Differential vulnerability to biological invasions: not all protected areas (and not all invaders) are the same

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Abstract

Protected areas (PAs) are fundamental for global biodiversity conservation but many are not delivering their conservation potential. In particular, the European Natura 2000 (N2K)—the largest coordinated network of PAs in the world—has insofar proved insufficient to achieve the EU’s biodiversity conservation targets. Despite the adoption of innovative legislation on the prevention and management of biological invasions, invasive alien species (IAS) remain a main threat to N2K. We explored whether the regulatory status of N2K has been efficient to prevent the establishment of regulated IAS (those under the scope of EU or national regulations) by conducting a case study in a highly biodiverse Mediterranean region of Spain. We: (1) analyzed whether the number of both regulated and unregulated IAS differ across adjacent unprotected areas (belt zones), N2K sites and N2K sites with additional protection as national park or nature reserve (APAs); (2) compared the spread pathways of regulated IAS present in areas with different protection status. While APAs hosted fewer regulated IAS, N2K sites did not perform better than belt zones. Specifically, there were fewer regulated IAS that spread through natural dispersal or intentional human assistance in APAs compared to N2K and belt zones, but those dispersing with unintentional human assistance were similarly distributed in PAs and belt zones. Further, protection level did not reduce the number of unregulated IAS. Thus, observed patterns indicate that the conservation obligations bound to the designation of an area as an N2K site are not sufficient to prevent or slow down biological invasions.

Keywords Natura 2000 Network · Invasive alien species · EU IAS Regulation · Socio-economic impacts · Invasion pathways · Conservation planning

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Introduction

Protected areas (PAs) play a critical role in global biodiversity conservation and consequently have experienced a pronounced expansion over time (Watson et al. 2014; Visconti et al. 2019). This trend was accelerated in the last decade, as the Aichi Target 11 of the Strategic Plan for Biodiversity 2011–2020 (Convention on Biological Diversity) called for conserving effectively through PAs at least 17% of terrestrial areas and inland waters, and 10% of coastal and marine areas by 2020. Following this line, proposals for the post-2020 conservation targets advocate the further growth of PA coverage worldwide to reverse negative biodiversity trends by 2030 (e.g., Woodley et al. 2019). However, some PAs do not achieve even basic goals, as biodiversity keeps declining within them (Watson et al. 2014).

Increasingly intense human pressure and environmental change appears the main underlying reason of low PA effectiveness (Watson et al. 2014; Jones et al. 2018; Schulze et al. 2018). Within impact drivers, invasive alien species (IAS) are perceived as one of the most serious threats to PAs globally (Pyšek et al. 2013; Schulze et al. 2018). Some studies conclude that PAs provide important protection from biological invasions, particularly those designated longer ago and with limited human activities (Gallardo et al. 2017; Liu et al. 2020). However, few PAs remain free from IAS, even when apparently unaffected by other anthropogenic disturbances (Pyšek et al. 2020). One reason might be that at large spatial scales the environmental and ecological factors that promote high native species richness in PAs might also promote high diversity of invaders (Lonsdale 1999; Stohlgren et al. 1999); that is, if native species and IAS respond similarly to favourable environmental and ecological conditions promoting high diversity, then PAs with high environmental heterogeneity and resource availability would allow more species of both invaders and natives to coexist (Peng et al. 2019). Particularly troublesome is the case of protected marine reserves, which according to the existing literature appear to have little effect on IAS or can even enhance the performance of IAS that were introduced before their establishment (Byers 2005; Burfeind et al. 2013). This may point to marine habitats being less resistant to biological invasions. PAs are not the first locations for the introduction of IAS, but once they colonize disturbed habitats in the surrounding belts, their spread into PAs is typical (Hiley et al. 2014; Holenstein et al. 2021). PAs with high human accessibility and population density are more vulnerable to biological invasions and require stronger management actions (Spear et al. 2013; Guerra et al. 2018; Pyšek et al. 2020).

