

## Article

# Updating the National Baseline of Non-Indigenous Species in Spanish Marine Waters

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**Abstract:** The introduction of new non-indigenous species (NIS) in Spanish marine waters is addressed under Descriptor 2 of the European Union's Marine Strategy Framework Directive. National baseline inventories of NIS have been compiled and updated for the three subregions (Western Mediterranean Sea, WMED; Bay of Biscay–Iberian Coast, ABI; Macaronesia, AMA) with data from 1800 to 2021. An overall of 574 species were identified with an alien, cryptogenic, crypto-expanding, or debatable status, mostly invertebrates (~65%) and primary producers (~22%). Of 412 alien species, 80.51% were reported in ABI, 67.82% in WMED, and 66.67% in AMA. Cryptogenic species are more abundant in the WMED (25.25%), compared to AMA (19.77%) and ABI (18.46%). ABI harbors more established species (62.56%) than AMA (45.2%) and WMED (43.56%), contrary to casual records (AMA 31.64%, WMED 23.76%, ABI 13.85%). Invasive species are more abundant (14.36%) in WMED. The 'transport-stowaway' pathway accounted for 142 (79.33%), 123 (67.58%), and 169 (85.21%) records in WMED, ABI, and AMA, respectively. The second most common pathway was 'transport-contaminant' related to mariculture (~10% of the total), prevalently in ABI with 42 species (23.08%). The Canary Islands stand out for species introduced through oil platforms from throughout the world. 'Unaided' was a relevant pathway of secondary introduction into the WMED, particularly of Lessepsian species progressing westwards. Temporal trends in newly introduced species show similar behavior among subregions.

**Keywords:** alien species; national NIS inventories; Mediterranean Sea; northeast Atlantic; Descriptor 2; Marine Strategy Framework Directive

## 1. Introduction

The ongoing increase in anthropogenic activities in the marine environment is critical for facilitating the introduction and dispersion of non-indigenous species (NIS) [1,2], which are considered a major threat to global biodiversity and ecosystem function [3,4]. Among NIS, some species show an invasive behavior that often implies severe impacts on the

ecosystem through rapid increases in their abundance and the extent of their distribution [5], also involving socio-economic and health impacts [6]. However, it is a challenging task to predict which species will become invasive during the early stages of the introduction process in order to mitigate the impact [5]. Still, positive effects may also be attributed to NIS by creating new ecosystem functions [7,8], or generating new values such as fishery resources [9], among others [10,11].

Anthropogenic pressures and related impacts on marine ecosystems are addressed in European policies such as the Marine Strategy Framework Directive (MSFD) (2008/56/EC) to achieve the good environmental status (GES) of Member States' marine waters through the assessment of 11 qualitative descriptors. Non-indigenous species introduced by human activities are considered under Descriptor 2 in order to assess levels that do not adversely alter the ecosystems (Commission Decision (EU) 2017/848). Specifically, the number of NIS which are newly introduced via human activity in the wild is addressed under the primary criterion of this descriptor (D2C1), in which Member States shall establish threshold values for the number of new introductions of NIS to analyze the trend per assessment period (six years) within the marine region or subregion concerned.

To implement the Member States' obligations, the MSFD (article 4) establishes European marine regions and subregions on the basis of geographical and environmental criteria. Within this classification, Spanish marine waters are incorporated into the subregions of the Western Mediterranean Sea (WMED), the Bay of Biscay and the Iberian Coast (ABI), and Macaronesia (AMA). A further categorization of five marine reporting units (MRU) is performed at the national level for Spanish coastal waters: (i) the Strait and Alboran, and (ii) the Levantine–Balearic MRU, both included in the WMED subregion; (iii) the North and (iv) the South Atlantic MRU, encompassed in ABI; and (v) the Canary Islands as part of the AMA subregion.

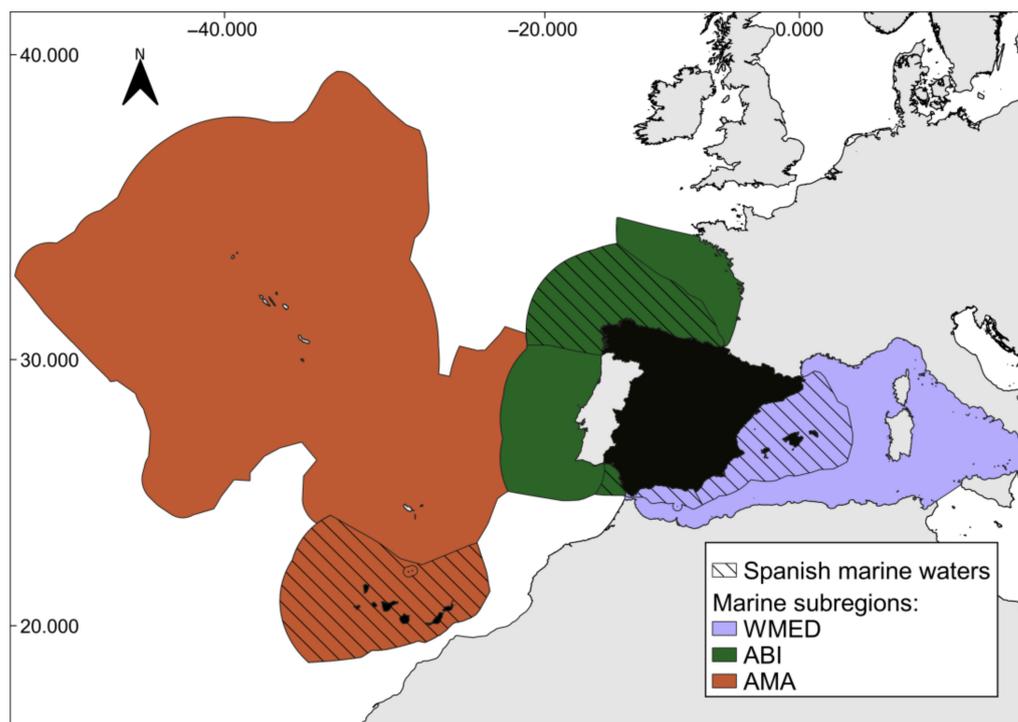
National baseline inventories of marine NIS are essential for analyzing trends in the introduction and associated pathways and serve to understand the invasion process at subregional [12] and regional scales [13,14]. In order to accomplish the GES of marine waters, Spain—through the collaboration of national NIS experts—participates in several established forums at a (sub)regional extent: (i) the Protection of the Marine Environment and Coastal Region of the Mediterranean (Barcelona Convention) that aims to implement the ecological approach process of Mediterranean countries in line with the MSFD, for which regional baselines are coordinated by the Regional Activity Centre for Specially Protected Areas; and (ii) the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention), which compiles and coordinates the data for the NE Atlantic region. Furthermore, the national inventories of EU countries are coordinated by the Joint Research Centre of the European Commission.

