

## Risk Assessment

## Risk screening of non-native macroinvertebrates in the major rivers and associated basins of Belarus using the Aquatic Species Invasiveness Screening Kit

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### Abstract

In invasive species ecology, risk identification is an essential first step of the overall risk analysis process. In this study, 24 non-native freshwater macroinvertebrate species were screened for their level of risk of invasiveness in the Dnieper, Pripyat and Neman rivers and associated basins of Belarus. Using the recently-developed Aquatic Species Invasiveness Screening Kit (AS-ISK), the threshold values of 12.25 for the Basic Risk Assessment (BRA) and 19.5 for the BRA + CCA (Climate Change Assessment) allowed identifying those species likely to be of high risk of invasiveness in the risk assessment area. For both the BRA and BRA+CCA, the highest-scoring species were zebra mussel *Dreissena polymorpha*, spinycheek crayfish *Orconectes limosus*, quagga mussel *Dreissena bugensis* and Caspian mud shrimp *Chelicorophium curvispinum*, and for the BRA only signal crayfish *Pacifastacus leniusculus*. For all species but one (namely, fragile ancyliid *Ferrissia fragilis* classified as low risk), the CCA resulted in an increase in score relative to the BRA, indicating that predicted future global warming conditions are likely to exacerbate the detrimental impacts of these non-native macroinvertebrates of Ponto-Caspian origin. The present application of AS-ISK confirms the predictive value of the tool, and benefits from incorporation of future climate change scenarios. This is a crucial step in the provision of guidelines for the successful management of non-native invasive species and for conservation of native faunas in the (invaded) habitats of Belarus.

**Key words:** AS-ISK, FI-ISK, climate change, Ponto-Caspian, Dnieper, Neman, Pripyat

### Introduction

Non-native invasive species (NIS) may often represent a major threat to native species due to their ability to alter ecosystem processes via direct predation and competition (Gozlan et al. 2013; Ricciardi et al. 2017). Control measures for those NIS responsible for the most severe impacts currently represent one of the major challenges in conservation ecology (e.g. Piria et al. 2018). Risk analysis is a key aspect in the management of NIS (Orr and Fisher 2009), and risk identification (or screening) in particular is an essential part of the whole decision-making process (EPA 1998; Andersen et al. 2004).

The distribution and abundance of NIS is not only a function of the abiotic and biotic components of the invaded habitat but also a result of human

activities and related impacts (Lipton et al. 1993; Dukes and Mooney 1999; Walther et al. 2009). In Belarus, the latter are especially severe (Semenchenko and Rizevski 2017), making the country quite vulnerable to the introduction and establishment of several non-native aquatic macroinvertebrates. This is because the main rivers of Belarus are part of the Central European invasive corridor (Bij de Vaate et al. 2002), which traverses the entire country and has been detrimentally affected since the mid-1900s by the intentional introduction of non-native macroinvertebrates as food source for fish originating from Lithuanian and Ukrainian reservoirs. In addition, since the late 1900s and early 2000s intensive shipping activities on the main rivers of Belarus have contributed to the expansion of NIS, particularly in river harbours through hull fouling (Semenchenko et al. 2011).



**Figure 1.** Map of study area (light green areas show the large woodlands).

The aim of this study was to determine the potential invasiveness of non-native macroinvertebrate species in the major rivers and associated basins of Belarus, and to assess whether predicted global warming conditions may enhance further their dispersal and potential impacts. For this purpose, a recently-developed, generic risk screening tool for aquatic species was employed to provide a preliminary evaluation of the non-native macroinvertebrate species that may cause ecological and economic impacts in the study area. It is anticipated that the present findings will provide for an important step towards the successful management of the macroinvertebrate species likely to be invasive, hence pose a threat to, the native freshwater ecosystems of Belarus. This knowledge is essential in view of overall management and conservation initiatives.

## Methods

### *Study area*

The three main rivers and associated basins of Belarus, namely the Dnieper, Pripyat and Neman, are transboundary. The Dnieper and Pripyat rivers are part of the Ponto-Caspian Basin, whereas the Neman River belongs to the Baltic Basin (Figure 1).

