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Assessment of the effectiveness of antifouling solutions for recreational boats in the context of marine bioinvasions

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ABSTRACT

The recreational boating sector is a major vector for the introduction of non-indigenous species (NIS) via biofouling. Despite applying control measures to prevent the growth of fouling communities, most vessels are NIS carriers. This study assessed the effectiveness of different antifouling strategies in a manipulative experiment by testing two common coating typologies (biocide-based and foul-release coatings), accompanied with simulated maintenance practices. The experiment was carried out in the Gulf of La Spezia (Italy) and samples were collected at two different periods. Results showed significant differences among antifouling treatments regarding community structure, diversity, coverage and biovolume of the sessile component, alongside a significant decrease in the performance of biocide-based coating with time. Interestingly, peracarid NIS/native species ratio was higher for biocide-based treatments, suggesting potential biocide resistance. This study highlights the urgent need to develop common and feasible biofouling management plans and provides insights towards identification of best practices for recreational vessels.

1. Introduction

The unwanted settlement and growth of organisms on artificial hard substrates partially or totally exposed to aquatic environments, namely biofouling, is a major issue for vessel navigation performance and artificial marine infrastructures, as well as for global biosecurity (Yebra et al., 2004; Dafforn et al., 2011; Schultz et al., 2011; Davidson et al., 2016). In fact, because of the increased drag in fouled surfaces, higher fuel consumption, hydrodynamic resistance and higher risk of structural damage are expected, leading to reduced operational efficiency and significantly increased costs (Schultz et al., 2011). Besides, ships' fouling, together with ballast water, is considered a major human-mediated vector for marine bioinvasions at global level (Hewitt et al., 2009; Georgiades et al., 2020; Ros et al., 2023).

In particular, recreational boats have been identified as one of the largest unregulated vectors of introduction and spread of non-indigenous species (NIS) (Clarke-Murray et al., 2011; Ferrario et al., 2017; Ashton et al., 2022) and, thus, a target for global biosecurity (MEPC, 2023). Recreational boating is, indeed, mainly responsible for secondary spread of NIS and offers frequent opportunities for transfers and high connectivity between locations, including areas of conservation and special interest (Ulman et al., 2019; Ashton et al., 2022). Despite the role of boats in the invasion dynamics, biosecurity measures are mostly focused on commercial vessels and ballast waters (Bailey, 2015; Drake et al., 2021), while biofouling of recreational boats is mostly based on recommendations and good practices that rely on self-management (IMO, 2012; GEF-UNDP-IMO GloFouling Partnerships, 2022).

Overall, effective antifouling strategies (AFS) to control fouling development usually require combining chemical, physical and even cultural approaches to maximize their performance (Wezenbeek et al., 2018; Xie et al., 2019; Culver et al., 2021).

Chemical approaches consist in the application of an antifouling coating (AF), whose action mechanism vary according to the coating category (Dafforn et al., 2011; Wezenbeek et al., 2018). They contain active compounds, such as enzymes or biocides, which hamper recruitment and/or affect early survival of foulers. On the other hand, physical approaches include not only a wide variety of cleaning devices

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and maintenance practices, but also coatings that alter surface properties and reduce the attachment strength, for instance, foul-release (FR) coatings (Xie et al., 2019). Yet, biocide-based (BC) is the most widespread coating typology and, currently, copper represents the most common active compound in combination with booster co-biocides (Jones and Bolam, 2007; Ytreberg et al., 2010). However, there is still uncertainty regarding their actual performance (Culver et al., 2021) and potential indirect effects on the environment (Amara et al., 2018; de Campos et al., 2021). Moreover, some target species have shown resistance to the biocides (Floerl et al., 2004; Piola and Johnston, 2006), potentially favouring their spread. Hence, alternatives that dodge the limitations of BC coatings are being explored, like the above-mentioned FR coatings, which have reached the market and are becoming a valuable alternative to the traditional BC coatings. Finally, cultural tactics integrate aspects of boat maintenance, such as planning and timing, adapting the frequencies of different maintenance activities (Culver et al., 2021), presence of supporting infrastructure for correct waste management, regulations and awareness, among others.

Different combinations of those approaches, however, can negatively alter the performance of selected AFS and increase environmental risks. For example, in-water cleaning of hulls can release propagules and viable fragments of biofouling taxa (Hopkins and Forrest, 2008; Kim et al., 2023), as well as favour the leaching of coating material (Schiff et al., 2004; Turner, 2010; Ralston and Swain, 2023) or antifouling paint particles (APPs), that keep acting as a source of biocides (Almeida et al., 2023). Thus, investigating the performance of different strategies is essential for a good biofouling management plan (Davidson et al., 2016).

The Mediterranean Sea is the second most visited destination worldwide for nautical and recreational tourism (Cappato et al., 2011; Ramieri et al., 2022). Previous studies in this region have shown that most boaters regularly apply antifouling measures with, at least, a yearly coating and cleaning frequency, out of which 50 % did so through professional services (Ferrario et al., 2016; Martínez-Laiz et al., 2019; Ulman et al., 2019). In addition to this, several Mediterranean boaters reported to carry out periodical manual cleaning during the boating season; these are usually carried out in-water with soft sponges, while the boat is anchored offshore. However, 71 % of the nearly 600 boat hulls surveyed by Ulman et al. (2019) across the Mediterranean hosted at least one NIS.

Altogether, we can reasonably hypothesize that the commonly recommended antifouling strategies, even if regularly followed, may be insufficient in the Mediterranean context. This study addresses this point by experimentally assessing the effectiveness of different antifouling strategies, based on commonly adopted boaters' practices. In particular, we apply and evaluate the performance of two selected antifouling coating typologies complemented with manual cleaning. The research aims to better understand the dynamics of fouling communities and their response to antifouling treatments, with a particular limelight on NIS recruitment, that serve as a baseline for future management plans applicable to the recreational boating sector.

2. Materials and methods

2.1. Study site

The experiment was conducted in the Gulf of La Spezia, located in the eastern side of the Ligurian coast, North-West Mediterranean Sea (Italy; Fig. 1). It has a semi-enclosed configuration given by the presence of a dam that protects the inner Gulf area (Gasparini et al., 2009). It hosts different urban settlements and many anthropogenic activities, including the presence of different industrial facilities, such as a big commercial and touristic harbour, a military base, several marinas, an electric power-plant and aquaculture installations for both finfish and shellfish (Gasparini et al., 2009). In particular, Fezzano and Le Grazie, located in the western part of the Gulf (Fig. 1), were selected for the study, based on the results of a previous NIS monitoring in the area (Tamburini et al., 2021).

