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Weak effects of habitat type on susceptibility to invasive freshwater species: an Italian case study

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ABSTRACT

1. Introduction of alien species is one of the major threats to aquatic biota and knowledge of the major correlates of their occurrence is pivotal in planning reliable conservation strategies.

2. To understand whether specific freshwater habitats are more likely to be invaded than others, a dataset on the occurrence of 1604 species in 54 taxonomic groups from 181 sites across the Italian peninsula was gathered.

3. The EUNIS habitat classification was used, selecting for the study's seven habitat types at the second EUNIS level, including lentic (EUNIS C1; 64 sites), lotic (EUNIS C2; 99 sites) and highly artificial (EUNIS J5; 18 sites) habitats.

4. The aim of the study was to test whether the overall number of alien species and the proportion of alien species for each taxonomic group differed between habitat types and could be explained by environmental, human-mediated, or climatic factors.

5. Using generalized linear mixed effect models to account for potential confounding factors, only average air temperature of the site was a significant positive predictor of the occurrence of alien species, regardless of habitat type, species richness, and other climatic variables.

6. A direct effect of temperature could be excluded given the origin of alien species, mostly from colder areas than Italy. Thus, an indirect effect could be hypothesized at the Italian latitudes, with warmer areas potentially

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more likely to be visited by tourists than colder areas. If this hypothesis is confirmed, the results of the analyses call for a compromise between the maintenance of recreational activities in the wild and the preservation of a natural environment to prevent the arrival and spread of alien species. On the other hand, no further recommendations can be implemented regarding habitat susceptibility to alien species.

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KEY WORDS: climate; environmental variables; fresh water; invasion; non-indigenous species; non-native species; propagule pressure

INTRODUCTION

One of the most important factors involved in species extinctions is the introduction of alien species that may outcompete local species, prey on them, or carry disease (Lockwood *et al.*, 2007; Willby, 2007; Richardson, 2011). Thus, understanding the mechanisms that drive the occurrence of alien species and the 'invasibility' of habitats with different characteristics is of paramount importance for managing and preserving natural ecosystems.

Freshwater habitats are known to host many of alien species (Strayer, 2010) that may have ecological or economic impacts. Recurring examples of global and local extinctions owing to introductions of alien species are documented in freshwater habitats, as for example the extinction of several species of cichlids in African lakes following the introduction of the Nile perch (Barel *et al.*, 1985); the extinction of the diving beetle *Dytiscus lapponicus disjunctus* endemic to a few high altitude lakes in the western Alps after the introduction of trout and char (Franciscolo, 1979); the extirpation of several populations of native freshwater crayfish (Barbaresi and Gherardi, 2000) and fish (Trumpickas *et al.*, 2011) after the introduction of alien species. Freshwater habitats represent a small proportion of the planet's surface (Gleick, 1993), but host a broad representation of the global biological diversity (Balian *et al.*, 2008). Therefore, threats to their integrity may be proportionately much more dangerous and with farther-reaching effects than in other habitats (Dudgeon *et al.*, 2006).

The aim of this study was to understand whether some freshwater habitats are more prone to invasion by alien species than others, in order to establish focused conservation priorities in the context of improving the efficacy of policy management. Thus, susceptibility to alien species invasion for different habitats was assessed with reference to the EUNIS habitat classification (http://eunis.eea.europa.eu/). A large amount of information was collected and analysed on the occurrence of alien and native species in different freshwater habitats across the Italian peninsula. The main expectation was that lentic habitats would have a higher number of alien species than lotic ones, because lakes and ponds are more attractive than streams for tourists who can deliberately or inadvertently introduce alien species (Muirhead and MacIsaac, 2005); moreover, more invertebrate species have resting stages for passive dispersal in lentic systems than in lotic systems (Radzikowski, 2013). Even within lentic habitats, the hypothesis is that more altered and eutrophic habitats (EUNIS code C1.3) would have more alien species because they are potentially more environmentally fragile and susceptible than pristine ones (C1.1) (Crawley, 1987). For the same reason, artificial habitats (J5) are also expected to be more prone to invasion than natural lentic (C1) and lotic (C2) habitats.

Climatic variables such as temperature and precipitation may also affect colonization processes of alien species in freshwater ecosystems, determining colonization patterns along latitudinal and altitudinal gradients. However, since warmer and drier areas as well as lowland and downstream areas are more affected by human activities and attract more visitors and tourists during the summer months (Scott and Lemieux, 2010), pure climate effects on the susceptibility of ecosystems and habitats to alien species invasions are difficult to detect.