In Belarus territory, the length of the Dnieper River is 595 km, current speed ranges from 0.3 to 1.2 m s<sup>-1</sup>, and intensive shipping occurs in the middle and downstream reaches; the length of the Pripyat River is 495 km, current speed ranges from 0.3 to 0.5 m s<sup>-1</sup>, and intensive shipping takes place along the entire water course; finally, the Neman River flows for 436 km in Belarus territory, and shipping occurs only in the downstream reaches. During the last fifteen years, suspended particulate matter in all three rivers has increased by ≈ 20% and average annual air temperature has increased by 1 °C (Loginov 2015). Notably, the Kiev Reservoir (Ukraine) and Kaunas Reservoir (Lithuania) are the main donors of non-native macroinvertebrates of mainly Ponto-Caspian origin to the Dnieper, Neman and Pripyat rivers in Belarus (Semenchenko and Rizevski 2017).

### *Risk screening*

In total, 24 species of aquatic macroinvertebrates were screened for their potential invasiveness in the Dnieper, Neman and Pripyat rivers and associated basins of Belarus. For the purposes of the present study, these will represent the Risk Assessment (RA) area. Species were chosen according to the following

criteria (number and relative percentage in brackets: see Supplementary material Table S1): (1) non-native with documented predation impact upon native species (3, 12.5%); (2) non-native modifying habitats of native species, including niche replacement (3, 12.5%); (3) non-native acting as hosts of potentially dangerous parasites (3, 12.5%); and (4) non-native without negative impacts (so far) reported (15, 27.5%) (Mastitsky 2007; Semenchenko et al. 2013, 2015; Semenchenko and Rizevski 2017). Notably, seven of these species (i.e. *Chaetogammarus warpachowskyi* Sars, 1897; *Chelicorophium mucronatum* (Sars, 1895); *Chelicorophium robustum* (Sars, 1895); *Dreissena bugensis* Andrusov, 1897; *Hemimysis anomala* Sars, 1907; *Pacifastacus leniusculus* (Dana, 1852) and *Pontogammarus (Turcogammarus) aralensis* (Uljanin, 1875)), albeit not recorded from the studied rivers, were included in this study as currently present in the Kaunas and Kiev Reservoirs, hence with potential to invade Belarus water courses.

The Aquatic Species Invasiveness Screening Kit (AS-ISK v1.4; Copp et al. 2016b: available for free download at <https://www.cefas.co.uk/nns/tools/> 09 March 2018) was used to identify potentially invasive macroinvertebrate species with respect to the RA area. AS-ISK is a generic replacement of the Fish Invasiveness Screening Kit (FISK: Copp et al. 2005, 2009; Lawson et al. 2013), which in turn is an adaptation from the Weed Risk Assessment screening tool for terrestrial plants (Pheloung et al. 1999; Gordon et al. 2008). AS-ISK includes the generic screening module of the European Non-native Species in Aquaculture Risk Analysis Scheme (Copp et al. 2016a), and incorporates the “minimum requirements” (Roy et al. 2014) for the assessment of NIS with regard to the recent EC Regulation on the prevention and management of the introduction and spread of NIS.

AS-ISK consists of 55 questions in total: the first 49 questions (adapted from FISK: Copp et al. 2016b) cover the Biogeography (Section 1) and Biology (Section 2) aspects of the taxon under screening, and these comprise the Basic Risk Assessment (BRA); the remaining six questions comprise Section 3 of the Climate Change Assessment (CCA), and require the assessor to evaluate how predicted climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. For each question, the assessor must provide a response, justification and level of confidence (see below), and the screened taxon eventually receives both a BRA and a BRA+CCA (composite) score. AS-ISK scores < 1 suggest that the taxon is unlikely to become invasive in the RA area, and is therefore classified as “low risk”; in contrast, score values > 1 classify the taxon as posing either a “medium risk”

or a “high risk” of becoming invasive. Distinction between medium and high risk levels depends upon setting a “threshold” value, which is typically obtained through RA area-specific “calibration” subject to the availability of a representative sample size (i.e. number of screened taxa). Finally, the ranked levels of confidence (1 = low, 2 out of 10 chances; 2 = medium, 5 out of 10; 3 = high, 8 out of 10; 4 = very high, 9 out of 10) associated with each question-related response in AS-ISK mirror the confidence rankings recommended by the Intergovernmental Panel on Climate Change (<http://www.environmental.org.uk>; see also Copp et al. 2016b).

