diversity

Article

Risk Screening and Distribution of the Invasive Amphipod *Dikerogammarus villosus* (Sowinsky, 1894) in the River Adda (Northern Italy)

Daniele Paganelli *, Maria Angela Cianci and Agnese Marchini

Department of Earth and Environmental Sciences, University of Pavia, Via Sant'Epifanio 14, 27100 Pavia, Italy
* Correspondence: daniele.paganelli@unipv.it

Abstract: In 2016, *Dikerogammarus villosus* (Sowinsky, 1894) (Crustacea, Amphipoda) was recorded for the first time in the River Adda, one of the main tributaries of the major Italian river, river Po. Here we investigate its distribution, population density and size classes distribution in the main course of the river, in the territory of the South Adda Regional Park. Furthermore, we defined its level of invasiveness using two different risk screening methods, in order to obtain a more comprehensive evaluation of its potential impacts: AS-ISK (Aquatic Species Invasiveness Screening Kit) and *Harmonia*$^+$. Finally, we compared the resulting invasiveness level with the output of the GISS (Generic Impact Scoring System) method, also used by IUCN. Our data confirm that the invasive amphipod is well-established in the downstream part of the River Adda, and its distribution seems related to hydrodynamism. Interestingly, its level of invasiveness was evaluated as low by *Harmonia*$^+$ and high by AS-ISK: this discrepancy is due to different evaluations of environmental and socio-economic impacts of a non-native species carried out by the two methods. Finally, we propose some possible actions to reduce the spreading rate of this invasive amphipod in this area.

Keywords: killer shrimp; invasiveness; impacts; management; crustaceans; native biodiversity; freshwater

check for updates

Citation: Paganelli, D.; Cianci, M.A.; Marchini, A. Risk Screening and Distribution of the Invasive Amphipod *Dikerogammarus villosus* (Sowinsky, 1894) in the River Adda (Northern Italy). *Diversity* **2022**, *14*, 838. https://doi.org/10.3390/d14100838

Academic Editors: Fabio Stoch and Michael Wink

Received: 1 August 2022
Accepted: 29 September 2022
Published: 5 October 2022

1. Introduction

Non-native species are organisms intentionally or unintentionally introduced outside their natural range of distribution by humans or human-mediated activities [1]. However, not all of them have high potential invasiveness (bio-ecological traits that enable them to spread and damage the environment) and, luckily, many of them remain innocuous (low potential invasiveness) [2,3]. Their impacts can vary from minimal or insignificant (no invasiveness) to substantial effects on the local ecosystem (high invasiveness) [4].

The Convention on Biological Diversity considers invasive species as one of the main threats to native biodiversity at a global level, as they can severely reduce local diversity and heterogeneity. In addition, they cause several ecological problems such as the alteration of ecosystem functioning, and negative interactions with native species, possibly leading to local extinctions [5–7]. Non-native species could also affect anthropogenic activities, causing costs of billions of euros [8–10] and, finally, they can directly or indirectly affect human health [1].

Freshwater ecosystems are particularly impaired by invasive species. Their native biota is characterised by high rates of endemism due to their nature of habitat-islands surrounded by lands; consequently, freshwater biodiversity is particularly threatened by anthropogenic impacts such as habitat alteration, pollution and over exploitation of the water resource [11–13]. In this context, non-native species intentionally or unintentionally introduced can find several empty niches and increase the biological homogenisation of these ecosystems [14].

In Italy, freshwater habitats host 150 non-native species, 43% of them being invertebrates (Tricarico, personal com). The high interconnection of hydrographic systems plays an important role in their dispersion, which is also increased by their high ecological adaptation [15,16]. The combination of these two conditions makes it very difficult to prevent the colonisation of non-native invertebrates, especially because most of them are usually small and their early detection is almost impossible [17]. Moreover, apart from a few examples such as freshwater bivalves or crayfish, the ecological impacts of non-native freshwater macroinvertebrates are poorly known, as well as their socio-economic impacts [18,19].

However, despite these difficulties, it is important to assess the risk of invasiveness of freshwater macroinvertebrates as well as their potential impacts on the newly colonised environment.

Dikerogammarus villosus (Sowinsky, 1894), a freshwater amphipod of Ponto-Caspian origin, has colonised several freshwater ecosystems in Europe, thanks to its high adaptability to environmental conditions [20–24]. In Italy it was known since 2003 from Lake Garda; from there, *D. villosus* spread in the river Mincio, then in the river Po [25–27]. In 2008, it was also detected in lake Bilancino in central Italy [28]. In 2016, it was found in the River Adda by the regional agency for the protection of the environment (ARPA Lombardia, unpublished data).

The main pathway of introduction and dispersal of this species could be identified as ships' ballast water, but it is also true that the interconnection of waterways and their active migration have contributed to the colonisation of the European territories [29–31]. Moreover, it was proved that *D. villosus* has the ability to resist out of the water and it could cling to waders, swimsuits and fishing equipment [32].

An aspect of the high ecological adaptability of *D. villosus* consists in its wide trophic niche: its diet varies from microalgae to invertebrates, up to fish and amphibian larvae and eggs [33–37], and it can opportunistically switch between feeding groups depending on available resources and season [38].

In this paper, we provide the first assessment of the distribution and population density of *D. villous* in a newly invaded area, namely the downstream part of the River Adda. Additionally, we provide data on its size class distribution from data collected at a single sampling event.

Beyond reporting novel distribution data of an invasive species, using this area as a risk assessment area (RA area), we define the level of potential invasiveness of the non-native amphipod by means of two different risk screening methods, in order to obtain a more comprehensive evaluation of its potential impacts: (i) the Aquatic Species Invasiveness Screening Kit—AS-ISK [39] and (ii) *Harmonia*$^+$ [40]. In particular, we selected these two screening tools following the results of a recent review of IAS assessment protocols proposed by [41] that indicated AS-ISK and *Harmonia*$^+$ as two out of the twenty-nine methods that meet the minimum standards of a risk screening tool should have. Moreover, these two methods are comparable because they quantify the invasiveness considering both bio-ecological traits and commercial impacts of IAS and they could be applied to aquatic species [42].

Then, we applied an impact assessment tool, namely Generic Impact Scoring System (GISS) [43,44], the base method used by IUCN for the evaluation of the impacts caused by non-native species [45], and compared all results.

To the best of our knowledge, this work represents the first attempt to quantify the invasiveness of this species in a newly invaded area using different approaches.

Finally, we suggested some possible actions to reduce the spreading rate of this invasive amphipod in this area.

2. Materials and Methods

2.1. Risk Assessment Area and Sampling Method

The River Adda, with a total length of 313 km and a mean flow rate of 187 m^3/s, is the longest tributary of the river Po (the major Italian river) and the fourth longest Italian river. It rises in the Alps near the border with Switzerland and flows through the densely populated and highly anthropized region of Lombardy (Northern Italy) (Figure 1).

