Science of the Total Environment 788 (2021) 147868

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

A global-scale screening of non-native aquatic organisms to identify potentially invasive species under current and future climate conditions

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https://doi.org/10.1016/j.scitotenv.2021.147868
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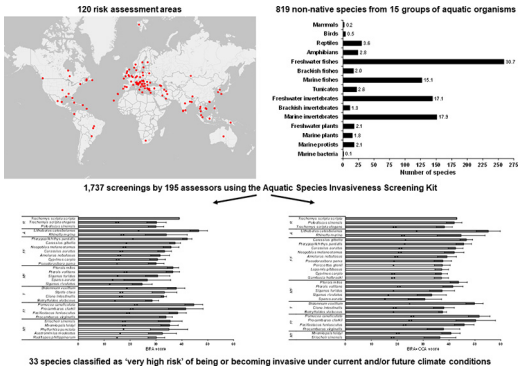
HIGHLIGHTS

- A global approach is required to identify invasive species posing high risk impact.
- 195 assessors screened 819 non-native species from 15 groups of aquatic organisms.
- Risk thresholds were identified for 14 aquatic organism groups.
- The resulting risk thresholds and rankings will help management and conservation.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 13 April 2021
Received in revised form 13 May 2021
Accepted 14 May 2021
Available online 20 May 2021

Editor: Damia Barcelo

Keywords:
Decision support tools
AS-ISK
Hazard identification
Non-native species
Risk analysis
Climate change

ABSTRACT

The threat posed by invasive non-native species worldwide requires a global approach to identify which introduced species are likely to pose an elevated risk of impact to native species and ecosystems. To inform policy, stakeholders and management decisions on global threats to aquatic ecosystems, 195 assessors representing 120 risk assessment areas across all six inhabited continents screened 819 non-native species from 15 groups of aquatic organisms (freshwater, brackish, marine plants and animals) using the Aquatic Species Invasiveness Screening Kit. This multi-lingual decision-support tool for the risk screening of aquatic organisms provides assessors with risk scores for a species under current and future climate change conditions that, following a statistically based calibration, permits the accurate classification of species into high-, medium- and low-risk categories under current and predicted climate conditions. The 1730 screenings undertaken encompassed wide geographical areas (regions, political entities, parts thereof, water bodies, river basins, lake drainage basins, and marine regions), which permitted thresholds to be identified for almost all aquatic organismal groups screened as well as for tropical, temperate and continental climate classes, and for tropical and temperate marine ecoregions. In total, 33 species were identified as posing a 'very high risk' of being or becoming invasive, and the scores of several of these species under current climate increased under future climate conditions, primarily due to their wide thermal tolerances. The risk thresholds determined for taxonomic groups and climate zones provide a basis against which area-specific or climate-based calibrated thresholds may be interpreted. In turn, the risk rankings help decision-makers identify which species require an immediate 'rapid' management action (e.g. eradication, control) to avoid or mitigate adverse impacts, which require a full risk assessment, and which are to be restricted or banned with regard to importation and/or sale as ornamental or aquarium/fishery enhancement.

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1. Introduction

The threat posed by invasive non-native species (NNS) worldwide to native species and ecosystems requires a global approach to identify species that pose a high risk of becoming invasive across varied geographies and climates (Gordon et al., 2008). Use of the same risk screening protocols worldwide can provide the larger-scale information with which to inform the decisions of policy makers and environmental managers in dealing with species invasions (Early et al., 2016; Shackleton et al., 2019). Indeed, reliable, evidence-based risk assessment (RA) methods are vital to decision-making in biosecurity management at national and international levels in order to prevent or mitigate unwanted biological invasions (Kaiser and Burnett, 2010). Ideally, the RA outcomes should compare and prioritise NNS according to their potential invasiveness under current and future climate conditions (Barney and DiTomaso, 2010), which aids in identifying the underlying factors associated with invasion hotspots (O'Donnell et al., 2012; Chapman et al., 2020).

The first step in the NNS risk analysis process is risk screening (i.e. hazard identification), which aims to identify the NNS that are likely to pose an elevated threat to native species and ecosystems and, therefore, warrant more comprehensive (i.e. full) RA (Copp et al., 2005b). Aquatic species that are likely to carry a high risk of becoming invasive, hence posing a threat to native species, usually possess life-history traits including frequent reproduction with a high incidence of ovoviviparity, large body size and a long life span, the ability to exploit opportunistically available food resources, a history of invasion success, and a close climate matching with the RA area (Statzner et al., 2008; Chan et al., 2021). In addition, these species often tolerate higher salinity, broader environmental temperatures and higher levels of organic pollution than native species (Leuven et al., 2009). Importantly, the identification of species posing a high risk of being (or becoming) invasive in a certain RA area provides a basis for advice to policy, decision-makers and other stakeholders regarding management options for existing and potential future invasive NNS (Copp et al., 2005b, 2016b; Mumford et al., 2010; David et al., 2013; David and Gollasch, 2018, 2019).

The objectives of the present study were to: (i) construct a global database of risk screenings that span the broadest range of aquatic organisms possible, given available resources, across the widest possible geographical spread; (ii) subject the global database of screenings to calibration and accuracy analysis; and (iii) generate global-scale risk thresholds at the organism group and climate class/marine ecoregion levels under both current and future climate conditions. The global-scale thresholds identified will provide a basis against which thresholds calibrated for specific RA areas may be interpreted and will also allow the 'rapid risk screening' of individual species for a certain RA area

whenever specific calibration is not possible. In addition, these global-scale thresholds will place RA area-specific risk screening calibrations within a broader, global context, also accounting for climate change predictions. In turn, this will enhance the value and scope of more localised calibrations to inform environmental policy and decision-makers of the relative risk rankings of aquatic NNS so as to facilitate the cost-effective allocation of management resources.

2. Materials and methods

2.1. Risk screening procedure

For the purposes of this study, 'invasive species' are defined, as per Copp et al. (2005c, p. 244), as those NNS "that spread, with or without the aid of humans, in natural or semi-natural habitats, producing a significant change in composition, structure, or ecosystem processes, or cause severe economic losses to human activities". Species were evaluated for their potential to become invasive in the assessor(s)-defined RA area using the Aquatic Species Invasiveness Screening Kit (AS-ISK: free download at www.cefas.co.uk/nns/tools/). This is a decision-support tool (Copp et al., 2016b, 2021) adapted from the Fish Invasiveness Screening Kit (Copp et al., 2009, 2005a), which itself was derived from the globally-applied Weed Risk Assessment of Pheloung et al. (1999). The AS-ISK comprises questions from 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., 2018) for the assessment of invasive NNS with regard to the 2014 EU Regulation 1143/2014 (European Union, 2014). As a taxon-generic toolkit, the AS-ISK is applicable to any aquatic species (other than parasites and pathogens) in virtually any climatic/marine ecoregion zone (Copp et al., 2016b; Table 1), and allows the screening of 27 groups of aquatic organisms in total (taxonomy after Ruggiero et al., 2015): mammals, birds, reptiles, amphibians, fishes (freshwater, brackish, marine), tunicates, lancelets, invertebrates (freshwater, brackish, marine), 'other' animals (freshwater, brackish, marine), plants (freshwater, brackish, marine), protists (freshwater, brackish, marine), fungi (freshwater, brackish, marine), and bacteria (freshwater, brackish, marine).

