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Article

# Cumulative Negative Impacts of Invasive Alien Species on Marine Ecosystems of the Aegean Sea

Konstantinos Tsirintanis<sup>1,\*</sup>, Maria Sini<sup>1</sup>, Michail Ragkousis<sup>1</sup>, Argyro Zenetos<sup>2</sup>, Stelios Katsanevakis<sup>1</sup>

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**Simple Summary:** Human activities and interventions, such as creating sea basin connections (e.g., the Suez Canal), shipping, and aquaculture can serve as pathways for marine species introductions to new ecosystems beyond their natural geographical range. These species are called alien and may become invasive, negatively impacting the recipient ecosystem. Effective management of invasive species requires understanding and assessing their impacts on the native biota. In this study, we mapped and quantified the cumulative impacts of invasive species on the marine habitats of the Aegean Sea, a Mediterranean ecoregion heavily affected by biological invasions. Our findings show that coastal habitats were more substantially impacted by invasive species than the open sea. South Aegean exhibited a higher frequency of strong impacts than the North, primarily due to alien fish and macrophytes. Shallow hard substrate was the most impacted habitat type, followed by shallow soft substrates, and seagrass meadows. The worst invasive species varied depending on the habitat type and metric used. Our study aligns with European guidelines for managing invasive species' impacts on native biodiversity and can serve as an essential tool for managing biological invasions and mitigating their impacts in the Aegean Sea.

**Abstract:** Biological invasions are a human-induced environmental disturbance that can cause major changes in ecosystem structure and functioning. Located in the northeastern Mediterranean basin, the Aegean Sea is a hotspot of biological invasions. Although the presence of alien species in the Aegean has been studied and monitored, no assessment has been conducted on their cumulative impacts on native biodiversity. To address this gap and identify the most highly impacted areas and habitats and the most impactful invasive species in the Aegean, we applied the CIMPAL index, a framework developed for mapping the cumulative impacts of invasive species. Coastal habitats showed stronger impacts than the open sea. The highest CIMPAL scores were four times more frequent in the South than in the North Aegean. Shallow (0–60 m) hard substrate was the most heavily impacted habitat type, followed by soft substrates and seagrass meadows. We identified *Caulerpa cylindracea*, *Lophocladia lallemandii*, *Siganus* spp. and *Womersleyella setacea* as the most impactful species across their range of occurrence in the Aegean but, ranking varied depending on the habitat and impact indicator applied. Our assessment can support marine managers in prioritizing decisions and actions to control biological invasions and mitigate their impacts in the Aegean Sea.

**Keywords:** Invasive species; alien; cryptogenic; impacts; CIMPAL; biodiversity; mapping; Aegean; management

## 1. Introduction

Human activities are causing significant changes in the oceans, leading to unprecedented disruptions in natural processes [1–3]. Biological invasions are regarded as one of the most severe human-induced disturbances as they reshape the structure and functioning of ecosystems worldwide [4,5]. The rate of alien species' introductions has accelerated since the 19<sup>th</sup> century [6] and is projected to intensify further in the 21<sup>st</sup> century, particularly in Europe [7]. Even under optimistic scenarios, the impacts of alien species on biodiversity will continue to grow, with significant consequences for communities and ecosystems [8].

Biological invasions are an integral part of the Anthropocene and contribute to global change [9]. There are ways to prevent new biological invasions by managing introduction pathways and mitigate the impacts of established species through targeted actions focusing on problematic species and priority areas [5,8]. However, managing marine alien species poses several challenges, including accurately assessing their negative impacts and their magnitude [10,11]. Not all alien species are invasive (i.e., have negative impacts on biodiversity, ecosystem services, or human health). Several established alien species have positive impacts, such as providing food for native species or humans, creating new habitats, and controlling other alien species or population explosions [10,12]. The impacts of alien species can vary across space [13] or time [14] and are greatly affected by the level of co-occurrence and interaction with other human disturbances [15-17]. Uncertainties, such as in predicting the distribution of alien species and the factors that define habitat invasibility, vulnerability, and resilience, reduce the reliability of bioinvasion studies and obscure the clarity needed to choose specific management actions [18]. Additionally, research on biological invasions is limited by weak evidence, frail correlations, and an overall lack of experimental studies [12,19].

Despite these limitations, the utilization of impact mapping techniques offers a promising tool to tackle the crisis of biodiversity loss and prioritize relevant management actions [20-22]. Although these methodologies are demanding, they provide a useful way to identify areas with the highest impacts or pressures, enabling marine managers to define priorities [23,24]. Impact mapping has been successfully applied at different spatial scales, including the Mediterranean Sea, and has even been used to map the impacts of invasive species [13,25].

The Mediterranean Sea has the highest number of alien species globally [26], which has caused significant adverse impacts on native marine biodiversity [12,13]. Over 1000 marine alien species have been introduced in the Mediterranean Sea, with more than 750 having established populations [27,28]. Assessing the impacts of alien and cryptogenic species in the Mediterranean, Tsirintanis et al. [12] assessed 76 species as invasive with significant negative effects on native biodiversity, ecosystem services, and human health. Moreover, the rate of establishment of alien species in the Mediterranean basin is accelerating, with no signs of levelling off [27,29]. The eastern Mediterranean Sea is particularly susceptible to biological invasions, with most new introductions occurring there [29,30]. The region's overall warmer climatic conditions, faster pace of sea-water temperature rise, the opening of the Suez Canal, and other human-induced disturbances enhance the introduction, establishment, and impacts of thermophilous Lessepsian immigrants on native ecosystems [31-33].

Located in the northeastern part of the Mediterranean Sea, the Aegean Sea is an ecoregion [34] that supports rich biodiversity but lacks effective conservation management of its marine resources [35]. As of 2019, 209 alien species have been recorded in the Aegean, of which 149 are established [36]. The Aegean also hosts 48 out of the 76 invasive species that negatively impact Mediterranean ecosystems [12], and the impact of some of these on local biodiversity has been well documented [12,19]. Accumulating evidence over the last 20 years in the area indicates an alarming increase in the abundance of records and the number of reported impactful cryptogenic and alien species (ICAS) [37].

Local climatic conditions, along with other human-activities that enhance the introduction of alien species, their natural expansion and their impacts, render the Aegean an ecoregion under siege that urgently needs effective management measures to eliminate local biodiversity loss within a proactive and adaptive systematic conservation planning approach. Regarding alien species in particular, such an approach requires continuous monitoring of alien species distribution, introduction pathways, and assessment of their impacts (negative or positive) [36,38]. Although the presence of alien species in the Aegean Sea has been continuously monitored over the past two decades [39,40] through scientific networks [41,42], citizen science initiatives [43], collections of unpublished records [44,45], and their introduction pathways have been assessed [31,46], there are no studies that address their cumulative impacts under a systematic framework.

To address this gap and assess the impacts of marine ICAS in the Aegean Sea, we used the CIMPAL index (Cumulative IMPacts of invasive ALien species), a framework developed for mapping the cumulative negative impacts of marine alien species on marine habitats [13]. Our goal was to

identify highly impacted areas and habitats, rank ICAS by their impacts, and assist the prioritization of future management decisions and actions to control and mitigate marine bioinvasions in the region.

## 2. Materials and Methods

We used CIMPAL to evaluate and map the impact of 26 ICAS (Table 1) present in the Aegean Sea, selected based on their negative effects on marine biodiversity, as reported by Tsirintanis et al. [12], and their abundance in the study area, as documented by Ragkousis et al. [37,45]. For the purpose of the analysis, targeted ICAS were classified into three groups: invertebrates, macrophytes, and fish. The foraminifer *Amphistegina lobifera* and the tunicate *Styela plicata* were included in invertebrates (Table 1).