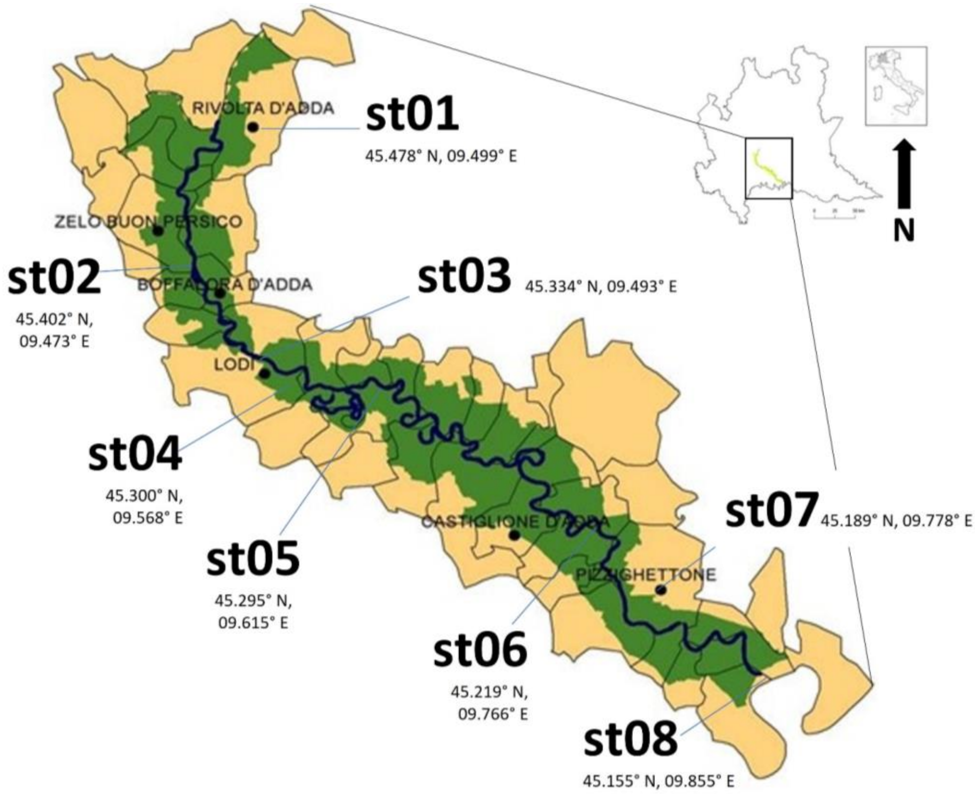


Figure 1. Study area: the main course of the River Adda in the administrative territory of the South Adda Regional Park. The coordinates are reported in WGS84.

In the administrative territory of the South Adda Regional Park (24.260 ha), the River Adda has an approximate length of 90 km, and it shows typical characteristics of a planitial river with a relatively low water current and meanders. Several natural, semi-natural and artificial canals originate from Adda and provide water to the surrounding agricultural fields and towns of different sizes.

In order to define the distribution of the non-native amphipod in the River Adda, we selected 8 sampling sites, from north to south, covering all the main course of the river in the administrative territory of the Park, qualitatively estimating the water current speed and the main substrate (Figure 1). Moreover, at each sampling time, we also recorded water temperature, oxygen concentration, percentage of oxygen saturation and conductivity using a multiparameter probe (HACH®, HQ40d).

On 31 August 2021, along a 10 m transect selected for habitat suitability and accessibility, we collected three sub-samples (following [46]) for each of the eight stations (one station every 12–15 km according to the accessibility to the water). Samples were collected by means of a modified hand net (950 μ mesh), placing a square frame (22 cm × 23 cm) covering a total area of 0.0506 m^2. Biological samples were preserved in 70% ethanol and transferred to the laboratory, where all gammarid specimens were counted and measured from the tip of the rostrum to the base of the telson using the software OPTIKA PROVIEW. Specimens smaller than 3 mm, which could not be unequivocally assigned to any gammarid species due to underdeveloped taxonomic characters, were not measured [24]. Subsequently, in order to describe the non-native amphipod populations, all specimens were sorted into 8 size classes according to their length: I, 3–6 mm; II, 6.01–9 mm; III, 9.01–12 mm; IV, 12.01–15 mm; V, 15.01–18 mm; VI, 18.01–21 mm; VII, 21.01–24 mm and VIII, 24.01–27.

As data were not normally distributed (Anderson-Darling test $p < 0.05$), the difference between the non-native amphipod density in each station was tested by means of a Kruskal–

Wallis test, followed by the Dunn test, a pairwise multiple comparisons procedure often used as a * post hoc * procedure following the Kruskal–Wallis test [47].

All the analyses were performed using the software R 4.2.1.

2.2. Risk Screening Tools

2.2.1. Aquatic Species Invasiveness Screening Kit (AS-ISK)

The Aquatic Species Invasiveness Screening Kit is a multilingual screening tool [48] available for free download at www.cefas.co.uk/nns/tools/ (accessed on 10 October 2021). It combines a Basic Risk Assessment (BRA) score, which evaluates the biogeographical, biological, and economic impacts, with a Climate Change Assessment (CCA) score, which evaluates the potential effects of future climate change on the risk of the species' introduction, establishment, dispersal, and socio-economic impacts. These are calculated from the answer to 55 questions, grouped into three sections (Biogeography/Historical, Biology/Ecology and Climate Change). The questions are also related to the sector affected by the species under screening, namely Commercial, Environmental and Species or population nuisance traits.

For each question, the reply of the assessor should be accompanied by a reference list (from scientific or grey literature), as well as by a confidence level, *sensu* Intergovernmental Panel on Climate Change [49], which is considered in the computation of the final risk value. Information on bio-ecological traits and socio-economic impact of the species were extracted from relevant literature obtained from Google Scholar and Web of Science (See Supplementary File S1).

A score > 1 indicates that an alien species may become invasive, causing medium or high risks, on the contrary, a score < 1 indicates that the alien species could not be considered invasive, and it is classified as "low risk".

For our assessment, we used the threshold for freshwater macroinvertebrates proposed by [50] that classify alien species with a BRA score > 13.25 as being "high risk".

2.2.2. Harmonia$^+$

Harmonia$^+$ is a risk screening tool for the definition of the potential invasiveness of animals or plants that may have an impact on the environment or human health [40], available for download at (https://www.biodiversity.be/2480/ (accessed on 10 October 2021)). It is composed of 41 questions divided into 10 modules (1: Introduction, 2: Establishment, 3: Spread, 4: Impacts on the Environment, 5: Impacts on Cultivated plants, 6: Impacts on Domestic animals, 7: Impacts on Human health, 8: impacts on Infrastructure, 9: Impacts on Ecosystem services, 10: effects of climate change on the screened species); only the first eight modules contribute to the final score and the authors of *Harmonia*$^+$ always recommend using the pessimistic answer in case of doubts [40].

As default settings, the arithmetic mean among the modules 1–3 scores provides the invasion score, while the maximum scores of modules 4–8 are aggregated in the impact score.

Similar to AS-ISK, the user should provide answers (all the information was collected from scientific literature as performed for AS-ISK, Supplementary File S2) as much as possible based on scientific literature as well as a level of confidence for each answer, following the framework for the consistent treatment of uncertainties by the Intergovernmental Panel on Climate Change [49].

The final *Harmonia*$^+$ score varies from 0 (lowest level of invasiveness) to 1 (highest level of invasiveness) and depends on the combination of the invasion score module, which evaluates the likelihood of introduction, establishment and spread of the species under assessment, and the impact score module, which includes all the impacts.

