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Buoys are non-indigenous fouling hotspots in marinas regardless of their environmental status and pressure

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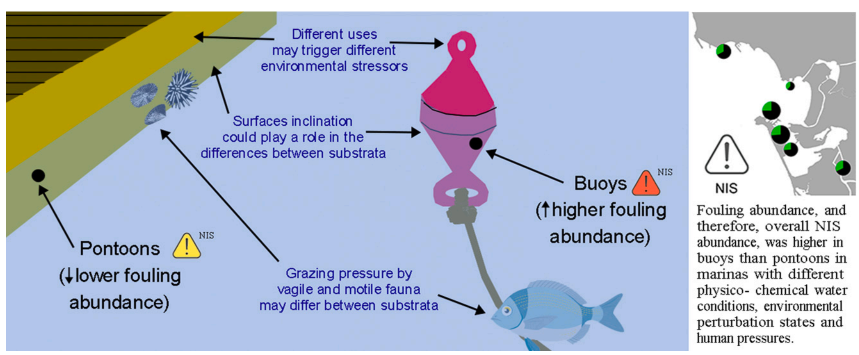
HIGHLIGHTS

- Floating structures fouling is a NIS hotspot in marinas, with fouling abundance being higher on buoys than pontoons.
- Buoys have an elevated risk of spreading NIS, as they are close to boat hulls and can detach and drift at sea.
- Marinas with low flushing capacity and high anthropic pressure are more likely to have high NIS abundance.
- Experimentally deployed settlement buoys could be a useful method to monitor NIS in artificial and natural areas.

GRAPHICAL ABSTRACT



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ABSTRACT

Marinas contribute to the degradation of coastal ecosystems, constitute non-indigenous species (NIS) hotspots and function as steppingstones in invasion processes. These often enclose highly modified water bodies that promote the concentration of pollutants and propagules, favoring NIS abundance. In these habitats, floating structures are often the most invaded by fouling NIS. This study aims to address the effect of floating substrate (buoys vs pontoons) on fouling assemblages, with special focus on NIS, in 6 marinas of Cadiz Bay during summer and winter seasons. Since the effect substrate type can depend on the water physicochemical conditions and environmental state and pressures of marinas, an environmental assessment was carried out for each marina using literature, physicochemical water measurements and environmental risk assessments. Despite the registered seasonal variation in fouling assemblages and the environmental variability among the studied marinas, the type of substrate played a key role in fouling assemblages' structure and abundance. The higher abundance of fouling assemblages in buoys than pontoons favor NIS prevalence in marinas and increase the risk of NIS dispersal, particularly considering that buoys are more likely to detach and drift at sea than pontoons. The results indicate that high-risk consideration should be given to this substrate type and that the potential environmental effects of biological pollution must be considered in risk assessments.

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1. Introduction

Coastal areas account for <15 % of Earth's surface, but host more than half of the world's population (Small and Nicholls, 2003). This, in combination with a continuous increase of human population in coastal areas, a high touristic pressure (up to 10 times higher than inland) and the effects of climate change, are demanding an increase in the construction of coastal defenses, land reclamations and the installation of coastal and offshore infrastructure (Batista e Silva et al., 2018; Sempere-Valverde et al., 2023a). The development of artificial shorelines is larger in ecosystems of higher interest, such as estuaries and bays, as they provide commercial opportunities and ecosystem services (de Andrés et al., 2017; Sempere-Valverde et al., 2023a). This has led to high coastal sprawl levels in these areas, with interventions that can have an important impact on water and wave dynamics and modify shoreline morphodynamics and water renewal capacity (Zarzuelo et al., 2020; Sempere-Valverde et al., 2023a). These impacts tense the conciliation between socioeconomic development and ecosystem conservation and emphasize the need of increasing conservation, mitigation, and restoration measures in the marine environment (Firth et al., 2016, 2020).

Within artificial shorelines, ports and marinas are among the interventions with higher physical footprint in the marine environment (Bugnot et al., 2020). These often involve extensive land reclamations, create highly modified water bodies, and concentrate human activity, which are related to habitat loss and environmental impacts that contribute to reduce the abundance and diversity of the biological communities inhabiting these artificial habitats (Heery et al., 2020; Momota and Hosokawa, 2021). As a result, the ecological state of coastal areas is reduced, which favors the colonization of these habitats by tolerant, opportunistic, cosmopolitan, and non-indigenous species (NIS) (Moreira et al., 2010; Megina et al., 2013; Lagos et al., 2017). Therefore, these structures contribute to the degradation of coastal ecosystems, constitute coastal NIS hotspots and function as steppingstones in invasion processes (Foster et al., 2016; Ros et al., 2020).

Environmental risk assessments can give an estimation of the human pressure and environmental state of the areas inside and around marinas, as well as an evaluation of the measures taken by the port to reduce human pressure and achieve international environmental standards (Gómez et al., 2017, 2019). Human pressure is estimated considering the land uses, operation, services, and navigation activity in the port and around it. State combines the susceptibility of the port waters (flushing capacity), its naturalness, or degree of hydromorphological alterations, and an estimation of the ecological value of the area where the port is located (Valdor et al., 2020). The environmental state and anthropic pressures in marinas can influence their biotic communities (Ruiz et al., 2009; Foster et al., 2016; Afonso et al., 2020). Therefore, it is important to better understand how these relate with the biotic assemblages inside marinas. This will allow us to improve risk-evaluation methods and limit the eco-environmental impacts in ports and marinas.

The abundance of NIS and the structure of fouling assemblages inside marinas are often influenced by a complex interaction of environmental and ecological variables (Ruiz et al., 2009; Foster et al., 2016; Afonso et al., 2020). These include environmental features that can be influenced by the design of the marina, such as substrate composition, roughness, morphology and orientation, and water dynamics, temperature, salinity, turbidity dissolved oxygen, nutrients, organic matter, and pollutants concentration; and ecological variables, such as the pressure of propagules, food availability, competition, facilitation, and trophic interactions (Sempere-Valverde et al., 2023a and references therein). According to Foster et al. (2016), medium-sized (with 200 to 550 m of seawalls) and semi-enclosed marinas tend to have higher NIS abundance than smaller and bigger marinas, as well as marinas with open and highly enclosed water bodies. This is because open marinas experience a lower recruitment of species than semi-enclosed marinas, in which the higher water residence time and modified water circulation limits larvae dispersal and facilitates the recruitment of fouling species, particularly NIS (Floerl and Inglis, 2003; Foster et al., 2016). On the contrary, highly enclosed marinas with reduced tidal flushing retain pollutants and sediment, reducing water quality, overall biotic richness, and the abundance of native and NIS (Guerra-García and García-Gómez, 2004, 2005; Chebaane et al., 2019).

