A Preliminary Assessment of the Impact of *Dikerogammarus villosus* on Ecological status for the Water Framework Directive

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Executive Summary

- *Dikerogammarus villosus* is an omnivorous predator that affects the native fauna of the ecosystems invaded, potentially affecting their WFD ecological status, and for which there are no effective eradication options available.
- The species is present in at least 16 European countries and is expected to continue its spread in Europe. The most recently invaded region is Great Britain, where it is present in four locations (Grafham reservoir and Barton Broad in England; Eglwys Nunydd reservoir and Cardiff Bay in Wales).
- This study aims: i) to locate the areas at national scale (England and Wales) under a highest risk of invasion (i.e. 'High risk' area); ii) to identify water-bodies having a WFD Good ecological status within the High risk area; iii) to assess their likelihood of invasion based on the catchment's local habitat characteristics; iv) to assess the potential impact of *D. villosus* on the WFD evaluation based on empirical data; and v) to propose management guidelines.
- Species distribution models suggest the species occurrence is favoured at a European scale by increasing human influence and population density, closeness to ports, decreasing altitude, minimum winter air temperature above -5˚C, intermediate alkalinity and low nitrate.
- The High risk area (i.e. high propagule pressure and climatic suitability) accounted for 26% of England and Wales.
- A total 1,883 WFD water-bodies (27% of the total) are located within this High risk area. 217 of them (3.0% of the total) are classified as Good ecological status. Most of them are located in the Anglian and Severn River Basin Districts.
- In the River Ouse catchment climate and water chemistry suitability are high for *D. villosus*. Grafham reservoir is strategically connected to the centre point of the catchment allowing both potentially upstream and downstream dispersal, and the high density of roads may facilitate accidental movement of the species. However, the substrate of the catchment is dominated by sand and silt, which is not likely to be invaded by *D. villosus*. Overall, the characteristics of the

catchment make it highly vulnerable to invasion, potentially affecting 12 Good and 82 Moderate status water-bodies.

- The Cotswolds and Bristol area also shows a high climatic match combined with appropriate water chemistry, boulder cover in the lower reaches, and high discharge in the main river courses. This region shows therefore the highest vulnerability levels after the River Ouse catchment, potentially affecting 36 Good and 110 Moderate ecological status water bodies. Because the region is not hydrologically connected with other invaded catchments, public awareness and control of aquatic activities are key to prevent the accidental introduction of the species.
- Of the invaded regions, South Wales showed the lowest climatic and water chemistry suitability for the species, concentrated in a strip along the southern coast. Because of the location of invaded water bodies –close to the coast— downstream dispersal is disregarded. Upstream dispersal is possible, overall assisted by the availability of hard substrata, which dominates the whole region. There are a notable number of Good ecological status water bodies in the region, though only 10 fall within the High risk area. Therefore, it is especially important to control both the upstream and the human-assisted spread of the species.
- The Broadlands Rivers area showed very high propagule pressure and climatic suitability for the species. Substrate texture –dominated by sand and silt—is not particularly suitable for *D. villosus* establishment, while water chemistry is in the suitable range of the species. Only 3 Good and 51 Moderate ecological status water bodies were identified in this region, though none of them directly downstream of the invaded Barton Broad and therefore reachable by natural dispersal. Because the invaded broad is directly connected both upstream and downstream the river Ant, there is an extremely high risk of dispersal of the species both by passive (drift) and active (swimming) dispersal.
- According to the literature, downstream drift in *D. villosus* is seasonal (maximum in spring and summer), related to the species life-cycle and density dependent. Upstream active dispersal is probably slower but may be important where the presence of hard substrata and vegetation assists the species. Because migration is usually density dependent, the spread detected along the Broadlands Rivers suggest the species has been long since established and is now probably beyond control. Consequently, it is first and foremost priority to control –and if possible— reduce the further spread of *D. villosus* in the Norfolk Broad. In the case of the three invaded reservoirs, it is vital to confine them to the invaded localities, and periodically check the closest river channels.

- The actual spread patterns of the species –with long spatial 'jumps'—suggests a human-mediated introduction. Public awareness and tougher controls of lake activities (fishing, angling, boating) are therefore necessary to control its spread, especially in catchments located within the High risk area, as those evaluated in this study.
- The biological element of the current WFD ecological assessment methods are likely to be impacted by the invasion of *D. villosus* in rivers and lakes within England and Wales. River monitoring methods (RICT) are likely to be more heavily affected by the invasion of *D. villosus* compared with lake monitoring methods (CPET and Phytoplankton), although the LAMM has the potential to be affected in lakes within pH range 6.7 and 8.8.
- For RICT, the presence of *D. villosus* is likely to deflate ASPT scores in areas of 'Good' ecological status by targeting high scoring groups and is likely to inflate ASPT scores in areas of 'Poor' ecological status as the relatively more tolerant *D. villosus* will be included within the Gammaridae.
- Differential effects on deep water and shallow water chironomidae in lakes following the invasion of *D. villosus* may affect the effectiveness of the CPET.
- Initial findings suggest that phytoplankton surveys may be unaffected by the invasion of *D. villosus* in lakes.

1. Introduction

Dikerogammarus villosus, commonly named the killer shrimp, is native to the Ponto Caspian region and in the last 15 years has invaded many countries in Europe thanks to a combination of natural and human-mediated dispersal (Pockl, 2009). Currently, the killer shrimp is present in at least 16 European countries and is expected to continue its spread in Europe. The most recently invaded region is Great Britain, where it was first located in September 2010 in the River Great Ouse catchment in eastern England (Grafham reservoir, latitude 52.29, longitude -0.31) (MacNeil et al., 2010a) and then in November 2010 its presence was confirmed at Cardiff Bay (latitude 51.46, longitude -3.16) and Eglwys Nunydd Reservoir, Port Talbot (latitude 51.54, longitude -3.73) in South Wales. The species has just been detected in March 2012 at Barton Broad, Norfolk (latitude 52.74, longitude 1.50). The presence of this species is of particular importance because it is an omnivorous predator known to dramatically affect the native fauna of the ecosystems invaded, including fish stocks (Casellato et al., 2007a), and for which there are no effective eradication options available once the species becomes established (Madgwick & Aldridge, 2011). For this reason, prevention and control of the spread of *D. villosus* are especially timely and relevant in Great Britain.

Species distribution models are used to measure the climate suitability for an invasive species, by projecting a model of the known species distribution into a region of interest (Guisan & Thuiller, 2005). For this reason, species distribution models have been used to locate the areas most climatically similar to the current range of *D. villosus* in Europe, and that are most susceptible to successful colonization in the event of an introduction (Gallardo et al., 2011). Apart from climate, other factors related to propagule pressure (e.g. human degradation, distance to ports and reservoirs) or habitat conditions (e.g. water chemistry) can be used to refine the models and locate areas where both propagule pressure and habitat suitability may facilitate invasion.

After the general areas under a high risk of invasion have been defined, local scale factors can be used to further narrow down the water-bodies at risk and the likely routes of introduction. For instance, while *D. villosus* is tolerant to a wide range of water chemistry conditions, it has been shown to have a competitive advantage over other gammarids under high salinity conditions (Wijnhoven et al., 2003). It predominantly lives in rocky shores and boulders (Boets et al., 2010); its establishment can be favoured by another Ponto Caspian invader, the zebra mussel (*Dreissena polymorpha*) that provides habitat and food sources to the species (Gergs & Rothhaupt, 2008); its natural dispersal is related to hydrological connectivity and water current (Van Riel et al., 2011); and its non-natural dispersal may be related to the facility of access to other water-bodies (i.e. transport connection) and their intensity of use (Gallardo et al., 2011). These local factors can be used to assess the potential dispersal of the species from the invaded locations in England and Wales towards other water-bodies, with particular attention to those classified as having a 'Good' ecological status under the WFD and where an eventual invasion of *D. villosus* may be more damaging.

Currently, the ecological status of UK surface waters is determined by measuring three general elements, (i) biological, (ii) chemical and physiochemical and, (iii) hydromorphological. Within these general elements, specific components of the system (e.g. fish abundance, dissolved oxygen, flow conditions) are measured, assessed against a reference, and scored to provide an overall measurement of the ecological status of a water body. Generally, for rivers and lakes, biological assessment uses four elements, (i) benthic macroinvertebrates, (ii) fish, (iii) phytoplankton and, (iv) macrophyte and phytobenthos. Current methods (some still under development) include the assessment of benthic invertebrates in rivers using a River Invertebrate Classification Tool (RICT) and in lakes using the Chironomid Pupae Exuvial Tool (CPET). The assessment of fish populations within rivers (Fisheries Classification System, FSC) is currently under development. The taxonomic composition of phytoplankton and the amount of chlorophyll *a* is used in the assessment of lakes. Diatoms are used for assessing river ecological status (DARES) and macrophytes are assessed using the macrophyte prediction and classification system (LEAFPACS) in both rivers and lakes.

In this risk assessment, our objectives are:

i) To locate the areas at regional scale (England and Wales) under a highest risk of invasion because of their propagule pressure and habitat suitability for the species ('High risk' area).

ii) To identify water-bodies having a Good ecological status according to the WFD evaluation in 2011 and for which a good status target has been set for 2015 located within the 'High risk' area.

iii) To assess the likelihood of invasion of those water bodies based on their hydrological connection with invaded water bodies, habitat characteristics (alkalinity, coverage of boulders and rocky shores, discharge), access facility (road connectivity) and presence of another Ponto-Caspian invader, the zebra mussel, which may facilitate *D. villosus*' invasion.

iv) To assess the potential impact of an eventual *D. villosus* invasion on the WFD evaluation based on the observed effects of the species in Grafham Water compared with two uninvaded reservoirs (Pitsford and Rutland Waters).

v) To propose management guidelines for the control of the species based on the above results.

2. Methodology

2.1 Species presence

Locations (latitude, longitude) where *D. villosus* is present in Europe were extracted from the Global Biodiversity Information facility (http://data.gbif.org, last accessed 15 February) and a comprehensive literature research (including locations from Dick & Platvoet, 2000; Devin et al., 2001; Bollache et al., 2004; Jażdżewski et al., 2005; Arbačiauskas & Gumuliauskaitė, 2007; Casellato et al., 2007a; Paunovic et al., 2007; Kinzler et al., 2009; Semenchenko et al., 2009) . When the species was reported from a particular stretch of a river (e.g. Danube estuary) a number of random points were manually located within the river stretch. A total of 323 occurrences were used for further analyses, distributed in 16 countries (Fig. 1).

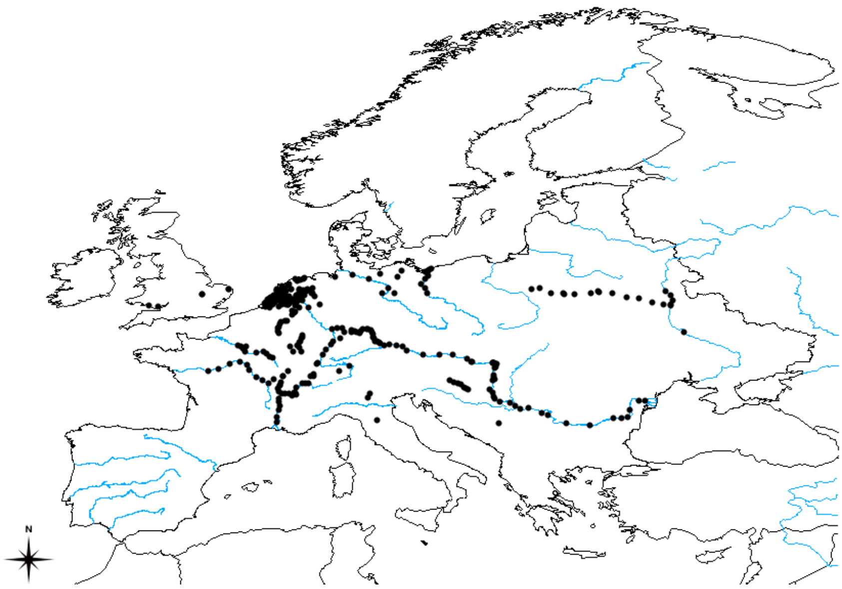


Figure 1: *D. villosus* occurrences used for modelling its potential distribution in England and Wales.

2.2 Socio-economic indicators

Socio-economic indicators are expected to reflect ecosystem disturbance and the intensity of use of ecosystems. Because invasive species' propagule pressure is directly related to these factors, we expect models based on socio-economic indicators to represent the likelihood of propagules being introduced across England and Wales. The four following predictors were used:

- *Population density*. Calculated as population per square kilometre, was obtained from the Oak Ridge National Laboratory (ORNL, www.ornl.gov) – Geographic Information Science and Technology database. Population density has been related to propagule pressure at the scale of Europe (Pyšek et al., 2010) and in Great Britain (Keller et al., 2009) (Fig. 2A).
- *Human influence*. Extracted from the Socio-Economic Data Applications Centre (sedac.ciesin.org/wildareas). Human influence combines population density, urban areas, night lights, land-use, and distance to roads, railways and navigable rivers. Each one of these layers is assigned a score that is later summed up to constitute the human influence layer (Sanderson et al., 2002), which ranges from 0=close to pristine locations, to 64= much degraded areas (Fig. 2B).
- *Distance to ports*. A list of commercial ports was obtained from the American Association of Port Authorities (www.aapa-ports.org, ports with > 30 megatonnes total cargo volume in 2009). The euclidean distance from each pixel to the closest port was calculated with the Spatial Analyst extension of ArcGIS 10 (Fig. 2C).
- *Distance to reservoirs*. A list of dams > 1 km^3 was extracted from the Global Water Systems Project (www.gwsp.org). The euclidean distance to the closest dam or reservoir was similarly calculated with the Spatial Analyst extension of ArcGIS 10 (Fig. 2D).