For the aim of this paper, we used the default setting so as not to introduce any further variability in the results and the thresholds proposed by [51]: high risk for scores > 0.66, the medium risk for scores between 0.33 and 0.66, and low risk for scores < 0.33.

2.3. Impact Assessment Tool

The Generic Impact Scoring System (GISS)

The Generic Impact Scoring System (GISS) evaluates the environmental and socio-economic impacts of alien species [43], according to 12 impact categories (1. Plants or vegetation, 2. Animals through predation, parasitism, or intoxication, 3. Other species through competition, 4. Transmission of diseases or parasites to native species, 5. Hybridization, 6. Ecosystems, 7. Agricultural production, 8. Animal production, 9. Forestry production, 10. Human infrastructure and administration, 11. Human health, 12. Human social life). In each category, the impact is scored according to a 6-level scale of intensity: from 0 (no impact or no evidence in scientific literature) to 5 (the major possible documented impact); each answer must be accompanied by a confidence level: 1 = low, 2 = medium, 3 = high [48].

The assessed species obtains a score, which varies between 0 (no evidence of impact) to 60 impact points (highest level of invasiveness) (Supplementary File S3).

As threshold values between medium and high impact levels, we used the limits proposed by [44]: impact scores of 10–19 are the limits for medium impact and scores of 20+ for high impact.

3. Results

3.1. Density, Distribution and Size Classes of *Dikerogammarus villosus*

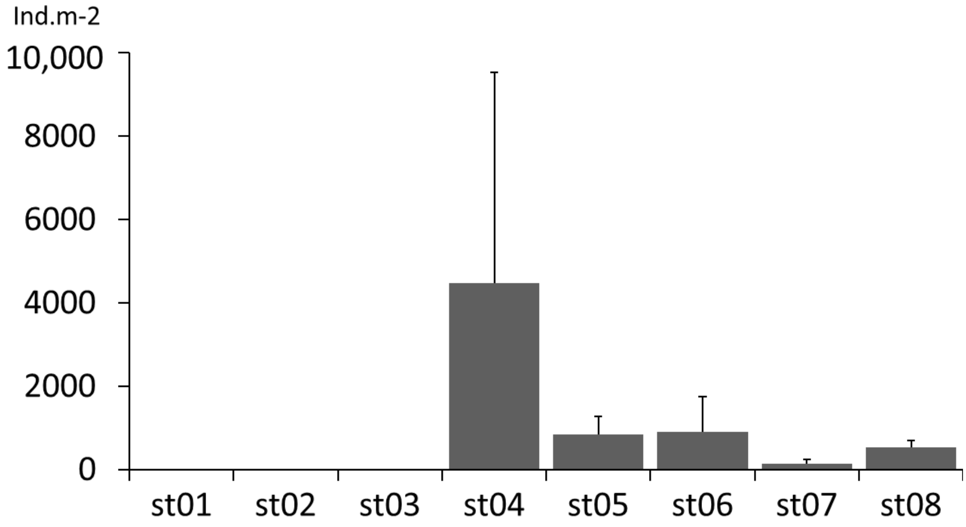
Dikerogammarus villosus was mainly present in the downstream part of the River Adda, especially in the sampling site st04 (4473 $\pm$ 5053 ind.m^{-2}), where a single ovigerous female was also found (Figure 2a). The population density was higher where the water current was less strong, and the bottom was mainly composed of finer-sized grains (Table 1).

Table 1. Main environmental parameters recorded during the sampling.

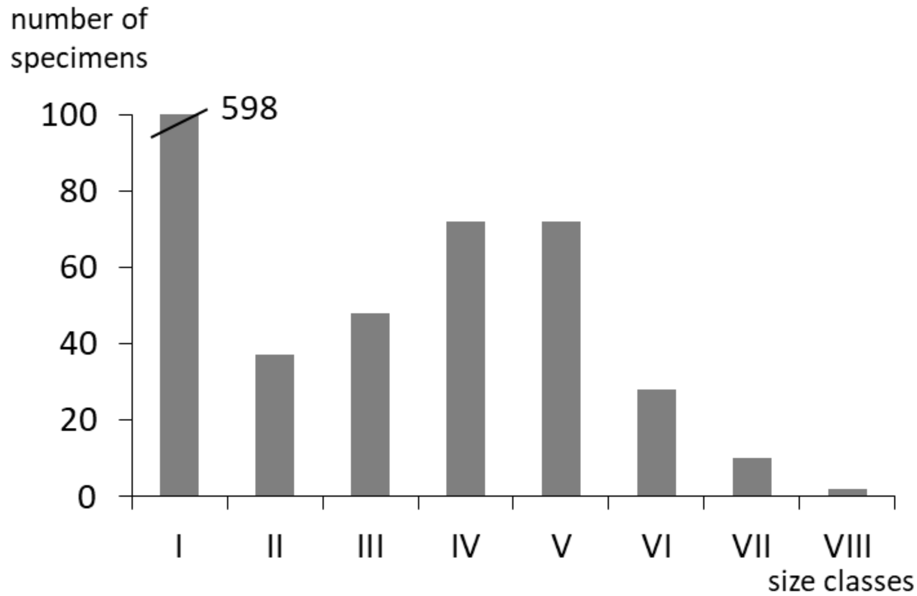
Station	Water Temperature (°C)	O_2 (mg L^{-1})	% of Saturation	Conductivity (µS m^{-1})	Main Substrates	Water Current
st01	19.9	9.59	105.7	241	Pebbles with periphyton	moderate
st02	20.1	8.61	95	285	Pebbles with periphyton	moderate
st03	20.4	10.41	115.4	328	Pebbles with periphyton and sand	moderate
st04	20.9	9.67	108.3	349	Stones, bryophytes, pebbles and sand	moderate
st05	21	11	123.4	374	Small pebbles with periphyton	moderate
st06	21.5	9.82	111	432	Small pebbles and sand	low
st07	23.2	7.69	89.8	359	Mud and macrophytes	low
st08	21.9	8.83	100.6	454	Small pebbles	low

The different density between sampling sites was tested using the Kruskal Wallis test and the result indicated that there is a statistically significant difference (DF = 7, H-Value = 21.36, p-Value = 0.003). We recorded the highest abundance of the amphipod in station st04, while in stations st01-02-03 we did not record any specimens; these differences were highlighted by the pairwise tests (Table 2).

Moreover, the size classes analysis indicated that the majority of specimens were in the first size class (3–6 mm) or in the fourth (12.01–15 mm) and fifth (15.01–18 mm) size classes (Figure 2b).



(**a**)



(**b**)

Figure 2. (**a**) Mean number of specimens (±standard deviation) collected in the eight stations in the River Adda. (**b**) Size-frequency distribution of the population sampled on 31 August 2021.

Table 2. Results of the pairwise Dunn test between *D. villosus* densities in each station. *: statistical significance, **: high statistical significance, ***: very high statistical significance.