2.2. Experimental design and sampling method

The effects of coating typology, maintenance practices and seasonality were examined, adapting the protocol developed by the Smithsonian Environmental Research Center for the purpose of the research (Marraffini et al., 2017; Tamburini et al., 2021). In total, 48 PVC plates with a dimension of 14 × 14 cm were sanded, coated if required, and attached to a brick, ensuring downward orientation of the coated surface, and deployed randomly at 1 m depth in floating pontoons or concrete docks in the selected marinas in May 2021.

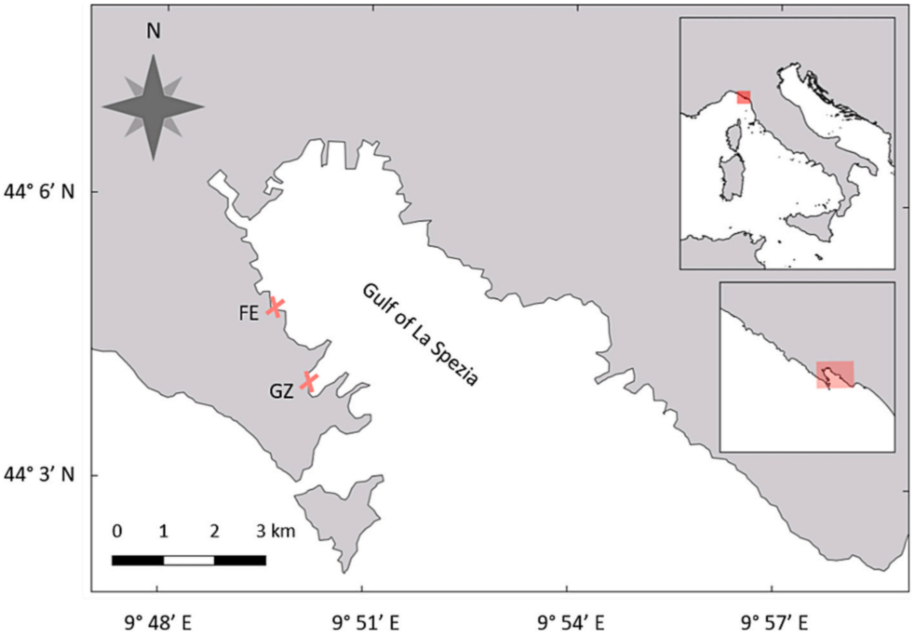

Fig. 1. Map of the study area. Sampling sites of Fezzano (FE) and Le Grazie (GZ) are indicated with a red X. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The effect of coating typology (factor "coating": three levels, including uncoated controls, referred to as C) was tested on two commercially available paints: a) a traditional biocide-based coating (BC) and b) a foul-release coating (FR) (see specifications in the SM). Two sampling periods (factor "season": two levels) were established, a first one immediately after the high boating season (T1: August 2021) and a second one at the end of the low season (T2: February 2022), before the preparations for the upcoming high season. Throughout the duration of the experiment, half of the plates were cleaned periodically (factor "maintenance": two levels) by means of a soft sponge, to simulate the common behaviour of Mediterranean boaters (monthly during T1; once every 2 months during T2). The remaining half was left untouched (no maintenance, NM; maintenance, M).

The combination of factors (coating and maintenance) resulted in six antifouling strategies (AFS: C + NM, C + M, BC + NM, BC + M, FR + NM, FR + M) that were tested for two different submersion periods (T1, T2), by deploying 4 plates per treatment and time (Fig. 2).

2.3. Biological sample analysis

Plates were retrieved and stored in zip-bags containing 70 % ethanol and preserved in the lab until their analysis. By means of a stereoscope (WILD Heerbrugg), fouling communities were morphologically analysed. For sessile assemblages, percentage cover of each the sessile species was assessed with a manual point count method, using a 7 × 7 point grid plus an extra random point (Chang et al., 2018). Although the adopted protocol is specifically designed for sessile fouling species, in this study we decided to also identify the associated mobile organisms. These were retrieved for specimen identification and direct counting of the individuals with a particular focus on peracarid crustaceans, due to the high occurrence of NIS within this taxon (Martínez-Laiz et al., 2019). Finally, the total biovolume of the sessile assemblages alone (excluding the mobile component) was determined by measuring the volume difference after adding the sample to an initial known volume (results included as SM).

Both sessile and mobile components were identified to lowest taxonomic level possible, their relative abundances noted and classified according to their potential origin (native, non-indigenous or cryptogenic; Chapman and Carlton, 1991), supported by available literature.

2.4. Statistical analyses

Statistical analyses were conducted using RStudio (`version 4.2.2`; R Core Team, 2022) and its following packages: `vegan` (Oksanen et al., 2022), `car` (Fox and Weisberg, 2019), `rcompanion` (Mangiafico, 2023), `indicspecies` (Cáceres and Legendre, 2009), `lmPerm` (Wheeler and Torchiano, 2016) and `ggplot2` (Wickham, 2016).

Differences in community composition based on the treatment type were tested using a multivariate approach with Permutational Analysis of Variance (PERMANOVA) (Anderson, 2001) and supported with the Analysis of Similarities (ANOSIM) (Clarke, 1993), both based on a dissimilarity matrix (Bray and Curtis, 1957). Furthermore, dispersion within treatment groups was checked with PERMDISP (Anderson, 2006). For the PERMANOVA, the function `adonis2` of the `vegan` package was used, applying the permutation test under the reduced model following a sequential addition of terms and 9999 free permutations. Non-metric Multidimensional Scaling (nMDS) was used as a representation of the pairwise distances among treatments and the species contributions and their association with specific treatments were determined by the Indicator Species Analysis, using the function `multipatt` of the package `indicspecies`.

Differences among treatments in univariate response variables such as coverage, diversity and NIS/native abundance ratio were tested with univariate analysis. Prior to the test, normality and homogeneity of variances were checked with Levene's (Levene, 1960) and Shapiro tests (Shapiro and Wilk, 1965), respectively. Whenever the assumptions were met, ANOVA (Chambers and Hastie, 1992) was performed, otherwise, permutational ANOVA (Wheeler and Torchiano, 2016) was applied. Both multivariate and univariate analyses were done with untransformed data.

3. Results

We retrieved 44 out of the 48 deployed PVC plates all four lost plates corresponding to the T2 sampling period. Biofouling organisms were classified into 129 taxa, out of which 76 were identified to species level, considering the sessile and mobile components of the community.