A paradigmatic example is the European Natura 2000 (hereafter ‘N2K’), the largest global network of PAs, consisting of 27,312 sites that occupy over 18% of European Union’s (EU) land area and almost 6% of its marine territory. The N2K Network was set to ensure the long-term survival of Europe’s most valuable and threatened species and habitats, and was built upon the EU Nature Directives—The Birds Directive 2009/147/EC and the Habitats Directive 1992/43/EEC. N2K sites are designated by EU Member States (MS), which must define conservation goals for the relevant habitat and species present in each site and establish the appropriate measures to achieve them. Specifically, MS are required to address the threats posed by IAS in compliance with the requirements of both the Nature Directives and the EU Regulation on the prevention and management of the introduction and spread of IAS (EU Regulation 1143/2014; hereafter ‘IAS Regulation’) (see Baquero et al. 2021a). In many cases, nature or marine reserves, national parks, or other PAs—established under national or regional law for a range of different purposes—might be also designated as N2K sites because they are relevant areas for species and habitats of EU importance as well (hereafter ‘N2K sites with an
additional protection status’). In such cases, the provisions of the EU Nature Directives apply, unless stricter rules are in place under national law.

Despite the strict regulatory framework, N2K sites remain vulnerable to bioinvasions because they (1) are typically embedded in highly human-dominated landscapes, and (2) adopt a more inclusive conservation approach that permits human activities, which are recognized as an integral part of ecosystem functioning (Baquero et al. 2021a). As a result, recent studies have called into question the efficiency of management plans to prevent the spread of IAS into N2K sites (Gallardo et al. 2017; Guerra et al. 2018; Mazaris and Katsanevakis 2018; Baquero et al. 2021a; Christopoulou et al. 2021). Indeed, IAS accumulation rate in the N2K Network has accelerated in the last decade (Baquero et al. 2021b), and this trend is likely to continue as the accumulation of alien species is projected to rise markedly in Europe in the next decades (64% increase from 2005 to 2050; Seebens et al. 2021). Enhancing and enlarging the network of PAs is a cornerstone of the new EU Biodiversity Strategy for 2030 (EC 2020) to halt biodiversity loss in EU’s ecosystems. However, the aim of safeguarding EU biodiversity could be hindered if the regulatory status of the current and projected network of legally PAs is insufficient to prevent or even slow down biological invasions.

In accordance with the Nature Directives, N2K management plans should address any IAS that negatively affect protected species and habitats. Yet the IAS Regulation only requires MS to take action against those producing the highest ecological and economic impacts, i.e. those included in the so-called Union list (so far, only 66 species), while leaving MS the option to regulate other high-impact IAS at the national level through lists of IAS of MS concern (Baquero et al. 2021a). Listed IAS are subject to very strict restrictions to prevent their entry, and surveillance systems must be established to facilitate their early detection and rapid eradication, or to control their spread in case introduction occurs (Baquero et al. 2021a). However, it is unknown whether the regulatory status of the N2K Network has reduced the rate of introduction and establishment of IAS, especially of those regulated at the EU or national levels (i.e., those included in the Union list or regarded as IAS of MS concern).

We explore this issue in central Spain, located in the highly biodiverse Mediterranean biogeographical region and considered as one of the 25 biodiversity hotspots of the world, but subject to high levels of human pressure (https://www.cbd.int/countries/profile?country=es#facts). We specifically analyze whether (1) the number of both regulated and unregulated IAS differ across unprotected areas, N2K sites and N2K sites with an additional protection status, and (2) the invasion pathways of detected regulated IAS differ across areas with different protection status.

We framed our study on comparing IAS richness in core areas of PAs to their belt zones (which include peripheries of PAs and the unprotected areas adjacent to them) because IAS presence in PAs is strongly driven by the surrounding landscape (Genovesi and Monaco 2013) and edge effects can facilitate invasions (e.g., Madalozzo et al. 2016). Previous studies have shown that the composition and richness of the community of IAS within PAs is affected by the IAS pool present in their surrounding areas (e.g., Spear et al. 2013; Holenstein et al. 2021). In this context, we hypothesise that PAs should:

1. Host fewer regulated IAS than their belt zones.
2. Accumulate fewer naturally dispersing regulated IAS because PAs are required to have monitoring plans coupled with rapid eradication schemes (according to IAS Regulation
and Spanish legislation), which are more easily tailored towards species already known to be dispersing.

