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Authors

Miller, Robert J
Adeleye, Adeyemi S
Page, Henry M
[et al.](#)

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Nano and traditional copper and zinc antifouling coatings: metal release and impact on marine sessile invertebrate communities

Robert J. Miller · Adeyemi S. Adeleye · Henry M. Page · Li Kui · Hunter S. Lenihan · Arturo A. Keller

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Abstract Artificial surfaces in coastal waters and offshore oceans, including boat hulls, docks, and offshore structures, are invariably colonized, or fouled, by a host of sessile species known collectively as fouling communities. Fouling has great economic impacts on shipping and other marine industries and plays an important role in the spread of marine invasive species across the globe. The main strategy to prevent fouling of artificial surfaces is application of antifouling coatings containing varying concentrations and mixtures of biocides. Presently, copper and zinc are popular antifouling biocides, and the latter is gaining in usage due to the known toxic characteristics of copper in the marine environment and consequent regulation and consumer opinion. Nanomaterials, including Cu and ZnO nanoparticles, have been explored as a way to efficiently deliver biocides from coating matrices. Here, we examine the efficacy and biocide release characteristics of several copper- and zinc-based antifouling coatings, including formulations containing traditional micron-sized Cu and ZnO particles and two containing copper and ZnO nanoparticles, respectively. Most of the antifouling coatings tested

significantly reduced the abundance and biodiversity of the fouling community in the three study locations across California. Invasive species were suppressed by most coatings at similar levels to natives, suggesting that in general, antifouling coatings do not favor invasive species. We found that zinc-based antifouling coatings were similar and in some cases better performing than copper, despite the generally lower toxicity of zinc to aquatic organisms compared with copper. The performance of zinc-based coatings, moreover, was not directly related to the amount of zinc released into the water or their zinc content. Nano-based coatings did not offer any clear advantages over non-nano coatings, either in the degree of Zn leaching or fouling suppression. Coating matrix properties clearly are an important factor affecting the efficacy and biocide leaching rate of antifouling coatings.

Keywords Marine ecosystems · Antifouling · Coastal pollution · Invasive species · Copper · Zinc · Nanoparticles · Environmental effects

R. J. Miller (✉) · H. M. Page · L. Kui
Marine Science Institute, University of California, Santa Barbara,
CA 93106, USA
e-mail: rjmiller@ucsb.edu

A. S. Adeleye
Department of Civil & Environmental Engineering, University of
California, Irvine, CA, USA

H. S. Lenihan · A. A. Keller
Bren School of Environmental Science and Management,
University of California, Santa Barbara, CA, USA

Introduction

Artificial surfaces in the ocean are invariably colonized by organisms, including exotic species from across the globe that have taken advantage of novel habitats. This “biofouling” increases drag, weakens structures, and includes non-native invasive species, and hundreds of millions to billions of dollars are spent annually on cleaning these organisms off and preventing their settlement using antifouling coatings (Wahl 1989; Callow and Callow 2011). Most antifouling coatings are biocide-containing paints, now typically employing copper as the active ingredient, sometimes with the addition of organic pesticides. Prior to the late 1980s, tributyltin, organic derivatives of tin, were used for antifouling, but due to severe toxic effects on non-target marine life, they were restricted and are now banned nearly worldwide (Lenihan et al. 1990; Alzieu 1998). Tributyltin was replaced largely by copper, which is less toxic, and although less toxic, it builds up in marine habitats and is now becoming recognized as negatively affecting native biota (Neira et al. 2011). As a result, new regulations on copper-based coatings are being implemented. For example, in 2011, Washington state enacted a phased-in ban on antifouling paints containing > 0.5% copper. California is also presently considering similar regulations, and local regulations have been enacted in areas like San Diego’s Mission Bay (Schiff et al. 2007). Internationally, the European Union recently enacted new regulations on application and residue levels of copper-containing biocides (McNeil 2018).

Alternative antifouling solutions are being developed due to new regulations and consumer environmental concern. Research is advancing on non-biocidal solutions, such as coatings that prevent settlement of organisms through their surface properties, e.g., micro- or nano-structured “self-cleaning” surfaces (Bixler et al. 2014), or via sloughing their outer layers (Ciriminna et al. 2015). Nevertheless, the primary response thus far has been movement towards alternative biocides, particularly zinc, which is considered less toxic than copper (Voulvoulis et al. 2002) but also has the potential to accumulate in the environment (Turner 2010). So-called booster biocides or co-biocides (e.g., zinc pyrithiones) are often added, and solubility of the coating binder is adjusted to enhance efficacy (Banerjee et al. 2011). Nanomaterial-based coatings have also been presented as a possible solution to the problem,

either via non-toxic surface interactions (Ciriminna et al. 2015) or more efficient delivery of biocides (Sathya et al. 2016). Copper oxide nanoparticles are marketed for use in antifouling paints and have been found in commercially available coatings (Adeleye et al. 2016). ZnO nanoparticle-based paint is also now available and like other zinc-based coatings is marketed as an environmentally friendly alternative to copper.

The potential environmental impact of zinc or nanotechnology-based antifouling coatings has received little attention. Because antifouling coatings are used on surfaces that are submerged in aquatic habitats, this application represents a much more direct exposure route to nanomaterials than is typical with diluted discharge-based exposures. Nanomaterials can be toxic to aquatic organisms through several mechanisms. Metal oxide nanoparticles, for example, typically dissolve to varying extents, releasing toxic metal ions into the water (Keller et al. 2010). Intact nanoparticles, moreover, can greatly increase the dose of metal to organisms that contact or ingest them (Jarvis et al. 2013).

Although antifouling coatings are a potential source of pollution, they also have potential environmental benefits in aquatic ecosystems as tools for prevention or mitigation of the dispersal and establishment of non-native species. Coastal marine habitats are heavily invaded by non-native species, which can have dramatic consequences on local ecosystem structure and function (Grosholz 2002), and threaten to homogenize marine communities, posing a threat to marine biodiversity worldwide (Molnar et al. 2008). Invasive species may expand through natural or human-assisted dispersal into adjacent habitats over short distances, but marine invasions are often initially facilitated by long-distance dispersal into regions far from the species’ native habitat (Richardson et al. 2016). Anthropogenic vectors, including boat and barge hulls, as well as ship ballast water, are an important dispersal mechanism for such long-distance transport of invasive species around the world (Grosholz 2002), particularly sessile invertebrates such as ascidians, bryozoans, and cnidarians (Carlton and Geller 1993; Lambert et al. 2001) that are prominent fouling organisms. Antifouling coatings prevent or reduce the transport of some of marine species (Nehring et al. 2001), while others may be resistant to biocides such as copper, facilitating transport and potentially giving them a competitive advantage in polluted habitats, such as harbors (Piola et al. 2009; McKenzie et al. 2012). Improvement and proper application of

antifouling coatings has been suggested as a management tool against invasive species in coastal marine habitats (Floerl and Inglis 2005; Piola et al. 2009).