The initial Spanish baseline was compiled by Tsiamis et al. [15] with data from 1970 to 2011 for the MSFD-D2 implementation, which was subsequently updated until 2017 [16]. For the present review, existing inventories were upgraded with new data up to 2021, according to recently published literature and major national reviews.

The current work provides an updated list of marine NIS in Spanish waters and analyzes trends of new introductions and pathways at subregional and national levels.

## 2. Materials and Methods

Records of NIS in Spanish marine waters are stored in a database fed by scientific publications, monitoring programs, and citizen science, as well as grey literature (e.g., technical reports, conference proceedings, and Ph.D and M.Sc. theses). Data were compiled for the Spanish coastline including the mainland and overseas territories (i.e., Canary Islands), and divided into each MSFD subregion comprising Spanish waters (i.e., WMED, ABI, and AMA; Figure 1).



**Figure 1.** Spanish marine waters delimitation according to the MSFD subregions. WMED = Western Mediterranean, ABI = Bay of Biscay–Iberian Coast, AMA = Macaronesia.

The dataset collected all detected NIS involving the date of first observation and related reference, status of the species, establishment success, and the most likely primary pathway of introduction into the subregion. Oligohaline species were included when that were recorded in estuarine or coastal areas of the marine subregion. The time period considered the first detected introduction in 1800 until December 2021. In cases where a time range was reported (e.g., sampling duration), the introduction date was set for the first year of the period. Additionally, whenever the exact date was not provided, the publication date was accepted as the first record date.

Species nomenclature was revised following the World Register of Marine Species [17] and the European Alien Species Information Network [18] was consulted for updates on the alien status of the species. Particular attention was paid to the alien status of each species in relation to their native distribution according to the following criteria adopted by UNEP/MAP [19]:

**Alien:** species with clear evidence of their non-native origin, even if they are native in a neighboring MSFD subregion, and strong indication of an anthropogenic mode of introduction.

**Cryptogenic:** species that cannot be demonstrably classified as native or non-indigenous in a particular region.

**Crypto-expanding:** species with some evidence of their non-indigenous status but uncertain due to an unclear mode of introduction from the native range (i.e., natural range expansion vs. human-mediated expansion).

**Debatable:** species with unresolved taxonomic status, e.g., species complexes, suspected undescribed native species, or species where taxonomic experts' opinions differ.

The establishment success of the species was further assessed at the subregional level with different categories:

**Established:** species with at least a self-maintaining population currently known to occur in the wild. Includes locally established species.

**Casual:** species with only a single or a few specimens recorded with no evidence of reproduction or spread.

**Invasive:** species with evidence of large populations, rapid spread, and potentially documented impacts on biodiversity and ecosystem services.

**Questionable:** species records with insufficient information or with uncertain identification, whose presence in the country needs to be confirmed (by re-examination of material if available).

**Unknown:** species with few records, where reporting lags may conceal their true establishment status as well as old records whose recent population status is not reported.

Suspected pathways of first introductions were assigned to each species at the sub-regional level following the Convention on Biological Diversity definitions [20], which categorization was reviewed by Pergl et al. [21], along with the certainty level of the primary pathway (i.e., high, medium, low):

**Release** in nature refers to the intentional introduction of living alien organisms for the purpose of human use in the natural environment (i.e., fishery in the wild—including game fishing—, other intentional releases), and the accidental or irresponsible release of live organisms from confinement into the natural environment (i.e., pet/aquarium/terrarium species—including live food for such species—).

**Escape** refers to the accidental escape of live organisms from confinement into the natural environment (i.e., aquaculture/mariculture, botanical gardens/zoos/aquaria—excluding domestic aquaria—, live food and live bait).

**Transport-Contaminant** refers to the unintentional movement of live organisms as contaminants of a commodity that is intentionally transferred through international trade, development assistance, or emergency relief (i.e., nursery material, contaminant on animals/plants—except parasites, species transported by host/vector—, parasites on animals—including species transported by host/vector—).

**Transport-Stowaway** refers to the moving of live organisms attached to transporting vessels, associated equipment and media (i.e., angling/fishing equipment, hitchhikers on ship/boat—excluding ballast water/hull fouling—, ship/boat ballast water, ship/boat hull fouling, organic packing material and other means of transport).

**Corridor** refers to the movement of alien organisms into a new region following the construction of transport infrastructures in whose absence spread would not have been possible (i.e., interconnected waterways/basins/seas).

**Unaided** refers to the secondary natural dispersal across borders of alien species that have been previously introduced by means of any of the foregoing pathways. Vagrant and range-expanding species (i.e., shifts in range distribution induced by climate change) were excluded from the inventories during the revision and validation process, and, therefore, not included in the analyses.

