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Sea-trial research on natural product-based antifouling paint applied to different underwater sensor housing materials

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ABSTRACT

Biofouling is a common challenge for underwater sensors, especially for long-term *in situ* monitoring in marine environments. In this study, we assessed the antifouling efficacy of a paint containing a natural product camptothecin (CPT) on six materials (316 L stainless steel, TC4 titanium alloy, 7075 aluminum alloy, polyoxymethylene, polyvinyl chloride, and Teflon), which are frequently used in the construction of underwater sensor housings. Additionally, a buoy-based sea-trial was performed to test the antifouling performance of the CPT-based paint on housings of three *in situ* sensors used for practical seawater monitoring applications, namely a spectrophotometer for chemical oxygen demand (COD) measurements and two fluorimeters for biochemical oxygen demand (BOD) and chlorophyll *a* (Chl *a*) concentration measurements. The results showed significantly lower macrofouling coverage on the areas painted with the CPT-based paint compared to the unpainted areas for each tested material over 9 months of seawater immersion. The CPT-based paint exhibited different antifouling performance for the different materials; in particular, it exhibited better antifouling performance on the plastic materials compared to the metal materials. Furthermore, when applied on submersible sensor housings in the sea-trial test, the CPT-based paint kept the housings of the COD sensor and the Chl *a* sensor clean for over 4 months. In addition, the paint prevented fouling of the BOD sensor housing even after 6 months of seawater immersion. Thus, our results suggest that the CPT-based paint could be used as a potential solution to control the biofouling of sensor housings for long-term *in situ* applications in marine environments.

1. Introduction

Submersible instruments are important for *in situ* ocean observations, marine investigations, scientific research, seawater quality monitoring, and emergency management, including a large number of chemical, acoustic, electrical, optical, and biological sensors. For instruments deployed underwater, biofouling can be a serious issue that affects their operation, maintenance, and data integrity (Delgado et al., 2021). In addition, biofouling on *in situ* sensor surfaces can shorten their operating lifetime, increase the cost and frequency of maintenance, and result in signal drift and data errors (Whelan. and Regan., 2006). This is especially true for long-term *in situ* monitoring sensors, and biofouling has been considered as a key limiting factor that affects deployment duration. Therefore, an effective method to control fouling is needed for applications that rely on *in situ* sensors in marine environments.

Several antifouling strategies have been proposed to protect the sensing surfaces of sensors (e.g., optical windows and filtration membranes), including the use of wipers, brushes, copper shutters, ultraviolet (UV) light irradiation, and ultrasonic treatment (Whelan. and Regan., 2006; Delauney et al., 2010; Delgado et al., 2021). As an important

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sensor component, sensor housing is also subject to biofouling problems. Whelan and Regan (2006) suggested that the need for biofouling protection of housing is equally important as the sensing surface, as the biofouling of housing can cause measurement interference, even if the sensing surface is clean. Because the biofouling of housing can disturb the biological and chemical properties of the seawater around the sensing surface, it can also modify the local underwater environment surrounding the sensors and introduce errors into the data (Whelan. and Regan., 2006; Lehaitre. and Compère., 2008; Delauney et al., 2010; Li et al., 2021). When the sensors are taken out from the seawater for maintenance after deployment, they have to be cleaned if fouling organisms are present on the sensor housing. Cleaning of the housing can change the status of the sensing surface, making a sensor response comparison before and after deployment difficult (Delauney et al., 2010; Delgado et al., 2021). In addition, cleaning of heavily fouled housing can be time-consuming and destructive to the housing surfaces, along with the risk of unexpected accidents and termination in observational data acquisition. Sensor housings are usually made of different materials from sensing surface. Thus, fouling control methods used for sensing surfaces are not appropriate for housings in practice (which usually have a comparatively larger surface area), due to additional power or infrastructure requirements. Hence, other methods are needed for the biofouling protection of housings (Whelan. and Regan., 2006).