3.1. Sessile community

Throughout the whole experiment, 59 sessile taxa had colonised the

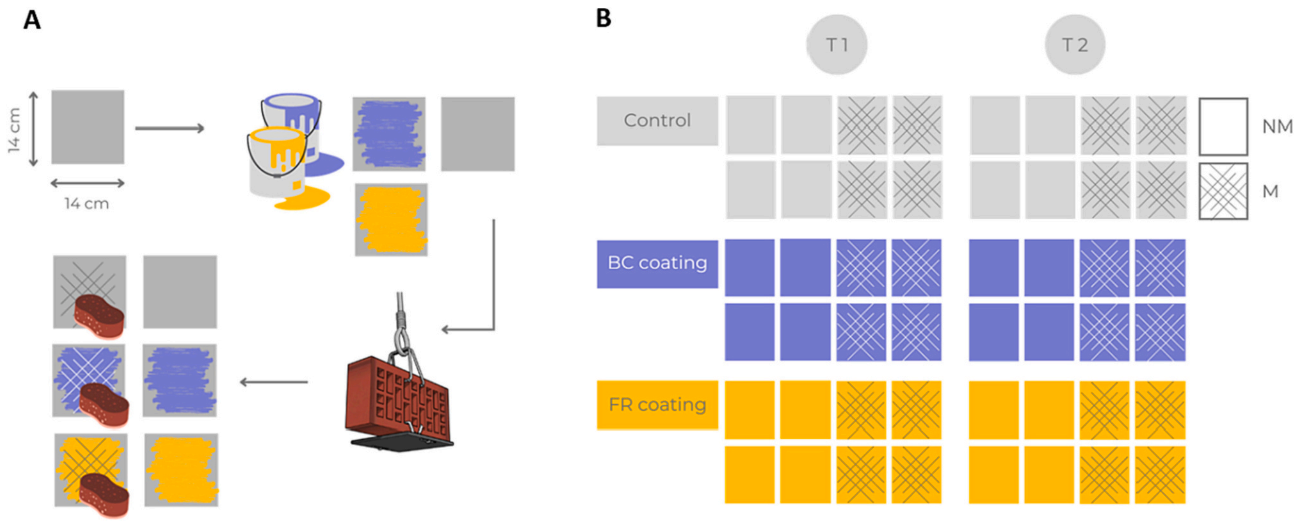

Fig. 2. A) Experimental procedure showing the coating, deployment and maintenance steps. B) Overview of the experimental design showing the combination of factors that result in different antifouling strategies (AFS). Colours indicate coating typology: control, biocide-based coating (BC) and foul-release coating (FR). Maintenance practices are indicated by the pattern (plain/patterned): uncleaned (NM) and cleaned (M) plates. Plates were sampled at two different periods (T1 and T2) and, in total, there were six AFS, with 4 plates per period each.

plates, out of which 47 were already present in uncoated plates during the first 3 months of submersion. In total, nine NIS species were identified (*Paraleucilla magna* Klautau, Monteiro and Borojevic, 2004; *Branchiomma luctuosum* (Grube, 1870); *Hydroides dirampha* Mörch, 1863; *Hydroides elegans* Haswell (1883); *Amathia verticillata* (delle Chiaje, 1822); *Celleporaria brunnea* (Hincks, 1884); *Tricellaria inopinata* d'Hondt & Occhipinti, 1985; *Watersipora arcuata* Banta, 1969; *Styela plicata* (Lesueur, 1823) and 37 native. Bryozoans and annelids represented the most abundant macrogroups of the sessile assemblages for the entire duration of the experiment (average abundance of bryozoans and annelids in control plates: 36.88 ± 4.19 % and 34.38 ± 3.68 %, respectively during T1; 21.53 ± 1.38 % and 33.68 ± 4.94 %, respectively during T2), while tunicates gained presence with time. However, the specific composition of those assemblages changed significantly with time, coating type and their interaction (*p*-values < 0.05; Table 1; Fig. 3).

Location (Fezzano vs Le Grazie) did not result a significant factor affecting the sessile community composition (p-value > 0.05), thus, it was excluded in the subsequent tests, as the communities of the two marinas can be considered highly similar (see also Tamburini et al., 2021). In fact, coating emerges as one of the main factors shaping the community composition, clearly clustering together samples of BC coated plates apart from any other coating treatment during T1, regardless maintenance (Fig. 3). However, the dissimilarity pattern becomes blurrier with time and BC's communities become more similar to untreated and FR-treated plates (Fig. 3). In particular, the Indicator Species Analysis (Table 2) identifies during T1 the bryozoan *Amathia gracilis* (Leidy, 1855) as the species most strongly related with BC coating, while the bryozoan *Schizoporella errata* (Waters, 1878) is the most related to FR coated plates. However, for T2, the most related species to BC coating changed (Table 2), being of particular interest the encrusting bryozoan *Watersipora subtorquata* (d'Orbigny, 1852).

Results on plate surface coverage (Fig. 4) and recruited biomass' volume (SM) support previous differences among treatments, being BC coated plates, irrespective of maintenance, significantly less fouled (lower percentage cover and biomass values) during T1 than the other two coating treatments (*p*-values < 0.05; Table 3).

Diversity seems unaffected by both coating and time, being maintenance the only significant factor (p-value = 0.0383; Table 3; Fig. 4), which tends to increase the diversity in cleaned plates. The serpulid *H. elegans* and the bryozoans *C. brunnea* and *T. inopinata* were the most frequent NIS found in the samples and *W. subtorquata* the most common cryptogenic species present. While differences in the overall sessile composition of communities were shaped by the coating typology, permutational ANOVA on NIS/native ratios as response variable determined maintenance as the factor driving differences among treatments (*p*-value = 0.03351; Table 3), being NIS presence higher in uncleaned plates, despite coating factor (Fig. 4). However, this effect dissipates with time.

3.2. Mobile community

Regarding the mobile component, Fig. 5 describes qualitatively the contribution of the major macrogroups, being peracarids the most abundant taxon across samples, accounting for at least the 85 % of the individuals during T1, although their relative abundances decreased with time. In fact, there are clear differences among mobile assemblages for T1 and T2 (*p*-value = 0.0001, Table 1), with a lower dominance of peracarids and clear shift towards more heterogeneous communities with time (Fig. 5). In mobile assemblages, coating is a significant factor (p-value = 0.0439; Table 1), however its effect is less evident than in sessile assemblages, and maintenance does not seem to have an impact. For longer submersion periods, there are no evident effects of any of the treatments (Fig. 6). However, location seems to be an important factor for the mobile communities (p-value = 0.0109; Table 1).

Analysing in deeper the mobile component, 33 species were identified in the peracarid community, out of which 8 were categorized as NIS, including *Jassa slatteryi* Conlan, 1990; *Caprella scaura* Templeton and Mills, 1836; *Stenothoe georgiana* Bynum and Fox, 1977; *Grandidierella japonica* Stephensen, 1938; *Paranthura japonica* Richardson, 1909; *Paracerceis sculpta* (Holmes, 1904); *Mesanthura romulea* Poore and Lew Ton, 1986 and *Laticorophium baconi* (Shoemaker, 1934). Besides, four species were categorized as cryptogenic: *Ericthonius brasiliensis* (Dana, 1853); *Ericthonius puntatus* (Spence Bate, 1857); *Elasmopus rapax* Costa, 1853; *Caprella equilibra* Say, 1818 (see Marchini and Cardeccia, 2017 for their classification as cryptogenic).