(3) Host fewer regulated IAS that mainly spread through intentional human assistance because their deliberate introduction, transport or confinement by humans is illegal under the EU and Spanish regulations, and consequently local authorities are enforced to impose stricter controls, especially in PAs.

(4) Not necessarily be more effective in holding back regulated IAS that spread through unintentional human assistance than their belt zones; such IAS are more difficult to detect and control before they are widely established because the exact paths through which they spread are typically less well documented or even unknown, being often identified only a posteriori (Essl et al. 2015).

Methods

Study area

The region of Castilla-La Mancha (CLM; 79,409 km²) includes 72 Special Areas of Conservation (SACs; designated under the Habitats Directive) and 39 Special Protection Areas (SPAs; designated under the Birds Directive) whose total area represents 23% of the regional land area (well above the 17% coverage required by Aichi Target 11) and 14% of the total area of Spanish N2K sites. Further, the N2K sites of the study area account for 2.3% of the whole European network, a contribution similar to that of countries such as Portugal (2.4%), Hungary (2.5%) or Croatia (2.6%), and higher than that of 14 other EU countries (Natura 2000 Barometer 2019, https://www.eea.europa.eu/data-and-maps/dashboards/natura-2000-barometer). Thirty per cent of the total area of N2K sites has an additional national or regional protection status, as they were declared national parks or nature reserves long before being included in the N2K Network (Fig. 1).

Data collection and species characterisation

We defined IAS as those species whose introduction and/or spread outside their natural past or present distribution threatens biological diversity, following the Convention on Biological Diversity of 5 June 1992. We built on the database developed and used by Guerra et al. (2018), wherein IAS occurrence records were collected from four sources: (1) biodiversity databases of environmental agencies that included up-to-date information from all wildlife monitoring programmes conducted by the CLM and Spanish governments. The database was updated with the most recent observations (2018–2021) from the regional monitoring schemes; (2) peer-reviewed scientific literature and reference books, and interviews with managers and experts for each taxonomic group; (3) existing global databases, such as the Global Biodiversity Information Facility (GBIF) and the European Information Network of Exotic Species (EASIN); and (4) extant management plans of analysed N2K sites. Occurrence data were geographically referenced to a grid of 905 10×10 km² cells (hereafter ‘IAS-presence map’).

We then grouped recorded IAS into four ‘Impact-Regulation Status’ categories: (1) IAS included in the Union list and thus regulated at the EU level (hereafter ‘Regulated-EU IAS’); (2) IAS not included in the Union list but listed in the Spanish Catalogue of IAS (Royal Decree 630/2013) and thus regulated at the national level (hereafter
Fig. 1 Map of the study area indicating the location of the Natura 2000 Network (N2K). N2K sites are shown in brown while sites with additional protection status (APAs) are shown in blue. The location of Castilla-La Mancha within Spain is displayed in the top-left map.

‘Regulated-Spain IAS’); (3) IAS identified by Nentwig et al. (2018) as those with the highest environmental and socioeconomic impacts, but not included in either the Union list or the Spanish Catalogue of IAS, and are therefore unregulated (hereafter ‘High-Impact Unregulated IAS’); and (4) other unregulated IAS that produce weaker impacts, that is, all those recorded IAS not included in any of the previous three categories (hereafter ‘Unregulated IAS’). We calculated the number of IAS from each Impact-Regulation Status category for each grid cell of the IAS-presence map.