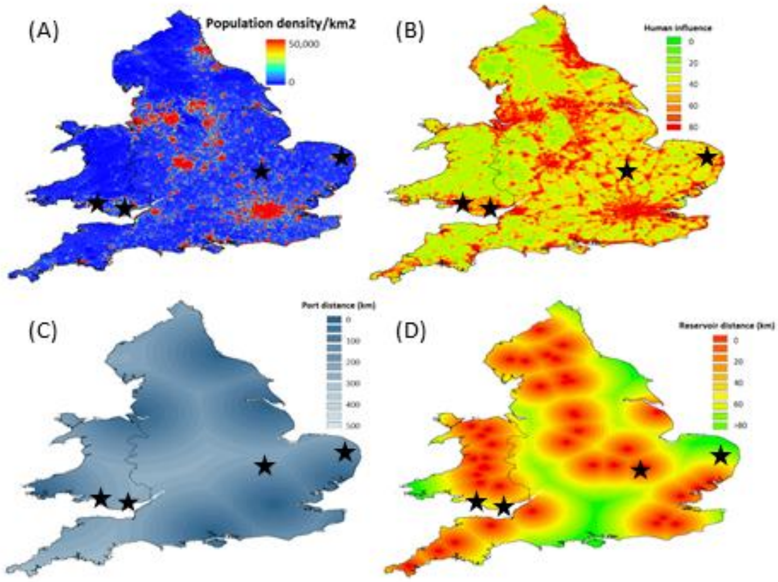


Figure 2: Socio-economic indicators used to assess the potential propagule pressure of *D. villosus* in UK. A: Population density per km^2. B: Human influence (0= pristine to 64= most degraded). C: Distance to main commercial ports. D: distance to closest reservoir/dam. Black stars correspond to the 4 locations where the species has been detected in England and Wales. Note that maps were used at the scale of Europe, although only England and Wales are shown in these examples.

2.3 Habitat indicators

Climate, geomorphological and water chemistry indicators were used to assess the likelihood of invasion based on the match between habitat conditions of places invaded by the species in Europe and those of Great Britain.

- *Climate*. Annual temperature (Fig. 3A), seasonality, temperature of the warmest and coldest months, annual precipitation (Fig. 3B), precipitation of driest and warmest months were extracted from WorldClim-Global Climate Data (www.worldclime.org). Temperature is known to directly affect the fecundity, reproduction, egg size and survival of gammarids (Sheader, 1996; Piscart et al., 2003; Pockl et al., 2003; Wijnhoven et al., 2003); it particularly affects the reproductive period and growth of *D. villosus* (Devin et al., 2003), and its oxygen consumption (Bruijs et al., 2001). Precipitation patterns determine the availability of water and the frequency of droughts and floods, which can have marked effects on gammarids.
- *Altitude*. Also extracted from WorldClim-Global Climate Data. Gammarids such as *D. villosus* usually inhabit lowlands so a negative relationship with altitude might be expected (Fig. 3C).
- *Water chemistry*. Electrical conductivity (Fig. 3D), alkalinity (Fig. 3E), nitrate (Fig. 3F), pH (Fig. 3G), dissolved organic carbon and sulphate concentration (Fig. 3H) were extracted from the

Geochemical Database of Europe (http://weppi.gtk.fi), the European Environment Agency (http://www.eea.europa.eu/themes/water), and England's Environment Agency. Data from these three monitoring networks was collated and interpolated across the whole of Europe using the Inverse Distance Weighting method available in ArcGIS 10. Water chemistry information was deficient for European Eastern countries as Belarus, Ukraine, Romania, Macedonia, Turkey and Russia. Therefore these countries were excluded from the interpolated model. For this reason, two habitat suitability models were generated: one based on climate and altitude including the whole Europe (35 countries, accounting for the native range of the species); and a second model based on water chemistry, calibrated using the 30 countries for which reliable water chemistry information was available. This second model consequently excluded part of the native range of the species.

All socio-economic and habitat maps were scaled to a 30 second resolution (approximately 1km^2) and WSG84 projection.

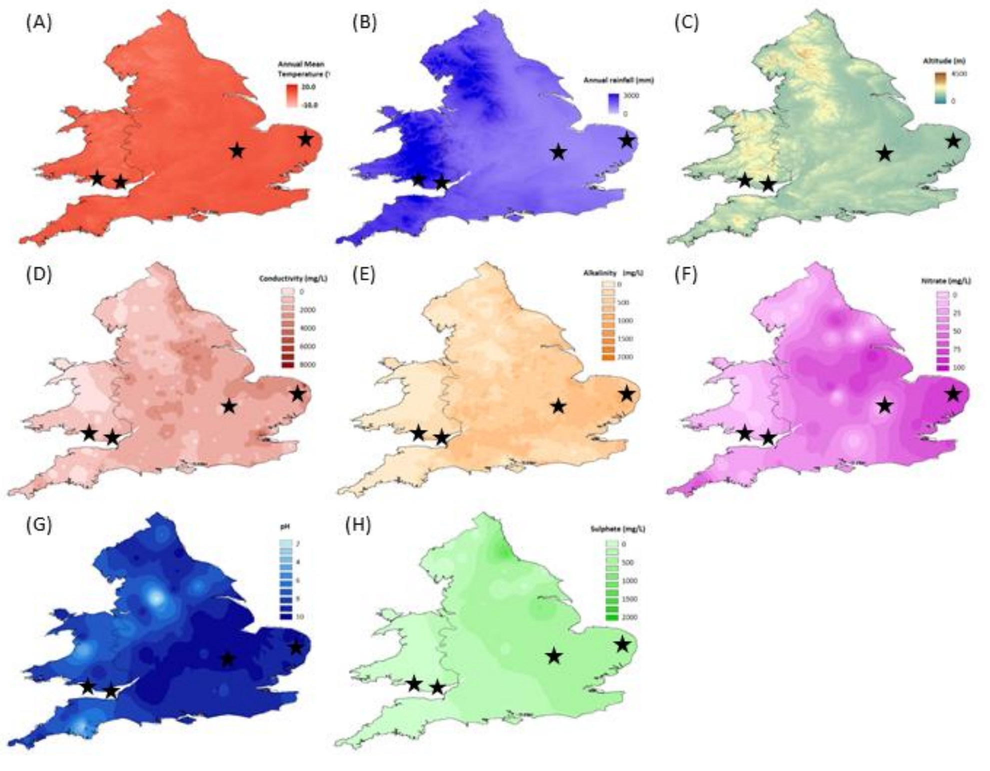


Figure 3: Habitat indicators used for modelling the potential distribution of *D. villosus* in England and Wales. A: Annual mean temperature. B: Annual rainfall. C: Altitude. D: Conductivity. E: Alkalinity. F: Nitrate. G: pH. H: Sulphate. Black stars correspond to the 4 locations where the species has been detected in England and Wales. Note that maps were used at the scale of Europe, although only England and Wales are shown in these examples.

2.4 Species distribution modelling

We used MaxEnt version 3.3 (www.cs.princeton.edu/~schapire/maxent) to develop species distribution models. MaxEnt is a machine-learning algorithm that minimizes the relative entropy between two probability densities (one estimated from the presence data and the other from the background) defined in covariate space (Elith et al., 2010). According to several studies comparing algorithms, MaxEnt is one of the highest performing methods for species distribution modelling (Elith et al., 2006; Phillips et al., 2006). For this study default modelling parameters (convergence threshold=10^5, maximum iterations= 500, regularization value B= auto) were used, following Phillips et al. (2006). However, to improve the transferability of models across space, we tried a regularization modifier between 1 and 5 (Phillips & Dudik, 2008). Regularization reduces the likelihood of overfitting models, thus increasing the ability of models beyond the training region (Medley, 2010).

For input, MaxEnt models used the dataset of *D. villosus* occurrences in Europe and the set of socio-economic or habitat factors that might limit the species' capabilities to survive. Data were split into two sets: 80% of the data was used for modelling and the remaining 20% to test the accuracy of the predictions. Because no absence data was available, a total of 10,000 pseudo-absences were generated from the Europe-wide background. To assess model performance we used the Area Under the ROC Curve (AUC) (Hanley & McNeil, 1982), which represents the probability that a random occurrence locality will be classified as more suitable than a random pseudo-absence. A model that performs no better than random will have an AUC of 0.5 whereas a model with perfect discrimination will score 1.

Three MaxEnt models were developed:

I. *Propagule pressure model*. Based on population density, human influence, distance to ports and reservoirs.
II. *Climate model*. Based on temperature, rainfall and altitude.
III. *Water chemistry model*. Based on nitrate, alkalinity, conductivity, sulphate, pH and dissolved organic carbon. This model excluded 5 eastern European countries covering part of the native range of the species because of insufficient data available.

After calibration, models were projected onto Europe to obtain suitability maps, ranging from 0= conditions completely different to those of the range of the species, to 1= complete match with current range of the species. Subsequently, we focused on England and Wales for closer examination. Finally, the threshold maximizing the sensitivity (i.e. number of presences correctly predicted) and specificity (i.e. number of absences correctly predicted) of the model was used to transform the suitability map into a predicted presence/absence map, and calculate the percentage of England and Wales' territory showing predicted presence according to each model. These values refer therefore to the total surface of England and Wales, and are provided as a general indicator of the potential threat posed by *D. villosus*.

In this study we consider that the areas where the propagule pressure and climatic models predict presence are under the highest risk because the two main conditions for the successful invasion of *D. villosus* occur: availability of propagules and habitat suitability. The area where both models predict the presence of the species is hereafter named 'High risk' area.

2.5 *Post-hoc* analyses

The Environment Agency dataset of water-bodies protected under the Water Framework Directive (WFD) was used to assess the potential impact *D. villosus* on the ecological status of England and Wales' water-bodies. Within the High risk area showing large-scale similarities with the species niche in Europe, the number of WFD water-bodies scoring a Bad, Poor, Moderate and Good status was calculated. We then assumed that water bodies under a Good ecological status and for which the Environment Agency has established a Good status target in 2015 are the most vulnerable to an eventual invasion of *D. villosus*, and are water bodies where its impacts would be most relevant. Therefore we analysed the spatial distribution of Good quality water bodies under risk, identifying the main river catchments where efforts to control the potential spread of the species should be concentrated. Finally, we focused on the four main geographical regions located within the High risk area: the River Ouse catchment (including the Cam, Ely and Bedford rivers and Northwest Norfolk), Costwolds and Bristol (Bristol, Avon, North Somerset streams, Costwolds, Severn Vale and Cheswell), South East Wales (including Ogmore, Tawe, Wye and Usk rivers) and the Broadland Rivers. For these four main geographical regions we used a number of local-scale indicators to assess the likelihood of invasion of *D. villosus* in Good status water bodies, including:

- Alkalinity/conductivity. While the species has been shown to tolerate a wide range of water chemistry conditions, *D. villosus* shows a competitive advantage over other Gammaridae at high salinities, which is directly related to conductivity and alkalinity.
- Substrate. *D. villosus* is known to inhabit preferably rocky shores and boulders. It has been also shown to live in zebra mussel beds, which provides it habitat and food resources.
- Discharge. Downstream dispersal of *D. villosus* depends mainly on river discharge. River dykes, dams and other barriers to flow can slow down the dispersal of the species, overall

upstream, although they are not likely to prevent it completely. Downstream dispersal of gammarids is density dependent (triggered by a maximum density that force organisms to find new locations to establish) and has been estimated to range between 20 and 100 km/year (Gallardo et al. 2011).

- Road network. Can indicate both the access facility to a water-body and its intensity of use; therefore the likelihood of propagules being accidentally translocated.

2.6 Literature review and data available

Two main sources of information were available to assess the potential effects of *D. villosus* invasion on biological assessment methods.

1. Peer reviewed literature was searched for information relating to biological effects of *D. villosus* invasion. Specifically, articles containing information relating to the direct effects of *D. villosus* on macroinvertebrates, fish, phytoplankton and macrophytes, as well as general effects on biomonitoring were referred to.

2. Ecologists at Anglian Water have collected information on deepwater macroinvertebrates and phytoplankton in Grafham Water (invaded reservoir) and two nearby uninvaded reservoirs (Pitsford and Rutland Waters).

