RISK ASSESSMENT SUMMARY SHEET

Dikerogammarus bispinosus

- A freshwater amphipod crustacean, similar to *D. villosus*.
- Spread across Europe, including to France and Netherlands, from the Ponto-Caspian region along shipping / canal routes.
- Modelling indicates it is capable of establishing across most of England, eastern Wales and eastern Scotland.
- Likely to be less invasive than *D. villosus*, but could displace native species and disrupt ecosystem processes (e.g. nutrient cycling).

Photograph: Denis Copilaș-Ciocianu

History in Europe

Not yet present in GB. It spread across Europe from the Black Sea basin though several invasion corridors following the connection of river basins by canal and reached the lower Rhine in 2008. From these water bodies introduction to GB may occur via ballast, on boats or with angling gear.

Global Distribution

Native to the Black Sea region. This species has invaded many European countries: areas in red (right) show distribution and year of first record.

It is not known to be introduced elsewhere.

Source: Copilaș-Ciocianu & Arbačiauskas, 2018
https://www.reabic.net/journals/bir/2018/3/BIR_2018_Copilas_Arbaciauskas.pdf

Impacts

Environmental: (moderate, medium confidence)

- None reported explicitly for *D. bispinosus.* However, strong competition and intra-guild predation is likely to lead to the displacement of shredding-efficient native amphipods from local habitats and disrupt nutrient flow.
- Less competitive than *D. villosus*.
- Potential to introduce novel parasites into British freshwaters and infect native amphipods.

Economic: (minor, medium confidence)

- None reported, but may have an adverse effect upon aquaculture by preying on eggs and larvae of several economically-important fish species.

Societal: (minimal, high confidence)

- None reported.

Introduction pathway

Most likely pathway is introduction as a hitchhiker with ballast, boats or angling equipment.

Spread pathway

Natural: (moderate, medium confidence) – moves through connected waterways through active swimming and passive drift.

Human: (major, high confidence) – similar to introduction pathways, spread is likely to be facilitated by angling and boating in GB.

Summary

	Response	Confidence
Entry	LIKELY	MEDIUM
Establishment	LIKELY	HIGH
Spread	RAPIDLY	HIGH
Impact	MODERATE	MEDIUM
Overall risk	MEDIUM	MEDIUM

GB NON-NATIVE ORGANISM RISK ASSESSMENT SCHEME

Name of organism: *Dikerogammarus bispinosus*
Author: Daniel Warren, Swansea University
Risk Assessment Area: Great Britain
Version: Draft 1 (May 2021), Peer review (Jul 2021), NNRAP 1 (Nov 2021), Draft 2 (Feb 2022), NNRAP 2 (Mar 2022), Draft 3 (Apr 2022), NNRAF (Dec 2022)
Signed off by NNRAF: December 2022
Approved by GB Committee: January 2024
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What is the principal reason for performing the Risk Assessment?

The GB Committee for non-native species is considering whether to add this species to the list of species of special concern. This assessment will form part of the evidence used to inform the Committee's decision. This species was selected for consideration following horizon scanning, in which *Dikerogammarus bispinosus* was ranked in the top 20 threats to biodiversity because of its potential to arrive, establish and cause negative biodiversity impact.

SECTION A – Organism Information	
Stage 1. Organism Information	**RESPONSE and COMMENT**
1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?	Yes. *Dikerogammarus bispinosus* (Martynov, 1925) – no common name Taxonomic Hierarchy: Animalia, Arthropoda, Crustacea, Malacostraca, Amphipoda, Gammaridae *D. bispinosus* was originally described as a subspecies of *D. villosus*, but was later recognised as a separate species, based on both morphological and molecular characteristics (Müller et al., 2002; Wattier et al., 2006). Differentiating between *D. bispinosus* and other Ponto-Caspian amphipod species in Great Britain (e.g. *D. villosus* and *D. haemobaphes*) is likely to be relatively simple, but will require adequate magnification to identify key morphological characteristics located on the first and second urosome (pointed dorsal protuberance with two main spines), second antenna (dense and long setation on the base (i.e. peduncle) and tip (i.e. flagellum) and gnathopods (long and dense setation on the propodus (i.e. palm); see Müller et al. (2002) for table of species-specific characteristics).
2. If not a single taxonomic entity, can it be redefined? (if necessary use the response box to re-define the organism and carry on)	NA
3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment)	No
4. If there is an earlier risk assessment is it still entirely valid, or only partly valid?	NA