Finally, we characterised the introduction and spread pathways of regulated IAS using the information provided in the European Environment Agency IAS portal (https://ias.eea.europa.eu/products/european-statistics). For each recorded IAS, we indicated whether the species predominantly entered/spread into/within Spain (1) through natural dispersal, (2) with unintentional human assistance, and (3) with intentional human assistance. (Note that these pathway categories are not mutually exclusive for IAS and a given species can enter into or spread across the territory through more than one general pathway; in this case, the species was counted in every category it belonged to.) We calculated the number of IAS characterised by each introduction and spread pathway for each grid cell of the IAS-presence map.

**Data analysis**

We conducted a gap analysis (Araújo 2004) to compare the actual performance of the N2K Network in the study area with the intended performance of the conservation network regarding biological invasions (preventing the spread and establishment of IAS to avoid the deterioration of natural habitats and the significant disturbance of the protected species for which the sites were designated). To do this, we overlapped a GIS layer of protected sites in the study area N2K Network and N2K sites with additional protection.
status (national parks and nature reserves) with the IAS-presence GIS map. To define the protection status of cells, we first calculated the proportion of each 10 × 10 km² grid cell covered by protected sites using GIS techniques (QGIS version 3.12.1; QGIS Development Team 2020). We considered a grid cell as ‘protected’ when this proportion was over 60% (Araújo 2004; Abellán and Sánchez-Fernández 2015). Within protected cells, we differentiated between those having (additional protection areas; APAs) and not having (N2K) extra protection status. We considered cells with overlapping values between 1 and 60% as ‘belt zones’. Cells totally outside the N2K Network (< 1%) were not used in the analyses.

We first examined whether a systematic spatial variation in IAS richness was present in the study area (that is, if IAS distributions were spatially clustered and thus not independent), which could affect statistical inference. We computed Moran’s I coefficient using the Ape R package version 5.5 (Paradis et al. 2021) as a measure of spatial autocorrelation in the distribution of IAS from each Impact-Regulation Status in the study area. (Moran’s I values range between −1 and +1, with values significantly below the expected value indicating negative autocorrelation, while values significantly over the expected value indicating positive autocorrelation.) We found a very weak (though significant; \( p < 0.05 \)) positive spatial autocorrelation pattern in the distribution of Regulated-EU (observed \( I = 0.039 ± 0.002 \) vs. expected \( I = −0.001 \)), Regulated-Spain (observed \( I = 0.072 ± 0.002 \) vs. expected \( I = −0.001 \)), High-Impact Unregulated (observed \( I = 0.046 ± 0.002 \) vs. expected \( I = −0.001 \)) and Unregulated IAS (observed \( I = 0.017 ± 0.002 \) vs. expected \( I = −0.001 \)). Since observed patterns were pretty weak, we did not account for spatial autocorrelation in subsequent analyses.

To compare the number of IAS from each Impact-Regulation Status, and introduction/spread-pathway category among APA, N2K and belt-zone cells, we performed Kruskal–Wallis rank sum tests and subsequent Dunn’s post hoc tests with Holmes adjustment of \( p \) values for multiple comparisons. There were large differences in sample size across cell types (39 APA cells and 60 N2K cells); thus, we randomly selected 60 out of the 452 belt-zone cells to perform each test in order to handle a more balanced design, as rank-based tests with unequal sample sizes can produce errors under some circumstances (e.g., Brunner et al. 2021). Since 87 out of the 99 protected cells included freshwater ecosystems, belt-zone cells were randomly selected maintaining the same proportion of land/freshwater habitats (88% of cells presenting freshwater ecosystems, 12% without freshwater ecosystems). We repeated the analyses 100 times to account for stochasticity in the selection of belt-zone cells. We also estimated the effect size of each test to enhance the interpretability of the magnitude of the effect of protection status on IAS numbers and thus provide an estimate of its biological importance (Nakagawa and Cuthill 2007); for this purpose, we used Glass’ rank-biserial correlation \( r_{rb} \), setting the cell type with the lowest protection status as the control group. We used the DescTools R package version 0.99.41 (Signorell 2021) to perform the statistical comparisons and the effectsize R package version 0.4.4-1 (Ben-Shachar et al. 2020) to compute the effect sizes. All statistical analyses were performed with R version 4.0.4 (R Core Team 2021).
Results

Effectiveness of PAs to prevent the establishment of IAS differing in their impacts and regulatory status

We compiled a grand total of 3706 georeferenced records for 96 IAS, out of which 8 were included in the Union list and 34 in the Spanish Catalogue of IAS, 6 were identified as High-Impact Unregulated IAS, and 48 as Unregulated IAS (Fig. 2; Online Resource 1).