Stations	st01	st02	st03	st04	st05	st06	st07
st02	0.500						
st03	0.500	0.500					
st04	0.001 ***	0.001 ***	0.001 ***				
st05	0.008 **	0.008 **	0.008 **	0.238			
st06	0.014 *	0.014 *	0.014 *	0.1868	0.429		
st07	0.143	0.143	0.143	0.022 *	0.096	0.129	
st08	0.025 *	0.025 *	0.025 *	0.129	0.339	0.406	0.186
				p-values			

3.2. *Definition of the Level of Invasiveness and Impacts of Dikerogammarus villosus*

According to AS-ISK, *D. villosus* obtains a BRA score of 40 (with a confidence of 0.88) and a BRA + CCA score of 44 (confidence level of 0.85) (see Supplementary File S1). Thus, considering the threshold used in this paper, the potential level of invasiveness for this species is high (Figure 3) and especially relevant in the species-population nuisance traits Sector (Table 3).

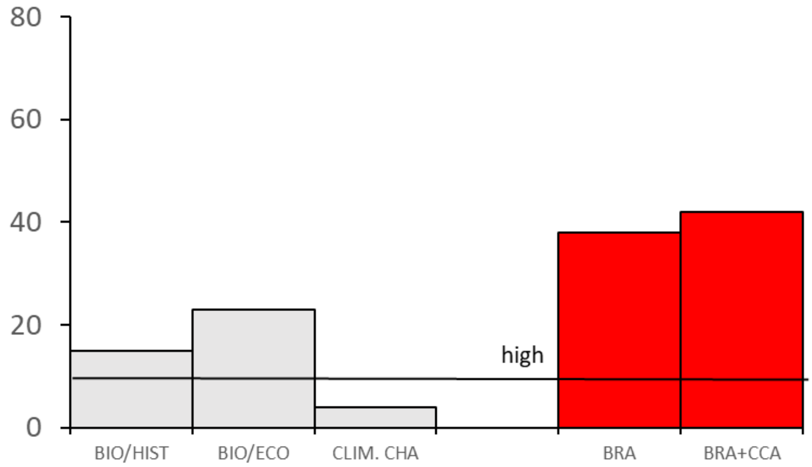


Figure 3. Score partition (in grey), BRA and BRA + CCA scores (in red) obtained applying AS-ISK for the evaluation of the invasiveness of *D. villosus*. BIO/HIST: Biogeography/Historical; BIO/ECO: Biology/Ecology; CLIM. CHA: Climate Change; BRA: Basic Risk Assessment; CCA: Climate Change Assessment. The colour red indicates a high level of invasiveness; the continuous line indicates the threshold between high/low level of invasiveness proposed by AS-ISK [50].

Table 3. AS-ISK statistics: score partition and sector affected.

AS-ISK Partition	Score
Biogeography/Historical	**17.0**
1. Domestication/Cultivation	0.0
2. Climate, distribution and introduction risk	3.0
3. Invasive elsewhere	14.0
Biology/Ecology	**23.0**
4. Undesirable (or persistence) traits	7.0
5. Resource exploitation	7.0
6. Reproduction	0.0
7. Dispersal mechanisms	4.0
8. Tolerance attributes	5.0
Climate change	**4.0**
9. Climate change	4.0
AS-ISK sector affected	
Commercial	14.0
Environmental	14.0
Species or population nuisance traits	22.0

The bio-ecological traits of this amphipod play a major role in the calculation of the final BRA score; the remaining points are assigned to the biogeography/historical section, while the lower score is related to the climate change adaptability of the species. In more detail, *D. villosus* shows high environmental adaptability and tolerance, it can modify trophic chains and its dispersion is boosted by more than one vector, hence resulting in a high bio-ecology score (Table 3).

The documented invasion of various European freshwater environments contributes to the score of the biogeography/historical partition (Table 3).

Conversely, according to *Harmonia*$^+$, *D. villosus* obtains a low level of invasiveness (Overall score = 0.187) (see Supplementary File S2).

The invasion score reaches 0.747 points, principally due to the high capability of the amphipod to be introduced and disperse in the wild by natural means. At the opposite end, the impact score reaches 0.250 points, mainly related to the interspecific competition with the native gammarids and the predatory effect of *D. villosus* on different organisms (Figure 4).

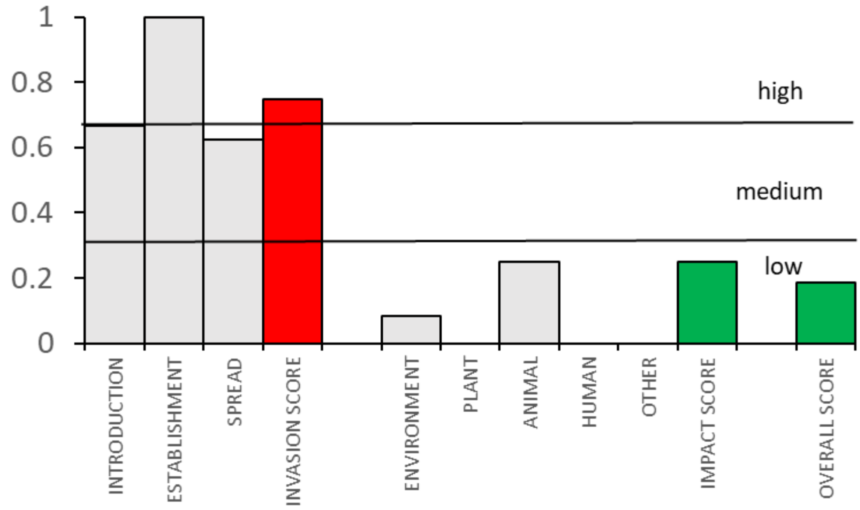


Figure 4. *Harmonia*$^+$ results applied to *D. villosus* in the River Adda. Continuous lines indicate the thresholds between high/medium/low level of invasiveness proposed by [51]: high risk, species with scores > 0.66; medium risk, between 0.33 and 0.66; low risk, <0.330. The colour red indicates high a level of invasiveness while the colour green indicates a low level of invasiveness.

Finally, the application of GISS indicates for *D. villosus* a medium level of impact, with 12 impact points and a confidence level of 2.92 (see Supplementary File S3). This result is mainly due to the impacts on the environment of the non-native gammarid (10 impact points, confidence level of 3); on the contrary, its impact on the economy reached only 2 impact points with a confidence level of 2.83 (Figure 5).

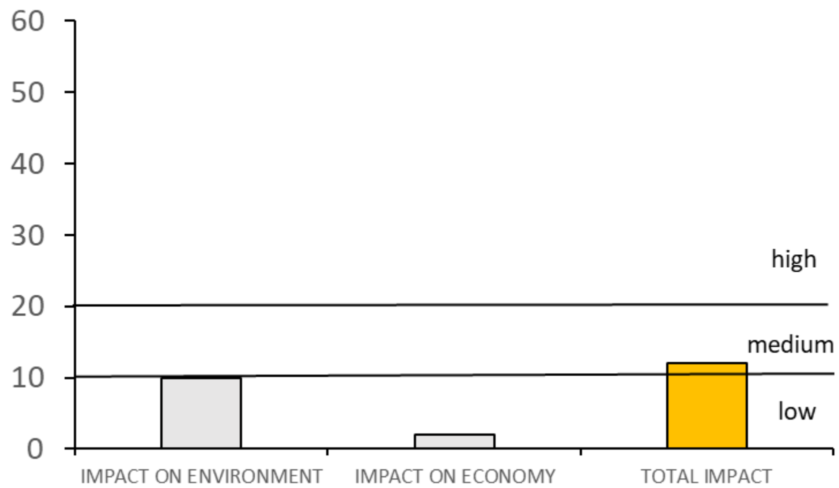


Figure 5. Results of the GISS impact assessment protocol on *D. villosus* in the River Adda. 0 = no impact; 60 = highest impact. Species with scores > 20 (high risk). The colour yellow indicates a medium level of invasiveness accordingly to the thresholds proposed by [44].