The results of the analysis of an extensive survey of freshwater biodiversity in continental Italy will provide evidence for the differential susceptibility to invasion of different habitat types and will provide a basis for setting conservation priorities, developing monitoring programmes and enforcing regulation. For example, if strong drivers of the occurrence of alien species are present, conservation efforts should focus mostly on minimizing their impact, or on enforcing more greater control on such drivers.

MATERIAL AND METHODS

Data and definitions

Data on the occurrence of freshwater species are from the Italian Long-term Ecological Research network (LTER-Italy, Bertoni (2012)) and from LifeWatch (http://www.lifewatch.eu). These networks collected and still collect data from different freshwater sites across the Italian peninsula. All the data on species occurrence were managed within the 'Alien species showcase' of LifeWatch, the European large infrastructure on biodiversity and ecosystem research, an e-science infrastructure offering ecological informatics services and tools to scientists and other public and private institutions involved in biodiversity and ecosystem research (Basset and Los, 2012). LifeWatch is organized in different consortia, five of which are represented here; consortia are responsible for data management from specific geographical areas and for the control of taxonomic reliability and consistency and of the homogeneity of the data. Most of the information on species presence of the different taxonomic groups comes from surveys during the period 1980 to 2012 and includes published papers and reports from universities and research institutions, as well as notes in technical reports from local authorities. The dataset included freshwater sites distributed across continental Italy, from the Alps in the north to the coasts in the south; sites were subsequently grouped into four main geographical areas merging two of the five consortia. The four main geographical areas are North-west (managed by CNR-ISE), North (managed by the University of Parma), Centre (managed by the Natural History Museum of the University of Firenze and the University of Perugia, and one site from the University of Parma), and South (managed by ARPA Puglia, and one site from the University of Parma) (Figure 1).

Definitions of 'alien' species vary in the literature (IUCN, 2000; Colautti and MacIsaac, 2004). The definition adopted here is a very general one, considering as alien any species deliberately or inadvertently introduced to Italy by human activities after the discovery of the New World by Columbus in 1492, similar to what plant invasion biologists call 'neophytes' (Pyšek, 1998). Given the problems involved in determining the impact of alien species on the environment (i.e. invasive species) (Junqueira, 2013) and their naturalization stage (Richardson *et al*., 2000), all alien species were considered equal, as alien in its broadest meaning.

Taxonomic groups were identified at levels that are commonly used in limnological analyses. It was not possible to use the same taxonomic rank for all groups, as all ranks above species level have no strict biological basis, and they do not correspond across the tree of life. Thus, some groups corresponded to the level of phylum or division (e.g. Chlorophyta, Cyanobacteria, Mollusca, Rotifera), others to the level of order or suborder (e.g. most insects such as Ephemeroptera, Hemiptera, Plecoptera and Trichoptera, and crustaceans such as Amphipoda, Cladocera, Copepoda and Isopoda) and others corresponded to families (e.g. the highly diverse groups of Coleoptera such as Curculionidae, Dytiscidae, Elmidae and Hydrophilidae). Others are paraphyletic groups, such as 'fishes', including both Agnatha and Actinopterygii, and 'macrophytes', including all plants growing in or near water such as emergent, submergent, or floating ones. The taxonomic groups have different rankings in the systematic hierarchy, but provide reliable clades for the analyses, represent the traditional focus of limnological studies, and group species with homogeneous ecological features.

Statistical analyses

The main aims were to test whether the number of alien species for each taxonomic group and the