The screening protocol consists of 55 questions (Copp et al., 2016b). The first 49 questions comprise the Basic Risk Assessment (BRA), which are concerned with the biogeographical and biological aspects of the species being screened. The last six questions address the Climate Change Assessment (CCA), which require the assessor to evaluate how future predicted climate conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact. To achieve a valid screening, for each question the assessor must provide a response, a level of confidence in the response, and a justification. In all cases, the assessor is a specialist in the biology/ecology of the aquatic organism under screening for the RA area under study. Upon completion of the screening, the species receives both a BRA score and a BRA + CCA (composite) score (ranging from −20 to 68 and from −32 to 80, respectively). Scores < 1 suggest that the species is unlikely to become invasive and is therefore classified as 'low risk' (Pheloung et al., 1999). Higher scores classify the species as posing either a 'medium risk' or a 'high risk' of becoming invasive. Distinction between medium-risk and high-risk levels depends upon setting a 'threshold' value (see Section 2.2 *Data processing and analysis*).

The ranked levels of confidence (1 = low; 2 = medium; 3 = high; 4 = very high) associated with each question-related response mirror the confidence rankings recommended by the International Programme on Climate Change (IPCC, 2005; see also Copp et al., 2016b). Based on the confidence level (CL) allocated to each response, a confidence factor (CF) is computed as:

$$CF = \sum (CL_{Qi})/(4 \times 55)\ (i = 1, \ldots, 55)$$

where CL_{Qi} is the confidence level for the *i*th Question (Q*i*), 4 is the maximum achievable value for confidence (i.e. very high: see above) and 55 is the total number of questions. Based on the 49 Qs comprising the BRA and the six Qs comprising the CCA, the CL_{BRA} and CL_{CCA} are also computed (out of the CL_{Total} for all 55 Qs).

2.2. Data processing and analysis

Data consisted of: (i) individual contributions to the present study by assessors invited to screen one (or more) NNS belonging to one or more aquatic organismal groups of choice (i.e. falling within their expertise) for a certain RA area; and (ii) datasets from more comprehensive screening studies of NNS for a certain RA area, both published (see Table 1) and unpublished. For each species screened, the scientific name used in the original contribution or study was updated to the most recent taxonomy after the World Register of Marine Species (www.marinespecies.org), else after the Integrated Taxonomic Information System (www.itis.gov/) or FishBase (www.fishbase.org). This was followed by 'cross-checking' for the existence of at least one peer-reviewed publication that used the updated scientific name in case of a change in taxonomy. A notable exception was the retention of the original name *Crassostrea gigas* instead of the recently proposed *Magallana gigas* for the Pacific oyster (see Bayne et al., 2017). Taxonomic details of the corresponding Order and Family were also retrieved for each species screened.

Except for marine regions, for each RA area the corresponding Köppen-Geiger climate class (i.e. Tropical, Dry, Temperate, Continental, Polar: Peel et al., 2007) was identified, noting that in several cases more than one climate class applied to the same RA area. For marine regions, the classification by Spalding et al. (2007) was used including: (i) Arctic, (ii) Temperate Northern Atlantic and Temperate Northern Pacific (grouped in the present study into 'Temperate marine'), and (iii) Central Indo-Pacific, Tropical Atlantic, Tropical Eastern Pacific and Western Indo-Pacific (grouped in the present study into 'Tropical marine').

The shape of the global distribution of the BRA and BRA + CCA scores was tested in R x64 v3.6.3 (R Development Core Team, 2020) using the package 'moments' v0.14 (Komsta and Novomestky, 2015), with normality, skewness and kurtosis evaluated by the Jarque-Bera (*JB*), D'Agostino and Anscombe tests, respectively. Computation of risk outcomes was based on receiver operating characteristic (ROC) curve analysis (Bewick et al., 2004). An ROC curve is a graph of sensitivity vs

Table 1
Published initial applications of the Aquatic Species Invasiveness Screening Kit (AS-ISK) by aquatic organismal group.

Aquatic organismal group	Reference(s)
Reptiles	Ruykys et al. (2021)
Amphibians	Ruykys et al. (2021)
Freshwater fishes	Glamuzina et al. (2017), Li et al. (2017), Tarkan et al. (2017a), Tarkan et al. (2017b), Dodd et al. (2019), Suresh et al. (2019), Interesova et al. (2020), Moghaddas et al. (2020), Zięba et al. (2020), Glamuzina et al. (2021), Haubrock et al. (2021), Kumar et al. (2021), Moghaddas et al. (2021), Radočaj et al. (2021), Ruykys et al. (2021), Wei et al. (2021)
Brackish fishes	Castellanos-Galindo et al. (2018), Clarke et al. (2020)
Marine fishes	Filiz et al. (2017a), Filiz et al. (2017b), Bilge et al. (2019), Clarke et al. (2020), Lyons et al. (2020), Uyan et al. (2020)
Tunicates	Clarke et al. (2020)
Freshwater invertebrates	Paganelli et al. (2018), Ruykys et al. (2021), Semenchenko et al. (2018)
Brackish invertebrates	Clarke et al. (2020), Ruykys et al. (2021)
Marine invertebrates	Clarke et al. (2020), Killi et al. (2020), Stasolla et al. (2020), Ruykys et al. (2021)
Freshwater plants	Ruykys et al. (2021)
Marine plants	Clarke et al. (2020), Ruykys et al. (2021)
Marine protists	Clarke et al. (2020)

1 – specificity for each threshold value, where in the present context sensitivity and specificity will be the proportion of a priori invasive and non-invasive species, respectively, correctly identified as such. For ROC curve analysis to be implemented, the species selected for screening must be categorised a priori as non-invasive or invasive using independent literature sources.

The a priori categorisation was as follows (see also Clarke et al., 2020): (i) a first search was made of FishBase for any reference to the species' threat, with the species categorised as non-invasive if listed as 'harmless', categorised as invasive if listed as 'potential pest', or scored as absent if either not evaluated or not listed in the above database; (ii) a second search was made of the Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI ISC: www.cabi.org/ISC) and the Global Invasive Species Database (GISD: www.iucngisd.org), with the species categorised as invasive if it appeared in any of such lists or scored as absent if not listed; (iii) a third search was made of the Invasive and Exotic Species of North America list (www.invasive.org), with the species categorised as invasive if it appeared in any of such lists or scored as absent if not listed; (iv) except for those species categorised as invasive in any (or all) of the previous three steps, a Google Scholar (literature) search was performed to check whether at least one peer-reviewed reference is found that 'demonstrates' (hence, not 'assumes') invasiveness/impact. The latter was then taken as 'sufficient evidence' for categorising the species as invasive; whereas, if no evidence was found, then the species was categorised as non-invasive. Overall, the advantage of this method is that, by virtue of its meta-analytical foundation, it draws upon and combines previous approaches into a multi-tiered protocol. This maximises the amount of information collectable about the NNS under screening, thereby increasing the accuracy of the screening outcomes (Vilizzi, Copp and Hill, unpublished).

A measure of the accuracy of the calibration analysis is the Area Under the Curve (AUC), which ranges from 0 to 1: a model whose predictions are 100% correct has an AUC of 1, one whose predictions are 100% wrong has an AUC of 0. In the former case there are neither 'false positives' (a priori non-invasive species classified as high risk, hence false invasive) nor 'false negatives' (a priori invasive species classified as low or medium risk, hence false non-invasive); in the latter case, the test cannot discriminate between 'true positives' (a priori invasive species classified as high risk, hence true invasive) and 'true negatives' (a priori non-invasive species classified as low or medium risk, hence true non-invasive). Following ROC curve analysis, the best threshold value that maximises the true positive rate and minimises the false positive rate was determined using Youden's *J* statistic.

