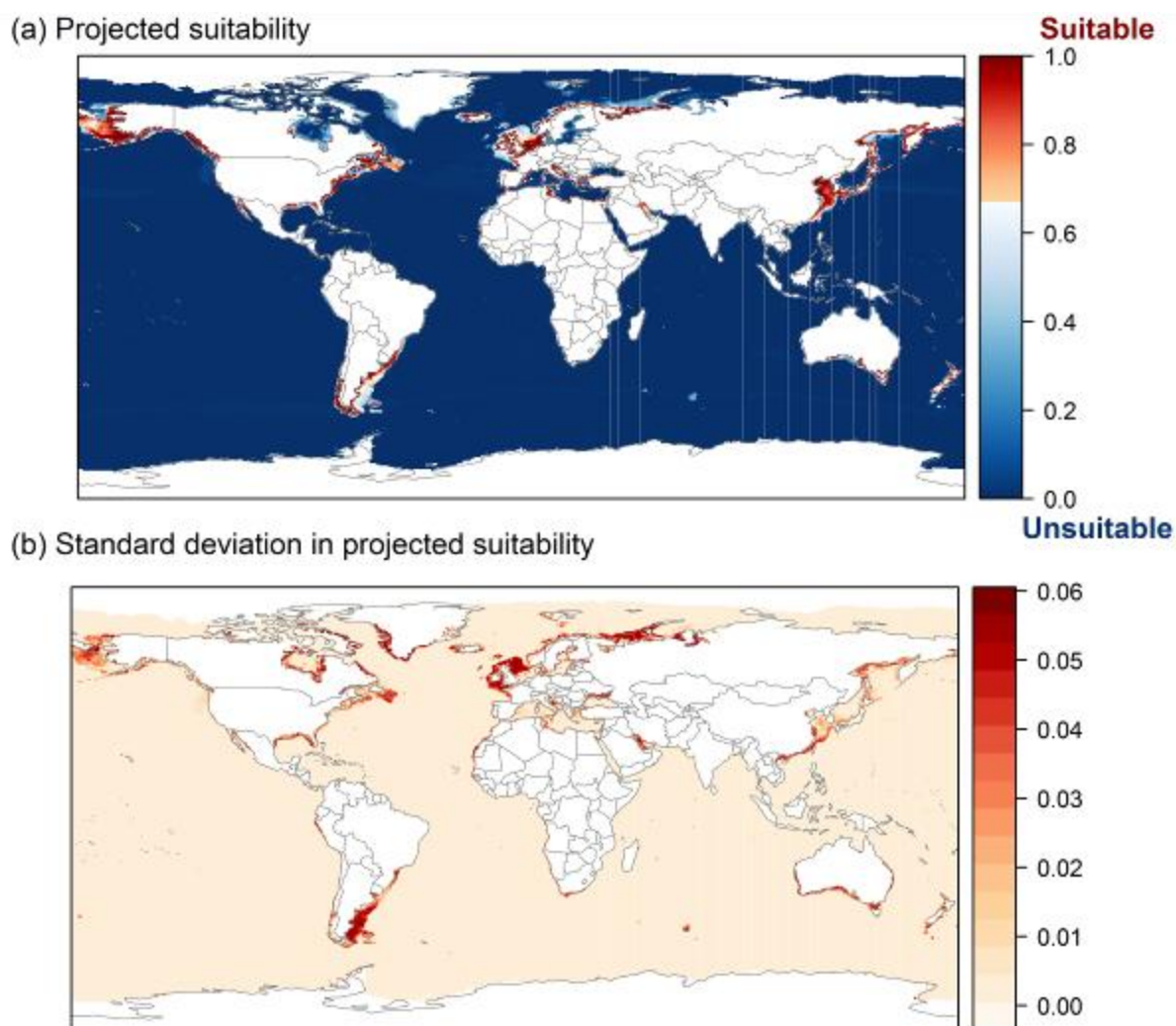


Figure 6. (a) Projected current suitability for *Asterias amurensis* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