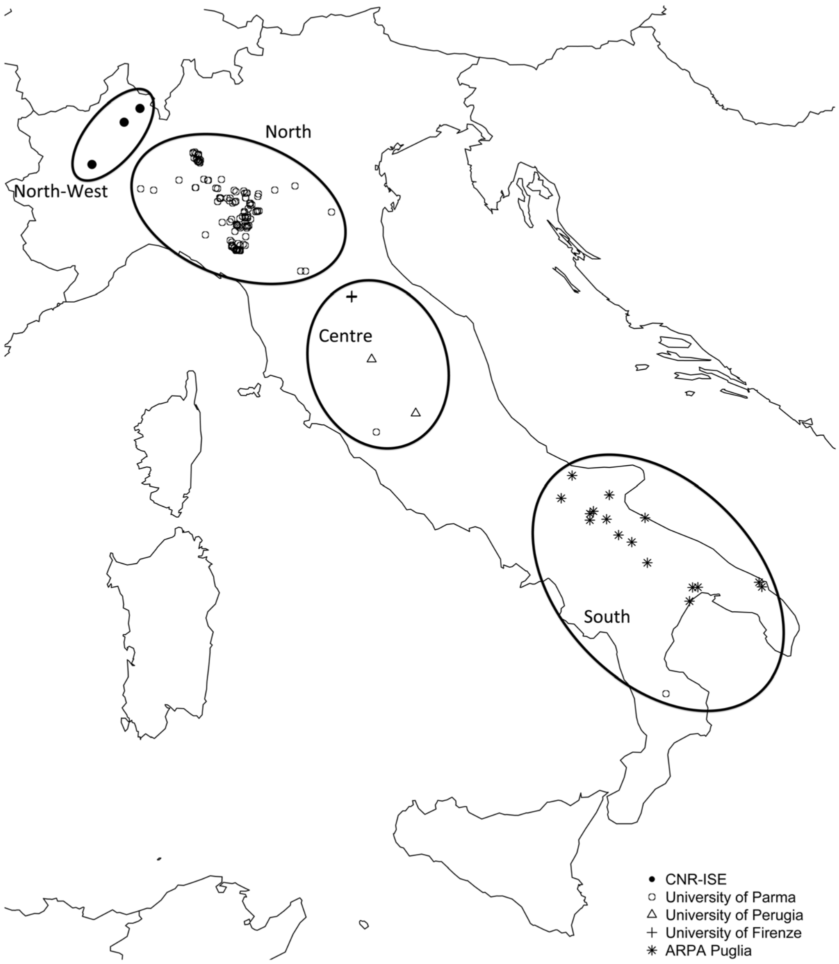

Figure 1. Geographical location of the analysed sites in Italy. Sites are coded according to the consortium of LifeWatch Italy handling and managing the data, CNR-ISE, Natural History Museum of the University of Firenze, University of Parma, University of Perugia, and ARPA Puglia. The four large circles identify four main geographical areas, North-west, North, Centre and South.

proportion of alien species in each taxonomic group were different between habitats. In order to improve the support and the reliability of the results, the models included additional explanatory variables that had the potential to affect the occurrence of alien species. A set of variables was thus explicitly included in the models, encompassing local species richness of each taxonomic group, and measures of precipitation and temperature. Another set of variables was included implicitly in the models, comprising taxonomic groups, sites and the four main geographical areas of Figure 1. Linear mixed effect models (LMEMs) (Zuur *et al.*, 2009) were used to account for the two sets of explicit (fixed effect) and implicit (random effect) variables. Only the results of the explicit variables are reported here, whereas the implicit variables are used to account for potential biases caused by data spatial correlation (e.g. closer sites would have similar temperatures and precipitation, and potentially also similar alien species), differences in taxonomic knowledge (e.g. different LifeWatch consortia include different taxonomists with different skills), and non-independence of the data (e.g. the same site hosts different taxonomic groups and different taxonomic groups are present in different sites). LMEMs have been designed for these types of analyses, with violations of the assumption of independence of observations (Bunnefeld and Phillimore, 2012). Taxonomic group and site identity, nested within the four main geographical areas, were used as cross random effects in all the statistical models.

Habitats were defined according to the first-level EUNIS classification system (Davies *et al*., 2004), including standing waters (C1: lentic habitats; 64 sites), running waters (C2: lotic habitats; 99 sites) and man-made water bodies (J5: highly artificial habitats; 18 sites). These main habitats were then divided within a more detailed classification scheme, according to the second EUNIS level. For standing waters (C1), the second-level EUNIS subcategories included in the dataset were permanent oligotrophic waters (C1.1), permanent mesotrophic waters (C1.2), permanent eutrophic waters (C1.3), and temporary waters (C1.6). For running waters (C2), the subcategories were springs (C2.1), fast turbulent watercourses (C2.2), and smooth-flowing watercourses (C2.3). For man-made water bodies (J5), the subcategories were highly artificial standing waters (J5.3), and highly artificial fast-flowing waters (J5.4 and J5.5). Such classification is very crude, but allows for the distinction of large categories of freshwater habitats, and in the case of standing waters even for their eutrophication level (Davies *et al*., 2004).

Species richness was calculated for each taxonomic group at each site directly from the dataset and used also to calculate the proportion of alien species as an explicit explanatory variable in the statistical models. Species richness of the receiving community is known to be an important variable in invasion ecology (Elton, 1958; Fridley *et al*., 2007), working as a buffer against alien invasion or favouring their arrival.

Data on precipitation and temperature for each site were obtained from the databases of WorldClim (http://www.worldclim.org/current) and United Nations Environmental Programme (http://www.grid.unep.ch/data/data.php). They represent the average precipitation and air temperature over a period approximately between 1950 and 2000.

Because the explanatory variables were measured at different scales and with different units, all continuous explanatory variables were standardized to optimize model fit before performing analyses, so that each variable had a mean of zero and unit standard deviation (Borcard *et al*., 2011). All statistical analyses were performed in R 2.15.0 (R Development Core Team, 2012), using the package vegan 2.0-3 (Oksanen *et al*., 2012) for standardization, the package lme4 0.999375-42 (Bates *et al*., 2011) for LMEMs, and the packages rgdal 0.7-8 (Keitt *et al*., 2012) and raster 1.9-70 (Hijmans and van Etten, 2012) for managing climatic variables.