**Unknown:** the primary pathway cannot be found in the literature.

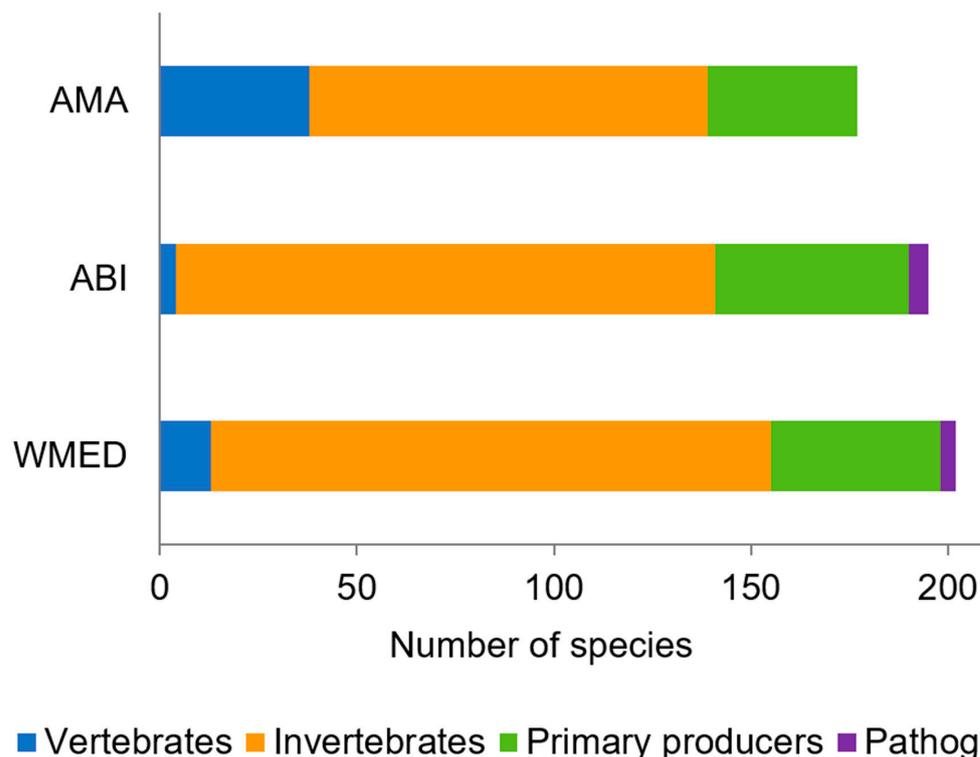
Lastly, the national experts who were appointed as coauthors of the current work reviewed the list of species, verified items such as first record citations, designated status, pathways of introduction, and any further records based on national data. The assessment of national inventories was further addressed by international experts from the D2-Online Working Groups in order to solve uncertainties at (sub)regional scales.

For the temporal trends study, all data regarding both the number of species introduced per year and the pathways of introductions since 1970 were included separately within each subregion, with no species status discrimination. To analyze the trend in the number of non-indigenous species introductions over the years, a generalized linear model (GLM) with a Poisson distribution was employed to investigate the relationship between the number of introductions as the dependent variable, and the year of introduction as the independent variable. A Chi-square test ( $\chi^2$ ) was applied to assess the goodness-of-fit of the models, at the 0.05 significance level. The GLM models were fitted using maximum likelihood estimation, with the year of introduction as a predictor variable. Data before 1970 were counted to represent the number of introductions before this period but were not included in the analysis. Patterns on the pathways of introductions over the years were graphically represented to visualize trends between subregions and among the years.

Statistical analysis and graphical representations were conducted using R version 4.2.2 [22], RStudio version 2023.3.0.386 [23], and the tidyverse package [24].

### 3. Results

An overall of 574 species were identified across Spanish marine waters by December 2021, including alien, cryptogenic, crypto-expanding, and debatable species (Supplementary Table S1). In general, the major proportion of introductions corresponded with invertebrates (~65%), followed by primary producers (macroalgae and microalgae: ~22%) (Figure 2).



**Figure 2.** Number of introduced species detected by 2021 in Spanish marine waters included in the subregions WMED = Western Mediterranean, ABI = Bay of Biscay–Iberian Shelf, and AMA = Macaronesia.

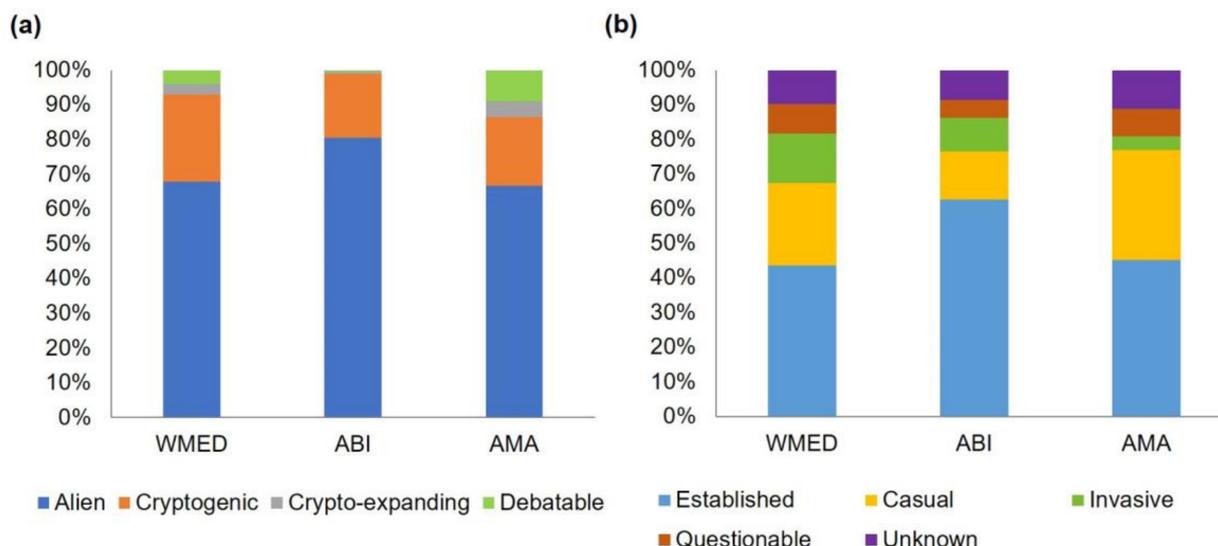
Marine waters encompassed in the WMED subregion registered 202 introductions with 179 species detected since 1970, including 142 invertebrates (70.30%), 43 primary producers (21.29%), 13 vertebrates (6.44%), and 4 pathogens (1.98%) (Table S1: WMED; Figure 2). Within invertebrates, annelids and mollusks constituted the main groups, both with 33 records.