In this study, we quantified the extent to which zinc and copper antifouling coatings, including coatings containing nanomaterial release metals, either Cu or Zn, into the surrounding water. We also examined the potential effect of these coatings on marine organisms, including community composition, invasive species dominance, and biodiversity, across a latitudinal gradient in California.

Materials and methods

Antifouling coatings

Six commercial Cu- and Zn-based antifouling coatings were used for this study that represented a range of biocide compositions. Plates were subjected to seven different treatments: control with no coating, four coatings containing zinc, and 2 copper-based coatings (Table 1). All coatings were blue in color and applied according to manufacturer's directions. Zinc coatings included one containing ZnO nanoparticles plus Zn omadine (Sea Hawk Mission Bay CSF copper-free antifouling coating 4502 [MBC], New Nautical Coatings Inc., Clearwater, FL), two containing micron-sized ZnO plus Zn omadine (Sea Hawk Mission Bay copper-free antifouling coating 4002 [denominated MB], Interlux Pacifica Plus YBB260 [PP], International Paint LLC), and one containing only Zn omadine (Petit Vivid TBT and copper-free 1262 [PV], Kop-Coat Marine Group, Rockaway, NJ). All Zn coatings were described as ablative and self-polishing, meaning they were designed to erode away and release biocide over time without excessive cleaning. Two epoxy-based Cu coatings were tested. Coppercoat antifouling coating (Aquarius Marine Coatings, UK) was obtained as a three-part product composed of epoxy resin, hardener, and dry Cu particles. The three parts are combined just before application. Scanning electron microscopy (SEM) analysis showed that the Cu particles included with Coppercoat had a size range of 2–40 μm . To test the effect of nanoparticulate Cu, in addition to preparing a Coppercoat mix with the original micron-sized (non-nano) Cu particles (CCb), another mix was prepared in which the micron-sized Cu particles were replaced with Cu nanoparticles (US Research Nanomaterials,

Houston, TX) with average particle size of 60 nm (CCn) (Adeleye et al. 2014).

Metal release from antifouling coatings

We tested the release rate of metals from antifouling coatings in the laboratory. Coatings were applied on small fiberglass-reinforced plastic plates (7 cm long \times 1.5 cm wide \times 0.3 cm thick, McMaster Carr, Los Angeles). Each plate was weighed (Denver Instrument SI-114) before and after coating and curing to measure the mass of coating applied. All coatings were thoroughly mixed and used without further amendment, except Coppercoat. Coppercoat mix was prepared as needed, in batches. To prepare each Coppercoat batch, 800 g of the Cu particles provided by the manufacturer was mixed with 200 mL of resin and 200 mL of hardener. To achieve a similar coating consistency using nanosized Cu, 100 g of nanosized Cu was mixed with 200 mL of resin and 200 mL of hardener. Using a higher mass of nanosized Cu resulted in a very thick consistency that could not easily be applied on plates. Although the coating thus prepared had only 12.5% of the mass of Cu in the Coppercoat mix, the consistency was similar, presumably due to the much higher surface area to volume ratio and resulting high reactivity of the nanoparticles. Coated plates were allowed to dry/cure for at least 48 h and then submerged in 300 mL of either seawater or simulated estuarine (brackish) water. Seawater was collected from the University of California, Santa Barbara, flow-through seawater system, which pumps Pacific Ocean water from 18 m water depth offshore, and is filtered (0.2 μm). Estuarine water was composed of 50% laboratory tap water and 50% filtered seawater. All treatments were applied in triplicate. Submerged coated plates were kept in conditions simulating natural surface waters, 14:10 light/dark at 20 ± 1 °C with moderate agitation (125 rpm rotary shaking).

Release of Zn or Cu from plates was monitored for 120 days; samples were taken at hours 0, 1, 3, 6, 24, 48, and 36, and then on days 7, 14, 30, 60, 90, and 120. At each sampling, aliquots were removed from all the treatments and analyzed for total Zn or Cu, dissolved Zn and Cu, and nanosized Zn or Cu present in the paint leachate, adopting techniques used in previous studies (Adeleye et al. 2016, 2018). To determine total Zn or Cu, an aliquot was digested with trace-metal grade HNO_3 (Fisher Scientific), followed by inductively coupled plasma atomic emission spectroscopy (ICP-

Table 1 Treatment coatings with metal content as stated on product Materials Safety Data Sheets

Components	Coating name					
	<i>Mission Bay</i>	<i>Pacifica Plus</i>	<i>Mission Bay CSF</i>	<i>Petit Vivid</i>	<i>Coppercoat</i>	<i>Epoxy Cu NP</i>
Zinc						
ZnO	35–50%	10–25%	5.4%			
ZnO NPs			20%			
Zn omadine	4–10%	1–10%	3.08%	4.95%		
Copper						
Cu					75%	
Cu NPs						9.4%

AES) analysis (Thermo Scientific iCAP 6300). To estimate dissolution, another aliquot was transferred into Millipore Amicon Ultra-4 3 kDa centrifugal filter tubes (nominal pore size 2 nm) and centrifuged with a swinging bucket rotor on a Sorvall RC 5B Plus (4000×g, 40 min) to separate the dissolved fraction. The filtrate containing the dissolved fraction was then acid-digested and measured using the ICP-AES. To measure the particulate fraction up to 200 nm, an aliquot was filtered with a 0.2-μm PVDF syringe filter (Thermo Scientific) and the filtrate was then analyzed via ICP-AES. Two hundred nanometers was chosen as the cutoff for nanosized Cu, similar to a previous study by Adeleye et al. (2016). Nanosized Zn/Cu was determined for each sample by subtracting dissolved Zn or Cu from the ≤ 200-nm fraction. Fractions > 200 nm were regarded as bulk Zn or Cu.