### Statistical analysis

The macroinvertebrate species selected for screening were assessed in conjunction by the first and second authors, who are knowledgeable in the freshwater macroinvertebrate fauna of Belarus (Semenchenko et al. 2013, 2015; Semenchenko and Rizevski 2017). For the CCA, the HadCM2 model adapted for Belarus (Loginov 2008) was used. According to this model, from 2010 to 2039 the mean rise in temperature by greenhouse gases alone is expected to be of 1.37 °C.

Following computation of the BRA and BRA+CCA scores, Receiver Operating Characteristic (ROC) analysis (Bewick et al. 2004) was used to assess the predictive ability of AS-ISK to discriminate between those macroinvertebrate species posing a high risk and those posing a medium or low risk of being invasive for the RA area. For ROC analysis to be implemented, species were categorised *a priori* in terms of their perceived invasiveness (i.e. non-invasive or invasive) based on information retrieved from the Black Book of Belarus (Semenchenko 2016), the European Network on Invasive Species (NOBANIS: <https://www.nobanis.org/> 09 March 2018), and the Global Invasive Species Database (GISD: <http://www.iucngisd.org/gisd/> 09 March 2018).

Statistically, a ROC curve is a graph of sensitivity vs 1 – specificity, where in the present context sensitivity and specificity will be the proportion of invasive and non-invasive macroinvertebrate species, respectively, that are correctly identified as such by AS-ISK. A measure of the accuracy of the calibration analysis is the Area Under the Curve (AUC), which typically ranges between 0.5 and 1.0, and the closer to 1.0 the better the ability (of AS-ISK) to differentiate between non-invasive and invasive species (for more details see e.g. Tarkan et al. 2017a, b). Following ROC analysis, the best AS-ISK threshold value that maximises the true positive rate (i.e. *a priori* invasive macroinvertebrate species classified as

invasive, hence “true positives”) and minimises the false positive rate (i.e. *a priori* non-invasive macroinvertebrate species classified as invasive, hence “true negatives”) was determined using Youden’s *J* statistic; whereas, the “default” threshold of 1 was set to distinguish between low risk and medium risk species (see *Risk screening*). Notably, species categorised *a priori* as invasive and classified as low risk will be “false negatives”; whereas, species categorised *a priori* as non-invasive and classified as high risk will be “false positives”.

ROC analysis was carried out with package pROC (Robin et al. 2011) for R x64 v3.4.3 (R Development Core Team 2017) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals). Based on the confidence level (CL) allocated to each response for a given species (see *Risk screening*), an overall confidence factor (CF<sub>Total</sub>) was then computed as:

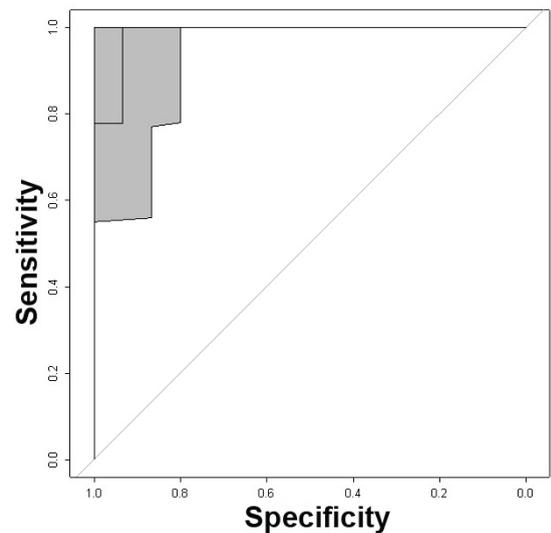
$$\sum(CQ_i)/(4 \times 55) \quad (i = 1, \dots, 55)$$