In 61% of replicated analyses, there were significant differences in the number of IAS regulated at the EU level among cell types ($p$ range 0.0007–0.10; Fig. 3), being significantly lower in APA than in belt-zone cells (mean ± s.d. 0.513 ± 0.885 vs. 0.959 ± 1.055).
and only marginally lower than in N2K cells (0.513 ± 0.885 vs. 0.900 ± 0.969; \(p_{\text{adjusted}}\) range 0.041–0.11); the mean effect sizes were moderate but significant (Table 1). The number of nationally regulated IAS was also lower in APA than in N2K (1.205 ± 0.864 vs. 2.083 ± 2.367) and belt-zone cells (1.205 ± 0.864 vs. 2.548 ± 2.183), although differences were statistically significant only in 15% of replicated analyses (\(p\) range 0.003–0.71; Fig. 3) and the mean effect sizes were small and not significant (Table 1). We did not detect significant differences in the number of High-Impact Unregulated IAS in any of replicated analyses (\(p\) range 0.066–1; Fig. 3), with similar values across APA, N2K and belt-zone cells (0.359 ± 0.707 vs. 0.333 ± 0.655 vs. 0.344 ± 0.582) and the mean correlations \(r_{\text{rb}}\) close to zero (Table 1). In 24% of replicated analyses, there were significant differences in the number of Unregulated IAS among cells (\(p\) range 0.0009–0.93; Fig. 3), being lower in belt-zone than in APA (1.291 ± 1.561 vs. 1.795 ± 1.765) and N2K cells (1.291 ± 1.561 vs. 2.050 ± 2.404), though mean correlation coefficients were small and not significant (Table 1).

**Effectiveness of PAs to prevent the establishment of IAS differing in their introduction and spread pathways**

Almost all regulated IAS recorded in the study area entered into Spain with intentional human assistance (38 out of 42 regulated species, although five of them also have other
Table 1  Rank-biserial correlation coefficients ($r_{rb}$) for the non-parametric tests of differences in the number of invasive alien species of each Impact-Regulation Status category and introduction/spread pathways between cells with different protection status (belt zones, Natura 2000 and Natura 2000 with additional protected areas)

| Variables                             | APA–N2K          | APA–Belts        | N2K–Belts         |
|---------------------------------------|------------------|------------------|------------------|
| Regulated-EU                          | $-0.26$ ($-0.45$ to $-0.06$) | $-0.25$ ($-0.44$ to $-0.05$) | $-0.03$ ($-0.21$ to $0.16$) |
| Regulated-Spain                       | $-0.16$ ($-0.36$ to $0.05$)   | $-0.11$ ($-0.30$ to $0.09$)   | $-0.05$ ($-0.25$ to $0.15$) |
| High-Impact Unregulated               | $-0.02$ ($-0.20$ to $0.16$)   | $0.00$ ($-0.18$ to $0.18$)    | $-0.02$ ($-0.18$ to $0.14$)  |
| Unregulated                           | $0.18$ ($-0.04$ to $0.39$)    | $-0.01$ ($-0.23$ to $0.21$)   | $0.17$ ($-0.02$ to $0.36$)   |
| Introduction natural dispersal        | $-0.03$ ($-0.12$ to $0.08$)   | $-0.02$ ($-0.10$ to $0.08$)   | $-0.01$ ($-0.10$ to $0.08$)   |
| Introduction unintentional human assistance | $0.03$ ($-0.16$ to $0.22$)    | $0.00$ ($-0.20$ to $0.19$)    | $0.03$ ($-0.13$ to $0.20$)    |
| Introduction intentional human assistance | $-0.21$ ($-0.42$ to $-0.01$)  | $-0.21$ ($-0.42$ to $-0.01$)  | $-0.03$ ($-0.23$ to $0.17$)   |
| Spread natural dispersal              | $-0.29$ ($-0.48$ to $-0.09$)  | $-0.17$ ($-0.38$ to $0.03$)   | $-0.12$ ($-0.31$ to $0.08$)   |
| Spread unintentional human assistance | $-0.12$ ($-0.32$ to $0.08$)   | $-0.09$ ($-0.29$ to $0.12$)   | $-0.03$ ($-0.22$ to $0.16$)   |
| Spread intentional human assistance   | $-0.19$ ($-0.39$ to $0.02$)   | $-0.19$ ($-0.39$ to $0.02$)   | $-0.02$ ($-0.22$ to $0.18$)   |