Study sites and field deployments

The field portion of the study was conducted at three harbors on the California coast (Fig. 1): Bodega Bay in the northern region (Spud Point Marina, 38.328°N, 123.057°W), Santa Barbara in the central region (Santa Barbara Harbor, 34.405°N, 119.689°W), and San Diego in the south (Mission Bay, 32.763°N, 117.237°W). Experimental plates ($N=12$ plates per treatment) with antifouling treatments were deployed at each site for 1 year, from June–July 2015 to June–July 2016. Plates were attached using plastic cable ties to 1 m × 1 m PVC pipe frames bolted to the northern facing side of the marina docks, in locations with similar hydrodynamic and light conditions. Plates were maintained vertically at a depth of approximately 1 m below the surface of the water, a similar depth to the fouling communities on

nearby boat hulls. After 12 months, plates were collected, placed in a pan of water, and photographed with a digital camera (Canon 6D). Sessile communities on the side of the plates facing the dock were quantified for consistency. Photos were uploaded to BisQue, a distributed, web-based platform for management and analysis of scientific imagery (Kvilekval et al. 2010). Proportional cover of all sessile organisms attached to the plates was estimated using point-contact sampling (Meese and Tomich 1992). A grid of 25 evenly distributed dots was placed over each photo, and the organism or bare space under each dot was identified and recorded. The number of contacts for each taxon was divided by 25 to determine the proportional cover and multiplied by 100 to estimate percent cover. Diversity was calculated as diversity of order 1, which corresponds to the exponential of the Shannon entropy index (H) (Jost 2006):

$${}^1D = \exp\left(-\sum_{i=1}^s p_i \ln p_i\right) = \exp(H) \quad (1)$$

where s is the total number of species and p_i is the percent cover of the i^{th} species. Diversity of order 1 represents the effective number of equally common species, weighting each species according to its percent cover, without favoring rare or common species (Jost 2006).

Data analysis

The dissolution rate of particles is described by Eq. 2, where C_s is the saturated concentration at particle surface and $C(t)$ is the concentration in the solution at time (t):



Fig. 1 Map of California showing study sites

$$\frac{dC}{dt} = k(C_s - C_t) \quad (2)$$

In our batch studies conducted in the laboratory, dissolution rate of Cu and Zn biocides decreased over time as the dissolved ion concentration (C_t) in the waters approached C_s . As a result, estimating the biocide dissolution rate over the entire course of the experiment may underestimate the actual release rate of the biocides in natural conditions where metal ions cannot locally accumulate, and C_t is always much lower than the saturation concentrations, C_s . Therefore, we estimated dissolution rate based on the early stage of the batch experiments.

Experimental dissolution data were fitted with Eq. 3, a modified first-order reaction kinetics equation. The model was only used to fit dissolution data obtained within 48 h (for all the Zn-based antifouling coatings) or 24 h (for Coppercoat and the Cu NP formulation since Cu has a lower saturation concentration than Zn):

$$C_t = C_s(1 - e^{-kt}) \quad (3)$$

where k is the dissolution rate coefficient (day^{-1}).

Two-way ANOVAs were used to test for an effect of coating type and site, and their interaction, on percent cover and species diversity (order 1) of fouling

invertebrates and percent cover of invasive species. Separate ANOVAs were run for Zn-based vs Cu-based coatings. Homogeneity of variance was tested with Levene's test. When ANOVA revealed significant differences among treatments, *post hoc* tests were conducted with Dunnett's method for multiple pairwise comparisons between each treatment and the control. To examine differences in community composition among the treatments at each site, we used principal coordinate analysis (PCO, metric MDS), followed by a canonical discriminant analysis (CDA). This approach to constrained ordination of ecological species abundance data is called canonical analysis of principal coordinates or CAP (Anderson and Willis 2003).

Results

Metal release from antifouling coatings

Initial metal dissolution rate

The estimated dissolution rate constants (Table 2) showed that higher salinity promoted the initial (48 h) dissolution of the Zn-based biocides. There were significant differences in initial Zn^{2+} release rates among the various formulations, with the highest rates seen for Mission Bay 4502 LT, which is based on ZnO nanoparticles plus Zn omadine. Results for copper-based coatings were mixed: non-nano Coppercoat exhibited similar dissolution rate constants in estuary and seawater, while the initial dissolution rate constant was much higher in estuarine water for the Cu NP formulation (Table 2). The initial release rate of Cu^{2+} was higher from the non-nano Coppercoat than from the NP formulation, probably due to the higher Cu content.

Total metal leached

Increasing amounts of total Cu or Zn (dissolved and particulate fractions) leached out of the antifouling paints into the media (estuary and seawater) over time (Figs. 2 and 3). Overall, more Cu was released from the non-nano Coppercoat than the Cu NP formulation (Fig. 2) as expected due to the higher mass of Cu particles in Coppercoat (Table 1). Total Cu leached from Coppercoat was higher in seawater than in estuarine water for up to 60 days, after which the concentration was relatively steady in seawater (Fig. 2a). Similarly, a

Table 2 Dissolution kinetic parameters (k) of zinc and copper from antifouling paints. Errors are standard deviation

Biocide type	Antifouling coating	Estuary		Seawater	
		k (day ⁻¹)	R^2	k (day ⁻¹)	R^2
Zinc					
	Pacifica Plus	0.292 ± 0.064	0.989	0.449 ± 0.14	0.990
	Mission Bay (4502 LT)	0.875 ± 0.046	0.995	1.502 ± 0.068	0.994
	Mission Bay (4002 LT)	0.271 ± 0.031	0.997	0.778 ± 0.025	0.995
	Petit Vivid	0.697 ± 0.211	0.999	0.792 ± 0.173	0.999
Copper					
	Coppercoat	0.923 ± 0.294	0.999	0.935 ± 0.253	0.998
	Nanoparticle formulation	0.834 ± 0.218	0.998	0.579 ± 0.203	1.000

slightly higher amount of total Cu leached from the Cu NP formulation in seawater than in estuarine water. In estuarine water, however, Cu continued to leach from non-nano Coppercoat, reaching 178.7 ± 43.7 mg/L after 120 days, compared to 33.1 ± 7.3 mg/L after 120 days in seawater (Fig. 2c).

