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Research article

The impacts of alien species on river bioassessment

Maria João Feio[a,*], Janine P. Silva[a], Robert M. Hughes[b,c], Francisca C. Aguiar[d], Carlos B.M. Alves[e], Sebastian Birk[f], Marcos Callisto[g], Marden S. Linares[e], Diego R. Macedo[h], Paulo S. Pompeu[i], Wayne Robinson[j], Christian Schürings[k], Salomé F.P. Almeida[l], Pedro M. Anastácio[m], Francis O. Arimoro[n], Min Jeong Baek[o], Mirian Calderón[p], Kai Chen[q], Peter Goethals[r], Marie Anne E. Forio[r], Jon S. Harding[s], Ben J. Kefford[t], Martyn G. Kelly[u,v], Unique N. Keke[w], Mark Lintermans[x], Renato T. Martins[y], Terutaka Mori[z], Keigo Nakamura[aa], Oghenekaro N. Odume[ab], Filipe Ribeiro[ac], Renata Ruaro[a], Sónia RQ. Serra[ad], Deep Narayan Shah[ae], Masanao Sueyoshi[af], Ram Devi Tachamo-Shah[ag]

[a] *Federal Technological University of Paraná, Laboratory of Biomonitoring and Applied Ecology, Department of Chemistry and Biology. Rua Deputado Heitor Alencar Furtado, 5000, 81280-340, Curitiba, PR, Brazil*
[b] *Amnis Opes Institute, Corvallis, OR, USA*
[c] *Oregon State University, Department of Fisheries, Wildlife, & Conservation Sciences, Corvallis, OR, USA*
[d] *Forest Research Centre, Associate Laboratory TERRA, School of Agriculture, University of Lisbon, Portugal*
[e] *Laboratório Nuvelhas, Projeto Manuelzão, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*
[f] *Department of Aquatic Ecology, Faculty of Biology, University of Duisburg-Essen, Germany*
[g] *Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Genética, Ecologia e Evolução, Belo Horizonte, MG, Brazil*
[h] *Universidade Federal de Minas Gerais, Instituto de Geociências, Departamento de Geografia, Belo Horizonte, Brazil*
[i] *Universidade Federal de Lavras, Departamento de Ecologia e Conservação. Lavras, MG, Brazil*
[j] *Gulbali Institute, Australia*
[k] *Department of Aquatic Ecology, Faculty of Biology, University of Duisburg-Essen, Universitätsstrasse 5, D-45141, Essen, Germany*
[l] *University of Aveiro, Campus de Santiago, Biology Department and GeoBioTec Research Centre, 3810-193, Aveiro, Portugal*
[m] *MARE – Marine and Environmental Sciences Centre/ ARNET—Aquatic Research Network, University of Évora, Portugal*
[n] *Department of Animal Biology, Federal University of Technology, P.M.B. 65, Minna, Nigeria*
[o] *National Institute of Biological Resources, South Korea*
[p] *INQUISAL-CONICET, Facultad de Química, Bioquímica y Farmacia, UNSL, Chacabuco, San Luis, Argentina*
[q] *School of Marine Biology and Aquaculture, & State Key Laboratory of Marine Resource Utilization in South China Sea, Hainan University, China*
[r] *Department Animal Sciences and Aquatic Ecology, Ghent University, Gent, Belgium*
[s] *School of Biological Sciences, University of Canterbury, Christchurch, New Zealand*
[t] *Centre for Applied Water Science, Institute for Applied Ecology University of Canberra, Australia*
[u] *Bowburn Consultancy, 11 Monteigne Drive, Bowburn, Durham, DH6 5QB, UK*
[v] *School of Geography, University of Nottingham, Nottingham, UK*
[w] *Department of Animal Biology, Federal University of Technology, Minna, Nigeria*
[x] *Centre for Applied Water Science, Institute for Applied Ecology, University of Canberra, Australia*
[y] *Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil*
[z] *Aqua Restoration Research Center, Public Works Research Institute, Kawashimakasada, Kakamigahara, Gifu, Japan*
[aa] *Japan Riverfront Research Center, Tokyo, Japan*
[ab] *Institute for Water Research, Rhodes University, P.O. Box 94, Makhanda, 6140, South Africa*
[ac] *MARE—Marine and Environmental Sciences Centre/ARNET—Aquatic Research Network, Faculty of Sciences, University of Lisbon, 1749-016, Lisbon, Portugal*

* Corresponding author.

E-mail addresses: mjf@ci.uc.pt (M.J. Feio), janine.silva@uc.pt (J.P. Silva), hughes.bob@amnisopes.com (R.M. Hughes), fraguiar@isa.ulisboa.pt (F.C. Aguiar), cbmalves@ufmg.br (C.B.M. Alves), sebastian.birk@uni-due.de (S. Birk), mcallisto13@gmail.com (M. Callisto), mslinares@ufmg.br (M.S. Linares), diegorm@ufmg.br (D.R. Macedo), pompeu@ufla.br (P.S. Pompeu), wrobinson@csu.edu.au (W. Robinson), christian.schuerings@uni-due.de (C. Schürings), salmeida@ua.pt (S.F.P. Almeida), anast@uevora.pt (P.M. Anastácio), f.arimoro@futminna.edu.ng (F.O. Arimoro), whitechiro@korea.kr (M.J. Baek), mrc_cali@yahoo.com.ar (M. Calderón), kaichen@hainanu.edu.cn (K. Chen), Peter.Goethals@ugent.be (P. Goethals), Marie.Forio@ugent.be (M.A.E. Forio), jon.harding@canterbury.ac.nz (J.S. Harding), Ben.kefford@canberra.edu.au (B.J. Kefford), MGKelly@bowburn-consultancy.co.uk (M.G. Kelly), n.keke@futminna.edu.ng (U.N. Keke), Mark.Lintermans@canberra.edu.au (M. Lintermans), martinsrt@gmail.com (R.T. Martins), t-mori@pwri.go.jp (T. Mori), fkmjp207@yahoo.co.jp (K. Nakamura), n.odume@ru.ac.za (O.N. Odume), fmribeiro@fc.ul.pt (F. Ribeiro), renataruaro@utfpr.edu.br (R. Ruaro), soniarqs@ci.uc.pt (S.RQ. Serra), dnshah@cdes.edu.np (D.N. Shah), m-sueyoshi55@pwri.go.jp (M. Sueyoshi).

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[ad] University of Coimbra, FCTUC/DCV, MARE - Marine and Environmental Sciences Centre/ARNET – Aquatic Research Network, Coimbra, Portugal
[ae] Central Department of Environmental Science, Tribhuvan University, Kirtipur, Nepal
[af] Aqua Restoration Research Center, Public Works Research Institute, Kakamigahara, Gifu, Japan
[ag] Department of Life Sciences, Kathmandu University, Dhulikhel, Nepal

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ABSTRACT

The extent of alien taxa impacts on river ecosystem health is unclear, but their frequency continues to rise. We investigated 1) the prevalence of including alien taxa in common bioindicators used in river bioassessment, 2) the effect of alien taxa on the richness and abundance of natives, and 3) whether including alien taxa in bioassessment tools increased their sensitivity to river degradation. In the 17 countries analyzed fish represented the greatest number of alien species (1726), followed by macrophytes (925), macroinvertebrates (556), and diatoms (7). Yet, alien species are only distinguished from natives in some fish and macrophyte indices. In addition, the analyses of 8 databases with fish, macroinvertebrate, or macrophyte data showed that abundance of alien taxa was associated with different stressors and pressures resulting in river degradation, and had a significant effect on native community composition. When alien species were accounted for, there was a strong negative correlation between the values of a fish index with alien richness and abundance while when alien taxa was not or only partially considered the results varied. Thus, we recommend: 1) Include specific metrics for alien species in biological quality indices. 2) Increase the investigation of alien taxa of small organisms (e.g. diatoms, small benthic invertebrates). 3) Eliminate sites with confirmed biological invasions for use as reference sites. 4) Remove alien from calculations of total richness and diversity. 5) Identify to the species level in biomonitoring programs. 6) Avoid legislation and management that protect alien species. 7) Encourage behaviors that prevent alien invasions of aquatic biota.

