Potential Disruptive Effects of Copper-Based Antifouling Paints On The Biodiversity of Coastal Macrofouling Communities

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Abstract

In recent years, after the ban on tributyltin (TBT)-based antifouling paints, copper-based paints have become the main coatings for boat hulls due to their efficiency and endurance. Copper(I) compounds like Cu_2O and CuSCN are used alone or in combination with booster biocides, i.e. Irgarol 1051, chlorothalonil and dichlofluanid. The expanded use of these paints has increased copper leaching into coastal environments, requiring attention and legislative restrictions for potential long-term effects on benthic populations. This study monitored the ecological succession of macrofouling communities on wooden and stainless steel panels immersed for 10 months in the southern basin of the Lagoon of Venice. The development of macrofouling communities on the panels coated with copper-containing antifouling paints was compared with those on the reference (uncoated) and TBT-coated panels. Series of biodiversity descriptors highlighted the preventing activity of the antifouling paints. The most active paints were those containing booster biocides and with self-polishing copolymers in the matrix. The macrofouling communities appeared dissimilar to those on the reference uncoated panels as regards the species richness, the coverage areas, and the biocoenosis structure. Generally, green algae, bryozoans and barnacles were the most tolerant taxa and a negative species selection occurred for sponges, serpulids and ascidians.

Introduction

Large amounts of antifouling paints are employed worldwide on vessels and permanently submerged structures to preserve them and reduce the associated economic costs. However, the severe damage caused to coastal ecosystems by the leaching of the biocidal substances contained in these paints has been well known since the 1980s for organotin compounds (Champ and Seligman, 1996).

Both paint efficacy and its potential for negative effects on the environment are related to content and release rate of the biocides. The latter is mainly controlled by the paint properties. The matrix is the most important component that incorporates the paint ingredients into the product. Apart from the biocide solubility, the matrix properties control both the manner and the rate at which the biocide is released into the water column (Kiil et al., 2006; Almeida et al., 2007). Therefore, the choice of the paint type, i.e. contact leaching or self-polishing paints, is based on the knowledge of the projected docking cycle and the speed and activity of the vessel, which determine the limitations of application. Contact leaching paint, also known as hard or long-life paints have an insoluble matrix, which does not erodes when immersed in seawater. Continuous biocide release is due to the high biocide concentration and, as the biocidal molecules are leached from the surface, microchannels are formed, which permit the release of biocide from deeper surfaces in the coating. Self-polishing paints are based on acrylic polymers, with which seawater reacts causing hydrolysis or ion exchange on the surface layer of the coating, allowing the biocide to be released into the water. The residual polymer backbone then becomes water-soluble and dissolves, exposing a fresh layer of active surface. The consequent constant and uniform surface renewal yields a self-smoothing paint surface with a steady release of biocidal molecules over the lifetime of the paint. Organotin copolymer paints, based on tributyltin methacrylate, were the first self-polishing antifouling coatings, in which the copolymer acted as both the paint matrix and biocide (Swain, 1999).

After the total ban on organotin compounds – the main compound used was TBT – by IMO-MEPC (1998) and, subsequently, by EC (ordinance No. 782/2003, 14 April 2003), the paint industry developed substitutive formulations. Among them, numerous commercial tin-free paints are now available as effective replacement, which are based on copper-based compounds (Yebra et al., 2004). Copper oxidule (Cu_2O) and copper thiocyanate (CuSCN) are the main copper(I) compounds typically employed at concentrations between 20 and 40% (Environment Agency, 1998; Brooks and Waldock, 2009; Pérez, et al., 2015). Although copper is the most important active compound of the antifouling paints that are being used, unlike the organotin paints, these copolymers do not generate sufficient biocide to be effective. Therefore, series of booster substances are incorporated in the formulations together with the principal copper-based biocide to increase the antifouling efficiency of the paints. The association with boosters has the role of increasing the antifouling activity and performance of the paints to a wide spectrum of target organisms tolerant to copper but have the potential to cause environmental damage, for which the risk evaluation is complex (Voulvoulis et al., 1999; Evans et al., 2000; Almeida et al., 2007; Ytreberg et al., 2021).

In all organisms, copper is an essential element because it plays a role in many enzymatic reactions. In mammals, copper has an important role in the formation of erythrocytes and in the development of bone, CNS and connective tissue (Underwood, 1977; WHO, 1996). However, an excess of copper can cause severe metabolic imbalances in humans, since copper has a very complex toxicity mechanism (Goyer, 1995; Ellingsen et al., 2015; Royer and Sharman, 2020). It is absorbed along the gastrointestinal tract and accumulates in the liver, brain, kidney and heart. Copper increases the membrane permeability, resulting in cell lysis, and inhibits glutathione reductase, causing oxidative stress. Consequently, the maximum copper concentration permissible for human health in drinking water is 1.3 mg/l (NRC, 2000). In natural environments, copper partly arises from the windblown dust and the volcanic eruptions (Nriagu and Davidson, 1986), and partly from run-off waters of mineral deposits, the contribution of which is minimal since its concentration generally does not exceed 1 mg/l (Bergvist and Sundbom, 1980; Davies and Bennett, 1985; Purves, 1985). Therefore, copper concentrations found in both freshwaters and coastal marine waters are of anthropogenic origin. The largest anthropogenic copper emissions into the environment (95%) are represented by extraction, production and processing of metals, wood and coal combustion, waste incineration, use of fertilisers and antifouling paints (Nriagu, 1979). In coastal marine waters, concentrations of copper higher than the mean natural concentration (0.003 mg l^{-1}) have been found in harbours and marinas due to the massive use of copper-based paints that began after TBT restrictions were enacted (Claisse and Alzieu, 1993; Srinivasan and Swain, 2007). The US EPA Clean Water Act (CWA) established that copper must not exceed the 3.1 mg/l limit in coastal waters, with regulatory compliance by 2022. Marine organisms that are able to accumulate high amounts of copper include different species of crustaceans and filter-feeding invertebrates such as bivalve molluscs (mussels, clams, oysters) and ascidians (NAS, 1977). On the one hand as edible species, this bioaccumulation could represent a risk for human health, and on the other hand as key coastal ecosystem species, their survival and reproductive capability could be negatively affected causing significant changes in communities (Addison et al., 2008). In these taxa, copper inhibits fertilisation and causes embryotoxicity, larval mortality, and immunotoxicity (Bellas et al., 2004; Zhang et al., 2010; Cima and Ballarin, 2012; Fabbri et al., 2014).

The aim of the present study was to evaluate the disturbing effects of a series of copper(I)-based paints found easily on the market due to their widespread use on boat hulls on the macrofouling communities on hard substrata in the Lagoon of Venice. The effects of antifouling paints on the ecological succession of these communities and the coverage areas of the settling species were analysed for 10 months on a series of wooden and stainless steel panels permanently submerged in the intertidal zone. The results were compared with reference (uncoated panels) and TBT-coated panels using a series of biodiversity descriptors.