Interestingly, coating, time and their interaction have significant effects on NIS/native peracarid ratio (*p*-values < 0.05; Table 3). In fact, during T1 this is significantly higher for BC coated samples, if compared to any other coating treatment, regardless maintenance (Fig. 7).

Table 1
Statistical summary of PERMANOVA test on community composition of both the sessile and mobile components. Significant factors (*p*-value < 0.05) are marked in bold and an asterisk.

	Ind. variable	DF	R SS	R2	F value	p-Value (>F)	
Sessile component	Coating	2	2.3366	0.20839	7.0453	**0.0001**	*
	Maintenance	1	0.3098	0.2763	1.8680	0.6606	
	Time	2	1.0838	0.09666	6.5359	**0.0001**	*
	Coating:Time	1	1.2734	0.11357	3.8395	**0.0001**	*
	Coating:Maint	2	0.2981	0.02658	0.8987	0.5596	
	Maint:Time	1	0.2727	0.02432	1.6446	0.0977	
	Coating:Maint:Time	2	0.3316	0.02957	0.9998	0.4312	
	Residuals	32	5.3065	0.47327			
Mobile component	Coating	2	0.8748	0.05754	1.5988	**0.0438**	*
	Maintenance	1	0.4408	0.02900	1.6114	0.0871	
	Time	1	2.0488	0.13477	7.4891	**0.0001**	*
	Location	1	0.6524	0.04291	2.3847	**0.0109**	*
	Coating:Maint	2	0.5091	0.03349	0.9305	0.5439	
	Coating:Time	2	0.6053	0.03982	1.1064	0.3205	
	Coating:Loc	2	0.3633	0.02390	0.6640	0.8930	
	Maint:Time	1	0.6380	0.04197	2.3321	**0.0110**	*
	Maint:Loc	1	0.6293	0.04140	2.3005	**0.0123**	*
	Time:Loc	1	0.7349	0.04834	2.6862	**0.0058**	*
	Coating:Maint:Time	2	0.5594	0.03680	1.0224	0.4251	
	Coating:Maint:Loc	2	0.4838	0.03183	0.8843	0.6155	
	Coating:Time:Loc	2	0.4767	0.03136	0.8712	0.6359	
	Maint:Time:Loc	1	0.2883	0.01897	1.0538	0.3817	
	Coating:Maint:Time:Loc	2	0.1517	0.00998	0.5547	0.9052	
	Residuals	21	5.7450	0.37792			

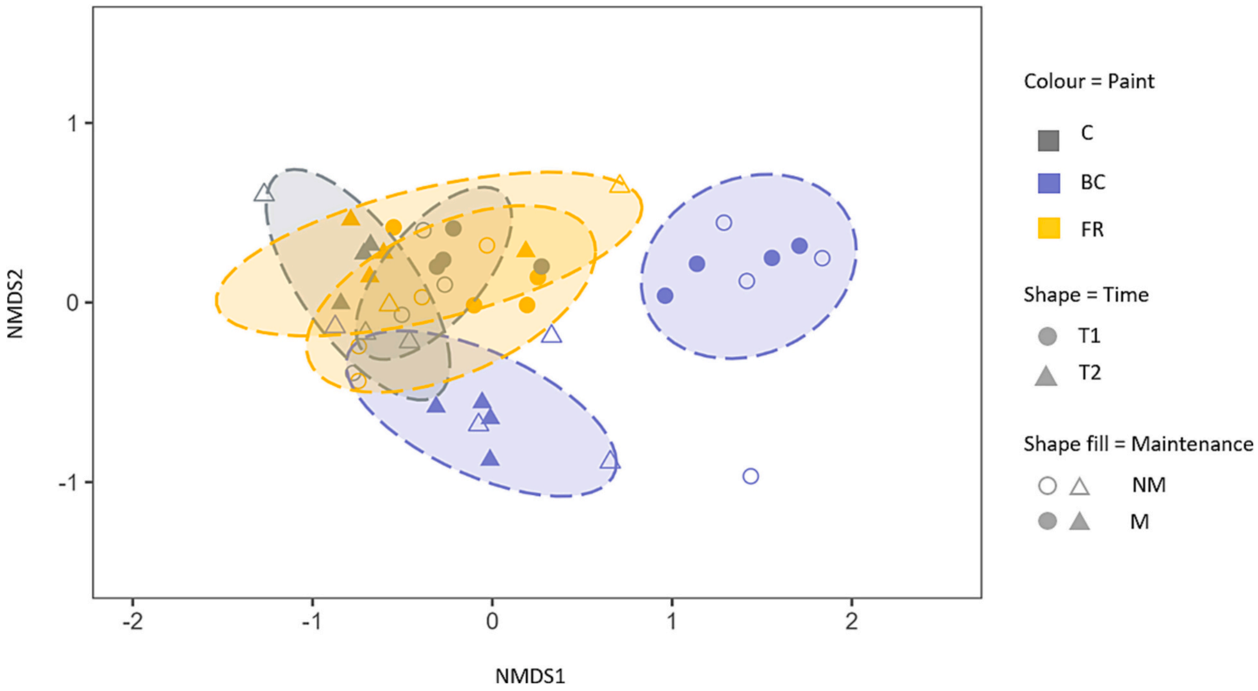

Fig. 3. nMDS of the sessile communities for T1 (dots) and T2 (triangles). Colours represent the coating factor: grey = control (C), purple = biocide-based coating (BC) and orange = foul-release coating (FR). Shape filling corresponds to maintenance practices: empty = unmaintained (NM); filled = maintained (M). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Statistical summary of the Indicator Species Analysis of the sessile communities with coating as grouping factor for both T1 and T2 with a significance level of 0.05.

Remarkable species per coating treatment	T1		T2	
	Statistic	p-value	Statistic	p-value
Group C (n° spp. = 8)				
Anomia ephippium Linnaeus, 1758	0.785	0.0002	0.519	0.0339
Branchiomma luctuosum	–	–	0.394	0.0008
Bugula neritina (Linnaeus, 1758)	–	–	0.671	0.0031
Ciona sp.	–	–	0.578	0.051
Cradoscrupocellaria bertholletii (Audouin, 1826)	0.502	0.0457	–	–
Crisia denticulata (Lamarck, 1816)	0.472	0.0064	–	–
Savignyella lafontii (Audouin, 1826)	0.451	0.0141	0.599	0.0075
Simplaria sp.	–	–	0.679	0.0055
Group BC (n° spp. = 2)				
Amathia gracilis (Leidy, 1855)	0.411	0.0078	–	–
Watersipora subtorquata	–	–	0.75	0.0009
Group FR (n taxa = 2)				
Actiniidae	0.784	0.0007	–	–
Schizoporella errata (Waters, 1878)	0.540	0.023	–	–

Laticorophium baconi represents most of the NIS countings across all treatments, but densities remain particularly high under BC treated plates (Fig. 5). This effect disappears with time, with no significant differences for T2 among treatments. The dominance and remarkable abundances of L. *baconi* in BC samples is supported also by the Shannon Index values of the peracarid communities (Fig. 7), significantly lower than the rest of the treatments during T1 (Table 3), indicating lower richness and a high relative abundance of that species.