**Table 1.** List of the targeted invasive cryptogenic and alien species (ICAS) species and their records in the Aegean Sea, for the application of the CIMPAL index.

Phylum, Class	Species	Status	Biotic group	Number of records
Arthropoda: Crustacea	<i>Callinectes sapidus</i>	Alien	Invertebrates	115
Bryozoa: Gymnolaemata	<i>Amathia verticillata</i>	Cryptogenic	Invertebrates	19
Chlorophyta: Ulvophyceae	<i>Caulerpa cylindracea</i>	Alien	Macrophytes	283
Chlorophyta: Ulvophyceae	<i>Codium fragile</i>	Alien	Macrophytes	22
Chordata: Ascidiacea	<i>Styela plicata</i>	Alien	Invertebrates	21
Chordata: Teleostei	<i>Fistularia commersonii</i>	Alien	Fish	171
Chordata: Teleostei	<i>Lagocephalus sceleratus</i>	Alien	Fish	254
Chordata: Teleostei	<i>Parupeneus forsskali</i>	Alien	Fish	70
Chordata: Teleostei	<i>Pempheris rhomboidea</i>	Alien	Fish	31
Chordata: Teleostei	<i>Pterois miles</i>	Alien	Fish	574
Chordata: Teleostei	<i>Sargocentron rubrum</i>	Alien	Fish	88
Chordata: Teleostei	<i>Siganus luridus</i>	Alien	Fish	919
Chordata: Teleostei	<i>Siganus rivulatus</i>	Alien	Fish	748
Chordata: Teleostei	<i>Upeneus pori</i>	Alien	Fish	23
Cnidaria: Anthozoa	<i>Oculina patagonica</i>	Cryptogenic	Invertebrates	51
Ctenophora: Tentaculata	<i>Mnemiopsis leidyi</i>	Alien	Invertebrates	77
Foraminifera: Globobulimina	<i>Amphistegina lobifera</i>	Alien	Invertebrates	26
Mollusca: Bivalvia	<i>Brachidontes pharaonis</i>	Alien	Invertebrates	35
Mollusca: Gastropoda	<i>Conomurex persicus</i>	Alien	Invertebrates	129
Mollusca: Bivalvia	<i>Pinctada radiata</i>	Alien	Invertebrates	159
Ochrophyta: Phaeophyceae	<i>Styopodium schimperi</i>	Alien	Macrophytes	67
Annelida: Polychaeta	<i>Hydroides elegans</i>	Alien	Invertebrates	24
Rhodophyta: Florideophyceae	<i>Asparagopsis</i> spp.	Alien	Macrophytes	117
Rhodophyta: Florideophyceae	<i>Lophocladia lallemandii</i>	Alien	Macrophytes	224
Rhodophyta: Florideophyceae	<i>Womersleyella setacea</i>	Alien	Macrophytes	24
Tracheophyta: Magnoliopsida	<i>Halophila stipulacea</i>	Alien	Macrophytes	132

To assess the impact of ICAS in the Aegean Sea, we partitioned the area into 1x1 km cells and estimated the percent cover of ten broad habitat types in each cell based on habitat maps produced by Sini et al. [35] and Topouzelis et al. [47]. The ten habitat types were: (1) seagrass meadows, (2) shallow soft substrates (0-60 m depth), (3) deep soft substrates (60-200 m depth), (4) soft substrates of the dysphotic zone (deeper than 200 m), (5) shallow hard substrates (0-60 m depth), (6) deep hard substrates (60-200 m), (7) hard substrates of the dysphotic zone (deeper than 200m), (8) submarine caves, (9) coralligenous formations, and (10) pelagic habitat. For the analysis, we classified habitats with depths of 0-200 m as coastal and those deeper than 200 m as open sea habitats. The pelagic habitat was considered part of both coastal and open sea habitats and covered the entire study area.

The CIMPAL index was calculated for each 1x1 km cell of the study area using the formula  $I_c = \sum_{i=1}^n \sum_{j=1}^m A_i H_j w_{i,j}$ . In this formula,  $A_i$  refers to the population state of species  $i$  in a given cell of the study area, standardized to range between 0 and 1. For each targeted ICAS, we estimated  $A_i$  using two approaches: (a) species distribution models (SDMs) (also known as ecological niche models or habitat suitability models) within the Aegean, as derived by Ragkousis et al. [48], and (b) ICAS presence/absence data acquired from Ragkousis et al. [37], updated with additional data from Ragkousis et al. [45].  $H_j$  is the percent cover of habitat  $j$  in a given cell of the study area. The impact weight  $w_{i,j}$  of ICAS  $i$  at habitat type  $j$  was estimated using an uncertainty-averse approach [13,49] (Figure 1), based on both the impact magnitude (Figure 2) according to [11,50] and the strength of the reported evidence (Figure 2) according to [19]. The impact magnitude and strength of evidence for every combination of ICAS and habitat type (Table S1) were retrieved from [12].

To rank ICAS based on their negative impacts, we estimated four different indicators: (D1) the total area of an ICAS occurrence, which was calculated as the total number of 1 x 1 km cells with at least one record of the specific ICAS; (D2) the number of cells with estimated impact score >0 for a given ICAS; (D3) the sum of impact score values of a specific ICAS across the entire study area; (D4) the average impact score across the ICAS's range of occurrence, excluding cells with score values lower than 0.1.

		Impact magnitude				
		Minimal	Minor	Moderate	Major	Massive
Strength of evidence	Robust	0	1	2	4	8
	Medium	0	0	1	2	4
	Limited	0	0	0	1	2

**Figure 1.** The estimation of impact weights  $w_{i,j}$  for species  $i$  at habitat  $j$  based on impact magnitude and strength of evidence, as defined in Fig. 2 (based on Katsanevakis et al. [13,19]).