Total Zn released into estuarine water was higher than that leached into seawater for all four Zn-based paints (Fig. 3). The ratio of total Zn leached into estuarine water to total Zn leached into seawater by day 120 was highest for the nano-based coating, Mission CSF, at 3.19. The ratio was similar for the non-nano Mission Bay (4002 LT) and Pacifica Plus coatings, at 2.23 and 2.25, respectively, and was slightly higher for the coating containing no particulate Zn, Petit Vivid (which only contains Zn omadine), at 2.40. By day 120, the highest amount of Zn was detected in the presence of Petit Vivid in both estuarine water and seawater, while the least amount of Zn leached from Pacifica Plus (which contains non-nano-ZnO and Zn omadine). Although the initial Zn release rate constant was highest in the nano-based Mission Bay CSF, the long-term release of Zn from the nano-enabled coating was not different from the release from the non-nano Zn coatings.

Metal size fractions

Concentration of dissolved Cu released from non-nano Coppercoat into seawater peaked within 24 h, after which the concentration was relatively steady. In estuarine water, however, dissolved Cu released from non-nano Coppercoat did not reach a maximum concentration until after 72 h. The relative amount of dissolved Cu detected in both media containing plates coated with

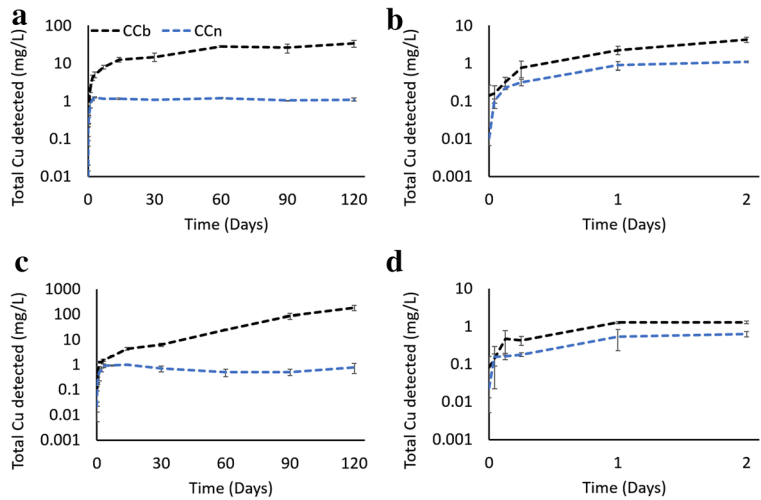
Coppercoat decreased with time while the relative amount of bulk Cu (defined as fraction >200 nm) increased steadily (Fig. 4a, b), suggesting that precipitation of dissolved Cu occurred in both estuarine water and seawater. Bulk Cu in media may have also been produced by direct release of Cu particles from the paint matrix. In contrast, dissolved Cu was a more dominant species detected in the leachate from the Cu NP formulation. The nanoparticulate Cu fraction (size ≈ 1–200 nm) released from Coppercoat was 0.01–13.7% of the total released Cu over 120 days, and from the Cu NP formulation, it was 0–7.50%.

Unlike the Cu-based antifouling coatings, dissolved Zn was the most dominant Zn fraction detected in both seawater and estuary after a few hours of submerging plates coated with the four Zn-based paints. This pattern persisted until the end of the study (Fig. 4c, d). The ratio of nanoparticulate Zn detected in media containing plates coated with the nano-ZnO-based Mission Bay CSF was not markedly different from the fraction detected in the other paints that contain non-nano-sized ZnO particles or just Zn omadine.

Field experiments

Sessile invertebrate communities on the plates responded to the coating treatments differently depending on site, and this resulted in significant coating × site interactions in all the ANOVA analyses (Table 3). Therefore, we conducted pairwise Dunnett's tests for each coating type against the controls within sites to evaluate the effects of the different coatings on fouling communities. Nevertheless, the results were broadly consistent across sites.

Fig. 2 Leaching of Cu from Coppercoat (CCb), and the Cu nanoparticle formulation (CCn) antifouling coatings. Total Cu detected in seawater over **a** 120 days and **b** 48 h. Total Cu detected in estuarine water over **c** 120 days and **d** 48 h. Dissolution kinetics (Table 2) were fit using the data from the first 48 h



Abundance and diversity of fouling organisms

In general, all the antifouling coatings lowered the diversity and abundance of fouling organisms at most sites. The percent cover of sessile invertebrates was very high, > 75%, on the control plates at all the sites, particularly Bodega Bay and Santa Barbara, where it was $\geq 90\%$ cover collectively (Fig. 5).

Invertebrate cover was significantly lower on the non-nano-ZnO plus Zn omdadine coating (Pacifica Plus) at all sites, followed by the Zn omdadine-only coating (Petit Vivid). Generally, the two ZnO plus omdadine coatings (Mission Bay and Mission Bay CSF) had much greater cover. The copper-based coatings also generally had high percent cover, particularly the nano-CuO formulation, although in