4. Discussion

The present study assessed the performance of different AFS that combined chemical and physical factors differently, aiming to elucidate their individual contribution to fouling management.

Overall, BC coating performance decreased in a relatively short period, with an efficiency time lesser than half of what stated by the manufacturer. FR coating showed low efficiency under stationary conditions, performing similarly to uncoated plates. These findings confirm what previously reported in other studies (Davidson et al., 2020; Culver et al., 2021; Lagerström et al., 2022). Regarding maintenance, it was only responsible of controlling particular components of the fouling, with little overall control of the total fouling biomass, as discussed more in detail below.

4.1. Sessile community

The high similarity among communities observed in the two investigated sites is easily explainable by the geographical proximity of the two marinas, promoting high connectivity of free-swimming larval stages of fouling taxa.

Results pointed out coating type as the main responsible of differences in sessile community composition and coverage among the different AFS. Biocide-based coating exhibits the greatest efficiency in the short-term; however, its performance considerably decreases with

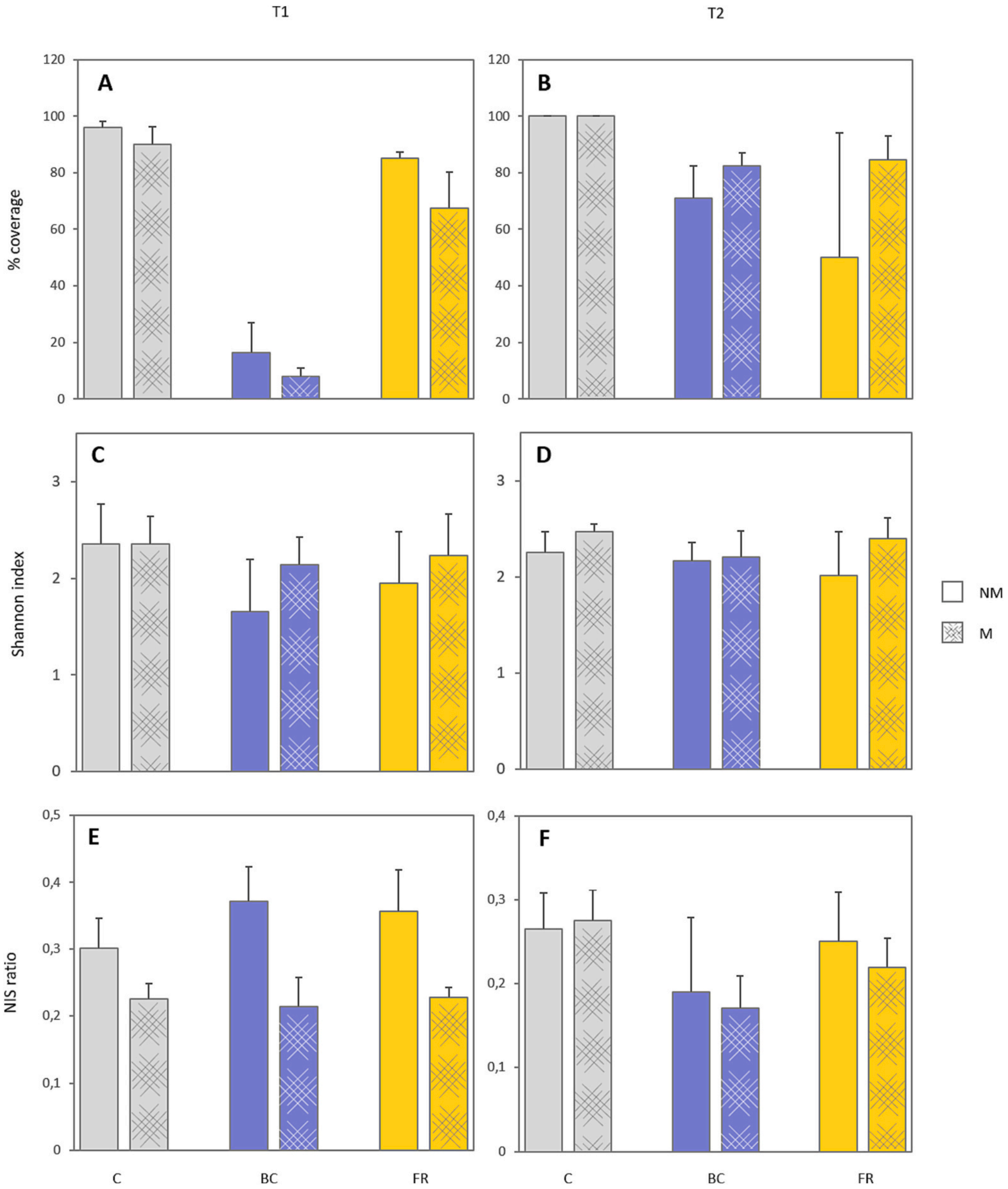

Fig. 4. Coverage (A, B), Shannon diversity index (C, D) and NIS/native ratio (E, F) of sessile communities per treatment and time (left = T1; right = T2). Colours represent the coating factor: grey = control (C), purple = biocide-based coating (BC) and orange = foul-release coating (FR). Bar pattern corresponds to maintenance practices: plain = unmaintained (NM); patterned = maintained (M). Variability is represented by the standard error. Summary of the statistical tests available in Table 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

time, as clearly evidenced by the results on coverage and community composition (see also Culver et al., 2021). Regarding FR coatings, they perform similarly to control plates, without significantly reducing total recruitment in terms of coverage and biovolume. Samples treated with FR exhibited a notable variability that can be explained by the spontaneous detachment of biofouling due to its weight, which should be more evident under dynamic conditions (Davidson et al., 2020). Actually, untouched FR plates ended up containing lower biovolume values than those periodically cleaned (Fig. S1), since the later ones did not accumulate enough biomass for self-release to happen. Yet, the strong variability observed among samples could also be responsible of blurring the effects of the coating typology, especially with time.