1. Introduction

Biological assessments are increasingly used globally for determining ecological status and trends in freshwater ecosystems (Dolédec and Statzner, 2010; Hering et al., 2004; Pont et al., 2006) with the goal of improving water body management, and conservation. Assemblage-based (e.g., fish, macroinvertebrates, macrophytes, diatoms) indices are widely used and effective tools for assessing those ecological conditions across large spatial extents (Birk et al., 2012; Feio et al., 2023). This is because of their capacities to capture complex taxonomic and functional ecosystem components (Karr et al., 2022; Ruaro et al., 2024). However, many of those indices do not distinguish native from alien species.

Alien species occur in many riverine ecosystems worldwide and their numbers continue to rise (Gallardo et al., 2016; Gubiani et al., 2018; Seebens et al., 2017), despite being considered a major cause of degraded ecological quality in rivers (Feio et al., 2023). Alien species include those occurring outside their natural ranges, dispersed because of human activities, whether intentionally or unintentionally (Walther et al., 2009). They occur widely in North America, Europe and Australia (Eriksen et al., 2021; Haubrock et al., 2022; Soto et al., 2023) but have also spread extensively across Southeast Asia (Peh, 2010), Africa (Makoni, 2020; van Wilgen et al., 2020), and Latin America (e.g., Bando et al., 2022; Gubiani et al., 2018; Jovem-Azevêdo et al., 2022). Rivers, especially those used for navigation and with significant hydrological modifications, are corridors that facilitate the spread of alien species (Espínola et al., 2022; Leuven et al., 2009; Minchin et al., 2019; Rocha et al., 2023). Species from other regions often enter rivers from the ballast water of ships, deliberate introductions for fisheries and biological control programs, aquarium releases, and water translocations (Gubiani et al., 2018; Olden et al., 2021; Weyl et al., 2020). When alien species establish and spread, they are invasive (Pyšek et al., 2020; Soto et al., 2023). Such species may be highly disruptive, competing for habitat and resources and sometimes causing physical injuries or death of native species and degrading ecosystem services (Britton et al., 2023; Manchester and Bullock, 2000).

Several factors influence the potential for successful establishment of a alien species in a new site: environmental conditions, biological traits (e.g., size, shape, reproduction cycles, physiological tolerance to pollutants), invasion history, and interactions with native and other alien species (Crooks et al., 2011; Galván et al., 2022; Michelan et al., 2010; Moyle and Light, 1996). These factors also influence native aquatic community composition and thus biological quality assessments. This raises the issue of how to assess alien species impacts on rivers. For example, in the European Union, alien species are a potential anthropogenic impact (UKTAG, 2013). In other countries, such as South Africa and the USA, alien species are specifically assessed by some biological quality indices (e.g., Joy and Death, 2004; Kleynhans and Louw, 2008; USEPA, 2016). However, there is insufficient evidence to determine the degree to which existing river bioassessment tools detect the effect of alien on ecosystem structure and function (but see Guareschi et al., 2021; Ruaro et al., 2024, 2021, 2018). Therefore, how bioassessment sampling protocols and indices integrate alien species and how reference databases are organized (e.g., whether alien species are flagged) influence the capacity to detect effects of alien species on ecosystem health (Haubrock et al., 2024; Kleynhans and Louw, 2008; Ruaro et al., 2018, 2021).

Moreover, the relevance of alien species depends on the biological groups used for bioassessment, their abundance and proportion to native species, risk of spreading and geographical region. Seebens et al. (2017) offered comprehensive global information about alien species but did not focus on the biological indicators used in river bioassessment. Briski et al. (2024) compiled a more comprehensive and accurate alien database and noted that freshwater invasions were less studied than terrestrial invasions. The effects of alien fish, crayfish, and molluscs on ecosystems are more evident and better studied, but the effects of other alien macroinvertebrates or most alien diatoms are largely unknown (e.g., Oliva-Paterna et al., 2021; Strayer, 2010). However, this may result from incomplete knowledge of the native ranges of those taxa, rather than the real extent of alien threats.

Therefore, we aimed to answer the following questions.

1. How prevalent are alien species among the most common bioindicator groups used in river bioassessment (e.g., macroinvertebrates, fish, macrophytes, diatoms)? We predicted that alien species would be most prevalent for fish because of deliberate widespread introductions of alien fish species.
2. Which bioassessment tools incorporate alien taxa? We predicted relatively few of those tools would explicitly include alien taxa.
3. Do existing bioassessment tools detect the effects of alien species? We predicted few of those tools would detect the effects of alien species.

4. Does the inclusion of alien metrics in bioassessment tools result in greater sensitivity to the general degradation of biological condition of rivers? We predicted that including alien would improve bioassessment sensitivity.

By answering these questions, we should be able to determine the degree to which existing river bioassessment methods need to be improved to detect alien species impacts on riverine ecosystems.

2. Methods

2.1. Biological data and indices

To answer questions 1 and 2, we used a two-step approach. i) We gathered information from local freshwater ecologists (co-authors), including their own datasets, national databases, unpublished studies and reports, and databases/studies published in non-English languages. ii) We complemented those data via searches in the Web of Science, Google Scholar, and other online-databases. Keywords used in the searches included, among others: river bioassessment, indices, macroinvertebrates, fish, macrophytes, microalgae, diatoms, alien species, exotic species, invasive alien species, freshwater, rivers, streams, lotic systems. Our review focused on 17 countries in Europe, Africa, Asia, North, Central, and South America, and Oceania, where the co-authors work. The regions cover a wide degree of environmental condtions and different experiences in river-bioassessment and alien species knowledge (Fig. 1). With the information collected we determined: 1) the number of alien species reported (including translocated species from other catchments in the same country; Tarkan et al., 2024); 2) the most detrimental alien species in each nation; and 3) whether the bioassessment indices used in each country included alien species.

To answer questions 3 and 4, we analyzed 8 bioassessment datasets and indices from 6 countries: 4 for fish, 3 for macroinvertebrates, and 1 for macrophytes (Table 1). Those datasets were selected because they were spatially extensive, had comprehensive site-specific biological data (data and biological index scores), and had co-authors familiar with the data, data analyses, and the field work used in generating those data. The datasets contained information on taxonomic composition and abundance of each taxa per site, and respective scores of biological quality indices and metrics used in each country for each biological element. For some countries the sites are located in different ecoregions or are grouped by river type (see Table 2). The river types have different