### 3. Results

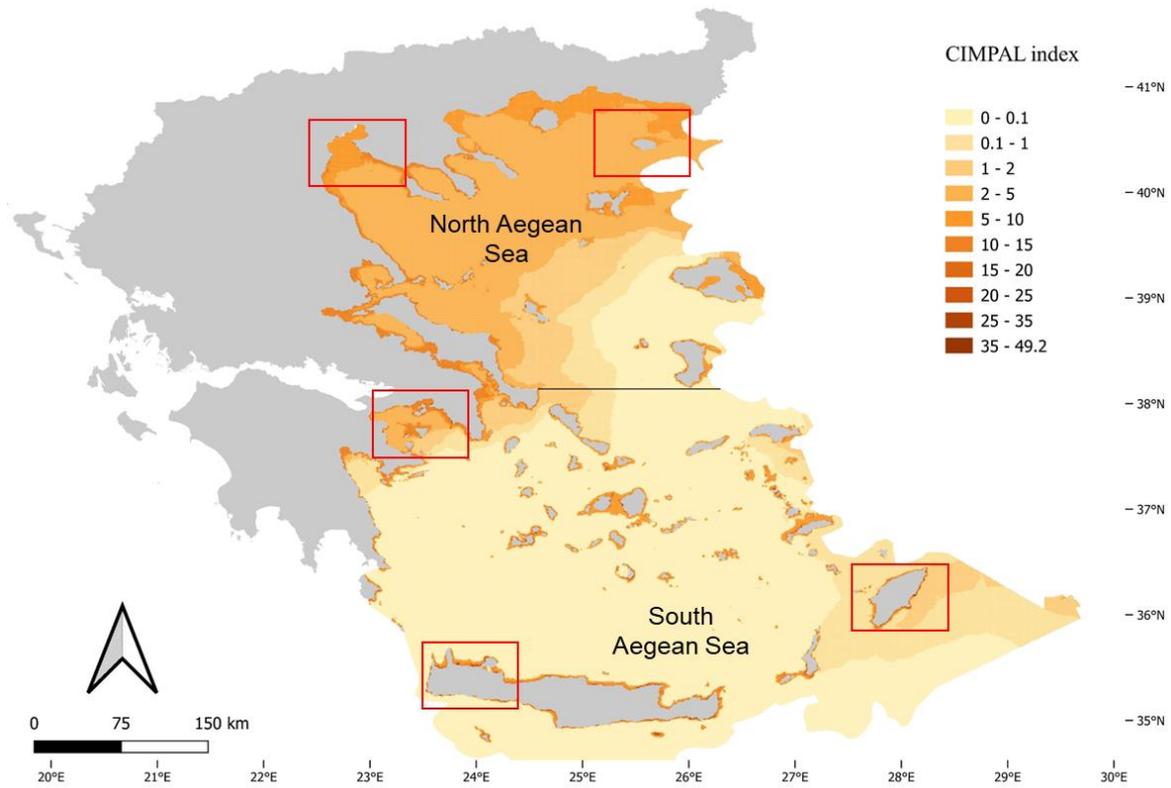
The use of CIMPAL to map the cumulative impacts of ICAS in the Aegean Sea revealed interesting spatial patterns of negative effects (Figure 3). The study area was made up of 321,346 grid cells, with a range of cumulative CIMPAL impact scores from 0 to 49.2 and a mean value of 1.35. The North Aegean covered 33% of the study area's grid cell surface, whereas the South Aegean covered the remaining 67% (Figure 3). Most grid cells (69%) corresponded to the open sea, while coastal habitats accounted for 31% of the Aegean Sea's surface. Coastal habitats at depths of up to 60 m displayed higher CIMPAL impact scores than deeper habitats or the open sea (Figure 3). Cumulative impacts on the open sea decreased from the north to the south (Figures 3 and 4). However, high CIMPAL scores (>20) only occurred in shallow coastal ecosystems (up to 60 m depth), appeared only locally without covering extensive surface areas, and were four times more frequent (79%) in the South Aegean (Figures 3 and 4).

The pelagic habitat contributed the most to the overall sum of CIMPAL scores for all grid cells in the Aegean Sea, among all impacted habitat types (Figure 5a). This was primarily due to the extensive coverage of this habitat type rather than because of the per-cell values of the CIMPAL index. The CIMPAL score for this habitat type ranged between 0 and 3.9, with a mean habitat impact value at 1.0 across the study area, ranking 4<sup>th</sup> in terms of average CIMPAL score (Figure 5b). The invasive ctenophore *Mnemiopsis leidyi* was the only invasive species that impacted the pelagic habitat.

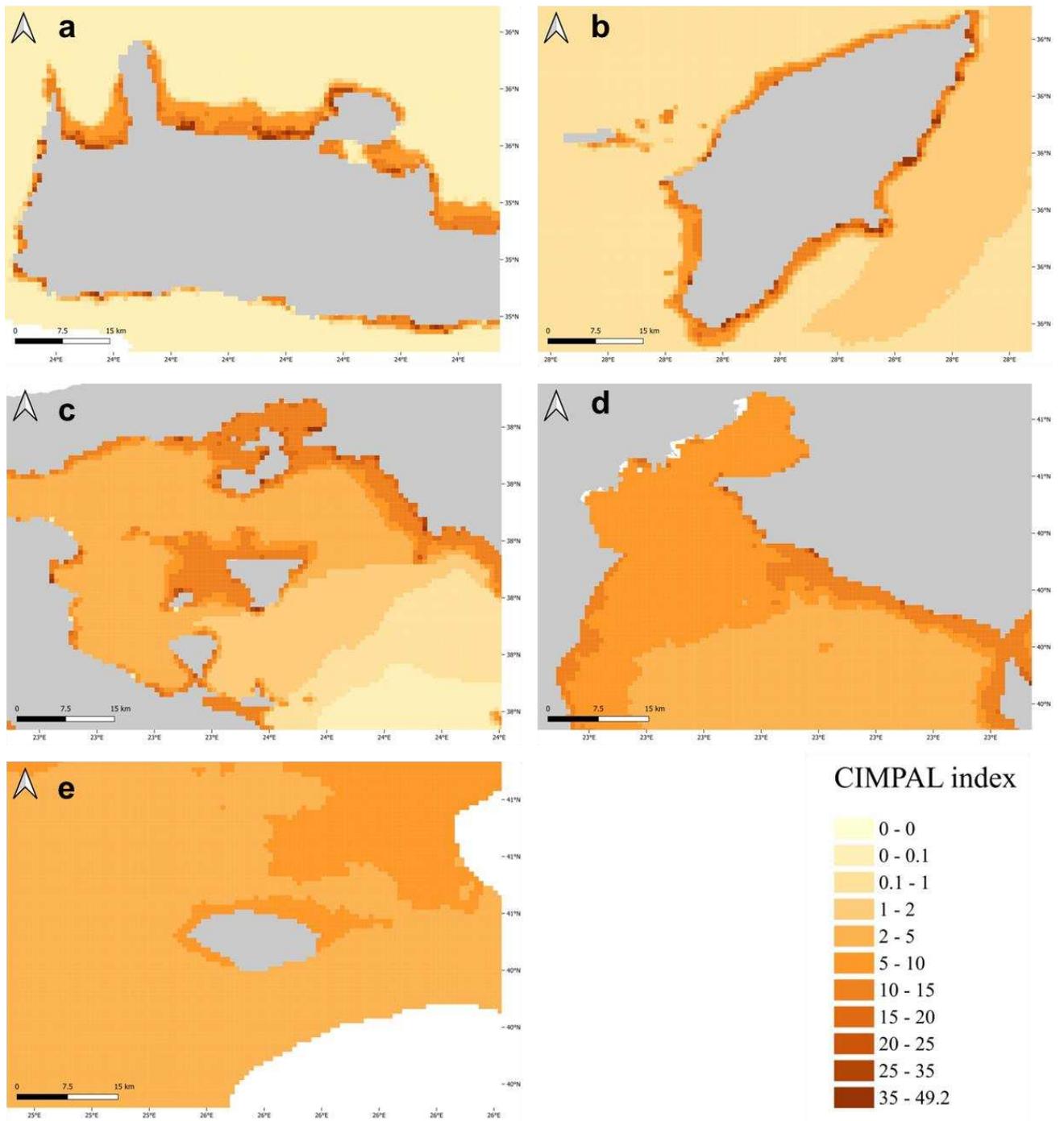
Strength of evidence	
<b>Robust</b>	The impact is estimated by experimental studies. The different types of experiments are <b>Manipulative Experiments</b> (field and lab experiments based on a random selection of experimental units and include treatments and controls) and <b>Natural Experiments</b> (field experiments based on natural selection of experimental units).
<b>Medium</b>	Impact assessment is derived from <b>Direct Observations</b> (direct observations or measurements of impact without doubt), <b>Modelling</b> (ecosystem models that simulate alien species impacts on biodiversity) and <b>non-experimental based correlations</b> (significant correlations between an alien species and an impact on native biodiversity, not based on an experimental design).
<b>Limited</b>	Qualitative Impact assessment based on <b>Expert Judgement</b> through the empirical knowledge of experts based on alien species' traits.
Impact magnitude	
<b>Massive</b>	Changes in community composition through local or global extinction (negative) because of competition, predation, grazing, parasitism, toxicity or bio-fouling; impact on ecosystem processes and ecosystem functioning resulting in species composition changes; or ecological engineering, resulting in change in community composition. Massive magnitude applies when under the hypothetical scenario of the alien species extinction, the induced changes are considered as irreversible within 10 years or within 3 generations of the extinct native taxon/a, whichever is longer.
<b>Major</b>	Changes in community composition through local or global extinction (negative) because of competition, predation, grazing, parasitism, toxicity or bio-fouling; impact on ecosystem processes and ecosystem functioning resulting in species composition changes; or ecological engineering, resulting in change in community composition. Major magnitude applies when under the hypothetical scenario of the alien species extinction, the induced changes are considered as reversible within 10 years or within 3 generations of the extinct native taxon/a, whichever is longer.
<b>Moderate</b>	Native species population changes due to competition, predation, grazing, parasitism, toxicity or bio-fouling, but without changes in community composition; or impact on ecosystem processes and ecosystem functioning resulting in population declines but no substantial change in community composition; or ecological engineering, resulting in population declines but no substantial change in community composition.
<b>Minor</b>	Impact on individual fitness of a native species due to competition, predation, grazing, parasitism, toxicity or bio-fouling without substantial population changes; minor impact on ecosystem processes and ecosystem functioning with no related population changes; any environmental or habitat alterations in chemical, physical or structural characteristics do not result in population changes.
<b>Minimal</b>	No effect on fitness of native species; negligible impact on native species due to competition, predation, grazing, parasitism, toxicity or bio-fouling; no effects on ecosystem processes and ecosystem functioning; no chemical, physical or structural impact on the ecosystem (not an ecosystem engineer).