Because of sample size constraints (see Vilizzi et al., 2019), group-specific thresholds for both the BRA and BRA + CCA were fitted to those groups of aquatic organisms for which >10 species were screened. Consequently, for mammals and birds for which there were low numbers of taxa a combined threshold was computed by pooling together the screened species for these groups with those screened for reptiles and amphibians. This rendered the respective thresholds statistically significant, permitting their use for distinguishing between high-risk and low-to-medium risk species until such time that RA-area-specific calibrations can be undertaken for those taxonomic groups. To highlight 'very high risk' species for the aquatic organismal groups with large enough sample sizes, ad hoc thresholds for the BRA and BRA + CCA were set weighted according to the range of scores for the high-risk species (see Clarke et al., 2020) and with the constraint that the species was screened for a 'representative' number of RA areas (i.e. weighted according to the corresponding organismal group). Additionally, climate class-specific thresholds were computed for freshwater fishes, and marine ecoregion-specific thresholds for marine fishes and invertebrates – the aquatic organismal groups with large enough sample size for successful computation of such thresholds. In all cases, ROC curve analysis was carried out with the package 'pROC' (Robin et al., 2011) for R x64 v3.6.3 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).

Following Smith et al. (1999), three measures of accuracy were defined:

1) for a priori invasive species: $A_i = (I_r/I_t) \times 100$, where I_r is the number of a priori invasive species rejected, and I_t the total number of a priori invasive species screened;
2) for a priori non-invasive species: $A_n = (N_a/N_t) \times 100$, where N_a is the number of a priori non-invasive species accepted and N_t the total number of a priori non-invasive species screened;
3) overall: $A_o = (N_a + I_r)/(N_t + I_t)$.

In all cases, values above 50% are indicators of the accuracy of the screening tool.

3. Results

In total, 1730 screenings were conducted by 195 assessors (the co-authors of this study) on 819 taxa comprising 798 species, nine subspecies, three hybrids, and nine genera (Supplementary data Table S1). Of these taxa (hereafter, loosely termed 'species'), 562 (68.6%) were categorised a priori as non-invasive and 257 (31.4%) as invasive (Supplementary data Table S1) and were screened relative to 120 RA areas (Supplementary data Tables S2 and S3) across all six inhabited continents (Fig. 1). The RA areas consisted of extensive geographical areas, regions, countries, parts of countries, states, other political entities, water bodies, river basins, lake drainage basins, and marine regions (Supplementary data Table S2). Screenings encompassed 15 groups of aquatic organisms (Fig. 2) in 104 Orders (Supplementary data Table S2), with 24 species assigned to two different groups depending on the RA area's aquatic habitat (Supplementary data Table S4).

The BRA scores ranged from −15.0 to 55.0, with a mean = 18.6, a median = 18.0, and 5th and 95th percentiles = −4.0 and 42.5. Their distribution was not normal ($JB = 36.664$, $P < 0.001$), not skewed (skewness = 0.076, $z = 1.230$, $P = 0.195$), but platykurtic (kurtosis = 2.305, $z = -9.359$, $P < 0.001$) (Fig. 3a). The BRA + CCA scores ranged from −27.0 to 67.0, with a mean = 22.3, a median = 22.0, and 5th and 95th percentiles = −5.6 and 51.1. Their distribution was not normal ($JB = 16.378$, $P < 0.001$), not skewed (skewness = −0.039, $z = -0.675$, $P = 0.499$), but platykurtic (kurtosis = 2.531, $z = -5.210$, $P < 0.001$) (Fig. 3b). The majority of delta values (i.e. differences between BRA + CCA and BRA scores, hence accounting for climate change predictions) were equal to 0, 4, 6 and 10 (>10% of the total in all cases), and overall the proportion of the positive differences was much larger than that of the negative differences (68.8% vs 16.7%) (Fig. 3c). Across all species, the mean CL values were: $CL_{Total} = 2.73 \pm 0.01$, $CL_{BRA} = 2.78 \pm 0.01$, and $CL_{CCA} = 2.25 \pm 0.02$, indicating in all cases medium to high confidence (Supplementary data Table S5).

Thresholds were computed for all screened groups of aquatic organisms except those represented by ≤10 species (Table 2). For reptiles, amphibians, freshwater and marine fishes, tunicates, freshwater and brackish invertebrates and marine protists, the BRA threshold was lower than the BRA + CCA one, whereas the opposite was true for brackish fishes, marine invertebrates, and freshwater and marine plants. Except for marine protists (BRA), the mean AUC values (in Table 2) were in all cases > 0.5 – this confirmed the ability of the toolkit to differentiate between a priori invasive and non-invasive species. After pooling, BRA and BRA + CCA thresholds could be computed for mammals and birds, and in both cases the BRA threshold was lower than the BRA + CCA one (Table 2).

Based on the aquatic organismal group-specific thresholds (excluding the pooled ones), all three measures of accuracy had a mean value ≥50% for all groups except tunicates (BRA + CCA only), marine plants and marine protists (both BRA and BRA + CCA) – a result of the relatively small sample sizes (Table 3). The number (and proportion) of true positives

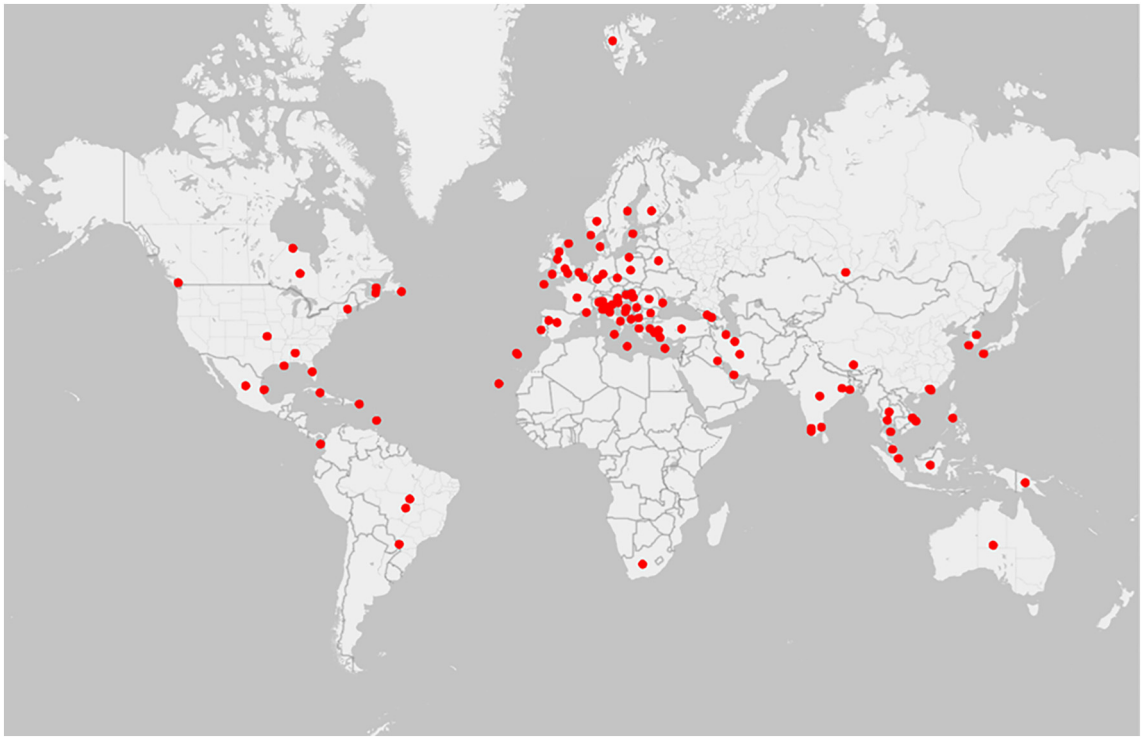

Fig. 1. Map of the risk assessment areas for which species were screened with the Aquatic Species Invasiveness Screening Kit (AS-ISK; see also Supplementary data Table S2).

was consistently larger than that of the false negatives, which in all cases accounted for only 0–5.6% of the screened species for each group (Table 4). Similarly, the proportion of false positives was in most cases smaller than that of the true positives, and the proportion of the medium-risk species was always relatively high. In total, 33 species were identified as carrying a very high risk of invasiveness: 26 species based on both the BRA and BRA + CCA, four on the BRA only, and three on the BRA + CCA only (Fig. 4a, b).