The ABI subregion hosts 195 species, 182 of which have been introduced since 1970. Most of them are invertebrates (137 taxa = 70.26%), principally represented by 42 species of crustaceans and 34 species of mollusks, followed by primary producers (49 taxa = 25.13%), pathogens (5 taxa = 2.56%), and vertebrates (4 taxa = 2.05%) (Table S1: ABI; Figure 2).

The Canary Islands, as part of the AMA subregion, reported 177 species, holding 101 invertebrates (57.06%), 38 vertebrates (21.47%), and 38 primary producers (21.47%) (Table S1: AMA; Figure 2); of these, 169 species have been detected since 1970. In this case, tunicates and bryozoans are the major components of the invertebrate fauna, with 29 and 27 taxa, respectively.

Regarding the validated status of the species (Figure 3a), the Spanish baseline included 412 alien species, of which 80.51% were reported in ABI, 67.82% in WMED, and 66.67% in AMA. On the contrary, cryptogenic species are more abundant in the WMED (25.25%), compared to AMA (19.77%) and ABI (18.46%). To a lesser extent, crypto-expanding species

were detected with more frequency in AMA (4.52%), followed by WMED (2.97%) and ABI (0.51%). It is also important to note that there is a larger number of debatable species in AMA (9.04%) that needs further assessment in order to resolve their alien status.



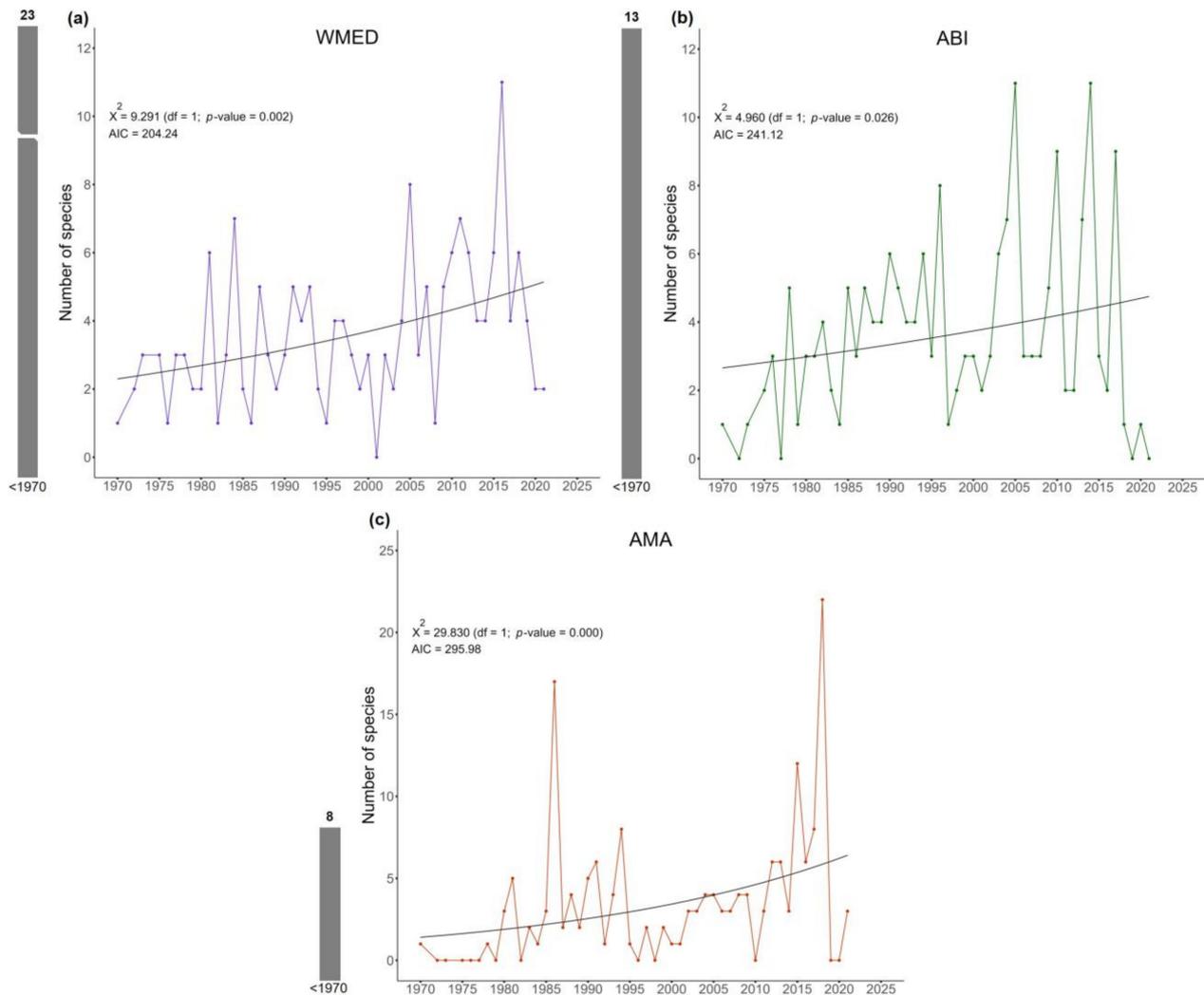
**Figure 3.** Proportion of NIS attending to (a) the status and (b) the establishment success validated by national experts for the Western Mediterranean (WMED), Bay of Biscay–Iberian Coast (ABI), and Macaronesia (AMA) subregions.