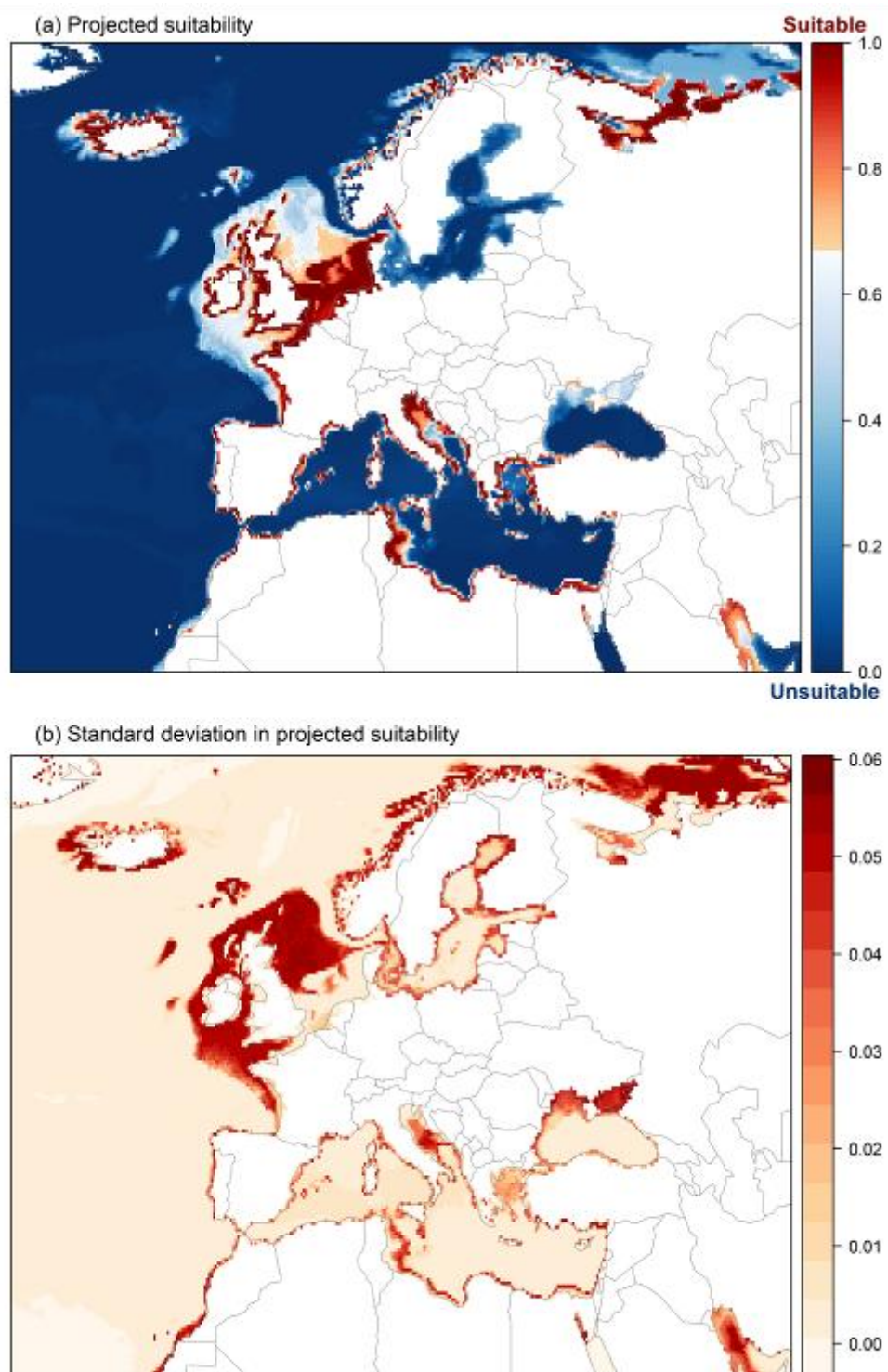


Figure 7. The most strongly limiting factors for *Asterias amurensis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