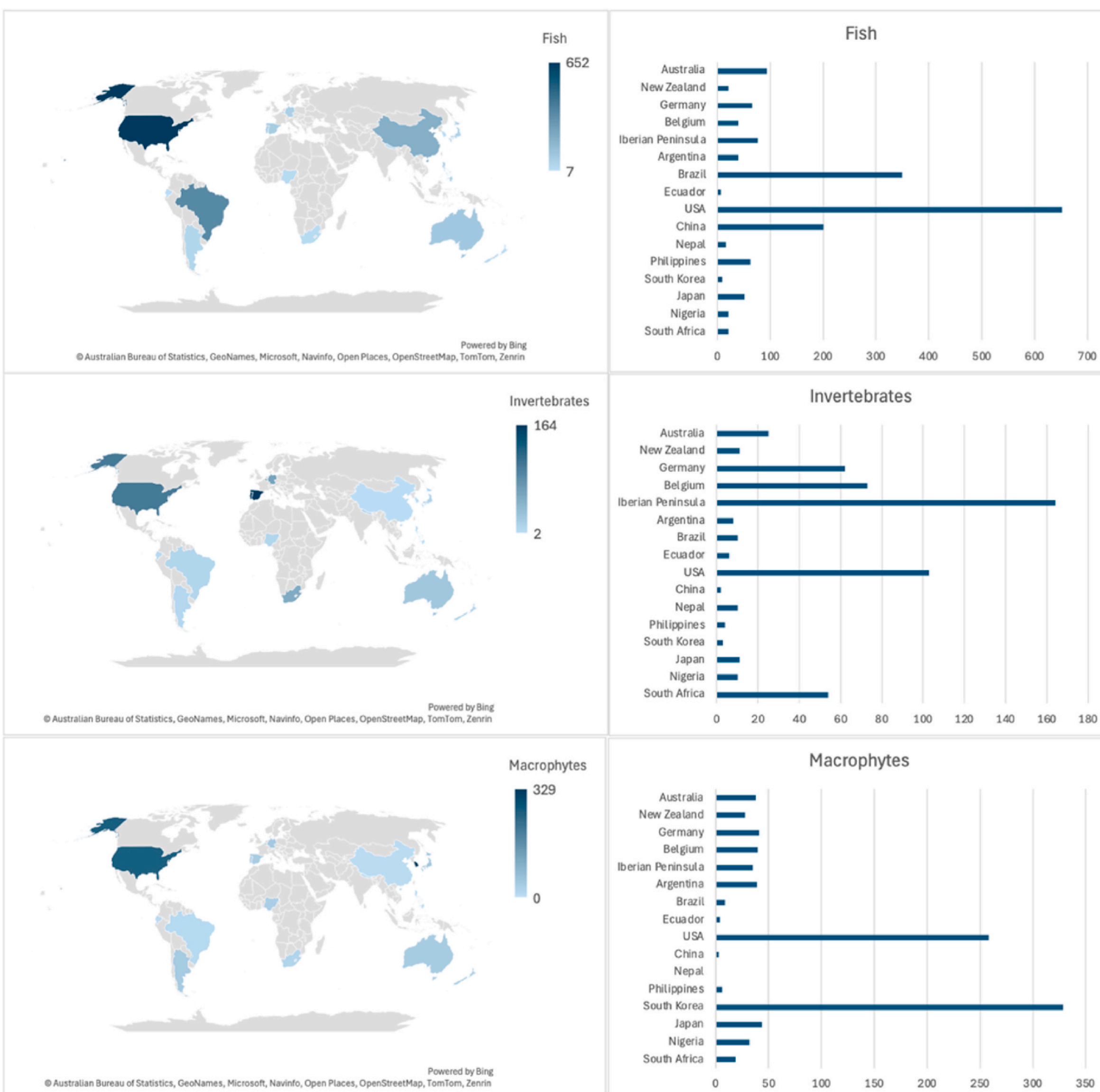

Fig. 1. Number of alien (including translocated) species extracted for the 17 countries included in our review.

Table 1
Description of the selected databases used for data analyses. RCA: method based on the Reference Condition Approach (i.e., boundaries adjusted to the reference conditions for that region/river type/environmental characteristics). Index type: multimetric index (MMI), predictive taxa richness model (PM), biotic index (BI, based on organic pollution tolerance), other (IBMR: Macrophyte Biological Index for Rivers). Alien included: the index includes a specific metric accounting for alien taxa.

Database	RCA	Sampling Method	Index type	Alien included	River types/Ecoregions	Sites	Native taxa	Alien taxa	Years
Fish Australia	–	electrofishing	PM	no	3	1510	28	13	2004–2013
Fish Brazil	x	hand net	MMI	partly	2	176	56–92	2–8	2009–2012
Invertebrates Brazil	x	kick-Net	MMI	no	4	44	89	2	2010–2019
Invertebrates Germany	x	hand net	MMI	no	7	430	936	40	2010–2019
Fish Portugal	x	electrofishing	MMI	yes	11	227	31	12	2019
Invertebrates Portugal	x	hand net	MMI	no	12	378	130	6	2019
Macrophytes Portugal	x	cover estimate	IBMR	partly	2	42	72	7	2004
Fish USA	–	electrofishing	MMI	yes	1	26	17	7	1983

Table 2
Spearman correlations (r_s) between alien taxa richness and abundance and the biological quality indices of the study sites from 8 databases for fish, macroinvertebrates, and macrophytes. *$p < 0.5$; **$p < 0.01$; ***$p < 0.001$.

Database	Alien richness	Alien abundance
Fish Australia	0.15***	0.09***
Fish Brazil	0.32***	−0.25**
Fish Portugal	−0.69***	−0.74***
Fish USA	−0.63***	−0.65***
Invertebrates Brazil	0.01	0.03
Invertebrates Germany	−0.16**	−0.15**
Invertebrates Portugal	−0.18***	−0.15***
Macrophytes Portugal	−0.2	−0.19

environmental conditions (e.g., climate, geology, catchment size), according to the European Water Framework Directive (EC, 2000).

Fish. We used 4 fish datasets. 1) **Australia:** 1449 sites from three ecoregions in the Murray Darling Basin, containing records of 28 native species and 13 alien, sampled between 2004 and 2013. Index scores were calculated by the number of collected species divided by the number of endemic species expected (by expert judgment) to be caught in a given sampling site, and did not include a specific metric for alien species. 2) **Brazil:** 155 sites from the Paraná River basin and 39 sites from the São Francisco River basin, sampled between 2009 and 2012, which contained records of 92 and 56 native species and 2 and 8 alien, respectively. The multimetric index used combined 6 metrics (% common species; % characiform individuals; % loricariid individuals; % trichomycterid individuals; % invertivore species, and % *Poecilia reticulata* individuals - an alien species) (de Carvalho et al., 2017). 3) **Portugal:** 227 sites from 11 river types sampled in 2019, containing records for 31 native species and 12 alien. The index used was the Fish Index of Biotic Integrity for Wadeable Rivers of Continental Portugal (F-IBIP; INAG, 2012; Oliveira et al., 2010). The F-IBIP includes the number of native species, % alien individuals, and feeding and reproduction guilds. 4) **USA**: 26 sites sampled in the Willamette River, Oregon, in 1983. In this river, 17 native and 7 alien species were captured (Hughes and Gammon, 1987). Their multimetric index included 2 alien metrics (% Common Carp individuals; % alien individuals) as well as metrics considering only native species richness and native cyprinid species richness.

Macroinvertebrates. Three datasets were used. 1) **Brazil**: 44 sites from Minas Gerais, comprising four catchments sampled between 2010 and 2019, which included 89 native and 2 alien taxa (Silva et al., 2017). The multimetric index was based on Ephemeroptera richness, % Gastropoda individuals, Shannon-Wiener diversity, % sensitive taxa richness, % scraper individuals, temporarily attached taxa richness, and gill respiration taxa richness. 2) **Germany:** 430 sites from 7 river types sampled between 2010 and 2019, containing records of 936 native and 40 alien taxa. The multimetric index combined several biodiversity-related indices (Hering et al., 2006). 3) **Portugal:** 378 sites representing 12 river types sampled in 2019, which included 130 native taxa and 6 alien. The multimetric index metrics described the number of families, taxonomic evenness (J'), the occurrence of sensitive taxa (Ephemeroptera, Plecoptera, Trichoptera), ASPT (Average Score Per Taxon), and abundance of selected families (mostly representative or sensitive taxa of northern or southern regions) (Feio et al., 2014; Ferreira et al., 2008).

Macrophytes. One dataset of 42 sites sampled in 2004 from Portugal represented 2 river types where 72 native taxa and 7 alien were collected. The Macrophyte Biological Index for Rivers (IBMR) estimated the cover of macrophyte species, which only partly accounted for alien taxa.

