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## Efficacy, microbial disruption, and algal toxicity of commercial antifouling coatings: A multi-level assessment

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### ABSTRACT

To mitigate marine biofouling, copper- and zinc-based antifouling (AF) paints are widely used, although their severe environmental toxicity is well established. Silicone-based fouling-release coatings (FRCs) have emerged as alternatives that prevent adhesion through surface properties rather than biocidal activity. However, their effectiveness and ecological impact across marine environments remain insufficiently assessed. This study compared the performance and environmental effects of five commercial coatings: two biocidal, two FRCs, and one inert coating. Panels were statically exposed for seven months at five European sites spanning salinity and temperature gradients. Fouling development was monitored monthly, and coating leachates were tested on red macroalgae and bacteria. Ecotoxicological assays included growth inhibition of *Ceramium tenuicorne*, *Aliivibrio fischeri* bioluminescence, *Escherichia coli* stress biosensors, quorum-sensing (QS) assays, and biofilm formation of marine bacteria.

Field experiments showed that FRCs consistently outperformed copper-based coatings in efficacy toward biofouling, even under static conditions. Copper-based leachates were highly toxic to *C. tenuicorne* ( $EC_{50} \approx 0.46\%$ ), whereas FRC leachates showed minimal effects and were about 100 times less toxic. Microbial assays revealed that all coatings—including the biocide-free formulations—altered microbial physiology and behavior: leachates induced protein-damage responses, and both QS signaling and biofilm formation were species-specific, confirming that biocide-free does not mean biologically neutral. These results demonstrate that antifouling leachates act not only as toxicants but also as chemical cues shaping microbial communication and early colonization. Integrating microbial-level responses into antifouling evaluations is therefore essential. Overall, FRCs remain the most environmentally acceptable option, combining strong antifouling performance with minimal toxicity.

### 1. Introduction

All submerged surfaces in the marine environment are rapidly colonized by bacteria, algae, and invertebrates, a process known as biofouling. This phenomenon represents a major ecological and economic challenge for the maritime sector, as it affects vessel speed, fuel consumption, and maneuverability, even at moderate fouling levels (Callow, 1990; Jin et al., 2022; Schultz et al., 2011). Increased fuel consumption directly translates into higher emissions of greenhouse gases, as well as nitrogen oxides (NO<sub>x</sub>), sulfur oxides (SO<sub>x</sub>), and

particulate matter (Cullinane and Cullinane, 2013). In addition, biofouling accelerates the corrosion of metal structures and acts as a vector for the spread of invasive species (Fernandes et al., 2016; Hewitt et al., 2009; Skovhus et al., 2017).

To mitigate these impacts, the dominant strategy relies on the use of antifouling (AF) paints, most of which release biocides. Contemporary biocidal AF coatings are largely based on inorganic copper compounds, primarily cuprous oxide (Cu<sub>2</sub>O), often combined with organic booster biocides to broaden antifouling efficacy (Finnie and Williams, 2009; Paz-Villarraga et al., 2022; Yebra et al., 2004). In addition, zinc oxide

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(ZnO) is commonly incorporated into formulations to regulate matrix erosion and biocide release. Although zinc is not classified as an active substance under the European Biocidal Products Regulation, its release from antifouling paints has been shown to induce toxic effects in non-target marine organisms (Karlsson et al., 2010; Watermann et al., 2005; Ytreberg et al., 2010). Beyond copper-based systems, several organic biocides and co-biocides have been used in antifouling formulations, including compounds such as tralopyril (Econea®), DCOIT, and copper pyrithione. While these substances can enhance antifouling performance, many exhibit high ecotoxicity, and some historically used booster biocides have been restricted or banned due to their environmental risks (de Campos et al., 2022; Paz-Villarraga et al., 2022). As a result, antifouling coatings may differ substantially in both efficacy and environmental risk depending on the composition and complexity of their biocide packages.

Although these coatings are effective against a wide range of organisms, their extensive use has raised major environmental concerns. The substances they release, particularly copper, are biologically active, affect non-target organisms, and accumulate in sediments and coastal environments (Amara et al., 2018; Boyle et al., 2016; Dafforn et al., 2011; Karlsson et al., 2010; Lagerström et al., 2020a; Thomas and Brooks, 2010; Ytreberg et al., 2010). Some antifouling biocides have been reported to be up to 400 times more toxic to non-target organisms than to their intended targets (de Campos et al., 2022). Furthermore, several studies have shown that AF paints can leach higher amounts of copper than the minimum required to ensure efficacy, resulting in unnecessary pollution (Lagerström et al., 2025; Lagerström et al., 2020b; Singh and Turner, 2009). The release of these biocides contributes to the deterioration of water quality and has negative effects on marine fauna across various ecosystems worldwide (Bighiu et al., 2017; Cima and Varello, 2023; Lagerström et al., 2020a; Martins et al., 2017; Soroldoni et al., 2018). In the Baltic Sea, a semi-enclosed sea with heavy maritime traffic and particularly vulnerable to contamination, antifouling paints account for nearly 40% of anthropogenic copper inputs, contributing to the non-compliance with the ‘good ecological status’ of European waters for the contaminant descriptor (EU-MSFD descriptor 8) (Korpinen et al., 2012; Ytreberg et al., 2022, Ytreberg et al., 2021).