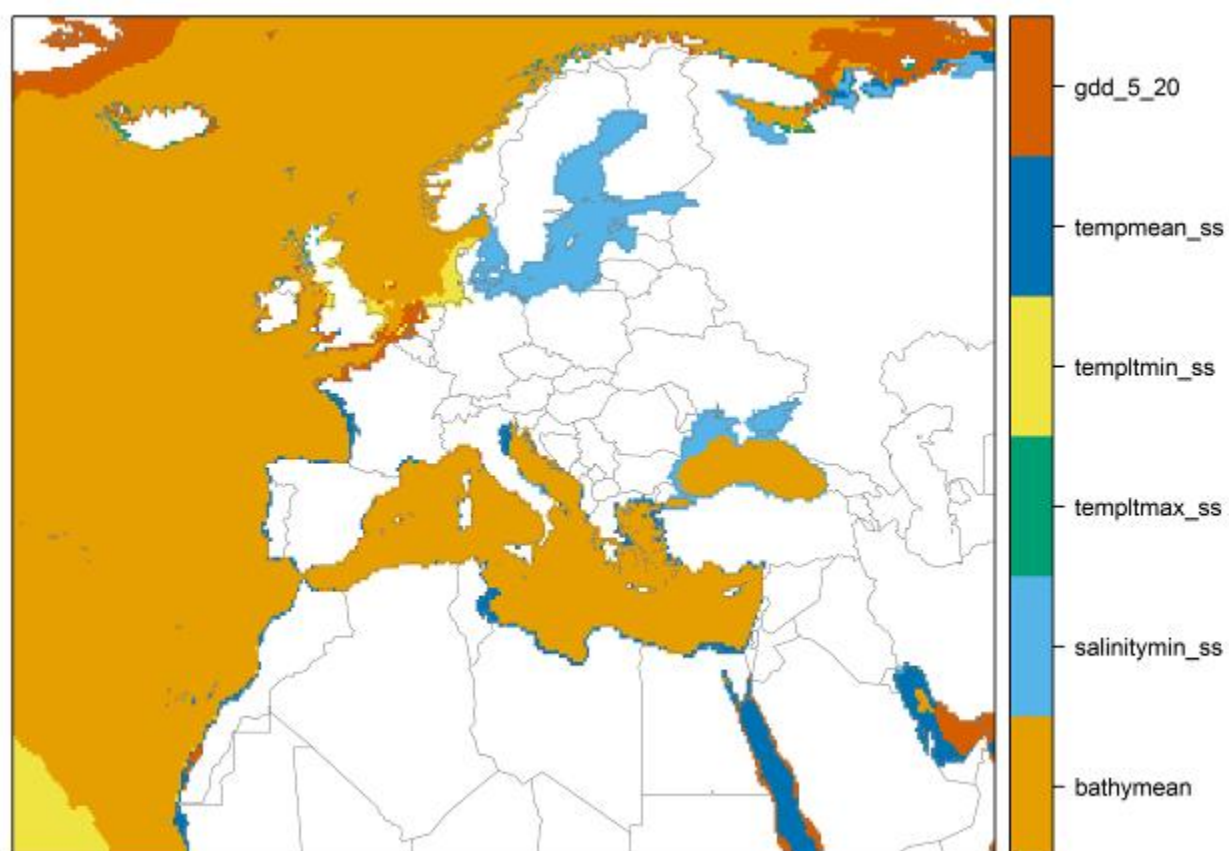


Figure 8. (a) Projected suitability for *Asterias amurensis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 6. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