Of the 82 non-marine region RA areas, 56 included one climate class. To these RA areas, an additional four were added for which the second climate class (namely, Continental) was only marginally represented (Supplementary data Table S2), whereas the only RA area represented by the Dry climate was removed from further analysis (noting that the Polar climate was found only in combination with the Temperate and/or Continental climates, hence could not be analysed separately regardless of sample size). In total, 59 non-marine region RA areas were therefore considered. For freshwater fishes in tropical, temperate and continental climates, both the BRA and BRA + CCA thresholds were higher for the tropical climate, lower for the temperate and even lower for the continental climate, whereas the BRA + CCA was similar to the BRA in all cases (Table 5). Of the 38 marine ecoregion RA areas, four fell within the Arctic ecoregion, 24 in the Temperate grouping (including 23 RA areas in the Temperate Northern Atlantic and one in the Temperate Northern Pacific ecoregions), and ten in the Tropical

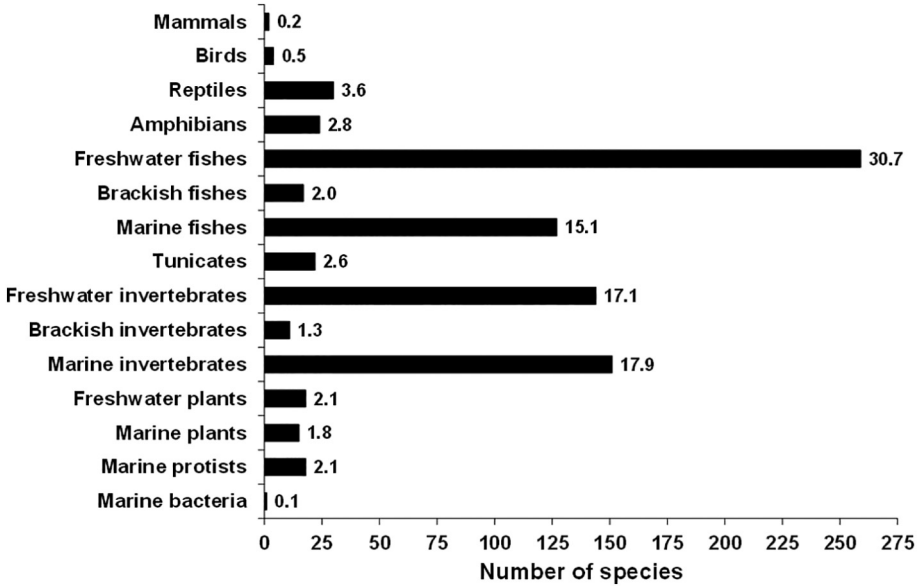

Fig. 2. Number (and corresponding percentage) of species screened according to aquatic organismal group.

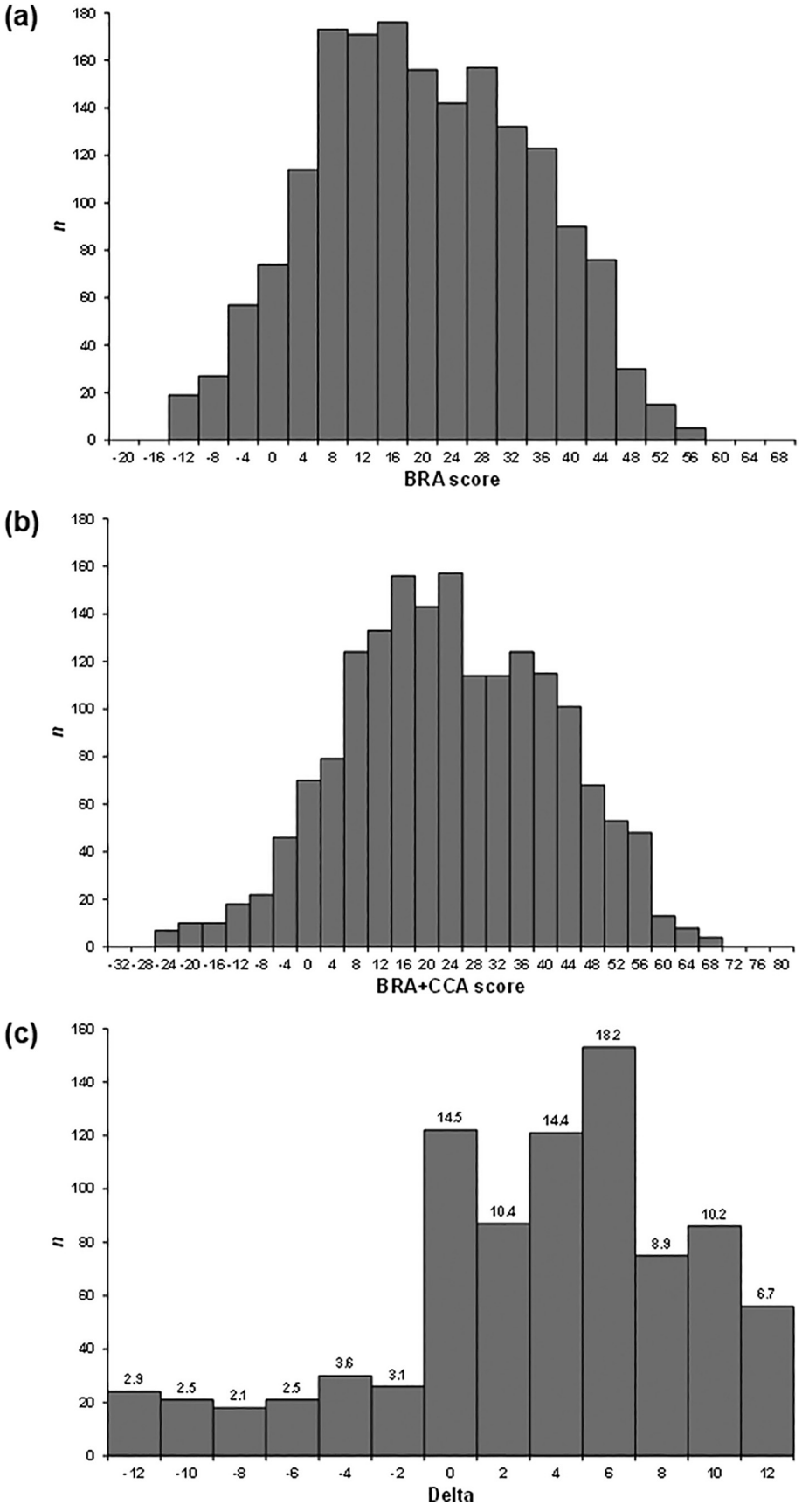

Fig. 3. (a) Frequency distribution of the Basic Risk Assessment (BRA) scores for the species screened with AS-ISK; (b) same for the BRA + CCA (Climate Change Component) scores; (c) same for delta CCA values (i.e. differences between BRA + CCA and BRA scores for each of the species screened) with corresponding percentage.