For establishment success (Figure 3b), ABI showed a higher proportion of established species (62.56%) than AMA (45.2%) and WMED (43.56%), opposite to casual records (AMA 31.64%, WMED 23.76%, ABI 13.85%). However, invasive species were more abundant in WMED, accounting for up to 14.36% of the overall records in the subregion, in contrast with ABI (9.74%) and AMA (3.95%). Lastly, questionable and unknown success summed around 7% and 10% in each subregion, respectively.

Temporal trends in newly introduced species showed similar behaviors among the subregions for Spanish marine waters (Figure 4), with slight differences between them. The number of newly introduced species varied considerably over the years. In WMED, the number of records varied from 0 in 2001 to 11 in 2016 (Figure 4a). In ABI, the range went from values of 0 records in 1972, 1977, 2019, and 2021 to 11 records in 2005 and 2014 (Figure 4b). AMA is the subregion with more years with 0 records, from 1972 to 1977, 1979, 1996, 2010, 2019, and 2020, but also reached the maximum number of newly introduced species from the 3 subregions, with 22 species in 2018 (Figure 4c). The results from the GLM model showed that there is a positive relationship between the number of species and the year from 1970 to 2021, for all three subregions. The estimated values for each subregion model were an average increase of 0.016 ( $p$ -value = 0.003; Std. Error = 0.005), 0.011 ( $p$ -value = 0.027; Std. Error = 0.005) and 0.030 ( $p$ -value =  $1.11 \times 10^{-7}$ ; Std. Error = 0.006) species per year for WMED, ABI, and AMA, respectively. The Chi-square test gave values of 9.291 ( $df = 1$ ;  $p$ -value = 0.002) for WMED, 4.96 ( $df = 1$ ;  $p$ -value = 0.026) for ABI, and 29.83 ( $df = 1$ ;  $p$ -value = 0.000) for AMA. Finally, the Akaike Information Criterion (AIC) was 204.24 for WMED, 241.12 for ABI, and 295.98 for AMA.

These results show that the predicted trends of the models were significant at the 0.05 significance level for all the three subregions, meaning an overall increasing trend of new introduced species over the years in WMED, ABI, and AMA (Figure 4). Yet, the estimated number of introductions per year represented low values for all the three subregions, indicating a slow rate of introductions per year. Furthermore, the Chi-square values validated the suitability of the GLM model with a Poisson distribution to analyze these trends, for the three study areas. The WMED GLM model was the one to better fit

the data, with the lowest AIC value, whereas AMA was the worst fitted model with the highest AIC value and ABI was in between.