Commercially available antifouling methods for sensor housings are mainly based on antifouling paints, which are widely used in the shipping industry (Delauney et al., 2010). Antifouling paints with metal-based antifoulants such as tributyltin and cuprous oxide have been extensively used to control biofouling (Yebra et al., 2004). However, concerns on the adverse environmental impacts of these antifoulants have led to bans and regulations on their use in antifouling paints (Thomas and Brooks, 2010; Price. and Readman., 2013). As an alternative to metal-based paint, fouling-release coatings have been developed, which are based on fluoro-copolymers and silicone polymers. The adhesion of these coatings to the fouling organisms are weak, which are removed by shear forces generated usually by movement in seawater (Callow and Callow, 2011; Hu et al., 2020). Unfortunately, this approach is problematic for many marine sensors, especially for those to be deployed in static state in seawater (O'Neill et al., 2016). Thus, new, effective, and environmentally friendly antifouling paints are needed for sensor housings.

Natural products with antifouling properties have been investigated as promising sources of environmentally friendly antifoulants (Liu et al., 2020), and a large number of natural product antifoulants have already been reported (Fusetani, 2011; Qian et al., 2015; Liu et al., 2020). However, most studies only employ laboratory tests to evaluate the antifouling efficacy of natural products (Qian et al., 2015). For practical application, they should be incorporated in coatings and tested using real sea trials. Recently, we found that camptothecin (CPT, isolated from plants such as *Camptotheca acuminate, Nothapodytes nimmoniana, Pyrenacantha klaineana*, and *P. volubilis* and previously known for its antitumor activity) exhibited effective antifouling activity in laboratory tests and field experiments. Furthermore, it exhibited lower toxicity on non-target aquatic organisms, compared to several commonly used commercial antifoulants, indicating the potential use of CPT as an environmentally friendly antifoulant (Feng et al., 2018). In this study, we evaluated the feasibility of using CPT-based paints for biofouling protection of sensor housings in real marine underwater environments. In our previous field experiments, the natural product-based paints were tested for antifouling efficacy by applying the coatings on epoxy panels immersed underwater in coastal sea environments (Feng et al., 2018). However, because diverse materials are used in the construction of sensor housings (Delgado et al., 2021), here the CPT-based paint was applied on panels made of six different types of materials often used for sensor housings, and the panels were subjected to static immersion tests in the sea for 9 months. Then, we evaluated the antifouling performance of the CPT-based paint on the housings of three *in situ* sensors, which were deployed in real underwater conditions on a moored surface buoy. In this study, we sought to address three questions. Are there observable differences in biofouling among the different materials used for sensor housings? Is the CPT-based paint effective in preventing biofouling on these materials? Could the CPT-based paint retain its antifouling properties when applied to sensor housings in practice?

2. Materials and methods

2.1. Preparation of panels made of materials commonly used in underwater sensor housings

In this study, the tested panels were made from six different materials, including three metals (316 L stainless steel, TC4 titanium alloy, and 7075 aluminum alloy) and three plastics (polyoxymethylene, polyvinyl chloride, and Teflon) (Table S1). These materials were chosen as they have been widely used for constructing underwater sensor housings. For the 7075 aluminum alloy panel, anodic oxidation was performed to improve its corrosion resistance, resulting in a layer of black anodic film on its surface. For the Teflon panel, Teflon was coated on the surface of the 7075 aluminum alloy. Three replicate panels were made for each material, which were 30 cm × 18 cm × 0.3 cm in size (length × width × thickness). The panels were then tied to the frames (made of 316 L stainless steel, 94 cm × 40 cm) by using thin Nylon bands through pre-drilled holes on the frame corners. Six frames were used in total, with each frame supporting three replicate panels for each tested material.