where  $CQ_i$  is the confidence level for Question  $i$  ( $Q_i$ ), 4 is the maximum achievable value for certainty (i.e. “very certain”), and 55 is the total number of questions comprising the AS-ISK tool. The CF<sub>Total</sub> ranges from a minimum of 0.25 (i.e. all 55 questions with certainty score equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Two additional confidence factors, namely the CF<sub>BRA</sub> and the CF<sub>CCA</sub>, were computed based on the 49 Qs of the BRA and the 6 Qs of the CCA, respectively.

Differences between mean confidence level and mean confidence factor for the BRA (CL<sub>BRA</sub> and CF<sub>BRA</sub>, respectively) and CCA (CL<sub>CCA</sub> and CF<sub>CCA</sub>, respectively) were tested by permutational analysis of variance (PERANOVA) based on a one-factor design (i.e. factor Component, with the two levels BRA and CCA). Analysis was in PERMANOVA+ v1.0.8 for PRIMER v6.1.18, with normalisation of the data, using a Bray-Curtis dissimilarity measure and 9999 unrestricted permutations of the raw data (Anderson et al. 2008) with statistical effects evaluated at  $\alpha = 0.05$ .

## Results

The ROC curves for the BRA and BRA+CCA provided an AUC of 0.9852 (0.9504–1.0000 95% CI), hence well above 0.5 (Figure 2). This indicated that AS-ISK was able to discriminate reliably between non-invasive and invasive macroinvertebrate species for the RA area. Youden’s *J* resulted in the “best”



**Figure 2.** Receiver Operating Characteristic curve for the AS-ISK based risk assessment of 24 macroinvertebrate species in the Dnieper, Neman and Pripyat river basins of Belarus.

thresholds of 12.25 for the BRA and 19.5 for the BRA+CCA, which were therefore chosen for calibration of the AS-ISK risk outcomes. Accordingly, the BRA threshold allowed to distinguish between medium risk species with scores within the interval [1, 12.25] and high risk species with scores within [12.25, 68]; the BRA+CCA threshold, between medium risk species with scores within [1, 19.5] and high risk species with scores within [19.5, 80]. Species classified as low risk were those with BRA and BRA+CCA scores within [−20, 1] and [−32, 1], respectively.

Based on the BRA threshold, 10 (41.7%) of the 24 species assessed were classified as high risk, 12 (50.0%) as medium risk, and the remaining two (8.3%) as low risk (Table S2). Of the high-risk species, nine were categorised *a priori* as invasive (hence, true positives) and the remaining one, namely the amphipod *C. mucronatum*, as non-invasive (hence, false positive); whereas, the two low-risk species were both categorised *a priori* as non-invasive (hence, true negatives), implying that no false negatives were detected. Based on the BRA+CAA threshold, 10 (41.7%) species were classified as high risk 13 (54.2%) as medium risk, and the remaining one (4.2%) as low risk. Of the high-risk species, similar to the BRA nine were true positives and one a false positive; whereas, there was only one true negative and, again, no false negatives. Finally, all medium-risk species for both the BRA and BRA+CCA were categorised *a priori* as non-invasive (Table S2).

For the BRA, the highest-scoring species (score  $\geq 20$ , chosen as an empirical relative “sub-threshold” to identify “very high risk” species) were zebra mussel *Dreissena polymorpha* (Pallas, 1771), spinycheek crayfish *Oreonectes limosus* (Rafinesque, 1817), quagga mussel *D. bugensis*, signal crayfish *P. leniusculus* and Caspian mud shrimp *Chelicorophium curvispinum* (Sars 1895)), in that order; whereas, the lowest-scoring species (i.e. classified as low risk) were the mysid *Limnomysis benedeni* Czerniavsky, 1882 and fragile ancyliid *Ferrissia fragilis* (Tryon, 1863). For the BRA+CCA, the highest-scoring species (score  $\geq 30$ , chosen as above) were again *D. polymorpha*, *O. limosus*, *D. bugensis* and *C. curvispinum*, in that order; whereas, *F. fragilis* was the only low risk species.