Dissimilarities in the assemblages of BC plates support the stated differences among treatments and suggest coating-specific composition of fouling communities. In fact, the Indicator Species Analysis greatly relates the presence of *W. subtorquata* to BC coated plates, implying some degree of resistance that favours colonization (Piola and Johnston, 2006) and, what is more, a facilitator effect as it acts as a foundation for other species to settle (Floerl et al., 2004).

As regards sessile NIS, coating fails to control their presence, being NIS/native ratios similar across coating treatments. In fact, NIS like *H. elegans* and *T. inopinata,* as well as the cryptogenic *W. subtorquata,* have shown tolerance to copper (Piola and Johnston, 2006; Piola and Johnston, 2008; Dafforn et al., 2011). Cleaning with a soft sponge, instead, does reduce the NIS presence during the first 3 months, regardless of coating, but does not reduce NIS in the long term. Abrasive methods could be more effective against cementing and encrusting organisms, like the main sessile NIS found in our plates (*H. elegans*, *T. inopinata* and *C. brunnea*) or the crytogenic *W. subtorquata*, but are less feasible to be conducted on a frequent basis. However, other studies have reported that cleared surfaces attract more organisms (Ralston and Swain, 2023) and, particularly, NIS, showing a positive correlation between disturbance and susceptibility to invasions (Altman and Whitlatch, 2007; McQuaid and Arenas, 2009). According to the intermediate

Table 3

Statistical outputs of the analysis of univariate responses, for both sessile and crustacean communities. Significant tests ($p < 0.05$) are marked in bold. The test is indicated for each case.

			Sessile community				Crustacean community				
	Ind. variable	DF	R SS	R mean sq	F value	p-value	R SS	R mean sq	Iter	p-value	
Diversity (Shannon Index)	Coating	2	0.743	0.3717	2.912	0.0689	1.2175	0.60874	1e+06	**0.00895**	*
	Maintenance	1	0.596	0.5958	4.667	**0.0383**	0.1147	0.11472	1e+06	0.31469	
	Coating:Maint	2	0.251	0.2505	1.962	0.1709	0.0548	0.02739	1e+06	0.78432	
	Time	1	0.101	0.0506	0.396	0.6761	1.7590	1.75904	1e+06	**0.00033**	*
	Coating:Time	2	0.147	0.0734	0.575	0.5683	2.2054	1.10269	1e+06	**0.00043**	*
	Maint:Time	1	0.012	0.0118	0.092	0.7634	0.4228	0.42277	1e+06	0.06152	
	Coating:Maint:Time	2	0.228	0.1140	0.893	0.4193	0.0829	0.04145	1e+06	0.69152	
	Residuals	32	4.085	0.1277			3.5439	0.11075			
	Statistical test		ANOVA				Permutational ANOVA				

	Ind. variable	DF	R SS	R mean sq	Iter	p-value	R SS	R mean sq	F value	p-value	
NIS ratio	Coating	2	0.00819	0.004097	1e+06	0.65115	0.2271	0.1135	3.804	**0.0330**	*
	Maintenance	1	0.04668	0.046679	1e+06	**0.03351**	0.0085	0.0085	0.284	0.5975	
	Coating:Maint	2	0.00633	0.003165	1e+06	0.71549	0.8551	08551	28.649	**7.1e-06**	*
	Time	1	0.03125	0.031252	1e+06	0.07801	0.1479	0.0740	2.478	0.0999	
	Coating:Time	2	0.02614	0.013068	1e+06	0.26587	0.4886	0.2443	8.185	**0.0014**	*
	Maint:Time	1	0.02994	0.029943	1e+06	0.08516	0.0001	0.0001	0.002	0.9609	
	Coating:Maint:Time	2	0.0014	0.000701	1e+06	0.92932	0.0020	0.0010	0.033	0.9676	
	Residuals	32	0.30337	0.00948			09551	0.0298			
	Statistical test		Permutational ANOVA				ANOVA				

	Ind. variable	DF	R SS	R mean sq	Iter	p-value
Coverage	Coating	2	20,062.5	10,031.2	1e+06	**2.2e-16**
	Maintenance	1	57.4	57.4	1e+06	0.678597
	Coating:Maint	2	221.7	110.8	1e+06	0.719082
	Time	1	4573.2	4573.2	1e+06	**0.000823**
	Coating:Time	2	11,036.3	5518.2	1e+06	**2.9e-05**
	Maint:Time	1	1780.7	1780.7	1e+06	**0.027656**
	Coating:Maint:Time	2	917.6	458.8	1e+06	0.264871
	Residuals	32	10,580	330.6		
	Statistical test		Permutational ANOVA			

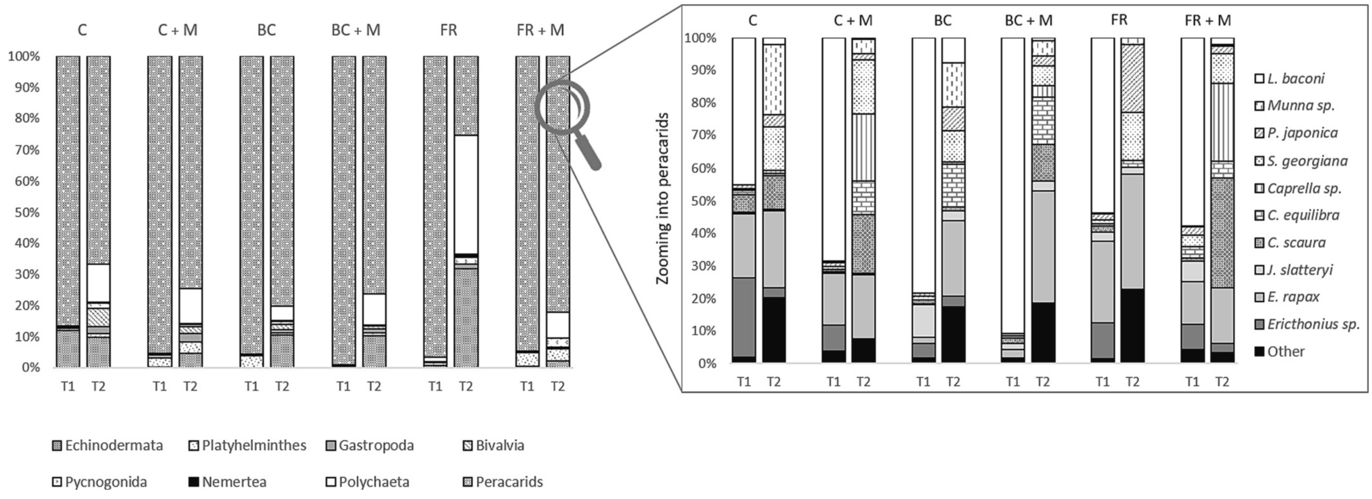

Fig. 5. Descriptive stacked bar chart of relative abundances of the mobile taxa categorized in macrogroups (left) and of peracarid species of the fouling community per treatment type and time. Peracarid species with relative abundances inferior to 10 % are considered as a unique group, namely, 'Other'.

disturbance hypothesis (Connell, 1978), more diverse fouling communities could also be expected on cleaned plates. Interestingly, our results show, indeed, a tendency to more diverse sessile communities in maintained plates (tendency to higher Shannon Indexes), however, in this case, the NIS/native species ratios were lower, contrasting with the previously mentioned susceptibility to invasions on disturbed surfaces.