Table 2
Basic Risk Assessment (BRA) and BRA + Climate Change Assessment (CCA) AS-ISK thresholds from receiver operating characteristic curve analysis for the groups of aquatic organisms screened in the present study (Marine bacteria added for completeness). For each aquatic organismal group, the following is provided: number of screened species (n), threshold (Thr) values for the BRA and BRA + CCA [the Area Under the Curve (AUC) values are >0.5, and therefore are statistically valid, including for Mammals, Birds, Reptiles and Amphibians – with the latter two groups pooled together with Mammals and Birds for computation of the thresholds], with corresponding mean, lower confidence interval (LCI) and upper confidence interval (UCI) for the AUC, very high risk threshold (VH Thr) and minimum number of risk assessment areas (RAAs) for selection of the very high risk species. Note that threshold values are given in increments of 0.05 and rounded to the first or second decimal (as applicable, following AS-ISK threshold notation).

Aquatic organismal group	n	BRA						BRA + CCA					
		Thr	Mean	LCI	UCI	VH Thr	RAAs	Thr	Mean	LCI	UCI	VH Thr	RAAs
Mammals	2	25.5	0.7180	0.5834	0.8525	–	–	31.6	0.6639	0.5223	0.8056	–	–
Birds	4	25.5	0.7180	0.5834	0.8525	–	–	31.6	0.6639	0.5223	0.8056	–	–
Reptiles	30	28.5	0.6550	0.4176	0.8924	–	2	36.5	0.5975	0.3489	0.8461	–	2
Amphibians	24	15.5	0.7983	0.6144	0.9822	–	3	19.5	0.7815	0.5934	0.9696	–	3
Freshwater fishes	259	14.7	0.8446	0.7957	0.8936	30	10	17.7	0.8213	0.7691	0.8735	36	10
Brackish fishes	17	38	0.7917	0.5542	1.0000	–	–	29.5	0.6875	0.3969	0.9781	–	–
Marine fishes	127	12.75	0.8254	0.7089	0.9420	–	3	19	0.7819	0.6541	0.9096	–	3
Tunicates	22	22.5	0.6417	0.3943	0.8890	–	2	36.75	0.5792	0.3119	0.8464	–	2
Freshwater invertebrates	144	13.25	0.8243	0.7185	0.9301	30	4	25.75	0.8199	0.7076	0.9322	36	4
Brackish invertebrates	11	15	0.6111	0.0247	1.0000	–	–	26.9	0.7222	0.4142	1.0000	–	–
Marine invertebrates	151	15.1	0.8842	0.8333	0.9351	30	3	14.25	0.8483	0.7859	0.9107	36	3
Freshwater plants	15	24.5	0.8611	0.6697	1.0000	–	–	12.5	0.9028	0.7522	1.0000	–	–
Marine plants	15	32	0.6161	0.3098	0.9224	–	–	27.25	0.6250	0.3197	0.9303	–	–
Marine protists	18	34	0.4545	0.1639	0.7452	–	–	42.75	0.5779	0.2899	0.8659	–	–
Marine bacteria	1	–	–	–	–	–	–	–	–	–	–	–	–

grouping (including three RA areas in the Central Indo-Pacific, four in the Tropical Atlantic, one in the Tropical Eastern Pacific, and two in the Western Indo-Pacific ecoregions) (Supplementary data Table S2). However, owing to low sample sizes, thresholds could not be computed for any aquatic organismal group in the Arctic ecoregion. For marine fishes, the BRA and BRA + CCA thresholds were higher for the temperate relative to the tropical ecoregion grouping, and the BRA + CCA threshold was higher than the BRA threshold in both cases (Table 5). For marine invertebrates, the BRA and BRA + CCA thresholds were higher for the tropical relative to the temperate ecoregion grouping, and the BRA threshold was similar to the BRA + CCA for the temperate ecoregion grouping but lower for the tropical ecoregion grouping (Table 5). In all cases, the mean AUC values were well above 0.5.

Based on the climate/marine ecoregion-specific thresholds, all three measures of accuracy had a mean value well above 50% in all cases and for both the BRA and BRA + CCA (Table 6). The number (and proportion) of true positives was consistently larger than that of the false negatives, which always accounted for only 0–1.6% of the screened species for each combination (Table 7). Similarly, the proportion of false positives was in most cases smaller than that of the true positives, and the proportion of medium-risk species was always relatively high.

Table 3
Accuracy measures for screenings on the groups of aquatic organisms for which BRA and BRA + CCA thresholds were directly computed (cf. Table 2). A_i = accuracy for a priori invasive species; A_n = accuracy for a priori non-invasive species; A_o = overall accuracy (see text for details). In italics, values < 50%.

Aquatic organismal group	BRA			BRA + CCA		
	A_i	A_n	A_0	A_i	A_n	A_0
Reptiles	70.0	75.0	73.3	70.0	75.0	73.3
Amphibians	100.0	70.6	79.2	50.0	50.0	50.0
Freshwater fishes	83.9	74.7	78.0	77.4	71.7	73.7
Brackish fishes	62.5	100.0	82.4	62.5	88.9	76.5
Marine fishes	88.9	75.2	77.2	72.2	79.8	78.7
Tunicates	80.0	58.3	68.2	*40.0*	91.7	68.2
Freshwater invertebrates	76.9	83.9	82.6	65.4	95.8	90.3
Brackish invertebrates	88.9	50.0	81.8	66.7	100.0	72.7
Marine invertebrates	86.4	78.3	81.5	83.1	75.0	78.1
Freshwater plants	83.3	100.0	88.9	58.3	100.0	72.2
Marine plants	*42.9*	87.5	66.7	*28.6*	75.0	53.3
Marine protists	*42.9*	36.4	38.9	71.4	*27.3*	*44.4*

4. Discussion

4.1. Risk screening extent

In this study, fishes and invertebrates represented the largest proportion of screened aquatic species, thus reflecting the composition of introduced animal species recorded for e.g. European waters (Alcaraz et al., 2005; Gherardi et al., 2009; Katsanevakis et al., 2013) but also the relative number of experts (cf. assessors) in the various aquatic organismal groups. After freshwater fishes, freshwater and marine invertebrates comprised the second most-widely screened group of aquatic organisms, with the marine invertebrates including a large proportion of Decapoda – an Order that comprises several of the world's worst invasive species (Lowe et al., 2000; Souty-Grosset et al., 2006). The large number of screenings for freshwater fishes in this study can be attributed to the importance of inland waters as providers of ecosystem services for human societies (e.g. Wilson and Carpenter, 1999) and to the fact that these habitats are under high human-induced pressure, including NNS introductions (e.g. Hughes et al., 1998; Rahel, 2000). The broad geographical spread of most of the screened freshwater fish species reflects the increasing homogenisation of aquatic fauna and flora as a result of worldwide introductions (e.g. McKinney, 1998; Rahel, 2000). Further, the taxonomic Orders that were more frequently screened are those usually ecologically flexible, able to withstand adverse ecological conditions, generally widespread over large spatial scales, and often of economic importance (e.g. Hulme, 2009).

Despite the large number of aquatic species screened in this study, bacteria were represented by only one species and no screenings for fungi were contributed. Risk screenings of these groups of aquatic organisms would require the participation of experts in the fields of microbiology and mycology, respectively. This points to the need for greater multi-disciplinarity in future risk identification/assessment studies, which is particularly important as both aquatic bacteria and fungi are known to exert in some cases severe ecological impacts once established and spread in their invasive range (Litchman, 2010), similar to their terrestrial counterparts (Alderman, 1996; Loo, 2008). Regardless, it must be noted that pathogenic and parasitic organisms are normally evaluated separately from other NNS using risk assessment protocols specific to infectious agents (e.g. Peeler et al., 2007; D'hondt et al., 2015; Copp et al., 2016a). In addition, the diminutive size of these taxa could cause their presence to go un-noticed, thereby limiting knowledge of their spread and extent of invasiveness.