**Figure 2.** Strength of evidence types (based on Katsanevakis et al., 2014) and impact magnitude categories (based on [11,50]).

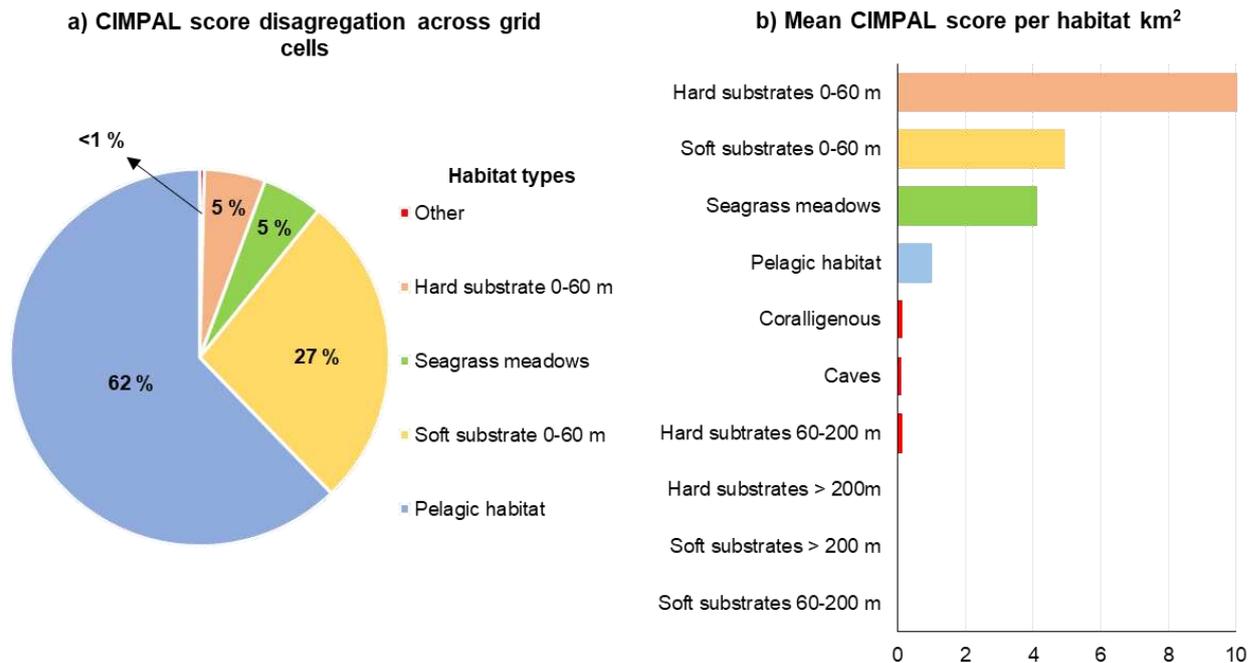
Shallow hard substrates (0-60 m depth) accumulated 5% of the CIMPAL scores (Figure 5a). This habitat covered 0.1% of the study area, exhibited the highest disaggregated CIMPAL impact scores, ranging from 0 to 47.5 and had an average CIMPAL score of 10.6 ranking as the most impacted habitat type (Figure 5b). High scores appeared more frequently in the South than North Aegean (Figure 6a). In total, 17 invasive alien species impacted this habitat, with *Siganus luridus*, *Siganus rivulatus*, and *C. cylindracea* being the most impactful (Figure 6b).



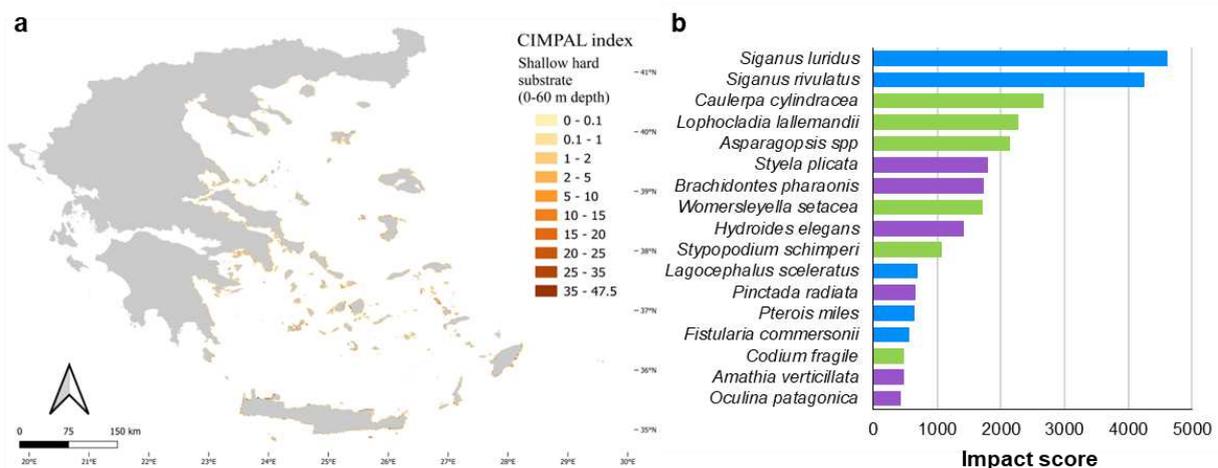
**Figure 3.** Mapping of ICAS cumulative impacts in the North and South Aegean Sea habitats using the CIMPAL index. Red rectangles highlight areas of the North and South Aegean illustrated in Figure 4.