5. Where is the organism native?	The Ponto-Caspian region, specifically the Black Sea basin (Cărăuşu et al., 1955; Jażdżewski & Konopacka,1988), and the lower stretches of the major rivers which drain into the Black Sea itself (e.g. lower Dnieper and Danube; Martynov, 1925; Borza et al., 2015). *D. bispinosus* appears to have experienced significant declines within its native range (e.g. lower Danube), recorded over the past decades, and has also become apparently absent in some regions (e.g. downstream of the Tisza estuary). *D. bispinosus* has also experienced considerable declines in some non-native regions, such as Lake Balaton where it was introduced in 1950 (Borza et al., 2015, 2017). Whilst there is no specific explanation as to these declines, it may be the result of antagonistic predatory (i.e. intraguild predation) and competitive interactions with other Ponto-Caspian native species, such as *D. villosus*, as well as cannibalism amongst conspecific amphipods (Kinzler et al., 2009).
6. What is the global distribution of the organism (excluding the risk assessment area)?	In addition to its natural range (i.e. Black Sea basin), *D. bispinosus* has been found in the Lower Rhine (Netherlands), Upper Rhine (France), Upper Danube (Germany, Austria), Middle Danube (Hungary, Slovakia, Croatia), Lower Danube (Romania), Lake Balaton (Hungary), Upper Dniester (Ukraine), Lower and Middle Dniester (Moldova/Ukraine), Odessa Gulf (Ukraine), Bug (Belarus; needs further confirmation), Lower Dnieper (Ukraine), Lower Don (Russia), Middle Volga (Russia), Lower Ural (Kazakhstan). In Western Europe, *D. bispinosus* has spread throughout the southern invasion corridor, invading the Rhine estuary via the Rhine-Main-Danube canal. In the late 1990's, *D. bispinosus* also invaded parts of European Russia, spreading from the Black Sea basin into the Caspian Sea basin along the northern invasion corridor (i.e. Volga-Don canal; Copilas-Coicianu & Arbačiauskas, 2018). This route of dispersal is supported by first reports from the Caspian basin in Saratov Reservoir (Volga river) between 2002 and 2006 (Yu & Yermokhin, 2004; Filinova & Sonina, 2012), and throughout the lower Don (Black Sea basin) in 2003 (Sayapin, 2003). From the Volga-Don canal, *D. bispinosus* has dispersed a significant distance eastward, having been found in the Ural River, located more than 800km from the Volga-Don canal, by Copilas-Coicianu & Arbačiauskas (2018).
7. What is the distribution of the organism in the risk assessment area?	No current distribution within Great Britain known

	Given the strong morphological similarities between *D. bispinosus* and *D. villosus*, it may be possible that *D. bispinosus* entered Great Britain with *D. villosus*, if translocated from a region with overlapping populations (e.g. Kley & Maier, 2005). However, with continued identification of *D. villosus* (and no reports of *D. bispinosus*) in invaded UK localities, including Grafham Water Reservoir (Cambridgeshire; Anglian Water, pers. comms.), Barton Broads (Norfolk; Bojko et al., 2013), Pitsford Reservoir (Northamptonshire; Clinton et al., 2018), and Cardiff Bay and Eglwys Nunydd (Glamorgan; MacNeil et al., 2012) – it is unlikely that *D. bispinosus* is already present in Great Britain.
8. Is the organism known to be invasive (i.e. to threaten organisms, habitats or ecosystems) anywhere in the world?	Yes. Laboratory-based studies have identified *D. bispinosus* as a highly adapted predatory omnivore, capable of outcompeting native European gammarids, such as *Gammarus roeselii*, for native prey species (Pöckl, 2012). *D. bispinosus* also appears to be a strong predator of smaller native amphipod species within mainland Europe (Pöckl, 2012). However, there have been no direct field studies to assess its impact within invaded regions.
9. Describe any known socio-economic benefits of the organism in the risk assessment area.	None

SECTION B – Detailed assessment

PROBABILITY OF ENTRY

Important instructions:

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

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.1. How many active pathways are relevant to the potential entry of this organism? (If there are no active pathways or potential future pathways respond N/A and move to the Establishment section)	few	high	
1.2. List relevant pathways through which the organism could enter. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 1.3 to 1.10 (copy and paste	i. Hitchhiking with desirable commodity species. ii. Hitchhiker in ship ballast / on hull or boating and / or angling equipment.		

additional rows at the end of this section as necessary).			
Pathway name:	i. Hitchhiking with a desirable commodity species (e.g. amphipods).		
i.1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)? (If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)	accidental	high	Historically, Ponto-Caspian amphipod species, such as *D. villosus* – and by extension *D. bispinosus* (likely mis-identified as *D. villosus*; see Müller et al., 2002; Wattier et al., 2006) – have been translocated and introduced into novel regions (e.g. the canals and rivers of Ukraine) as a resource species for commercially valuable fish (i.e. fish food; Grigorovich et al., 2002 and references therein). To-date, some amphipod species are still considered to be important resources for aquaculture (e.g. Jiménez-Prada et al., 2018; Vargas-Abúndez et al., 2021). In Great Britain, this is unlikely to take place through the activities of organisations with high environmental awareness, however, there is a small, yet possible, risk of independent aquaculturists/fisheries importing amphipods from mainland Europe, to bolster stock populations. For example, the introduction of *D. villosus* into Pitsford Reservoir was supposedly linked to the intentional release of invasive amphipods, removed from Grafham Water, by anglers (Anglian Water, pers. comms.).
i.1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?	unlikely	high	Based on previous observations, recorded in relation to other Ponto-Caspian amphipod species (e.g. *Pontogammarus robustoides*), it is possible that a large number of organisms will be translocated within a single event. For example, Arbačiauskas & Rakauskas (2010) reported the establishment of three Ponto-Caspian amphipod species (*P. robustoides*, *Obesogammarus crassus*, and *Chaetogammarus warpachowskyi*) in a reservoir in Lithuania, following the introduction of 1600 individuals in the early 1960's.

Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.			The introduction of *D. bispinosus* along with a commodity species seems unlikely, but if it were to occur then it is possible that high numbers of animals (including *D. bispinosus*) would be introduced.
i.1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	very high	If *D. bispinosus* is accidentally translocated as a contaminant, alongside desirable invertebrate stock, it is highly likely that conditions during transfer will be very good, allowing a large proportion of amphipods to survive translocation from source populations. Reproduction during transit is possible, although unlikely. However, amphipods brood their young, and it is likely that brooding females, carrying up to 40 eggs (Kley & Maier, 2006), would be introduced via this pathway, leading to the release of young during and after transit.
i.1.6. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	very high	If *D. bispinosus* is being released alongside desirable commodity species, existing management practices will ensure high probability of survival.
i.1.7. How likely is the organism to enter the risk assessment area undetected?	very likely	very high	Should the release of *D. bispinosus* occur alongside a commodity species then it is likely that both the commodity species and *D. bispinosus* would go undetected until an abundant population became established in the wild. For example, when *D. villosus* was first identified in Grafham Water, a large population of amphipods had already become established (MacNeil et al., 2010). Non-detection is likely to be especially true for *D. bispinosus*, if misidentified as another *Dikerogammarus* species (e.g. *D. villosus*;