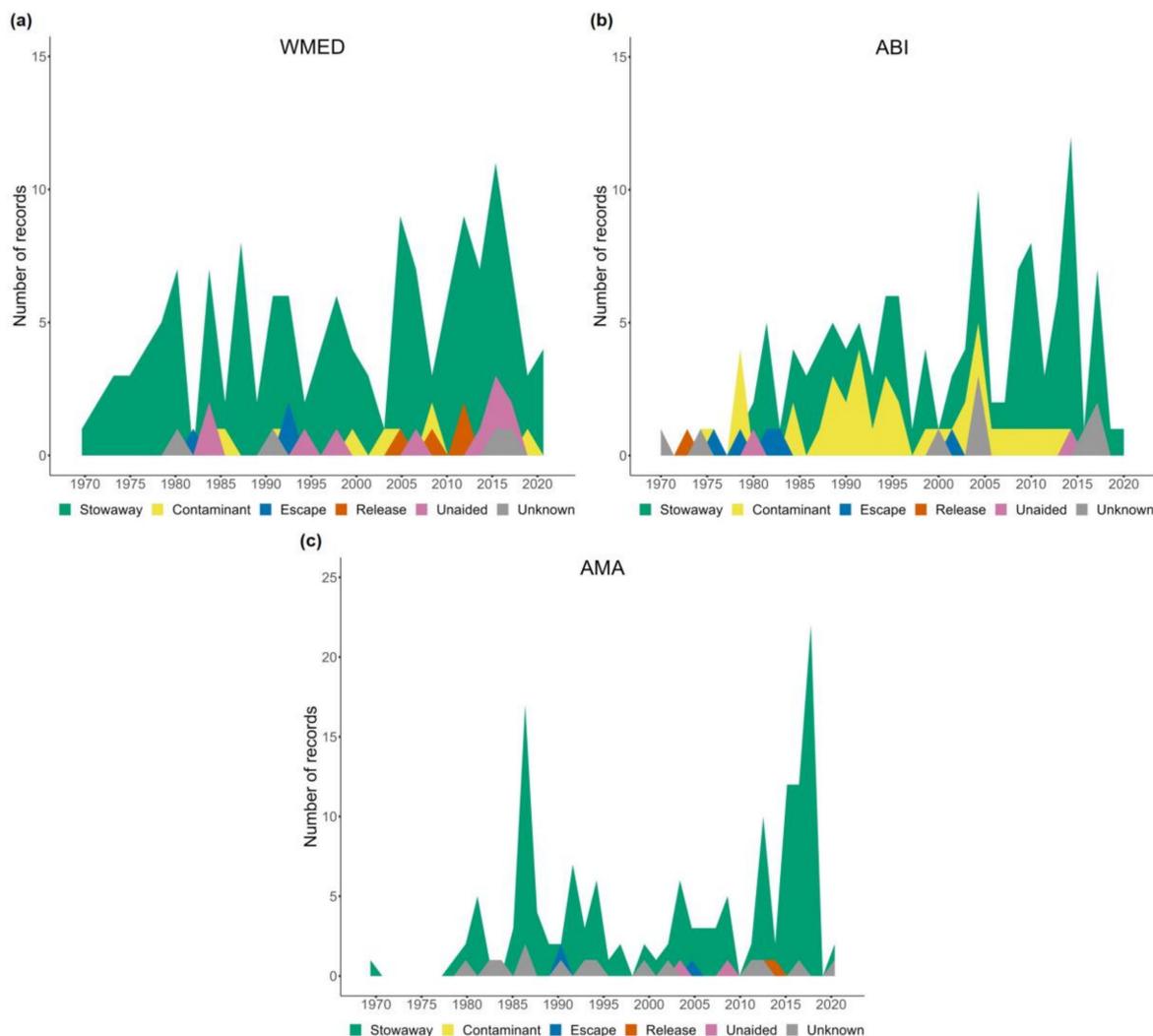


**Figure 4.** Temporal trend in newly introduced species for Spanish marine waters in (a) WMED = Western Mediterranean, (b) ABI = Bay of Biscay–Iberian Coast, and (c) AMA = Macaronesia. The black line represents the GLM model output. The grey bar shows the amount of introduced species before 1970.

More than half of the total recorded species were introduced to Spanish marine waters by ‘transport-stowaway’ (Figure 5), which entails the main primary pathway of the records analyzed since 1970, accounting for 142 (79.33%), 123 (67.58%), and 169 (85.21%) records in WMED, ABI, and AMA, respectively. The second most common pathway of introduction was ‘transport-contaminant’ (~10% of the total), yet in this case with a large heterogeneity among subregions. ABI was the one to enhance the overall values for this pathway with a total of 42 species (23.08%; Figure 5b), followed by 12 species in WMED (6.70%; Figure 5a), mostly related to the mariculture activity accomplished in these subregions. On the other hand, less than 1% of the registered species in AMA since 1970 were introduced as contaminants, with only one species (Figure 5c).

The rest of the pathways had moderate to low relevance depending on the subregion. ‘Escape’ was the second most common pathway in AMA with 4 species (2.37%), of which 3 were introduced for aquaculture purposes; while in ABI it represented the third pathway (5 species = 2.75%) and even the fourth in WMED (5 species = 2.79%). ‘Unaided’ was considered the third pathway in WMED, with 11 species (6.15%), but it was barely represented

by 3 species in AMA (1.78%) and 2 species in ABI (1.10%). ‘Release’, along with ‘unaided’, was the third most common pathway in AMA, whereas it represented a lower proportion of species introduced in WMED and ABI, with 5 (2.79%) and 1 species (0.55%), respectively.



**Figure 5.** Temporal trends in primary pathways of introduction assigned to recorded species in (a) WMED = Western Mediterranean, (b) ABI = Bay of Biscay–Iberian Coast, and (c) AMA = Macaronesia.

In the three subregions, there are still species with unknown pathways of introduction. AMA was the one holding the larger amount with 14 species (8.28%), followed by ABI with 9 species (4.95%), and WMED with 4 species (2.23%).

#### 4. Discussion and Conclusions

The present work intends to update the Spanish baseline of marine non-indigenous species (NIS) until 2021, with special attention to the status of the species in order to comprehend the trend of new introductions in Spanish marine waters. On this matter, NIS have mostly increased their presence on our coasts during the last 20 years, mainly aided by human-mediated activities such as maritime transport and aquaculture. Waters included in the Western Mediterranean (WMED) subregion have experienced the highest number of introductions until 2021 compared to the Bay of Biscay–Iberian Coast (ABI) and Macaronesia (AMA), which is in accordance with previous studies at regional [25,26] and European levels [14].

The arrival of new species is a complex process that implies many anthropogenic and ecological factors such as maritime traffic density, coastal human activities, dispersal capacity of the species, habitat suitability, etc. Indeed, the number of new introductions per year has shown highly irregular progress over the years for all the Spanish subregions. Despite the heterogeneity of data over the years and the consequent difficulty to establish future assumptions, our results suggest that the number of NIS introduced is increasing over time in all three subregions for the last decades. Furthermore, the differences in the estimated values and AIC values for the three subregions suggest that the drivers of NIS introduction may differ among the subregions. However, it has to be taken into consideration that many yearly variations within a subregion, or differences among subregions, might also be influenced by variables such as the uneven sampling effort during the assessed periods and throughout locations.

The dominance of the ‘transport-stowaway’ pathway across all the three subregions is consistent with previous studies that have identified shipping as a significant vector for the introduction of NIS in marine environments [12,27]. The high number of species introduced via this pathway highlights the need for effective management measures to prevent the introduction and spread of NIS through shipping activities, as recommended by the International Convention for the Control and Management of Ships’ Ballast Water and Sediments (BWM 2004). Furthermore, the AMA subregion, which is represented by the Canary Islands, serves as a significant hub for the transportation of oil platforms from several locations around the world [28,29]. This unique vector of introduction, classified within the ‘stowaway’ category, presents peculiarities in terms of shipping, being enormous structures with slow navigation speeds that provide ample opportunities for many species of algae, invertebrates, and fishes to adhere to them or use them as a shelter and, therefore, travel long distances [28,30]. As a result, the Canary Islands boast a remarkable number of species whose introduction occurred through oil platforms [31–33], comprising around 20% of the registered species by stowaway within the AMA subregion. Established populations have been reported for species such as *Abudefduf hoefleri*, *Abudefduf saxatilis*, *Hypoleurochilus pseudoaequipinnis*, *Oculina patagonica*, and *Tigrigobius zebrellus*; while *Cronius ruber*, *Tubastraea coccinea*, and *Tubastraea tagusensis* showed invasive behavior [33,34].