Using these two data sets we critically assessed the potential effects of *D. villous* invasion on the following techniques:

1. RICT (River Invertebrate Classification Tool) Literature Review

2. CPET (Chironomid Pupal Exuviae Tool) Literature Review & Anglian Water data analysis

3. LAMM (Lake Acidification Macroinvertebrate Metric) Literature Review

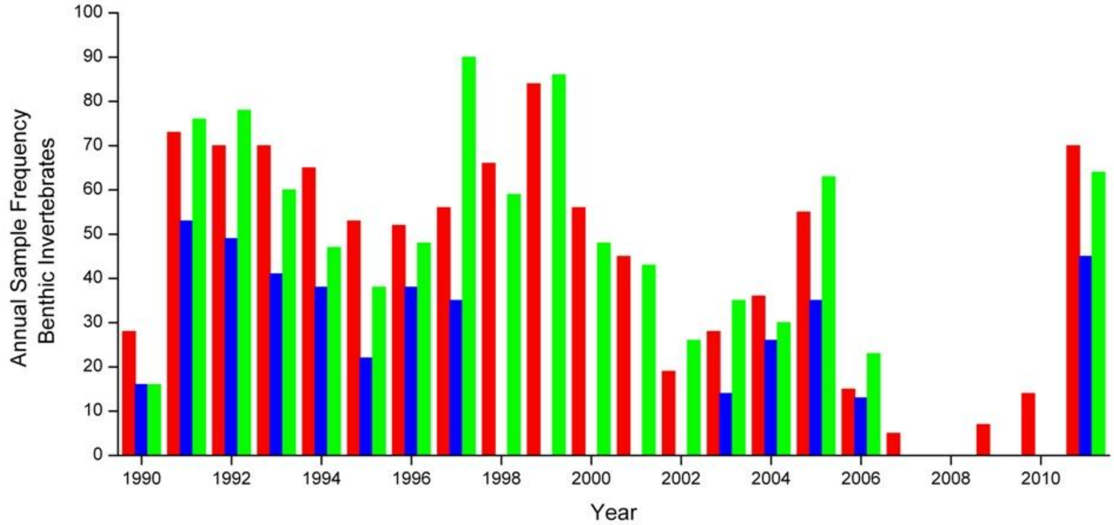
4. Phytoplankton Literature Review & Anglian Water data analysis

Biological information from Grafham, Pitsford and Rutland Waters

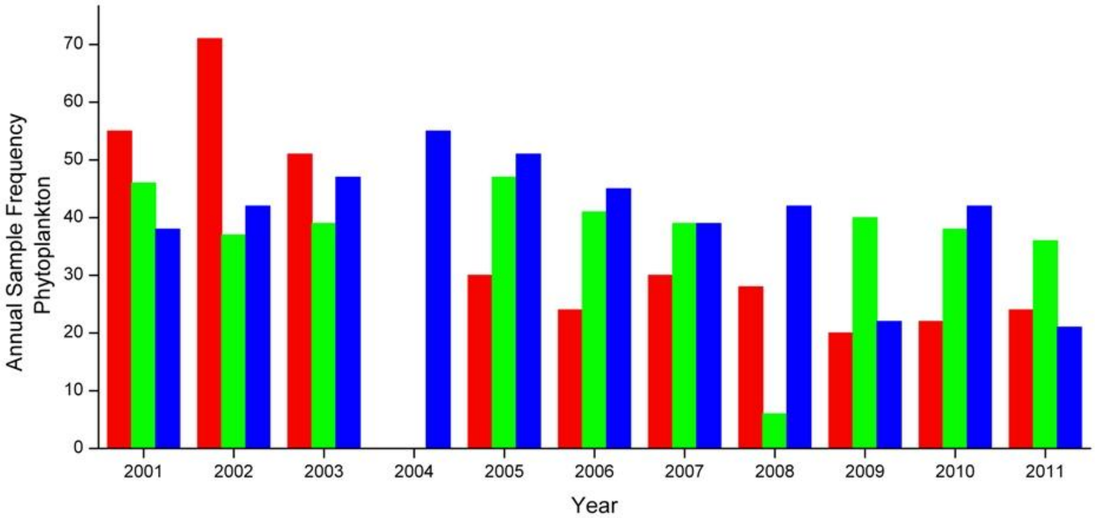
We assumed that the invasion of *D. villosus* happened sometime in the very late 2000's as, if present within the reservoir, *D. villosus* was at levels below detection in 2008 (zu Ermgassen, pers. comms.). Confirmation of the presence of the species in Grafham Water in 2010 (MacNeil, et al., 2010) and the highly contiguous nature of its distribution and the high levels of abundance at this time, we give a nominal year of 2009 as time at which the species is likely to have a biological effect within the reservoir.

Anglian Water Dataset

Deep water (c. >2m) samples of benthic invertebrates and samples of phytoplankton have been collected at Grafham, Pitsford and Rutland Waters by Anglian Water ecologists as part of their reservoir monitoring program. Samples of benthic invertebrates have been collected since 1990 and collections of phytoplankton have been made since 2001. As annual sampling frequency was not consistent across the time series and sample frequency differed in timing within each year (mean annual sampling frequency 6.8 ±0.4 for deep water invertebrate samples and 10.1 ±0.4 for phytoplankton; Figure 4) standardisation of the dataset resulted in information on deep water benthic samples available from five sites in each reservoir and phytoplankton information at three sites in each reservoir. To account for seasonal sampling differences we included a harmonic function (sine / cosine) in our analysis. Following a standard protocol, collected samples were sub-sampled and animals identified to a minimum of taxonomic family level and genus level for phytoplankton, by the Ecology Unit at Anglian Water. As the grab sampling unit changed over the time series (Eckman grab, dimensions 140mm x 140mm 1990 until 2003; Eckman grab, dimensions 150mm x 150mm, 2004 until 2010; Van Veen grab, dimensions 150mm x 170mm 2011 onwards) the abundance of animals in each sample was standardised to number of individuals per meter squared ($N\ m^{-2}$).



(A)



(B)

Figure 4: Annual sample frequency (number per year) for (A) benthic invertebrate samples and, (B) phytoplankton within the three reservoirs monitored by Anglian Water. Red= Grafham Water, Green= Rutland Water, Blue= Pitsford reservoir.

Following the invasion of Grafham Water, potential changes to the deep water macroinvertebrate and phytoplankton community were assessed by comparing general characteristics of the community at Grafham Water with those of Pitsford and Rutland Waters. Three general characteristics of the community were measured on a sample basis; total invertebrate abundance, community richness and community diversity. Total invertebrate abundance was the total abundance of animals (standard N m^{-2}, see above) in a sample, community richness was the total number of taxonomic families (benthic) and genera (phytoplankton) present in a sample and, community diversity was the invertebrate diversity of a sample measured using the Shannon Index (Shannon & Weaver, 1949). We also assessed the potential impact of *D. villosus* invasion on the abundance of Chironomidae in the benthic samples, as it may be likely that any impact on this family specifically may influence the potential effectiveness of the CPET technique.

Each of the characteristics were analysed with respect to reservoir and invasion status (i.e. pre 2009 or 2009 onwards) separately using a linear mixed effects model incorporating a random effect to account for pseudo-replication associated with multiple site sampling and a harmonic (sine + cosine) function to account for seasonality. All data were transformed (x'=log(x+1)) to improve normality and all statistical analysis was performed in R version 2.11.1 (R Development Core Team, 2010).

Data Limitations

We fully acknowledge the limitations of the long-term data here analysed. Firstly, while benthic samples have been standardised to number of animals per square meter, the changes in the sampling equipment used is likely to have affected the sample of the invertebrate community collected. Secondly, as the invasion of Grafham occurred in the late 2000's, there is very little information available relating to community structure post invasion and as such the structure of the data is not balanced (cf. pre invasion information 18 years benthic, 7 years phytoplankton and post invasion 1 year benthic and 3 year phytoplankton). The results from this analysis must therefore be viewed with this in mind and it is likely that more information gathered from future sampling will be required to provide robust analysis of any patterns.

3. Results

Average climatic and environmental conditions of water bodies invaded by *D. villosus* across Europe are summarized in Table 1. Although the species tolerates a wider range of conditions, average values show a preference for lowland cool water bodies with high alkalinity, and high nitrate and organic carbon concentration usually associated with polluted waters.

Table 1. Habitat values registered in *D. villosus* location points in Europe. The minimum, maximum and average values are shown. Data extracted from the interpolated water chemistry maps. PP: precipitation. T: Temperature.

	Average	Min	Max		Average	Min	Max
Alkalinity (mg/L)	242.7	51.6	483.8	**Annual PP (mm)**	731.2	435.0	1110.0
Conductivity (mg/L)	271.0	16.1	3342.3	**Driest month PP (mm)**	43.7	24.0	69.0
Nitrate (mg/L)	12.3	0.5	38.9	**PP seasonality**	18.3	8.0	37.0
DOC (mg/L)	7.5	1.1	20.3	**Annual T (°C)**	9.8	7.2	14.0
pH	7.7	6.7	8.8	**T seasonality**	6.1	4.6	8.6
SO4 (mg/L)	62.4	7.4	697.9	**Min T (°C)**	-1.47	-8.6	2.1
Altitude (m)	91.5	0	472.0	**Max T (°C)**	23.41	19.9	29.6

3.1 Species Distribution Modelling

Results from the three separate species distribution models performed for *D. villosus* are summarized in Table 2.

The propagule pressure model is expected to reflect the probability of *D. villosus* propagules being introduced into different parts of England and Wales (Fig. 5 A-B). Propagule pressure values were high in all locations were the species is already present (0.51-0.69). The most important variable in the propagule pressure model was human influence followed by distance to main commercial ports (Table 2). The species suitability increased exponentially with both human influence and population density, although it decreased at the highest population densities, as the species is not likely to be present in populated urban areas. *D. villosus* suitability was high closest to ports and reservoirs and dropped at a distance between 100 and 300 km.

The climatic model yielded the highest AUC value and provided the most ecologically meaningful prediction (Table 2, Fig. 5 C-D). Most important predictors included minimum temperature, altitude and rainfall in the driest month (Table 2). The highest climate suitability was obtained in this case in Barton Broad (0.59) while Eglwys Nunydd reservoir obtained the lowest (0.18). Grafham reservoir and Cardiff bay scored 0.32 and 0.30 respectively. As expected, *D. villosus* suitability showed a negative relationship with altitude; while a unimodal response to temperature and precipitation was observed, with highest suitability found at minimum temperature between -5 and +5 °C, and precipitation of the driest month between 30 and 70 mm.

The water chemistry model showed a lower AUC score than the previous two models (Table 2, Fig. 5 E-F). Alkalinity was the most important predictor of *D. villosus* presence, followed by nitrate and dissolved organic carbon concentration (Table 2). The minimum suitability of places where the species is already present ranged from 0.15 at Barton Broad to 0.49 at Cardiff Bay. Grafham and

Eglwys Nunydd reservoirs scored 0.32 and 0.30 respectively. The prediction was affected by the interpolation method used to generalize the water chemistry data across Europe, so results should be taken with caution. *D. villosus* showed a unimodal response to alkalinity with maximum suitability scores at 500 mg/L; suitability decreased with increasing nitrate concentration and was highest at 10 mg/L dissolved organic carbon.

Table 2: Results from the species distribution models performed with socio-economic and habitat factors. AUC ranges from 0.5 (no better than random model) to 1 (perfect prediction). Threshold: minimum suitability used to transform the logistic map into a presence-absence map. % predicted refers to the total area of England and Wales where the models predict the species presence. Predictors' contribution was calculated as the increase in regularized gain to the model. Variables in bold are the most important model contributors.

	Propagule pressure	**Climate**	**Water chemistry**
AUC	0.88	0.95	0.85
Threshold	0.35	0.21	0.29
% predicted	67.13	27.33	29.18
Population density	42.2		
Port distance	39.8		
Human influence	16.9		
Reservoir distance	1.1		
Min. Temp.		48.3	
Altitude		16.7	
Prec. Driest month		10.4	
Temp. seasonality		8.1	
Max. temp.		7.7	
Precip. Seasonality		3.8	
Annual precipit.		1.4	
Alkalinity			40.4
NO_3			25.1
DOC			13.7
Sulphate			9.9
pH			6.9
Conductivity			1.6

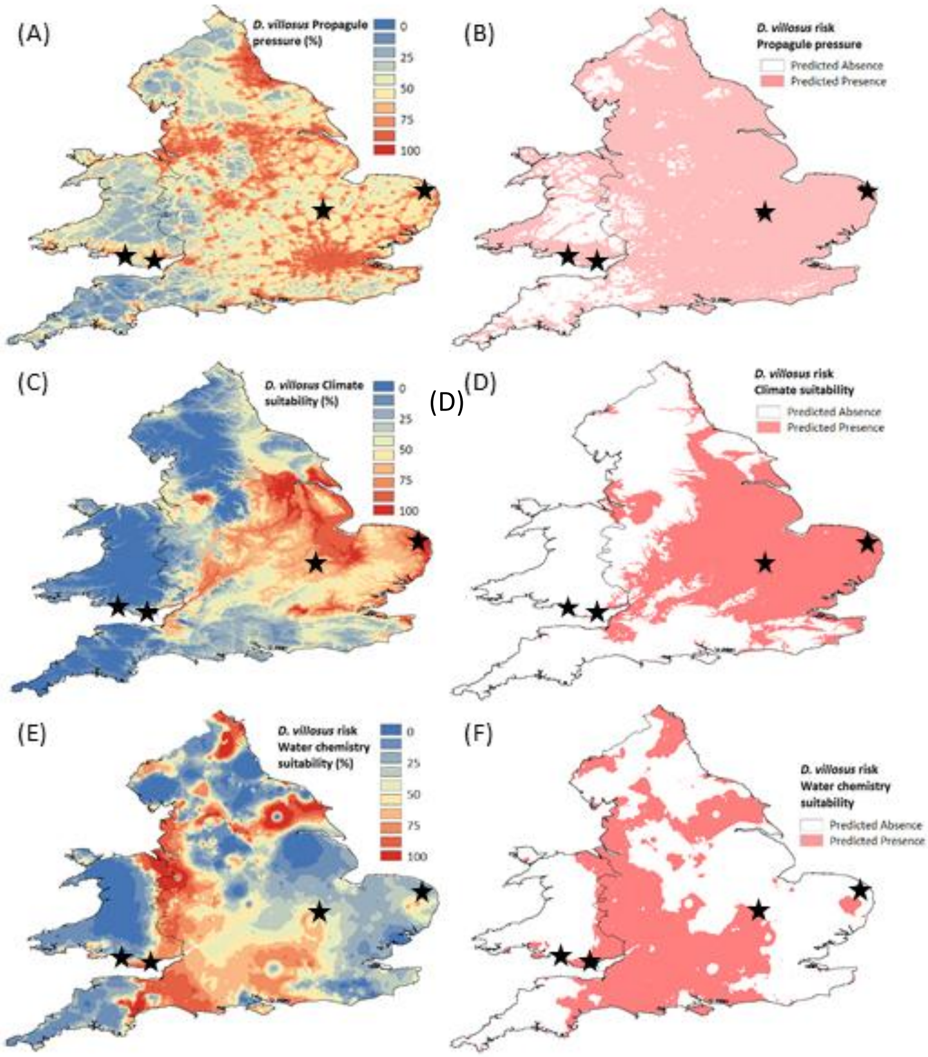


Figure 5: suitability maps obtained with MaxEnt using *D. villosus* presence in Europe and a suit of socio-economic factors (A-B), climate plus altitude (C-D) and water chemistry (E-F). Logistic maps (A, C and E) were converted into predicted presence/absence (B, D and F) using the threshold maximizing the sensitivity and specificity of the model. Black stars correspond to the 4 locations where the species has been detected in England and Wales.