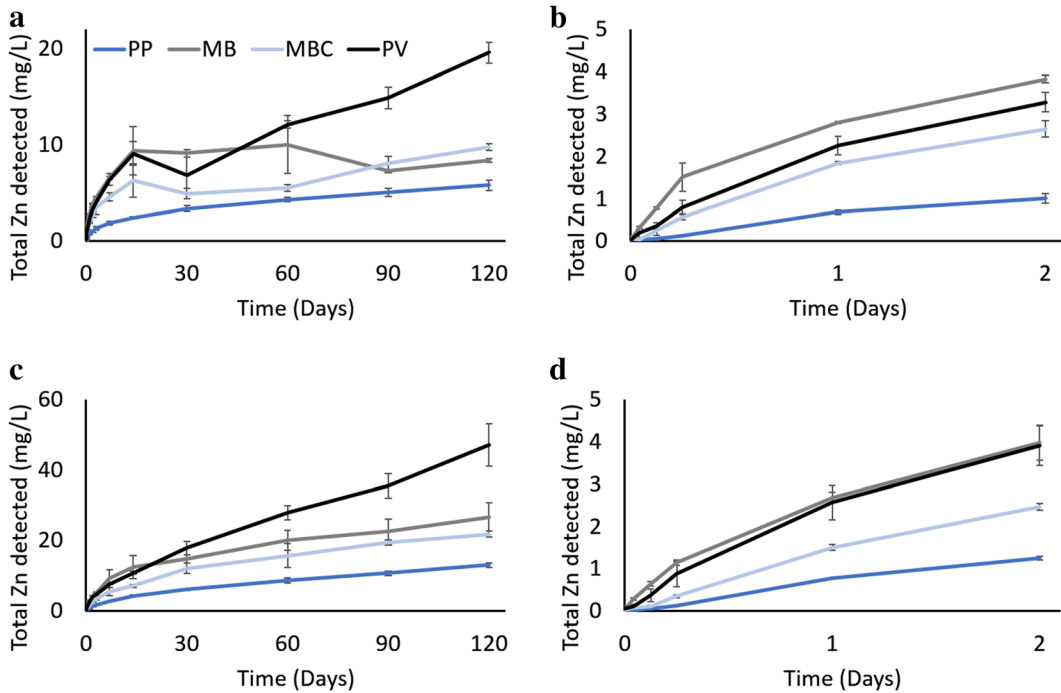


Fig. 3 Leaching of Zn from Pacifica Plus (PP), Mission Bay (MB), nano-based Mission Bay CSF (MBC), and Petit Vivid (PV) antifouling coatings. Total Zn detected in seawater over **a**

120 day and **b** 48 h. Total Zn detected in estuarine water over **c** 120 days and **d** 48 h. Dissolution kinetics (Table 2) were fit using the data from the first 48 h

Fig. 4 Relative abundance of different forms of Cu detected in **a** seawater and **b** estuary water in the presence of plates coated with Coppercoat; relative abundance of different forms of Zn detected in **c** estuary water and **d** seawater in the presence of plates coated with Pacifica Plus. Results for other Zn-based coatings were similar

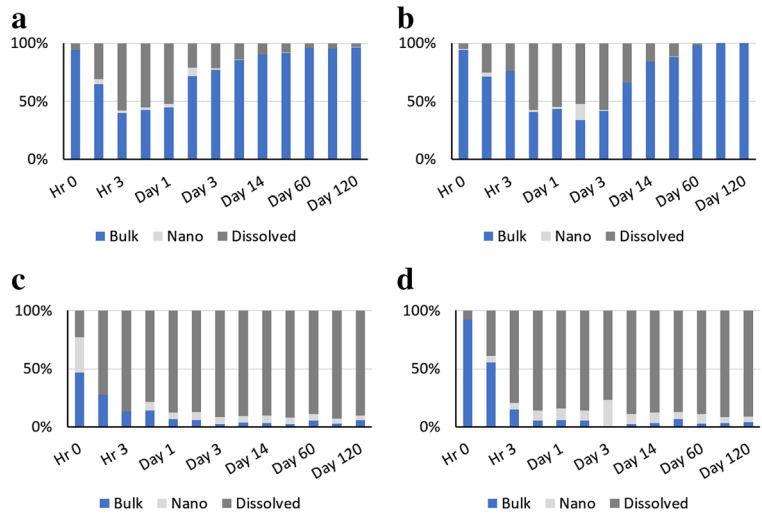


Table 3 Results for the two-way ANOVAs evaluating the effects of coating treatment and site on percent cover and species diversity of fouling invertebrates and percent cover of invasive species

Dependent variable	Biocide type		Df	Sum Sq	Mean Sq	F value	P value
Percent cover	Zinc	treatment	3	287,961	95,987	498.13	< 0.001
		site	2	5646	2823	14.65	< 0.001
		Treatment × site	6	17,488	2915	15.13	< 0.001
		Residuals	276	53,184	193		
	Copper	treatment	3	210,502	70,167	270.65	< 0.001
		site	2	32,350	16,175	62.39	< 0.001
		treatment × site	6	49,479	8246	31.81	< 0.001
		Residuals	276	71,555	259		
Diversity order 1	Zinc	Treatment	3	396.1	132.04	127.59	< 0.001
		Site	2	22.1	11.07	10.7	< 0.001
		Treatment × site	6	39	6.5	6.28	< 0.001
		Residuals	276	285.6	1.03		
	Copper	Treatment	3	429.7	143.22	155.29	< 0.001
		Site	2	36.1	18.05	19.57	< 0.001
		Treatment × site	6	64.5	10.75	11.66	< 0.001
		Residuals	276	254.6	0.92		
Percent cover of invasive species	Zinc	Treatment	3	58,218	19,406	35.49	< 0.001
		Site	2	89,432	44,716	81.77	< 0.001
		Treatment × site	6	117,051	19,509	35.67	< 0.001
		Residuals	276	150,928	547		
	Copper	Treatment	3	146,296	48,765	133.58	< 0.001
		Site	2	99,409	49,704	136.15	< 0.001
		Treatment × site	6	85,503	14,250	39.04	< 0.001
		Residuals	276	100,757	365		

Bodega Bay, the non-nano CuO Coppercoat had the lowest cover of all treatments (Fig. 5).

Species richness also tended to be highest on the control plates and was quite similar across sites, averaging about 6 species (Bodega Bay 6.5 ± 0.3 , Santa Barbara 5.9 ± 0.3 , San Diego 6.2 ± 0.3). Decreasing cover correlated generally with lower diversity, as can be seen for the Pacifica Plus, Petit Vivid, and non-nano CuO Coppercoat coatings (Fig. 6). In contrast, the Mission Bay and nano-CuO Copper Coat had high diversity, in some cases on par with the control.

Abundance of invasive species

The percent cover of known invasive species relative to the total community was very high on the control plates at all three sites, comprising the majority of the community

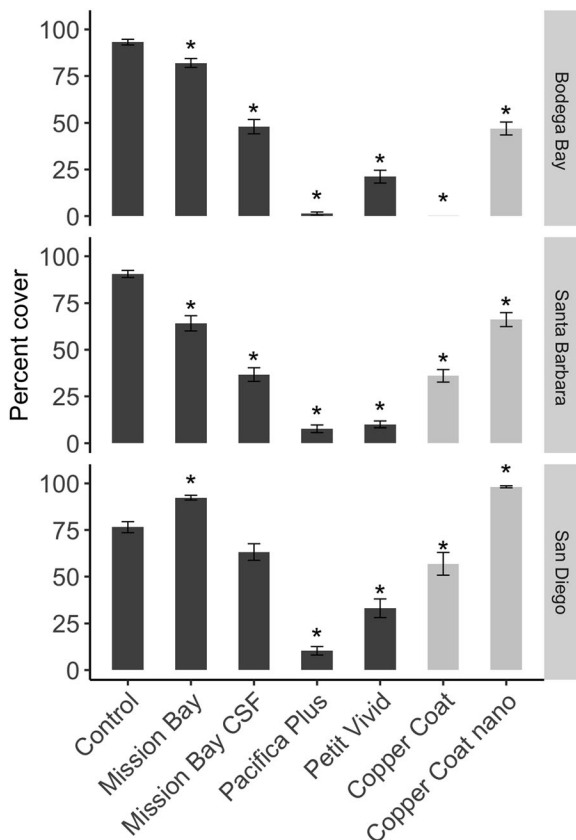


Fig. 5 Mean percent cover of sessile invertebrates on controls and treatment coatings for each of the three study sites. Error bars are standard errors. Asterisks indicate significance in post hoc comparisons against site-specific controls using Dunnett’s tests. Dark gray bars indicate Zn-based coatings; light gray bars are Cu-based coatings