Artificial substrates provide surfaces for colonization by fouling assemblages and their associated fauna (Connell, 2001). Among artificial substrates, floating structures in marinas (e.g., pontoons) are considered as hotspots of NIS (Connell, 2001; Dafforn et al., 2009). These create novel habitats with unique physicochemical and environmental characteristics that condition assemblages' composition (Connell, 2000; Holloway and Connell, 2002). For instance, plastic pontoons can stimulate *Bugula* species, as its larvae prefer to settle on plastic surfaces rather than wood and concrete (Pinochet et al., 2020). Pontoons can host unique trophic cascades, as they often host grazers, such as sea urchins and limpets, and cast a shadow that can reduce fouling-feeding fish abundance and feeding performance (Glasby, 2001; Munsch et al., 2017; Giachetti et al., 2020). Furthermore, pontoons can facilitate the transport of invasive species, as their surfaces are close to vessels hulls, therefore, playing a significant role as steppingstones in invasive processes (Connell, 2000; Megina et al., 2016). Therefore, these structures should be prioritized when monitoring and managing NIS inside marinas and for the mitigation of coastal ecosystem degradation.

Buoys constitute the most conspicuous floating structure inside marinas. In fact, buoys are present in marinas of microtidal regions, such as the Mediterranean, where floating pontoons are uncommon. Like pontoons, buoys are often close, or in direct contact with boat hulls, which can increase the transfer of NIS between these structures. This is applicable to vagile fauna (Molina et al., 2017), but also semi-sessile species such as mussels and sabellids, which have limited movement in their adult form, including the ability to detach from the substrate. Moreover, larvae and propagules from sessile species could arrive and settle in higher densities, and some colonies of ascidians and bryozoans could grow and spread between substrates that are close or in direct contact (Minchin and Gollasch, 2003). However, our knowledge on the fouling assemblages on buoys inside marinas is limited (Sempere-Valverde et al., 2023a). This study aims to address the effect of floating substrate (buoys vs pontoons) on fouling assemblages, with special focus on sessile NIS. However, the effect of the type of substrate could also depend on the structure of marinas, water physicochemical conditions and environmental state and pressures. To address this issue, an environmental assessment was carried out for each marina using literature, physicochemical water measurements and an environmental risk assessment.

2. Methodology

2.1. Study location and characterization of the marinas

Samplings were conducted in six marinas of Cadiz Bay (NE Atlantic, Spain), a densely populated and vulnerable ecosystem with intense maritime traffic and a NIS hotspot (Ros et al., 2013; Reverter-Gil and Souto, 2019). The marinas were located within three water basins with different environmental conditions: Outer Bay, Puntales channel, and Sancti Petri channel, which constitutes a second connection of the inner bay with the open sea (Fig. 1) (Zarzuelo et al., 2020, 2021). The main forcings governing the hydrodynamics of this estuarine bay are tides, wind and waves, which are predominantly westerlies from the Atlantic Ocean (Zarzuelo et al., 2021). Among the studied basins, Sancti Petri channel had the most extreme temperature values through the year, while the lowest salinity levels occur in the Inner Bay (see Zarzuelo et al., 2021).

An Environmental Risk Assessment (ERA) was conducted for each marina using the environmental state (St) and environmental pressure (Pr) indexes (Gómez et al., 2019). On the one hand, the St index is the sum of the flushing capacity, calculated using the Complexity Tidal

Range index (CTRI, see Gómez et al., 2017), the Ecological Value or number of ecological singular elements closer than 1 km from the marina, and the Naturalness, which indicates the level of modification of the marina's water body (see Gómez et al., 2019). For the variables used to calculate the CTRI, the water masses total area, maximum length, and entrance width of the marinas were obtained using Google Earth. The medium tidal range was set on 1.8 m for all marinas due to their geographical proximity (Puertos del Estado, 2023). The ecological value was computed as the number of natural protected areas in a 1 km radius around the marina and the naturalness was estimated using Google Earth and following Gómez et al. (2019) guidelines. On the other hand, the environmental pressure index is the sum of the Navigation Activity or density of boats (berths/m^2), the Port Activity, which indicates the presence of gas stations and dry docks, the Dredging Activity or dredging probability, and the External activity, which depends on the main land use within a 1 km radius from the marina (see Gómez et al., 2019 for more details). This information was obtained from the marinas' websites (Puertos de Andalucía, 2023), the urban zoning plans of the studied municipalities (e.g., Ayuntamiento de Cádiz, 2023; Ayuntamiento de Rota, 2023), consulting Google Earth, and following Gómez et al. (2019) guidelines.

Water temperature, salinity, pH, and turbidity were measured in three haphazardly chosen sites inside each marina water body, in both 2016 March (winter) and September (summer) using a conductometer LF 323-A WTW, pH-meter PH 330i WTW, and turbidimeter TURB355IR. Moreover, three water samples were taken from each marina to measure the concentration of organic and inorganic carbon, total Nitrogen, and chemical elements (Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, and Zn) using inductively coupled plasma optical emission spectrometry (ICP-OES). Organic carbon and nitrogen were quantified with a Total Organic Carbon Analyzer (TOC-VCPH) and Shimadzu ASI-V Autosampler.

2.2. Sampling methodology

Samplings were conducted by haphazardly selecting five buoys and pontoons inside each studied marina. The fouling present in the surface of these structures was studied in an area of 25 × 15 cm per structure. This area was delimited using a frame that was haphazardly located on the submerged vertical surfaces of each floating structure, just below the water level (0–15 cm). Samplings were carried out on surfaces within arm's reach from the pontoons, and buoys were extracted from the water when necessary. Samplings were repeated in two seasons: 2016 March (winter) and September (summer). A total of five replicates were sampled per season, marina, and substrate (buoys and pontoons).

The frame used for delimitating each replicate's surface area was divided into fifteen 5 cm^2 sub-quadrats using thin ropes. Sessile species presence on each of these sub-quadrats was counted to obtain species frequency of occurrence in a 0-to-15 scale on the surface of the buoys and pontoons as an estimation of species abundance. When necessary, specimens were collected and stored in 95 % ethanol for their morphological identification using specialized literature. Identified taxa were assigned to non-indigenous species (NIS) or no NIS based on their biogeographical distribution in accordance with literature and actualized databases.

2.3. Statistical analyses

Principal Component Analyses (PCAs) were used for the ordination of marinas based on the normalized physicochemical data collected during winter and summer. On the other hand, species frequency of occurrence was square root transformed and used to calculate a Bray-Curtis resemblance matrix, which was used to conduct a Permutational Multivariate Analysis of Variance (PERMANOVA) and a Principal Coordinates Ordination (PCO) (Clarke and Gorley, 2006; Anderson et al., 2008). The design for PERMANOVA was orthogonal with the factors Season (fixed: winter and summer), Substrate (fixed: buoys and pontoons), and Marina (random: 6 levels). Finally, a resemblance matrix for the centroids of Season × Marina × Substrate, obtained as output from the PCO, was used to conduct a Similarity Profile Analysis (SIMPROF) to highlight statistically homogeneous groups within the assemblages (p threshold = 0.05).

Species richness and abundance, NIS richness and abundance, and NIS frequency (percent NIS abundance from total abundance) were tested using univariate PERMANOVA on the same orthogonal design than multivariate analyses. Assemblages' abundance on quadrats was obtained as the sum of all species frequencies of occurrence, and NIS relative abundance was calculated as the percentage of NIS abundance within the assemblages. Analyses were conducted with Primer-e v.6 + PERMANOVA software (Clarke and Gorley, 2006; Anderson et al., 2008).