In response to these concerns, fouling-release coatings (FRCs), primarily silicone-based, have been developed as a non-biocidal alternative to copper- and zinc-based antifouling (AF) paints. Their effectiveness relies on the creation of low-energy, low-friction surfaces that reduce organism adhesion and facilitate detachment under hydrodynamic forces (Brady and Singer, 2000; Hu et al., 2020; Lejars et al., 2012; Townsin and Anderson, 2009). Some studies have reported that FRCs can match, or even surpass, copper-based paints in antifouling performance, while being generally less toxic to marine organisms (Lagerström et al., 2022; Oliveira and Granhag, 2020). However, knowledge remains limited regarding their effectiveness across environmental gradients (temperature, salinity, fouling pressure), particularly under static conditions, which contributes to the continued widespread use of copper-based antifouling paints despite growing environmental concerns. Moreover, ecotoxicological studies suggest that certain commercial FRCs can release toxic compounds during the initial immersion or through progressive polymer erosion (Feng et al., 2012; Muller-Karanassos et al., 2021; Piazza et al., 2018; Truby et al., 2000; Watermann et al., 2005). In some cases, these coatings may also contain additional functional additives, including booster biocides or fluorinated polymers, which have been associated with persistent environmental effects and increased ecological concern. These emissions have been associated with adverse effects ranging from slight toxicity in marine bacteria (Watermann et al., 2005) to severe developmental impairments in sea urchin and fish embryos (Feng et al., 2012), sometimes persisting even after pre-immersion treatments. The potential release of microplastics and polymer degradation by-products (Karlsson and Eklund, 2004; Piazza et al., 2018; Tamburri et al., 2022) further intensifies ecological pressure. Such emissions may disrupt

marine ecosystems at multiple levels, impacting not only macro-organisms but also microbial communities that play a key role in biofilm structuring and succession. A deeper understanding of these release mechanisms and their consequences is therefore essential for assessing the actual potential of FRCs as a sustainable alternative to biocidal AF paints.

This study aims to compare the effectiveness and environmental impacts of commercial copper- and zinc-based antifouling paints used on recreational boats with silicone-based biocide-free FRC coatings. To achieve this, we combined: (i) field experiments conducted under static conditions at multiple European sites differing in salinity and temperature, to assess antifouling performance; (ii) a suite of ecotoxicological assays designed to evaluate coating toxicity and microbial interactions, including growth and viability tests with the red macroalga *Ceramium tenuicorne*; acute toxicity assessment using the luminescent bacterium *Aliivibrio fischeri*; bacterial biosensors targeting specific stress and metabolic pathways; and assays of biofilm formation and quorum sensing — a cell-to-cell communication mechanism that regulates collective microbial behaviors, including biofilm development.

## 2. Materials and methods

### 2.1. Antifouling coatings tested

Five commercially available antifouling coatings were selected to represent a range of technologies currently authorized for use on leisure boats under 12 m in Sweden. The set comprised two biocidal paints containing Cu<sub>2</sub>O and ZnO, two biocide-free FRCs, and one inert non-biocidal coating based on TecCel® technology, which does not provide antifouling functionality (Table 1). The detailed composition of each coating is provided in Table A.1, according to manufacturer safety data sheets (SDS).

### 2.2. Field evaluation of coating performance

#### 2.2.1. Panel preparation and exposure sites

Antifouling performance was evaluated under natural marine conditions at five coastal sites spanning strong gradients in salinity, temperature, and fouling pressure. Square PVC panels (100 × 100 × 2 mm) were lightly abraded and coated with the appropriate epoxy primer—Hempel Light Primer 45559 for Hempel coatings and International Gelshield 200 for International products—applied with a roller following manufacturer instructions. For FRCs, a dedicated tie-coat (Silic-One TieCoat 27450 or B-Free TieCoat YBF900) was applied over the primer, followed by two topcoat layers of the antifouling formulation.

Panels were deployed between late May and early June 2024 on static frames positioned vertically 1 m below the surface (Lagerström et al., 2022). Immersion was staggered by one week across sites, and four replicate panels per coating were randomly placed on each frame

**Table 1**

Summary of commercial antifouling coatings tested, including manufacturer, coating type, and main biocidal components.