Problems arise related to the lack of water chemistry data in Europe at the same spatial resolution as in the UK and the interpolation method used, which resulted in a distorted water chemistry prediction model (Fig. 5E). For this reason, we combined the propagule pressure and climate models only, to identify the areas in England and Wales where both factors may facilitate invasions (High risk area). This map showed that a 26.32% of England and Wales territory shows the minimum socio-economic and climatic conditions necessary for the species to establish (Fig. 6).

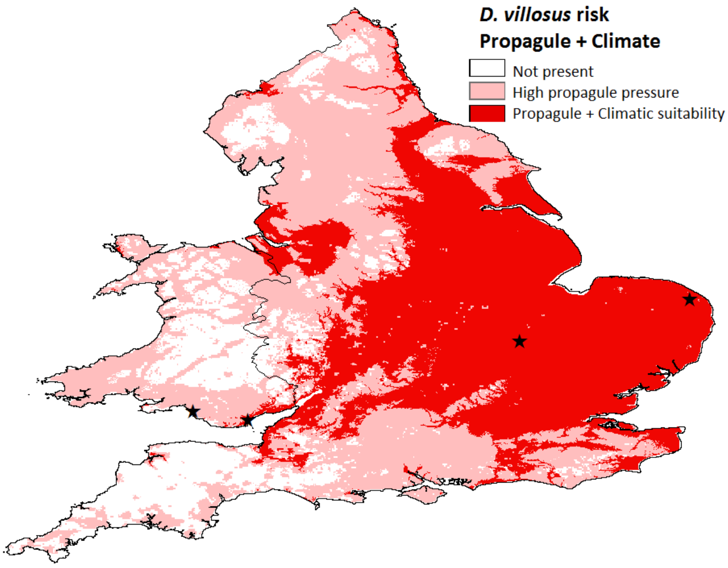


Figure 6: High risk area for *D. villosus* invasion according to species distribution models. Stars represent the four water bodies already invaded by the species.

Within this High risk area, a total of 1,889 WFD water bodies are located, which is a 26.58% of all monitoring water bodies located in rivers, lakes, canals, coasts and transitional waters of England and Wales (total= 7,105). A summary by River Basin District of water bodies within the High risk area showing Good, Moderate, Poor or Bad ecological status in 2010 is provided in Table 3; while in Table 4 summaries are provided by management catchment. From those 1,889 water bodies within the High risk area, 54.20% (1,024 water bodies) obtained a Moderate ecological status according to the last WFD evaluation in 2011; another 30.17% (570) had Poor ecological status and a 4.13% (78) Bad. Water bodies considered presenting a Good ecological quality accounted for 11.49% (217) of the water bodies within the risk area, which corresponds to a 3.05% of the total number of water bodies in England and Wales. Most of those Good ecological status water bodies are located in the Anglian River Basin District, which encompasses catchments already invaded by the species (Ouse river, and Broadland rivers). The Severn River Basin District also included a considerable number or Good ecological status water bodies under a high risk of being invaded, including the South East Valleys where Cardiff Bay is located. In contrast, only one Good status water-body present in the Ogmore to Tawe catchment –where Elgwys Reservoir is located—was under risk of being invaded according to distribution models.

Table 3: Number of water bodies within the High risk area defined in Figure 6. Data summarized by River Basin Districts (RBD), ordered by name. Highlighted in grey, RBD with the highest number of Good status water bodies affected. The total number of water bodies monitored in England and Wales is 4,653.

	Ecological Status			
RBD Name	**Good**	**Moderate**	**Poor**	**Bad**
Anglian	50	278	120	12
Dee	2	6	2	0
Humber	47	291	158	22
North West	13	76	37	19
Northumbria	7	23	18	3
Severn	47	141	75	7
Solway Tweed	0	3	2	0
South East	7	55	35	2
South West	7	14	7	1
Thames	35	133	116	12
Western Wales	2	4	0	0
Total	217	1024	570	78

Table 4: Number of water bodies within the High risk area (Fig. 6). Data summarized by management catchment, ordered by name. Highlighted in grey, catchments with the highest number of Good status water bodies affected. The total number of water bodies monitored in England and Wales is 4,653.

	Ecological Status					**Ecological Status**			
Catchment Name	**Good**	**Moderate**	**Poor**	**Bad**	**Catchment Name**	**Good**	**Moderate**	**Poor**	**Bad**
Adur and Ouse	2	11	15	0	North Norfolk	1	2	2	0
Aire and Calder	2	19	8	0	North West Norfolk	2	6	3	0
Alt or Crossens	1	7	1	2	North West Wales	1	1	0	0
Arun and Western Streams	1	15	4	1	Northumberland Rivers	4	5	3	0
Bristol Avon and North Somerset Streams	13	32	21	1	Ogmore to Tawe	1	2	0	0
Broadland Rivers	3	51	19	2	Old Bedford including the Middle Level	2	8	1	0
Cam and Ely Ouse (including South Level)	7	35	21	3	Ribble	0	2	0	0
Cherwell	7	9	11	0	Roding, Beam and Ingrebourne	0	5	5	1
Colne	2	10	8	0	Rother	1	12	3	0
Combined Essex	3	43	26	1	Severn Uplands	0	3	0	0
Conwy and Clwyd	0	1	0	0	Severn Vale	7	32	7	0
Cotswolds	9	8	15	2	Shropshire Middle Severn	0	0	1	0
Cuckmere and Pevensey Levels	0	1	0	0	Soar	3	18	20	3
Darent	0	3	2	0	South and West Somerset	3	9	7	1
Derbyshire Derwent	4	8	7	0	South East Valleys	0	5	1	0
Derwent (Humber)	2	18	7	3	South Essex	0	1	2	0

Don and Rother	7	37	22	2	Staffordshire Trent Valley	1	12	9	1
Dorset	2	2	0	0	Stour	0	5	10	0
Douglas	0	4	2	0	Swale, Ure, Nidd and Upper Ouse	2	17	8	1
Dove	2	5	1	1	Tame Anker and Mease	3	29	19	2
East Hampshire	0	2	1	1	Tees	1	5	8	3
East Suffolk	4	11	18	2	Teme	2	4	0	0
Eden and Esk	0	1	2	0	Test and Itchen	1	1	1	0
Esk and Coast	2	0	0	0	Thame and South Chilterns	3	13	11	3
Hull and East Riding	2	24	6	3	Tidal Dee	2	0	0	0
Idle and Torne	1	19	13	2	Tyne	2	6	1	0
Irwell	2	6	3	1	Upper and Bedford Ouse	10	32	10	2
Isle of Wight	0	1	0	0	Upper Dee	0	0	1	0
Kennet and Pang	2	5	1	0	Upper Lee	0	6	14	0
Loddon	0	3	1	0	Upper Mersey	1	13	10	1
London	0	17	11	3	Usk	2	3	1	0
Louth Grimsby and Ancholme	2	11	4	1	Vale of White Horse	4	13	5	0
Lower Trent and Erewash	3	32	30	3	Warwickshire Avon	7	38	28	4
Maidenhead to Sunbury	0	11	6	0	Waver or Wampool	0	2	0	0
Medway	1	8	13	1	Wear	0	5	6	0
Mersey Estuary	0	18	4	6	Weaver and Gowy	6	23	16	9
Middle Dee	0	6	1	0	Welland	5	18	7	1
Mole	0	8	8	1	Wey	2	9	2	0
Nene	7	28	4	0	Wharfe and Lower Ouse	3	10	3	0
New Forest	2	1	0	0	Witham	6	32	9	1
North Devon	2	1	0	0	Worcestershire Middle Severn	3	11	11	2
North Kent	0	1	1	1	Wye	7	4	4	0

3.2 Local environmental conditions facilitating the establishment of *D. villosus*

A review on the species water chemistry ranges was performed (Table 5), which confirmed the wide tolerance of the species to a range of conductivity (from 75 µS/cm in the Netherlands to 3,830 µS/cm in France) and pH (from 6.0 in the Netherlands to 8.9 in France and Belgium). These ranges would only exclude from invasion areas in the North and West of England and Wales; where anyway the species was predicted absent in the distribution models. In comparison with the UK mean values for Gammaridae published by the Centre for Intelligent Environmental Systems (www.cies.staffs.ac.uk), there are no major differences between the range of *D. villosus* and that of other gammarids in UK.

Table 5: Water chemistry values reported for *D. villosus.*

Region	Conductivity (μS/cm)	DO (mg/L)	pH	Temp (˚C)	Reference
Flanders (Belgium)	247-1870	2.6-15.3	6.9-8.9	7-26.5	Boets et al. 2010
Moselle river (France)	145-3830		6.7-8.9		Devin et al. 2001
The Netherlands	75-267		6.0-8.1	Max. 31	Wijnhoven et al. 2003
Poland	600-1100				Grabowski et al. 2009
Lake Garda (Italy)	223-317	7.6-10.9	7.37-8.82	6.5-23	Casellato et al. 2008
Grafham reservoir (UK)	808.6		8.58	12.15	McNeil et al 2010
UK (Gammaridae)	667.8	10.62	7.87	11.48	www.cies.staffs.ac.uk

In this study we have identified at national scale the broad areas that show the necessary climatic suitability for the species to establish, and the WDF-Good ecological status water bodies under risk within those areas. After this, local factors including water chemistry, substrate type, discharge, hydrological connectivity and road connectivity were used to further assess the likelihood of invasion of Good ecological water bodies within the following catchments: River Ouse (Bedford, Cam, Ely, Ouse and North-Norfolk), Bristol (Bristol, Avon, North Somerset streams, Costwolds, Severn Vale and Cheswell), South Wales (South Valeys, Ogmore, Tawe, Wye and Usk) and Broadland Rivers (Fig. 7). A summary of the main characteristics of each of these regions can be found in Table 6.

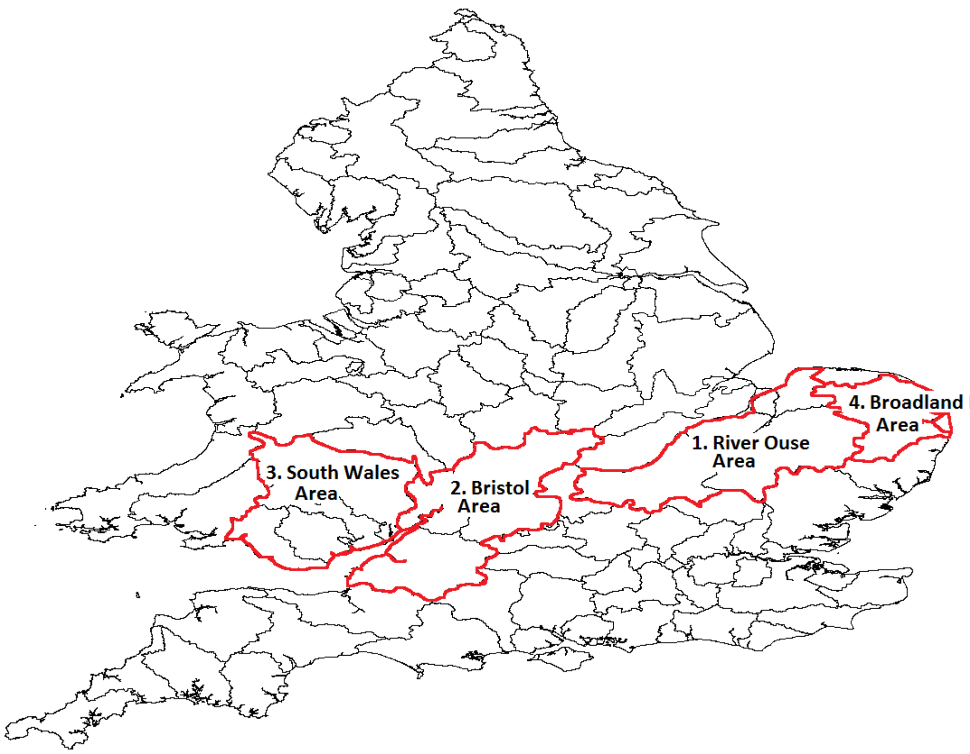


Figure 7: Location of catchments grouped into 3 main areas used to assess the probability of invasion of *D. villosus.*

1. The River Ouse catchment. This area included the Bedford, Cam and Ely streams as well as North West Norfolk management catchment at the River Ouse mouth. According to the species distribution models, both propagule pressure and climate suitability is high for *D. villosus* in the River Ouse catchment, associated to the low altitude, high population density and human influence of the catchment (Fig. 8). At least 21 water bodies were classified as having a Good ecological status in 2011 in the River Ouse catchment, 71 Moderate, mainly located in the upper reaches (Fig. 8, Table 6). The dominating substrate in the catchment is sand and silt, with boulders accounting for less than 20% of the coverage. Water chemistry values registered in the catchment were in the range of those reported in the literature for *D. villosus*, suggesting water chemistry is suitable for the species while substrate texture is not. Grafham reservoir is strategically connected to the middle River Ouse channel, which could potentially facilitate the spread of the species downstream. Drift would be also assisted by the high discharge of the main River Ouse channel. At least 8 Good ecological status water bodies are directly located downstream the invaded reservoir, or very close to the main River Ouse channel and would therefore be under highest risk of being invaded. From all the characteristics studied, the climatic and water chemistry match with the range of the species and the strategic hydrological connectivity of Grafham reservoir are identified as the main factors of risk in the River Ouse catchment. The fact that the zebra mussel is spread over much of this catchment further reinforces the idea of a high risk of spread and establishment.