			Müller et al., 2002; Wattier et al., 2006).
i.1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	very high	*D. bispinosus* is capable of breeding throughout most of the year, excluding autumn/winter months (October-December) when reproductively active females often become absent from regions (Kley & Maier, 2006). Propagules translocated during spring/summer months (April – August), are likely to contain a relatively high proportion of brooding (ovigerous) females, particularly during April/May (Kley & Maier, 2006).
i.1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very likely	very high	The intentional introduction of amphipod sub-populations will release *D. bispinosus* into a new habitat, if accidentally collected along with desirable amphipod species.
i.1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very unlikely	medium	
End of pathway assessment, repeat as necessary.			
Pathway name:	ii. Hitchhiker in ship ballast (water and/or sediment) or attached to hull, or boating and/or angling equipment (e.g. mooring ropes, fishing nets).		
ii.1.3. Is entry along this pathway intentional (e.g. the organism is imported for trade) or accidental (the organism is a contaminant of imported goods)?	accidental	high	Release as a hitchhiker is likely to be the most important point of entry for invasive freshwater invertebrates (Knight et al., 2017). If *D. bispinosus* populations occur in regions where there is shipping/boating activity, then *D. bispinosus* may be accidentally taken up in ballast water (collected in freshwater ports), or become attached to the hull and/or submerged shipping/boating equipment,

(If intentional, only answer questions 1.4, 1.9, 1.10, 1.11)			such as mooring ropes (again in freshwaters). Indeed, unintentional introductions via shipping activities has been identified as a main form of entry into freshwaters across Western Europe – via the Rhine-Main-Danube canal, as well as European Russia via the Volga-Don canal (Copilas-Ciocianu & Arbačiauskas, 2018). Similarly, if recreational activities, such as angling, take place in regions colonised by *D. bispinosus*, it may also be possible for multiple individuals to become attach to, and be translocated by, various pieces of angling equipment (e.g. fishing nets). Equipment fouling has been shown to be a key route of invasion for other invasive amphipod species, including *D. villosus* (Anderson et al., 2014; Smith et al., 2020). As such, this route is also likely to be important for *D. bispinosus*.
ii.1.4. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year? Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place.	likely	medium	Depending on the volume of water taken up in ship/boat ballasts, a relatively large sub-population of amphipods may be collected, particularly within sediment and/or aquatic vegetation. Translocation on the hulls and/or equipment of commercial/recreational boats/ships is likely to involve a relatively small sub-population of amphipods - depending on how successful they are to attaching and persisting on materials. For example, previous studies have demonstrated that other *Dikerogammarus spp.* (e.g. *D. haemobaphes*) can effectively attach to lengths of sailing rope, with a relatively high percentage of amphipods (24%) remaining on the ropes, even after attempts to remove them via rope shaking (Bacela-Spychalska, 2015). Probability of release for large numbers of amphipod organisms within a single year will be largely dependent on the volume of

			traffic between regions (e.g. Anderson et al., 2014; Smith et al., 2020).
ii.1.5. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	likely	very high	*D. bispinosus* is a freshwater/brackish-tolerant species and is therefore likely to survive translocation in the absence of ballast exchange. *Dikerogammarus* species are often euryoecious, with some species exhibiting a wide-ecophysiological tolerance to conditions such as low oxygen concentration, high temperature (up to 30^{o}C), and salinities up to 20%, making them adapted to survive transport in freshwater/brackish ballast water (Bruijs et al., 2001; Rachalewski et al., 2018). *D. bispinosus* is also tolerant to high levels of alkalinity, water conductivity (linked to salinity), and pollution (e.g. nitrates/sulphates; Gallardo & Aldridge, 2013). Previous literature has also reported effective long-term survival in *Dikerogammarus* species whilst attached to angling/boating equipment, again in the absence of management. For example, *D. villosus* has been shown to survive outside of water for up to 16 days, if kept in damp conditions (Anderson et al., 2015).
ii.1.6. How likely is the organism to survive existing management practices during passage along the pathway?	moderately likely	medium	If ballast water exchange takes place at sea (i.e. salinity > 3%; Bruij et al., 2001) then *D. bispinosus* is unlikely to survive because it is a freshwater/brackish-tolerant species. Controlling the spread of potentially invasive species via ballast water has improved somewhat, following the implementation of the International Convention for the Control and Management of Ballast Water and Sediments (BWM Convention, 2004), which aims to improve maritime biosecurity through the installation of management systems, by conducting regulated ballast water exchanges at sea under strict conditions (at least 200 nautical miles from land, and at least 200 metres deep), and by maintaining accurate records of ballast water exchanges for monitoring and management purposes.