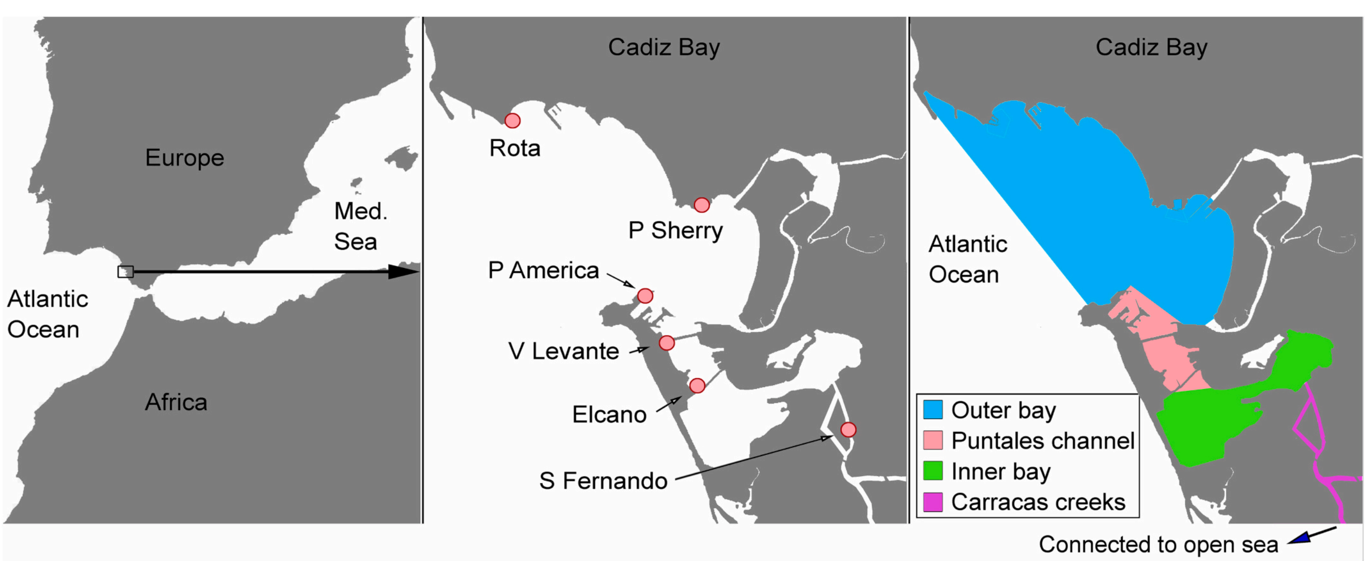

Fig. 1. Study area (36° 32′ 15″ N; 6°, 15′ 52″ W), including the location of the sampled marinas and the different basins of -Cadiz bay (Afonso et al., 2020; Zarzuelo et al., 2020, 2021). Med. Sea = Mediterranean Sea; Sancti Petri = Sancti Petri channel; P Sherry = Puerto Sherry; P América = Puerto América, V Levante = Viento de Levante; S Fernando = San Fernando.

Table 1
Environmental risk assessment (ERA = Pr * St; Gómez et al., 2019) results for the six studied marinas of Cadiz Bay, including the results for the environmental pressures (Pr = NV_i + PT + DG + EX) and states (St = $CTRI_i$ + EV + NA) (grey columns). The colors assigned to ERA indicate risk-score ranges: lower than 2 (red), from 2 to 3 (yellow) and higher than 4 (green). NV_i = relative density of boats; PT = Port operations; DG = Dredging probability; EX = external activity (land use); CTRI = Complexity Tidal Range Index (Gómez et al., 2017); EV = Ecological value; NA = Naturalness.

Marina	NV_i	PT	DG	EX	Pr	CTRI	$CTRI_i$	EV	NA	St	ERA
Rota	0.25	1	0.2	0.5	1.95	15.85	0.64	0	0.5	1.14	2.23
P Sherry	0.35	1	0.2	0.5	2.05	10.47	0.43	0	0.5	0.93	2.90
P America	0.37	1	0.5	1	2.87	10.28	0.42	0	0	0.42	1.20
V Levante	1	0.5	0.2	1	2.70	4.12	0.17	0	0.5	0.67	1.80
Elcano	0.60	0.5	0.5	1	2.60	20.92	0.85	0	0	0.85	2.21
S Fernando	0.41	0.5	0	0.5	1.41	24.63	1	1	1	3	4.23

3. Results

3.1. Environmental characterization

According to the Environmental Risk Assessment, San Fernando was the marina with highest likelihood of suffer adverse effects because of environmental stressors, while Puerto America had the lowest environmental risk (Table 1). The higher vulnerability in San Fernando was due to its high environmental state (St) explained by the proximity of this marina to the protected area of Bahia de Cadiz Natural Park (EV), its low amount of hard infrastructure (NA), and its relatively high flushing capacity ($CTRI_i$). Due to its high naturalness, it was also the marina with lower environmental pressure risk (Pr). On the other hand, Puerto America was the marina with lower St, due to its low $CTRI_i$ and naturalness and, along with the other marinas in Puntales Channel (Viento de Levante and Elcano), had the highest environmental pressure (Pr) scores. This was due to the high demographic density and industrial land use in this area (EX). The variables used to calculate the density of boats (NV) and the Complexity Tidal Range Index (CTRI) can be found at supplementary materials (Table S1).

Water environmental parameters inside the marinas had a high seasonal variation (Fig. 2; see Table S2). The marinas from Outer Bay had the lowest water turbidity and acidity in both seasons (Rota: turbidity = 3.76, pH = 8.15; Puerto Sherry: turbidity = 2.86; pH = 8.16); while the innermost one, San Fernando, was among the marinas with higher water turbidity (18.24), lower pH (7.98), and higher concentrations of Al (0.49), B (0.59), Ba (0.02), Fe (0.26), S (0.14) and Sr (0.71).