Materials And Methods

Study site

The study site (i.e., the sampling station) was a mobile wharf consisting of joined plastic floats located along the Sottomarina channel (Lat. 45° 14’ N, Long. 12° 17’ E) near the port inlet of Chioggia (Venice, Italy) in the southern basin of the Lagoon of Venice. The study area is a low-boat-traffic zone that represents a subtype of euhaline waters and contains an unconfined microenvironment of the lagoon biome that is particularly rich in biodiversity. The basin has a tidal range > 50 cm and a depth < 1.5 m. During the experiment, temperature ranged between a minimum of 3.2°C in January and a maximum of 30.0°C in June. Salinity varied from 13.42 to 38.15 PSU depending on the rainfall trend. For the geographical location near the port inlet, the site, although with low hydrodynamicity, is greatly exposed to water circulation and tides that increased the oxygenation. Dissolved oxygen concentrations usually ranged from 7.03 mg l^{-1} in summer months to 7.92 mg l^{-1} in winter months (Irato et al., 2007).

Experimental setup

Twenty panels, each with an exposed surface of 20 x 15 cm, were deployed in the study site separately installed every 60 cm along the mobile wharf. Each panel was vertically anchored with a nylon rope to a brick basement on the bottom and to the floating pier on the top so that the panel remained constantly submerged with tides by approximately 50 cm never contacting the bottom. The close binding with the rope maintained the vertical orientation of the panels to limit rotation and exposition, which could influence fouling colonisation by shading or floating disturbance. Therefore, all panels had the same light-exposed colonisable area of 300 cm^2, which was considered for observations and analysis of fouling settlement.

The substrata of the panels were larch wood and stainless steel. Four substratum references were represented by two replicates of unpainted wood and stainless steel panels, respectively. The remaining

16 panels were pairs of replicates of 8 wooden and 8 stainless steel panels coated with four ('Paints A–D') copper(I)-based antifouling paints designed for boat hulls (Table 1).

These paints were chosen because of their widespread utilisation in the North Adriatic Sea. With the exception of Paint B, which contained only copper(I) compounds and was based on hard-matrix technology, the other paints were based on self-polishing matrices and differed due to the booster biocide additives they contained. In particular, Paint A contained a mix of organotin (TBT) compounds, Paint C contained a fungicide member of the sulfamide class (dichlofluanid), and Paint D contained both an s-triazine herbicide (Irgarol 1051) and an organochlorine fungicide (chlorothalonil).

The panels were submerged from March to January and monitored monthly over a period of 10 months beginning in April. Photographs of the panel surfaces were taken with a Nikon Coolpix 995 digital camera (Nikon Corporation, Tokyo, Japan). All the operations were performed on the panels, which were immediately and gently air-exposed to avoid withering, drying up and destroying collapse of biofoulers. The analysis of the changes in ecological succession that occurred on the coated panels in comparison with the reference panels was carried out with a series of biodiversity descriptors. The 'species richness' was the total number of species, by month, present on all panels of the same type. The 'coverage-abundance area' was a quantitative analysis (percent cover) of the settlement capacity of the various species on panel areas calculated from digital photos using the Infinity Analyze Application v. 5.0.0 software (Lumenera Co. 2002–2009). The 'biocoenosis structure' was the percentage of coverage for each taxon, namely the set of species belonging to the same taxonomic group, in respect of the total coverage of the whole community (100%) on panels of the same type.

Data analysis

Statistical analysis were performed using PRIMER 6 (PRIMER-E Ltd, Plymouth, UK), and the level of significance was set at $p < 0.05$ for all statistical tests. To investigate significant differences among the covering surfaces of each fouling species on the various types of paint coating, the measures of the areas (cm^2) per month were compared using permutational multivariate analysis of variance (PERMANOVA plus; Anderson, 2001) considering one fixed factor, i.e., the type of paint coating, and one random factor, i.e., the monitoring month. All analyses were carried out using 9999 permutations. To test the hypothesis that the species composition of the community on different types of paint coating was significantly different, an agglomerative hierarchical cluster analysis using Bray-Curtis dissimilarity was used considering the presence/absence data of species (Kruskal and Wish, 1978). Bray-Curtis, clustering was performed with the R package 'clustsig' (Whitaker and Christman, 2015). Communities that are similar in terms of their species composition have been clustered together and represented through a dendrogram.

Results

On both the wooden and steel panels coated with antifouling paints, delays in the appearance of primary biofilms were observed, so these panels lacked the settlement of any kind of macrofouling organism until June or July. Generally, the settlement inhibition was more evident on wooden panels coated with the antifouling paints, which showed a less number of species and, in the case of Paints A and D, the absence of any recognisable ecological succession than the corresponding stainless steel panels.

Every detail of the trends and fluctuations of the coverage areas (Figs. 1, 2), those of species richness (Fig. 3) and those of the biocoenosis structure, the latter considered on the wooden panels as a case in point (Fig. 4), over the experimental immersion are reported for comparative considerations.

Reference panels

The early colonisation arose from a few species of pioneer organisms, which appeared in spring and included green algae as *Lychaete pellucida* (Hudson) Wynne, 2017 and *Ulva intestinalis* Linnaeus, 1753, red algae as *Ceramium ciliatum* Ducluzeau, 1806, and small calcareous tubeworms (serpulids) as *Janua heterostropha* (Montagu, 1803).

The coverage extension occurred in the summer months by a higher number of species. They were represented by green algae (*U. intestinalis* and *Ulva rigida* Agardh, 1823), serpulids (*J. heterostropha* and *Hydroides dianthus* (Verrill, 1873)), bush-like bryozoans (*Bugula neritina* (Linnaeus, 1758) and *Bugulina stolonifera* (Ryland, 1960)), and benthic tunicates, i.e., ascidians (*Ascidiella aspersa* (Müller, 1776), *Molgula socialis* Alder, 1863, *Ciona robusta* Hoshino & Tokioka, 1967, *Styela plicata* (Lesueur, 1823), *Botryllus schlosseri* (Pallas, 1766), *Botrylloides leachii* (Savigny, 1816), *Diplosoma listerianum* (Milne Edwards, 1841)).

In autumn, a competition for the substratum occurred. The main species involved were green algae (*U. rigida*), red algae (*C. ciliatum*, *Gracilariopsis longissima* Steentoft, Irvine & Farnham, 1995 and *Polysiphonia sertularioides* (Grateloup) Agardh, 1863), bivalves (*Mytilus galloprovincialis* Lamarck, 1819), barnacles (*Amphibalanus amphitrite* (Darwin, 1854)), and spotted sponge areas of *Aplysina aerophoba* (Nardo, 1833), *Halichondria panicea* (Pallas, 1766), *Haliclona (Reniera) cinerea* (Grant, 1826), and *Leucosolenia variabilis* Haeckel, 1870. Ascidians persisted both with solitary (*C. robusta*, *S. plicata*) and colonial (*B. schlosseri*, *B. leachii*) species until winter, when significant areas of settlement were recognisable.

Comparing the wooden panels with the stainless steel panels, the early colonisation appears more represented on steel panels but the following coverage trend is similar (Fig. 1) with the exception of the absence of bivalves on stainless steel panels (Fig. 2). The peak corresponding to the maximum species richness occurred from August to September on the wooden (value = 15) and stainless steel (value = 13) panels, respectively (Fig. 3), with biocoenosis structures formed of green algae, sponges, serpulids, bryozoans, and ascidians (Fig. 4).