Table 4
Risk outcomes (given as number of screened species and corresponding percentage) for the BRA and BRA + CCA for the groups of aquatic organisms for which risk thresholds were directly computed. Species are categorised a priori as either Non-invasive or Invasive (see Supplementary data Table S2). Medium-risk and high-risk outcomes for each group are based on the thresholds given in Table 2, whereas low-risk outcomes are based on a 'default' threshold of 1.

Aquatic organismal group	BRA				BRA + CCA			
	Non-invasive		Invasive		Non-invasive		Invasive	
	n	%	*n*	%	*n*	%	*n*	%
Reptiles								
Low	0	0.0	1	3.3	0	0.0	1	3.3
Medium	15	50.0	2	6.7	15	50.0	2	6.7
High	5	16.7	7	23.3	5	16.7	7	23.3
Amphibians								
Low	1	4.2	0	0.0	12	50.0	0	0.0
Medium	11	45.8	0	0.0	5	20.8	7	29.2
High	5	20.8	7	29.2	17	70.8	7	29.2
Freshwater fishes								
Low	43	16.6	2	0.8	50	19.3	3	1.2
Medium	81	31.3	13	5.0	69	26.6	18	6.9
High	42	16.2	78	30.1	47	18.1	72	27.8
Brackish fishes								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	9	52.9	3	17.6	8	47.1	3	17.6
High	0	0.0	5	29.4	1	5.9	5	29.4
Marine fishes								
Low	36	28.3	1	0.8	33	26.0	1	0.8
Medium	46	36.2	1	0.8	54	42.5	4	3.1
High	27	21.3	16	12.6	22	17.3	13	10.2
Tunicates								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	7	31.8	2	9.1	11	50.0	6	27.3
High	5	22.7	8	36.4	1	4.5	4	18.2
Freshwater invertebrates								
Low	14	9.7	0	0.0	1	0.7	0	0.0
Medium	85	59.0	6	4.2	112	77.8	9	6.3
High	19	13.2	20	13.9	5	3.5	17	11.8
Brackish invertebrates								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	1	9.1	1	9.1	2	18.2	3	27.3
High	1	9.1	8	72.7	0	0.0	6	54.5
Marine invertebrates								
Low	15	9.9	0	0.0	16	10.6	0	0.0
Medium	57	37.7	8	5.3	53	35.1	10	6.6
High	20	13.2	51	33.8	23	15.2	49	32.5
Freshwater plants								
Low	1	5.6%	0	0.0%	4	22.2%	1	5.6%
Medium	5	27.8%	2	11.1%	2	11.1%	4	22.2%
High	0	0.0%	10	55.6%	0	0.0%	7	38.9%
Marine plants								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	7	46.7	4	26.7	6	40.0	5	33.3
High	1	6.7	3	20.0	2	13.3	2	13.3
Marine protists								
Low	0	0.0	0	0.0	0	0.0	0	0.0
Medium	4	22.2	4	22.2	3	16.7	2	11.1
High	7	38.9	3	16.7	8	44.4	5	27.8

4.2. Risk outcomes under current and future climate conditions

The high proportion of positive 'delta' values (i.e. after accounting for climate change predictions: Fig. 3c) is in line with findings that climate change is likely to exacerbate the risk of introduction, establishment, dispersal and impact of several NNS, though some species might respond negatively to changes in climate conditions (Kernan, 2015). With predicted warmer temperatures, reduced lake ice cover, altered flow regimes, increased salinity due to changes in precipitation and saltwater intrusion, and increased environmental disturbances, climate change is likely to favour the spread of NNS along their invasion pathways as these conditions present opportunities for enhanced survival and lower invasion resistance of the invaded habitats (Rahel and Olden, 2008). Further, climate change may result in altered transport and/or introduction mechanisms or reduced effectiveness of control strategies (Hellmann et al., 2008).

Several of the top (i.e. 'very high risk') species under current climate conditions achieved an even higher score under conditions of climate change (Fig. 4). Of these species, most are either primarily warm-water/tropical or have wide thermal tolerances. For example, the red-eared slider *Trachemys scripta scripta* is a very common semi-aquatic turtle native to the south-eastern USA (Florida to south-eastern Virginia: Powel et al., 1991) and in its introduced range occurs in a wide variety of habitats, including slow-flowing rivers, floodplain swamps, marshes, seasonal wetlands and permanent ponds (Scriber et al., 1986). Both goldfish *Carassius auratus* and gibel carp *Carassius gibelio* are known to establish across a wide geographical and climatic range. These species are widespread and locally invasive both in Europe's more northerly parts, such as Finland and Poland (e.g. Grabowska et al., 2010; Puntila et al., 2013), across the Mediterranean region (e.g. Crivelli, 1995; Tarkan et al., 2012), and further afield in Australia (e.g. Beatty et al., 2017) and the Americas (Magalhães and Jacobi, 2013; Halas et al., 2018). The common lionfish *Pterois miles* is another highly invasive species, especially since its invasion of the western Atlantic and Mediterranean Sea, which has been unprecedentedly rapid (Bariche et al., 2017; Schofield, 2010). The channelled applesnail *Pomacea canaliculata* is native to South America and has been introduced as an ornamental species in Europe and the Mediterranean area, but also elsewhere in the world through aquaculture (https://www.cabi.org/isc/datasheet/68490). *Phyllorhiza punctata* is native to the tropical Western Pacific, i.e. from Australia to Japan (Rippingale and Kelly, 1995) and has been reported across the Mediterranean Region (Abed-Navandi and Kikinger, 2007; Boero et al., 2009; Çevik et al., 2011; Gueroun et al., 2014; Deidun et al., 2017). An in-depth, species-specific description of all the 'very high risk' species identified in this study (Fig. 4) is provided in the Supplementary data, Appendix A1.

Overall, the responses to climate-change questions tended to increase scores as well as thresholds for most taxonomic groups in most climatic regions. Species with broad distributions tended to possess broad thermal tolerances, suggesting that those species are likely to be able to expand their ranges, and thus impacts, poleward under future climate conditions. For example, six freshwater fish species not native to Great Britain were predicted to benefit from the forecasted future climate conditions, thus offering the potential to expand their ranges (Britton et al., 2010), which in Great Britain would be northward. Such poleward shifts in marine species distributions were initially deemed likely (VanDerWal et al., 2013), based in part on shifts observed in previous warm periods (Drinkwater, 2006), and they have been predicted for some freshwater fishes, e.g. channel catfish *Ictalurus punctatus* (McCauley and Beitinger, 1992). Indeed, poleward shifts have been documented in a recent meta-analysis of marine species distributions (Chaudhary et al., 2021). As such, tropical species are likely to expand poleward into temperate regions (e.g. Quero, 1998; Scavia et al., 2002), and temperate species to expand poleward into continental regions (Root et al., 2003; Hickling et al., 2006). Whereas, species with more limited thermal tolerances are likely to undergo shifts in their distributions (and thus impacts), which are characterised by range recessions and range expansions in a poleward direction (Roessig et al., 2004; Rahel and Olden, 2008; Eissa and Zaki, 2011; Renaud et al., 2012). Wide thermal tolerances may be enhanced by local adaptation, such as is apparent in the cold-adapted population of eastern mosquitofish *Gambusia holbrooki*, which has established a self-sustaining population in Normandy, France (Beaudouin et al., 2008). This reflects the wide geographical distribution of *Gambusia holbrooki* in its native range, which