			If boating/sailing equipment is adequately decontaminated, via thermal exposure (e.g. hot water spray/steam; Anderson et al., 2015; Shannon et al., 2018; Bradbeer et al., 2020, 2021), application of chemical disinfectants (e.g. Virkon; Bradbeer et al., 2020), followed by an appropriate period of drying, then *D. bispinosus* is unlikely to survive. However, it is important to note that survival depends on the consistency with which management practices are applied. National initiatives, such as "Check, Clean, Dry", aim to improve biosecurity capacity amongst stakeholders, industry, and the public. Whilst these initiatives are often readily adopted by stakeholders with high environmental awareness (e.g. Environment Agency, Anglian Water, etc.), consistent application of risk reducing measures by the public can vary, particularly in regular water-users which frequently visit multiple sites within a short period of time (e.g. two weeks; Smith et al., 2020). With appropriate training (e.g. E-learning), signage, access to biosecurity equipment, and monitoring/management at sites, awareness of these initiatives may increase (Shannon et al., 2020), although efficacy and consistency of implementation remains unclear, but is expected to vary.
ii.1.7. How likely is the organism to enter the risk assessment area undetected?	very likely	high	In the absence of surveillance, it is likely to go undetected. Detection is possible if there is appropriate inspection of ballast tanks, boat hulls and equipment. However, if amphipods are situated within aquatic vegetation, or within equipment (e.g. weave of mooring rope), then it may be more difficult to detect invaders.
ii.1.8. How likely is the organism to arrive during the months of the year most appropriate for establishment?	very likely	high	*D. bispinosus* is capable of breeding throughout most of the year, excluding autumn/winter months (October-December) when reproductively active females often become absent from regions (Kley & Maier, 2006). Propagules translocated during spring/summer

			months (April – August), are likely to contain a relatively high proportion of brooding (ovigerous) females, particularly during April/May (Kley & Maier, 2006). As such, large numbers of young invaders may be released into novel regions.
ii.1.9. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	very likely	high	Ballast exchange within freshwater systems will release *D. bispinosus* into novel regions. The use of contaminated angling/boating equipment will provide opportunity for release.
ii.1.10. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	likely	low	Probability of entry as a hitchhiker is likely, having been frequently cited as a primary invasion pathway for other Ponto-Caspian amphipod species (e,g. *D. villosus*, *D. haemobaphes*), both in Great Britain and in Europe. However, probability of release is likely to be dependent on the volume of traffic between regions (e.g. Anderson et al., 2014; Smith et al., 2020).
End of pathway assessment, repeat as necessary.			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways (comment on the key issues that lead to this conclusion).	likely	medium	The overall probability of *D. bispinosus* entering Great Britain is high/likely, expected to be facilitated by two main entry routes: release alongside a commodity species and as a hitchhiker. Release as a hitchhiker from ballast water or equipment is the more likely route of entry, having been identified as a primary invasion pathway for other Ponto-Caspian species. Accidental release alongside an internationally traded commodity species (e.g. fish) is less likely to facilitate its entry into Great Britain, but may facilitate its spread nationally if domestic exchanges between fisheries does occur. However, this form of translocation is expected to be driven by unofficial activities by independent aquaculturists only, rather than by the actions of organisations with high environmental awareness. Given its long-term presence in the Netherlands – a region from which entry is likely due to high volumes of trade – and evidence

			indicating that the time between first recordings of invaders in the Netherlands and then Great Britain has reduced significantly over recent years, it is likely that *D. bispinosus* may enter Great Britain in the near future, if not already (see Gallardo & Aldridge, 2015).

PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already well established in the risk assessment area, only complete questions 1.15, 1.21 and 1.28 then move onto the spread section. If uncertain, check with the Non-native Species Secretariat.

QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.12. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions in the risk assessment area and the organism's current distribution?	likely	high	Climatic conditions in Great Britain are similar to those in invaded European regions. Climatic models have identified large areas of Britain which demonstrate a high degree of suitability for invasion, based on conditions such as temperature and precipitation; particularly Thames, Anglian, Severn and Humber River basin districts (Gallardo & Aldridge, 2013). As climatic conditions are predicted to continue to change, it is expected that the potential invasion range may increase further, as regions become more suitable (Gallardo & Aldridge, 2020).
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions in the risk assessment area and the organism's current distribution?	likely	high	When compared to other *Dikerogammarus* species (e.g. *D. villosus*), *D. bispinosus* exhibits a greater tolerance to variable water/current velocities (i.e. rheotolerant; Borza et al., 2017), and as such is likely to survive in various lotic freshwater systems. There are numerous slow flowing and lentic freshwater habitats in Great Britain that are similar to the native range, as well as those in invaded European regions. Water chemistry is also a strong predictor associated with the potential for *D. bispinosus* to become established in Great Britain, particularly alkalinity. Similarities in alkalinity (>120mg/L), recorded in Great Britain, the Ponto-Caspian region, and invaded areas of Europe, suggest that most of England, the eastern part of Wales, and the east coast of Scotland are predicted to be highly suitable for *D. bispinosus* establishment (Gallardo & Aldridge, 2013). Similarities in abiotic

			conditions, such as water conductivity, salinity, nitrate concentrations and dissolved organic carbons, are also likely to facilitate establishment of *D. bispinosus* and other Ponto-Caspian amphipods (Gallardo & Aldridge, 2013; Cuthbert et al., 2020).
1.14. How likely is it that the organism will become established in protected conditions (in which the environment is artificially maintained, such as wildlife parks, glasshouses, aquaculture facilities, terraria, zoological gardens) in the risk assessment area? Subnote: gardens are not considered protected conditions	likely	high	If *D. bispinosus* is introduced to an aquaculture facility (e.g. commercial fisheries), for example as a contaminant alongside resource species, it is likely to become established.
1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?	widespread	high	Freshwater lakes, reservoirs, rivers and streams are all very common in Great Britain. Refer to comments already provided in response to 1.12 and 1.13.
1.16. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such	NA	NA	