4. Discussion

The first goal of this article was to describe the distribution of *D. villosus* in the main course of the River Adda, in the territory of the South Adda Regional Park. Our results indicate that the non-native gammarid is established in the downstream part of the river, especially in microhabitats with lower hydrodynamism and the presence of aquatic plants or bryophytes (such as in st04), where all size classes were well represented during our single sampling event. This result is consistent with observations from other invaded hydrographic regions of Europe, where *D. villosus* has been shown to monopolise lentic microhabitats and downstream regions of large watercourses [21,52].

At the opposite end, in the upstream part of the River Adda, where the water current is stronger and the bottom is mainly composed of pebbles, we only found native gammarids, namely *Echinogammarus stammeri* (S. Karaman, 1931). Since the source population of *D. villosus* is very likely the Po River, it can be expected that in the Adda River *D. villosus* will stay confined in the downstream area, while native gammarid species can persist in fast-flowing areas of rivers [21,52]. However, we cannot rule out the hypothesis that the invasive amphipod has just begun its invasion in the Adda River and will spread upstream in the next future.

Clearly, the magnitude of the impact of *D. villosus* is strictly related to its abundance and population dynamics but, despite *D. villosus* is not included in the list of non-native species of Union concern, its invasiveness is considered very high in the scientific literature, due to its high ecological adaptability, high tolerance, high reproduction rate and consequently, it interferes at various level of the trophic chain [20,53–55].

Our second goal was to quantify its potential impact in the main course of the River Adda through two different screening tools, AS-ISK and *Harmonia*$^+$. Apparently, the two methods provided opposite results: AS-ISK classifies *D. villosus* with a high level of invasiveness in the risk assessment area, contrary to *Harmonia*$^+$ which assigns it a low level of invasiveness. Actually, analysing in detail the two components which determine the final classification of the invasiveness level in *Harmonia*$^+$ (namely, invasion and impact scores), it emerged that the invasion score of *Harmonia*$^+$ classified *D. villosus* with a high level of invasiveness, in accordance with AS-ISK and the literature.

However, the overall *Harmonia*$^+$ impact score was not particularly high because the non-native amphipod does not have documented socio-economic impacts in the South Adda Regional Park (Figure 4), which is consistent with AS-ISK results.

Therefore, different final outcomes depend on the different construction of the two tools, whose final result strongly depends on two main factors: the characteristics of the assessment area (in terms of habitat availability and socio-economic value) and threshold values employed which determine the final invasiveness ranking of the evaluated species [48]. Regarding the risk assessment area, the downstream part of the River Adda is an environment with few peculiar species that could be directly affected by the presence of *D. villosus*. For example, it can predate larvae and eggs of amphibians, such as *Rana latastei* (Boulenger, 1879) [35], as well as fish [33,36]. Moreover, the recreational fishing activity could be impacted by *D. villosus*: when the amphipod reaches high abundance, it could also cause damage to fishing baits (Agency for the Protection of the Environment of the Lombardy Region, personal communication).

The definition of the thresholds is the second issue in an assessment: in order to obtain discriminant threshold values between high level and low level of invasiveness, it is necessary to pass through an intercalibration process. For AS-ISK, the threshold value (BRA score > 13.25 as invasive) was obtained by intercalibrating the results of 144 freshwater species [50], so it should be considered highly reliable, although restrictive [56].

On the other side, despite *Harmonia*$^+$ protocol being an accurate method, it does not provide any specific threshold value and its sporadic application to freshwater species limits the interpretation of our results. The thresholds adopted here, following [51], should in fact be discussed over a much larger set of applications.

Finally, GISS classified *D. villosus* as a species with a medium level of impact. Additionally, in this case, the impact on the environment was much higher than the impact on the economy mainly because this freshwater species produces impact at the ecosystem level, but it does not have any impact on agricultural, forestry and animal production as well as human infrastructure or health. Nevertheless, even for this method, there is a potential problem related to the thresholds: as stated by Nentwig and colleagues [57], these values are partially the result of an intercalibration process, and they are mainly derived from expert judgement. Therefore, also, in this case, different threshold values could lead to a different ranking of the assessed species and, consequently, to different management actions.

Recently, Wood and co-authors reviewed several methods to possibly control the invasive amphipod. In their article, they identify three main categories of control: biological, chemical and physical [58].

Biological control uses potential predators or pathogens already present in the assessment area or introduced to contrast non-native species. Amphipods are commonly predated by fish or aquatic insects [59,60]; however, the lower activity and "aggressivity" of *D. villosus* compared to other gammarids could prevent it from being captured [61,62].

Another option to contrast the expansion of *D. villosus* could be the introduction of enemies from its native area, for example, Microsporidia *Cucumispora dikerogammari* Ovcharenko, Bacela, Wilkinson, Ironside, Rigaud and Wattier, 2009, which is known to cause high mortality to the invasive gammarid [63] but low infection rate on non-target species [64]. However, such management solutions should be preceded by experimental studies to assess their environmental feasibility and to explore possible unknown impacts on other non-target species.

Chemical control is the most generic method to control an invasive organism, even for aquatic invertebrates [65]. However, the use of nonselective chemical compounds threatens non-target species [66] and is therefore not applicable in a natural habitat such as the River Adda.

The last category of control methods is physical control, which includes trapping and habitat alteration or manipulation, both very expensive in terms of time and effort. The trapping method is more suitable for closed habitats and for bigger species (e.g., non-native crayfish), while habitat modification (e.g., artificial barriers or substrate replacement) may cause permanent changes to the environment [67]. Furthermore, both methods could have impacts on non-target species such as fish or amphibians and thus, for these reasons, both methods are not feasible in the River Adda.

To contrast the further spreading of the non-native species in the River Adda it is necessary to act on its anthropogenic vectors of dispersion. *Dikerogammarus villosus* has the capability to survive out of the water for quite a long time and it has been demonstrated to cling to fishing gears, boats [32] and even on scientific tools (personal observation). So, a meticulous cleaning of the equipment could reduce the spreading rate of this species, as also proposed in the project Check-Clean and Dry protocol [68,69].

5. Conclusions

Our data report the presence of *Dikerogammarus villosus* in the southern part of the River Adda, beyond its previously known invaded range in Italy. Other than this, we also quantify the potential impact of this species in the newly invaded area. All the risk-screening and impact assessment tools employed confirmed its dangerousness for the environment and local biodiversity.

However, finding the perfect tool for predicting the impact of all types of non-native species on all possible habitats is a challenge. In our opinion, both *Harmonia*$^{+}$ and AS-ISK are screening methods that share similar characteristics and allow the user to predict the level of invasiveness of a species. For this reason, they can be considered useful tools for the management of an invasive species. However, due to the fact that *D. villosus* is a small freshwater species, difficult to spot in the wild and, as highlighted by GISS, its impacts are not so evident (especially on socio-economic aspects), thus the assessment and consequently, the management of this species, is extremely challenging.