**Figure 4.** Cumulative impacts on areas of the South (a, b, c) and North Aegean (d, e). These are a) Chania, Crete, b) Rhodes Island, c) Saronikos Gulf, d) Thermaikos Gulf, e) Samothrace Island.



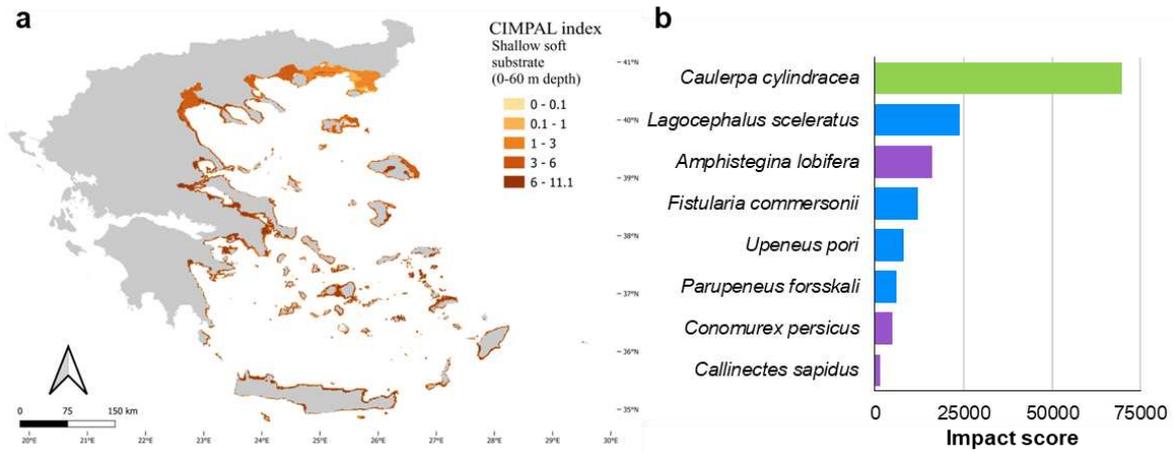
**Figure 5. a)** Disaggregation of the total CIMPAL score for all cells in the study area by habitat type, **b)** average CIMPAL scores per habitat type.



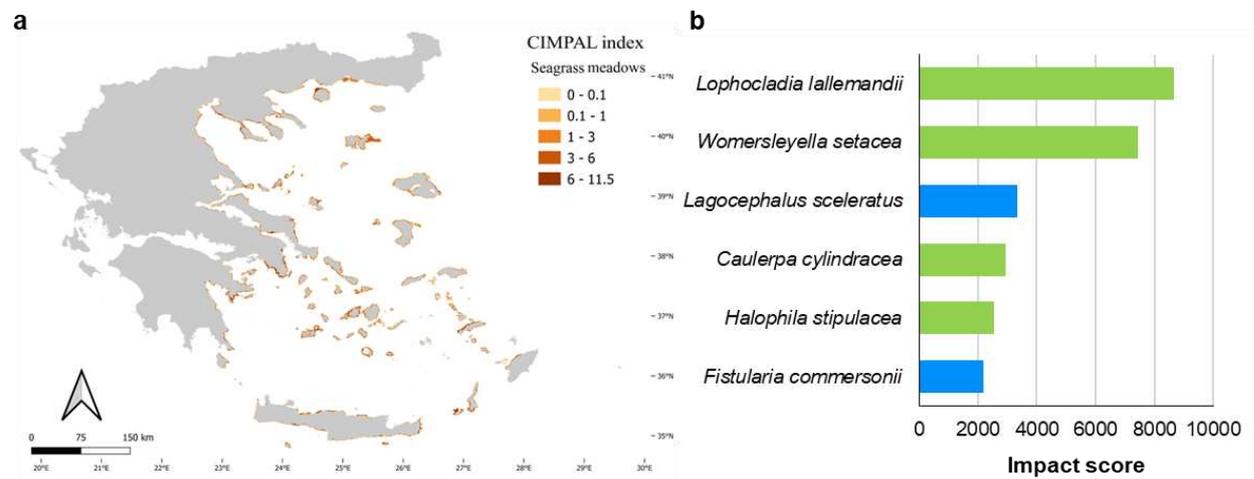
**Figure 6. a)** Estimation of CIMPAL index on shallow hard substrates between 0-60 m depth in the Aegean Sea. **b)** Impacting ICAS on this habitat type, ranked by the estimated total impact score (summed over all grid cells). Green color for macrophytes, blue for fish and purple for invertebrates.

Shallow soft substrates (0-60 m depth) covered 6.5% of the Aegean Sea's surface and accounted for 27% of the cumulative CIMPAL impact scores (Figure 5a). The impact values for this habitat type ranged from 0 to 11.1, with a mean CIMPAL score of 4.9 ranking as the 2<sup>nd</sup> most impacted habitat type on average (Figure 5b). Scores were higher in the South than North Aegean (Figure 7a). This habitat type was impacted by eight invasive species, with the invasive green alga *Caulerpa cylindracea* having the highest impact score, followed by the invasive fish *Lagocephalus sceleratus* and the foraminifer *Amphistegina lobifera* (Figure 7b).

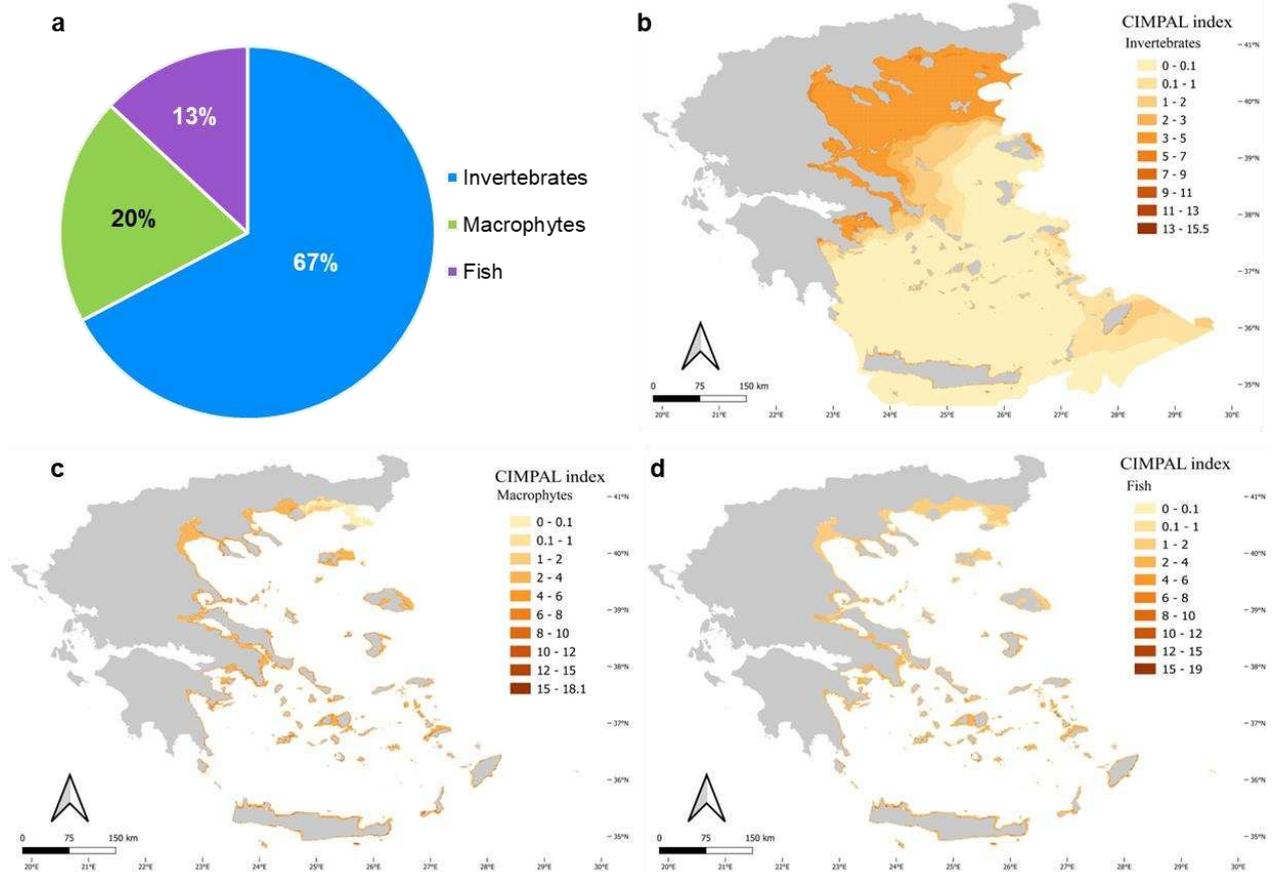
Seagrass meadows accounted for 5% of CIMPAL scores (Figure 5a) and covered 0.8% of the study area. The average CIMPAL score for seagrass meadows was 4.2 (Figure 5b), with values ranging from 0 to 11.5. The CIMPAL score exhibited increased heterogeneity across the study area, without clear geographical patterns (Figure 8a). The red algae *Lophocladia lallemandii* and *Womersleyella setacea* were the ICAS with the highest CIMPAL scores on seagrass meadows (Figure 8b).