species in the risk assessment area?			
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	moderately likely	medium	*D. bispinosus* may become established in freshwaters inhabited by competing species (e.g. *Gammarus pulex*). Lab studies show *D. bispinosus* to be a strong competitor against other amphipod species (Pöckl, 2012). As such, there is likely to be some niche partitioning, with *D. bispinosus* colonising regions comprising of simple habitat and sparse vegetation, whereas native *G. pulex* is likely to occupy complex habitat consisting of dense vegetation. *D. bispinosus* is also likely to exist at greater depths, although if *D. villosus* is already present in an invaded region, this may prevent establishment of *D. bispinosus*, as *D. villosus* is an even stronger competitor (Kley & Maier, 2005). Similarly, competition in regions containing *D. haemobaphes* may prevent establishment of *D. bispinosus*, with both *Dikerogammarus* species often occupying deeper waters (Grabowski & Bacela, 2005), however, this might subsequently lead to *D. bispinosus* becoming established further upstream (see Kley & Maier, 2005).
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	likely	medium	*Dikerogammarus* species demonstrate highly flexible, effective, antipredator responses, providing a competitive advantage over native amphipod species, which are more likely to be preyed upon (Briffa et al., 2016; Jermacz & Kobak, 2018; Rolla et al., 2020). *D. bispinosus* may potentially acquire some parasites from native species. For example, Bojko et al. (2019) reported the potential for *D. haemobaphes* and *D. villosus*, currently present in Great Britain, to acquire *Cucumispora ornata*, a microsporidian which has been detected in native *G. pulex*. Although this fungus negatively affects host

			survival, it is not very common amongst *Dikerogammarus* species, and the effects are not very strong.
1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?	very likely	high	There are no management practices that would remove it. However, some biosecurity initiatives, such as "Check, Clean, Dry" may be effective in preventing the initial introduction of amphipods into waterways, thereby preventing their establishment along recreational pathways.
1.20. How likely are management practices in the risk assessment area to facilitate establishment?	unlikely	high	*D. bispinosus* is likely to invade wild freshwater habitat. Therefore, management practices are unlikely to affect establishment. However, the unlicensed movement of amphipods from Europe, as fish food, may inadvertently facilitate establishment in GB waters.
1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?	likely	high	There are few possible mechanisms of eradication following the establishment of *D. bispinosus* in invaded regions, such as the treatment of whole water courses with pyrethroids (e.g. lambda-cyhalothrin). However, given the higher tolerance of some *Dikerogammarus* species to pyrethroids when compared to native amphipods (5-fold higher tolerance; Bundschuh et al., 2013), it is highly likely that some animals would survive the treatment of an area. Moreover, treatment of these areas using highly toxic chemicals, is likely to have a significant, adverse, and long-lasting impact on native flora and fauna.
1.22. How likely are the biological characteristics of the organism to facilitate its establishment?	likely	high	*D. bispinosus* is a strong competitor of native European amphipods, capable of intraguild predation (Pöckl, 2012). *D. bispinosus* also demonstrates high reproductive capacity, particularly in Spring/Summer months, when ovigerous females produce relatively large eggs (up to 0.15mm^3 in volume), carrying between 10 and 40 eggs per clutch, and exhibit high fecundity and reproductive effort (Kley & Maier, 2006). However, estimates of clutch size and egg volume are comparable to those of native *G. pulex*, which generate

			between 6 and 29 eggs per clutch, with each egg measuring at approximately 0.11mm^3 in volume (Sutcliffe, 1992).
1.23. How likely is the capacity to spread of the organism to facilitate its establishment?	likely	high	Natural dispersal via interconnected waterways has been identified as a primary mode of spread and establishment for *D. bispinosus*, and other *Dikerogammarus* species, in some European countries (Grigorovich et al., 2002; Arbačiauskas et al., 2011; Copilas-Ciocianu & Arbačiauskas, 2018). However, accidental anthropogenic movement, either in ballast water or attached to boating/angling equipment, is likely to contribute far more to the spread of *D. bispinosus* within Great Britain (Anderson et al., 2014; Knight et al., 2017; Smith et al., 2020).
1.24. How likely is the adaptability of the organism to facilitate its establishment?	likely	high	A wide eco-physiological tolerance to conditions such as current velocity, temperature, alkalinity, salinity, water conductivity and pollution is likely to aid in the establishment of *D. bispinosus* in Great British freshwaters (Gallardo & Aldridge, 2013; Borza et al., 2017). As with other *Dikerogammarus* species, *D. bispinosus* is likely to demonstrate flexible omnivory, capable of consuming a wide range of prey items (Dick et al., 2002).
1.25. How likely is it that the organism could establish despite low genetic diversity in the founder population?	very likely	high	Whilst there is no published evidence regarding the effects of post-invasional genetic bottlenecking on the establishment of *D. bispinosus*, there is some published literature concerning other invasive amphipods, particularly *Dikerogammarus* species. Many invasive amphipod species have experienced post-invasional genetic bottlenecking, which does not appear to have impacted upon establishment. *Dikerogammarus haemobaphes*, *Gammarus tigrinus*, and *Echinogammarus ischnus* have undergone varying degrees of genetic bottlenecking whilst spreading throughout Europe, and North America (Muller et al., 2002; Cristescu et al. 2004; Kelly et al., 2007).

1.26. Based on the history of invasion by this organism elsewhere in the world, how likely is to establish in the risk assessment area? (If possible, specify the instances in the comments box.)	very likely	high	There have been numerous instances of establishment in new regions within mainland Europe including the Rhine estuary, parts of European Russia, and the Caspian Sea Basin via the Volga-Don canal (Copilas-Coicianu & Arbačiauskas, 2018). Environmental conditions in Great Britain are similar to those of several invaded regions, hence establishment is likely if introduced.
1.27. If the organism does not establish, then how likely is it that transient populations will continue to occur? Subnote: Red-eared Terrapin, a species which cannot re-produce in the risk assessment area but is established because of continual release, is an example of a transient species.	likely	medium	Repeated introductions of *D. bispinosus* sub-populations, via the invasion pathways described above, are likely to lead to new or transient populations.
1.28. Estimate the overall likelihood of establishment (mention any key issues in the comment box).	likely	high	If introduced, it is likely to become established in Great Britain.