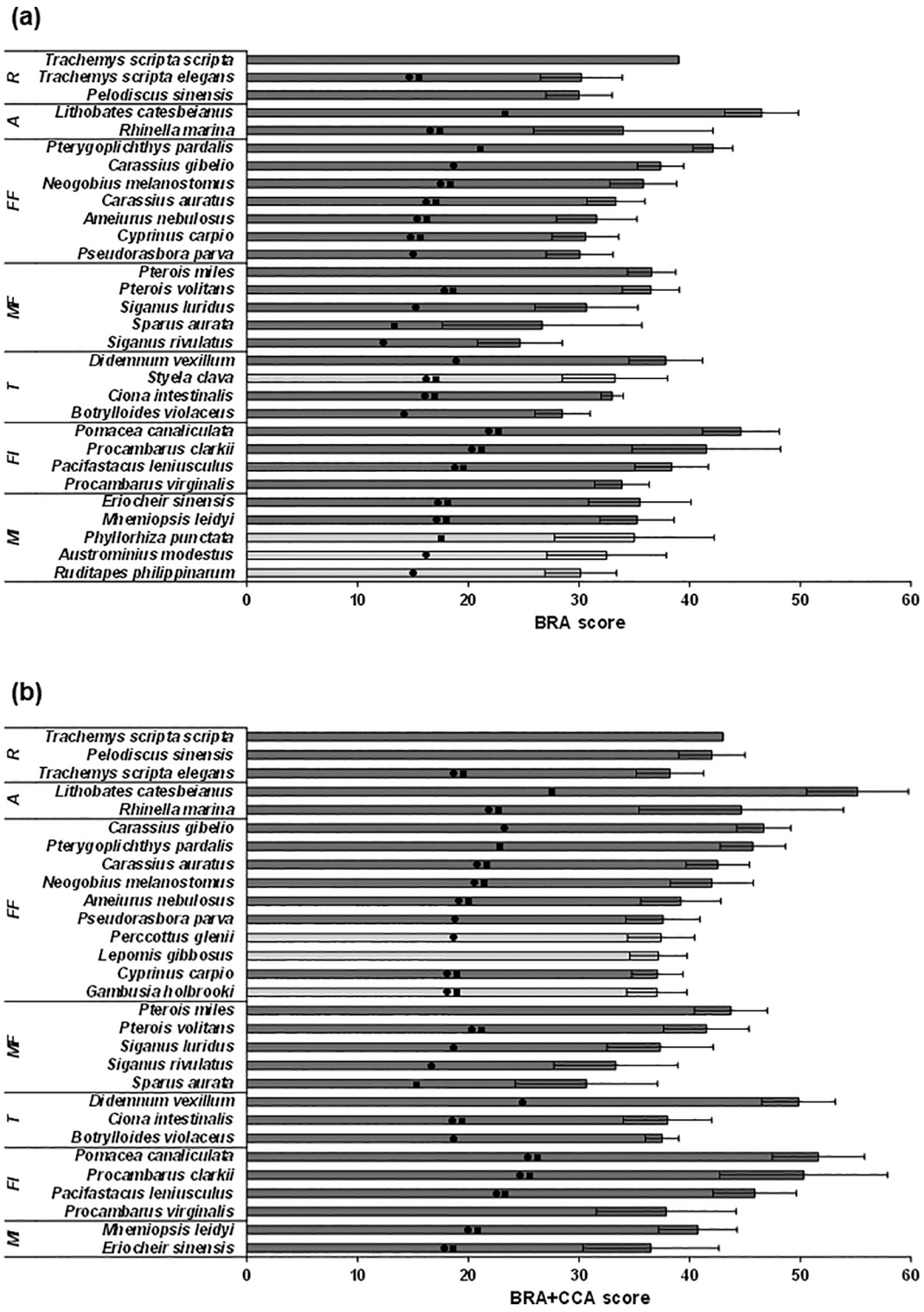

Fig. 4. (a) Very high risk species based on the BRA score (±SE) and screened for a representative number of risk assessment areas; (b) Same for the BRA + CCA. In dark gray, very high risk species for both the BRA and BRA + CCA. Black circle (•): listing in the Centre for Agriculture and Bioscience International Invasive Species Compendium (CABI ISC); black square (▪): listing in the Global Invasive Species Database (GISD). R = Reptiles; A = Amphibians; FF = Freshwater fishes; MF = Marine fishes; T = Tunicates; FI = Freshwater invertebrates; MI = Marine invertebrates. Within each aquatic organismal group, species are ordered according to decreasing score. See Table 2 for very high risk thresholds and number of risk assessment areas criteria.

extends along the Mississippi River basin from the Gulf of Mexico northward to midway up the states of Indiana and Illinois (Aislabie et al., 2019).

Thus, in many cases, increased scores for BRA + CCA for freshwater species are warranted. Further, this phenomenon, which is based on the interaction of climate and physiology, should pertain also to species in different aquatic environments such as brackish and marine systems. For example, red lionfish *Pterois volitans* scores in Florida (USA) increased slightly from the BRA to the BRA + CCA, with modest increases mainly due to a greater potential for the species to survive inshore in the northern Gulf of Mexico during winter, thereby extending annual impacts (Lyons et al., 2020). Conversely, within tropical climate zones, warmer conditions under climate-change scenarios are not likely to

Table 5
BRA and BRA + CCA thresholds from receiver characteristic curve analysis for freshwater fishes relative to three climate classes (after Peel et al., 2007) and for marine fishes and invertebrates relative to two marine ecoregion groupings (modified after Spalding et al., 2007). Abbreviations and notation as in Table 2.

Climate/marine ecoregion	*n*	BRA				BRA + CCA			
		Thr	Mean	LCI	UCI	Thr	Mean	LCI	UCI
Freshwater fishes									
Tropical	63	18.4	0.9153	0.8370	0.9936	19.6	0.8986	0.8088	0.9885
Temperate	200	15.9	0.8685	0.8197	0.9174	16.0	0.8493	0.7974	0.9012
Continental	58	12.9	0.7844	0.6579	0.9110	13	0.7387	0.5992	0.8782
Marine fishes									
Temperate	46	19.5	0.8083	0.6422	0.9745	31.5	0.8208	0.6589	0.9828
Tropical	83	12.5	0.8521	0.6994	1.0000	23.4	0.8016	0.6390	0.9643
Marine invertebrates									
Temperate	97	15.1	0.9236	0.8643	0.9829	15.6	0.8871	0.8153	0.9588
Tropical	63	35.75	0.7386	0.6085	0.8688	23.25	0.7279	0.5895	0.8662

incite large alterations to the spread, abundance, or impacts of tropical species. The same can be said for continental climate zones with regard to cold-water species due to their existing adaptations to that climate types. Whereas range recession of a species' distribution could occur should a future temperature regime exceed the species' thermal tolerances (Scavia et al., 2002).

Despite the general emphasis on range expansion and greater impacts of invasive species due to warmer conditions (Rahel and Olden, 2008; Bradley et al., 2010), climate change is a complex issue for NNS risk assessment. When providing responses to CCA questions and ranking their confidence in those responses, the assessor must consider a great breadth of information, including climate-match model predictions (e.g. Britton et al., 2010), if available for their RA area, as well as emissions scenarios, climate-model outputs, and time frames (Kennedy, 1990). The IPCC (2014) presents a variety of scenarios based on future emissions levels, with the extremes represented by RCP8.5 and RCP2.6. Further, there are numerous climate models that may be used for guidance, though these may differ in profound ways from one another in terms of predicted future temperature and precipitation regimes (e.g. Kirtman et al., 2017). Other anomalies such as Artic warming, which are expected to lead to harsh, cold winters in mid-latitude areas of North America and Asia (Cohen et al., 2014; Kug et al., 2015), or non-analogue climates (Fitzpatrick and Hargrove, 2009) occur. Extreme events can set back or cancel species range expansion (Canning-Clode et al., 2011; Rehage et al., 2016) and thus may influence future risk estimates. Multi-directional range shifts are not only possible, but likely (VanDerWal et al., 2013). The time frame for such predictions is an important variable as well, given that the potential outcomes of range expansion, contraction, or oscillation in size are relative to current NNS ranges. In view of the complexity of climate change interactions with biologically important factors such as physiology, dispersal, demography, species interactions, and evolution, not all changes in climate may result in greater spread or heighten NNS impacts (Urban et al., 2016).