In conclusion, the actions proposed summed to a biological control and to an increase in the awareness of the problems caused by *D. villosus* to the local biodiversity, could represent an integrated approach for the management of the non-native amphipod in the River Adda.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d14100838/s1, File S1: AS-ISK report, File S2: *Harmonia*$^+$ report, File S3: GISS report.

Author Contributions: Conceptualization, D.P. and A.M.; methodology, D.P.; software, D.P.; validation, D.P. and A.M.; formal analysis, D.P. and M.A.C.; investigation, D.P. and M.A.C.; data curation, D.P. and M.A.C.; writing—original draft preparation, D.P.; writing—review and editing, A.M.; visualization, D.P.; supervision, A.M. All authors have read and agreed to the published version of the manuscript.

Funding: No funds were received to carry out this research.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Manuela Marchesi and Natale Pizzochero of the Regional Agency for the Protection of the Environment of Lombardy Region (ARPA LOMBARDIA) for communicating the record of the species in the area and providing recommendations on the study area.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. IUCN; IUCN Species Survival Commission (SSC); IUCN Species Survival Commission (SSC), Invasive Species Specialist Group. *IUCN EICAT Categories and Criteria: The Environmental Impact Classification for Alien Taxa (EICAT)*, 1st ed.; IUCN: Gland, Switzerland; Cambridge, UK, 2020. [CrossRef]
2. Leung, B.; Roura Pascual, N.; Bacher, S.; Heikkilála, J.; Brotons, L.; Burgman, M.A.; Dehnen Schmutz, K.; Essl, F.; Hulme, P.E.; Richardson, D.M.; et al. TEASIng apart alien species risk assessments: A framework for best practices. *Ecol. Lett.* **2012**, *15*, 1475–1493. [CrossRef] [PubMed]
3. Guareschi, S.; Wood, P.J. Biological Invasions of River Ecosystems: A Flow of Implications, Challenges, and Research Opportunities. In *Imperiled: The Encyclopedia of Conservation*; Dominick, A., Della, S., Goldstein, M.I., Eds.; Elsevier: Amsterdam, The Netherlands, 2022; pp. 485–498. [CrossRef]
4. Occhipinti-Ambrogi, A.; Galil, B.S. A uniform terminology on bioinvasions: A chimera or an operative tool? *Mar. Pollut. Bull.* **2004**, *49*, 688–694. [CrossRef] [PubMed]
5. Charles, H.; Dukes, J.S. Impacts of invasive species on ecosystem services. In *Biological Invasions*; Nentwig, W., Ed.; Springer: Berlin/Heidelberg, Germany, 2007; pp. 217–237.
6. Langor, D.W.; Sweeney, J. Ecological impacts of non-native invertebrates and fungi on terrestrial ecosystems. *Biol. Invasions* **2009**, *11*, 1–3. [CrossRef] [PubMed]
7. Kenis, M.; Auger-Rozenberg, M.A.; Roques, A.; Timms, L.; Péré, C.; Cock, M.J.W. Ecological effects of invasive alien insects. *Biol. Invasions* **2009**, *11*, 21–45. [CrossRef]
8. Kettunen, M.; Genovesi, P.; Gollasch, S.; Pagad, S.; Starfinger, U.; ten Brink, P.; Shine, C. Technical Support to EU Strategy on Invasive Species (IAS)—Assessment of the Impacts of IAS in Europe and the EU, 2009, Final Report. Available online: https://ec.europa.eu/environment/nature/invasivealien/docs/Kettunen2009IASTask%201.pdf (accessed on 29 September 2022).
9. Vilà, M.; Basnou, C.; Pysek, P.; Josefsson, M.; Genovesi, P.; Gollasch, S.; Nentwig, W. How well do we understand the impacts of alien species on ecosystem services? A pan-European cross-taxa assessment. *Front. Ecol. Environ.* **2009**, *8*, 135–144. [CrossRef]
10. Haubrock, P.; Turbelin, A.; Cuthbert, R.; Novoa, A.; Taylor, N.; Angulo, E.; Ballesteros-Mejia, L.; Bodey, T.; Capinha, C.; Essl, F.; et al. Economic costs of invasive alien species across Europe. *NeoBiota* **2021**, *67*, 153–190. [CrossRef]
11. Cox, J.G.; Lima, S.L. Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators. *Trends Ecol. Evol.* **2006**, *21*, 674–680. [CrossRef] [PubMed]
12. Gallardo, B.; Clavero, M.; Sánchez, M.I.; Vilà, M. Global ecological impacts of invasive species in aquatic ecosystems. *Glob. Chang. Biol.* **2016**, *22*, 151–163. [CrossRef]
13. Rinaldo, A.; Gatto, M.; Rodríguez-Iturbe, I. *River Networks as Ecological Corridors: Species, Populations, Pathogens*; Cambridge University Press: Cambridge, UK, 2020; p. 438.