2.2. Preparation of the CPT-based antifouling paint

The CPT-based paint was prepared by mixing the following ingredients in a high-speed disperser (ingredients expressed as weight percentage): 10% CPT, 10% rosin, 14% chlorinated polyether resin, 5% Fe_2O_3, 1% bentonite, 15% zinc oxide, 10% talcum powder, and 35% dimethylbenzene. First, rosin was added to dimethylbenzene and dispersed until the rosin was completely dissolved. Then, the chlorinated polyether resin was added to the solution and dispersed for 30 min. Subsequently, the other ingredients were added and dispersed until a fineness of ca 80 μm was achieved. The prepared paint was then used in the sea trials.

2.3. Sea trial with testing panels

The prepared CPT-based paint was brushed onto the panels described above, and the painted area was ca 15 cm × 18 cm for each panel (except for each Teflon panel, where the painted area was 12 cm × 18 cm). Three layers of paint were applied and allowed to dry between each application, resulting in a dry film with a thickness of ca 150 μm. The unpainted panel areas were used as control. The panels were hung on a floating raft in Xiamen Bay, China (24°52′N, 118°17′E) from July 7, 2019 at a depth of 1 m in seawater for 9 months. The panels were photographed at 3-, 6-, and 9-month intervals during submersion. The surface areas colonized by the fouling organisms on the painted and unpainted areas of each panel were then analyzed using Adobe Photoshop CS6.

2.4. Sea trial with underwater sensors

In this study, we also used three underwater sensors, including a spectrophotometer for chemical oxygen demand (COD) measurement, a UV fluorimeter for biochemical oxygen demand (BOD), and a visible fluorimeter for chlorophyll *a* concentration measurements. The housings of the spectrophotometer for COD measurement and the visible fluorimeter for chlorophyll *a* concentration measurement, and their shared wiper system axle were all made of 316 L stainless steel. The housing of their wiper system and the housing of the UV fluorimeter were made of polyoxymethylene. The housings were washed with tap water to remove dust on the surfaces, and after the housings were air-dried, we applied a layer of epoxy tie coat along with a subsequent application of the CPT-based paint, which was sprayed on the housing surfaces to improve paint adhesion. After the epoxy tie coat dried, the CPT-based paint was sprayed and allowed to dry, forming a dry film with a thickness of ca 200 μm. Then, the sensors were installed under a moored surface buoy platform in Dapeng Ao Cove of Daya Bay, Shenzhen, China (22°120′N, 114°31′E) on June 22, 2020 (Fig. 1). The sensors were then inspected and photographed after 4 and 6 months' deployment.

2.5. Statistical analysis

Differences in macrofouling coverage between the treatments were analyzed via one-way ANOVA, followed by a Tukey post-hoc test using SPSS version 22.0, and the significance level was set at $P < 0.05$. PRIMER V7.0.21 software (PRIMER-E Ltd, Lutton, Ivybridge, UK) was used to perform multivariate analyses of the compositions and covering of the biofouling organisms. Cluster analysis and two-dimensional non-metric multi-dimensional scaling (nMDS) ordinations were produced from Euclidean distance matrices. The data were then separated into different groups at 60%–80% similarity based on Euclidean distance, and the results were superimposed on the nMDS ordination plots. The major taxa that explaining the grouping on the nMDS plots were identified by SIMPER analysis.