2.2. Abiotic data

The eight biological datasets outlined above were complemented with available abiotic data for each sampling site, including different variables depending on the study region (Table S1). Consequently, we sought to harmonize variables across the datasets to improve consistency in the statistical analyses (see below). To do this, we sorted abiotic variables into two main categories. i) Stressor variables included dissolved oxygen (mg/L), pH, conductivity (μS/cm), total nitrogen (mg/l), and total phosphorus (mg/l)). ii) Pressure variables included percentage of pastureland, cropland, urban areas, and stream hydromorphology. The hydromorphological modifications were assessed through the River Habitat Survey protocol in Portugal, which results in two indices: Habitat modification Score (HMS) and Habitat Quality Assessment (HQA) (Environmental Agency, 2003). The HMS assesses the degree of modification of the channel, (e.g., artificial walls, channel, existence of barriers). The HQA measures the naturalness, quality, diversity, richness and rarity of the channel physical structure. It includes information on flow, channel substrate, channel features, bank features, bank vegetation structure, in-stream channel vegetation, and land-use (Feio et al., 2016). A Local Disturbance Index (LDI) was included as an additional variable in the analysis of the Brazilian macroinvertebrate dataset. The LDI is the equivalent of the USEPA W1_hall metric, which measures the degree of anthropogenic impacts in the riparian zone of wadeable streams (Kaufmann et al., 1999; Ligeiro et al., 2013). This metric is based on the occurrences and proximity of walls, dikes, revetments, riprap or dams, buildings, pavement or cleared lots, roads or railroads, inlet or outlet pipes, landfills or trash, parks or maintained lawns, row crops, pastures, rangelands, hay fields, or evidence of livestock, logging, and mining in the site riparian zone. It ranges from 0 (undisturbed area without anthropogenic disturbances) to 7 (riparian zone with intensive anthropogenic activities and suppressed riparian vegetation).

2.3. Data analyses

For comparison purposes, the proportion of native taxa abundance to total abundance (NAR), and the proportion of native taxa richness to total richness (NRR) were calculated for all databases. Spearman rank correlation (r_s) coefficients were calculated to examine the relationships between alien taxa richness and abundance with the biological quality index scores. Estimated correlation values were classified as very weak:

$r_s < 0.20$; weak: $0.20 < r_s < 0.39$; moderate: $0.40 < r_s < 0.59$; strong: $0.60 < r_s < 0.79$; and very strong: $r_s > 0.80$ (Evans, 1996). The correlation tests were performed on all available data for each dataset using the 'rstatix' package (v0.7.2; Kassambra, 2023) with a two-tailed test for statistical inference.

In addition, we conducted a Wilcoxon rank sum test with a continuity correction to compare the index scores between sites affected by alien taxa and sites not affected by alien taxa. This test was chosen because of the inability to achieve homogeneity of variance within some datasets following several transformation attempts. Wilcoxon rank sum tests were performed with the 'stats' package (v4.2.2; R Core Team, 2022). Bar plots were made with the 'ggplot2' package (v3.4.4; Wickham, 2016). To perform this test and assure balanced comparisons, 5 to 10 sites with and without alien taxa from each river type/ecoregion were randomly selected from each dataset. We chose to subsample from the total number of sites because, for some river types/ecoregions this was the maximum number of sites with alien taxa that could be compared to sites of the same river type without alien taxa. River types/ecoregions with fewer than 5 sites where alien taxa were present or absent were removed from this analysis, as these did not offer comparable information on both alien taxa-affected and unaffected areas (detailed information of the number of sites analyzed per river type/-ecoregion can be consulted on Table S2).

The association between alien taxa and the general degradation of rivers (considering their stressors, pressures) and the variation in native assemblages was explored using Canonical Correspondence Analysis (CCA) or Redundancy Analysis (RDA) (ter Braak, 1986) separately for each dataset (except for the Australia fish dataset due to the absence of abiotic data; see Table S1. Although the analyses may be different, the outputs are comparable and interpretations are similar. Specifically, a unimodal ordination method (i.e., CCA) was selected for the Brazilian, German and Portuguese datasets, and a linear ordination method (i.e., RDA) was selected for the USA dataset after a preliminary Detrended Correspondence Analysis showed a gradient length larger than 4 SD (Standard Deviation) and less than 3 SD, respectively. Partial CCA was used to examine the influence of environmental variables and alien taxa abundance independently of the influence of river type/ecoregion to account for potential variation from the natural attributes of sampling sites. Environmental variables were standardized to zero mean and unit variance to ensure comparability between variables. A stepwise forward selection of the variables was used, and river/type was included as a conditional variable. Highly correlated variables ($r_s > 0.75$) were excluded from the models. Abundance data were log(x+1) transformed to reduce the weight of very abundant taxa and increase the weight of rare taxa in the data analyses. For these analyses, we considered only the river types/ecoregions included in the Wilcoxon rank sum tests and further removed sites lacking abiotic data (see Table S2 for details). The statistical significance of the final models was tested with Monte Carlo tests under 999 permutations. CCAs and the RDA were built with the 'vegan' R package (v2.6-4; Oksanen et al., 2022).

3. Results

3.1. Alien species by biological group

Our review showed that the highest number of alien taxa reported for the 17 countries analyzed were for fish (a total of 1726 compiled here), followed by macrophytes (925), and macroinvertebrates (556) and diatoms (7) (Table S3). These numbers varied between regions, with the highest numbers of alien recorded for fish in large countries such as the USA, followed by Brazil, China and Australia; invertebrates in the Iberian Peninsula (Portugal and Spain), followed by the USA, Belgium, and Germany; and macrophytes in South Korea, followed by the USA (Table S3). There was little information on alien diatom species except for *Didymosphenia geminata*, which originated in the Northern Hemisphere and is now widespread in parts of New Zealand and South America. Several alien species can readily establish permanent populations, harmful impacts on ecosystems, or other relevant factors (Table S3).

3.2. Associations between alien species and river quality

The direction and magnitude of canonical coefficients along the axes of the Canonical Correspondence Analysis (CCA) and Redundancy Analysis (RDA) axes indicate associations between alien abundances and river degradation, including water pollution, hydromorphological alterations, and habitat degradation (Fig. 2). As expected, no single cause of degradation was associated with alien species abundance across all datasets. This may have resulted from regionally differing importance of stressors and pressures, insufficient information on all relevant stressors, and the co-occurrence of multiple pressures.

Furthermore, alien abundance showed a statistically significant negative association with native assemblage composition in most datasets, and this was generally very significant (Monte Carlo: $p < 0.001$). Specifically, in the Brazilian fish dataset, the first two axes of the CCA explained 38% of the constrained variation in taxa abundance. Axis 1 was mainly defined by alien abundance ($\lambda = 0.69$), palm swamp ($\lambda = 0.52$) and agriculture ($\lambda = 0.37$). In axis 2, the positive coefficient of alien abundance ($\lambda = 0.16$) was aligned with local disturbance ($\lambda = 0.37$) and temperature ($\lambda = 0.36$). Constrained and conditioned effects included in the model explained 17% of the total variation in fish assemblage composition. Alien abundance, palm swamp, agriculture, eucalyptus forest, and conductivity showed highly significant associations ($p < 0.001$) with assemblage composition.

The first two axes of the CCA for the Brazilian macroinvertebrate dataset explained 37% of the constrained variation, and constrained and conditioned effects included in the model explained 48% of the total variation in assemblage composition. We found a positive coefficient for alien abundance along axis 1 ($\lambda = 0.07$), which was mainly related to temperature ($\lambda = 0.53$), pH ($\lambda = 0.37$), and total nitrogen ($\lambda = 0.23$). Axis 2 was mainly defined by alien abundance ($\lambda = -0.56$), chlorophyll *a* ($\lambda = 0.38$), pH ($\lambda = 0.37$), and temperature ($\lambda = 0.35$). In this case, alien abundance, conductivity, total phosphorous, and total nitrogen showed significant statistical associations ($p < 0.05$) with assemblage composition.

In the Portuguese fish dataset, the first two axes explained 47% of the constrained variation in assemblage composition. The positive coefficient for alien abundance along the first axis was associated with total suspended solids ($\lambda = 0.58$) and biochemical oxygen demand ($\lambda = 0.54$), and negatively associated with habitat quality ($\lambda = -0.49$). Axis 2 was mainly related to ammonia ($\lambda = -0.70$), nitrite ($\lambda = -0.56$), and orthophosphate ($\lambda = -0.41$), and negatively with alien abundance ($\lambda = -0.09$). Constrained and conditioned effects explained 22% of the total variation in native assemblage composition. Alien abundance, ammonia, total suspended solids, habitat modification, and nitrate were significant variables in this model ($p < 0.05$).