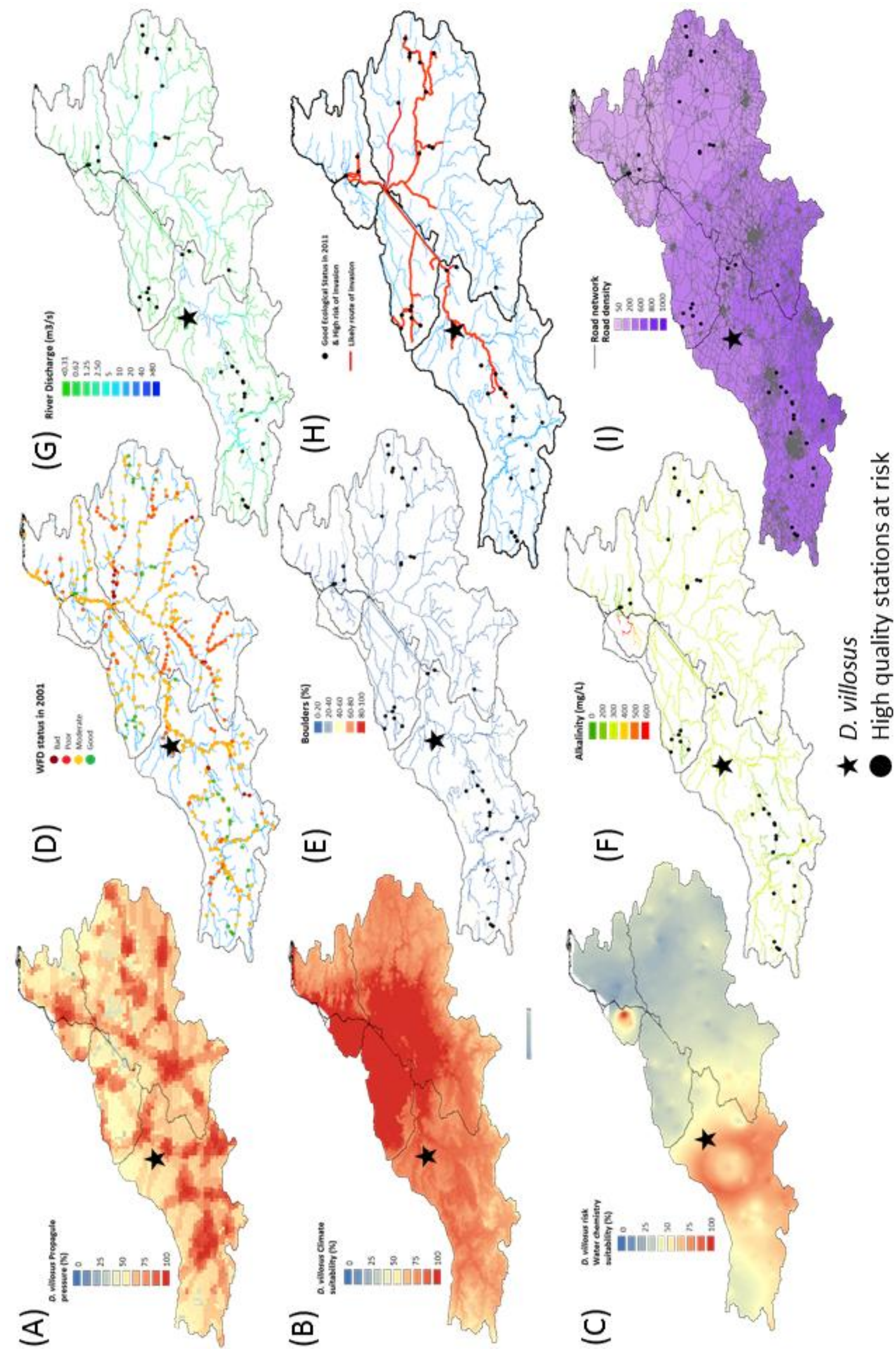


Figure 8. Analysis of risk in the River Ouse catchment. A-C: results of species distribution models performed with propagule pressure, climate and water chemistry factors respectively. D: Ecological status (in 2011) of monitoring water bodies located in the catchment. E: Percentage of boulder substrate. F: Alkalinity of waters. G: River discharge. H: likely routes of natural dispersal from invaded water bodies towards 'Good' ecological status water bodies under risk. I: road network and road density as indicators of access facility and potential human-mediated transport.

2. Cotswolds and Bristol area. The Bristol area analysed here included the Bristol, Avon and North Somerset streams, Cotswolds, Severn Vale and Cheswell management catchments. Within the North-East, the climate and water chemistry models yielded high values in the Cotswolds and Bristol area; while propagule pressure was only high in very populated areas and across transport corridors (roads) (Fig. 9). The High risk area was concentrated in this case in North-East, overall in Warwickshire Avon and Cheswell catchments. A total of 36 Good and 110 Moderate ecological status water bodies were located within the High risk area, a figure much higher than that of the River Ouse and Broadland rivers. Alkalinity, conductivity and organic carbon in the Cotswolds and Bristol area were similar or slightly lower than in the River Ouse; while nitrate and pH were lower. The coverage of boulder substrate was considerable, overall in the lower reaches of the Severn Vale and Bristol catchments. River discharge was also high in the main river channels. A high climate matching with the species range, along with adequate water chemistry, coverage of the species preference substrate and high river discharge suggest that this region might be suitable for the establishment of *D. villous* in the event of an introduction. The spread of the zebra mussel within the Cotswolds and Severn Vale further supports this idea. As *D. villosus* has not appeared yet and there is not direct hydrological connection to the invaded catchments, no potential routes of natural dispersal could be described, although the extensive network of roads may facilitate human mediated access to rivers and wetlands and therefore the spread of the species.

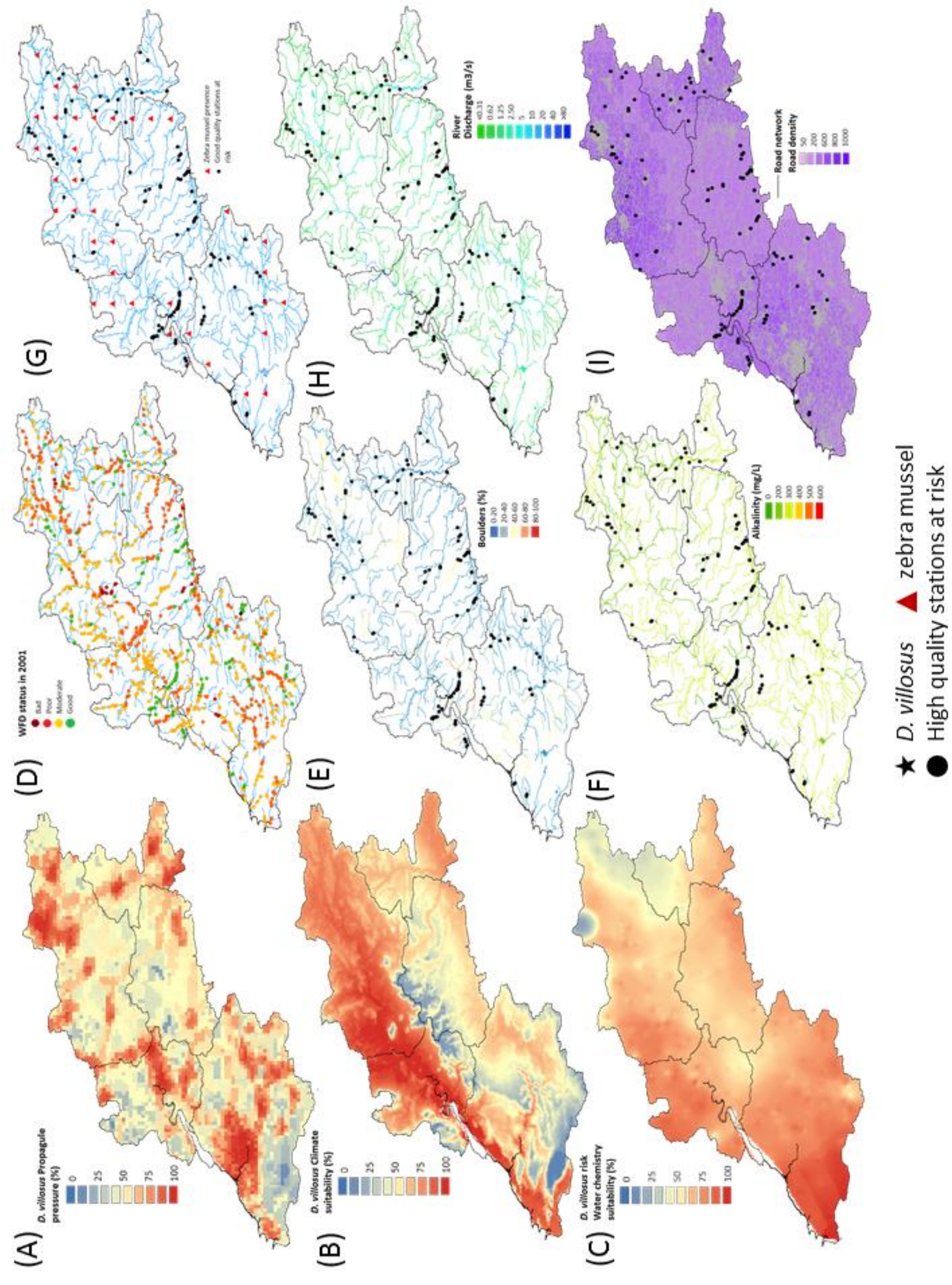


Figure 9. Analysis of risk in the Cotswolds and Bristol area. A-C: results of species distribution models performed with propagule pressure, climate and water chemistry factors respectively. D: Ecological status (in 2011) of monitoring water bodies located in the catchment. E: Percentage of boulder substrate. F: alkalinity of waters. G: River discharge. H: hydrological connectivity, location of 'Good' ecological status water bodies and presence of zebra mussel. I: road network and road density as indicators of access facility and potential human-mediated transport.

3. *South Wales.* The south-eastern part of Wales showed a relatively low propagule and climate suitability for *D. villosus*, which was nevertheless high in a strip along the south coast and the Severn Vale (Fig. 10). Water chemistry suitability was also high in this region, overall in the eastern part, where the zebra mussel is also present. Contrary to the previous catchments studied, here the substrate is dominated by boulders, the preferred habitat for *D. villosus* (Hesselschwerdt, et al. 2008; Boets, et al., 2010). Although the number of monitoring water bodies classified as having a Good ecological status is considerable (Table 6) only 10 water bodies were included within the High risk area delimited by species distribution modelling. As the two invaded water bodies (Eglwys Nunydd reservoir and Cardiff bay) are very close to the mouth of the rivers, the natural dispersal of the species towards Good quality water bodies is low, even if the species can actively swim upstream. Finally, road connectivity was very high close to Cardiff Bay, which implies an increased facility of access and involuntary transport to other locations. In summary, despite the climatic conditions of the area being not particularly suitable for *D. villosus* and the natural spread of the species being unlikely, the location of numerous Good ecological status water bodies in the area along with the dominance of the habitat this species preferably colonizes make this region especially vulnerable to an eventual accidental introduction of the species.