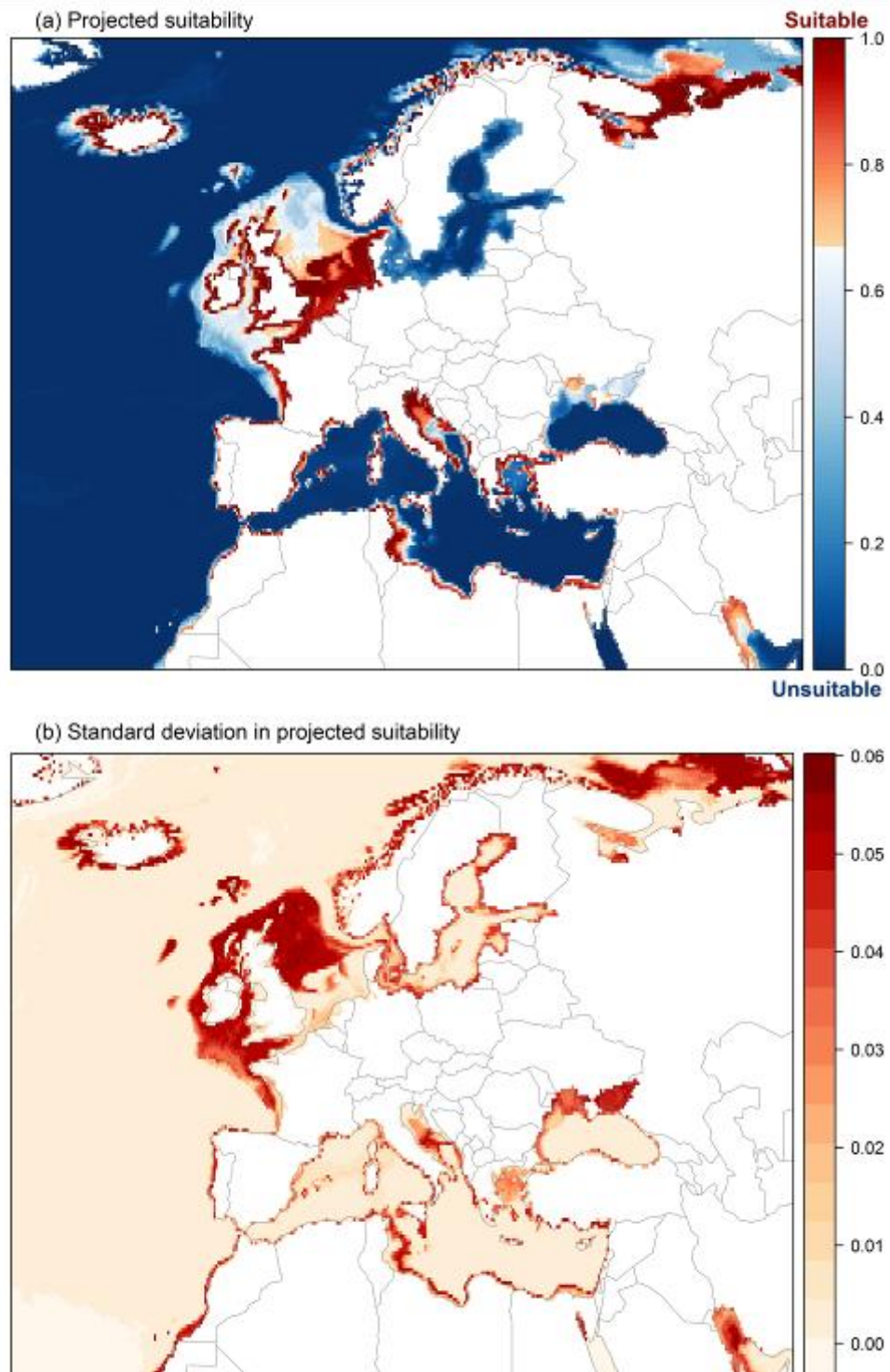


Figure 9. (a) Projected suitability for *Asterias amurensis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 6. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