The first two axes of the CCA for the Portuguese macroinvertebrate dataset explained 45% of the constrained variation, and constrained and conditioned effects explained 15% of the total variation in the data. This CCA showed a negative coefficient for alien abundance along axis 1, which was mainly defined by nitrate ($\lambda = -0.72$), nitrite ($\lambda = -0.60$), biochemical oxygen demand ($\lambda = -0.55$), orthophosphate ($\lambda = -0.54$), versus habitat quality ($\lambda = 0.53$). Axis 2 was mostly related to ammonia ($\lambda = 0.60$), nitrite ($\lambda = -0.53$), and alien abundance ($\lambda = 0.37$). Nitrate showed the strongest statistical association ($p < 0.001$) with species composition, followed by alien abundance ($p < 0.01$).

The first two axes of the CCA for the German macroinvertebrate dataset explained 35% of the constrained variation in the data. This analysis showed a positive coefficient for alien abundance along the first axis ($\lambda = 0.19$) and a negative coefficient along the second axis ($\lambda =$ -0.07). Axis 1 was mainly defined by chloride ($\lambda = 0.82$) and dissolved oxygen ($\lambda = -0.46$). Axis 2 was related to cropland ($\lambda = -0.59$), urban

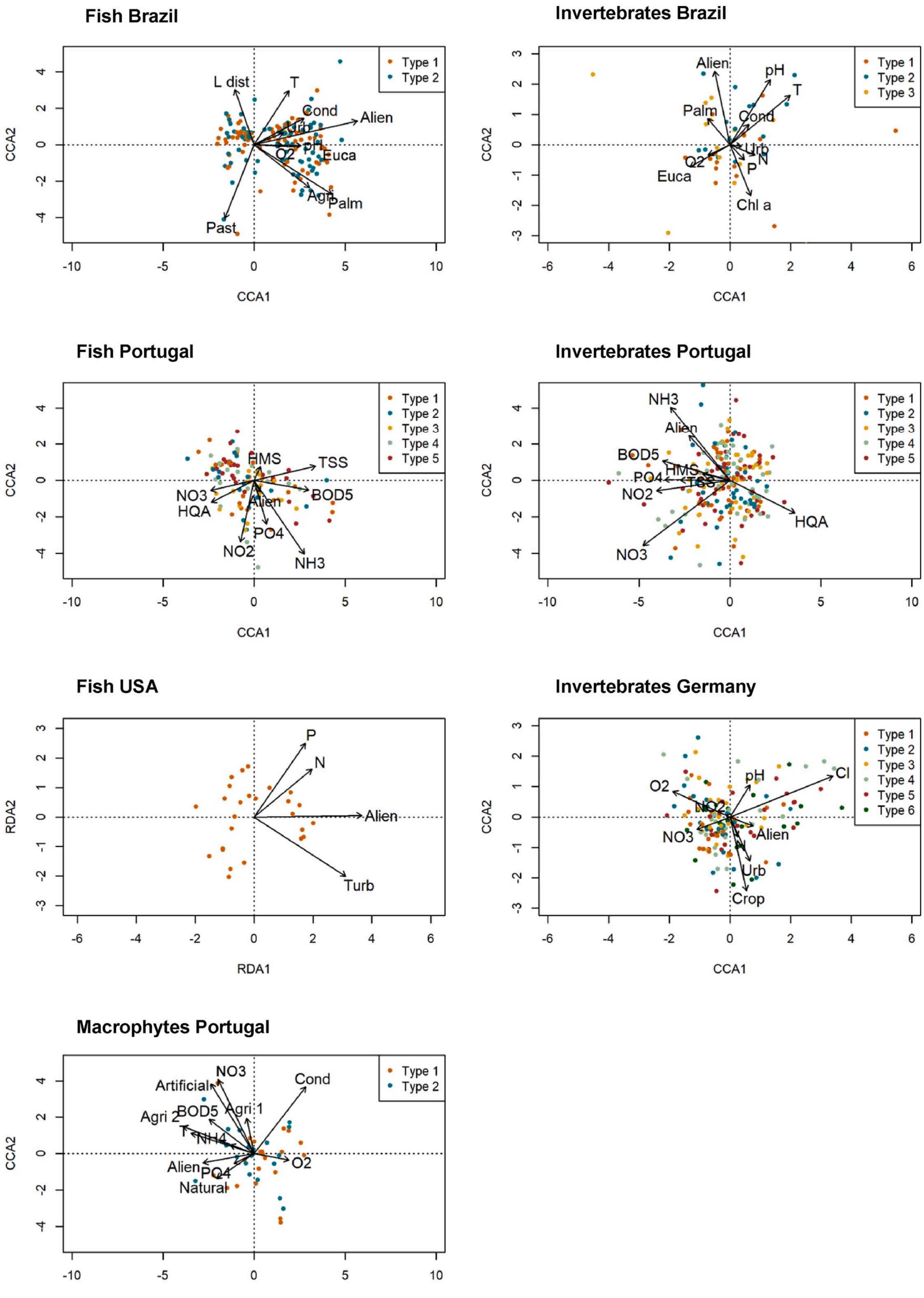

Fig. 2. Ordination diagrams of the Canonical Correspondence Analysis (Brasil, Portugal, and Germany) and Redundancy Analysis (USA) showing the influence of environmental factors and the abundance of alien taxa on native assemblages conditioned on river type/ecoregion for each database. Colored dots represent sites of each river type/ecoregion. Agri 1: Intensive agriculture in the catchment (%); Agri 2: Extensive agriculture in the catchment (%); Agri: Agriculture (%); Alien: total abundance of alien taxa; Artificial: Artificial areas in the catchment (%); BOD5: Biochemical Oxygen Demand (mg/L); Chl a: Clorophyl a (mg/L); Cl: Chloride (mg/L); Cond: Condutivity (μS/cm); Crop: Cropland (%); Euca: Eucalyptus forest (%); HMS: Habitat modification score; HQS: Habitat quality assessment score; L dist: Local Disturbance Index; N: Total nitrogen (mg/L); Natural: Natural areas in the catchment (%); NH3: Ammonia (mg/L); NH4: Ammonium (mg/L); NO2: Nitrite (mg/L); NO3: Nitrate (mg/L); O2: Dissolved oxygen (mg/L); P: Total phosphorus (mg/L); Palm: Palm swamp (%); Past: Pasture (%); PO4: Orthophosphate (mg/L); T: Temperature (°C); TSS: Total suspended solids (mg/L); Turb: Turbidity (NTU); Urb: Urban (%) Details on environmental variables are in Supplementary Table 1.

land use (λ = −0.35), and chloride (λ = 0.32). Constrained and conditioned effects explained 15.49% of the total variation in assemblage composition. Chloride, nitrite, total nitrogen, dissolved oxygen and cropland were highly significant variables in this model ($p < 0.001$).

For the Portuguese macrophyte dataset, the first two axes of the CCA explained 31% of the constrained variation, and constrained and conditioned effects explained 47% of the total variation in assemblage composition. Alien abundance showed a negative coefficient along axis 1 (λ = −0.40) and axis 2 (λ = −0.07). Axis 1 was associated with extensive agriculture (λ = −0.55), temperature (λ = −0.49), and conductivity (λ = 0.40). Axis 2 was related to nitrate (λ = 0.57), artificial areas (λ = 0.53), and conductivity (λ = 0.52). Temperature, conductivity, and extensive agriculture were the most significant variables ($p < 0.001$), although alien abundance was also statistically significant ($p < 0.05$).