3. Results

3.1. Sea trial with panels of different materials

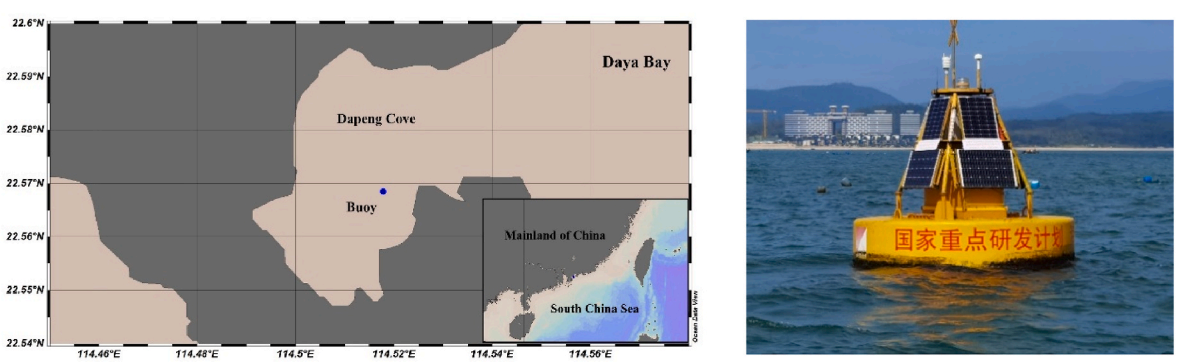
As shown in Figs. 2 and 3, after 3 months of immersion in seawater, almost no macrofouling was observed on the painted area of the panels for each material, while substantial macrofouling was observed on the unpainted areas of each panel. This indicated the outstanding antifouling efficacy of the CPT-based paint for all the materials under test. Among all the panels, the 316 L stainless steel, TC4 titanium alloy, and polyvinyl chloride panels all showed 100% macrofouling coverage on the unpainted areas. Macrofouling coverage was comparatively lower on the unpainted areas of the panels made from the other three materials, in particular Teflon, which had a macrofouling coverage of 83.93%. We observed that barnacles were the main settlers on the unpainted areas of the 316 L stainless steel, TC4 titanium alloy, polyoxymethylene, and polyvinyl chloride panels. By contrast, on the 7075 aluminum alloy, the main settlers were bryozoans, and for Teflon, the coverage by barnacles (43.38%) was similar to that by bryozoans (38.38%). We observed differences in macrofouling coverage and the main settlers among the unpainted areas of the different materials at 3 months.

After immersion for 6 months, all unpainted areas of the six materials were completely covered with biofoulers, including bryozoans, barnacles, oysters, tubeworms, and solitary ascidians. By contrast, the painted areas of the six materials all showed significantly lower fouling coverage than the unpainted areas ($P < 0.05$). As shown in Figs. 2 and 3, the coverage percentages on the painted areas of the three plastics (polyoxymethylene, polyvinyl chloride, and Teflon) were significantly lower than those on the painted areas of the three metals (316 L stainless steel, TC4 titanium alloy, 7075 aluminum alloy) ($P < 0.05$), suggesting that the CPT-based paint was more effective in protecting the three plastics from biofouling. This trend of lower fouling coverage on the painted areas of the three plastics (compared to the three metals) persisted for 9 months. After 9 months of submersion, the CPT-based paint still exhibited good antifouling performance for TC4 titanium alloy, 7075 aluminum alloy, polyoxymethylene, polyvinyl chloride, and Teflon, reducing biofouling by 73.33%–96.41% compared to the control unpainted areas (100% coverage). For the 316 L stainless steel, the coverage percentage on the painted area reached 71.62% (although it was lower than the control unpainted area), suggesting low antifouling efficacy of the CPT-based paint for this material at 9 months. During the field experiment, seasonal changes in fouling communities were observed, with the abundance of barnacles decreasing over time, while the abundance of bryozoans and ascidians increased.

As shown in Fig. 4, multivariate analysis of composition and coverage of macrofouling organisms on the panels of the tested materials showed distinct groups at different immersion times. SIMPER analysis showed that this was most likely due to the continuous increase in bryozoans and continuous decrease in barnacles over time. Distinct groupings were also observed for the painted and unpainted areas (except for the painted area of 316 L stainless steel at 9 months, 60% similarity based on Euclidean distance), indicating that the CPT-based paint was effective in preventing biofouling of the tested materials during the field experiment (except for 316 L stainless steel at 9 months). Furthermore, there were distinct groups for painted areas of the three plastics and those of the three metals, at 6 and 9 months (80% similarity based on Euclidean distance). This suggested that the CPT-based paint exhibited different antifouling performance between the plastic and metal materials.