Panels coated with antifouling Paint A

In June, the green alga *L. pellucida* appeared as the first pioneer species. Neither ecological succession (Fig. 1) nor stable biocoenosis structures (Fig. 4) were recognisable, and only the appearance of spotted areas colonised by organisms that are resistant to biocidal action, such as the red algae *P. sertularioides* and *G. longissima*, barnacles (*A. amphitrite*), and ascidians remained until the end of the observation period on stainless steel panels (Fig. 2). Generally, in the various studied months, the species richness was scarce (Fig. 3), with maximum values of 2 and 7 on the wooden and steel panels, respectively. PERMANOVA revealed significant negative effects for serpulid (*J. heterostropha*) and bryozoan (*B. stolonifera*) settlements on both wooden and steel panels (Tables 2 and 3). Negative effects were much more evident on the wooden panels than on the stainless steel panels due to the complete absence of the green alga *U. rigida*, the red alga *G. longissima*, serpulids (*H. dianthus*), sponges, and ascidians.

Panels coated with antifouling Paints B and C

On panels coated with Paints B and C, the observed ecological successions resulted in less coverage (Fig. 1) and less representative taxa (Fig. 4) compared with those observed on the reference panels. On all the panels, stable fouling organism coverage occurred beginning in July, when the biocide substance efficiency, which was higher in the first months of panel immersion, progressively decreased. Before July, only temporary organism settlements appeared, represented by the temporary presence of green algae or sponges and bryozoans. After July, the biocoenosis structures showed the presence of green and red algae, bryozoans, serpulids, barnacles and ascidians. The latter, mainly including *C. robusta*, *S. plicata* and botryllids, progressively extended their coverage areas until the winter months (Fig. 2).

Regarding Paint B, the peak species richness was observed in autumn (October), with maximum values of 7 and 9 on the wooden and stainless steel panels, respectively (Fig. 3). PERMANOVA showed significant inhibition of settlements of the red algae *G. longissima* and *P. sertularioides*, and of the ascidian *B. leachii* on both the wooden and stainless steel panels (Tables 2 and 3), and of the bivalve *M. galloprovincialis* on the wooden panels (Table 3).

Regarding Paint C, the peak species richness was observed in late summer (August-September), with maximum values of 9 and 12 on the wooden and stainless steel panels, respectively (Fig. 3). PERMANOVA did not reveal significant differences in types of species settlements between the coated and reference panels except for the late settlement of the serpulid *H. dianthus*, the bryozoan *B. neritina* and the ascidian *B. schlosseri* on the stainless steel panels (Table 3). This exception was shared with Paint B and could be related to the scarce adhesion onto stainless steel panels, which caused a high leaching of the paints and a progressive increase of antifouling uncoated area favouring some taxa.

Panels coated with antifouling Paint D

Paint D showed strong antifouling action, as the macrofouling settlements on panels coated with these paints were transitory and very limited in both the coverage areas (Figs. 1, 2) and numbers of species (Fig. 3) throughout the entire observation period. The presence of species, mainly represented by macroalgae, bryozoans and barnacles, never exceeded one month; often, primary biofilms again appeared and replaced the organisms that had previously settled. Therefore, the settlements appeared to be random and to lack any stabilised biocoenosis structure (Fig. 4). The species richness was much more limited on the wooden panels (maximum value = 1) than on the stainless steel panels (maximum value = 4) (Fig. 3). The PERMANOVA confirmed the negative effects of the paint on settlements of species belonging to serpulids, bryozoans, molluscs and ascidians (Tables 2 and 3).

Discussion

Modality and time of development of a biofouling community depend on climate, immersion site, and physico-chemical characteristics of both water column and substratum. A temporal succession, a seasonal succession and a biotic succession of the principal taxa can be always distinguished (Redfield and Delvy, 1952; Scheer, 1954). In the present study, the ecological succession resulting from the monthly analysis of the reference panels from spring to winter was similar to those reported in previous research works confirming the trend of settlement and growth of macrofouling organisms of hard substrata in the Lagoon of Venice (Cornello and Manzoni, 1999; Cornello, and Occhipinti Ambrogi, 2001; Cima and Ballarin, 2013).

Generally, macrofouling was observed two-months later on the panels treated with antifouling paints in comparison with the reference panels. The resulting areas of such settlements were much smaller in comparison with those of the reference panels, appearing as spotted distributions. All antifouling paints significantly inhibited the settlement of the ascidian *A. aspersa* and the sponges *H. cinerea* and *L. variabilis* on the wooden panels and of the red alga *C. ciliatum* and the sponge *H. panicea* on the stainless steel panels. These results support previous negative effects of copper-containing antifouling paints observed on sponges (Rejeki et al., 2010), ascidians (Osborne et al., 2018), and red algae (Ytreberg et al., 2010).

The macrofouling communities observed on the various experimental panels coated with antifouling paints appeared dissimilar in taxa composition to those on the reference uncoated panels (Fig. 5). Paints A, B and D generally inhibited the settlement of sponges. Both Paints A and D also inhibited serpulids and ascidians on wood panels but only serpulids on steel panels, and Paint D prevented molluscs on wood panels as well. Paint B inhibited red algae and molluscs on wood panels. Overall, the most tolerant taxa to these antifouling paints were represented by green algae, bryozoans and barnacles, which were found on all panels. This observation confirms previous study on taxa with the most tolerance to copper, in particular the genus *Enteromorpha* (Correa et al., 1996), the bryozoan *B. neritina* (Piola and Johnston, 2006), and the barnacle *A. amphitrite* (Qiu et al., 2005). Based on the dissimilarity of the communities, the comparison of the negative effects can lead to orders of antifouling efficiency and consequent disruptive potentials on native community of the copper-based paints examined herein as follows: Paint D ≥ Paint A > Paint B > Paint C for wooden panels, and Paint B ≥ Paint D > Paint A > Paint C for stainless steel panels. A higher dissimilarity due to a higher performance of antifouling paints was in general present for communities on wooden panels than on stainless steel panels. The more extensive coverage of biofoulers on stainless steel panels than on wooden panels is far difficult to interpret. Different adhesion capability of antifouling paints on different types of substrata and the progressive wearing off of the paint coating must be considered, but other factors could be involved. A primary bacterial film on a metal surface immersed in natural waters could create electrochemical conditions which accelerate metal corrosion (Dexter, 1993). Microbiologically influenced corrosion and marine biofouling are closely related to the biofilms in dictating the subsequent biofouler settlement (Li and Ning, 2019), although the response of fouling organisms to materials is not universal and different taxa can be differently affected depending on physicochemical factors at the metal surface. As a result, metallic substrata are more susceptible to biofouling settlement than some non-metallic substrata such as fibreglass, wood and plastics (Pomerat and Weiss, 1946).

The efficiency of both Paint A and Paint D was assessed by the fact that no ecological succession was recognisable as well as few taxa that were temporary resistant to biocidal action appeared several times with limited coverage. Paint D demonstrated lasting biocidal power, which was higher than that of the TBT-based Paint A because the taxa settlements always appeared to be random and never stabilised. This particular antifouling effect may be related to both the high copper oxidule content of the paint and the synergistic effect with boosters. Chlorothalonil is toxic to various aquatic invertebrates (Cima et al., 2008; Dumollard et al., 2017; Amara et al., 2018), and Irgarol 1051 is a photosynthesis-inhibiting herbicide used in agriculture and therefore able to withstand seaweed growth (Dahl and Blanck, 1996; Hall et al., 1999; Johansson et al., 2012).