The mean value of the 100 replicates and their 95% confidence intervals (CI) are shown. Significant correlations (zero not included in the CI) are marked in bold.
There were no significant differences in the number of IAS introduced through natural dispersal ($p$ range 0.089–0.94) or unintentional human assistance ($p$ range 0.24–1) across cell types (Fig. 4), and the mean correlations $r_{rb}$ were close to zero (Table 1). In 25% of replicated analyses there were significant differences across cell types in the number of IAS introduced with intentional human assistance ($p$ range 0.0001–0.26; Fig. 4), which were lower in APA than in N2K cells ($1.564 \pm 1.334$ vs. $2.833 \pm 3.065$; $p_{\text{adjusted}}$ range 0.065–0.31) and significantly lower than in belt-zone cells ($1.564 \pm 1.334$ vs. $3.366 \pm 3.548$), the mean effect sizes being moderate but significant in both cases (Table 1).

Most regulated IAS generally spread through two (27 species) or all three (7 species) general pathways and only 8 species spread through a unique pathway (Online Resource 1). In 55% of replicated analyses, the number of regulated IAS that spread through natural dispersal differed across cell types ($p$ range 0.0004–0.31; Fig. 4), being significantly lower in APA than in belt-zone cells ($0.974 \pm 1.328$ vs. $2.647 \pm 3.020$), showing a moderate but significant mean effect size (Table 1). Likewise, in 18% of replicated analyses ($p$ range 0.0008–0.32; Fig. 4), APA cells contained significantly fewer regulated IAS that spread with intentional human assistance than belt-zone cells ($1.513 \pm 1.254$ vs. $3.268 \pm 3.387$), although in this case the mean effect size was smaller and marginally significant (Table 1). By contrast, we did not detect significant variations in the number of regulated IAS that spread with unintentional human assistance ($p$ range 0.054–0.75; Fig. 4), and the mean correlations $r_{rb}$ were small and not significant (Table 1).

**Fig. 4** Number of IAS of each introduction (I) and spread (S) pathways categories—natural dispersion, unintentional and intentional human assistance (HA)—in the 10 $\times$ 10 km² grid cells from belt zones (Belts), Natura 2000 sites (N2K), and Natura 2000 with APAs. The graphic displays one of the 100 replicates, representing the most frequent distribution of belt-zone values. The graphic shows the median value and the first and third quartiles, which correspond with the lower and upper hinges of the box; the whiskers extend from the hinges 1.5 times the inter-quartile range. White circles indicate records beyond those limits. The maximum number of IAS of each category recorded in a belt, N2K or APA cell was 2 (I Nat Disp), 3 (I Unintentional HI), 15 (I Intentional HI), 12 (S Nat Disp), 6 (S Unintentional HI) and 14 (S Intentional HI).
Discussion

Effectiveness of protected areas and regulatory status of IAS

Our first hypothesis—areas with higher protection levels would host fewer regulated IAS—was only partially corroborated, as we observed a weak pattern of APAs hosting fewer regulated IAS than N2K sites and their belt zones, but no differences between N2K sites and their surrounding belts. Our analyses on IAS regulated at EU or national levels are in line with the study of Gallardo et al. (2017) on the 100 IAS with the highest impacts (irrespective of their regulatory status) at the European scale, which found that nationally designated PAs hosted on average less than half the number of IAS than N2K sites.