PROBABILITY OF SPREAD			
Important notes: • Spread is defined as the expansion of the geographical distribution of a pest within an area.			
QUESTION	**RESPONSE**	**CONFIDENCE**	**EDITED COMMENT**
2.1. How important is the expected spread of this organism in the risk assessment area by natural means? (Please list and comment on the mechanisms for natural spread.)	moderate	medium	Once established in Great British freshwaters, it is possible that *D. bispinosus*, as with other *Dikerogammarus* species, may disperse naturally along interconnected waterways (Grigorovich et al., 2002; Arbačiauskas et al., 2011; Copilas-Ciocianu & Arbačiauskas, 2018). Natural dispersal is likely to be a combination of active (i.e. swimming) and passive (drift) mechanisms (Gallardo et al., 2012). It is also possible that active dispersal may be promoted by interactions with strong competitors, such as other invasive amphipods (e.g. *D. villosus*; Kobak et al., 2016). Other potential modes of natural dispersal might include spread by migratory waterfowl, which has been reported for other amphipods (Swanson, 1984), and suggested for other *Dikerogammarus* species (Gallardo et al., 2012).
2.2. How important is the expected spread of this organism in the risk assessment area by human assistance? (Please list and comment on the mechanisms for human-assisted spread.)	major	high	The spread of *D. bispinosus* within Great Britain is likely to occur via the same vectors associated with initial entry (excluding ballast water). Human-assisted dispersal is likely to be a major driver of spread for *D. bispinosus* within Great Britain, providing multiple opportunities for introductions into novel habitats. The movement of commercial / recreational vessels (e.g. canal boats, narrowboats, kayaks, canoes, sailing dinghies) between and within freshwater systems could facilitate the spread of *D. bispinosus* sub-populations, as hitchhikers attached to hulls and/or boating equipment (e.g. mooring ropes; (Anderson et al.,

			2014; Bacela-Spychalska, 2015 Copilas-Ciocianu & Arbačiauskas, 2018). Similarly, if recreational activities, such as angling, take place in regions colonised by *D. bispinosus*, it may also be possible for sub-populations to attach to, and be spread by, various pieces of angling equipment (e.g. fishing nets), as reported in other *Dikerogammarus* species (Anderson et al., 2014; Smith et al., 2020).
2.3. Within the risk assessment area, how difficult would it be to contain the organism?	very difficult	high	*D. bispinosus* is also unlikely to be detected until a sufficiently large population has been established. As such containment is likely to be very difficult.
2.4. Based on the answers to questions on the potential for establishment and spread in the risk assessment area, define the area endangered by the organism.	see comment	high	A large area of Great Britain, particularly the Thames, Anglian, Severn and Humber river basin districts. "At risk" regions also include most of England, the eastern part of Wales, and the east coast of Scotland. Suitability of at-risk regions is based on predictions reported by Gallardo & Aldridge (2013), which considered climatic conditions, and water chemistry characteristics (e.g. alkalinity).
2.5. What proportion (%) of the area/habitat suitable for establishment (i.e. those parts of the risk assessment area were the species could establish), if any, has already been colonised by the organism?	0-10	high	*D. bispinosus* is not believed to be currently present in Great Britain.
2.6. What proportion (%) of the area/habitat suitable for establishment, if any, do you expect to have been invaded	0-10	medium	The likelihood of introduction into Great Britain is probably quite low. However, if introduced, the potential spread of *D. bispinosus* within Great Britain is unlikely to be contained.

by the organism five years from now (including any current presence)?			
2.7. What other timeframe (in years) would be appropriate to estimate any significant further spread of the organism in the risk assessment area? (Please comment on why this timeframe is chosen.)	40	high	Based on the predicted spread of invasive freshwater organisms within Great Britain, it is likely that, following the introduction of *D. bispinosus* into British freshwaters, a relatively large area of Britain may become invaded over the next 30 – 40 years. For both *D. villosus* and *D. haemobaphes*, Gallardo & Aldridge (2020) predicted a potential range increase of 15 – 18% (best-case scenario) and 24 – 28% (worst-case scenario) by 2050. With large areas of Great Britain identified as potentially suitable for invasions by *D. bispinosus* (see Gallardo & Aldridge, 2013), similar rates of spread may be expected by *D. bispinosus* over a similar time frame, once established in Great Britain.
2.8. In this timeframe what proportion (%) of the endangered area/habitat (including any currently occupied areas/habitats) is likely to have been invaded by this organism?	10-33	low	See comments for section 2.7
2.9. Estimate the overall potential for future spread for this organism in the risk assessment area (using the comment box to indicate any key issues).	rapidly	high	If *D. bispinosus* becomes established in Great British freshwaters, any dispersal within/between systems is likely to be largely attributed to human-mediated translocation**,** with a relatively large area of Great Britain identified as highly suitable. Future, post-establishment spread is also likely to occur through interconnected waterbodies/waterways.