Regarding Paints B and C, in the first 3–4 months of the study period the few taxa settled with spotted areas forming not-well structured communities. However, beginning in the summer months the taxa settled on the panels coated with both paints showed increasing coverage areas on the antifouling-coated panels, suggesting a time-dependent loss of efficiency. The scarce effects observed in the case of Paint B – a unique paint based on contact-leaching technology and containing only copper oxidule without boosters – on species settlement and growth supported the importance of both the type of matrix with biocidal exposure and the co-presence of boosters for enlarging the performance of copper-containing paints. Antifouling life is not simply a function of product formulation, but also of the system application, the biocide package in the matrix, the biocide reservoir within the paint, and the rate of release (Ivče et al., 2020). With time, the soluble biocidal compounds are released from the contact leaching paints, leaving behind a depleted, porous matrix. As this depleted layer increases in thickness, the diffusion of biocides from the paint film decreases exponentially over time and effective life is limited (Zhou, 2015). The low efficiency of Paint C could be related to the different copper(I) compound (CuSCN) contained in this paint type and/or its lower concentrations compared to those in Cu_2O-based paints. The presence of a less effective booster, such as dichlofluanid (Konstantinou and Albanis, 2004), could also be considered as a cause of this result. The scarce antifouling effect of Paint C is an example of the paradox regarding balancing the development of paints with low copper contents and compliance with EU Regulation 1143/2014 concerning the need to prevent the growth and minimise the transport of invasive alien species on hulls of pleasure craft (AMOG Consulting, 2001).

Conclusion

Copper-based paints mainly differ in their copper(I) concentrations, presence of booster biocides and type of matrix (i.e., contact leaching or self-polishing). Their commercial formulations must meet with two requirements, which might seem in contrast for the impossibility to develop targeted systems: to have the most efficient antifouling properties for organisms that settle on immersed structures, and simultaneously be safe for non-target marine organisms (Rossini et al., 2019).

Copper compounds were considered ideal for use as antifoulants since the chemistry of copper changes in the marine environment, thereby affecting its bioavailability (and therefore its toxicity) by rapid organic complexation. A key factor in the environmental aspect of copper-based antifoulants is that the active ionic form of copper exists only briefly while it is on the coated surface. During that time, it performs its efficacy by impeding the settlement of fouling organisms. However, the co-presence of organic booster biocides and the type of polymeric matrix can significantly increase the performance of the paint. The synergistic effects of these compounds are still largely unknown together with their behaviour and fate in the coastal ecosystems and their potential to cause deleterious effects on various benthic organisms (Brooks and Waldock, 2009).

Although antifouling products are regulated under the Biocidal Products Regulation in the EU (BPR, EU Regulation 528/2012) as regards their application, production and marketing, most EU countries have not developed any restrictions or bans regarding the use of copper-based antifouling paints (Lagerström et al., 2020). Some countries have independently limited the emissions of copper from antifouling paints in their coastal waters, or the application of paints with copper biocides has been restricted only to recreational craft. Considering the wide use of these antifouling paints together with the continuous leaching of biocidal substances into the seawater column and sediments, the dangerousness of these xenobiotic compounds to coastal communities – in particular to fragile ecosystems such as those of the Lagoon of Venice – must be considered. In this study, results with copper-based paints revealed unpredictable effects on the settlement and growth of key species of macrofouling of hard-substrata, which have the potential to alter the native structure of the communities. Continue pollution of copper antifoulants can cause a reduction of diversity and has differential effects on recruitment of more tolerant non-indigenous species increasing the invader dominance (Dafforn et al., 2008; Piola and Johnston, 2008). Consequently, greater control and monitoring of risk assessments before the introduction of so-called “eco-friendly” copper-based formulations in commerce is critically essential to avoid long-term irreversible changes on benthic populations and coastal trophic networks. The problem of limiting the release of copper into the environment should be solved by researching new technologies at the matrix level. The amount of copper in the formulation of the paint could be significantly reduced with a more resistant matrix useful to both prevent the rapid oxidation of copper and long-time maintain the antifouling action by contact with biofoulers. In such way, the antifouling action should be limited to the surfaces to be protected without leaching of biocides into the water column causing a minor impact on coastal ecosystems.

Declarations

Ethical approval

The authors followed all applicable international, national, and/or institutional guidelines for the care and use of animals.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Conflict of interests/Competing interests

The authors declare that they have no conflict/competing interests.

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Authors’ contributions

RV and FC contributed equally to the content of this manuscript. RV: conceptualization, methodology, statistical analysis, data analysis, writing original data preparation; FC: supervision, reviewing, and editing. All authors read and approved the final manuscript.

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Tables

Table 1

Copper-based antifouling paints used to coat the panels submerged at the sampling station

Paint		Biocides	Cu (wt%)	Matrix	Use
A	Sigmaplane HB Antifouling	Cu_2O (28 wt%) TBT methacrylate (19 wt %) TBTO (0.5 wt %)	24.8	Self-polishing copolymers	Steel and wooden hull of fishing boats and cargo vessels more than 25 m in length
B	Sikkens Vinyl Antifouling 2000	Cu_2O (41 wt %)	36.41	Contact leaching (hard or insoluble)	Steel (not aluminium), wooden and polyester hull of sailing boats and yachts
C	Sikkens Self-polishing Antifouling 2000	CuSCN (20 wt %) Dichlofluanid (9 wt %)	10.4	Self-polishing copolymers	Steel, aluminium wooden and polyester hull of sailing boats and yachts
D	Baseggio Sirena Antivegetativa Universale	Cu_2O (42 wt %) Chlorothalonil (7 wt %) Irgarol 1051 (1.1 wt %)	37.3	Self-polishing copolymers	Steel, wooden and fibreglass hull of fishing boats

Table 2

PERMANOVA results of species coverage on wooden panels. For each species, pseudo-F values (indicated as F) and Monte Carlo p-values (indicated as $P_{(MC)}$) are reported for “month”, “antifouling paint”, and “month x antifouling paint interaction”. Statistically significant effects ($p < 0.05$) are indicated in bold.