3.2. Fouling assemblages

Sixty-one taxa were identified on quadrats, from which 13 were identified as NIS (Table 2). Macroalgae and tunicates were the richest groups, with 14 species each, followed by cnidarians (13 spp.) and bryozoans (12 spp.). Bryozoa was by far the most abundant group, with a frequency of occurrence of 12 out of 15, which indicates presence in 80 % of the quadrats' surfaces, followed by ascidians (45 %), annelids (32 %) and macroalgae (30 %). From those, macroalgae was more

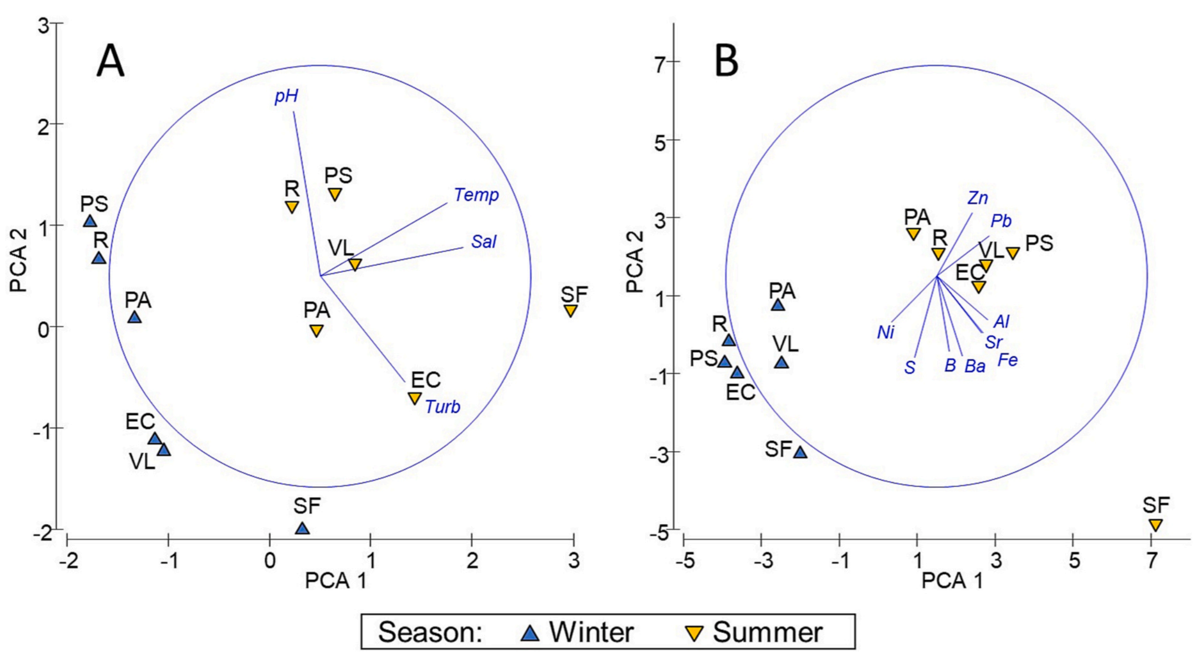

Fig. 2. Principal Coordinates Analyses (PCA) conducted using the environmental variables averaged across seasons and marinas. Only those variables with a Pearson correlation higher than 0.3 with any of the PCA axes are included as vectors (in blue), with vector length indicating the correlation strength. The blue circumference indicates the maximum correlation limit (1). A: PCA-A includes the in-situ measurements pH, temperature (Temp), salinity (Sal) and turbidity (Turb). B: PCA-B includes the water samples analyses for water carbon and chemical elements content. R = Rota; PS = Puerto Sherry; PA = Puerto America; VL = Viento de Levante; EC = Elcano; SF = San Fernando.

Table 2
List of the identified species and their average abundances for each season and substratum (± Standard Error). Abundance is expressed as number of sub-quadrats in which the species was present (from 0 to 15). * = Non-indigenous species; Oc = Ochrophyta; Ch = Chlorophyta; Po = Porifera; Ant = Anthozoa; Hy = Hydrozoa; Ann = Annelida; Ar = Arthropoda.

Taxa		Winter		Summer	
		Buoy	Pontoon	Buoy	Pontoon
Rhodophyta	*Ceramium* sp.	0	0.8 (±0.4)	0	0.1 (±0.1)
	Polysiphonia sp.	1.6 (±0.8)	0.4 (±0.3)	0.2 (±0.1)	0.6 (±0.2)
	Red filamentous algae	1.6 (±0.7)	1.5 (±0.5)	0	0
	Ellisolandia elongata (J.Ellis & Solander) K.R.Hind & G.W.Saunders, 2013	0	2.3 (±0.6)	0.1 (±0.1)	1.4 (±0.6)
	Caulacanthus ustulatus (Mertens ex Turner) Kützing, 1843 *[1]	0	0	<0.1	0.7 (±0.4)
	Chondracanthus acicularis (Roth) Fredericq, 1993	0	<0.1	0	0
Oc	*Dictyota* sp.	0.2 (±0.2)	2.2 (±0.6)	0	0
	Colpomenia sinuosa (Mertens ex Roth) Derbès & Solier, 1851	<0.1	0	0	0
	Ectocarpus siliculosus (Dillwyn) Lyngbye, 1819	0	<0.1	0	0.2 (±0.2)
Ch	*Bryopsis plumosa* (Hudson) C.Agardh, 1823	0.7 (±0.5)	0.3 (±0.3)	0	0
	Derbesia sp.	0	0.2 (±0.2)	0	0.3 (±0.2)
	Cladophora sp.	0	0	0	<0.01
	Ulva sp.	0.9 (±0.4)	1.4 (±0.4)	0	0.4 (±0.2)
	Valonia utricularis (Roth) C.Agardh, 1823	0	0	0	<0.1
Po	*Cliona* sp.	<0.1	0	0	0
	Haplosclerida	0.3 (±0.3)	0	0	0
	Mycale sp.			0.3 (±0.2)	
Ant	*Eunicella singularis* (Esper, 1791)				<0.1
	Exaiptasia diaphana (Rapp, 1829)				<0.1
	Astrangia sp.	0.3 (±0.2)		0.3 (±0.2)	
	Caryophyllia (Caryophyllia) inornata (Duncan, 1878)	<0.1			
Hy	*Ectopleura crocea* (Agassiz, 1862) *[2]	1.7 (±0.8)	0.7 (±0.5)	<0.1	
	Eudendrium spp.	1.1 (±0.7)	0.2 (±0.2)	0.6 (±0.3)	1.1 (±0.5)
	Pennaria disticha Goldfuss, 1820	0.8 (±0.6)	0.7 (±0.4)		
	Plumularid hydrozoan	<0.1	0.9 (±0.5)		1.0 (±0.6)
Mollusca	*Magallana gigas* (Thunberg, 1793) *[3]	1.2 (±0.5)	0.9 (±0.5)	2.5 (±0.7)	1.9 (±0.5)
	Mytilus galloprovincialis Lamarck, 1819	<0.1	0.3 (±0.2)	0.1 (±0.1)	0.3 (±0.2)
	Patella depressa Pennant, 1777		0.1 (±0.1)		0.2 (±0.1)
	Siphonaria pectinata (Linnaeus, 1758)		0.2 (±0.1)		0.2 (±0.1)
	Littorina sp.				<0.1
Bryozoa	*Bugula neritina* (Linnaeus, 1758) *[4]	4.2 (±0.9)	2.5 (±0.7)	2.3 (±0.7)	0.5 (±0.3)
	Bugulina calathus (Norman, 1868)	2.7 (±0.8)	<0.1	1.7 (±0.5)	
	Bugulina stolonifera (Ryland, 1960) *[4]	0.3 (±0.2)			
	Chartella papyracea (Ellis & Solander, 1786)		<0.1		
	Hippopodina feegeensis (Busk, 1884)			0.1 (±0.1)	
	Savignyella lafontii (Audouin, 1826)			0.4 (±0.4)	
	Tricellaria inopinata d'Hondt & Occhipinti Ambrogi, 1985 *[5]	1.0 (±0.4)	0.5 (±0.3)	1.4 (±0.6)	0.7 (±0.4)
	Schizobrachiella sanguinea (Norman, 1868)	0.8 (±0.4)	0.4 (±0.2)	<0.1	
	Schizoporella errata (Waters, 1878)	4.0 (±0.9)	0.7 (±0.2)	8.5 (±0.9)	3.8 (±0.6)
	Watersipora subatra (Ortmann, 1890) *[6]	4.5 (±0.9)	0.1 (±0.1)	3.2 (±0.9)	0.4 (±0.2)
	Biflustra cf. *tenuis* (Desor, 1848)			0.2 (±0.1)	
	Amathia verticillata (delle Chiaje, 1822) *[7]		<0.1	2.7 (±0.8)	0.5 (±0.2)
Ann	*Sabella spallanzanii* (Gmelin, 1791)	<0.1	0.3 (±0.2)	<0.1	0.2 (±0.1)
	Branchiomma luctuosum (Grube, 1870) *[8]	1.4 (±0.4)	0.5 (±0.2)	2.6 (±0.6)	0.9 (±0.4)
	Sabellidae	4.4 (±0.8)	0.3 (±0.2)	6.0 (±1.0)	2.7 (±1.0)
Ar	*Chthamalus stellatus* (Poli, 1791)		<0.1		<0.1
	Perforatus perforatus (Bruguière, 1789)	2.4 (±0.5)	1.8 (±0.5)	3.6 (±0.7)	4.6 (±0.7)
Tunicata	*Aplidium* sp.				<0.1
	Clavelina lepadiformis (Müller, 1776)	2.9 (±0.9)	0.4 (±0.2)	0.8 (±0.3)	0.2 (±0.1)
	Clavelina oblonga Herdman, 1880 *[9]			0.8 (±0.6)	0.6 (±0.3)
	Cystodytes sp.	1.2 (±0.4)	<0.1	0.1 (±0.1)	<0.1
	Didemnum vexillum Kott, 2002 *[10]	0.1 (±0.1)		<0.1	
	Diplosoma listerianum (Milne Edwards, 1841)			0.3 (±0.2)	
	Ascidiella aspersa (Müller, 1776)	1.0 (±0.5)	<0.1		
	Ecteinascidia turbinata Herdman, 1880			3.1 (±0.9)	1.0 (±0.4)
	Perophora sp.			0.6 (±0.4)	
	Phallusia fumigata (Grube, 1864)			1.0 (±0.6)	0.2 (±0.1)
	Botrylloides spp.	2.6 (±0.6)	<0.1	1.9 (±0.5)	1.3 (±0.5)
	Botryllus schlosseri (Pallas, 1766)	0	0	0.3 (±0.2)	0
	Polyandrocarpa zorritensis (Van Name, 1931) *[11]	0.5 (±0.4)	0	0	0
	Styela plicata (Lesueur, 1823) *[12]	1.5 (±0.3)	1.2 (±0.3)	2.2 (±0.5)	1.1 (±0.3)