14. Gozlan, R.E.; Britton, J.R.; Cowx, I.; Copp, G.H. Current knowledge on non-native freshwater fish introductions. *J. Fish Biol.* **2010**, *76*, 751–786. [CrossRef]
15. Paganelli, D.; Pandolfi, A.; Sconfietti, R.; Marchini, A.; Vilizzi, L. Potential invasiveness by non-indigenous macrozoobenthos in the secondary hydrographic system of a temperate-climate river catchment. *Ecol. Indic.* **2018**, *88*, 274–281. [CrossRef]
16. Strayer, D.L. Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshw. Biol.* **2010**, *55*, 152–174. [CrossRef]
17. Olden, J.D. Biotic homogenization: A new research agenda for conservation biogeography. *J. Biogeogr.* **2006**, *33*, 2027–2039. [CrossRef]
18. Laverty, C.; Wolfgang, N.; Dick, J.T.A.; Lucy, F.E. Alien aquatics in Europe: Assessing the relative environmental and socioeconomic impacts of invasive aquatic macroinvertebrates and other taxa. *Manag. Biol. Invasions* **2015**, *6*, 341–350. [CrossRef]
19. Diagne, C.; Leroy, B.; Gozlan, R.E.; Vaissière, A.C.; Assailly, C.; Nuninger, L.; Roiz, D.; Jourdain, F.; Jarić, I.; Courchamp, F. InvaCost, a public database of the economic costs of biological invasions worldwide. *Sci. Data* **2020**, *7*, 277. [CrossRef] [PubMed]
20. Boets, P.; Pauwels, I.S.; Lock, K.; Goethals, P.L.M. Using an integrated modelling approach for risk assessment of the 'killer shrimp' *Dikerogammarus villosus*. *River Res. Appl.* **2014**, *30*, 403–412. [CrossRef]
21. Kobak, J.; Jermacz, Ł.; Marcińczyk, J.; Bartoszyńska, E.; Rutkowska, D.; Pawłowska, K. Abiotic factors affecting habitat selection by two invasive gammarids *Dikerogammarus villosus* and *Pontogammarus robustoides*. *Hydrobiologia* **2017**, *797*, 247–263. [CrossRef]
22. Minchin, J.D.; Arbačiauskas, K.; Daunys, D.; Ezhova, E.; Grudule, N.; Kotta, J.; Molchanova, N.; Olenin, S.; Višinskienė, G.; Strake, S. Rapid expansion and facilitating factors of the Ponto-Caspian invader *Dikerogammarus villosus* within the eastern Baltic Sea. *Aquat. Invasions* **2019**, *14*, 165–181. [CrossRef]
23. Rewicz, T.; Wattier, R.; Grabowski, M.; Rigaud, T.; Bącela-Spychalska, K. Out of the Black Sea: Phylogeography of the Invasive Killer Shrimp *Dikerogammarus villosus* across Europe. *PLoS ONE* **2015**, *10*, e0118121. [CrossRef]
24. Devin, S.; Piscart, C.; Beisel, J.N.; Moreteau, J.C. Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. *Arch. Hydrobiol.* **2003**, *158*, 43–56. [CrossRef]
25. Casellato, S.; La Piana, G.; Latella, L.; Ruffo, S. *Dikerogammarus villosus* (Sowinsky, 1894) (Crustacea, Amphipoda, Gammaridae) for the first time in Italy. *Ital. J. Zool.* **2006**, *73*, 97–104. [CrossRef]
26. Casellato, S.; Visentin, A.; La Piana, G. The predatory impact of *Dikerogammarus villosus* on fish. In *Biological Invaders in Inland Waters: Profiles, Distribution and Threats*; Gherardi, F., Ed.; Springer: Berlin/Heidelberg, Germany, 2007; pp. 495–507. [CrossRef]
27. Casellato, S.; Masiero, L.; La Piana, G.; Gigliotti, F. The alien amphipod crustacean *Dikerogammarus villosus* in Lake Garda (N-Italy): The invasion continues. *Neobiota* **2008**, *7*, 115–122.
28. Tricarico, E.; Mazza, G.; Orioli, G.; Rossano, C.; Scapini, F.; Gherardi, F. The killer shrimp, *Dikerogammarus villosus* (Sowinsky, 1894), is spreading in Italy. *Aquat. Invasions* **2010**, *5*, 211–214. [CrossRef]
29. Jazdzewski, K.; Konopacka, A. Invasive Ponto-Caspian species in waters of the Vistula and Oder basins and the southern Baltic Sea. In *Invasive Aquatic Species of Europe: Distribution, Impacts and Management*; Leppäkoski, E., Gollasch, S., Dordrecht, O., Eds.; Kluwer Academic Publishers: Amsterdam, The Netherlands, 2002; pp. 384–398.
30. de Vaate, A.B.; Jazdzewski, K.; Ketelaars, H.A.M.; Gollash, S.; van der Velde, G. Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Can. J. Fish. Aquat. Sci.* **2002**, *59*, 1159–1174. [CrossRef]
31. Cuthbert, R.N.; Kotronaki, S.G.; Dick, J.T.; Briski, E. Salinity tolerance and geographical origin predict global alien amphipod invasions. *Biol. Lett.* **2020**, *16*, 20200354. [CrossRef] [PubMed]
32. Bacela-Spychalska, K.; Grabowski, M.; Rewicz, T.; Konopacka, A.; Wattier, R. The 'killer shrimp' *Dikerogammarus villosus* (Crustacea, Amphipoda) invading Alpine lakes: Overland transport by recreational boats and scuba-diving gear as potential entry vectors? *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2013**, *23*, 606–618. [CrossRef]
33. Dick, J.; Platvoet, D.; David, K. Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea: Amphipoda). *Can. J. Fish. Aquat. Sci.* **2002**, *59*, 1078–1084. [CrossRef]
34. Platvoet, D.; Dick, J.; Konijnendijk, N. Feeding on micro-algae in the invasive Ponto-Caspian amphipod *Dikerogammarus villosus* (Sowinsky, 1894). *Aquat. Ecol* **2006**, *40*, 237–245. [CrossRef]
35. Taylor, N.G.; Dunn, A.M. Size matters: Predation of fish eggs and larvae by native and invasive amphipods. *Biol. Invasions* **2017**, *19*, 89–107. [CrossRef]
36. Warren, D.A.; Bradbeer, S.J.; Dunn, A.M. Superior predatory ability and abundance predicts potential ecological impact towards early-stage anurans by invasive 'Killer Shrimp' (*Dikerogammarus villosus*). *Sci. Rep.* **2021**, *11*, 4570. [CrossRef]
37. Koester, M.; Bayer, B.; Gergs, R. Is *Dikerogammarus villosus* (Crustacea, Gammaridae) a 'killer shrimp' in the River Rhine system? *Hydrobiologia* **2016**, *768*, 299–313. [CrossRef]
38. Maazouzi, C.; Piscart, C.; Pihan, J.C.; Masson, G. Effect of habitat-related resources on fatty acid composition and body weight of the invasive *Dikerogammarus villosus* in an artificial reservoir. *Fundam. Appl. Limnol.* **2009**, *175*, 327. [CrossRef]
39. Copp, G.H.; Vilizzi, L.; Tidbury, H.; Stebbing, P.D.; Tarkan, A.S.; Miossec, L.; Goulletquer, P. Development of a generic decision-support tool for identifying potentially invasive aquatic taxa: AS-ISK. *Manag. Biol. Invasions* **2016**, *7*, 343–350. [CrossRef]
40. D'hondt, B.; Vanderhoeven, S.; Roelandt, S.; Mayer, F.; Versteirt, V.; Adriaens, T.; Ducheyne, E.; Martin, G.S.; Grégoire, J.; Stiers, I.; et al. Harmonia$^+$ and Pandora$^+$: Risk screening tools for potentially invasive plants, animals and their pathogens. *Biol. Invasions* **2015**, *17*, 1869–1883. [CrossRef]