SPECIES	**MONTH (MO)**	**ANTIFOULING PAINT (AP)**	**MO x AP**
Lychaete pellucida	$F_{(9.99)} = 1.411$ $P_{(MC)} = 0.292$	$F_{(8.99)} = 1.001$ $P_{(MC)} = 441$	$F_{(72.99)} = 1.479$ $P_{(MC)} = 0.249$
Ulva intestinalis	$F_{(9.99)} = 0.389$ $P_{(MC)} = 0.912$	$F_{(8.99)} = 0.928$ $P_{(MC)} = 0.497$	$F_{(72.99)} = 0.312$ $P_{(MC)} = 0.998$
Ulva rigida	$F_{(9.99)} = 3.944E-2$ $P_{(MC)} = 1$	$F_{(8.99)} = 4.728$ $P_{(MC)} = 0.0003$	$F_{(72.99)} = 7.3717E-2$ $P_{(MC)} = 1$
Ceramium ciliatum	$F_{(9.99)} = 7.6253E-2$ $P_{(MC)} = 0.999$	$F_{(8.99)} = 0.854$ $P_{(MC)} = 0.555$	$F_{(72.99)} = 0.126$ $P_{(MC)} = 1$
Polysiphonia sertularioides	$F_{(9.99)} = 1.137$ $P_{(MC)} = 0.415$	$F_{(8.99)} = 1.960$ $P_{(MC)} = 0.061$	$F_{(72.99)} = 0.182$ $P_{(MC)} = 1$
Gracilariopsis longissima	$F_{(9.99)} = 0.185$ $P_{(MC)} = 0.988$	$F_{(8.99)} = 2.324$ $P_{(MC)} = 0.0259$	$F_{(72.99)} = 0.139$ $P_{(MC)} = 1$
Aplysina aerophoba	$F_{(9.99)} = 0.267$ $P_{(MC)} = 0.970$	$F_{(8.99)} = 1.9756$ $P_{(MC)} = 0.063$	$F_{(72.99)} = 0.455$ $P_{(MC)} = 0.969$
Halichondria panicea	$F_{(9.99)} = 3.719$ $P_{(MC)} = 0.025$	$F_{(8.99)} = 0.9$ $P_{(MC)} = 0.531$	$F_{(72.99)} = 2.222$ $P_{(MC)} = 0.082$
Haliclona cinerea	$F_{(9.99)} = 2.6144E-2$ $P_{(MC)} = 1$	$F_{(8.99)} = 13.5$ $P_{(MC)} = 0.0001$	$F_{(72.99)} = 4.4444E-2$ $P_{(MC)} = 1$
Leucosolenia variabilis	$F_{(9.99)} = 3.9216E-2$ $P_{(MC)} = 1$	$F_{(8.99)} = 6$ $P_{(MC)} = 0.0001$	$F_{(72.99)} = 6.6667E-2$ $P_{(MC)} = 1$
Hydroides dianthum	$F_{(9.99)} = 0$ $P_{(MC)} = 0$	$F_{(8.99)} = 6.677$ $P_{(MC)} = 0.0001$	$F_{(72.99)} = 0$ $P_{(MC)} = 0$
Janua heterostropha	$F_{(9.99)} = 0.109$ $P_{(MC)} = 0.999$	$F_{(8.99)} = 2.711$ $P_{(MC)} = 0.011$	$F_{(72.99)} = 0.158$ $P_{(MC)} = 1$
Bugula neritina	$F_{(9.99)} = 0.195$ $P_{(MC)} = 0.989$	$F_{(8.99)} = 5.402$ $P_{(MC)} = 0.0001$	$F_{(72.99)} = 0.138$ $P_{(MC)} = 1$

SPECIES	**MONTH (MO)**	**ANTIFOULING PAINT (AP)**	**MO x AP**
Bugulina stolonifera	$F_{(9.99)}$ = 5.5438E-2 $P_{(MC)}$ = 1	$F_{(8.99)}$ = 3.812 $P_{(MC)}$ = 0.001	$F_{(72.99)}$ = 8.774 E-2 $P_{(MC)}$ = 1
Mytilus galloprovincialis	$F_{(9.99)}$ = 0.424 $P_{(MC)}$ = 0.889	$F_{(8.99)}$ = 2.71 $P_{(MC)}$ = 0.0104	$F_{(72.99)}$ = 0.186 $P_{(MC)}$ = 1
Amphibalanus amphitrite	$F_{(9.99)}$ = 0 $P_{(MC)}$ = 0	$F_{(8.99)}$ = 5.589 $P_{(MC)}$ = 0.0001	$F_{(72.99)}$ = 0 $P_{(MC)}$ = 0
Ascidiella aspersa	$F_{(9.99)}$ = 5.5099E-2 $P_{(MC)}$ = 1	$F_{(8.99)}$ = 1.676 $P_{(MC)}$ = 0.003	$F_{(72.99)}$ = 9.3668E-2 $P_{(MC)}$ = 1
Ciona robusta	$F_{(9.99)}$ = 1.566 $P_{(MC)}$ = 0.250	$F_{(8.99)}$ = 6.107 $P_{(MC)}$ = 0.0002	$F_{(72.99)}$ = 0.414 $P_{(MC)}$ = 0.985
Molgula socialis	$F_{(9.99)}$ = 5.8824E-2 $P_{(MC)}$ = 0.999	$F_{(8.99)}$ = 1 $P_{(MC)}$ = 0.445	$F_{(72.99)}$ = 0.1 $P_{(MC)}$ = 1
Styela plicata	$F_{(9.99)}$ = 31.803 $P_{(MC)}$ = 0.0001	$F_{(8.99)}$ = 5.900 $P_{(MC)}$ = 0.0001	$F_{(72.99)}$ = 22.147 $P_{(MC)}$ = 0.0001
Botrylloides leachii	$F_{(9.99)}$ = 3.9216E-2 $P_{(MC)}$ = 1	$F_{(8.99)}$ = 10.907 $P_{(MC)}$ = 0.0001	$F_{(72.99)}$ = 5.5729E-2 $P_{(MC)}$ = 1
Botrylloides violaceus	$F_{(9.99)}$ = 3.405E-2 $P_{(MC)}$ = 0.999	$F_{(8.99)}$ = 8.275 $P_{(MC)}$ = 0.0001	$F_{(72.99)}$ = 5.7884E-2 $P_{(MC)}$ = 1
Botryllus schlosseri	$F_{(9.99)}$ = 3.007 $P_{(MC)}$ = 0.052	$F_{(8.99)}$ = 4.869 $P_{(MC)}$ = 0.0003	$F_{(72.99)}$ = 0.806 $P_{(MC)}$ = 0.718
Diplosoma listerianum	$F_{(9.99)}$ = 1.371 $P_{(MC)}$ = 0.314	$F_{(8.99)}$ = 0.941 $P_{(MC)}$ = 0.491	$F_{(72.99)}$ = 1.146 $P_{(MC)}$ = 0.439