First, tests for the correlates of the absolute number and then of the proportion of alien species for each group at each site were performed, looking for differences between the three main habitats: lentic, lotic, and artificial. Second, more detailed differences within the main habitat types were explored (i.e. at the second-level EUNIS categories). The models with absolute numbers of alien species were performed with a Poisson error distribution, whereas the models with proportions were performed with a binomial error.

RESULTS

The dataset contained 181 freshwater sites distributed across different areas in the Italian peninsula (Figure 1) with information on the occurrence of 1604 species belonging to 54 taxonomic groups. Average air temperature for the sites ranged between 4.6 °C and 16.5 °C; average annual rainfall ranged between 482 mm and 1129 mm; species richness for each taxonomic group at each site ranged between 1 and 151.

In total, 42 alien species were found in 11 taxonomic groups; alien species represented 2.6% of the total richness of the analysed freshwater systems (lentic, lotic, and artificial). No taxonomic groups with alien species were found in the 18 sampled artificial water bodies (Figure 2), represented by three different habitats according to EUNIS second-level classification: J5.3 – highly artificial standing waters, and J5.4 and J5.5 – highly artificial fast-flowing waters. These 18 artificial sites were discarded from the subsequent analyses to avoid confounding the results.

The main models both for alien species richness and proportion implicitly controlled for differences between taxonomic groups, for spatial structure, and for other potential biases. The model analysing the overall number of alien species for each group supported a positive correlation of alien species with species richness of the taxonomic group in the

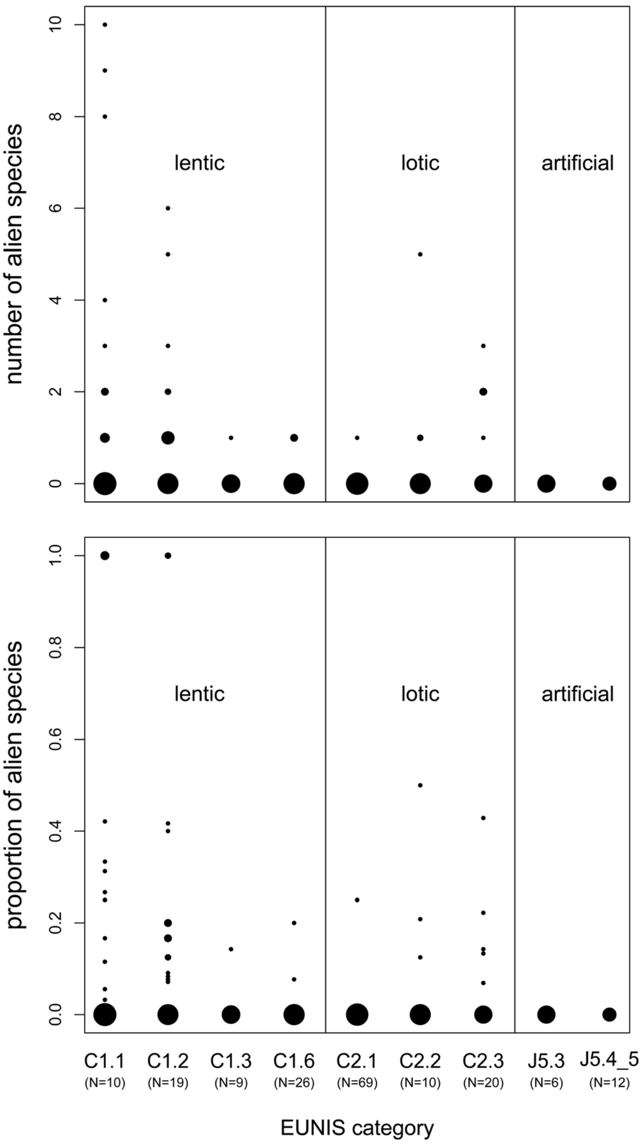

Figure 2. Plots of the distribution of the number of alien species and of the proportion of alien species in each site in each taxonomic group for the analysed freshwater habitats in Italy. The size of the black circles is proportional to the number of sites for each group on a logarithmic scale. The smallest circles correspond to 1 and the largest to 84. The two vertical lines divide the three first-level EUNIS habitats: lentic, lotic, and artificial. EUNIS categories are C1.1: permanent oligotrophic waters; C1.2: permanent mesotrophic waters; C1.3: permanent eutrophic waters; and C1.6: temporary waters for a total of 64 lentic sites; C2.1: springs; C2.2: fast turbulent watercourses; and C2.3: smooth-flowing watercourses for a total of 99 lotic sites; J5.3: highly artificial standing waters; and J5.4_5: highly artificial fast-flowing waters for a total of 18 artificial sites.

receiving community and with local air temperature (Table 1). No difference was found between lentic and lotic habitats for the number of alien species (Table 1). The model analysing the proportion of alien species for each group confirmed the effect of temperature, whereas the effect of richness of the receiving community disappeared (Table 1).