Except for *F. fragilis* for which there was no change in score, the CCA resulted in an increase in the BRA+CCA relative to the BRA score for all other species. This increase was highest for *D. bugensis*, *D. polymorpha* and the amphipod *Pontogammarus robustoides* (Sars, 1894) (i.e. 12 points, equal to the maximum achievable score for the five corresponding CCA Qs). Notably, for no species was the BRA+CCA lower than the corresponding BRA score.

Mean confidence level for all Qs (i.e.  $CQ_{1-55}$ ) was  $3.70 \pm 0.02$  SE, and for the BRA Qs (i.e.  $CQ_{1-49}$ )  $3.79 \pm 0.01$  SE, hence within the category “very high” in both cases; whereas, for the CCA,  $CQ_{50-55}$  was  $2.97 \pm 0.09$  SE, hence at the top range of the “high” confidence level. Similarly, the mean values for  $CF_{Total} = 0.93 \pm 0.01$  SE and  $CF_{BRA} = 0.95 \pm 0.01$  SE were higher than the mean value for the  $CF_{CCA} = 0.74 \pm 0.02$  SE. Notably, mean confidence level and factor were significantly higher for the BRA compared to the CCA (PERANOVA:  $F_{1,46}^{\#} = 81.96$ ,  $P^{\#} < 0.001$ ;  $\# =$  permutational). However, in all cases the narrow standard errors indicated overall similarity in both confidence levels and factors across the species assessed.

## Discussion

In this study, the ten macroinvertebrate species classified by AS-ISK as posing a high risk of invasiveness (and categorised *a priori* as invasive) in the three main rivers and associated basins of Belarus, three are currently listed as invasive in GISD (i.e. *D. bugensis*, *D. polymorpha* and *P. leniusculus*), four in NOBANIS (i.e. *C. curvispinum*, killer shrimp *Dikerogammarus villosus* (Sowinski, 1894), *O. limosus* and *P. robustoides*), and two in both databases (i.e. *D. polymorpha* and *P. leniusculus*), with *D. polymorpha* being additionally listed as one of the 100 world’s worst invasive alien species ([http://www.iucngisd.org/gisd/100\\_worst.php](http://www.iucngisd.org/gisd/100_worst.php) 09 March 2018). In

addition, the observed increase in score (i.e. BRA+CCA relative to BRA) after accounting for predicted climate change conditions indicates that the expected impact of the species classified at high risk of invasiveness for the RA area is likely to increase further. In this respect, the spread of invasive aquatic macroinvertebrates in general to new areas has been reported to benefit from increasing temperatures, ultimately facilitating their establishment (Walther et al. 2009). Also, increased amounts of suspended particulate matter, as observed for the water courses of the RA area and regarded as an indirect effect of global warming and reservoir construction, is believed to contribute further to the establishment of non-native macroinvertebrate species, and especially those of Ponto-Caspian origin (bij de Vaate et al. 2002).

*Dreissena polymorpha* is currently the most widely-spreading invasive macroinvertebrate species not only in European waters but also in North America. Whilst in Belarus the low pH ( $< 7.0-7.5$ ) and calcium content concentrations ( $< 24$  mg/l) of fresh waters (Karatayev et al. 2003) may represent a limiting factor for its expansion, this species has spread dramatically in the Dnieper River on Ukrainian territory following construction of the Dnieper Reservoir, which has led to an increase in suspended particulate matter and temperature coupled by an overall decrease in water current speed (Son 2007; Semenchenko et al. 2015). Regardless, in Belarus the number of lakes with *D. polymorpha* has been dramatically increasing at an average of 23 invaded lakes every ten years (Karatayev et al. 2011). In Europe, the main pathway of introduction for *D. polymorpha* is shipping (by hull fouling), even though fisheries activities in general (i.e. fishermen’s nets and boats) are known to have contributed to the spread of the species across several lakes of Belarus (Karatayev et al. 2003). The documented negative impacts of *D. polymorpha* are not only related to ecosystems processes (i.e. altered trophic interactions and reduced availability of food for native species) but also to engineering works through clogging of pipes especially in cooling reservoirs.