Table 3

PERMANOVA results of species coverage on stainless steel panels

SPECIES	**MONTH (MO)**	**ANTIFOULING PAINT (AP)**	**MO x AP**
Lychaete pellucida	$F_{(9.99)}$ = 6.0166E-2 $P_{(MC)}$ = 0.999	$F_{(8.99)}$ = 0.878 $P_{(MC)}$ = 0.535	$F_{(72.99)}$ = 0.111 $P_{(MC)}$ = 1
Ulva intestinalis	$F_{(9.99)}$ = 5.4926E-2 $P_{(MC)}$ = 0.999	$F_{(8.99)}$ = 1.129 $P_{(MC)}$ = 0.347	$F_{(72.99)}$ = 0.100 $P_{(MC)}$ = 1
Ulva rigida	$F_{(9.99)}$ = 0.425 $P_{(MC)}$ = 0.893	$F_{(8.99)}$ = 1.170 $P_{(MC)}$ = 0.325	$F_{(72.99)}$ = 0.440 $P_{(MC)}$ = 0.978
Punctaria latifolia	$F_{(9.99)}$ = 0 $P_{(MC)}$ = 0	$F_{(8.99)}$ = 1 $P_{(MC)}$ = 0.445	$F_{(72.99)}$ = 0 $P_{(MC)}$ = 0
Ceramium ciliatum	$F_{(9.99)}$ = 5.2288E-2 $P_{(MC)}$ = 0.999	$F_{(8.99)}$ = 2.25 $P_{(MC)}$ = 0.035	$F_{(72.99)}$ = 8.8889E-2 $P_{(MC)}$ = 1
Polysiphonia sertularioides	$F_{(9.99)}$ = 0.325 $P_{(MC)}$ = 0.944	$F_{(8.99)}$ = 2.051 $P_{(MC)}$ = 0.048	$F_{(72.99)}$ = 0.100 $P_{(MC)}$ = 1
Gracilariopsis longissima	$F_{(9.99)}$ = 0.163 $P_{(MC)}$ = 0.995	$F_{(8.99)}$ = 2.117 $P_{(MC)}$ = 0.046	$F_{(72.99)}$ = 8.874E-2 $P_{(MC)}$ = 1
Aplysina aerophoba	$F_{(9.99)}$ = 13.804 $P_{(MC)}$ = 0.0002	$F_{(8.99)}$ = 1.242 $P_{(MC)}$ = 0.290	$F_{(72.99)}$ = 8.701 $P_{(MC)}$ = 0.0006
Halichondria panicea	$F_{(9.99)}$ = 0.821 $P_{(MC)}$ = 0.619	$F_{(8.99)}$ = 3.330 $P_{(MC)}$ = 0.003	$F_{(72.99)}$ = 0.759 $P_{(MC)}$ = 0.763
Hydroides dianthum	$F_{(9.99)}$ = 8.555 $P_{(MC)}$ = 0.001	$F_{(8.99)}$ = 5.561 $P_{(MC)}$ = 0.0001	$F_{(72.99)}$ = 1.330 $P_{(MC)}$ = 0.322
Janua heterostropha	$F_{(9.99)}$ = 0.811 $P_{(MC)}$ = 0.618	$F_{(8.99)}$ = 2.287 $P_{(MC)}$ = 0.032	$F_{(72.99)}$ = 0.474 $P_{(MC)}$ = 0.964
Bugula neritina	$F_{(9.99)}$ = 6.495 $P_{(MC)}$ = 0.0034	$F_{(8.99)}$ = 2.509 $P_{(MC)}$ = 0.016	$F_{(72.99)}$ = 1.229 $P_{(MC)}$ = 0.388
Bugulina stolonifera	$F_{(9.99)}$ = 0.183 $P_{(MC)}$ = 0.991	$F_{(8.99)}$ = 7.084 $P_{(MC)}$ = 0.0001	$F_{(72.99)}$ = 0.263 $P_{(MC)}$ = 0.999
Electra monostachys	$F_{(9.99)}$ = 5.8824E-2 $P_{(MC)}$ = 0.999	$F_{(8.99)}$ = 1 $P_{(MC)}$ = 0.451	$F_{(72.99)}$ = 0.1 $P_{(MC)}$ = 1

SPECIES	MONTH (MO)	ANTIFOULING PAINT (AP)	MO x AP
Amphibalanus amphitrite	$F_{(9.99)} = 0$ $P_{(MC)} = 0$	$F_{(8.99)} = 3.886$ $P_{(MC)} = 0.0008$	$F_{(72.99)} = 0$ $P_{(MC)} = 0$
Ascidiella aspersa	$F_{(9.99)} = 5.4155E\text{-}2$ $P_{(MC)} = 0.999$	$F_{(8.99)} = 1.862$ $P_{(MC)} = 0.079$	$F_{(72.99)} = 9.2063E\text{-}2$ $P_{(MC)} = 1$
Ciona robusta	$F_{(9.99)} = 0.635$ $P_{(MC)} = 0.745$	$F_{(8.99)} = 6.819$ $P_{(MC)} = 0.0001$	$F_{(72.99)} = 0.317$ $P_{(MC)} = 0.998$
Molgula socialis	$F_{(9.99)} = 1.313$ $P_{(MC)} = 0.337$	$F_{(8.99)} = 0.681$ $P_{(MC)} = 0.708$	$F_{(72.99)} = 0.697$ $P_{(MC)} = 0.812$
Styela plicata	$F_{(9.99)} = 1.423$ $P_{(MC)} = 0.297$	$F_{(8.99)} = 6.655$ $P_{(MC)} = 0.0001$	$F_{(72.99)} = 0.296$ $P_{(MC)} = 0.998$
Botrylloides leachii	$F_{(9.99)} = 0.126$ $P_{(MC)} = 0.997$	$F_{(8.99)} = 3.086$ $P_{(MC)} = 0.0047$	$F_{(72.99)} = 0.131$ $P_{(MC)} = 1$
Botrylloides violaceus	$F_{(9.99)} = 2.150$ $P_{(MC)} = 0.125$	$F_{(8.99)} = 1.246$ $P_{(MC)} = 0.291$	$F_{(72.99)} = 3.830$ $P_{(MC)} = 0.012$
Botryllus schlosseri	$F_{(9.99)} = 7.785$ $P_{(MC)} = 0.002$	$F_{(8.99)} = 2.617$ $P_{(MC)} = 0.013$	$F_{(72.99)} = 1.0389$ $P_{(MC)} = 0.520$
Diplosoma listerianum	$F_{(9.99)} = 0.720$ $P_{(MC)} = 0.677$	$F_{(8.99)} = 1$ $P_{(MC)} = 0.434$	$F_{(72.99)} = 0.283$ $P_{(MC)} = 0.999$

Figures

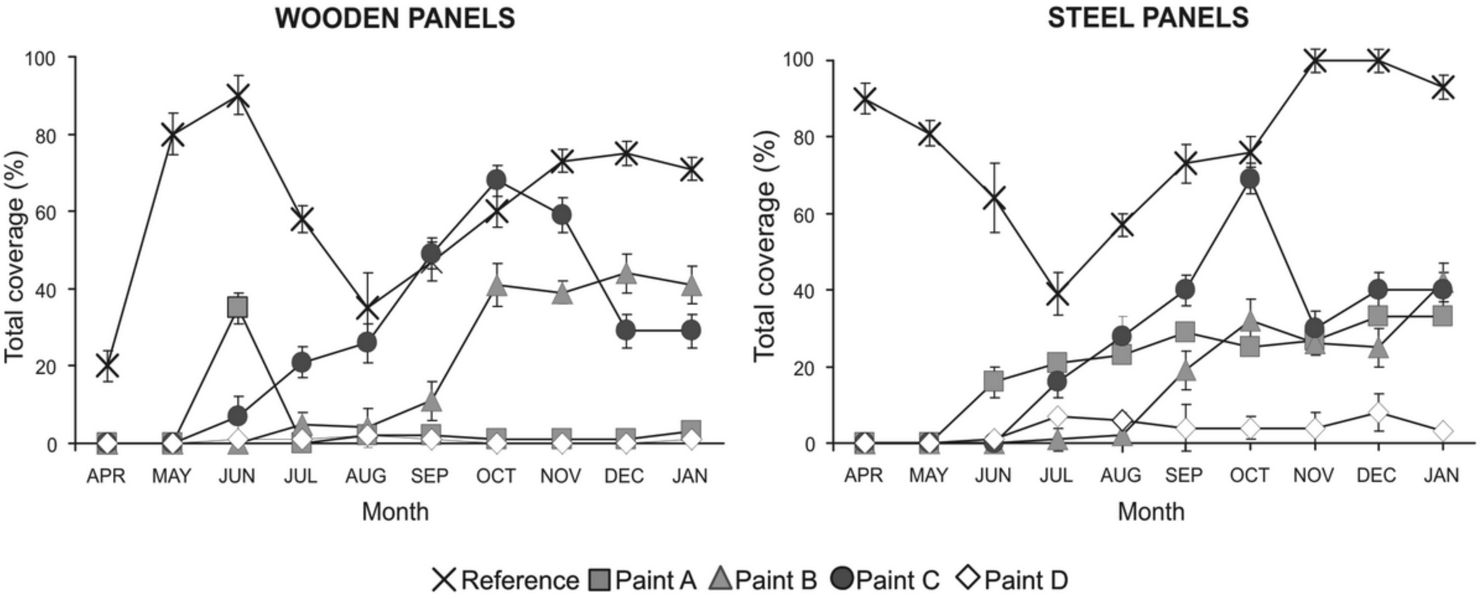

Figure 1

Trend of percentage of total coverage dynamic monitored monthly as average ± standard deviation on replicates (n = 2) of the different panels.