In the RDA for USA fish, the first two axes explained 82% of the constrained variation in assemblage composition. The first axis was mainly associated with alien abundance ($r = 0.94$) and turbidity ($r = 0.80$), whereas the second axis was mostly defined by total phosphorus ($r = 0.64$) and turbidity ($r = 0.52$). The environmental variables included in the model explained 32% of the total variation in the species data. Alien abundance showed the strongest statistical association ($p < 0.001$) with assemblage composition.

Biological quality scores were generally lower in sites where alien taxa were present, versus those where they were absent, but the differences were often small and not statistically significant (Fig. 3; Table 3). Significant decreases were found in fish biotic indices that partly (Brazil) or fully (Portugal, USA) accounted for the effect of alien taxa, but effects were inconsistent in the German macroinvertebrate dataset (river types 2, 3, 4) that did not account for alien taxa. Conversely, increases in biotic index scores in sites affected by alien taxa were found in indices without a specific metric to account for alien taxa, namely in the macroinvertebrate datasets from Brazil (ecoregion types 1, 3, and 4), Germany (river types 5 and 6), and Portugal (river type 5), and the Australian fish dataset (all ecoregions), although none were statistically significant (Fig. 3; Table 3).

Sites with lower NAR and NRR were not accompanied by a consistent decrease in their biotic index scores (Fig. 3). Whereas NRR and NAR showed a sharp decrease from the reference value of 1 (sites lacking alien taxa), the biotic indices showed only slight or even negligible decreases. This was particularly noticeable when the biotic indices did not include a specific metric for alien taxa (e.g., macroinvertebrates from Brazil and Portugal, fish from Australia). Alternatively, and as expected, the Portuguese and USA fish biotic indices, which specifically accounted for alien taxa, showed a strong decrease in sites where alien taxa were present, broadly following the trends of NAR and NRR.

When biotic indices included a alien metric, there were strong correlations between the indices and the alien richness and abundance. Even though this was expected, we acknowledge a potential inflation of the values of these correlations due to the fact that index scores are calculated using metrics that reflect the occurrence of alien taxa (e.g., percentage of alien fish, number of alien taxa). There was a strong negative correlation between the biotic index scores and alien richness ($r_s = -0.69$, $p < 0.001$) and abundance ($r_s = -0.74$, $p < 0.001$) for the Portuguese fish dataset. Similarly, the USA fish biotic index scores were negatively correlated with alien richness ($r_s = -0.63$, $p < 0.001$) and abundance ($r_s = -0.65$, $p < 0.001$).

Results for biotic indices that partly included a metric for alien taxa were inconsistent. The Brazilian fish biotic index showed a weak positive correlation with alien richness ($r_s = 0.32$, $p < 0.001$) and a weak negative correlation with alien abundance ($r_s = -0.25$, $p < 0.01$). The Portuguese macrophyte index was not significantly correlated with alien richness ($r_s = 0.2$, $p = 0.21$) or abundance ($r_s = -0.19$, $p = 0.22$).

Correlations with biotic indices that did not account for alien taxa also showed no or weak correlations with alien taxa. For the Australian fish dataset, the biotic index was very weakly correlated with alien richness ($r_s = 0.15$, $p < 0.001$) and abundance ($r_s = 0.09$, $p = 0.001$). The Brazilian macroinvertebrate biotic index was not correlated with alien richness ($r_s = 0.01$, $p = 0.93$) nor with abundance ($r_s = -0.19$, $p = 0.83$). The German macroinvertebrate biotic index showed a very weak negative correlation with alien richness ($r_s = -0.16$, $p > 0.01$) and abundance ($r_s = -0.15$, $p = 0 < 0.01$). Lastly, the Portuguese macrophyte index showed a very weak negative correlation with alien richness ($r_s = -0.18$, $p > 0.001$) and abundance ($r_s = -0.15$, $p = 0 < 0.01$).

4. Discussion

We found that all biological indicator groups used in river monitoring have known alien species in the studied nations, with a larger number reported for fish, followed by macrophytes, then macroinvertebrates, and finally diatoms. That difference was not reported by Strayer (2010), who listed more alien macroinvertebrate species than fish in North America and Europe. The greater number of alien fish species is likely biased for five reasons. 1) There is more information for fish than for the other groups, including their historical native ranges. 2) More fish species are deliberately translocated. 3) The numbers of alien macroinvertebrate are probably underestimated in most regions, but less so for Crustacea and Mollusca, which have commercial value. 4) Taxonomic resolution is usually poorer for macroinvertebrates, resulting in alien species being grouped into an order or family. 4) The extent of alien diatoms is probably underestimated, except for the *Didymosphenia geminata* that form large mats, because of their small size, frequent nomenclature changes, misidentifications, and lack of historical databases for many regions (Padisák et al., 2016; Spaulding et., al 2010; but see Buczkó et al., 2022; Coste and Ector, 2000).

By accounting for only 17 countries, our review underestimated the numbers of alien species on those nations' continents. Yet, other studies have noted great numbers of alien species in Europe (Haubrock et al., 2023; Zamora-Marín et al., 2023), North America (Cranberg and Keller, 2023), and globally (Seebens et al., 2021), although populations of some alien taxa may be saturated in Europe (Soto et al., 2023).

For all the groups studied, invasive species are considered harmful for the maintenance of native species. Multiple negative effects in ecosystems by alien species have been reported, from heavy predation leading to extinction, alterations in river bank stability, increased water turbidity, decreased water flows, increasing siltation, and anoxic conditions (Britton, 2023; Bunn et al., 1998; Crystal-Ornelas and Lockwood, 2020; Emery-Butcher et al., 2020; Strayer, 2012). Nonetheless, not all alien species are equally damaging to aquatic ecosystems, depending on their relative invasiveness and life histories (Bolpagni, 2021; Emery-Butcher et al., 2020).

However, none of these issues are necessarily reflected in biological quality indices. Indeed, there were clear and significant decreases in fish biotic index scores only for fish indices that included metrics that partly or fully accounted for alien taxa. Guareschi et al. (2021), comparing data from before and after invasions, were able to detect the effect of an invasive species, *Dikerogammarus haemobaphes,* through a decrease in one macroinvertebrate biotic index (WHPT - an abundance-based adaptation of BMWP; UKTAG, 2023) and in functional richness and redundancy. We thus conclude that it is important to consider specifically the presence of alien species in assemblage bioassessment indices. Aparicio et al. (2023) also added a alien species metric to a Pan-European fish index. This metric, Alien Fish Pressure, consists of the proportion of alien species and the proportion of alien individuals in the sample. In our results, however, it was unclear whether alien richness or alien abundance better indicated alien species impacts. Therefore, combining both seems useful.

Our results also showed for all datasets, that the presence of alien species is often associated with other anthropogenic impacts, resulting in the overall loss of ecosystem quality. A similar association was reported by Sage (2020). This association could mean that other anthropogenic effects are favoring the spread of alien species, but could also