Plague-carrying *O. limosus* is native to North America. Following its introduction to Europe, this species has spread via rivers and canals and in some cases through accidental release by human-related activities (Holdich and Pöckl 2007). The first records of *O. limosus* in the Neman River Basin date back to 1997 (Alekhnovich et al. 1999). The species’ rate of spread has since been increasing at  $12$  km year<sup>-1</sup> in an upstream direction (Alekhnovich and Razlutskiy 2013), and the species is expected to reach also the Pripyat River Basin in the near future. This species is encountered under different environmental

conditions and occurs in different types of water bodies, including heavily polluted ones with decreased oxygen concentrations (Holdich et al. 2006). In the Neman River Basin, *O. limosus* prefers muddy substrata with macrophyte beds (i.e. *Elodea canadensis* Michx. and *Potamogeton* spp.), but can also inhabit harder substrata (Alekhnovich and Razlutskiy 2013). This decapod is characterised by high fecundity (>400 eggs) and rapid maturation compared to native crayfishes (Alekhnovich and Buřič 2017). Spread of *O. limosus* in Europe and Belarus has led to adverse impacts on native crayfish populations of the narrow-clawed crayfish *Astacus leptodactylus* (Eschscholtz, 1823) and broad-fingered crayfish *Astacus astacus* (Linnaeus, 1758). These have been caused not only by transmission of the crayfish plague, but also by direct competition for food and habitat resources (Peay 2009; Alekhnovich and Buřič 2017). Finally, in some rivers of Belarus native crayfishes have disappeared following introduction of *O. limosus* (A. V. Alekhnovich, personal communication).

Together with *D. polymorpha*, *D. bugensis* is regarded as one of the most successful freshwater invaders of Europe and North America, even though in the latter region the rate of colonisation of inland lakes is less intensive compared to the former (Karatayev et al. 2015). *Dreissena bugensis* can reproduce at lower temperatures, prefers lotic conditions compared to *D. polymorpha*, and inhabits predominantly reservoirs (Son 2007). *Dreissena bugensis* is able to colonise silty sediments, especially in the profundal zones of deep, large lakes (Karatayev et al. 2015), and can survive at depths up to 140 m, although it prefers living at 4–10 m below the water surface (Mills et al. 1996). Similar to *D. polymorpha*, *D. bugensis* can alter trophic chains by decreasing food availability for macroinvertebrates and fish, and especially so in the profundal zone (Nalepa et al. 2009a, b). In some shallow lakes and cooling reservoirs, decreased abundance of *D. polymorpha* has been paralleled by an increase in the abundance of *D. bugensis* (Karatayev et al. 2015). Finally, there is evidence that *D. bugensis* may cause less severe impacts on unionid mussels compared to *D. polymorpha* (Sherman et al. 2013).

*Pacifastacus leniusculus* is a North American crayfish that was introduced into Europe in the 1960s and has since spread to neighbouring countries with Belarus (i.e. Latvia, Lithuania and Poland). Adult size can reach 16–20 cm, sexual maturity is achieved at 6–9 cm at two-to-three years of age, egg numbers range from 200 to 400, and longevity can be up to 20 years (Alekhnovich and Buřič 2017). This species can adapt to warm waters and pH values < 6.5

(Holdich et al. 1997), and occurs in brackish waters with salinities as high as 20‰. *Pacifastacus leniusculus* can be found in a variety of habitats including rocky substrata, although most commonly in association with aquatic vegetation. This species is a polytrophic feeder whose diet consists of aquatic insects, plant material (in adults) and fish eggs (Lewis 2002). *Pacifastacus leniusculus* is resistant to the crayfish plague *Aphanomyces astaci* (Schikora 1906) and is a vector of transmission of this disease in European water bodies. Notably, the crayfish plague fungus of which the species is a carrier has caused large-scale mortalities amongst indigenous European crayfish populations (Holdich 1999).