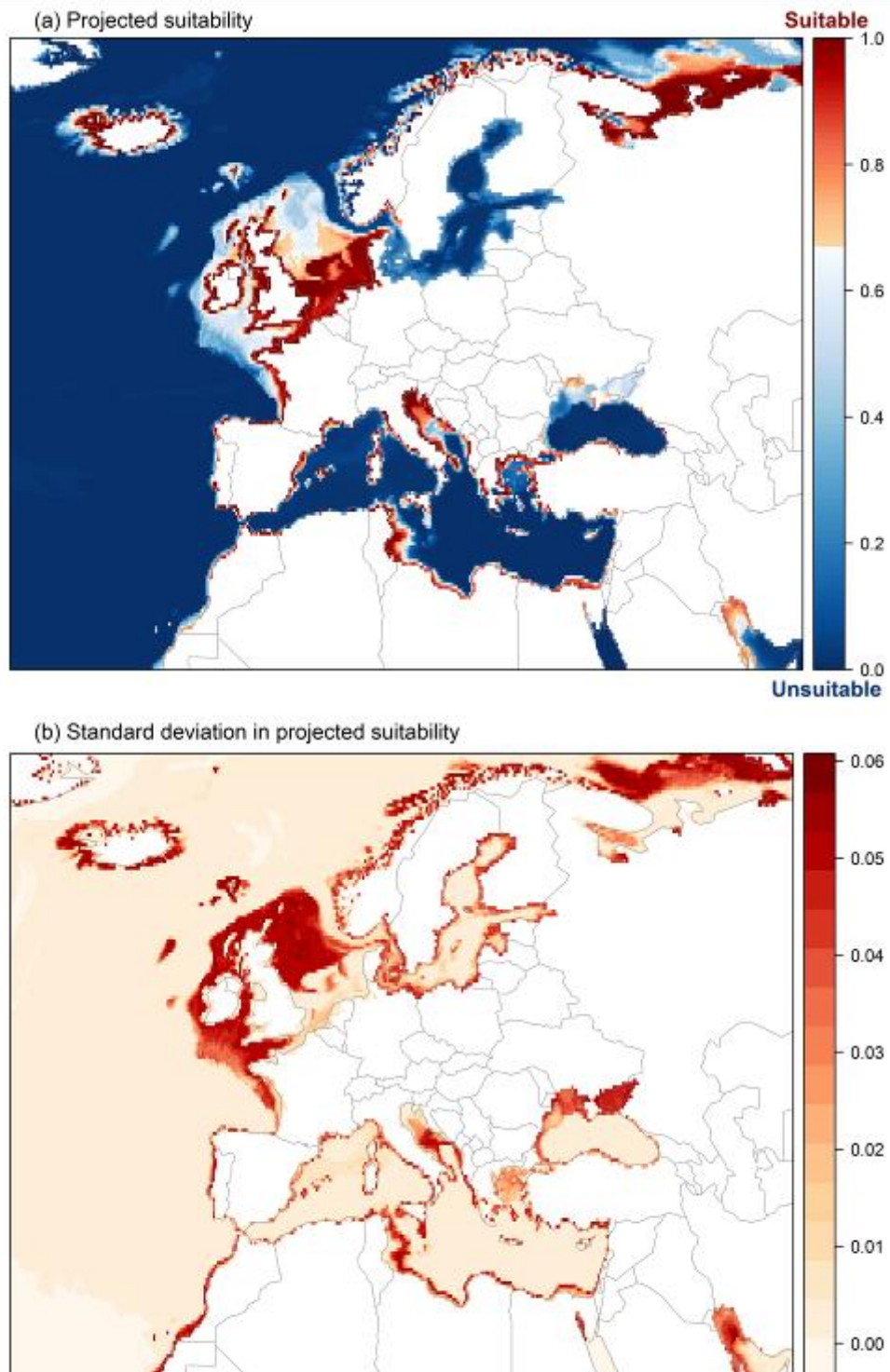


Figure 10. Variation in projected suitability for *Asterias amurensis* establishment among marine subregions of Europe. The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. Macaronesia is excluded as it is not part of the study area.