3.2. Sea trial with underwater sensors

As shown in Fig. 5a, the housings of the underwater spectrophotometer, the Chl *a* sensor (fluorimeter), and their shared wiper system, which were painted with the CPT-based paint, remained clean after 4 months of deployment under a surface buoy in the marine environment. By contrast, fouling organisms (mainly barnacles) settled on the unpainted stainless-steel frames for supporting and protecting these



(a) (b)

Fig. 1. Location of the moored surface buoy (a) and photo of the buoy platform (b).

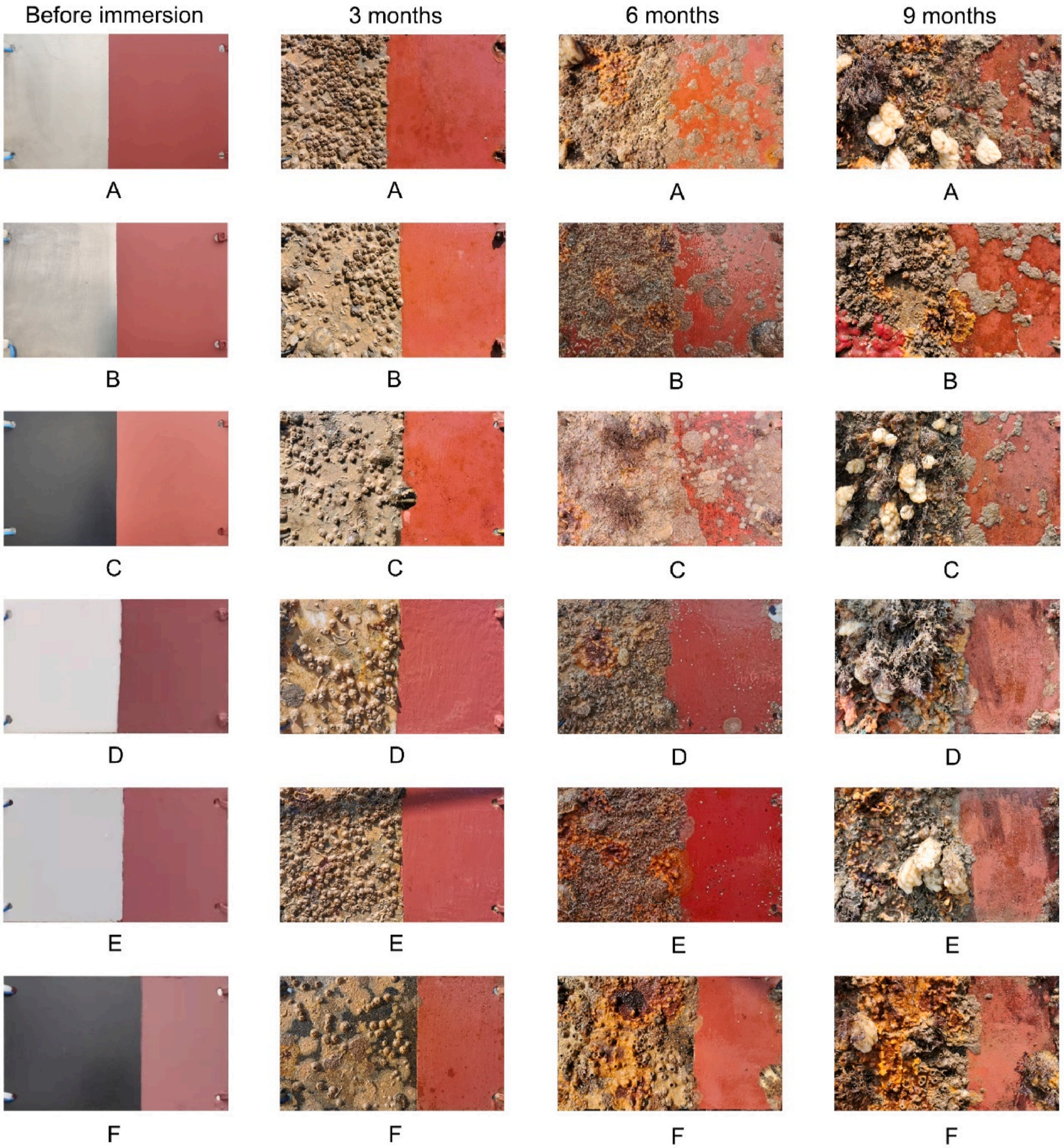


Fig. 2. Test panels before and after immersion in seawater at different time points. A: 316 L stainless steel, B: TC4 titanium alloy, C: 7075 aluminum alloy with a black anodic film on the surface, D: polyoxymethylene, E: polyvinyl chloride, and F: 7075 aluminum alloy coated with Teflon. Each panel was randomly selected from three replicates for each treatment and immersion time.

underwater instruments. Likewise, the painted housing of the BOD sensor (a UV fluorimeter) remained clean after immersion in the same sea area for 6 months, while the unpainted frames were severely fouled (mainly by tubeworms, barnacles, and bryozoans, Fig. 5b). Thus, the CPT-based paint exhibited outstanding antifouling performance when applied to the submersible sensor housings in practice.

4. Discussion

Although many underwater sensors are designed to obtain long-term *in situ* measurements, they are actually easy to be affected by biofouling in the short term (sometimes in less than 1 week), especially in sea areas with a high fouling pressure (Delauney et al., 2010). Most studies on techniques to combat biofouling have only employed bioassay(s) with only one or several fouling species in laboratory to test antifouling