4.3. Management implications

The very low proportion and, in most cases, near or total absence of false negatives across the representative groups of aquatic organisms screened in the present study is an indicator of the accuracy of the risk screenings (cf. Kumschick and Richardson, 2013). The management consequences of this elevated accuracy could be that of a large number

Table 6
Accuracy measures for screenings on freshwater fishes relative to three climate classes and for marine fishes and invertebrates relative to two marine ecoregion groupings (see Table 5). Abbreviations and notation as in Table 3.

Climate/marine ecoregion	BRA			BRA + CCA		
	A_i	A_n	A_0	A_i	A_n	A_0
Freshwater fishes						
Tropical	84.6	94.6	90.5	84.6	89.2	87.3
Temperate	82.5	75.0	78.0	87.5	64.2	73.5
Continental	81.1	66.7	75.9	83.8	57.1	74.1
Marine fishes						
Temperate	80.0	77.8	78.3	80.0	83.3	82.6
Tropical	91.7	81.7	83.1	66.7	88.7	85.5
Marine invertebrates						
Temperate	88.5	90.1	89.7	84.6	85.9	85.6
Tropical	38.6	100.0	57.1	65.9	78.9	69.8

Table 7
Risk outcomes (given as number of screened species and corresponding percentage) for the BRA and BRA + CCA for freshwater fishes relative to three climate classes and for marine fishes and invertebrates relative to two marine ecoregion groupings (see Table 5). See Table 4 for details.

Climate/marine ecoregion	BRA				BRA + CCA			
	Non-invasive		Invasive		Non-invasive		Invasive	
	n	%	*n*	%	*n*	%	*n*	%
Freshwater fishes								
Tropical								
Low	10	15.9	1	1.6	8	12.7	1	1.6
Medium	25	39.7	3	4.8	25	39.7	3	4.8
High	2	3.2	22	34.9	4	6.3	22	34.9
Temperate								
Low	31	15.5	0	0.0	38	19.0	1	0.5
Medium	59	29.5	14	7.0	39	19.5	9	4.5
High	30	15.0	66	33.0	43	21.5	70	35.0
Continental								
Low	8	13.8	0	0.0	8	13.8	0	0.0
Medium	6	10.3	7	12.1	4	6.9	6	10.3
High	7	12.1	30	51.7	9	15.5	31	53.4
Marine fishes								
Temperate								
Low	1	2.2	0	0.0	1	2.2	0	0.0
Medium	27	58.7	2	4.3	29	63.0	2	4.3
High	8	17.4	8	17.4	6	13.0	8	17.4
Tropical								
Low	33	39.8	1	1.2	31	37.3	1	1.2
Medium	25	30.1	0	0.0	32	38.6	3	3.6
High	13	15.7	11	13.3	8	9.6	8	9.6
Marine invertebrates								
Temperate								
Low	15	15.5	0	0.0	12	12.4	0	0.0
Medium	49	50.5	3	3.1	49	50.5	4	4.1
High	7	7.2	23	23.7	10	10.3	22	22.7
Tropical								
Low	0	0.0	0	0.0	4	6.3	1	1.6
Medium	19	30.2	27	42.9	11	17.5	14	22.2
High	0	0.0	17	27.0	4	6.3	29	46.0

of species ultimately warranting comprehensive (full) RA. In this study, accuracy was measured explicitly and represented the 'pragmatic' approach, given that a full (comprehensive) RA, which might follow for species identified as potentially posing a high risk of invasiveness, would normally involve a major economic commitment. This is contrary to the 'idealistic' approach, which would involve assessing both medium- and high-risk species, given the even higher economic commitment of accounting for both. An even more pragmatic approach could be therefore to base management decisions (or species' rankings) on the risk screening outcomes until such time that a full RA can be undertaken. This approach has been employed by the UK's Alien Species Group in its 'impact' ranking of aquatic NNS with regard to waterbody classification under the EU Water Framework Directive (https://ec.europa.eu/environment/water/water-framework/index_en.html). Within this context, aquatic NNS are listed provisionally as being of low, medium or high impact (UK-TAG ASG, 2021) pending the outcome of a full, and in some cases rapid, RA commissioned by the Great Britain Non-native Species Secretariat (www.nonnativespecies.org). The categorisation of the species is then subsequently confirmed or changed according to the outcome of the full or rapid RA.

As shown in the present study, invasive and non-invasive species could be distinguished accurately across aquatic organisms to a greater degree than would be expected by chance alone. Calibrated thresholds could be computed for several taxonomic groups, and for freshwater and marine fishes and for marine invertebrates also based on climate/ecoregion. In RA areas for which no calibration is possible, e.g. due to a statistically insufficient number of screenings and/or to the requirement of screening only one target species (or small group thereof), these generalised thresholds (i.e. at the organism group or climate class level) can be reliably used in future risk screening applications for distinguishing between species that pose a high risk of being invasive from those posing a low-to-medium risk. Use of these thresholds may be therefore of particular relevance in cases of individual species being risk screened (e.g. Castellanos-Galindo et al., 2018; Zięba et al., 2020), including 'rapid risk assessment' studies, or for RA areas where the number of NNS is too limited for a valid calibration to be undertaken (e.g. Filiz et al., 2017b; Paganelli et al., 2018; Semenchenko et al., 2018; Dodd et al., 2019; Lyons et al., 2020).

As is common in NNS risk analysis (e.g. Caley et al., 2006; Barry et al., 2008), the available scientific information (both peer-reviewed and gray literature) about the species being screened was reflected in the confidence rankings assessors attributed to their responses. As such, given the robust confidence levels, the present study provides a means for existing risk rankings to be adjusted and a stronger evidence base to identify: (i) which species require an immediate 'rapid' management action (e.g. eradication, control) to avoid or mitigate adverse impacts; (ii) which to subject to a full RA; and (iii) which to restrict or ban with regard to importation and/or sale as ornamental or aquarium/fishery enhancement. To this end, biological monitoring programmes may explicitly search for high-scoring species because the sooner these species are found in new areas the more likely are eradication programmes to be successful. Such monitoring could be supported by developing techniques such as DNA metabarcoding (Brown et al., 2016) and eDNA surveys (Holman et al., 2019). Risk identification therefore plays an important role in the provision of advice to policy makers, for the development of appropriate legislation, and associated regulation and management pertaining to NNS. In this perspective, the present study has also provided a means of fine-tuning NNS risk analysis procedures in countries that encompass more than one climatic class by the computation of generalised thresholds. In conclusion, the present study provides a comprehensive baseline to help identify (through risk screening using AS-ISK) for management priority high risk species across a range of taxonomic groups and geographical/climatic regions, even where existing information on such species invasiveness/impact is limited.

CRediT authorship contribution statement

LV and GHC designed the concept, co-wrote and edited the manuscript; LV analysed the data, and all other authors contributed data to and participated in the composition and editing of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study is dedicated to our co-author, the late Prof. Olaf Weyl, whose sudden passing during field work in November 2020 was received by us with great sadness. This study represents a contribution to the term of reference 'd' ("Advance knowledge base to further develop indicators to evaluate the status and impact of non-indigenous species in marine environments") of the ICES working group on Introductions and Transfers of Marine Organisms. The participation of GHC was supported by the Cefas Science Excellence fund. AW and ÁF were supported by TKP2020-NKA-16 project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.147868.

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