Despite the prominent importance of ‘transport-stowaway’ as the primary pathway of introduction, it is essential to note that the number of species introduced as a ‘transport-contaminant’ is also significant and cannot be overlooked. Combined with introduced species through ‘escape’ from confinement highlights the relevance of closely monitoring aquaculture facilities in order to prevent and mitigate accidental or irresponsible escapes [35].

‘Unaided’ has also been shown to be a relevant pathway of secondary introduction into the WMED subregion due to the natural spread of NIS that have been previously introduced in neighboring areas by anthropogenic means. Specifically, it is considered a recurrent issue in relation to Lessepsian species that expand their distribution within the Mediterranean Sea undergoing a process of meridionalization in the Western Mediterranean from the southern sectors [36], as happened with the cornetfish *Fistularia commersoni*, the toadfish *Lagocephalus sceleratus*, the goatfish *Upeneus pori*, or the most recent register of the lionfish *Pterois miles* [37].

On the other hand, the low frequency of introduction via ‘release’ suggests that the regulation of intentional releases of alien organisms for human use in the natural environment is generally effective. However, the risks associated with this practice should be continuously monitored and evaluated to prevent unintended consequences, since Mediterranean countries in particular could face a significant problem with this form of introduction in the near future [38]. Finally, the results for the ‘unknown’ records highlight the need for further research to identify and understand the pathways of introduction of the related NIS.

The number of species of which the native or introduced status is uncertain is relatively high in Spain; cryptogenic species ranged between 18 and 25% (ABI and WMED, respectively) of the total species listed. This result is in line with previous works that recently reported introduced species in different European regions (e.g., [39,40]). For some of these species, such as the red algae *Polysiphonia delicata* and *P. radiata*, or the parasite *Haplosporidium pinnae*, their ecological traits strongly suggest that they were introduced, but their native distribution is unknown [41,42]. For other species, their historical introduction makes it difficult to recognize them as introduced [43]. It has been widely recognized that enhancing knowledge about global marine biodiversity, particularly using molecular information, is needed to ameliorate the problem of cryptogenesis and, more generally, to improve our ability for detecting NIS.

Discrepancies regarding the alien status of the recorded species have been found among the analyzed subregions, in particular for *Oculina patagonica*, which is considered cryptogenic within the Mediterranean Sea, but alien in Macaronesia. The scleractinian coral *O. patagonica* has been thought to be introduced into the Mediterranean Sea from the western coast of South America since 1966 [44] and has been reported throughout the whole basin [45,46], expanding its distribution through maritime transport. It was first observed in 1972 along the southeast coast of Spain [47] and it has been documented to be present and increasingly abundant throughout the Mediterranean Iberian coast pointing to a westward expansion [48]. It was originally described from Patagonia based on fossil materials [44]; however, recent studies showed genetic differences between Mediterranean and Atlantic populations [49], therefore, the origin of the species is still considered dubious [50]. Even though *O. patagonica* is considered cryptogenic within the Mediterranean, the invasive coral is reported as an alien species in the Canary Archipelago due to its introduction by means of oil platforms [33,51].

Other species have been found to be partially native within a subregion [14], but with different alien status when analyzing them between national MRU. The location of the Mediterranean Iberian coast close to the Strait of Gibraltar facilitates the entrance of Atlantic species predominantly from northwest Africa. This fact implies that some species present in the Strait and Alboran MRU, such as the gastropod *Cymbium olla*, might be considered naturalized in the area as part of a range expansion process. Nonetheless, their presence in the Levantine–Balearic MRU might result from anthropogenic activities, hence assessing the same species as introduced at the subregional level. At the regional scale, the gastropod *Steromphala albida* is considered native to the Mediterranean Sea as a range expansion from the Adriatic [25]; however, it is considered to have been introduced into the Spanish Mediterranean coast as an anthropogenic translocation [52].

In Atlantic waters, the Mediterranean mollusks *Bolinus brandaris* and *Hexaplex trunculus* are considered partly native species in the ABI subregion, appearing naturally in the southwest coast of Spain (South Atlantic MRU) but having been introduced into the North Atlantic MRU as unintentional co-transport of larvae/juveniles into commercial bivalve cultures [53]. Also in Atlantic waters, the presence of the teleosts *Argyrosomus regius*, *Dicentrarchus labrax*, and *Sparus aurata* in the Canary Archipelago (AMA) is noteworthy where they have a restricted distribution to the eastern islands [54] but have been introduced as an aquaculture species in the central and western islands [55]. Native but locally absent species might involve negative effects in the environment whenever escapes of farmed individuals happen; for instance, affecting natural fish assemblages by competition for the resources [56], and spread of diseases and/or parasites [57], among others [35].

Additionally, the range expansion of thermophilic species is a relevant driving force registered in Spanish waters and assisted by the increase in sea temperature. Particularly, the natural spread of warm-affinity species from the tropical East Atlantic are more frequently recorded in the Canary Islands in a process known as tropicalization [58]. Some of these species have been also recorded associated with human activities, such as oil platforms [32] and, therefore, are considered as partially introduced into the AMA subre-

gion [28]. This is the case of the surgeonfish *Acanthurus monroviae* [59] and the butterflyfish *Prognathodes marcellae* [31].