Analysing lentic and lotic waters separately, it was possible to test for the effect of the more detailed differences in habitat types. For lentic systems, the dataset included four different habitats according to EUNIS (C1.1, C1.2, C1.3, and C1.6). The overall number of alien species was positively related with richness and temperature, but not with habitat type (Table 2). The positive effect of temperature and the lack of differences between habitats were shown also by the model analysing the proportion of alien species in lentic waters (Table 2).

For lotic systems, the dataset comprised three different habitats in EUNIS (C2.1, C2.2, and C2.3). Only species richness was correlated with the number of alien species, whereas the difference between habitats was not clearly significant (Table 3). When analysing the proportion of alien species, the results highlighted significant differences between habitats, with smooth-flowing watercourses (C2.3) hosting a higher proportion of alien species than springs (C2.1) (Table 3).

The large number of taxonomic groups without alien species (~80% of the groups) might have created problems in the analyses owing to the high number of zero data (absence of aliens). Thus, the analyses were repeated including only the 11 taxonomic groups with alien species, namely Annelida, Cladocera, Copepoda, Cyanobacteria, Decapoda, fishes, macrophytes, Mollusca, Ostracoda, Platyhelminthes and Rotifera (Figure 3). This reduced dataset consisted of 163 of the 181 original sites. The results of all the previous models, either including all natural systems, only lentic habitats, or only lotic habitats, were qualitatively confirmed by the results of the analyses from the reduced dataset (Tables 4–6), demonstrating no biases in the analyses.

DISCUSSION

Alien species list

The number of alien species in the analysed dataset (42, of which 36 were animal species) is about one third of the 112 animal species reported for the Italian freshwater habitats by Gherardi *et al.* (2008)

Table 1. Results of the linear mixed effect models explaining the absolute number of alien species and the proportion of alien species in natural freshwater habitats of the Italian peninsula (estimates, standard errors, and *P*-values are reported). Taxonomic group and site nested within geographic area were used as cross- random effects

	Number of species			Proportion of species		
	Estimate	SE	*P*	Estimate	ES	*P*
(intercept)	-6.630	1.190	<0.0001	-6.358	0.770	<0.0001
habitat	-0.086	0.398	0.8289	-0.603	0.376	0.1091
richness	0.777	0.204	0.0001	0.148	0.177	0.4012
precipitation	-0.013	0.138	0.9196	0.149	0.124	0.2277
temperature	1.455	0.302	<0.0001	1.112	0.266	<0.0001

Table 2. Results of the linear mixed effect models explaining the absolute number of alien species and the proportion of alien species in lentic habitats of the Italian peninsula (estimates, standard errors, and *P*-values are reported). Taxonomic group and site nested within geographic area were used as cross-random effects. The effects of the different habitats are not shown explicitly, but only 'EUNIS habitat' as a variable is reported

	Number of species			Proportion of species		
	Estimate	SE	*P*	Estimate	SE	*P*
(intercept)	-6.121	1.145	<0.0001	-6.924	1.019	<0.0001
EUNIS habitat	---	---	>0.3	---	---	>0.4
richness	0.877	0.260	0.0008	0.039	0.269	0.8851
precipitation	-0.074	0.137	0.5890	-0.088	0.151	0.5594
temperature	2.108	0.586	0.0003	2.057	0.626	0.0010

Table 3. Results of the linear mixed effect models explaining the absolute number of alien species and the proportion of alien species in lotic habitats of the Italian peninsula (estimates, standard errors, and *P*-values are reported). Taxonomic group and site nested within geographic area were used as cross-random effects

	Number of species			Proportion of species		
	Estimate	SE	*P*	Estimate	SE	*P*
(intercept)	-10.672	4.004	0.0077	-7.502	1.701	<0.0001
C2.1-C2.2	-0.209	1.877	0.9111	0.001	1.967	0.9998
C2.1-C2.3	2.467	1.298	0.0573	2.972	1.357	0.0285
C2.2-C2.3	2.257	1.674	0.1775	2.972	1.890	0.1159
richness	2.220	0.673	0.0009	-0.085	0.374	0.8205
precipitation	0.459	0.715	0.5209	1.165	0.770	0.1305
temperature	0.141	0.813	0.8626	0.369	0.893	0.6793

because of the different scope of the datasets. Whereas Gherardi *et al*. (2008) aimed at listing all the alien animal species known for freshwater habitats in Italy, the present analysis focused on the occurrence of alien species at freshwater sites where reliable lists of all the species, both native and alien, for each taxonomic group were available. Moreover, the analysed dataset did not include all the taxonomic groups of the extensive survey of Gherardi *et al*. (2008), according to species occurrence at the study sites considered here. The same reasoning applies to the lower number of alien freshwater species found in the analysed dataset than in other European countries, ranging between 60 and 90 (Minchin and Eno, 2002; Gollasch and Nehring, 2006; Gherardi, 2007).