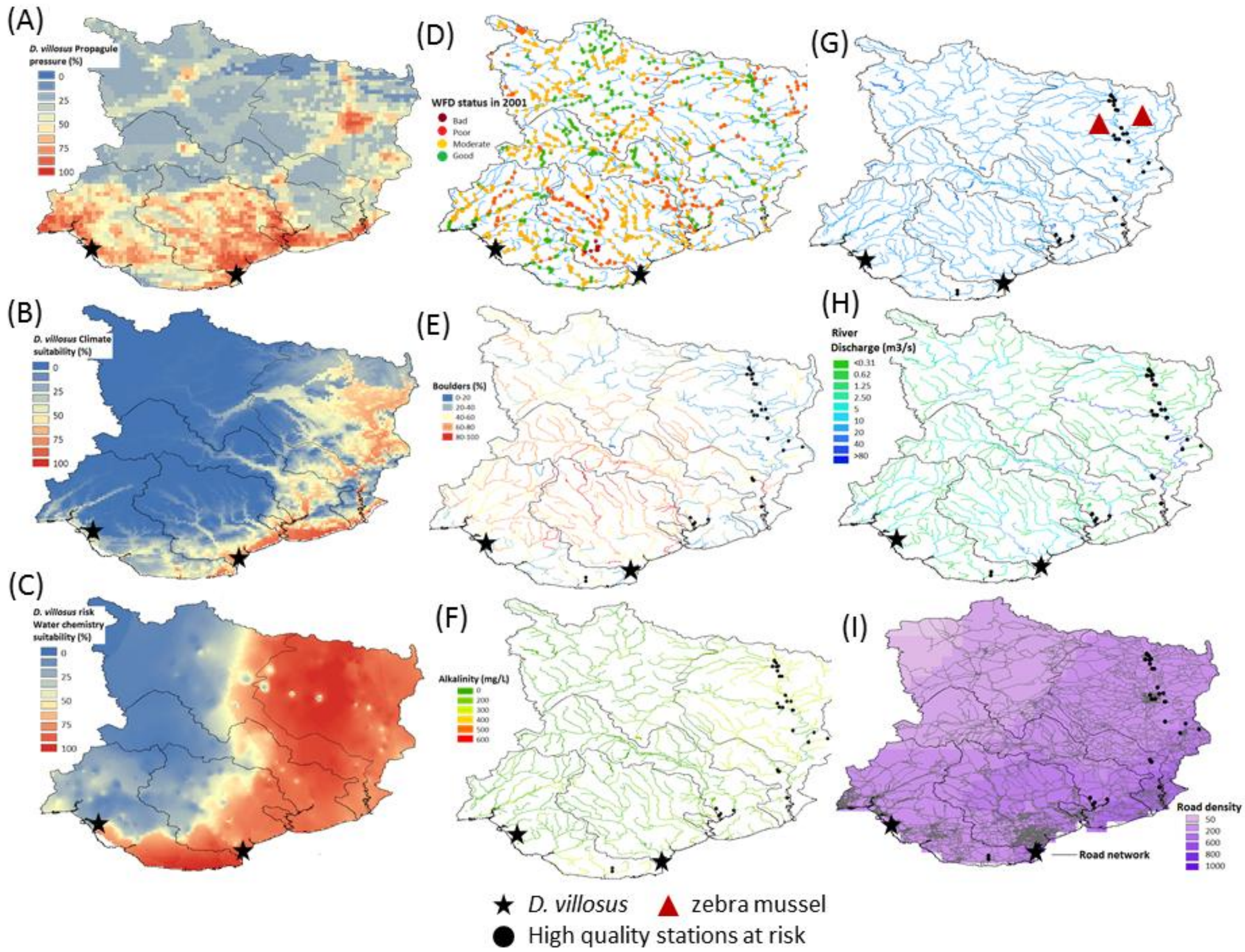


Figure 10. Analysis of risk in South Wales. A-C: results of species distribution models performed with propagule pressure, climate and water chemistry factors respectively. D: Ecological status (in 2011) of monitoring water bodies located in the catchment. E: Percentage of boulder substrate. F: alkalinity of waters. G: Hydrological connectivity and presence of zebra mussel. H: River discharge. I: road network and road density as indicators of access facility and potential human-mediated transport.

4. Broadland Rivers. The Broadlands Rivers area showed very high propagule pressure and climatic suitability for the species, while water chemistry suitability was lower than in the River Ouse catchment (Fig. 11). Similar to the Ouse case, substrate texture –dominated by sand and silt—is not particularly suitable for *D. villosus* establishment, while water chemistry is in the suitable range of the species. Only 3 Good ecological status water bodies were identified within the High risk area in this region. These water bodies are very unlikely to be invaded by natural means as they are located upstream of different river courses. However the high road density close to 5 of the Good ecological status water bodies and closeness to invaded sites may facilitate their access and thus the accidental introduction of the species. Finally it is worth noting the presence of zebra mussels within locations close to Barton Broad may facilitate spread. In summary, a high climatic match with the range of the species, high conductivity and nitrate concentration, road connectivity and recreational boating are the most important risk factors in the area, although the spread of the species towards Good ecological water bodies is unlikely, at least in the short term (Table 6).

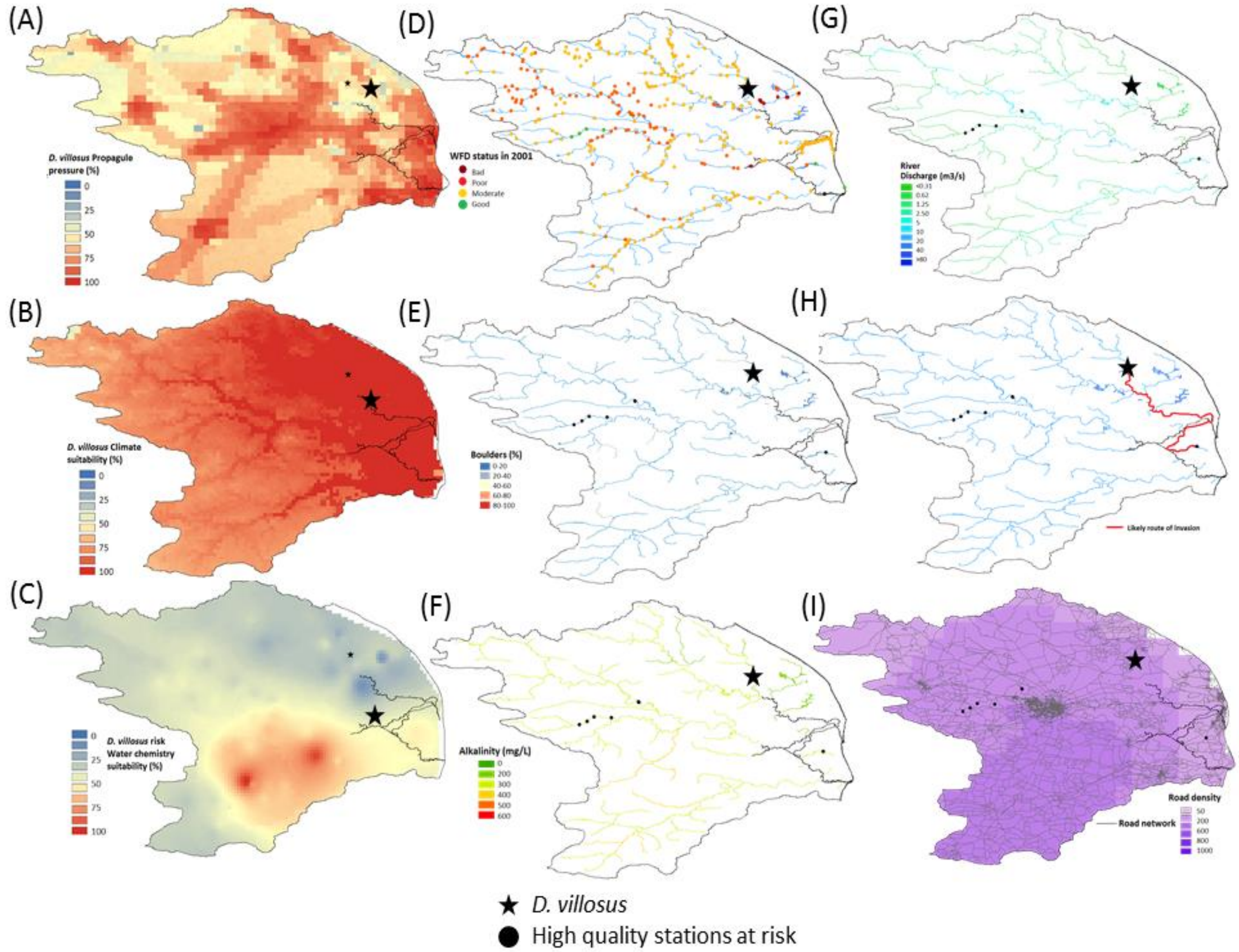


Figure 11. Analysis of risk in the Broadland Rivers. A-C: results of species distribution models performed with propagule pressure, climate and water chemistry factors respectively. D: Ecological status (in 2011) of monitoring water bodies located in the catchment. E: Percentage of boulder substrate. F: alkalinity of waters. G: River discharge. H: likely routes of natural dispersal from invaded water bodies towards High quality water bodies under risk. I: road network and road density as indicators of access facility and potential human-mediated transport.

When the four regions are compared, the River Ouse and Cotswolds and Bristol areas seem to yield high risk scores for most of the local scale factors examined. Both of them show a high climatic and water chemistry match with the range of the species, boulder substrate in some of the reaches, widespread presence of zebra mussels and a dense road network that suggest environmental degradation and high accessibility to the public.

Table 6. Summary of risk factors associated with *D. villosus* at the local scale. Catchments are divided into geographical regions according to Figure 6. Numbers of Good, Moderate, Poor and Bad waterbodies refer to those within the High risk are defined through species distribution models (Figure 2).

	Ouse River	**Cotswolds and Bristol**	**South Wales**	**Broadland Rivers**
Propagule	High	Intermediate	Low. Only high close to the coast	High
Climate	High	High. Overall at the North East	Low. Only high close to the coast	High
Chemistry	High in Upper and Bedford Ouse	High	High in the East part	Low
Combined	High	Intermediate	Low. Only high close to the coast	High
Good quality water bodies	21	36	10	3
Moderate quality water bodies	71	110	14	51
Poor quality water bodies	35	71	6	19
Bad quality water bodies	5	7	0	2
Alkalinity (mg/L)	104.0-541.1	106.8-331.0	66.86-277.4	103.9-433.3
Conductivity (µS/cm)	168.8-1817	197.9-1779	209.9-687.9	168.8-5781
Nitrate (mg/L)	2.90-42.71	2.37-28.72	8.09-18.06	2.90-42.9
pH	8.13-8.64	7.72-8.49	7.58-8.11	7.55-8.98
DOC (mg/L)	1.29-7.43	1.51-8.34	4.83-6.20	1.29-7.43
Sulphate (mg/L)	57.31-126.8	19.11-89.28	11.59-43.14	57.3-125.5
Substrate	Boulders > 50% only in Upper and Bedford Ouse. Rest < 20%	Boulders > 40% in lower reaches of Severn Vale and Bristol catchments	Boulders > 40 %	Boulders < 20%
River discharge	High in the main channel and upper reaches	High in the main channel and lower reaches	High in the river Wye	Generally low
Zebra mussel presence	All over the catchment, overall in the upper and middle reaches	Present in all sub-catchments	Limited to a few locations in the Severn Vale and the Wye river	Limited to a few locations in the lower reaches
Hydrological Connectivity	High possibility of downstream and upstream dispersal dispersal. Several 'Good' ecological status water bodies directly connected	Not hydrologically connected to any invaded region	Upstream dispersal facilitated by boulders	High downstream and upstream connectivity of invaded sites
Road connectivity	High in Upper and Bedford Ouse	High	High close to the coast including Cardiff Bay	High in central Broadlands
Overall evaluation	High risk	Intermediate risk	Low risk	High risk

3.3 Potential Effects on Biomonitoring

Literature Review

Using the Web of Science database (ISI web of Knowledge, 2010; accessed on 08.03.2012) the term "*Dikerogammarus villosus*" returned 158 journal articles published between 1978 and 2011. Refinement of this search, by reading through the article looking specifically for information detailing taxa affected by the presence of *D. villosus*, provided four articles (Table 7) containing information relating to experimental and field observations of the effects of *D. villosus* presence on freshwater invertebrate fauna (Table 7).

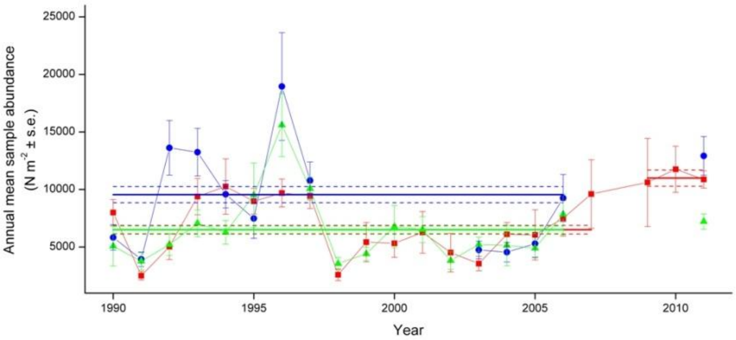
Table 7: Details of species affected by the presence of *D. villosus*

Study Type	Species affected	Weakly / not affected species	Reference
Field	*Gammarus tigrinis* *Gammarus dubenii*		Dick & Platvoet, 2000
Experiment	*Caenis robusta* *Asellus aquaticus* *Ischnura elegans* *Sigara sp.* *Eurycercus lamellatus* *Chironomus sp.* *Chaoborus sp.*	*Pisciola geometra*	Dick et al., 2002
Experiment	*Tubifex sp.* *Asellus aquaticus* *Chironomid larvae* *Simuliid larvae* *Ephemerella sp. larvae*	*Hydropsyche sp. larvae*	Krisp & Mailer, 2005
Field	*Chelicorophium curvispinum*	*Dendrocoelum sp.* *Ancylus fluviatilis* *Gastropoda spp.*	van Reil, et al., 2006

Benthic Invertebrates

For the benthic invertebrate community, all four models of community characteristics (i.e. total invertebrate abundance, community richness, community diversity and Chironomidae abundance) invasion status, reservoir and an interaction between the invasion status and reservoir were highly significant in explaining differences in the four community characteristics. All four models indicated Pitsford and Rutland Waters had significantly higher total invertebrate abundance ($F_{(2,2314)}$=26.56, p<0.001), community richness ($F_{(2,2314)}$=88.83, p<0.001), community diversity ($F_{(2,2314)}$=54.76, p<0.001) and Chironomidae abundance ($F_{(2,2314)}$=19.99, p<0.001) than Grafham Water. All characteristics were significantly higher post 2009 (abundance, $F_{(1,2314)}$=83.34, p<0.001; richness, $F_{(1,2314)}$=178.96, p<0.001; diversity, $F_{(1,2314)}$=44.46, p<0.001; Chironomidae. $F_{(1,2314)}$=38.50, p<0.001) and all two-way interactions were significant except for chironomidae (Figure 12).