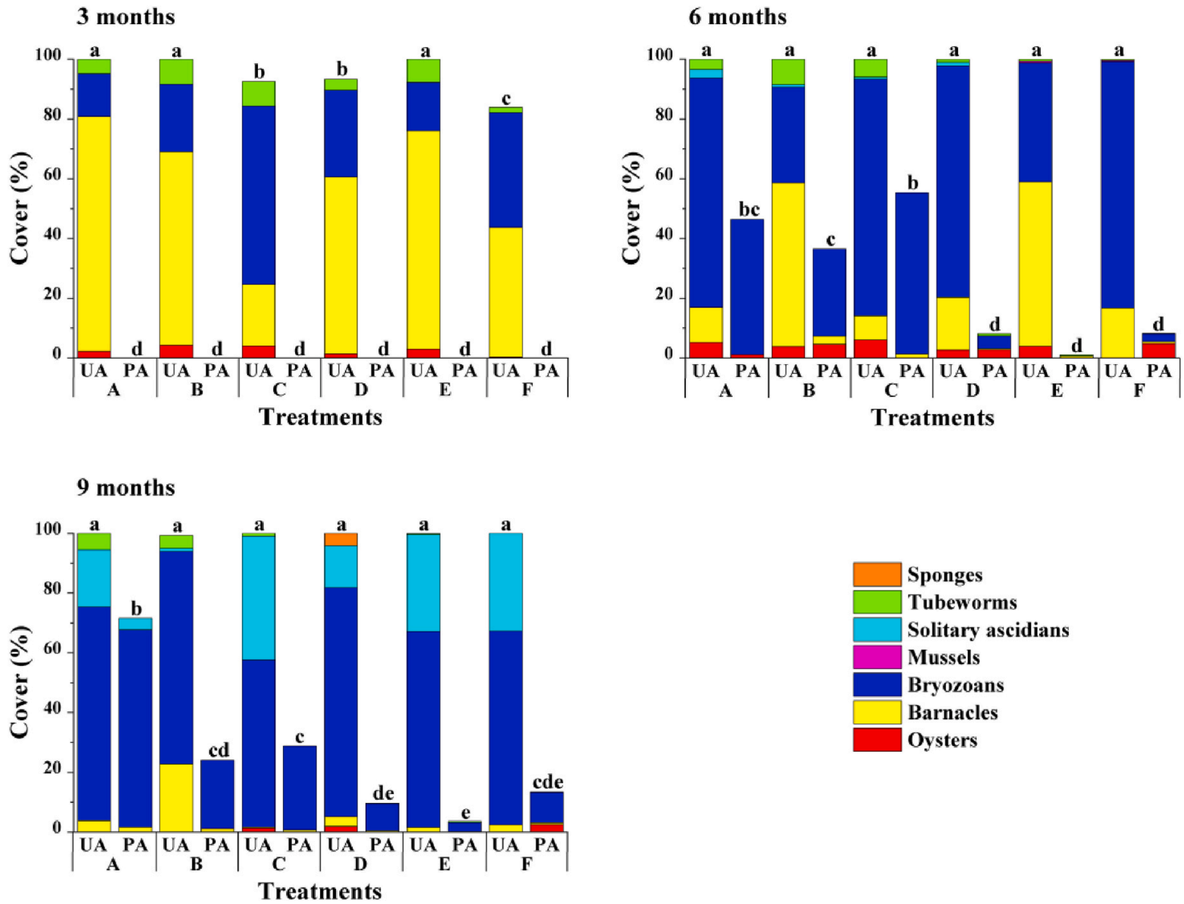


Fig. 3. Macrofouling coverage on the panels made of different materials after different immersion time. A: 316 L stainless steel, B: TC4 titanium alloy, C: 7075 aluminum alloy with a black anodic film on the surface; D: polyoxymethylene, E: polyvinyl chloride, F: 7075 aluminum alloy coated with Teflon, UA: unpainted area, and PA: painted area. Data shown are the mean of three replicates, and the different letters above the columns indicate significant differences in macrofouling coverage among the various panels ($P < 0.05$).

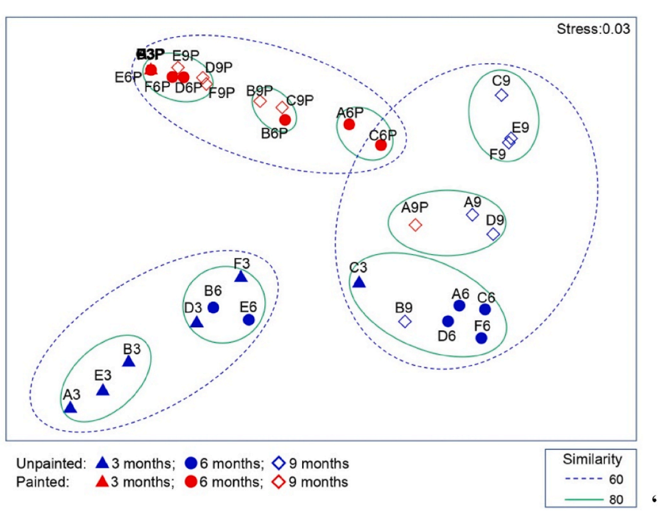


Fig. 4. nMDS plots of the compositions and coverage of macrofouling organisms on the panels of different materials with painted and unpainted areas after different immersion times. Each point represents the average composition and coverage of the macrofouling organisms within a given material, and the lines encircling the treatment groups have a similarity of 60% and 80%. A: 316 L stainless steel, B: TC4 titanium alloy, C: 7075 aluminum alloy with a black anodic film on the surface, D: polyoxymethylene, E: polyvinyl chloride, and F: 7075 aluminum alloy coated with Teflon.

efficacy, without verification in sea trials (Qian et al., 2015; Carve et al., 2019). Marine environments contain a diversity of fouling organisms (Gu et al., 2020), and field testing is a critical step for evaluating the efficacy of antifouling methods for sensors in real environments. Therefore, in this study, the 9-month field test indicated that the CPT-based paint was effective in protecting the sensor housing materials against various fouling organisms. Consistently, the sea-trial with three *in situ* sensors confirmed the stable antifouling efficacy of this coating for sensor housings. Furthermore, the CPT-based paint was environmentally friendly (Feng et al., 2018) and could be easily applied on housing surfaces to protect them from biofouling for several months with no power requirements. Therefore, this paint has promising potential as an antifouling strategy for sensor housings.