**Figure 7. a)** Estimation of CIMPAL index on shallow soft substrates between 0-60 m depth in the Aegean Sea. **b)** Impacting ICAS on this habitat type, ranked by the estimated total impact score (summed over all grid cells). Green color for macrophytes, blue for fish and purple for invertebrates.



**Figure 8. a)** Estimation of CIMPAL index on seagrass meadows in the Aegean Sea. **b)** Impacting ICAS on this habitat type, ranked by the estimated total impact score (summed over all grid cells). Green color for macrophytes, blue for fish and purple for invertebrates.



**Figure 9.** (a) Disaggregation of the total CIMPAL score for all cells in the study area by biotic group (fish: blue color, macrophytes: green, invertebrates: purple), and maps of the impacts of ICAS in the Aegean Sea for (b) invertebrates, (c) macrophytes, and (d) fish.

Impacts on caves, coralligenous, and hard substrates between 60-200 m depth accounted for less than 1% (Figure 5a) of the cumulative impact score. No species impacting the rest habitat types were documented.

Invertebrates were the biotic group with the highest contribution to the cumulative ICAS impacts in the Aegean Sea, accounting for 67% of the overall CIMPAL score (Figure 9a). Nevertheless, the invasive ctenophore *Mnemiopsis leidyi*, which only affects the pelagic habitat, was responsible for 92% of the invertebrate CIMPAL impact score in the Aegean. Invertebrate impacts showed a decreasing CIMPAL score gradient in the open sea, from the North Aegean to the South (Figure 9b). Macrophytes accounted for 20% of the overall CIMPAL score in the Aegean (Figure 9a). Their impact did not show any strong spatial pattern across the study area, except for lower CIMPAL scores in the northeastern part of the Aegean Sea (Figure 9c). Higher CIMPAL scores (>10) were more frequent (78%) in the South than in the North Aegean. Fish accounted for 13% of the cumulative impacts (Figure 9a) and had a stronger impact on the coastal habitats of the South Aegean (Figure 9d); high CIMPAL scores (>10) were much more frequent in the South Aegean (88%).

The impact ranking of ICAS varied depending on the indicator used (Figure 10). Excluding *M. leidyi* from all rankings, the herbivorous fishes *Siganus luridus* and *S. rivulatus* and the lionfish *Pterois miles* were the top three ranking species according to D1. According to D2, the fishes *Lagocephalus sceleratus*, *Fistularia commersonii*, and *Parupeneus forsskali* were the top three ranking species. D3 identified the green alga *Caulerpa cylindracea* as the most impactful ICAS in the Aegean, with an impact score approximately three times higher than the second-ranking species, *Lagocephalus sceleratus*. The top 10 most impactful invasive species, according to D3, included five fish, three macrophytes, and two invertebrates (Figure 10). *C. cylindracea* was also ranked as the most impactful invasive species in the Aegean by D4, with a significant difference from the second-ranking

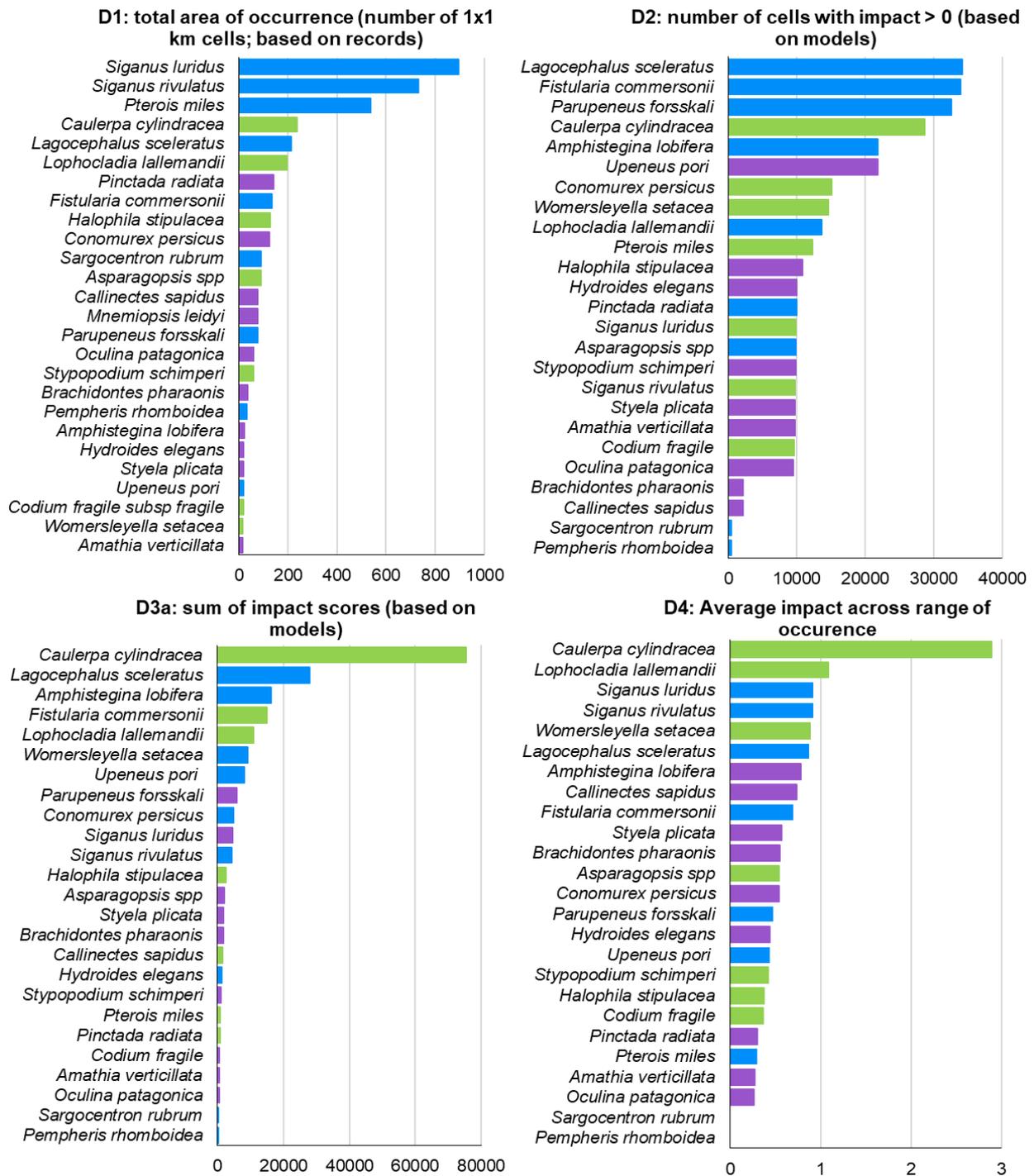
*Lophocladia lallemandii*. D2 and D3 exhibited high similarity, with nine common species in their top ten (Figure 10) although with different rankings.