There is a great concern about the growing expansion of NIS in European Seas [14] since their introduction can also cause serious damage to the economy [60], and even to public health [61]. In order to accomplish the established environmental objectives of the Marine Strategy Framework Directive (MSFD), the Spanish Catalogue of Invasive Exotic Species (Real Decreto 216/2019) provides a list of relevant species that might constitute a serious threat to native species, habitats, or ecosystems, or for the economic resources associated with the use of natural heritage. In this respect, all the marine macroalgae included within the national catalogue have been recorded in our inventories for Spanish marine waters: *Acrothamnion preissii*, *Asparagopsis armata*, *Asparagopsis taxiformis*, *Caulerpa cylindracea*, *Caulerpa taxifolia*, *Codium fragile*, *Gracilaria vermiculophylla*, *Grateloupia turuturu*, *Lophocladia lallemandii*, *Rugulopteryx okamurae*, *Sargassum muticum*, *Stypopodium schimperi*, *Undaria pinnatifida*, and *Womersleyella setacea*.

As non-arthropod invertebrates, the mollusk *Crepidula fornicata* and the polychaete *Ficopomatus enigmaticus* have been reported both in the WMED and ABI subregions. However, the ctenophore *Mnemiopsis leidyi* has been only found in WMED, particularly in the Levantine–Balearic MRU. Considered an opportunistic invader [62], *M. leidyi* might alter the zooplankton community due to its voracious feeding habits, and, therefore, may affect fish stocks through competition within the food web but also by directly consuming fish eggs and larvae [63,64].

As part of the concerned crustaceans, the mitten crab *Eriocheir sinensis* and the mud crab *Rhithropanopeus harrisi* are included in ABI, having been firstly detected in the Miño and Guadalquivir estuaries, respectively. The presence of the crab *Percnon gibbesi* in Spanish Mediterranean waters is noteworthy, where it has rapidly proliferated in the last 20 years [65,66], probably aided by shipping but also natural spread from its native range in the subtropical NE Atlantic. In WMED, the mud crab *Dyspanopeus sayi* is also relevant, with established populations in the Levantine–Balearic MRU.

Lastly, among the fish species reported in the Spanish Catalogue, the mummichog *Fundulus heteroclitus* is remarkable in the Levantine–Balearic MRU (Ebro River Delta) and the South Atlantic MRU (Guadalquivir estuary), where it was introduced as a biocontrol agent of mosquito populations and can be found in brackish and coastal waters, including estuaries and salt marshes.

Of the aforementioned invasive species, exclusively the mitten crab *Eriocheir sinensis* and the brown alga *Rugulopteryx okamurae* are also of concern for European waters, included in the Regulation (EU) 2016/1141. The marine macrophyte *R. okamurae* is abundant in infralittoral rocky bottoms of the southern Spanish marine subregions, with records down to the bathyal zone [67]. Since its first detection in 2015 in the Strait and Alboran MRU, it has rapidly expanded its distribution northward along the Spanish Mediterranean and Atlantic coasts [68], and southward to Macaronesia [69]. This invasive species induces extreme impacts on fisheries and marine protected areas by producing structural and functional changes in the ecosystem [70]. Furthermore, it generates secondary compounds with bioactive and anti-grazing activities [71] that contribute to the invasiveness success of *R. okamurae*. Taking advantage of this invasiveness, secondary compounds of the alga have been found to show anti-inflammatory potential for biomedical purposes [72].

*Eriocheir sinensis* is an oligohaline thermophilic species present in river systems and estuarine environments with significant adverse ecological and socio-economic impacts [73]. Management actions by the Andalusian Regional Government were implemented to contain and reduce the population of this invasive crab in the Guadalquivir estuary (South Atlantic MRU), aiming also to prevent its dispersal to neighboring natural protected areas [74]. Still, comparable scenarios and optimization of management protocols are needed to be developed.

Oligohaline species such as *E. sinensis* and *F. heteroclitus* are usually found in transitional waters with salinity between 0.5 and 5 ppt and are considered under the Water

Framework Directive. Even though transitional waters are not taken into consideration under the MSFD, oligohaline species have been reported by several Member States when they were found in estuarine or coastal systems of the marine region, regardless of their oligohaline/marine/freshwater status [16]. Furthermore, the list of invasive species of European concern presents a bias for species of fresh and brackish waters, which are frequent in the northern areas of the northeastern Atlantic region but unusual in marine waters [75].

In summary, this study aimed at providing insights into the process of introduction of NIS in the Spanish marine waters included in three of the European subregions. The data obtained confirmed that the introduction of NIS is a multifaceted process that involves various factors such as the region of suitable introduction, the available pathway of introduction, and even possible differences over the years in other variables. While the common primary pathway of introduction for all subregions was found to be ‘transport-stowaway’, the heterogeneity of data among the subregions revealed regional differences in the mechanisms of introduction. As such, tailored management strategies based on the specific mechanisms of introduction in each subregion may be necessary to prevent future introductions. By doing so, the risks of introducing new species and the subsequent environmental and economic potential impacts could be minimized.

Our study emphasizes the importance of implementing effective management and conservation policies to control the introduction of NIS through anthropogenic pathways. The increasing trend of the number of NIS arriving each year over the last few decades underscores the importance of adopting a proactive approach to NIS management.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d15050630/s1>, Table S1: List of NIS and the first year of detection at subregional levels comprising Spanish marine waters. WMED = Western Mediterranean, ABI = Bay of Biscay–Iberian Shelf, AMA = Macaronesia. Group: VER = vertebrates, INV = invertebrates, PP = primary producers, INV/par = parasites, PP/micro = microalgae.

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