Although maintained BC plates did not show greater decrease in their performance, as expected due to potential coating damage and faster loss of active compounds, interactions between maintenance and coating performance still need to be clarified.

4.2. *Mobile community*

Contrarily to what observed for the sessile component, the associated

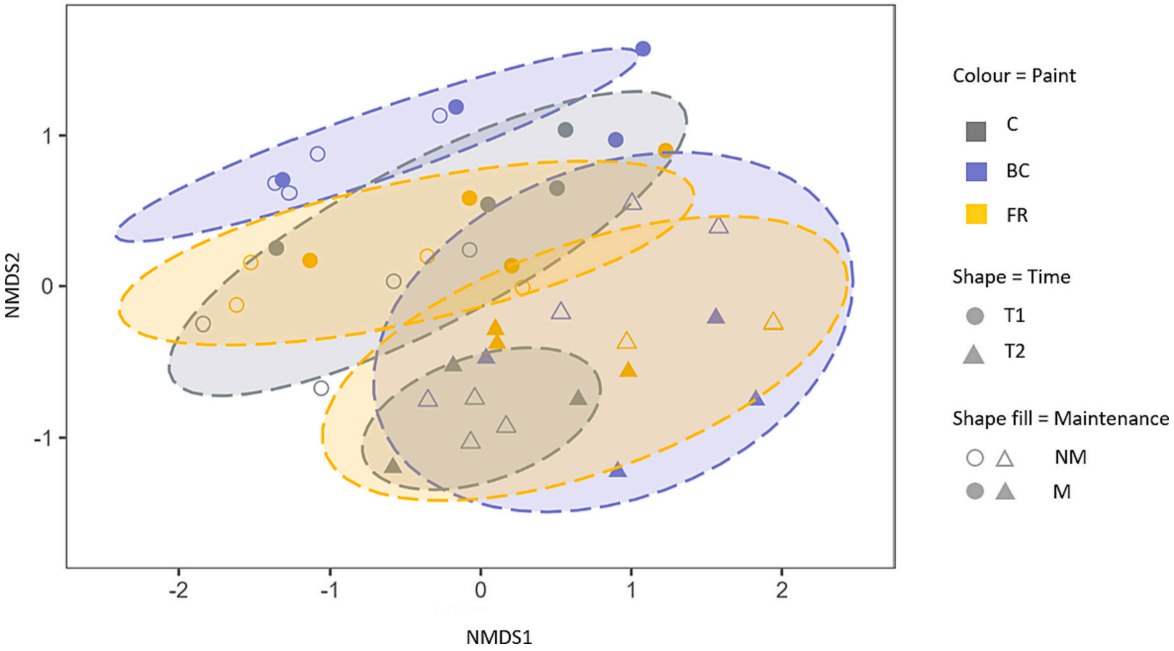

Fig. 6. nMDS of the mobile communities for T1 (dots) and T2 (triangles). Colours represent the coating factor: grey = control (C), purple = biocide-based coating (BC) and orange = foul-release coating (FR). Shape filling corresponds to maintenance practices: empty = unmaintained. (NM); filled = maintained (M). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

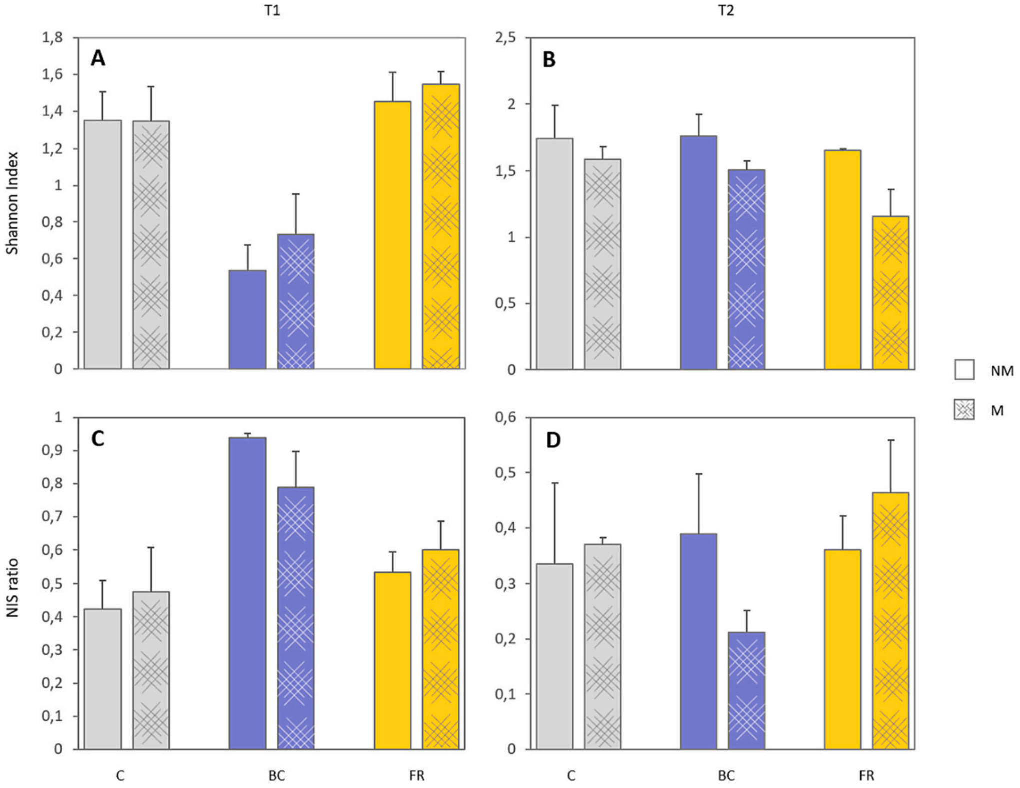

Fig. 7. Shannon diversity index (A, B) and NIS/native ratio (C, D) of peracarid communities per treatment and time (left = T1; right = T2). Colours represent the coating factor: grey = control (C), purple = biocide-based coating (BC) and orange = foul-release coating (FR). Bar pattern corresponds to maintenance practices: plain = unmaintained (NM); patterned = maintained (M). Variability is represented by the standard error. Summary of the statistical tests available in Table 3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mobile communities significantly differed between locations. Prior studies (Martínez-Laiz et al., 2019; Saenz-Arias et al., 2022) have repeatedly shown high beta-diversity in peracarid assemblages, often linked to local-scale variability in environmental characteristics (Kenworthy et al., 2018). Besides, peracarids, indeed, lack pelagic larvae and instead present extended parental care (Thiel, 2003), resulting in low dispersion capacity and gregarious life-style. Some of them, such as corophiids and ischyrocerids, even lead a semi-sessile life (Moore and Eastman, 2015), contributing to prodigious population densities locally (Thiel, 2003; Moore and Eastman, 2015).