Information gathered from littoral macroinvertebrate samples collected by the Environment Agency at Grafham and Rutland Waters in 2004 and 2005 were also made available. The numbers of Chironomidae collected in the 2004/5 samples were compared with 2012 numbers of chironomidae in littoral samples at these two reservoirs using data gathered as part of a Natural England funded project (Dodd & Aldridge, 2012). At total of 580 and 470 Chironomidae were collected at Grafham and Rutland respectively in 2004/5 and in 2012, 8 Chironomidae were collected at Grafham and 60 at Rutland. The relative difference in Chironomidae numbers between the two reservoirs was significantly different in each time period (χ^2=47.75, p<0.001; Yates corrected for low sample size).



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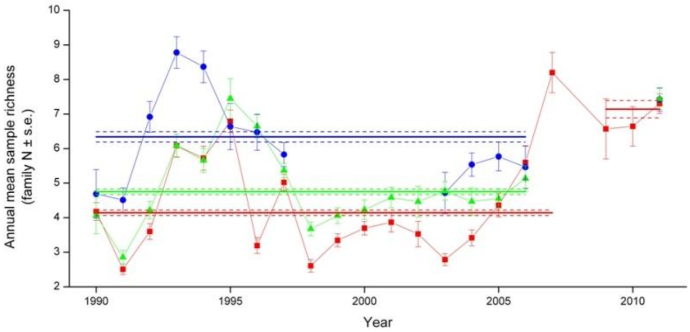

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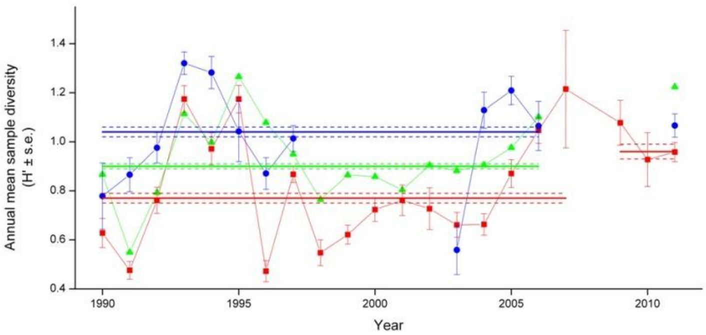

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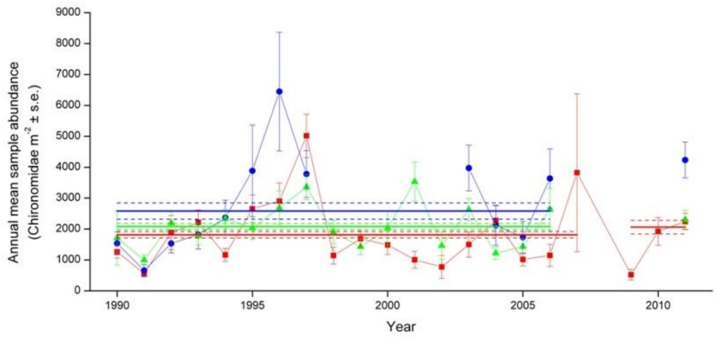
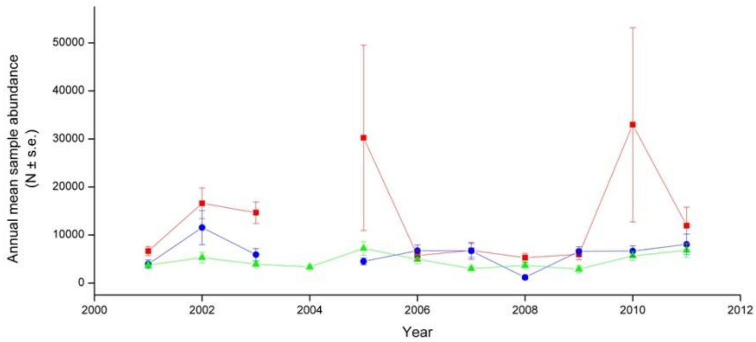


(D)

Figure 12: Community characteristics derived from the long-term timeseries of deep water (c. >2,m) invertebrate samples collected by Anglian Water; (A) total community abundance, (B) community richness, (C) community diversity and, (D) Chironomidae abundance. Grafham Water, red squares; Pitsford Water, blue circles; Rutland Water, green triangles; pre 2009 mean and 2009 onwards reservoir mean solid line (± se, dashed line).

Phytoplankton

For phytoplankton samples, differences in reservoir-specific community characteristics were only evident for abundance ($F_{(2,1162)}$=32.01, p<0.001), with Grafham Water having significantly greater total phytoplankton abundance compared with the other two reservoirs. Phytoplankton diversity and richness was not significantly different between the three reservoirs and phytoplankton community abundance, richness and diversity were all significantly greater post 2009 (abundance $F_{(1,1162)}$=17.12, p<0.001; richness $F_{(1,1162)}$=17.37, p<0.001; diversity $F_{(1,1162)}$=25.44, p<0.001) (Figure 13).



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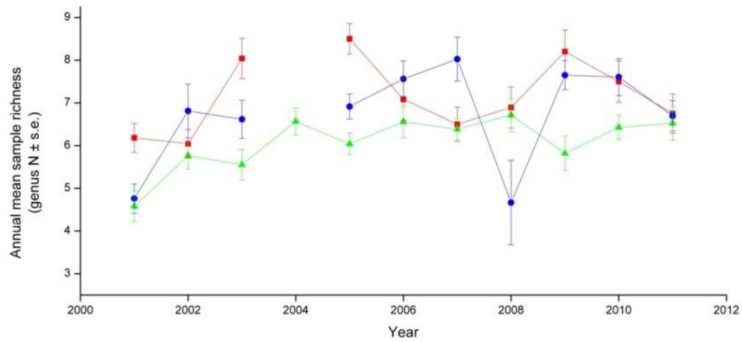
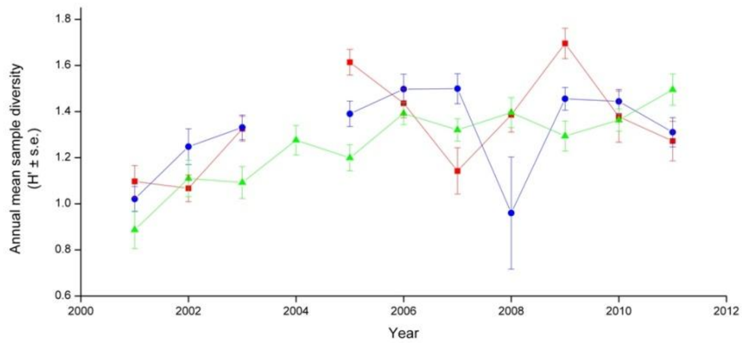


(B)



(C)

Figure 13: Community characteristics derived from the long-term timeseries of phytoplankton samples collected by Anglian Water; (A) total community abundance, (B) community richness and, (C) community diversity and. Grafham Water, red squares; Pitsford Water, blue circles; Rutland Water, green triangles.

4. Discussion

4.1 Risk of invasion of *D. villosus* at the scale of England and Wales

In this study three species distribution models have been developed to assess the potential risk of invasion of *D. villosus* in England and Wales. The three models are expected to reflect different aspects related to the process of invasion: the introduction of propagules, large-scale climatic constraints and local-scale water chemistry constraints. The first model used socio-economic factors which are expected to be related to the vectors of introduction of the species (e.g. ports, reservoirs, roads) and the intensity of use of aquatic ecosystems (e.g. population density, human influence). In a pan-european study, Pysek et al. (2010) highlighted the density of population and economic wealth of countries as main predictors of the number of invasive species in a given country. This relationship was further confirmed by Keller et al. (2009) at the scale of Great Britain. Ports are also important gateways of aquatic invaders introduced with ballast water or through hull fouling, which according to Stretfaris et al. (2005) could be as many as 10,000 species every day. Human influence and distance to ports were indeed two of the main predictors in the propagule pressure model. Propagule pressure is predicted to be highest in the London area, the north-east of England –close to commercial ports—and the Midlands. The climatic model offered the highest AUC score and in our opinion the most ecologically meaningful prediction. *D. villosus* showed in this case a negative response to altitude, as it might be expected for a freshwater species; and it was limited by a minimum winter temperature of -5˚C. Minimum temperature is certainly important for the survival of freshwater species, although the winter survival of only a few individuals may be enough to maintain the population. South-East England, East Anglia, and the Midlands obtained highest suitability scores under the climatic model.

Water chemistry is an important factor affecting the distribution of freshwater species, though the prediction offered by the water chemistry model showed the lowest AUC score and was deficient in several aspects. Firstly, the interpolation of water chemistry data across Europe provided distorted maps with particularly high or low values generating artificial spherical structures that warped the prediction map. Second, water chemistry values are very variable depending on seasonality and human activities (e.g. agricultural and urban runoff). Thus, to be reliable, maps should encompass intra- and inter-annual changes in water chemistry (e.g. climatic data used in this study are means for the 1960-1990 period). Third, no data was available for eastern European countries (e.g. Belarus, Ukraine) where *D. villosus* is native, which further limits the ability of the model to reflect the range of the species. Although results should be taken with caution, highest water chemistry scores were found in the Cotswolds and North England; and contrary to the propagule pressure and climate models, water chemistry suitability scores were low in South East England and East Anglia.

Because of the limitations associated with the water chemistry model, only the first two models were combined in order to find the areas where both propagule pressure and climatic suitability may facilitate invasion. This High risk area comprised 26% of the area of England and Wales, which is lower than the 60% obtained in a previous study (Gallardo et al., 2011). Differences in the High risk area between both models are due to the different algorithm used for modelling (Support Vector Machines vs. MaxEnt), the threshold used to transform the continuous predictions into a predicted presence-absence map (more conservative in the present study) and the area used as reference to calculate percentages (Great Britain vs. England and Wales). The total percentages of England and Wales suitable for the species given above refer to the total territory and not to the extension of waterbodies, so they should be taken as a simple approximation. Because waterbodies are separated by extended land patches, the colonization of climatically suitable habitats will depend on the ability of the species to disperse through interconnected waterbodies and the role played by vectors (e.g. animals, anglers and boaters). In England, the extensive Midland canal system, constructed predominantly in the 18th and 19th centuries, provides widespread and interconnected hard substrates along which *D. villosus* can spread and reach the regions most climatically suitable or with more appropriate water chemistry (Gallardo et al., 2011). Moreover, the areas where climate suitability for *D. villosus* is especially high already support a well-established and abundant population of another Ponto-Caspian species, the zebra mussel (*Dreissena polymorpha*) (Aldridge, 2010), which is known to facilitate the dispersal of *D. villosus*. These two species have co-evolved over a long period of time, being commonly found in the same assemblages (Casellato et al., 2007b). Zebra mussel populations may help the colonization of *D. villosus* by providing habitat complexity through the production of byssus threads and shells, and food material through biodeposition (Gergs & Rothhaupt, 2008). Currently the zebra mussel is present in much of England, and localised parts of Wales; and it is experiencing an increase in abundance and spread in relation to increasing water quality and waterway connectivity (Aldridge et al., 2004). Approximately 3% of the WFD monitoring water bodies showing Good ecological status in England and Wales are located within the High risk area. The ecological status of these water bodies may be especially vulnerable to an eventual invasion of *D. villosus*, which may prevent achieving the WFD 2015 and 2025 targets. The majority of these Good ecological water bodies at risk were located in the Cotswolds and Bristol area, the Severn Vale, Broadland Rivers and the River Ouse catchment.