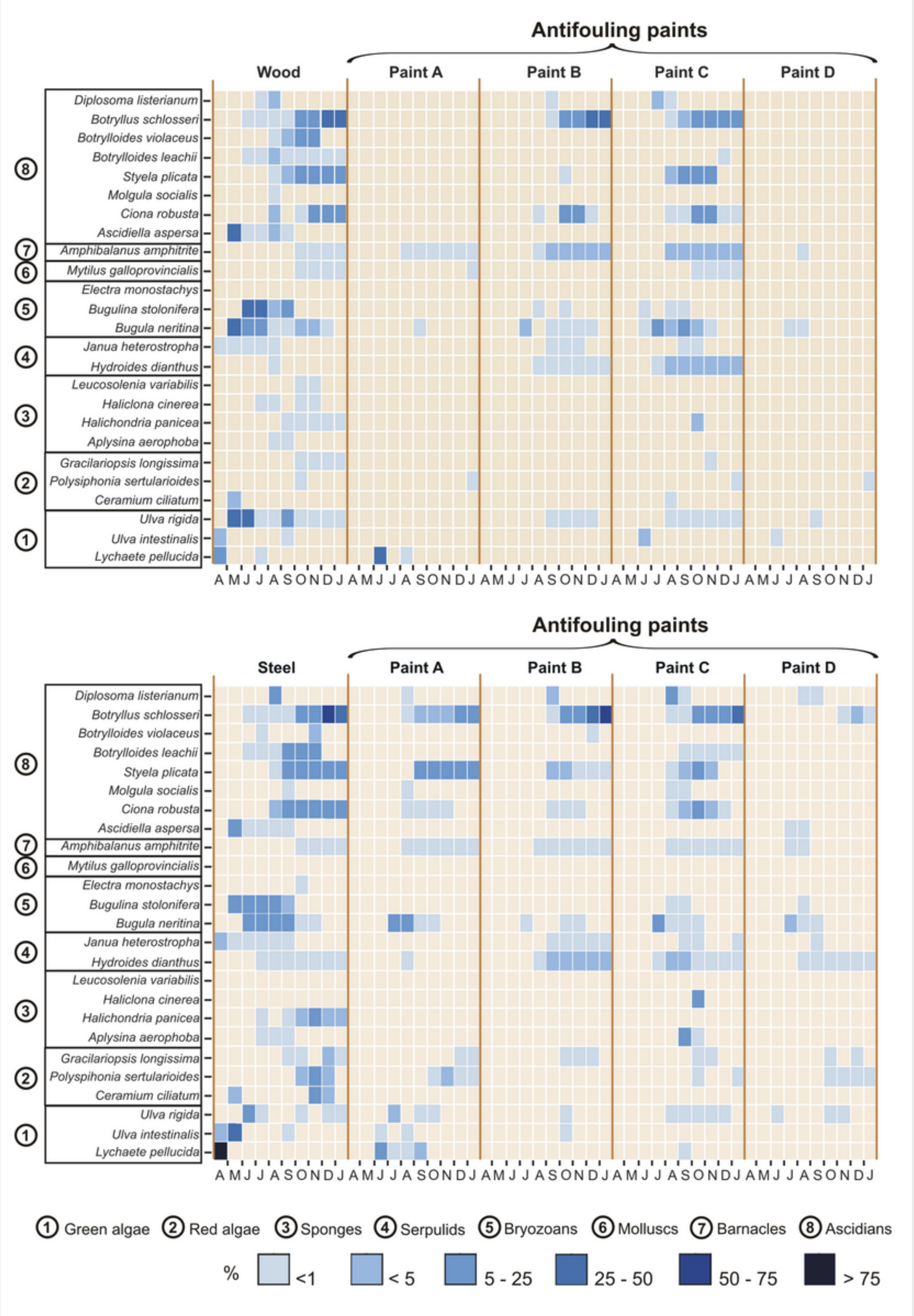

Figure 2

Trend of the biodiversity descriptor ‘coverage-abundance area’ as the total percent area of each species measured monthly using photos of replicates (n = 2) of the wooden (top scheme) and stainless steel (lower scheme) panels. Species are clustered in taxonomic groups (number in small ring).

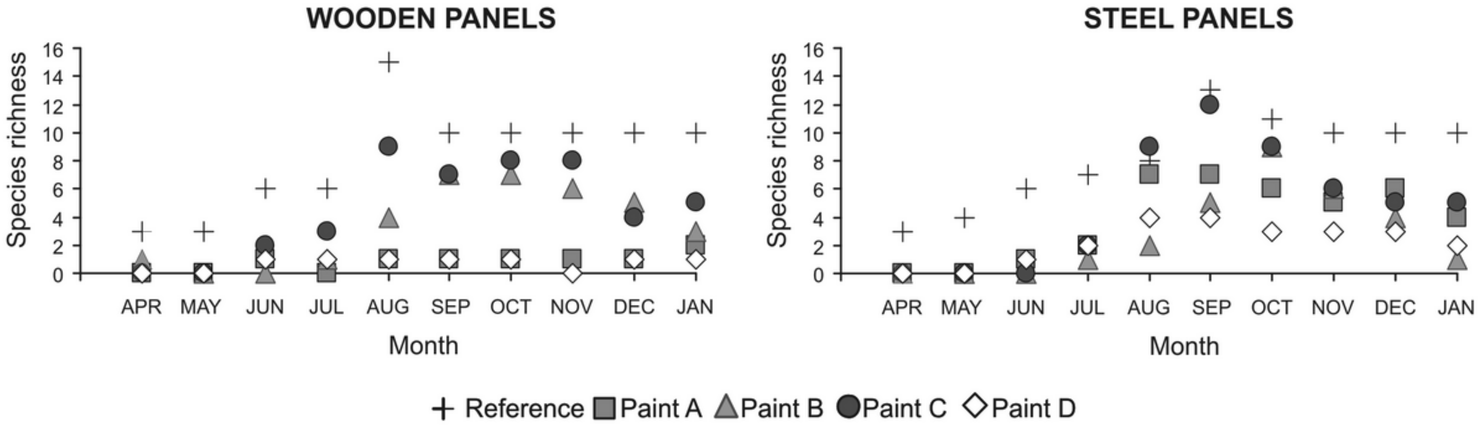

Figure 3

Trend of the biodiversity descriptor ‘species richness’ considered as the total number of species found monthly on the replicates (n = 2) of the different panels.