abundance at all sites (Fig. 7). Surprisingly, the invasive species responded differently to the coatings relative to the response of the native invertebrates (Fig. 7). The non-nano CuO Coppercoat was generally the most effective in limiting coverage of invasives. The nano-based Coppercoat was moderately effective in San Diego and Santa Barbara but had no effect in Bodega Bay. The ZnO with Zn omadine coatings (Mission Bay, Mission Bay CSF, Pacifica Plus, and Petit Vivid) significantly reduced the cover of invasive species in San Diego and Santa Barbara (except the Pacifica Plus at this site), but only Pacifica Plus was effective in suppressing invasives at Bodega Bay (Fig. 7).

Community structure

At all sites, communities on the control (uncoated) plates were quite different from those on treated plates, as revealed

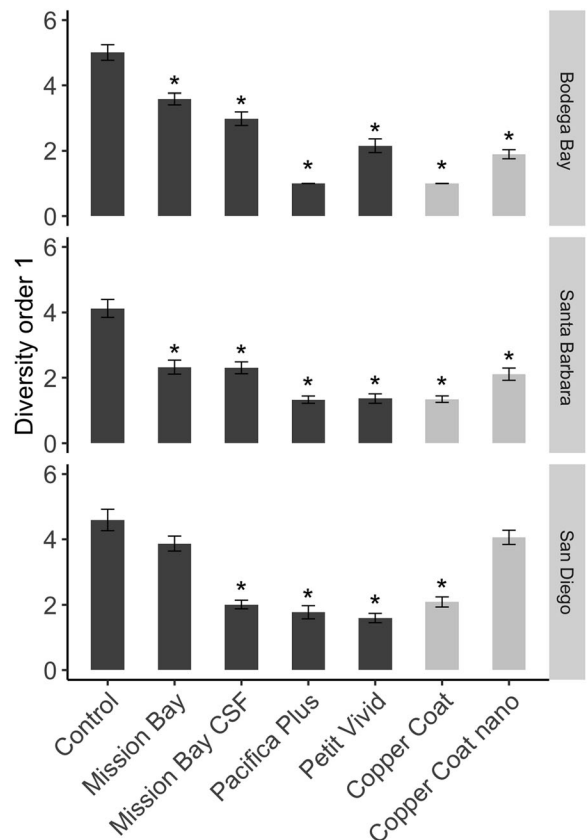


Fig. 6 Mean diversity of sessile invertebrates on controls and treatment coatings for each of the three study sites. Error bars are standard errors. Asterisks indicate significance in post hoc comparisons against site-specific controls using Dunnett’s tests. Dark gray bars indicate Zn-based coatings; light gray bars are Cu-based coatings

by CAP analysis (Fig. 8). While the communities in the antifouling treatments tended to be clustered more closely, there was a fairly consistent gradient (Fig. 8). Generally, the Petit Vivid and nano-CuO Coppercoat were more distant from the control, while the two Mission Bay coatings were in the middle. At San Diego (Fig. 8), this pattern was driven by high abundance of the invasive tunicates *Sympyegma reptans* and *Ciona intestinalis* on all three treatments (Fig. 8). The strong difference in the controls at Bodega Bay was driven by relatively low abundance of the invasive bryozoan *Watersipora subatra* on the controls (Fig. 8).

Discussion

Our results showed that Cu- and Zn-based antifouling coatings had quite different long-term effectiveness at

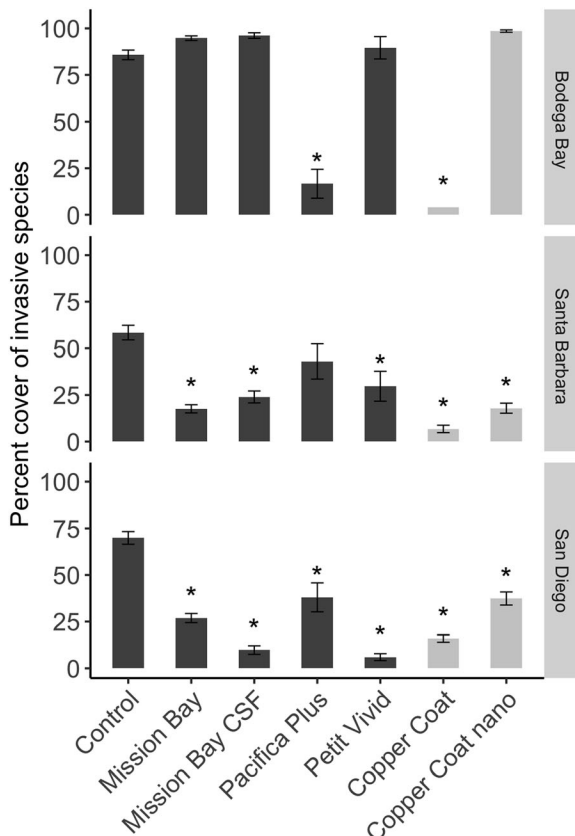


Fig. 7 Mean percent cover of invasive species on controls and treatment coatings for each of the three study sites. Error bars are standard errors. Asterisks indicate significance in post hoc comparisons against site-specific controls using Dunnett's tests. Dark gray bars indicate Zn-based coatings; light gray bars are Cu-based coatings

controlling fouling across sites and suppressing the relative abundance of non-native species, with marked differences between formulations. Despite zinc's generally lower toxicity compared to copper, zinc-based coatings were as or more effective than copper at reducing fouling. The presence of nanoparticles in the various formulations was not necessarily a formula for successful antifouling action. Boat hulls and other mobile fouled surfaces, such as barges and buoys, are major vectors of transport for invasive fouling organisms (Carlton and Geller 1993), and our results suggest that antifouling coatings can help prevent this, potentially slowing or preventing the spread of non-native sessile invertebrate species. Although some invasive marine invertebrates have been shown to be resistant to copper and other biocides (Piola et al. 2009; McKenzie et al. 2012), our results suggest that this was not the case overall. Moreover, fouling communities were generally similar on the different coating types, suggesting that the type of biocide used does not markedly shape the spread of specific invasive groups (Fig. 8).