Notwithstanding the lower number of species in the analysed dataset compared with other more extensive but qualitative studies, the proportion of alien species to the total richness is very similar, if not slightly higher in the currently analysed dataset than in the dataset by Gherardi *et al*. (2008) (2.6% and 2.0%, respectively). Thus, the fact that these figures are similar supports the reliability and representativeness of the dataset used for the analyses in the present study, for the taxonomic groups that have been considered.

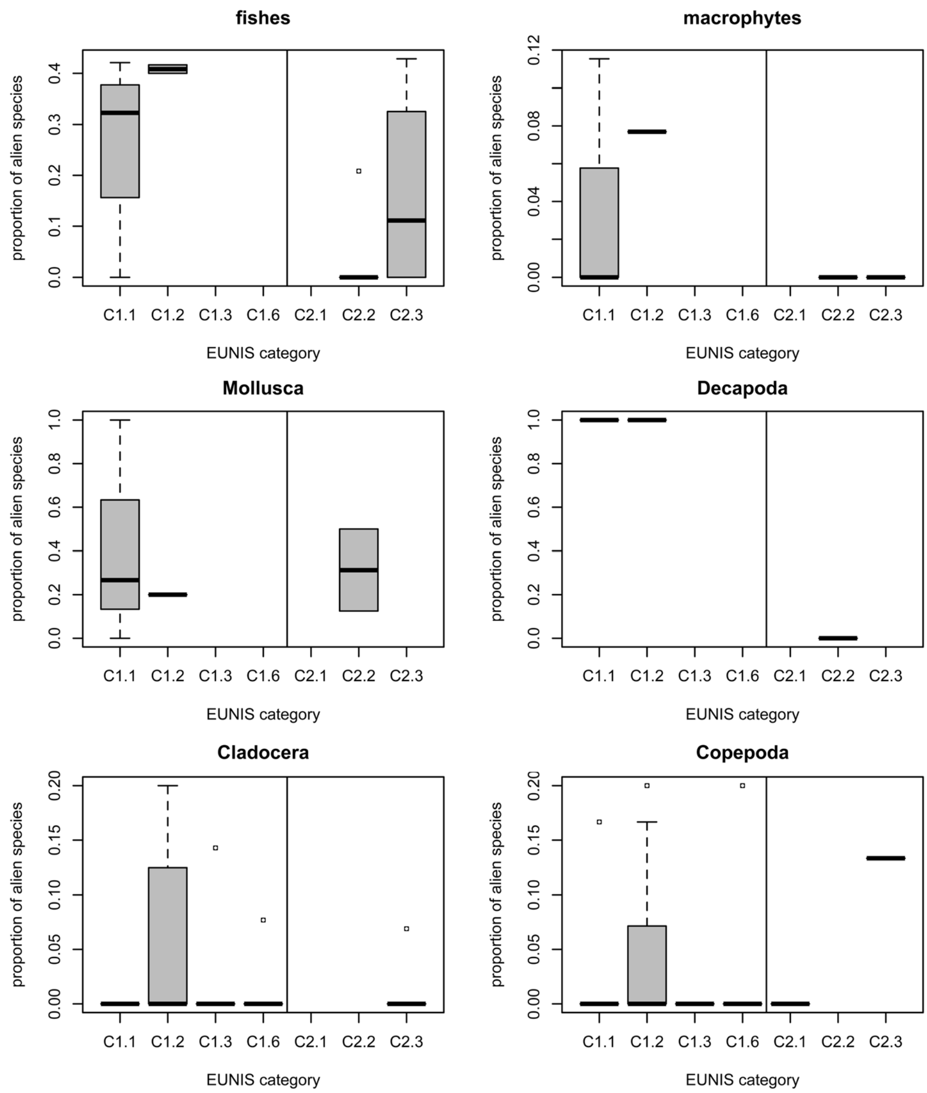

Figure 3. Boxplots of the distribution of the proportion of alien species in each site in each of the six taxonomic groups with the highest number of alien species for lentic and lotic freshwater habitats in Italy. EUNIS codes and sample size as in Figure 2. For each EUNIS category, the thick horizontal line represents the median of the distribution, the box includes 50% of the data, the whiskers reach the highest and lowest value within 95% of the distribution, and the circles represent single values outside 95% of the distribution. The vertical line divides the two first-level EUNIS habitats: lentic and lotic.