Statistical results highlight that coating type significantly affects mobile community composition; and together with time, it is the main factor driving differences among communities. In fact, mobile species in fouling communities strongly depend on the presence of three-dimensional complex structures that host them (Martínez-Laiz et al., 2022; Tempesti et al., 2022), which increases with time and explains the difference in the composition of the communities (Vicente et al., 2021). Although manual removal of the coverage could affect recruitment of vagile organisms and lead to the differences across treatments, maintenance does not seem to have an impact on the community composition of these assemblages.

Among the mobile organisms, peracarids were the most abundant group and L. *baconi* a remarkable species, due to the considerable densities and the significance of its finding. *Laticorophium baconi* was first described in the coast of California (Shoemaker, 1934), in the northeast Pacific Ocean, its likely native range, and considered exotic in the coast of East Asia, Australia and Atlantic Ocean (Hirayama and Morton, 1986; Valério-Berardo and De Souza, 2009; Ahyong and Wilkens, 2011). *Laticorophium baconi* has recently been reported in European Mediterranean waters (Gouillieux and Sauriau, 2019), although its occurrence could date back earlier, unreported due to misidentification with *Apocorophium acutum* (Chevreux, 1908). Its presence has also been documented in other European coasts, Western Australia and New Caledonia (Guerra-García et al., 2023).

This study shows the impressive colonization capacity of *L. baconi* on bare surfaces: it resulted the most abundant species in T1 plates, with notable abundances in BC coated plates, which are of particular concern, as they correspond to the high season of boating and thus, the period of greatest mobility and connectivity between locations (Ulman et al., 2019). These great abundances are reflected in the NIS/native ratio and Shannon Index values of the different treatments, which reflect a low diversity and a NIS/native ratio near one, implying the dominance of L. *baconi* in BC freshly coated plates. In all treatments, the presence of L. *baconi* decreases with time, with the development of the fouling communities and arrivals of other species, coinciding with low season and winter period. These results, together with those from *W. subtorquata*, add to the pre-existing reports on NIS tolerance to biocides, particularly to copper (Floerl et al., 2004; Piola and Johnston, 2006; Culver et al., 2021), which could not be effective enough in limiting their dispersion.

4.3. Challenges and future perspectives

Many factors contribute to fouling dynamics and, when it comes to its prevention, additional factors, such as the strategies followed, need to be included in the equation. Establishing clear links among abiotic factors in a certain context, boat type, including travel habits, and antifouling efficiency, is key for effective biofouling management (Acosta et al., 2010; Parretti et al., 2020). Furthermore, certain environmental parameters, such as salinity or temperature (Singh and Turner, 2009; Lagerström et al., 2020) are known to affect biocide-leaching rate and, thus, determine coating performance and suitability of paint selection. Additionally, cleaning practices and tools can also play a role in determining coating functionality (Oliveira and Granhag, 2020). Although in-water cleaning can be effective in reducing the overall biofouling coverage, unintended side effects that pose biosecurity risks and environmental hazards might happen: a) some species may persist (Davidson et al., 2008); b) viable propagules or complete individuals may be released; c) spawning could be triggered; and d) increased biocide discharge and diminished coating performance could be expected (Morrisey et al., 2013; Kim et al., 2023).

The interaction among all these factors contributes to uncertainty on the efficiency of different biofouling management approaches and their combination. In warm seas, like the Mediterranean, where temperatures are favourable in extended periods for biofouling growth, and salinity levels are relatively higher, antifouling performance could be compromised (Kiil et al., 2001; Dobretsov, 2009). Indeed, our study suggest that the widely used BC coatings have lower performance than expected under the current experimental conditions. Coatings that rely on surface alterations rather than chemical leaching could be a potential alternative.

Likewise, the duration of stationary lay-up periods are a determining factor for the development of fouling communities and, thus, a limitation for the optimal performance of AF coatings. While coatings are designed to perform best under movement, lay-ups are common and unavoidable; hence, understanding their implications for biofouling management is essential to develop workable post-lay-up approaches (Davidson et al., 2020). The results of this study reflect fouling dynamics under different treatments in motionless conditions, which is an important factor to consider that limits the outcome to idle period simulation.

Therefore, understanding the complex interactions involved in biofouling management is crucial to develop effective control plans, biosecurity standards, risk assessment procedures and realistic regulations, in accordance to the regional context (Sylvester et al., 2011).

Despite the evidences of fouling from recreational vessels as an important vector for the spread of NIS (Ferrario et al., 2017; Ashton et al., 2022), there is a lack of a regulatory framework specifically addressing the issue and establishing clear fouling management protocols. Boating, yachting and other forms of maritime tourism constitute an important sector (Cappato et al., 2011), particularly in the Mediterranean, and many efforts of sustainable development focus on it (Ramieri et al., 2022). However, their role in the spread of NIS has been overlooked to the extent that biofouling management within this sector relies on guidelines and good practices (MEPC, 2011; MEPC, 2023). Additionally, the wide commercial offer of solutions with different approaches, the lack of standards and knowledge on the factors influencing fouling dynamics, remarkably contribute to the haziness of the problem and leave the responsibility to boat owners.

5. Conclusions

The tested antifouling strategies are insufficient to control biofouling development under the current experimental conditions. Coatings performed as expected in the short term, during summer season. However, after longer idle-periods, the performance of BC coatings dropped significantly, without reaching half of its service life. It is of particular concern the potential tolerance of certain species, which puts at risks biosecurity strategies. Thus, there is an urgent need for efficient antifouling technologies and development of management policies that tackle the issue of biofouling from an integrative approach (economic, ecological, and cultural perspectives), considering the factors influencing the effectiveness of AFS, with a special focus on recreational boats, implementation feasibility and assessment standards.

CRediT authorship contribution statement

Mar Santos-Simón: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Jasmine Ferrario:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Beatriz Benaduce-Ortiz:** Investigation, Formal analysis, Data curation. **Maren Ortiz-Zarragoitia:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Agnese Marchini:** Writing – review & editing, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

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 archived at Mendeley data repository (DOI: 10.17632/w82cx6g48w.1) and accessible using the following link: https://data.mendeley.com/preview/w82cx6g48w?a=ff986fe1-1412-4824-bcac-95fa1b0e6da3

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116108.

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