*Chelicorophium curvispinum* was first recorded from Belarus in 1914 (Wolski 1930). This species is possibly the most widespread Ponto-Caspian amphipod throughout the RA area. In the Dnieper River, the species' abundance can reach > 5000 ind. m<sup>-2</sup> on muddy substrata with *Dreissena* spp. shells and rocks, even though in the Rhine River maximum densities of 642,000 ind. m<sup>-2</sup> have been reported (Rajagopal et al. 1998). *Chelicorophium curvispinum* is characterised by rapid growth rate and its reproductive season begins in March, with three generations produced per year and a mean brood size of 25–35 eggs (Rajagopal et al. 1998; Grabowski et al. 2007). Unlike other amphipods, *C. curvispinum* is a filter feeder with food items consisting mainly of phytoplankton and detritus. Also, a positive correlation has been found between amphipod's density and phytoplankton concentration (van den Brink et al. 1993). *Chelicorophium curvispinum* has the ability to build mud tubes on hard substrata, making it an "ecological engineer" (Jones et al. 1994). Abundance of this species is affected not only by the presence of "soft" macroinvertebrates, but also by food competition with *D. polymorpha* (van den Brink et al. 1993; Rajagopal et al. 1998). *Chelicorophium curvispinum* is an active migrator moving ≈15 km year<sup>-1</sup>, whose spread is further aided by dredging activities especially in river ports (Josens et al. 2005; Semenchko et al. 2015).

Amongst the other species classified as carrying a high risk of invasiveness, the amphipod *P. robustoides* has been successfully introduced to the Kiev and Kaunas reservoirs as food source for fish, and it has been present since the 1960s (Arbachauskas 2005). Since 2007, this species has rapidly spread upstream of the Pripyat River at a rate > 12 km year<sup>-1</sup> (V. Semenchko, unpublished data). *Pontogammarus robustoides* can reach 12 mm in length and produces three generations per year. In Latvian lakes, this species prefers low-flow to stagnant, shallow near-shore waters with different substrata and environmental conditions (Paidere et al. 2016). In the Dnieper

and Pripyat rivers, *P. robustoides* occurs on rocky substrata with sand and on macrophyte beds, with preference for *Ceratophyllum* sp. This species is invasive in Baltic countries (i.e. Estonia, Lithuania and Latvia), where it acts as a predator (Arbachauskas 2005; Gumuliauskaite and Arbaciauskas 2008). Invasion of this species from the Kaunas Reservoir into the Neman River Basin in Belarus is expected to occur in the near future.

The other high-risk amphipod *D. villosus* is amongst the 100 worst invasive alien species in Europe (DAISIE 2009). This species has wide environmental tolerances: it can survive at temperatures from 0 to 35 °C (Bruijs et al. 2001; Wijnhoven et al. 2003; van der Velde et al. 2009), salinities up to 12–20‰, as well as low oxygen concentrations (with a lethal concentration of 0.35 mg O<sub>2</sub> L<sup>-1</sup>, (Dedyu 1980). *Dikerogammarus villosus* reproduces all year round in its native range (Mordukhai-Boltovskoi 1969), and in its invasive areas is characterised by a long reproductive period, early sexual maturity, short generation and life span, and brief duration of the embryonic development (Pöckl 2009). This species prefers coastal waters with vegetated areas consisting of *Potamogeton* sp. and *Ceratophyllum* sp. mats near or on the water surface, but also occurs on stony substrata and zebra mussel shells, and occasionally on sand (Devin et al. 2003; Semenchenko et al. 2013). It is an omnivorous species that kills its prey by biting and shredding. Interactions (i.e. competition and predation: Dick et al. 2002; Kinzler et al. 2009) between *D. villosus* and native gammarids can result in the displacement or local extinction of the latter, thereby reducing biodiversity (Kley and Maier 2005; Semenchenko et al. 2013). Also, predation by *D. villosus* leads to alteration in macroinvertebrate assemblage composition and abundance. Finally, this species has been observed attacking small fish and their progeny (Casellato et al. 2007), thereby disturbing the natural interactions at multiple levels in the food web (van Riel et al. 2006).