Shading legend:	0 – 0.9	1 – 1.9	2 – 3.9	4 – 7.9	8 – 15

*[1] Smith et al. (2014); *[2] Schuchert (2010); *[3] Des et al. (2022); *[4] Ryland et al. (2011); *[5] Occhipinti-Ambrogi and Savini (2003); *[6] Reverter-Gil and Souto (2019); *[7] Marchini et al. (2015); *[8] Fernández-Romero et al. (2021); *[9] Ordóñez et al. (2016); *[10] Ordóñez et al. (2015); *[11] Brunetti and Mastrototaro (2004); *[12] de Barros et al. (2009).

abundant during winter in pontoons (Fig. 3). Bryozoans and ascidians were abundant in both seasons, although they were more abundant in buoys than pontoons. As these two groups add up to over 75 % of the identified NIS (Table 2), NIS abundance followed the same pattern as bryozoans and ascidians, being higher in buoys than pontoons.

Fouling assemblages varied across marinas, seasons, and substrates; except for San Fernando, where no differences between buoys and pontoons were observed during summer season (Table 3). Overall, the soft-bodied macroalgae *Dictyota* and *Polysiphonia* were more abundant in winter, while the prostrate calcareous invertebrates *Schizoporella errata* and *Perforatus perforatus* were more abundant in summer than winter (Fig. 4). Buoys were mostly characterized by a relatively stable assemblage composition with high abundance of sessile filter feeders in both winter and summer (SIMPROF group c in Fig. 4). Pontoons, however, had a higher abundance of macroalgae than buoys, particularly *Dictyota* and *Ellisollandia elongata* (SIMPROF group b in Fig. 4).

Fouling abundance was higher in buoys than pontoons in all marinas (Table 4; Fig. 5). Also, total species richness, NIS richness, and NIS abundance were generally higher in buoys than pontoons, although with differences among marinas. Despite differences in NIS abundance, the proportion of NIS within the assemblages (percent NIS abundance from total abundance) did not differ between substrate (pseudo-$F_{1,96} = 6.909$, P(perm) = 0.055; see Fig. S1).

4. Discussion

The variability of assemblages across marinas is driven by complex processes, including their environmental status, pressures, ecological interactions, and biogeographical processes related to non-indigenous species (NIS) spread across geographical areas and latitudinal gradients (Bishop et al., 2015; Gestoso et al., 2017, 2018; Leclerc et al., 2019; Sedano et al., 2020a; Susick et al., 2020). In this study, all marinas were in the same biogeographical area, so their differences were likely influenced by local processes, such as estuarine and coastal dynamics, and their environmental state and pressures (Kocak et al., 1999; Floerl and Inglis, 2003; Kenworthy et al., 2018). These differences were reflected in most water parameters, particularly between the inner marinas of San Fernando, in Sancti Petri channel, and the marinas in Outer Bay (Rota and Puerto Sherry), which had a relatively lower abundance of bryozoans, ascidians and NIS. The innermost Sancti Petri channel is an area more affected by water runoff, which could be related with San Fernando having a high turbidity and chemical elements concentration in water, despite of its better environmental state. Finally, the marinas in Puntales Channel were in a high-density residential and industrial area and had the lowest environmental states and highest pressures, particularly Puerto America and Viento de Levante. Interestingly, these were the marinas with higher ascidians, bryozoans, and NIS abundance. Nevertheless, substrate type played a key role in fouling assemblages' structure and abundance despite the variation in fouling assemblages among the studied seasons and marinas.