Coating	Producer	Type of coating	Copper (I) Oxide (Cu <sub>2</sub> O) (%)	Zinc oxide (ZnO) (%)
Ecopower racing 76460	Hempel	Hard coating	/	/
Micron Superior YBD204	International	Biocide	31.93	10–15
Mille NCT 7173 A	Hempel	Biocide	17.1	5–10
Silic One 77450	Hempel	FRC	/	/
B-Free Explore YBF304	International	FRC	/	/

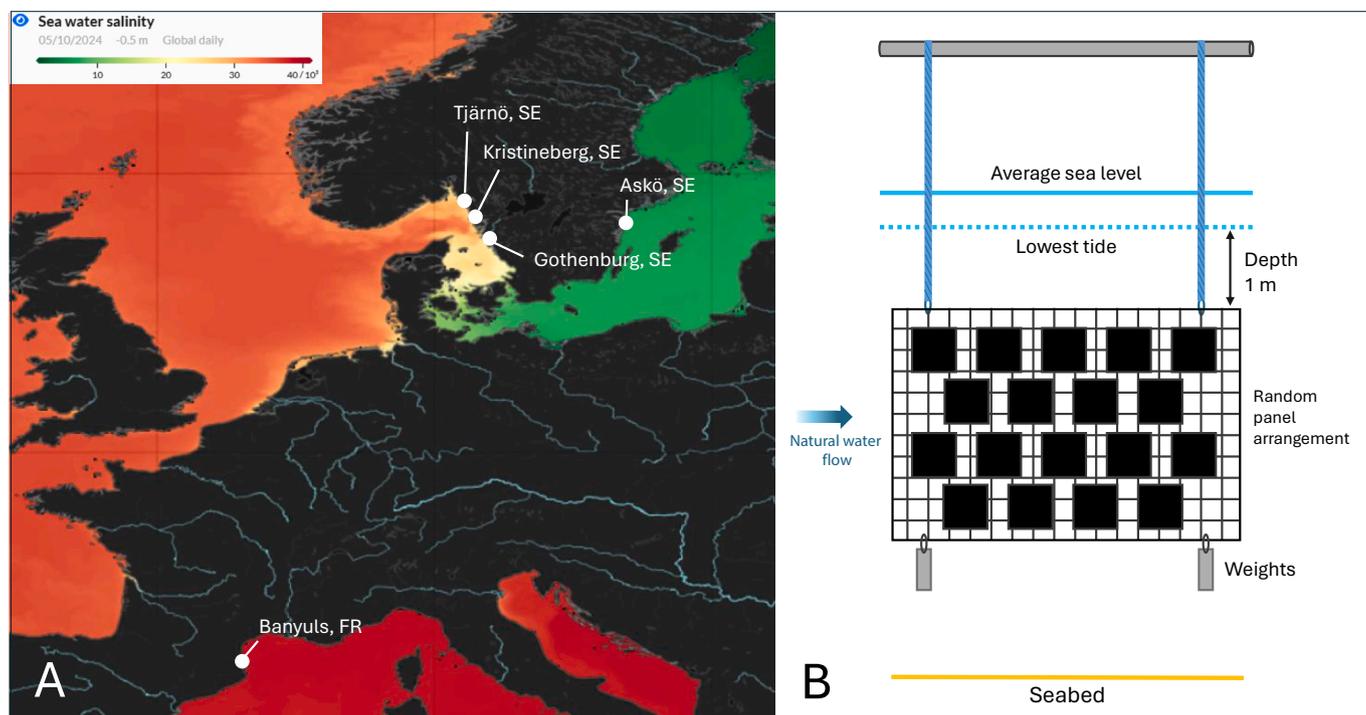


Fig. 1. Locations of the marine exposure sites (A) and overview of the experimental setup (B). FR = France, SE = Sweden.

(Fig. 1). These sites ranged from low-salinity Baltic conditions (Askö, 6 PSU) to fully marine Mediterranean waters (Banyuls, 38 PSU) (Table 2). Temperature and salinity at 1 m depth were obtained from in situ sensors at Kristineberg and Banyuls or obtained using E.U. Copernicus Marine Service Information (CMEMS) for Askö, Gothenburg, and Tjärnö. Each panel was photographed once per month during the seven-month immersion period. These images were used to quantify fouling type and surface coverage, and to assess coating performance under contrasting environmental conditions.