Table 4. Results of the linear mixed effect models explaining the absolute number of alien species and the proportion of alien species in natural freshwater habitats of the Italian peninsula including only the 11 taxonomic groups with alien species (estimates, standard errors, and *P*-values are reported). Taxonomic group and site nested within geographic area were used as cross-random effects

	Number of species			Proportion of species		
	Estimate	SE	*P*	Estimate	SE	*P*
(intercept)	-2.087	0.574	<0.0001	-3.462	0.559	<0.0001
habitat	-0.753	0.461	0.1021	-0.597	0.370	0.1074
richness	0.462	0.115	<0.0001	0.075	0.127	0.5562
precipitation	0.507	0.163	0.0018	0.139	0.109	0.2010
temperature	1.441	0.329	<0.0001	1.100	0.264	<0.0001

Table 5. Results of the linear mixed effect models explaining the absolute number of alien species and the proportion of alien species in lentic habitats of the Italian peninsula including only the 11 taxonomic groups with alien species (estimates, standard errors, and *P*-values are reported). Taxonomic group and site nested within geographic area were used as cross-random effects. The effects of the different habitats are not shown explicitly, but only 'EUNIS habitat' as a variable is reported

	Number of species			Proportion of species		
	Estimate	SE	*P*	Estimate	SE	*P*
(intercept)	-2.853	0.844	0.0007	-4.543	0.863	<0.0001
EUNIS habitat	---	---	>0.2	---	---	>0.3
richness	0.642	0.189	0.0007	0.040	0.207	0.8464
precipitation	-0.052	0.117	0.6551	-0.054	0.127	0.6713
temperature	2.079	0.572	0.0003	1.954	0.572	0.0006

Table 6. Results of the linear mixed effect models explaining the absolute number of alien species and the proportion of alien species in lotic habitats of the Italian peninsula including only the 11 taxonomic groups with alien species (estimates, standard errors, and *P*-values are reported). Taxonomic group and site nested within geographic area were used as cross-random effects

	Number of species			Proportion of species		
	Estimate	SE	*P*	Estimate	SE	*P*
(intercept)	-4.831	1.484	0.0011	-5.655	1.295	<0.0001
C2.1-C2.2	1.581	1.793	0.3782	1.917	1.389	0.1676
C2.1-C2.3	2.698	1.434	0.0598	2.859	1.272	0.0246
C2.2-C2.3	1.117	1.438	0.4371	0.942	1.032	0.3613
richness	0.117	0.234	0.6168	-0.197	01237	0.3920
precipitation	0.567	0.731	0.4386	0.267	0.541	0.6209
temperature	0.116	0.825	0.8881	-0.031	0.630	0.9607

Correlates of invasibility

Temperature was the most important correlate of invasibility both for the models on species richness and those on proportions. Sites in warmer areas hosted more alien species than those in colder areas. This could be due to a direct effect of temperatures, favouring alien species, or to indirect effects, mediated by the tourist preference to frequent warm areas. A direct effect of temperature could be due to warmer sites facilitating invasion processes, being able to host a larger number of alien species compared with colder sites. Nevertheless, this hypothesis could hold true only if the majority of alien species originally come from warmer areas than the invaded areas. The native geographical ranges of the alien species do not fit with the hypothesis of such a direct effect, as only 20% of the alien species come from warm areas, while most of the other species have their original distribution areas at in more northerly latitudes in Europe or in North America than in the Italian sites (Pignatti, 1982; Gherardi *et al*., 2008). This scenario, with most species coming from northern latitudes, suggests that, at present, invasibility does not reflect the effects of global warming (Walther *et al*., 2009); that would have been supported if alien species came mostly from warmer areas than Italy.

One alternative explanation for the positive effect of temperature could be an indirect effect related to higher numbers of tourist visits to sites with high annual average temperatures (Bigano *et al*., 2005; Scott and Lemieux, 2010). Temperature could thus be used as a proxy of propagule pressure. This result would reinforce the role of propagule pressure attested by Colautti *et al*. (2006) and by Blackburn *et al*. (2013). Of course, the positive relationship between tourism and temperature excludes winter tourist activities, but freshwater sites are not used for winter sports in Italy (e.g. ice-skating, skiing on lakes) compared with fresh waters in higher latitudes (Bigano *et al*., 2005).