Surprisingly, the effectiveness of the zinc-based coatings was not directly related to the concentration of zinc that they contained. For example, non-nano Mission Bay had the highest nominal ZnO concentration, and released significant amounts of total zinc in both estuarine and sea water over the 120-day laboratory experiment, yet it had among the highest abundance and diversity of fouling organisms. In contrast, the non-nano Pacifica Plus released the least amount of total zinc in the experiments, yet was among the most effective at suppressing abundance and diversity of fouling organisms at all sites. Given that copper generally has higher toxicity than zinc in ecotoxicology assays (Mu et al. 2018), these results suggest that zinc-based coatings are preferable to copper, and that carefully formulated coatings could minimize release of zinc into the environment.

Our results suggest that zinc-based coatings can be just as effective in suppressing fouling organisms, including invasive species, apparently contradicting the species sensitivity data from the literature. Part of the reason for this could be a lack of data for marine fouling taxa. Toxicity assays for marine species are much more limited than their freshwater counterparts, and marine model species for toxicity are more taxonomically restricted (Leung et al. 2001; Raisuddin et al. 2007). Fouling communities like those in this study are dominated by tunicates (Chordata), bryozoans (Ectoprocta),

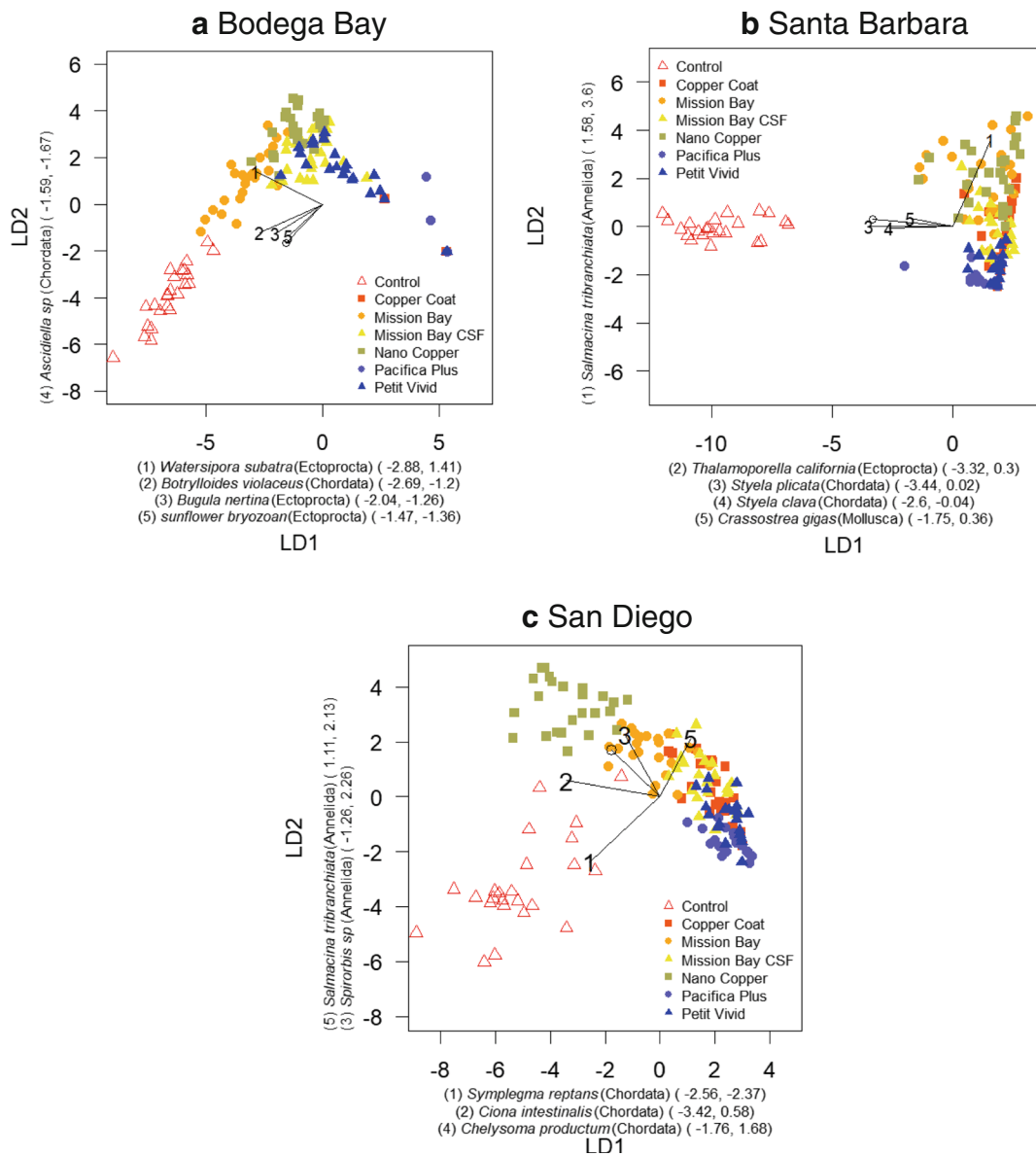


Fig. 8 Results of canonical analysis of principal coordinates using Euclidean distances. The top 5 species driving separation between treatments are displayed on the graphs with numbers

corresponding to the lines showing direction of influence. Line lengths are proportional to each species' influence, and species names are listed alongside the axis they had the most influence on

hydroids (Cnidaria), polychaete worms (Annelida), and other sessile invertebrates that are not represented in the assortment of marine model species used in toxicity assays, which are dominated by crustaceans and fish (Leung et al. 2001). The close contact of fouling organisms with the coatings, moreover, means that immediately upon settlement, they are likely exposed to relatively high concentrations of the active ingredients at the immediate surface of the coating, as well as being in

direct contact with it. Early life stages can be more sensitive to toxic effects, and even sublethal effects early in development can strongly impact post-settlement survival (Bellas et al. 2005; Contreras et al. 2007; Girling et al. 2015).