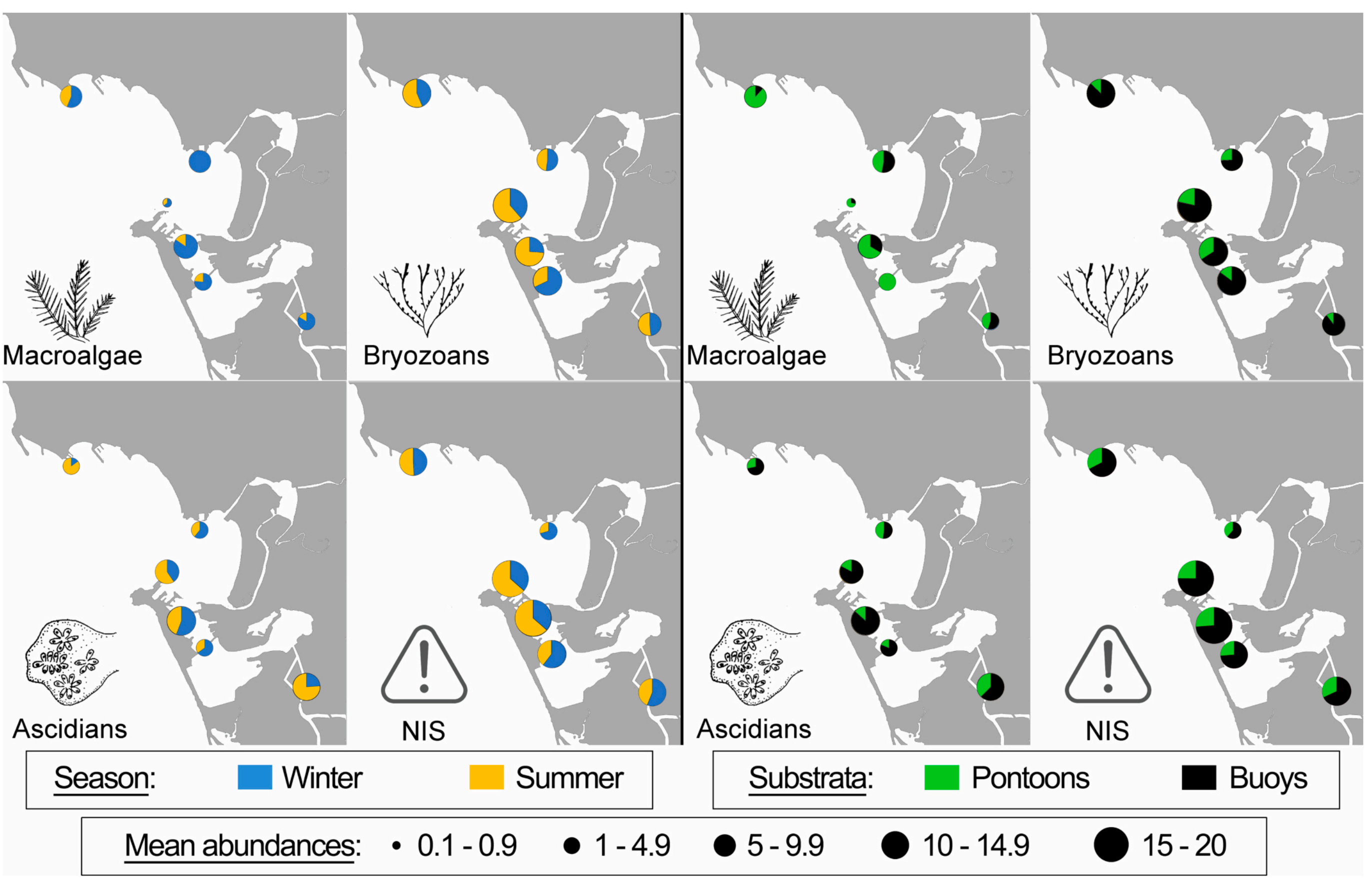

Fig. 3. Mean abundances (piecharts size) for macroalgae, bryozoans, ascidians and non-indigenous species (NIS) in each of the studied marinas in Cadiz Bay. For each group, abundances were obtained by summing species' frequency of occurrence (from 0 to 15). Colors indicate the relative contribution of Season (winter and summer) and Substrate (buoys and pontoons) to the displayed coverage.

Table 3
Multivariate PERMANOVA results on taxa frequencies of occurrence (from 0 to 15). Bold font indicates significant results. Pairwise comparisons (sig. level = 0.05): Se × Ma × Su = all marinas differed seasonally (winter ≠ summer), and all marinas had variation between substrates (buoys ≠ pontoons) in all seasons, except San Fernando in summer season (buoys = pontoons). Se = Season; Ma = Marina; Su = Substrate; Df = Degrees of freedom; MS = Mean Square sum; Res = Residual variation.

Source	Df	MS	Pseudo-F	P(perm)
Season	1	25,357	3.96	**0.014**
Marina	5	15,460	10.01	**0.001**
Substrate	1	31,148	6.52	**0.008**
Se × Ma	5	6410	4.15	**0.001**
Se × Su	1	4677	1.54	0.232
Ma × Su	5	4429	3.09	**0.001**
Se × Ma × Su	5	3048	1.97	**0.001**
Res	96	1544		

The different assemblages composition and higher sessile invertebrate's abundance in buoys than pontoons could be related to the inclination of each structure surfaces (Glasby, 2001; Glasby and Connell, 2001). The rounded shape of buoys imply that their underwater surfaces are slightly inclined downwards, promoting a more shadowed habitat than pontoons, which lateral surfaces are mostly vertical. This could explain the higher abundance of macroalgae in pontoons, particularly during winter, and the higher abundance of bryozoans and ascidians in buoys, including the NIS *Watersipora subatra* and *Bugula neritina*, although there are other factors that could have contributed to these differences. For instance, buoys could be subjected to higher disturbances than pontoons, as they move and rotate on their vertical axis, and can be temporarily extracted from the water during use. Buoys can be in close contact with both pontoons and boat hulls, which might increase their risk as NIS dispersion vector. Another factor that might affect sessile assemblages' composition is differences in the plastic material of buoys and pontoons, although hollow hard plastic buoys and pontoons, such as the ones sampled in this study (e.g., Fig. S2), are usually made from HDPE plastic. Nevertheless, pontoons can be coated with concrete and buoys can be made from plastic foam. In any case, material type seems to play a minor role in structuring fouling assemblages within marinas (Sempere-Valverde et al., 2023a, 2023b). Therefore, it is likely that the differences between substrates are due to the differences in the eco-environmental conditions of these floating structures (e.g., surface inclination, grazing pressure, and structure mobility and size). For example, trophic processes could differ between buoys and pontoons, as the high mobility and small size of buoys might hamper the grazing intensity of fouling-feeding fish, and their surfaces might not sustain a permanent vagile grazing community (e.g., limpets), so the assemblages in buoys and lines would be exposed to a lower predation pressure than those in pontoons, favoring fouling abundance and modifying assemblages' structure (Marić et al., 2017; Giachetti et al., 2020; Chebaane et al., 2022).