PROBABILITY OF IMPACT

Important instructions:

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

QUESTION	RESPONSE	CONFIDENCE	EDITED COMMENT
2.10. How great is the economic loss caused by the organism within its existing geographic range **excluding the risk assessment area**, including the cost of any current management?	minimal	medium	There are currently no studies that indicate any economic losses as a result of *D. bispinosus*. However, based on previous studies concerning other *Dikerogammarus* species, there may be an expected economic cost imposed by *D. bispinosus* towards aquaculture, particularly fisheries. For example, Taylor & Dunn (2017) reported a potentially significant predatory impact imposed by invasive *D. villosus* towards the eggs and larvae of several economically important UK fish species; the native brown trout (*Salmo trutta*), and the non-native ghost carp (*Cyprinus carpio*). Greater predation, when compared to native amphipods (*G. pulex*), was predicted to adversely affect fish recruitment, negatively impacting upon economically valuable activities, such as angling (Mawle & Peirson, 2009).
2.11. How great is the economic cost of the organism **currently** in the risk assessment	minimal	very high	*D. bispinosus* is not believed to be currently present in Great Britain.

area **excluding management** costs (include any past costs in your response)?			
2.12. How great is the economic cost of the organism likely to be **in the future** in the risk assessment area **excluding management** costs?	minor	medium	Whilst there are currently no studies concerning the potential economic cost of *D. bispinosus* in British freshwaters, this invader might be expected to have an adverse effect upon aquaculture (i.e. fisheries), as predicted in other *Dikerogammarus* species already present in Britain (see Taylor & Dunn, 2017).
2.13. How great are the economic costs **associated with managing** this organism **currently** in the risk assessment area (include any past costs in your response)?	minimal	very high	*D. bispinosus* is not believed to be currently present in Great Britain.
2.14. How great are the economic costs **associated with managing** this organism likely to be **in the future** in the risk assessment area?	minor	medium	There is no standard, ecologically sound method prescribed for the eradication of Ponto-Caspian amphipods from British freshwaters. Any economic costs associated with the future management of *D. bispinosus* in Great Britain are likely to be attributable to schemes to improve national biosecurity, and therefore prevent the spread of *D. bispinosus*, if it becomes established. "Check, Clean, Dry" is a nationally recognised initiative promoted by various non-/governmental organisations, including the non-native species secretariat, which focusses on low-cost, easy-to-implement management procedures to effectively eliminate invaders, and decontaminate clothing, as well as commercial/recreational equipment (GB NNSS, 2021). Dissemination of these biosecurity practices at a national scale, amongst stakeholders (e.g. Environment Agency), site managers (e.g. Anglian Water) and recreational water users (e.g. anglers), is likely to incur some economic costs.

2.15. How important is environmental harm caused by the organism within its existing geographic range **excluding the risk assessment area**?	minor	medium	There have been no published studies which explicitly evaluate the environmental impact of *D. bispinosus* within invaded regions. However, based on previously published laboratory-based evidence of potential ecological impact by *D. bispinosus*, it is possible that this Ponto-Caspian invader may disrupt some environmental processes. As with other *Dikerogammarus* species, strong competition and intra-guild predation is likely to lead to the displacement of native amphipods (MacNeil et al., 2011; Kobak et al., 2016). Amphipods are keystone detritivores in freshwater ecosystems, which process primary basal energy resources (e.g. submerged leaf litter), releasing nutrients for other aquatic organisms (e.g. filter feeders). As such, displacement of shredding-efficient native amphipod species (i.e. *G. pulex*) by *D. bispinosus* is likely to disrupt nutrient flow (MacNeil et al., 2011). This may be further exacerbated if *D. bispinosus* consumes other native shredders.
2.16. How important is the impact of the organism on biodiversity (e.g. decline in native species, changes in native species communities, hybridisation) **currently** in the risk assessment area (include any past impact in your response)?	minimal	very high	*D. bispinosus* is not believed to be currently present in Great Britain.
2.17. How important is the impact of the organism on biodiversity likely to be in the **future** in the risk assessment area?	moderate	medium	The largest impact to biodiversity may be towards native amphipods, which could be locally excluded from habitats.

			Laboratory-based studies have identified *D. bispinosus* as a highly adapted predatory omnivore, capable of outcompeting native European gammarids, such as *Gammarus roeselii*, for native prey species (Pöckl, 2012). *D. bispinosus* also appears to be a strong predator of smaller native amphipod species within mainland Europe (Pöckl, 2012). As with other *Dikerogammarus* species, *D. bispinosus* may consume various native macroinvertebrate species (Dick et al., 2010; Dodd et al., 2014). Predation may also extend to some vertebrate species, with previous studies having reported the potential for *D. villosus* to consume the early life stages of several fish (Taylor & Dunn, 2017), and amphibian species (Warren et al., 2021). However, given the paucity of empirical evidence concerning the predatory impact of *D. bispinosus* towards non-amphipod species, it is unclear as to the extent of impact within invaded regions; particularly when compared to other *Dikerogammarus* species. As *D. bispinosus* is omnivorous, it is unlikely to have a major impact on prey diversity. When compared to invasive *D. villosus*, impacts imposed by *D. bispinosus* are likely to be lower, potentially more akin to those of *D. haemobaphes*. This, in part, is likely to be due to having a comparably smaller body size (up to 16mm in length), when compared to *D. villosus* (up to 30mm in length; Dobson, 2012); which is considered to be the largest freshwater gammarid in Europe (Devin et al., 2003). A substantially larger body-size has previously been shown to explain the ecological impact of *D. villosus* towards freshwater macroinvertebrates (see Dodd et al., 2014), but also early-stage vertebrates as well (see Taylor & Dunn, 2017; Warren et al., 2021).