The high-risk amphipod *C. mucronatum* (which resulted as a false positive in the present study) is a small species with adult body length of 5–6 mm that produces two to three generations per year depending on water temperature (Dedyu 1980). In the Dniester estuary, although *C. mucronatum* prefers low salinities of 0.3–3.0‰, it can survive at levels of 3–5‰ (Grigorovich and MacIsaac 1999). This amphipod inhabits both lotic and lentic waters (Birshtein and Romanova 1968) and occurs on different types of substrata including mud, sand, stones and shells of *D. polymorpha* (Dedyu 1980). Population densities can reach 4660 ind. m<sup>-2</sup> on silty–sandy bottoms (Dedyu 1980). However, no evidence is currently

available about potential impacts of this species on the native macrozoobenthos.

Focusing on the species classified as low risk, *L. benedeni* was found to occur in high abundances (176 ind. m<sup>-2</sup>) in the littoral zone of the Pripyat River at depths of 0.2–0.3 m with macrophyte beds (Semenchenko et al. 2007); whereas, *F. fragilis* is a typical limnetic species of North American origin that co-exists with other molluscs (Beran and Horsák 2007), but for which no evidence is available on potential impacts on the native faunas.

### Concluding remarks

Similar to the recent AS-ISK applications on freshwater fishes (Tarkan et al. 2017a, b; Glamuzina et al. 2017; Li et al. 2017), the present application on freshwater macroinvertebrates has proven the ability of the tool to discriminate reliably between non-invasive and invasive species, indicating the tool's overall predictive value and accuracy. As regards the latter, the identification in the present study of no false negatives and of only one false positive, combined with the finding that all species categorised *a priori* as invasive were classified as high risk and all medium-risk species were categorised *a priori* as non-invasive, is a notable and positive outcome. In fact, this is in agreement with the original purpose of the WRA (from which AS-ISK inherits through its predecessor FISK “family” of tools: Copp 2013) to predict that virtually all major invaders would have a high probability of becoming invasive, and incorrectly predict that non-invaders would become invasive less than 10% of the time (Gordon and Gantz 2011). Clearly, from a risk-based perspective, it may be argued that rejecting a (potentially) non-invasive species is likely to pose, in the majority of cases, less serious consequences in terms of ecological and economic costs compared to accepting an invasive species (hence, likely to cause detrimental effects) – even though proper cost-benefit analyses would need to be implemented in case of rejection of a (potentially) non-invasive species of particular value to e.g. fisheries and/or aquaculture activities (e.g. Tarkan et al. 2017a).

Except for a recent (“indirectly” calibrated, due to low sample sizes) application of AS-ISK to the non-indigenous macrozoobenthos of the River Ticino catchment in north-west Italy (Paganelli et al. 2018), previous risk screening studies on non-native macroinvertebrates have relied upon the Freshwater Invertebrates Invasiveness Screening Kit (FI-ISK, as adapted from FISK to this category of aquatic organisms: Copp 2013), and have contributed to provide guidelines for the successful implementation

of management and regulation protocols in several RA areas across Eurasia (Tricarico et al. 2010; Škraba et al. 2013; Patoka et al. 2014, 2017; Uderbayev et al. 2017; Vodovsky et al. 2017). This is also an expectation of the outcomes of the present study, which, compared to previous FI-ISK based applications, has further benefited from the incorporation of climate change considerations and related uncertainty. This additional feature in AS-ISK has provided for wider scope and, possibly, overall predictive ability relative to its predecessor tool.

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## Supplementary material

The following supplementary material is available for this article:

**Table S1.** Macroinvertebrate species screened for their potential invasiveness in the Dnieper, Neman and Pripyat rivers and associated basins of Belarus.

**Table S2.** Macroinvertebrate species assessed for the Dnieper, Neman and Pripyat river and associated basins of Belarus using the AS-ISK tool.

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