The higher abundance of fouling assemblages in buoys than pontoons favor NIS prevalence in marinas, given that NIS are abundant on floating substrates, and increase the risk of NIS dispersal, particularly considering that buoys are more likely to detach and drift at sea than pontoons (Astudillo et al., 2009; Ivkić et al., 2019; Leclerc et al., 2019). This can also be applied to buoys and other floating structures from outside marinas, such as buoys from aquaculture facilities (see Astudillo et al., 2009; Leclerc et al., 2019). NIS can be more abundant in marinas with low environmental state (Floerl and Inglis, 2003; Gómez et al., 2019), such as Viento de Levante and Puerto America (see Table 1 and Fig. 3). Unlike environmental pollution, biological pollution might spread and impact local habitats, both artificial and natural. Therefore, the management of port areas should not only be assessed environmentally but ecologically, as more disturbed structures usually have higher biological pollution risk (Guerra-García et al., 2021a, 2021b;

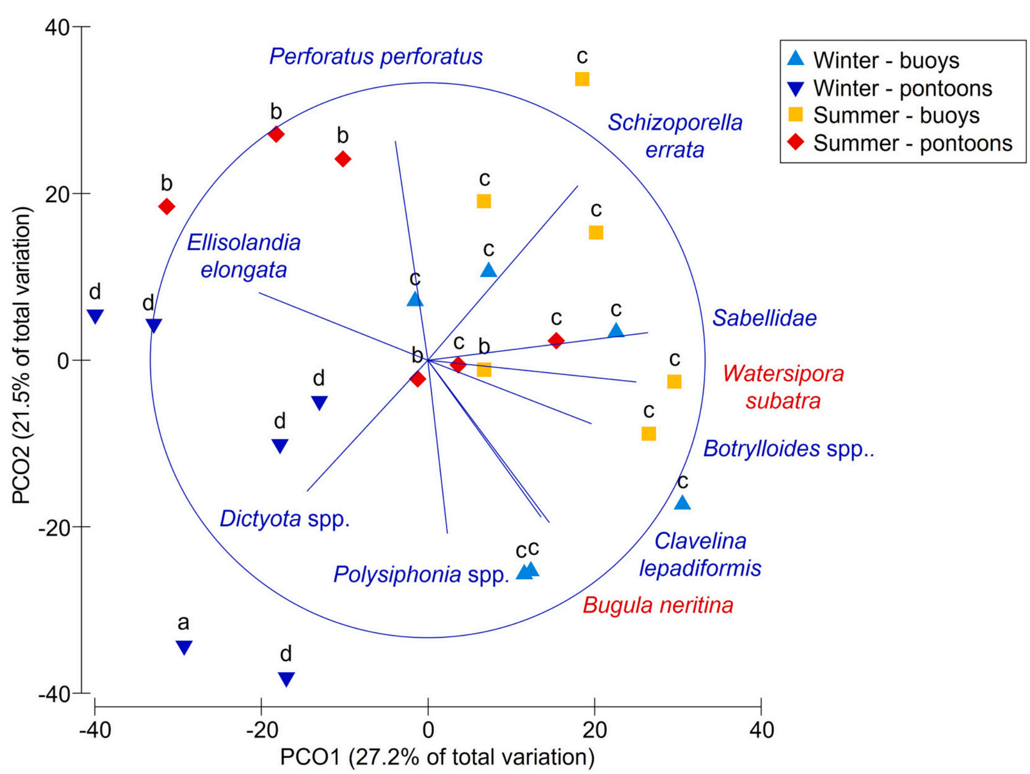

Fig. 4. Principal Coordinates Ordination (PCO) displaying the centroids for Season × Marina × Substrate. Those taxa with a Pearson correlation higher than 0.6 with any of the PCO axes are included as vectors, with vector length indicating the correlation strength and the blue circumference the maximum correlation limit. Letters over symbols indicate the four different assemblage types segregated by SIMPROF (sig. level < 0.05). Taxa font color indicates geomorphological status (blue = native and unresolved; red = non-indigenous and cryptogenic taxa).

Table 4
Univariate PERMANOVA results on total species richness, NIS richness, the sum of all species abundance (total assemblages' abundance) and the sum of NIS abundances (NIS abundance). Bold font indicates significant results (P(perm) <0.05). Pairwise comparisons for Se × Ma (for total abundance) and Se × Ma × Su (total species richness), NIS richness and NIS abundance are indicated in Fig. 5. Se = Season; Ma = Marina; Su = Substrate; Df = Degrees of freedom; MS = Mean Square sum; Res = Residual variation.

Source	Df	Total species richness			NIS richness		
		MS	pseudo-F	P(perm)	MS	pseudo-F	P(perm)
Season	1	11.41	0.46	0.520	2.41	0.74	0.431
Marina	5	29.95	6.11	**0.001**	9.89	6.04	**0.001**
Substrate	1	190.01	22.44	**0.007**	66.01	33.54	**0.006**
Se × Ma	5	24.83	5.06	**0.001**	3.25	1.98	0.089
Se × Su	1	1.41	0.10	0.781	0.68	0.11	0.752
Ma × Su	5	8.47	1.73	0.145	1.96	1.20	0.313
Se × Ma × Su	5	14.39	2.93	**0.014**	5.96	3.64	**0.007**
Res	96	4.91			1.64		

Source	Df	Total abundance			NIS abundance		
		MS	pseudo-F	P(perm)	MS	pseudo-F	P(perm)
Season	1	710.5	0.708	0.458	33.08	0.186	0.665
Marina	5	1168	9.047	**0.001**	393.1	5.956	**0.002**
Substrate	1	14,919	33.053	**0.003**	3111	23.92	**0.005**
Se × Ma	5	1003	7.773	**0.001**	178.1	2.699	**0.020**
Se × Su	1	48.10	0.212	0.674	4.41	0.029	0.872
Ma × Su	5	451.4	3.496	**0.008**	130.1	1.971	0.091
Se × Ma × Su	5	226.8	1.757	0.132	153.5	2.321	**0.047**
Res	96	129.1			344.5		

Saenz-Arias et al., 2022). For example, Puerto America is near an international cruises dock, which has been related to higher rates of species introduction and NIS abundances (Tempesti et al., 2022). In addition, organic carbon inputs from urban effluents, agriculture and aquaculture could promote a high abundance of invertebrates inside marinas, including NIS (Arias et al., 1984; Kenworthy et al., 2018). Finally, marinas in ecologically singular areas, such as saltmarshes and estuaries, have different NIS dynamics than marinas in open coasts and should be considered as a priority in coastal management (Wetzel et al., 2014; Afonso et al., 2020). This is because the proliferation of NIS can have trophic cascade effects and impact ecosystem services in closed systems, such as estuaries and lagoons (Katsanevakis et al., 2014; McQuaid and Griffiths, 2014).