4.2 Risk of invasion of *D. villosus* at the catchment scale

Although species distribution models are useful to risk assess England and Wales at large-scale, accurate predictions at the local scale should consider other habitat-related factors. In the case of *D. villosus*, hard artificial substrate (boulders), a high oxygen saturation (2.6-15.3 mg/L) and low to intermediate conductivity (223-2820 µS/cm) have been identified as important suitability factors (Boets et al., 2010). For this reason, we selected four major geographical regions in England and Wales to further investigate how local environmental factors may potentially affect the spread and establishment of *D. villosus*: the River Ouse catchment, Cotswolds and Bristol area, and South Wales. Amongst the four areas, the River Ouse catchment showed the highest risk scores in terms of climate suitability and water chemistry. However, hard substrata are rare in the River Ouse catchment, dominated by sand and silt, which may prevent the colonization of *D. villosus*. Nevertheless, because there is a considerable number of Good ecological status water bodies directly downstream of Grafham reservoir, efforts to control the spread of the species to the main River Ouse channel are particularly relevant. The Cotswolds and Bristol area also showed high risk scores, including a greater coverage of boulder substrata in the lower reaches, which suggest that this region is under a particularly high risk of invasion in the event of an introduction. Because the species has not yet been detected, and there is not direct hydrological connection with invaded catchments, its introduction must be necessarily human-mediated. For this reason, public awareness is vital to prevent the accidental introduction of the species in the Cotswolds and Bristol region. Finally the South Wales region showed the lowest suitability scores, both in terms of climate and water chemistry, which suggest that the spread of the species in this area is very unlikely. The two invaded water bodies, Elgwys Nunydd reservoir and Cardiff Bay, are located close to the coast (in the strip showing high climatic suitability) and therefore downstream dispersal can be disregarded. However, upstream dispersal might allow the species to access Good ecological water bodiewater bodies if the species spread to the main river channel is not controlled, especially considering the wide coverage of boulders in this region.

In summary, climatic and water chemistry suitability may allow the establishment of *D. villosus* in the River Ouse and the Cotswolds and Bristol regions; while in South Wales only a strip along the south coast is suitable for the species. *D. villosus* is known to inhabit preferably hard artificial substrata such as boulders, which are abundant in South Wales, the upper and lowest reaches of the Bedford and Ouse rivers and the lower reaches of the Severn Vale. Substrate may therefore strongly limit species dispersal. River discharge was logically higher in the main river channels of these catchments than in secondary reaches. However, because water current is not always directly related to river discharge, we cannot assess how it is likely to affect the species dispersal.

4.3. Dispersal considerations of *D. villosus*

Downstream natural dispersal in the case of *D. villosus* is likely to be achieved through active drift (Bilton et al., 2001; van Riel et al., 2006). In the Rhine river for instance, *D. villosus* along with another Ponto-Caspian invader, *Chelicorophium curvispinum*, accounted for 90% drifting invertebrates (Van Riel et al., 2011). According to the authors, drift activity occurred mainly at night, it was lowest in winter and most intense in summer. Organisms were able to colonize the stony substrate directly from the water column. Higher drift in spring in summer can be triggered by changes in abiotic conditions (food availability, occurrence of spring floods) or by a higher overall macroinvertebrate abundance and reproduction, reflecting particular life-cycle dynamics (Cellot, 1996). In this sense, a strong relationship between drift and the density of shrimps in the benthos has been noted in *Gammarus pulex* (Elliott, 2002) which suggests that higher densities of *D. villosus* will result in a higher probability of downstream drift. In this regard, high densities (ca. 400 individuals/m^2) of *D. villosus* have been observed throughout Grafham reservoir, especially in the margins (MacNeil et al., 2010b); as well as the two Welsh locations (up to 4,000 individuals/m^2 , S. Ormerod unpubls. data). Gammarids often move upstream against the water current, although the importance of such dispersal is not clear. Hancock and Hughes (1999) documented very restricted movement upstream by adult shrimps only while juvenile and larvae moved downstream. In contrast, Elliott (2003) found gammarids to move predominantly upstream (up to 2 km per year). The natural spread of the species is therefore not constant and depends on a variety of biotic (density, presence of predatory fish and invertebrates) and abiotic (water current, temperature, oxygen, seasonality) factors.

As noted before, limited dispersal through unsuitable habitats may hamper the ability of the species to spread. However, as highlighted by Gherardi (2007) the high inherent dispersal ability of aquatic species often allows them to swim or drift through unsuitable areas, making aquatic habitats especially vulnerable to biological invasions. Consequently, while aquatic species would need external vectors to invade new catchments at a large scale, unsuitable patches at the catchment scale may only slow down their dispersal. Because the species is in three out of four locations confined to reservoirs not directly linked to the main hydrological network, dispersal through human activities becomes especially relevant. The spread of *D. villosus* can be facilitated by human activities such as fishing, angling or boating (Leuven et al., 2009). Surfaces such as waders, boats and equipment are vulnerable to fouling and could transport the species between water bodies (MacNeil et al., 2010b). The species could also be transferred with movements of fish stocks or foraging water birds. Certainly, in the case of England and Wales the spread of *D. villosus* is characterized by long jumps, which suggests human-assisted introduction rather than slow dispersal by natural means. In addition, the widespread distribution of the zebra mussel suggests that aquatic invaders may be able to overcome the limitations imposed by unsuitable land patches or confinement. This highlights the importance of a rapid implementation of prevention measures to minimize the human-related spread of aquatic invaders between catchments.

The new population recently discovered in the Norfolk broad is especially worrisome, since the broad is connected both upstream and downstream to the main Ant river channel. *D. villosus* needs to reach a maximum density to start spreading and colonizing the rest of the river channel both upstream and downstream. The species has been recently found in new locations close to the one initially reported on 14th March, which suggests the species is long since established in the Broads, has already reached such critical density and is now beyond control. With the limited information currently available, we can't identify the origin of the invasion of the Broads and direction of spread. However, the clustering of invaded sites upstream the river Ant suggests a downstream drift from there to the downstream invaded sites. In a previous study (Gallardo et al., 2011) we assumed the species can disperse at a velocity between 20 and 100 km/year downstream and 2-10 km/year upstream, depending on biotic and abiotic factors including the river discharge and substrate texture. Even considering the most conservative scenario (20 km/year downstream), this would lead to a complete invasion of the river Ant catchment in a relatively short period of time.

4.4 Potential Effects on WFD Monitoring

4.4.1 RICT – River Invertebrate Classification Tool

Evidence from the published literature has shown the wide range of prey species affected by the presence of *D. villosus* (Table 1). Generally groups of invertebrates which were less affected by the presence of *D. villosus* were Gastropoda spp. (snails & limpets, including *A. fluviatilis*) and *P. geometra* (fish leech). These groups are well armoured (snails and limpets have shells and leeches have a high chitin content) and are low scoring groups in the RICT (e.g. Hirudinea score 3 and molluscs generally score 3). Groups of invertebrates which were affected strongly by the presence of *D. villosus* were mayflies (e.g. Caenidae and Ephemerellidae), Simuliidae and Gammaridae. These species are less well armoured and generally higher scoring in the RICT (e.g. Ephemerellidae score 10, Caenidae score 7, Simuliidae and Gammaridae score 6). Predatory and competitor effects of the presence of *D. villosus* in riverine macroinvertebrate communities are likely to have a differential effect on the presence of high and low scoring families. A reduction in the number of high scoring families and little or no effect on low scoring families will result in both lowering of the Average Score per Taxon (ASPT) and a lowering in the number of scoring taxa (NTaxa)in water of high and good quality.

Additionally, the presence of *D. villosus* within the macroinvertebrate community may affect the RICT, as *D. villosus* individuals would score as Gammaridae under the RICT. Emerging evidence is that *D. villosus* has the capacity to withstand greater environmental extremes compared with the native *Gammaurs pulex* (Devin & Beisel, 2007). An increased tolerance of high temperature and low dissolved oxygen may potentially allow *D. villosus* to survive under conditions which would normally exclude Gammaridae. The effects of invasive Gammaridae on the BMWP system has been highlighted from the Isle of Man, where the presence of the invasive species resulted in an over inflation of the ASPT score in poor quality areas (MacNeil & Briffa, 2009).

Overall, the presence of *D. villosus* in river sections may affect the output from RICT in two ways. Firstly by reducing ASPT scores in river sections of high and good quality and secondly, by inflating ASPT score in sections with poor quality.

4.4.2 CPET - Chironomid Pupal Exuvial Tool

Initial results from the long-term data analyses suggest that invertebrate communities in deeper waters may escape some of the impact of *D. villosus* invasion. However, the interpretation of this data analysis must be considered in light of the limitations of the data series mentioned previously. One potential reason supporting this result is the lack of coarse substrate present at the depths from which the deep water invertebrate samples were collected in Grafham Water. As *D. villosus* shows a significant preference for coarse substrate (Boets, et al., 2010, Hesselschwerdt, et al. 2008) it may be likely that the deep water fauna present in Grafham reservoir has, to date, escaped detectable effects of *D. villosus* invasion. Information relating to the invertebrate community structure gathered from the margins of Grafham, Pitsford and Rutland have shown that the littoral Chironomidae community in Grafham may be further reduced as a result of the recent *D. villosus* invasion, potentially lowering the number of exuviae available for the classification tool. The differential effect of *D. villosus* on the deep water and shallow water invertebrate communities may have implications for CPET monitoring of still waters.

4.4.3 LAMM - Lake Acidification Macroinvertebrate Metric

Effects on *D. villosus* invasion on lake monitoring using the LAMM are likely only to be apparent in lakes with a pH within the range 6.7 to 8.8. As *D. villosus* is does not tolerate acidic conditions it is likely that invasion of this species will only occur in lake systems that are either not affected by lowered pH or have recovered pH levels to that falling within the range suitable for D. villosus survival. The relatively high altitude (upland) location of many of the acid-sensitive water bodies also makes them unlikely candidates for the establishment of *D. villosus*. It is unlikely that *D. villosus* has the potential to establish within lake systems currently monitored for acid-sensitivity, however the species does have the potential to impact the metric produced by LAMM in lakes which meet the species' environmental requirements.

4.4.4 Phytoplankton

Results from the long-term data analysis suggest that there is (at present) no detectable effect on the diversity or abundance of phytoplankton following the invasion of *D. villosus*. It is likely therefore that this monitoring technique may remain effective following the invasion of *D. villosus*.

Current monitoring methods used in the WFD will likely detect the ecological impacts of *D. villosus* invasion; specifically the RICT would be highly sensitive. The ecological effects of *D. villosus* invasion in lakes may not be easily detected with the current assessment methods, although the long-term effects of *D. villosus* invasion are yet to be established. Littoral samples of macroinvertebrates in lakes would almost certainly detect an ecological signal following the invasion of *D. villosus* in lakes falling within the environmental tolerance range for this species. There is evidence that *D. villosus* will consume micro algae (Platvoet, et al., 2006) as such there is a potential that both phytoplankton and diatom assessment methods may be affected by the presence of *D. villosus*. Information relating to *D. villosus* utilising this resource is sparse and no general conclusions can be drawn regarding effects on these monitoring methods.

Based on our assessment of the published literature and the biological data available, the invasion of *D. villosus* within water bodies which are currently at a status less than good is likely to affect the potential of the water body to reach good status in river systems. For lakes, the potential to achieve good ecological status in biological terms may be possible. However, as there may be secondary effects on the other two elements (physicochemical and hydromorphological), as a result of feedback loops within the system (Odling-Smee, et al., 2003), good ecological status may be prevented through secondary effects of *D. villosus* invasion within the system. For example, *D. villosus* has been shown to consume zooplankton (Stoffels, et al., 2011). Impacts on zooplankton may have knock on effects on the status through increased abundance of phytoplankton and a resultant decrease in dissolved oxygen.

4.4.5 Fisheries Classification System (FCS)

Although not assessed directly, the impacts on the FCS monitoring tool may be impacted by the invasion of *D. villosus*. As *D. villosus* has been shown to predate on *Cottus perifretum*, bullhead, eggs (Platvoet, et al., 2009) and larval fish (Platvoet, et al., 2009), it may be likely that the invasion of *D. villosus* may affect this classification tool through a reduction in fish abundance as a result of D. villosus predation on the early life history stages of fish species used in the classification tool.

4.5 Management guidelines for the control of *D. villosus* in England and Wales

The results from this study have shown that the movement of *D. villosus* in England and Wales is characterised by long- jumps, strongly suggesting that current dispersal of this species is human mediated. This relationship is exemplified by both the lack of evidence of *D. villosus* colonising the River Ouse through natural drift and, the recent colonisation of Barton Broad which is approximately 130 km from Grafham Water (the nearest known *D. villosus* population).

However, because Barton Broad is directly connected with the main river channel, natural dispersal may be relevant in this last location. Considering the dispersal abilities of the species, we may assume *D. villosus* is long since established in the river Ant broads, reached a critical density and started drifting downstream, although upstream dispersal cannot be disregarded. Considering the relevance of Barton Broad for the conservation of natural biodiversity, the control and –if possible eradication —of the species should be prioritized in this area. As the species drift is maximum in early spring and summer, it is vital to implement action as soon as possible, definitely before summer, to stop the further spread of the species.

Current investigation of control methods have shown that many of the very effective control agents would prove difficult to be implemented at infected water bodies (Stebbing, et al., 2011) due to the stringent nature and associated licensing problems of some chemicals for public use and the associated risk involved use near potable water sources. Education, physical removal, drainage of infected water bodies, and control of boat movement are therefore likely to be most crucial in limiting further spread of this species.

The potential effectiveness of current WFD monitoring strategies could be improved to include a measurement of bio-contamination within biological communities. These methods are being developed (Arbačiauskas, et al., 2008; MacNeil, et al., 2010) and their implementation in ecological monitoring being discussed (Cardoso & Free, 2008). Generally evidence of bio-contamination (the presence of non-native species) within invertebrate communities reduces the resultant overall ecological status of the water body under assessment. Incorporating these methods may provide a more accurate assessment of ecological status.

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