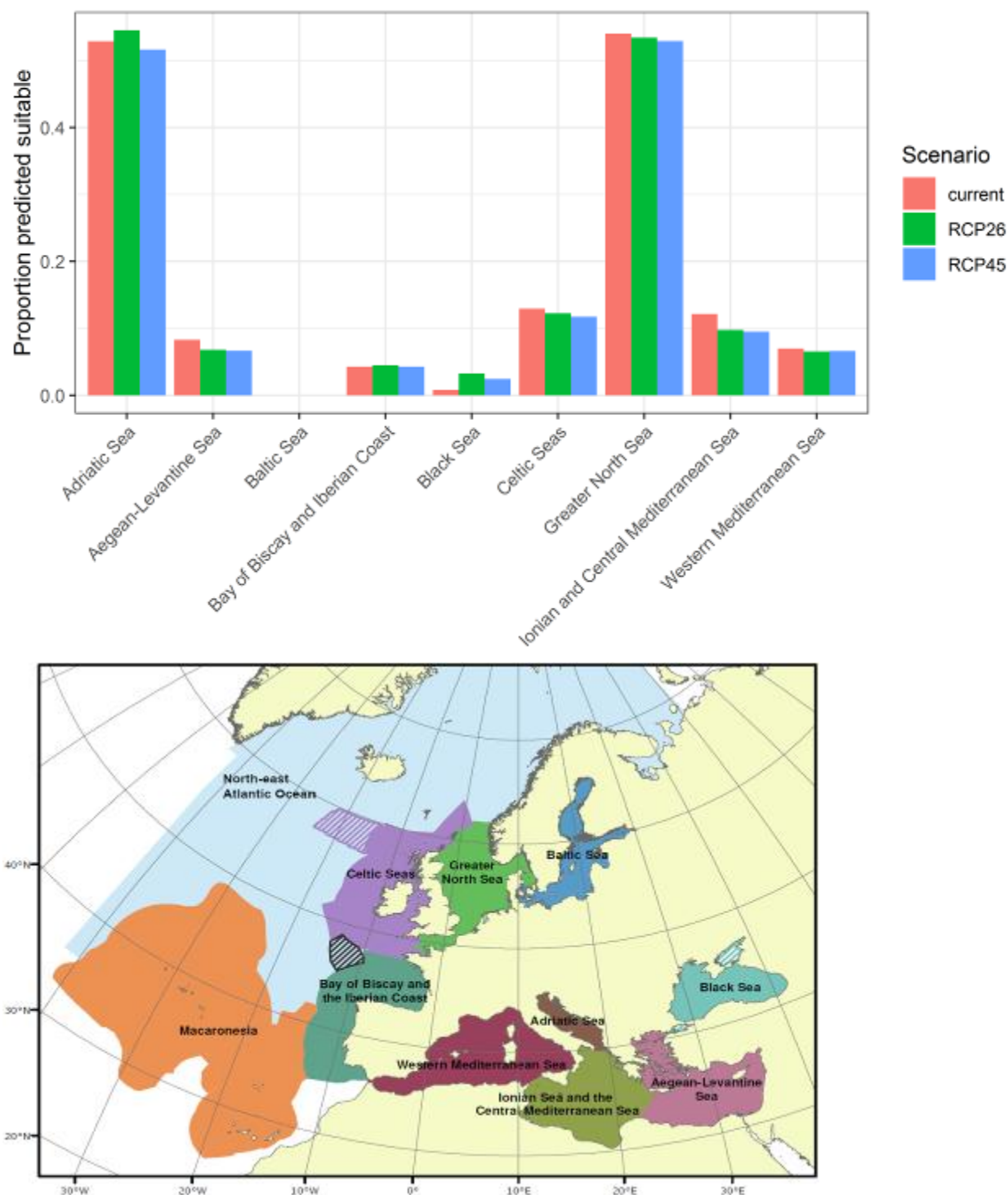