Moreover, indicator D3 was also estimated based on actual ICAS records – D3b – (Figure 11) instead of their modelled distribution – D3a – (Figure 10). D3b ranked six fish, two macrophytes, and two invertebrates in its top 10. Although both methods had seven species in common in their top 10, there were some differences in the rankings. For instance, the two invasive siganids ranked 10th and 11th in D3a, but ranked 2nd and 3rd in D3b. The invasive foraminiferan *Amphistegina lobifera* ranked 3rd in D3a and 12th in D3b. The lionfish *P. miles* ranked 19th in D3a and 10th in D3b (Figures 10 & 11).

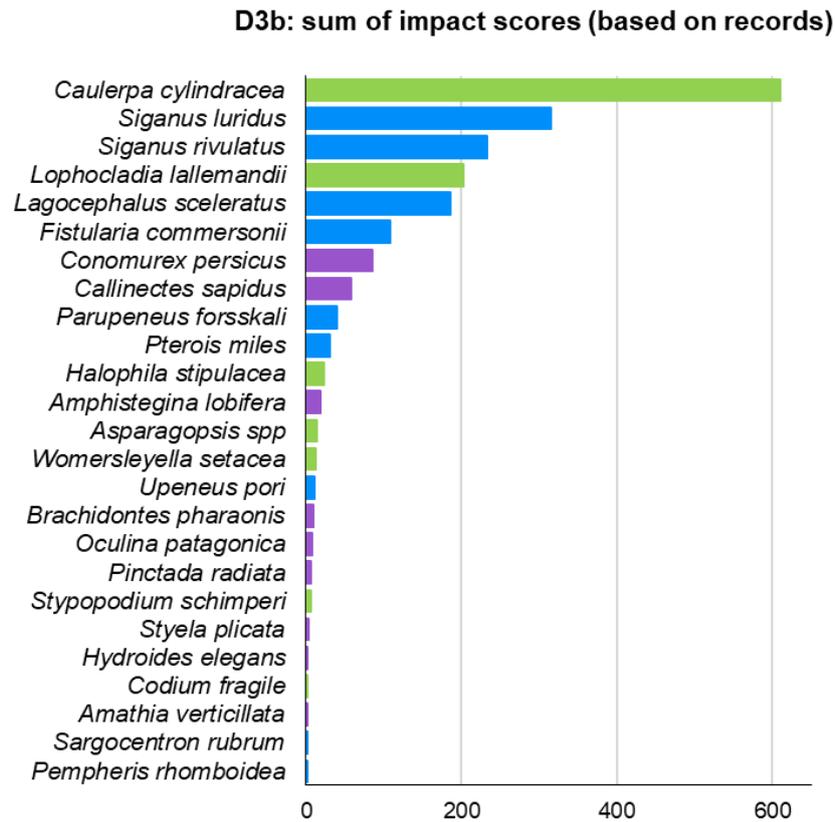
#### 4. Discussion

According to CIMPAL, the impact scores of ICAS in the Aegean Sea are higher in coastal habitats than in the open sea. The invasive ctenophore *Mnemiopsis leidyi* is the only ICAS that extends its distribution to the open sea, whereas the impacts of the other ICAS appear to be confined to coastal habitats. Even within coastal waters (0-200 m depth), ICAS impacts were higher in habitats up to 60 meters deep. In a pan-Mediterranean biodiversity assessment, Coll et al. [51] indicated that shallow coastal waters have higher biodiversity values and a higher concentration of alien species. In the application of CIMPAL at Mediterranean scale, Katsanevakis et al. [13] also found that ICAS mainly impacted coastal habitats. These results may be attributed to the fact that most alien species introduced in the Mediterranean Sea are demersal or benthic species that prefer shallower and warmer waters [31].

However, the observed patterns of some ICAS impacts may partly reflect the spatial distribution of research effort, which is primarily conducted in shallow coastal ecosystems, in habitats that are easily accessible by scientific divers or citizen scientists (e.g. rocky reefs and seagrass meadows in waters <60 m), and in areas that represent important introduction pathways (e.g., large ports) or that are systematically monitored by scientific institutes or other marine conservation agencies [13,31,37]. This is a commonly reported problem in studies assessing biodiversity in the Mediterranean Sea. Marine research in the region has traditionally been restricted to shallow waters, whereas large parts of the deep sea remain understudied [35,51]. Research effort bias may have also affected the current mapping of ICAS in the open sea. For example, the spatial distribution of *Mnemiopsis leidyi* was mainly based on sampled data gathered during early summers from 2004-2006 and 2008. However, the sampling was only conducted in open-sea surveys in the North Aegean [52]. Consequently, the resulting habitat suitability modelling depicted its occurrence throughout the North Aegean, but its presence in the South Aegean is largely unknown. Since the species has been reported as far south as Syria and Israel [53,54], its presence in the South Aegean is possible. Furthermore, for several planktonic or hard-to-identify ICAS that are underrepresented in the respective datasets, there is high uncertainty regarding their origin, distribution, and impacts, and as a result, they often cannot even be included as alien species in official lists [18,55–57].



**Figure 10.** Ranking of ICAS impact in the Aegean Sea according to the four CIMPAL indicators D1-D4 (excluding *M. leidyi*). Macrophytes: green color, fish: blue, and invertebrates: purple.



**Figure 11.** ICAS impacts ranking using D3 indicator but with presence/absence data derived from field observations instead of modelled data.

The CIMPAL results revealed cumulative impacts of ICAS on coastal habitats throughout the Aegean Sea, with a higher frequency of stronger impacts in the south Aegean. Upon examining the impact maps of the distinct biotic ICAS groups (Figure 9), it became apparent that invasive fish impacts were the primary cause of the higher coastal impact scores in the south. Recent studies on the spatiotemporal distribution patterns of marine ICAS throughout the Greek Seas reported similar patterns, with a higher ICAS richness and abundance of records observed in the South Aegean areas and a corresponding decrease towards the north [31,37]. These distribution trends are believed to be related to specific species traits and introduction pathways [31,58]. For example, the majority of alien fish in the Mediterranean are thermophilous species of Indo-Pacific origin, introduced through the Suez Canal in the southeastern part of the Mediterranean [31], and thus are favored by the higher mean sea water temperatures of the southern Aegean [59].