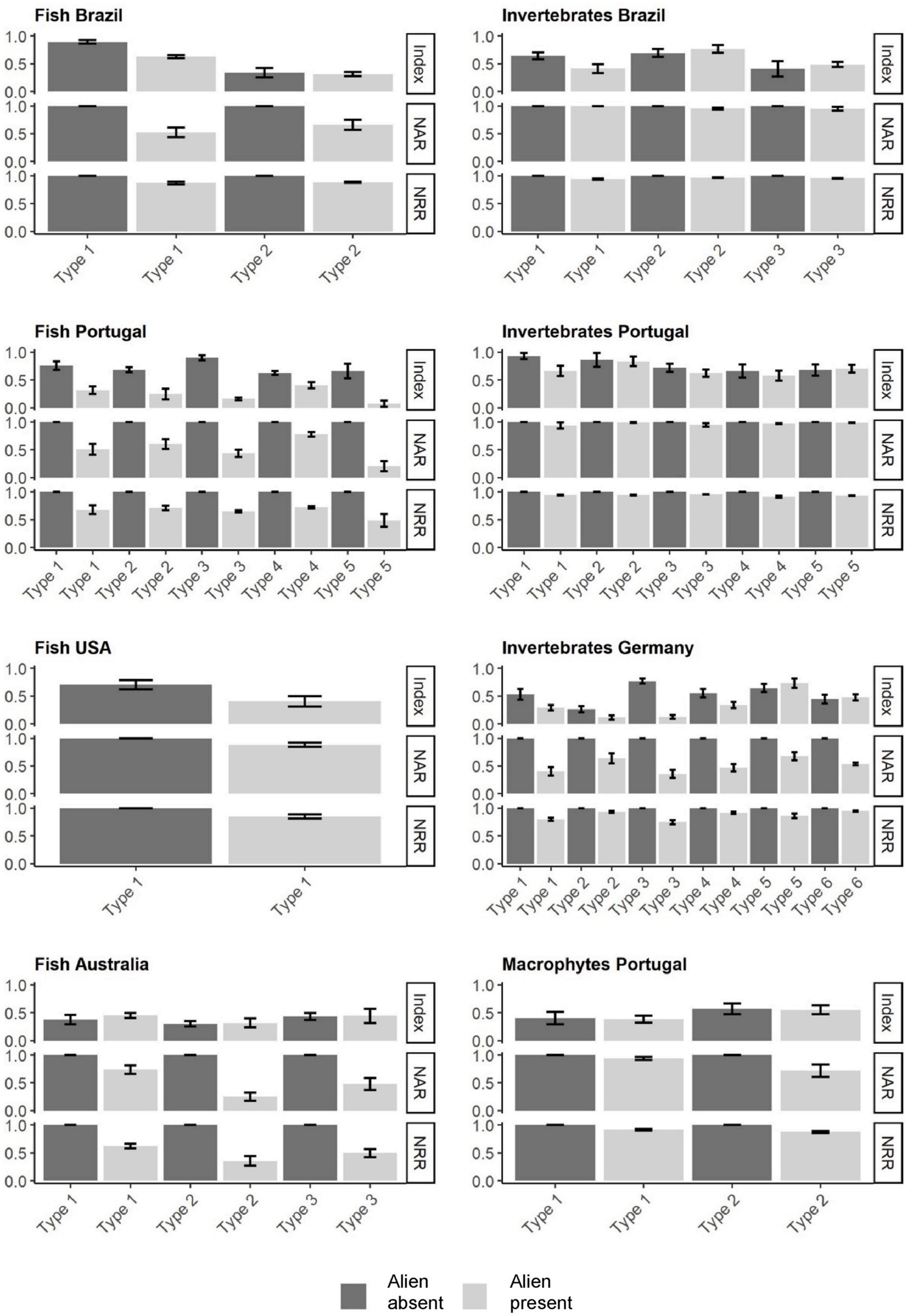

Fig. 3. Mean biotic indices (Index), proportion of native abundance to total abundance (Native abundance ratio: NAR), and proportion of native taxa richness to total richness (Native richness ratio: NRR) for each database and respective river type/ecoregion. Dark grey bars represent sites where alien taxa were absent and light grey bars represent sites where alien taxa were present. Error bars show standard errors.

Table 3
Comparison of the index scores between sites affected and not affected by alien taxa using the Wilcoxon Rank Test (W, p) with Continuity Correction for each database. Statistically significant differences are in bold.

Database	River type/Ecoregion	Wilcoxon Rank Test (W, p)
Fish Australia	1	W = 62.5; p = 0.363
	2	W = 55; p = 0.732
	3	W = 44; p = 0.678
Fish Brazil	1	**W = 2.5; p=<0.001**
	2	W = 49; p = 0.970
Fish Portugal	1	**W = 4; p = 0.001**
	2	W = 22.5; p = 0.238
	3	**W = 0; p = 0.001**
	4	**W = 3; p = 0.002**
	5	**W = 2; p = 0.002**
Fish USA	1	**W = 23; p = 0.043**
Invertebrates Brazil	1	W = 28; p = 0.281
	2	W = 14; p = 0.090
	3	W = 43; p = 0.464
	4	W = 28; p = 0.756
Invertebrates Germany	1	W = 27.5; p = 0.096
	2	**W = 20; p = 0.000**
	3	**W = 0; p = 0.000**
	4	**W = 19; p = 0.021**
	5	W = 62; p = 0.383
	6	W = 55; p = 0.734
Invertebrates Portugal	1	**W = 22; p = 0.038**
	2	W = 40; p = 0.713
	3	W = 39; p = 0.427
	4	W = 35; p = 0.689
	5	W = 27; p = 0.854
Macrophytes Portugal	1	W = 30; p = 1.000
	2	W = 31; p = 0.957

indicate that alien species are driving the loss of ecosystem quality (Poikane et al., 2024). Anthropogenic pressures in the rivers caused by hydraulic infrastructures such as dams, artificial banks and channels, result in altered flows, the loss of habitats or creation of novel ones, modify biotic interactions (e.g., predation and competition), lead to alterations in water temperature regimes and poorer water quality, which facilitate the establishment of alien and invasive species (e.g. Shea and Chesson, 2002, Catford et L. 2014, Paillex et al., 2017, Gois et al., 2019). In our study water quality and morphological alterations were always associated to the alien species at our sites. However, different stressors associated with the presence of alien species, partly because different stressors were analyzed in each region but also because the importances of individual stressors vary regionally (Herlihy et al., 2020; Schinegger et al., 2016). Adopting a full characterization of rivers in all countries, as required by the Water Framework Directive by (EU, 2000) and the USA Clean Water Act objectives (EPA, 1972), would yield more consistent pressure and stressor datasets. That is, measuring multiple biological elements, multiple water quality variables, hydromorphological alterations, physical habitat structure, riparian conditions, and catchment land use with common protocols are essential to improve our understanding of this issue (Hughes and Peck, 2008).

4.1. Inclusion of alien species in bioassessment datasets and biological indices

The treatment of alien species in river bioassessment differs among countries and organism groups. Individuals of alien species can be i) not collected or recorded; ii) collected and quantified but not used in index calculation; iii) treated the same as native species; or iv) used in calculating specific alien metrics.

Often large alien macroinvertebrate species (e.g., the red swamp crayfish, *Procambarus clarkii, Corbicula fluminae,* or the Chinese mitten crab, *Eriocheir sinensis)* are not adequately collected through common macroinvertebrate sampling methods (e.g., hand nets or Surber samplers), because they are too large or escape, meaning that their numbers are underestimated (Soto et al., 2023). In many cases a alien species is not recognized as alien. This happens more often with macroinvertebrates and diatoms because native ranges are poorly known and the taxonomic keys and expertise can be inadequate to identify them to species, especially in the tropics (Buss et al., 2015; Feio et al., 2021).

In other cases, individuals of alien species are recorded but not used in the calculation of bioassessment indices. This is a common situation with macrophytes and macroinvertebrates. Biotic indices such as the BWMP (Biological Monitoring Working Party index, Armitage et al., 1983) and its variants (e.g., Alba-Tercedor et al., 2002; Ministry of Environment, 2017; Stark, 1985) are based on the sensitivity of listed invertebrate taxa (often families) to water pollution. This means that if a alien family is not listed or discriminated against, it will not be accounted for in the final score.

Alien species are also used in a non-intentional way (i.e., not as alien species). This is the case of Multimetric Indices (MMI) based on macroinvertebrates, such as the ICM-STAR and derived indices (Buffagni et al., 2006; Feio et al., 2014) and others (Feio et al., 2021; Ruaro et al., 2021) where no specific metric addresses alien species. Nonetheless, these are included in other metrics regardless of their alien status. In the Portuguese macroinvertebrate index (IPtI, Feio et al., 2014; Ferreira et al., 2008), the metrics that assess total richness, evenness, the ASPT and family abundances can include alien taxa if they were present at least-disturbed sites when sampled and used to define reference values. The Asian clam, *Corbicula fluminea,* is a case in point. A similar situation may happen with predictive models where the expected taxa (calculated based on assemblages sampled at reference sites) at a site are compared with observed taxa (e.g., Feio et al., 2009; Feio and Poquet, 2011; Reynoldson et al., 1995; Simpson and Norris, 2000). It is also the case of the Benthic Macroinvertebrate Index (BMI) from South Korea which treats native species data the same as that from three alien species (red-veined darter, *Sympetrum fonscolombei,* acute bladder snail, *Physella acuta,* and golden apple snail, *Pomacea canaliculata*). In the IPS (Indice de Polluosensibilité Spécifique, Coste in Cemagref, 1982), which is widely used for diatom bioassessment in Europe, some alien species have a sensitivity and indicator value, and are used in calculating the index as if they were native species.