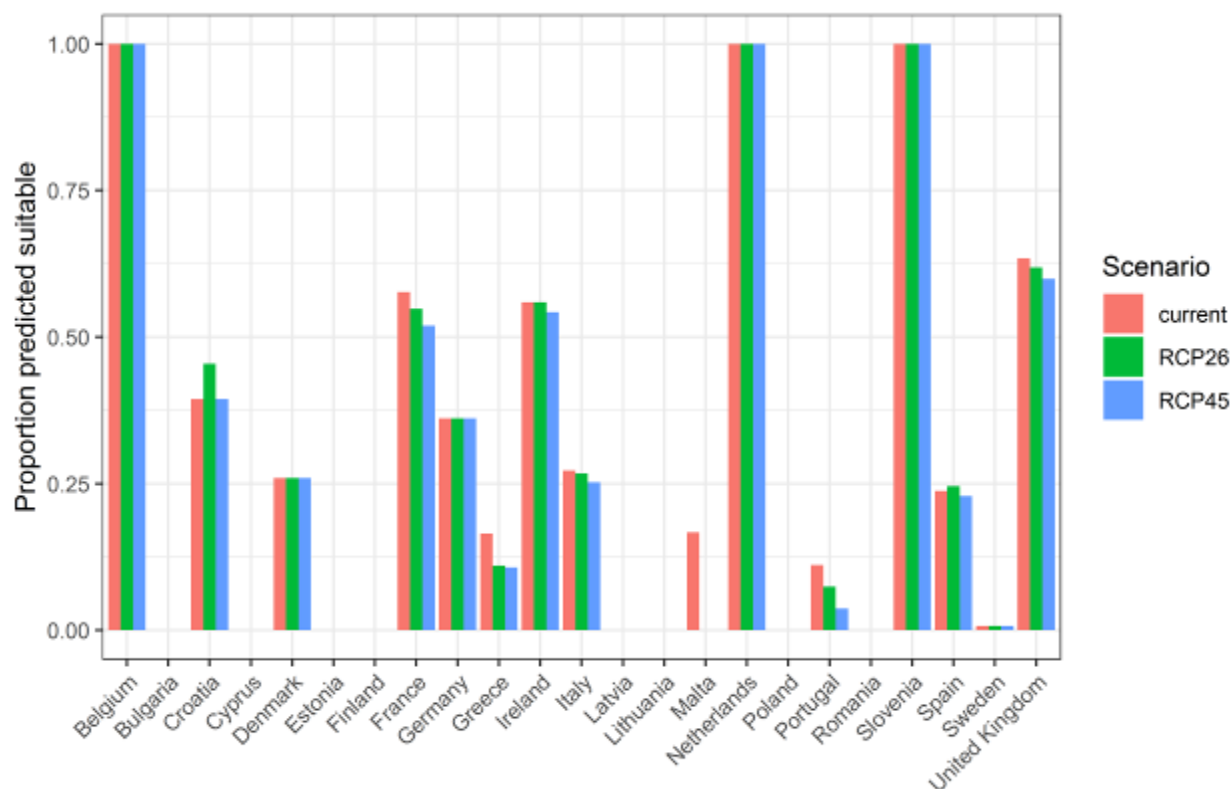