Among the six materials tested in this study, we observed differences in macrofouling coverage and community composition after immersion for 3 months. Studies have shown that the chemical and physical properties of a material can influence the settlement of fouling organisms (Carve et al., 2019; Chen et al., 2021). Here there was significantly lower macrofouling coverage on Teflon compared to the other tested materials, possibly due to the superior hydrophobic nature (low surface energy) of Teflon (Dhanumalayan and Joshi, 2018). Hydrophobic surfaces have been previously found to reduce the settlement of some fouling organisms, such as barnacles (Aldred et al., 2010). It should also be noted that when the immersion time increased to 6 and 9 months, 100% fouling coverage was observed in all treatment materials including Teflon, indicating that although Teflon itself may reduce marine macrofouling to a certain extent during the initial immersion period, this effect expired when subjected to high fouling pressure after

(a) (b)

Fig. 5. Application of the CPT-based paint on housings of the COD sensor, the Chl *a* sensor and their wiper for 4 months (a) and on the housing of the BOD sensor for 6 months (b). White arrows indicate the instrument housings painted with CPT-based paint, and blue arrows indicate unpainted frames with fouling organisms settled on their surfaces. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

long time immersion.

Interestingly, the CPT-based paint showed a different antifouling performance for different materials, suggesting that the material type should be taken into account when applying CPT-based paint on sensor housings. Furthermore, this result suggests the need for future studies on testing whether the material type also affects the efficacy of other antifouling coatings. This is especially important as with the development of the marine economy and the advancement of materials science, more man-made structures composed of different materials will be used in marine environments. For marine sensors, material selection is affected by the system reliability requirements, availability, cost, and manufacturing capability (Delgado et al., 2021). The present study indicates that performance of antifouling coatings should also be considered during material selection. We observed better antifouling performance of the CPT-based paint on the three plastic materials compared to the three metal materials; however, the reasons are unclear. One possibility could be the interactions between the CPT-based paint and the substratum materials, which affected the antifouling performance of the coating. Another possibility could be invisible corrosion on the three metal materials, which affected the antifouling performance of the coating. The two metals, 316 L stainless steel and TC4 titanium alloy, were supposed to be corrosion resistant. Furthermore, for the 7075 aluminum alloy, anodic oxidation was used to improve its corrosion resistance. Studies have previously found that 316 L stainless steel and titanium alloys retained good corrosion resistance after exposure to seawater for 180 days (Al-Muhanna and Habib, 2016). However, other studies have shown that 316 L stainless steel can undergo corrosion by some bacteria (such as sulfate-reducing bacteria) (Xu et al., 2007). Regardless, the mechanism responsible for the differences in antifouling performance exhibited by the different materials coated with CPT-based paint requires further exploration.

In addition to the application on underwater sensors, it is worth noting that the CPT-based paint also has great potential for other artificial submerged structures in the marine environment, such as ship hulls, oil platforms and aquaculture facilities. Colonization by fouling organisms is a serious problem for these structures, causing large penalties to the efficient operation of the structures and huge economic loss (Abioye et al., 2019; Kumar et al., 2021). It would be interesting to investigate the practical effectiveness of the CPT-based paint on these structures by long-term *in situ* sea tests as performed in this work.

In conclusion, the results confirmed that the CPT-based paint was effective in preventing biofouling on the six different materials used for manufacturing marine sensors. Furthermore, the practical application of this coating on *in situ* sensors showed good antifouling performance in a real marine environment. As it becomes increasingly important to obtain long-term ocean observations (Lehaitre. and Compère., 2008), and a major limitation of *in situ* sensors for long-term monitoring is their vulnerability to biofouling (macrofouling has been found even on long-term deep-sea instrumentation) (Blanco et al., 2013), treatment with CPT-based paint provides a potential solution for managing the challenges of biofouling.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2022.105400.

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