Despite the fact that threats of IAS on PAs is accelerating worldwide (Pyšek et al. 2020), PAs in general have proved effective in resisting biological invasions, especially those designated earlier (Pyšek et al. 2003; Gallardo et al. 2017; Liu et al. 2020)—typically found in isolated locations free from anthropogenic disturbance (Gaston et al. 2008), or subject to intensive and sustained conservation management (Genovesi and Monaco 2013; Shackleton et al. 2020). Vulnerability of PAs to bioinvasions is more likely a function of the magnitude of propagule pressure than biotic resistance of resident communities (Hulme et al. 2014; Peng et al. 2019). Therefore, low human accessibility (Foxcroft et al. 2011; Gallardo et al. 2017; Guerra et al. 2018) and restriction of human activities and management of their impacts both within PA boundaries and their surrounding belts (Genovesi and Monaco 2013; Liu et al. 2020) are key conditions to keep IAS at bay.

In our study region, APAs were national parks or nature reserves declared long before being designated as N2K sites, so they had remained under limited human influence and been intensively managed for conservation over several decades. By contrast, the similar number of regulated IAS registered in N2K sites and their belt zones could be explained by their recent designation, high human accessibility, and permissive management that allows low-intensity activities. However, due to the correlational nature of our study, we cannot ascribe the observed patterns to any specific mechanism.

We also observed that PAs hosted more alien species of low impact that their belt zones. Thus, taken as a whole, the total number of IAS were similar in protected areas and unprotected belt zones. In general, ecological factors that promote high native biodiversity may also facilitate biological invasions, so that higher richness of both native and alien species is expected in ecosystems of high resource availability and heterogeneity (e.g., Stohlgren et al. 2003; Landi et al. 2020). For example, Carpio et al. (2017) showed that PAs in the Iberian Peninsula are conflict hotspots for terrestrial vertebrates—as they are highly diverse and accommodate a high number of alien species—and thus must be intensively managed. We hypothesize that stricter conservation and management in APAs might have contributed to control the number of regulated IAS, while that was not the case in N2K sites. However, this is difficult to assess because the year of first record of IAS in PAs and adjacent belts are not available; therefore, it is not possible to establish whether APAs are actually holding back biological invasions or slowing them down, or simply some IAS at the belts have not had yet the time to colonize the adjacent APAs.

It is also important to note that we have studied IAS richness but not abundance (as those data are not available for most species), so we cannot contrast the invasion levels across cells with different protection status. Further, we cannot know the regulatory status of the most abundant IAS. Holenstein et al. (2021) detected that the most invasive species, having features that permit rapid colonization and spread, were four times more abundant
within Norwegian PAs’ surrounding belts than other alien species, and consequently were also overrepresented within PAs. Therefore, assessing the effectiveness of the protection status of PAs to hold back biological invasions in our study area would require the analysis of not only richness of regulated IAS but also of their relative abundance compared to unregulated IAS.