Table 2. Variation in projected suitability for *Asterias amurensis* establishment among marine subregions of Europe (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Region	Current climate	RCP26	RCP45
Adriatic Sea	0.53	0.55	0.52
Aegean-Levantine Sea	0.08	0.07	0.07
Baltic Sea	0.00	0.00	0.00
Bay of Biscay and Iberian Coast	0.04	0.04	0.04
Black Sea	0.01	0.03	0.02
Celtic Seas	0.13	0.12	0.12
Greater North Sea	0.54	0.53	0.53
Ionian and Central Mediterranean Sea	0.12	0.10	0.10
Western Mediterranean Sea	0.07	0.07	0.07

Figure 11. Variation in projected suitability for *Asterias amurensis* establishment among the 12-nautical-mile national waters of European Union countries and the UK. The bar plots show the proportion of grid cells in each country's waters classified as suitable in the current climate and projected climates for the 2070s under two RCP emissions scenarios. The location of each region is also shown.



12-nautical-mile national waters of European Union countries and the UK

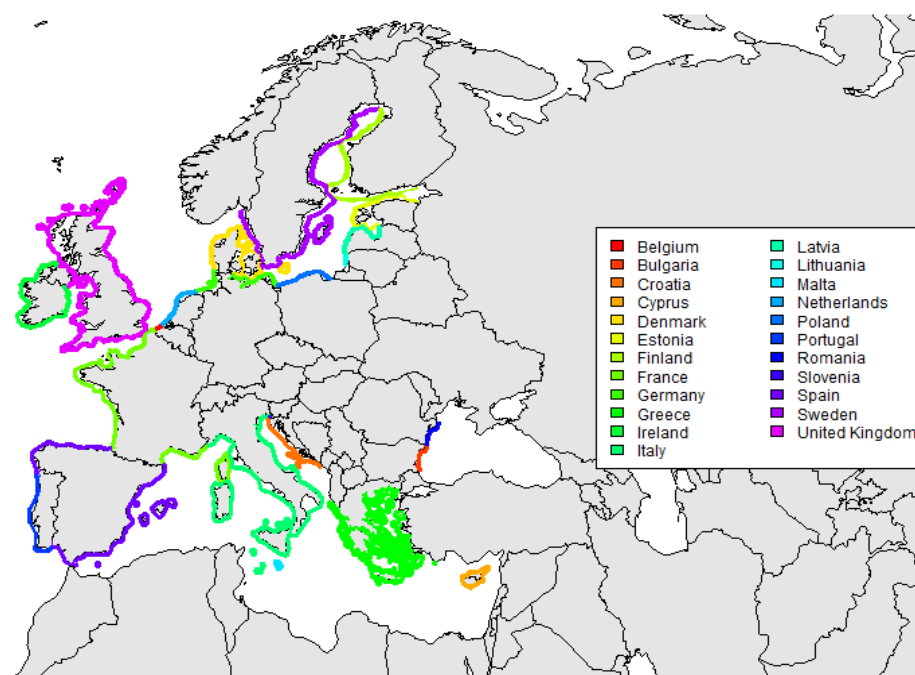


Table 3. Variation in projected suitability for *Asterias amurensis* establishment among 12-nautical-mile national waters of European Union countries and the UK (numerical values of Figure 11 above). The numbers are the proportion of grid cells in each country's waters classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Country	Current climate	RCP26	RCP45
Belgium	1.00	1.00	1.00
Bulgaria	0.00	0.00	0.00
Croatia	0.39	0.45	0.39
Cyprus	0.00	0.00	0.00
Denmark	0.26	0.26	0.26
Estonia	0.00	0.00	0.00
Finland	0.00	0.00	0.00
France	0.58	0.55	0.52
Germany	0.36	0.36	0.36
Greece	0.17	0.11	0.11
Ireland	0.56	0.56	0.54
Italy	0.27	0.27	0.25
Latvia	0.00	0.00	0.00
Lithuania	0.00	0.00	0.00
Malta	0.17	0.00	0.00
Netherlands	1.00	1.00	1.00
Poland	0.00	0.00	0.00
Portugal	0.00	0.00	0.00
Portugal	0.00	0.00	0.00
Portugal	0.11	0.07	0.04
Romania	0.00	0.00	0.00
Slovenia	1.00	1.00	1.00
Spain	0.00	0.00	0.00
Spain	0.24	0.25	0.23
Sweden	0.01	0.01	0.01
United Kingdom	0.63	0.62	0.60

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Asteroidea records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 4). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as underwater vegetation were not included in the model.

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