Zinc may also act more subtly to inhibit biofouling. For example, zinc is known to be toxic to microalgae, including diatoms (Sunda and Huntsman 1996; Miller et al. 2010, 2017), and diatoms and other microalgae,

along with bacteria, are often early successional colonizers of artificial surfaces. Such biofilms can facilitate recruitment of other sessile species (Dahms et al. 2004; Qian et al. 2007). Further research on the effect of zinc-based antifouling coatings could reveal whether inhibition of biofilms is an important mechanism of their efficacy.

The efficacy of metal-based antifouling coatings is accompanied by release of these toxins into coastal waters, particularly estuaries. Dissolution of ionic metals is affected by water chemistry. For example, the dissolution of Cu particles is driven by pH and complexing anions such as Cl^- that form soluble Cu complexes (King 2002; Adeleye et al. 2014; Conway et al. 2015; Keller et al. 2017; Adeleye et al. 2018). As a result, we expected more rapid leaching of Cu from the plates submerged in seawater, which had more Cl^- ($\text{Cl}^- \approx 19.3 \text{ g/L}$) than the estuarine water ($\text{Cl}^- \approx 9.70 \text{ g/L}$) (Adeleye et al. 2016)). The dissolution rate constant was similar, however, in seawater and estuarine water. This behavior suggests that the matrix of the paint, not simply media chemistry, plays an important role in the fate of the biocide.

The mechanism of particulate ZnO dissolution in seawater is not as well understood as that of Cu (or Cu_2O) but it is believed to be controlled by particle size and morphology, surface properties, and media chemistry (Lee and Piron 1995; Yebra et al. 2006; Zhou and Keller 2010). While Yebra et al. (2006) hypothesized that the dissolution of ZnO in seawater is driven by Cl^- , experimental results showed either no clear effect of Cl^- concentrations on dissolution (Ytreberg et al. 2017; Lagerström et al. 2018) or negative effects of Cl^- on ZnO dissolution (Singh and Turner 2009; Yung et al. 2017). In this study, we found that total Zn released into estuarine water was higher than total zinc leached into seawater for all four Zn-based paints. Apart from the chemical composition of biocides, the release rate of biocides from antifouling coatings is also controlled by the paint binder reaction and paint polishing processes but these were not the primary focus of this study and thus were not studied (Ytreberg et al. 2017). Among the different zinc-based coatings, for example, the trend of initial dissolution rate was different from the overall trend of Zn release, which suggests the composition of the coating matrix plays an important role in the release rate of biocides over time.

Attenuation of dissolution over time in the incubation experiments was caused mainly by (1) saturation and (2)

surface passivation of Cu particles by OH^- , which causes the deposition of duplex $\text{Cu}_2\text{O}/\text{CuO}$, $\text{Cu}(\text{OH})_2$ film on the surface of Cu particles in alkaline conditions (King 2002). In addition, the Cu (II) ions formed by the oxidation of Cu(I) complexes are precipitated into $\text{Cu}(\text{OH})_2$ at concentrations above the solubility product of copper hydroxide ($1 \times 10^{-8} \text{ M}$) (King 2002; Albrecht et al. 2011; Adeleye et al. 2014; Adeleye 2015; Conway et al. 2015), controlling dissolved ion concentration in marine systems.

Unlike the Cu-based antifouling coatings, dissolved Zn was the most dominant Zn fraction detected in both seawater and estuarine water after a few hours of submerging plates coated with the four Zn-based paints until the end of the study. Zn^{2+} was the dominant species at the Zn concentrations ($\approx 10^{-4} \text{ M}$) and pH ($\approx \text{pH } 8.1$) of our media (Albrecht et al. 2011). The highest amounts of particulate Zn (nano and bulk) were detected within the first few hours of the study, perhaps due to release of surface ZnO or Zn omadine particles not strongly bound to the paint matrices. But these particles dissolved rapidly, and there was no evidence of precipitation of Zn ions, which occurs mainly above pH 8.7 (Albrecht et al. 2011), over the course of the study. The ratio of nanosized Zn detected in media that contained plates coated with the nano-based Mission Bay CSF paint was not remarkably different from the fraction detected in the other paints that contain larger-sized Zn particles, likely due to the fast dissolution of ZnO, regardless of particle size (Franklin et al. 2007; Heinlaan et al. 2008).

We found no clear advantage of nanoparticle-containing coatings in either preventing fouling or limiting metal release, relative to non-nano formulations, for either zinc or copper. The ZnO nanoparticle-containing coating had similar patterns of Zn release to other Zn-based coatings. The zinc released was largely dissolved regardless of whether the coating contained nanomaterials. Although the mass of Cu in Coppercoat was 8 times higher than in the Cu NP formulation, the ratio of total Cu released from Coppercoat to total Cu released from Cu nanoparticle formulation only exceeded 8 after 2 weeks (in seawater) or 1 month (in estuarine water). This points to an initially higher total Cu release rate from the non-nano Cu formulation. Initial Zn release rate was higher in the nano-ZnO paint than in the non-nano coatings. The higher initial release of Cu or Zn did not however translate to a higher long-term antifouling efficacy in the field tests.

Conclusions

We found no clear advantage of nanoparticulate Zn or Cu in antifouling coatings, relative to traditional micro-sized formulations, either in their efficacy or toxin release characteristics. The release rate of Zn²⁺ from nano-ZnO was similar to the non-nano formulations. Release rate of Cu²⁺ was initially higher from the non-nano Cu formulation, but after 2–4 weeks, release was similar from the nano coating and proportional to Cu content. Adding nano Cu to the Coppercoat mixture had to be limited, since it significantly increased the viscosity of the mixture. This limitation could likely be overcome by adjusting the coating matrix. There was no significant difference in the long-term antifouling effectiveness between nano and non-nano formulations.

Clearly, both the efficacy of antifouling coatings and their release of metals into coastal ecosystems are both highly dependent on coating formulation and water characteristics, rather than simply biocide type and concentration. Unfortunately, formulation data are typically not available for commercially available coatings, making individual product testing necessary for acquiring information about leaching and efficacy. More research and transparency on formulation composition or characteristics, therefore, would be needed to achieve more predictable outcomes from regulation of antifouling coatings.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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