**Effectiveness of protected areas and introduction and spread pathways of regulated IAS**

Again, our second and third hypotheses were corroborated only for APAs but not N2K sites. There were fewer species that spread through natural dispersal or intentional human assistance in APAs than in N2K and belt zones. However, the trend in IAS that disperse intentionally assisted by humans was weak and the magnitude of the effect only marginally significant. The 80% of regulated invasive alien animals (both vertebrates and invertebrates) recorded in CLM that spread through either of these general pathways live in, or in connection to, aquatic habitats (Online Resource 1). Therefore, the failure of N2K sites—and the barely moderate success of APAs—to prevent or slow down IAS introduction and establishment might be linked to the freshwater ecosystems therein (88% of protected grid cells), since most of the N2K sites in CLM were designated on the basis of threatened aquatic species or habitats (Sánchez-Fernández et al. 2021). Water bodies such as rivers, lakes or wetlands are documented corridors for IAS colonization of PAs (Foxcroft et al. 2011, 2019; Vardarman et al. 2018; Holenstein et al. 2021), and controlling this pathway requires intensive management outside PA boundaries (Genovesi and Monaco 2013). Invasive fishes that were historically introduced for recreational fishing and are currently widespread and dispersing unaided clearly illustrate this issue (Muñoz-Mas and García-Berthou 2020).

In general, the extensive presence of many regulated IAS in CLM is a legacy of the past; they were deliberately (though legally) released under more permissive regulations by authorities long ago for economic, recreational or ornamental reasons (as it was often the case everywhere; Pyšek et al. 2020), and illegally by other stakeholders (e.g., anglers). Those IAS are currently dispersing through natural mechanisms that are hard to control and management plans in N2K sites (designated just a few decades ago) have been ineffective to eradicate them, partly because of inadequate monitoring and data quality (Guerra et al. 2018).

In contrast to the aforementioned general pathways, we did not detect differences in the number of regulated IAS spreading with unintentional human assistance between protected and unprotected areas—as we had hypothesised. Most of these IAS are also dispersing naturally, with almost all regulated invasive alien plants (IAP) falling into this casuistry. Most regulated IAP were introduced as ornamentals and the propagule pressure from outside PAs is now difficult to hold back; they disperse naturally and also assisted unintentionally by humans, especially through farming, road networks and tourism.

**Conclusion and implications for conservation and management**

Our study reveals a pattern towards nationally or regionally designated PAs hosting fewer regulated IAS than their surrounding belts, at least of those species that are dispersing unaided or with intentional human assistance. However, we detected in general many
regulated and unregulated IAS in belt zones, which could represent a risk of invasion in the future—assuming that study PAs are not the focal points of IAS introduction but are being colonized from their belts (which we have not assessed). The increasing pressure of IAS in areas surrounding PAs is a global phenomenon (Liu et al. 2020) and their relevant role in shaping communities inside PAs through colonization events (Vardarman et al. 2018; Holenstein et al. 2021) emphasises the need for prompt actions beyond PA boundaries (Genovesi and Monaco 2013; Donaldson et al. 2017), especially for those PAs close to highly populated areas (Spear et al. 2013). Therefore, management plans of PAs should consider the establishment of regulated buffer areas of land managed to impede bioinvasions.

Contrary to APAs, we did not observe a better performance of N2K sites compared to their adjacent unprotected areas in terms of IAS richness, in agreement with previous studies (Gallardo et al. 2017; Mazaris and Katsanevakis 2018; Christopoulou et al. 2021). In N2K, where human activities such as farming, forestry, tourism, fishing, hunting and other recreational uses are allowed, identifying and controlling intentional and unintentional pathways of IAS introduction and spread is critical. While the EU IAS Regulation is certainly a pathway-focused policy, it requires MS to take priority action only on pathways of IAS regulated at the EU level, which does not cover the management needs at the local level. Thus, managing pathways successfully calls for the establishment and enforcement of social norms, best practices and codes of conduct within involved stakeholders and local communities (Tollington et al. 2017; Baquero et al. 2021b). Pathway management must be coupled with intensive surveillance to rapidly detect new IAS incursions (Baquero et al. 2021a). In this context, initiatives to raise public awareness of social responsibility and to increase citizen engagement and contribution to collaborative surveillance are necessary to enhance IAS management in N2K (Tollington et al. 2017; Baquero et al. 2021b).

Finally, the fact that neither APAs nor N2K sites were effective against High-Impact Unregulated IAS emphasises the need to develop legally binding lists of IAS of national concern that are sufficiently representative and dynamic to manage the IAS of high impact present in a territory.

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Data availability The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare.
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