Many macroinvertebrate biotic or multimetric indices require only family-level identification, meaning that the index cannot distinguish native from alien species of the same family. For example, the invasive brown planarian, *Girardia tigrina,* is found in polluted rivers in Japan, whereas a native planarian, *Dugesia japonica,* is typically found in clean water environments. Because of challenges in field identification, confusing the two species can alter the results of the indices (e.g. the Japanese BMWP, Iwasaki, 2007). The alien *P. antipodarum* in Europe, North America, Australia and Japan was previously in the family Hydrobiidae and thus included in the calculation of the BMWP (Armitage et al., 1983) and derived indices. Now it is in the family Tateidae and is no longer used to calculate those indices, which has implications in temporal comparisons.

Finally, alien species metrics are used in some fish indices and at least one macrophyte index because of growing concerns with the negative impacts that alien species have on indicator assemblages, as well as their indication of anthropogenic disturbance. In the NPS-MF (2020), New Zealand introduced a metric for alien fish in the Fish Index of Biotic Integrity (Joy and Death, 2004). In Portugal, the proportion of alien species in a sample is used as a metric (Oliveira et al., 2010; Feio et al., 2019). Also, in South Africa the impacts of alien are explicitly quantified by a metric in the Fish Response Assessment Index (FRAI) version 2 (Kleynhans and Louw, 2008), in terms of their predatory, habitat modifying effects, and effects on native fish species. In Brazilian wadeable streams it is only the presence and abundance of a guppy, *Poecilia reticulata*, that is retained in a metric addressing alien species (Casatti et al., 2009; de Carvalho et al., 2017; Ferreira and Casatti, 2006), whilst in the USA, two specific alien metrics (% Common Carp individuals; % alien individuals) were used in a multimetric index (Hughes and Gammon, 1987). Whittier et al. (2007) included % alien individuals in their MMIs for western USA streams and the USEPA uses % alien individuals or % native taxa in its National Rivers & Streams Assessment. Finally, for macrophytes, the Portuguese index also includes some alien species such as the water fern, *Azolla filiculoides*, but not others with similar detrimental effects, such as parrot's-feather watermilfoil, *Myriophyllum aquaticum*.

Even when not used in biological indices, alien information in datasets is nevertheless highly relevant and efforts should be made to keep consistent records and maintain adequate sampling procedures. This information can be used to detect the spread of alien species spatially and temporally (Haubrock et al., 2022) and how the climate crisis favors or mitigates the invasions (Zhang et al., 2020), thereby facilitating better management strategies (Januchowski-Hartley et al., 2011). Several countries are assessing alien species and retaining this information in datasets, but it is usually an independent process from the ecological assessment of rivers. In Japan, all alien species collected by the National Census on River Environments (NCRE) have been documented in a database since 1990. However, the sampling protocols and number of monitoring sites within each river have changed over different periods (Sueyoshi et al., 2016). This poses a challenge when comparing changes in the distribution of alien species across these different times. In New Zealand, there is no national monitoring of fish, but some regional governments survey pest fishes and *Didymosphenia geminate* (Jellyman et al., 2011). In the USA, the U.S. Geological Survey maintains a nonindigenous aquatic species database for invertebrates, vertebrates, and plants (https://nas.er.usgs.gov). Regular bioassessment can contribute to the detection and follow-up of invasive alien species. Although alien assessments of larger organisms, such as fish and macrophytes, can focus on specific species, entire samples of diatoms and smaller macroinvertebrates must be processed.

Alien species are sometimes viewed as being beneficial. This is the case for anglers and fishery managers who intentionally promote and stock alien fish (de Leaniz et al., 2010; Pelicice et al., 2017; Rowe and Wilding, 2012). In addition, legislation has been proposed to designate alien fishes as naturalized to enhance their use in aquaculture (Azevedo-Santos et al., 2017). However, such species harm river ecosystem functions and their native species, i.e., a fish assemblage that is good for angling is not beneficial to fish assemblage condition (Lomnicky et al., 2021). Thus, better environmental education and legislation are needed regarding the control of NNs.

5. Conclusion

We offer 7 recommendations for managers and policymakers working in the fields of freshwater conservation and environmental assessment based on our findings. 1) Include specific metrics for alien species in the biological indices used in river bioassessment. 2) Increase the investigation of alien taxa of small organisms (e.g. diatoms, small benthic invertebrates). 3) Where possible, eliminate sites with confirmed biological invasions from reference databases. 4) Remove alien species from calculations of total richness metrics. 5) Identify to species level in biomonitoring programs where alien species are likely to occur. 6) Avoid legislation and management that protect alien species, even if they have high economic value. 7) Encourage behaviors that prevent alien invasions of all aquatic biological elements.

CRediT authorship contribution statement

Maria João Feio: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Janine P. Silva:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Robert M. Hughes:** Writing – review & editing, Investigation, Data curation. **Francisca C. Aguiar:** Writing – review & editing, Validation, Investigation, Data curation. **Carlos B.M. Alves:** Writing – review & editing, Validation, Investigation, Data curation. **Sebastian Birk:** Writing – review & editing, Validation, Investigation, Data curation. **Marcos Callisto:** Writing – review & editing, Validation, Investigation, Data curation. **Marden S. Linares:** Writing – review & editing, Validation, Investigation, Data curation. **Diego R. Macedo:** Writing – review & editing, Validation, Investigation, Data curation. **Paulo S. Pompeu:** Writing – review & editing, Validation, Investigation, Data curation. **Wayne Robinson:** Writing – review & editing, Validation, Investigation, Data curation. **Christian Schürings:** Writing – review & editing, Validation, Investigation, Data curation. **Salomé F.P. Almeida:** Writing – review & editing, Validation, Investigation. **Pedro M. Anastácio:** Writing – review & editing, Validation, Investigation. **Francis O. Arimoro:** Writing – review & editing, Validation, Investigation. **Min Jeong Baek:** Writing – review & editing, Validation, Investigation. **Mirian Calderón:** Writing – review & editing, Validation, Investigation. **Kai Chen:** Writing – review & editing, Validation, Investigation. **Peter Goethals:** Writing – review & editing, Validation, Investigation. **Marie Anne E. Forio:** Writing – review & editing, Validation, Investigation. **Jon S. Harding:** Writing – review & editing, Validation, Investigation. **Ben J. Kefford:** Writing – review & editing, Validation, Investigation. **Martyn G. Kelly:** Writing – review & editing, Validation, Investigation. **Unique N. Keke:** Writing – review & editing, Validation, Investigation. **Mark Lintermans:** Writing – review & editing, Visualization, Investigation. **Renato T. Martins:** Writing – review & editing, Validation, Investigation. **Terutaka Mori:** Writing – review & editing, Validation, Investigation. **Keigo Nakamura:** Writing – review & editing, Validation, Investigation. **Oghenekaro N. Odume:** Writing – review & editing, Validation, Investigation. **Filipe Ribeiro:** Writing – review & editing, Validation, Investigation. **Renata Ruaro:** Writing – review & editing, Validation, Investigation. **Sónia RQ. Serra:** Writing – review & editing, Validation, Investigation. **Deep Narayan Shah:** Writing – review & editing, Validation, Investigation. **Masanao Sueyoshi:** Writing – review & editing, Validation, Investigation. **Ram Devi Tachamo-Shah:** Writing – review & editing, Validation, Investigation.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.123874.

Data availability

Data will be made available on request.

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