The occurrence of alien species was not different between EUNIS first-level habitats (lentic vs lotic). This pattern was unexpected, as the original hypothesis was that lakes are visited more than rivers by tourists, and thus would be more prone to invasion. The lack of significance of habitat type in the statistical models could be because lentic waters were in areas with significantly lower air temperature than lotic waters (ANOVA: $F_{1,161} = 64.4$, $P < 0.0001$). It is impossible to ascertain from the dataset whether there are no differences in invasibility between habitats, or if these two opposite effects of temperature and habitat may balance each other out, producing a spurious lack of significance of the role of habitat type in the analyses. Nevertheless, when repeating all the analyses without including temperature as an explanatory variable, small but significant differences were found between habitats in both the number and the proportion of alien species ($P \sim 0.03$) with lentic sites richer in alien species than lotic sites, as expected. No differences were found between habitats within lentic and lotic systems, even when excluding temperature from the analysis. Thus, the effect of habitat type on alien species may be worth further exploration with larger datasets from other geographic areas covering wider environmental gradients than in the present study.

While searching for correlates of invasibility in terrestrial habitats, a significant effect of variables representing habitat properties, climate, and propagule pressure was found by Chytrý *et al.* (2008). Nevertheless, habitat properties seem to be more important in explaining occurrence of alien species in terrestrial than in freshwater organisms; for example, it is a common feature that disturbed and human-altered habitats host more alien species than natural ones in terrestrial systems (Crawley, 1987; Vilá *et al.*, 2007; Chytrý *et al.*, 2008), but this was not confirmed by the analysis performed on Italian fresh waters. Overall, the lack of taxonomic groups with alien species in the artificial water bodies in the dataset could be due to sampling bias and to a lower number of artificial water bodies. Nevertheless, even within lentic waters, no differential occurrence of aliens could be ascribed to human-mediated eutrophication. It is of interest to note also that permanent and temporary water bodies were not significantly different in the occurrence of alien species.

Another correlate that is often discussed in invasion ecology is species richness of the invaded community; the debate moves around whether rich communities would act as a buffer against invasion or as facilitators (Elton, 1958; Fridley *et al.*, 2007). The present analysis does not support any of these hypotheses, as the proportion of alien species was never influenced by species richness, either positively, or negatively.

Unexpectedly, no alien species were found in artificial habitats. These habitats were excluded from the analyses, and it is difficult to understand whether such absence could be due to artefacts (e.g. sampling bias) or to actual properties of the habitats (e.g. stochastic processes related to the short time scale of artificial lakes, energetic constraints to detritus-based food chains, which take time to be fully structured, additional human disturbance on the water regime to which alien species might not manage to adapt).

Perspectives

This study is, to our knowledge, the first describing the role of environmental and human variables across a wide diversity of freshwater organisms and habitats, in order to set conservation priorities. It is true that not all alien species will have economic or environmental impacts, and several alien species would not pose any threat to the conservation of biological diversity (Junqueira, 2013). Nevertheless, more species will have the probability of becoming invasive if more alien species were present, simply by chance, owing to numerical effects. Thus, it is pivotal to understand the large-scale mechanisms behind the occurrence of alien species, as attempted in the present study.

The strong influence of a variable such as temperature that may be correlated with propagule pressure on the occurrence of alien species in Italian freshwater habitats confirms previous indications that biological invasions may be potentially controlled and limited only by mitigating human activities in the environment (Pyšek *et al.*, 2010). This finding will pose socio-economic problems difficult to overcome, with requests for compromise between the maintenance of recreational activities in the wilderness and the preservation of a natural environment to prevent the arrival and spread of alien species. The results of the present analysis suggest that conservation strategies for freshwater habitats in temperate areas such as Italy should focus on the warmest and most accessible sites, regardless of habitat type, to maximize their efficiency and efficacy. Given that the present analysis could not disentangle the direct and the indirect effects of temperature on the occurrence of alien species, more focused analyses should try to assess their effect on invasibility. This will be the best way to understand whether conservation efforts should call for a compromise between the accessibility to water bodies for recreation and the need to prevent the spread of alien species, or if pure climatic variables have such a strong direct effect in overcoming the role of propagule pressure (Catford *et al.*, 2009).

Occurrence of alien species was similar in lentic and lotic habitats, thus their spread should be controlled with equal efforts in the two habitats, regardless of their different appeal for tourists and visitors (Muirhead and MacIsaac, 2005). Both pristine and polluted sites should also be equally monitored for freshwater invasive species, contrary to the expectations of increased susceptibility of disturbed habitats based on analyses of terrestrial plant communities (Crawley, 1987).

The effect of biological diversity either buffering or favouring alien species was not detected (Elton, 1958; Fridley *et al.*, 2007) in the present study since the probability of occurrence of alien species was not related to local species richness for any of the taxonomic groups. An additional implication of the present macro-ecological analysis is that conservation priorities should thus focus on all freshwater habitats without concentrating only on biologically rich water bodies.

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