Macrophytes impacts did not show specific, large-scale spatial patterns across the study area, possibly due to the species' contrasting spatial distribution. Specifically, certain species, such as *Codium fragile*, are primarily recorded in the North Aegean, while *Halophila stipulacea* and *Styopodium schimperi* are mainly found in the South Aegean. Other species, such as *Asparagopsis spp.*, *Caulerpa cylindracea*, and *Lophocladia lallemandii*, are observed throughout the study area [37,48]. Katsanevakis et al. [31] also depicted a heterogenous spatial distribution of macrophyte alien species in the Aegean Sea. In that study, aquaculture and shipping were reported as the two main pathways for macrophyte introduction in the Mediterranean Sea, indicating no common thermal affinity contrary to the case of Lessepsian species. The varying patterns of macrophyte distribution and their associated impacts are more likely related to their different ecological traits and niches, such as their varying thermal preferences [60, 61].

Invertebrate ICAS impacts were generally higher in coastal habitats compared to the open sea, particularly in South Aegean. Katsanevakis et al. [31] identified the Suez Canal and shipping as the two main pathways for invertebrate introductions in the Mediterranean Sea. Therefore, the pool of alien invertebrate species in the region includes both thermophilic species from the Red Sea that tend

to be more abundant in the South Aegean and vessel-introduced species with various thermal preferences. The latter are often observed in or near ports [37]. Shipping and ports have been globally associated with bioinvasions [62,63], and shipping is regarded as one of the primary introduction pathways in the Mediterranean Sea [64-66]. Invertebrates account for a significant portion of the alien diversity within Mediterranean ports [67]. In the South Aegean Sea, the Saronikos Gulf is considered a biological invasions hotspot due to Piraeus, the largest Greek port. Several of the assessed ICAS, such as *Styela plicata*, *Brachidontes pharaonis*, *Hydroides elegans*, *Pinctada radiata*, *Amathia verticillata*, are found in high abundance within Mediterranean ports [64], and some of the depicted CIMPAL impacts are related to them.

The coastal habitats with the highest average cumulative impact were shallow hard and soft substrates between 0-60 m depth and seagrass meadows. Shallow hard substrates between 0-60 m depth were the most impacted habitat type. The main species responsible for the impacts on this habitat type were the invasive herbivorous fish *Siganus luridus* and *Siganus rivulatus*. Several studies in the Aegean Sea have linked the presence of *Siganus* spp. with adverse grazing effects on macroalgal communities [68-71] and a degraded rocky reef ecological state [72,73]. Three macroalgal ICAS follow in the list of the most impactful species on shallow hard substrates: the green alga *C. cylindracea*, and the red algae *Lophocladia lallemandii* and *Asparagopsis* spp. The invasive green alga impacts biodiversity in these habitats through various mechanisms such as competition, overgrowth, increased sedimentation, and ecosystem engineering [12]. The invasive red algae can adversely impact native species through competition for resources [74-76] or through the creation of novel habitats that alter benthic community composition to a more impoverished state [77,78]. The invasive ascidian *Styela plicata* is the invertebrate ICAS with the most negative impacts on this habitat type. It forms dense aggregations that can cover the available hard substrate, outcompeting native species [79].

Soft substrates between 0-60 m depth exhibited the highest sum of cumulative impacts on coastal ecosystems. *Caulerpa cylindracea*, the sole macrophyte among the eight ICAS impacting this habitat type, ranked first as the most impactful. This invasive green alga can form dense mats on shallow sediments, leading to major structural changes to the resident soft-bottom faunal communities and ecosystem functioning [80-82]. Half of the species impacting soft substrates between 0-60 m depth were fish. *Lagocephalus sceleratus* and *Fistularia commersonnii* are considered among the most impactful alien Mediterranean fish, being voracious predators that prey upon several species of the resident biota [83-86]. Ecological modelling approaches have also indicated that *L. sceleratus* is an important competitor of native carnivores with high retention rates [87]. *Amphistegina lobifera* ranked 3<sup>rd</sup> among species impacting shallow soft substrates. This invasive foraminifer can dominate sediment communities and introduce significant shifts in community composition [88, 89] or even alter habitat structure by creating a novel habitat with its accumulating shells [90].

Seagrass meadows in the Aegean Sea were found to be impacted by six ICAS. Among these, the red algae *L. lallemandii* and *Womersleyella setacea* have been identified as the most impactful, forming dense epiphytic mats on *Posidonia oceanica* meadows and impacting their fitness [91-93] and the associated communities [94,95]. The pelagic habitat is exclusively impacted by *Mnemiopsis leidyi*. This invasive ctenophore preys opportunistically on planktivorous organisms [96,97] and can adversely impact the structure of zooplanktonic communities [98].

CIMPAL has been effectively used as an impact assessment tool for alien species in European Seas [13,99] and inland waters [100]. One of the concerns in applying the CIMPAL index is related to the quality of habitat mapping and assessments of population state [13]. In its present application for the Aegean Sea, using a 1x1 km grid and with high-resolution shallow water habitat maps was sufficient to depict spatial patterns. The impact of each studied species was assessed based on its recorded impacts within the Mediterranean [12]. The impact weights used were based on the worst documented impacts for each species, irrespective of the location and timing of the report. However, ICAS impacts vary in space and time, and the fact that no spatiotemporal impact variance has been considered may have led to an overestimation of ICAS impacts. Finally, the current impact

assessment utilizes a list of impactful ICAS derived from a recent Mediterranean review [12], which refers to documented impacts in the literature; unpublished impacts may have been missed.

This study is the first comprehensive evaluation of the cumulative adverse impacts of ICAS on the marine habitats of the Aegean Sea. The EU Regulation 1143/2014 stipulates specific measures “to prevent, minimize and mitigate the adverse impact on biodiversity of the introduction and spread within the Union, both intentional and unintentional, of invasive alien species”. Article 19 of the Regulation pertains to managing widely spread invasive alien species of Union concern, and it mandates that the management measures should be proportional to the severity of the environmental impact. Greece enforced the Regulation in February 2021. It was soon realized that a national list of IAS (HELLAS-ALIENS) should be supported by a database with all important information about species origin, traits, status, habitats, pathways of introduction, potential impacts, and geographical distribution. All marine species included in HELLAS-ALIENS [101] were considered in this study. Therefore, this study provides an essential foundation for future management and mitigation actions concerning ICAS. It identifies the most heavily impacted areas, the most affected habitats, and the most impactful ICAS in the region, through the application of CIMPAL and its associated indicators.

## 5. Conclusions

According to the CIMPAL index, the Aegean Sea’s ecosystems are impacted by ICAS in various spatial patterns. Coastal habitats have higher cumulative impacts compared to the open sea, and the South Aegean has four times more frequent high cumulative impact scores compared to the North. The most heavily impacted habitat is shallow hard substrate (0-60 m), followed by shallow soft substrate (0-60 m) and seagrass meadows. The study identified the most impactful species both overall and by habitat.

Biological invasions have become a significant challenge for the Aegean Sea, substantially affecting its marine ecosystems. As an eastern Mediterranean ecoregion, the Aegean Sea is a biological invasions and climate change hotspot but also has invaluable biodiversity that requires conservation. This study provides an essential tool aligned with relevant European guidelines that can contribute to the prioritization of future management actions and impact mitigation measures for biological invasions in the Aegean Sea.

**Supplementary Materials:** The following supporting information can be downloaded at: [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1), Table S1: Matrix of impact weights  $w_{i,j}$  for all combinations of the targeted 26 invasive alien and cryptogenic species and the 10 studied habitat types.

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