This study constitutes, to our knowledge, a pioneer effort in the quantitative study of fouling on buoys in port areas. The results indicate that high-risk consideration should be given to this substrate type for NIS management. The high NIS abundance and diversity on buoys (e.g., Fig. S2) highlights the importance of this substrate for NIS monitoring and management. Given the importance of biological pollution in coastal areas and their environmental effects, it is imperative that NIS are catalogued and monitored in marinas and considered as a risk factor in environmental risk assessments (Foster et al., 2016; Ferrario et al., 2017; Gómez et al., 2017, 2019; Guerra-García et al., 2021a, 2021b). Moreover, buoys could be ideal experimental substrates for NIS monitoring in marinas, as they can host a high sessile species abundance and diversity. These buoys would be deployed in a similar manner as settlement plates to recruit and study fouling assemblages (see Marraffini et al., 2017). Furthermore, buoys are common in marinas, they are not a hazard to boats or navigation and can include monitoring devices such as data loggers or cameras (Chebaane et al., 2022). Moreover, they can be installed in natural areas without the need for additional structures. This is an advantage with respect to settlement plates, which are hung from overhangs and floating structures and are difficult to install in natural areas and marinas without pontoons (e.g., marinas in microtidal regions). Finally, a better understanding on the effects of population density, land use, port operations, maritime traffic, pollution levels, artificial structure density, substrate type, marina flushing time and the design and use of port infrastructure on fouling communities will improve our capacity to manage NIS, mitigate the impacts of coastal sprawl, and reduce coastal ecosystem degradation (Floerl and Inglis, 2003; Foster et al., 2016; Firth et al., 2016, 2020; Leclerc et al., 2019; Sedano et al., 2020b; Susick et al., 2020).

5. Conclusions

While seasonal variation and the environmental variability among marinas played a role in shaping fouling assemblages, the type of substrate, specifically buoys and pontoons, emerged as a key factor in determining the structure and abundance of assemblages. The higher sessile invertebrate abundance on buoys than pontoons can favor an increase of species richness, as well as NIS richness and abundance per unit of area, as it occurred in some of the studied marinas and seasons. This not only increases the risk of NIS dispersal but highlights the significance of this substrate type for NIS monitoring and management. Buoys, with their high sessile species abundance and diversity, can complement settlement plates as experimental substrates for NIS monitoring, offering advantages such as ease of installation, compatibility with monitoring devices, and applicability in both artificial and natural areas. Moreover, these can be used to experiment eradication methods, such as manual removal, periodic replacement, and timed emersion (desiccation stress). Overall, improving our understanding of the various factors influencing fouling communities will enhance our capacity to manage NIS, mitigate the impacts of coastal development, and preserve coastal ecosystems.

CRediT authorship contribution statement

Juan Sempere-Valverde: Formal analysis, Data curation, Writing – original draft, Visualization. **María D. Castro-Cadenas:** Conceptualization, Methodology, Investigation, Data curation, Writing – review & editing. **José Manuel Guerra-García:** Conceptualization, Methodology, Resources, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Free Espinosa:** Writing – review & editing, Supervision. **José Carlos García-Gómez:** Writing – review & editing, Funding acquisition. **Macarena Ros:** Conceptualization, Methodology, Resources, Investigation, Writing – review & editing, Supervision, Funding acquisition.

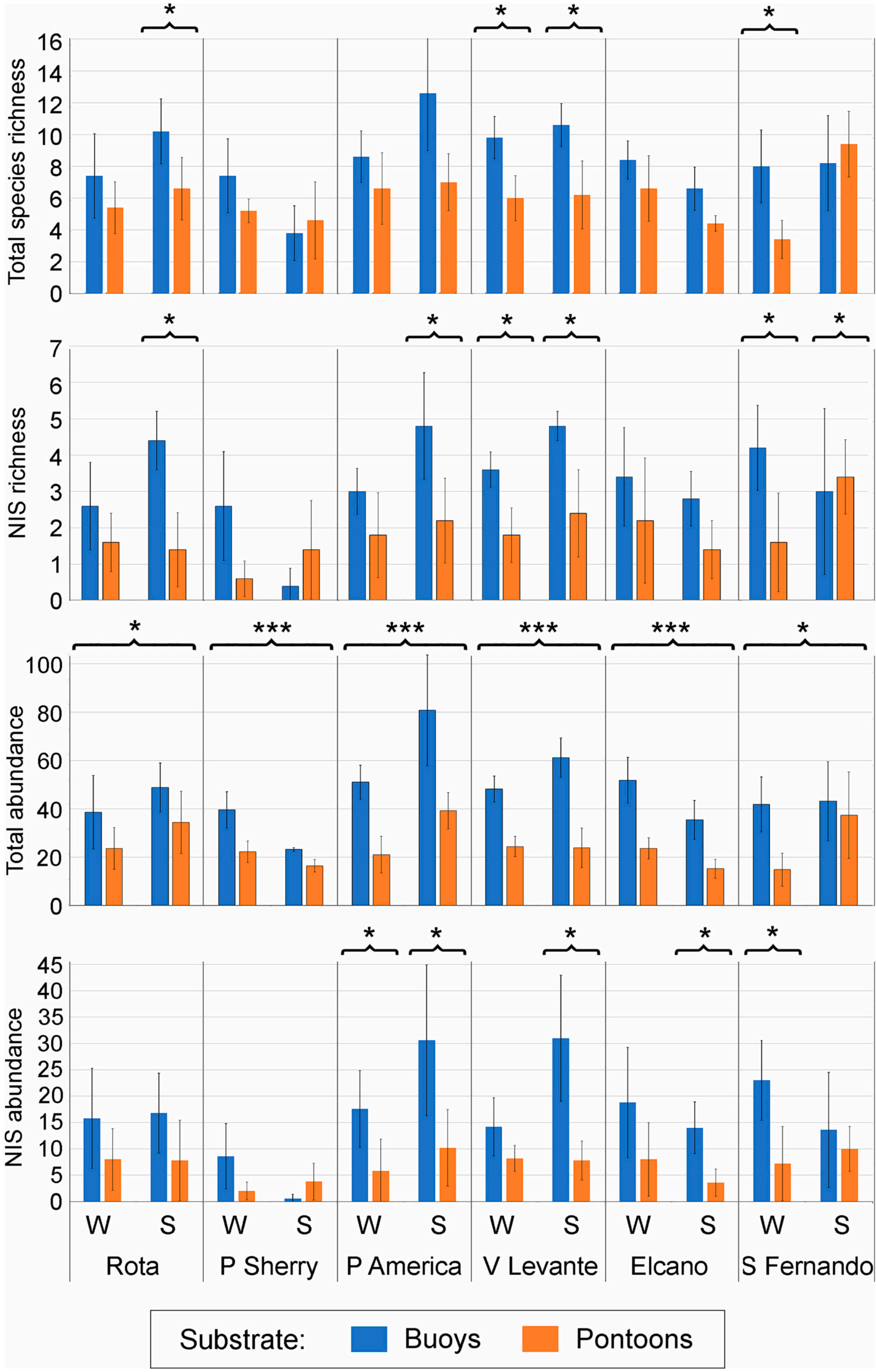

Fig. 5. Average and standard deviation error bars for all species richness, non-indigenous species (NIS) richness, the sum of all species abundance (total assemblages' abundance) and the sum of NIS abundances (NIS abundance) in each marina, substrate and season. Asterisks indicate statistically significant results for pairwise comparisons of the PERMANOVAs in Table 4. W = Winter; S = Summer; * = P(perm) <0.05; *** = P(perm) <0.001.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168301.

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