41. Roy, H.E.; Rabitsch, W.; Scalera, R.; Stewart, A.; Gallardo, B.; Genovesi, P.; Essl, F.; Adriaens, T.; Bacher, S.; Booy, O.; et al. Developing a framework of minimum standards for the risk assessment of alien species. *J. Appl. Ecol.* **2018**, *55*, 526–538. [CrossRef]
42. Srėbalienė, G.; Olenin, S.; Minchin, D.; Narščius, A. A comparison of impact and risk assessment methods based on the IMO Guidelines and EU invasive alien species risk assessment frameworks. *PeerJ* **2019**, *7*, e6965. [CrossRef]
43. Nentwig, W.; Kuhnel, E.; Bacher, S. A generic impact-scoring system applied to alien mammals in Europe. *Conserv. Biol.* **2010**, *24*, 302–331. [CrossRef]
44. Nentwig, W.; Bacher, S.; Pyšek, P.; Vilà, M.; Kumschick, S. The generic impact scoring system (GISS): A standardized tool to quantify the impacts of alien species. *Environ. Monit. Assess.* **2016**, *188*, 315. [CrossRef]
45. Tarkan, A.S.; Emiroğlu, Ö.; Aksu, S.; Başkurt, S.; Aksu, İ.; Vilizzi, L.; Yoğurtçuoğlu, B. Coupling molecular and risk analysis to investigate the origin, distribution and potential impact of non-native species: An application to ruffe *Gymnocephalus cernua* in Turkey. *Eur. Zool. J.* **2022**, *89*, 102–114. [CrossRef]
46. Voshell, J.R.; Laiton, R.J.; Hiner, S.W. Field techniques for determining the effect of toxic substances on benthic macroinvertebrates in rocky-bottomed streams. In *Aquatic Toxicology and Hazard Assessment*; Cowgill, U.M., Williams, L.R., Eds.; ASTM: Philadelphia, PA, USA, 1989; Volume 12, pp. 134–155.
47. Dunn, O.J. Multiple comparisons using rank sums. *Technometrics* **1964**, *6*, 241–252. [CrossRef]
48. Copp, G.H.; Vilizzi, L.; Wei, H.; Li, S.; Piria, M.; Al-Faisal, A.J.; Almeida, D.; Atique, U.; Al-Wazzan, Z.; Bakiu, R.; et al. Speaking their language—Development of a multilingual decision-support tool for communicating invasive species risks to decision makers and stakeholders. *Environ. Model. Softw.* **2021**, *135*, 104900. [CrossRef]
49. Mastrandrea, M.D.; Field, C.B.; Stocker, T.F.; Edenhofer, O.; Ebi, K.L.; Frame, D.J.; Held, H.; Kriegler, E.; Mach, K.J.; Matschoss, P.R.; et al. *Guidance Note for Lead Authors of the IPCC Fifth Assessment Report on Consistent Treatment of Uncertainties*; Intergovernmental Panel on Climate Change (IPCC): Geneva, Switzerland, 2010. Available online: https://www.ipcc.ch/pdf/supporting-material/uncertainty-guidance-note.pdf (accessed on 29 September 2022).
50. Vilizzi, L.; Copp, G.H.; Hill, J.E.; Adamovich, B.; Aislabie, L.; Akin, D.; Al-Faisal, A.J.; Almeida, D.; Azmai, M.N.A.; Bakiu, R.; et al. A global-scale screening of non-native aquatic organisms to identify potentially invasive species under current and future climate conditions. *Sci. Total Environ.* **2021**, *788*, 147868. [CrossRef] [PubMed]
51. Lemmers, P.; Collas, F.P.L.; Gylstra, R.; Crombaghs, B.H.J.M.; van der Velde, G.; Leuven, R.S.E.W. Risks and management of alien freshwater crayfish species in the Rhine-Meuse river district. *Manag. Biol. Invasions* **2021**, *12*, 193–220. [CrossRef]
52. Borza, P.; Huber, T.; Leitner, P.; Remund, N.; Graf, W. How to coexist with the 'killer shrimp' *Dikerogammarus villosus*? Lessons from other invasive Ponto-Caspian peracarids. *Aquat. Conserv. Mar. Freshw. Ecosyst.* **2018**, *28*, 1441–1450. [CrossRef]
53. Grabowski, M.; Bącela, K.; Konopacka, A.; Jażdżewski, K. Salinity-related distribution of alien amphipods in rivers provides refugia for native species. *Biol. Invasions* **2009**, *11*, 2107–2117. [CrossRef]
54. Grabowski, M.; Bacela, K.; Konopacka, A. How to be an invasive gammarid (Amphipoda: Gammaroidea)–comparison of life history traits. *Hydrobiologia* **2007**, *590*, 75–84. [CrossRef]
55. Rewicz, T.; Grabowski, M.; MacNeil, C.; Bącela-Spychalska, K. The profile of a 'perfect' invader—The case of killer shrimp, *Dikerogammarus villosus*. *Aquat. Invasions* **2014**, *9*, 267–288. [CrossRef]
56. Paganelli, D.; Kamburska, L.; Zaupa, S.; Garzoli, L.; Boggero, A. Impacts analysis of macroinvertebrate alien species in the hydrographic system of a subalpine lake on the Italian-Swiss border. *Water* **2021**, *13*, 3146. [CrossRef]
57. Nentwig, W.; Bacher, S.; Kumschick, S.; Pyšek, P.; Vilà, M. More than "100 worst" alien species in Europe. *Biol. Invasions* **2018**, *20*, 1611–1621. [CrossRef]
58. Wood, L.E.; Smith, E.R.C.; Bojko, J.; Stebbing, P. Options for the control of *Dikerogammarus villosus* (killer shrimp) and other invasive amphipods. *Manag. Biol. Invasions* **2021**, *12*, 662–684. [CrossRef]
59. Balcombe, S.R.; Bunn, S.E.; McKenzie-Smith, F.J.; Davies, P.M. Variability of fish diets between dry and flood periods in an arid zone floodplain river. *J. Fish Biol.* **2005**, *67*, 1552–1567. [CrossRef]
60. Bajer, P.G.; Ghosal, R.; Maselko, M.; Smanski, M.J.; Lechelt, J.D.; Hansen, G.; Kornis, M.S. Biological control of invasive fish and aquatic invertebrates: A brief review with case studies. *Manag. Biol. Invasions* **2019**, *10*, 227–254. [CrossRef]
61. Kinzler, W.; Maier, G. Selective predation by fish: A further reason for the decline of native gammarids in the presence of invasives? *J. Limnol* **2006**, *65*, 27–34. [CrossRef]
62. Kobak, J.; Jermacz, Ł.; Płąchocki, D. Effectiveness of zebra mussels to act as shelters from fish predators differs between native and invasive amphipod prey. *Aquat. Ecol.* **2014**, *48*, 397–408. [CrossRef]
63. Ovcharenko, M.O.; Bacela, K.; Wilkinson, T.; Ironside, J.E.; Rigaud, T.; Wattier, R.A. *Cucumispora dikerogammari* n. gen. (Fungi: Microsporidia) infecting the invasive amphipod *Dikerogammarus villosus*: A potential emerging disease in European rivers. *Parasitology* **2010**, *137*, 191–204. [CrossRef]
64. Bacela-Spychalska, K.; Wattier, R.A.; Genton, C.; Rigaud, T. Microsporidian disease of the invasive amphipod *Dikerogammarus villosus* and the potential for its transfer to local invertebrate fauna. *Biol. Invasions* **2012**, *14*, 1831–1842. [CrossRef]
65. Freeman, M.A.; Turnbull, J.F.; Yeomans, W.E.; Bean, C.W. Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. *Aquat. Conserv.* **2010**, *20*, 211–223. [CrossRef]
66. Sanchez-Bayo, F.P. Insecticides mode of action in relation to their toxicity to non-target organisms. *J. Environ. Anal. Toxicol.* **2012**, *2012*, 1–9. [CrossRef]

67. Stebbing, P.; Irving, S.; Stentiford, G.; Mitchard, N. *A Review of Potential Methods to Control and Eradicate the Invasive Gammarid, Dikerogammarus villosus from UK Waters*; Cefas: Weymouth, UK, 2012; pp. 4–47.
68. De Ventura, L.; Weissert, N.; Tobias, R.; Kopp, K.; Jokela, J. Identifying target factors for interventions to increase boat cleaning in order to prevent spread of invasive species. *Manag. Biol. Invasions* **2017**, *8*, 71–84. [CrossRef]
69. Shannon, C.; Quinn, C.H.; Stebbing, P.D.; Hassall, C.; Dunn, A.M. The practical application of hot water to reduce the introduction and spread of aquatic invasive alien species. *Manag. Biol. Invasions* **2018**, *9*, 417–423. [CrossRef]