2.18. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services, caused by the organism **currently** in the risk assessment area (include any past impact in your response)?	minimal	very high	*D. bispinosus* is not believed to be currently present in Great Britain.
2.19. How important is alteration of ecosystem function (e.g. habitat change, nutrient cycling, trophic interactions), including losses to ecosystem services, caused by the organism likely to be in the risk assessment area in the **future**?	moderate	low	Gammaridean amphipods are omnivorous and are important shredders, processing detritus which then becomes available to other invertebrates. Displacement of native amphipod species, and a reduction in the abundance of shredding prey species, may disrupt nutrient flow within invaded regions (MacNeil et al., 2011; Pöckl, 2012).
2.20. How important is decline in conservation status (e.g. sites of nature conservation value, WFD classification) caused by the organism **currently** in the risk assessment area?	minimal	very high	*D. bispinosus* is not believed to be currently present in Great Britain.
2.21. How important is decline in conservation status (e.g. sites of nature conservation value, WFD classification) caused by the organism likely to be in the **future** in the risk assessment area?	minor	medium	
2.22. How important is it that genetic traits of the organism could be carried to other species, modifying their genetic nature and	minimal	medium	No reports of cross breeding with other amphipods (although no explicit tests have been documented). Cross breeding is very unlikely as *D. bispinosus* is phylogenetically distant from native amphipods.

making their economic, environmental or social effects more serious?			
2.23. How important is social, human health or other harm (not directly included in economic and environmental categories) caused by the organism within its existing geographic range?	minimal	high	NA
2.24. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?	minor	low	There are no published studies which examine the microbiome of *D. bispinosus*, either within its natural range or within invaded regions. However, there is some literature pertaining to *D. villosus* and *D. haemobaphes*. It may be possible that *D. bispinosus* may introduce novel parasites into British freshwaters, such as *Cucumispora dikerogammari*, a microsporidian identified in *D. villosus* (Ovcharenko et al., 2009), and found to infect native amphipods, including *G. pulex* (Bacela-Spychalska et al., 2012). Similarly, Bojko et al. (2015, 2019) identified another microsporidian (*C. ornata*), which can infect *Dikerogammarus* species, as well as native amphipods, and is known to adversely affect host survival.
2.25. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the comment box)	NA	NA	
2.26. How important are the expected impacts of the organism despite any natural control by other organisms, such as	moderate	medium	Predation by native fish species (e.g. trout) may regulate *D. bispinosus* within Great Britain.

predators, parasites or pathogens that may already be present in the risk assessment area?			
2.27. Indicate any parts of the risk assessment area where economic, environmental and social impacts are particularly likely to occur (provide as much detail as possible).	See comment	high	A large area of Great Britain particularly the Thames, Anglian, Severn and Humber river basin districts. "At risk" regions also include most of England, the eastern part of Wales, and the east coast of Scotland. Suitability of at-risk regions based on predictions reported by Gallardo & Aldridge (2013), which considered climatic conditions, and water chemistry characteristics (e.g. alkalinity).
2.28. Estimate the overall impact of this organism in the risk assessment area (using the comment box to indicate any key issues).	moderate	medium	There is little likelihood of economic or societal impacts within Great Britain. Ecological/environmental impact may be possible through displacement of native species, although this requires further research.

RISK SUMMARIES			
	RESPONSE	**CONFIDENCE**	**COMMENT**
Summarise Entry	likely	medium	Hitchhiking in ballast tanks, on boat hulls, and on boating/angling equipment are the most likely routes of entry into Great Britain.
Summarise Establishment	likely	high	Should entry occur, a large area of Great Britain is expected to be suitable for establishment.
Summarise Spread	rapidly	high	Should entry and establishment occur, spread is likely, facilitated by the movement of contaminated boating/angling equipment.
Summarise Impact	moderate	medium	There is little likelihood of economic or societal impacts within Great Britain. Ecological/environmental impact may be possible through displacement of native species, although this requires further research.
Conclusion of the risk assessment	medium	medium	Risk is considered to be high based on the significant expansion of this invader in mainland Europe, its long-term presence in the Netherlands – a potential gateway to the UK – and the projected suitability for *D. bispinosus* in GB freshwaters. If introduced, *D. bispinosus* is likely to exhibit a similar invasion potential to that of *D. haemobaphes*, spreading extensively throughout GB's highly interconnected water network, in a relatively short amount of time. The potential for establishment is likely to be hindered in regions colonised by other Ponto-Caspian amphipods (e.g. *D. villosus* and *D. haemobaphes*), with competition preventing colonisation; although this in turn may facilitate further spread in search of unoccupied regions. Within areas colonised by *D. bispinosus*, native amphipod are likely to be most heavily affected, with competition and intraguild predation leading to declining numbers and/or local extinctions. As with other *Dikerogammarus* species, currently present in the UK, *D. bispinosus* may also be an effective predator of native macroinvertebrates, although field-based evidence of this is lacking. Given the scarcity of empirical data regarding the ecological impacts that can be directly

			attributed to *D. bispinosus* invasions in European freshwaters, and the fact that some invasive populations appear to be limited/declining, impacts imposed by *D. bispinosus* are likely to not be as severe as those anticipated for *D. villosus*.

Additional questions are on the following page ...

ADDITIONAL QUESTIONS - CLIMATE CHANGE			
QUESTION	**RESPONSE**	**CONFIDENCE**	**COMMENTS**
3.1. What aspects of climate change, if any, are most likely to affect the risk assessment for this organism?	Ambient temperature, precipitation, water chemistry	medium	
3.2. What is the likely timeframe for such changes?	50 years	medium	
3.3. What aspects of the risk assessment are most likely to change as a result of climate change?	Probability of establishment and spread.	medium	
ADDITIONAL QUESTIONS – RESEARCH			
4.1. If there is any research that would significantly strengthen confidence in the risk assessment please summarise this here.	Further research concerning the ecological impact of *D. bispinosus* towards native freshwater communities, either through competition or predation, would provide greater insights into this invader's impact potential. It would also enable in-depth comparisons between *D. bispinosus* and other *Dikerogammarus* species currently present in the UK.	high	

Please provide a reference list on the following page ...

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