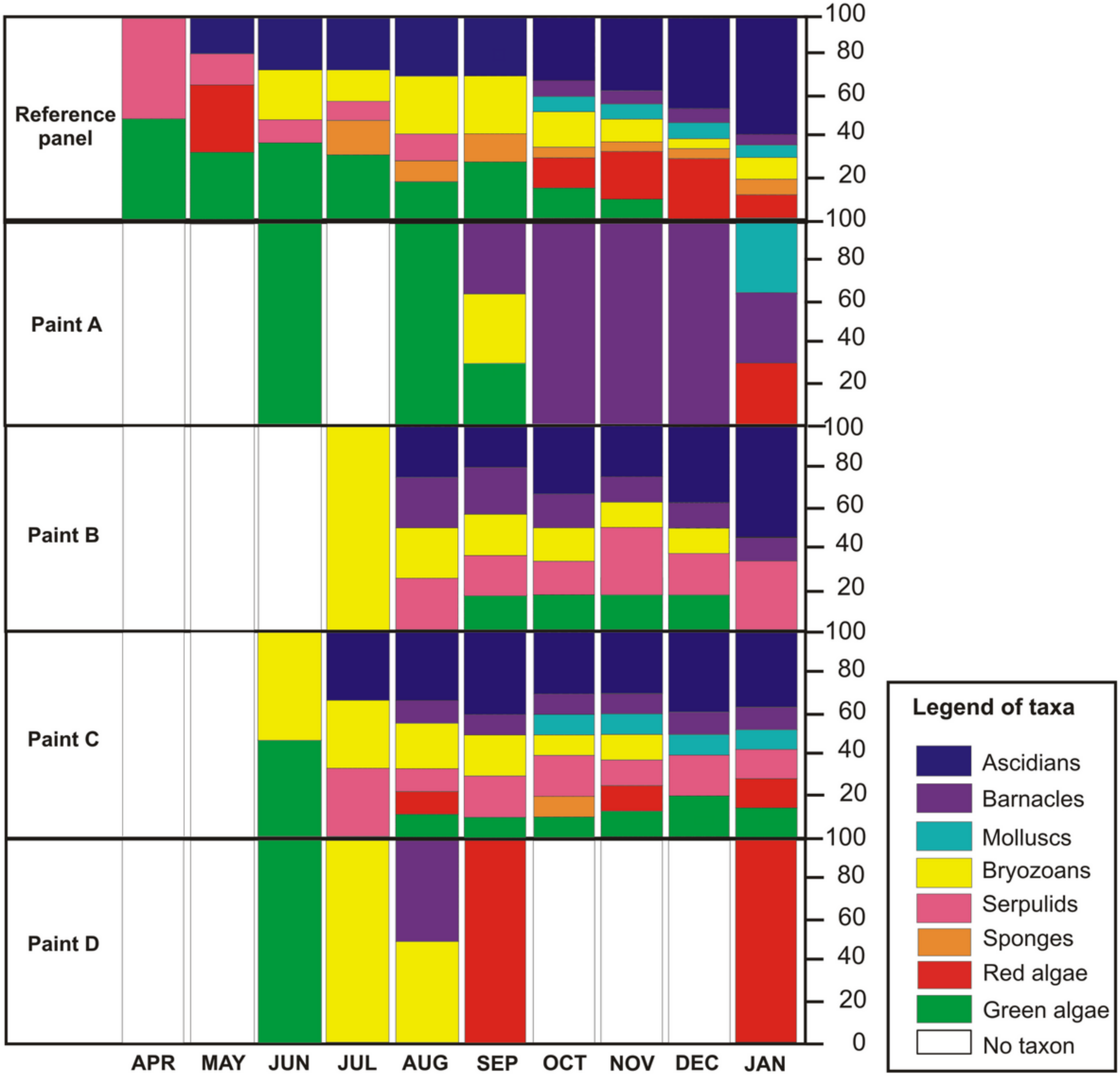

Figure 4

Changes in the biodiversity descriptor 'biocoenosis structure' on the reference and paint-coated wooden panels throughout the experimental immersion in the Lagoon of Venice. The percent value of each taxonomic group is expressed as the total value obtained from the pool of two replicates.

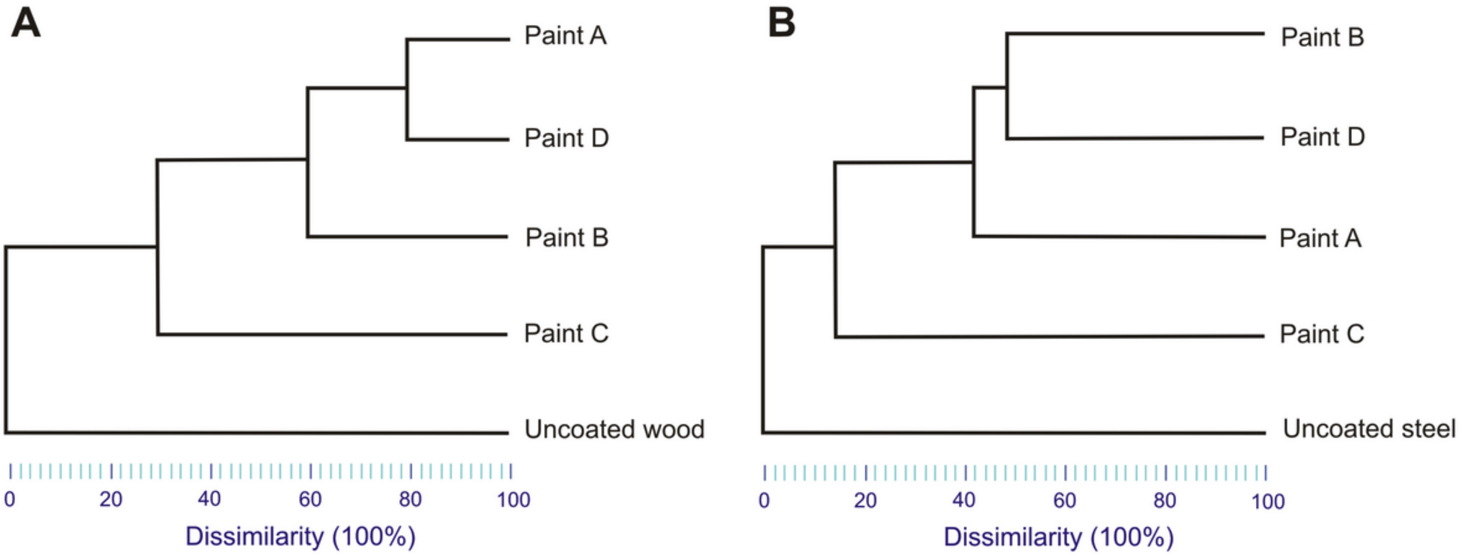

Figure 5

Dendrograms obtained from cluster analysis using the Bray-Curtis percentage dissimilarity value of assemblages based on the presence or absence of taxonomic groups for all coating treatments. Clusters were obtained from pooled sets of species belonging to the same taxonomic groups settled on wooden (A) and stainless steel (B) panels throughout the 10-month experimental immersion in the Lagoon of Venice.