### 2.2.2. Fouling assessment methodology

Antifouling effectiveness was assessed based on the percentage of biofouling coverage and organism identification. Fouling was rated on a 0–100 scale following the US Navy's Naval Ships' Technical Manual (NSTM) (2006) and detailed in Lagerström et al. (2020b), where bryozoans are assigned a score of 40 (Lagerström et al., 2020b). The surface area occupied by each category was estimated according to (ASTM, 2005), with the outer 13-mm margin excluded from the analysis. The inert coating Ecopower Racing served as the reference condition, representing the local fouling pressure at each site, against which the performance of biocidal and fouling-release coatings was evaluated. For overall comparisons among treatments, a fouling rate (FR) was calculated for each replicate panel (Oliveira and Granhag, 2020). The score (0–100) of each fouling category was multiplied by its corresponding surface coverage (%) and summed. FR values were analyzed using a Kruskal–Wallis test, followed by Dunn's post-hoc test, to compare copper-based and fouling-release coatings. All statistical analyses were

Table 2

Field exposure sites. SE: Sweden, FR: France.

Site	Location	Coordinates	Salinity (PSU)
Askö	Baltic Sea (SE)	58.8233°N, 17.6355°E	6
Gothenburg	Kattegat (SE)	57.6926°N, 11.8328°E	17–24
Kristineberg	Skagerrak (SE)	58.2498°N, 11.4442°E	20–28
Tjärnö	Skagerrak (SE)	58.8818°N, 11.1340°E	24–28
Banyuls-sur-Mer	Mediterranean (FR)	42.4812°N, 3.1359°E	38

performed in RStudio with a significance level of 5% ( $\alpha = 0.05$ ).

### 2.3. Assessment of environmental impact of antifouling coating

#### 2.3.1. Preparation of leachates

Leachate water was generated for each coating to assess their environmental effects under controlled conditions. Leaching was carried out in natural seawater (NSW), selected for its ecological relevance due to the presence of dissolved organic matter, which influences biocide speciation and release behavior (Karlsson et al., 2010). NSW was collected at the Kristineberg Marine Research Station (Sweden) from depth via pumping system then filtered through 0.2  $\mu\text{m}$  filters and sterilized in an autoclave. The same batch of sterilized NSW, adjusted to a salinity of 25 PSU, was used for all leaching and ecotoxicological experiments to ensure consistency across assays.

Leaching followed the procedure described by Karlsson and Eklund (2004) (Karlsson et al., 2006; Karlsson and Eklund, 2004). Paints were applied with a fine roller onto the outer lower surface of sterile plastic Petri dishes, covering 10  $\text{cm}^2$  squares using the same painting protocol as for the field experiment. The paints were allowed to dry for 48 h, after which the coated dishes were immersed in 1 L of NSW for 1 h to remove any loose paint flakes. They were then transferred to autoclaved glass beakers containing 1 L of NSW at 25 PSU, covered with aluminum foil to prevent evaporation and photosynthetic growth. To simulate gentle water movement, beakers were placed on a shaking table (at 30 rpm) for 14 days at room temperature ( $22 \pm 2$  °C). As a control, an unpainted Petri dish was leached under identical conditions to verify the absence of compounds emitted by the plastic and to confirm that Aged NSW (natural seawater after 14 days of agitation) showed no intrinsic toxicity. This control was used as a reference in all ecotoxicological assays. Leachate samples were stored at 4 °C until testing.

#### 2.3.2. Algal growth inhibition assay (*Ceramium tenuicorne*)

The growth-inhibition assay on the red alga *C. tenuicorne* was carried out in accordance with Eklund (2005) and ISO 10710:2008 (Eklund, 2005; Karlsson and Eklund, 2004; ISO, 2008). The algal strain was obtained from the Algal Bank (GUMACC) at the University of Gothenburg

<https://www.gu.se/en/marina-vetenskaper/about-us/algal-bank-guma> cc. Prior to testing, *C. tenuicorne* was mass-cultured for a month under conditions described by Eklund (2005) to ensure sufficient biomass for the bioassay (Eklund, 2005).

During the experiment, uniform apical pieces of algae (1–2 mm) were exposed to the leachate for one week. Algae length was measured at the beginning and end of the test. Growth rates in the various test dilutions were calculated and compared with those of a control (0% leachate). EC<sub>50</sub>, EC<sub>20</sub> and EC<sub>10</sub> values, defined as the concentrations inducing 10%, 20%, and 50% effects, respectively, were calculated when a significant dose–response was observed. In addition, the NOEC (No Observed Effect Concentration), defined as the highest tested concentration showing no significant effect, and the LOEC (Lowest Observed Effect Concentration), defined as the lowest concentration inducing a significant effect compared to the control, were determined. The leachates tested were diluted with autoclaved NSW at 25 PSU supplemented with nitrogen (3.46 mg·L<sup>-1</sup>), phosphorus (0.78 mg·L<sup>-1</sup>) and iron (0.10 mg·L<sup>-1</sup>). For each leachate, 8 different leachate concentrations (0; 0.1; 0.33; 1; 3.33; 10; 33; and 100%) were tested. The test was carried out in sterile Petri dishes, and four replicates were used per treatment with two pieces of algae in each; six replicates were used for the 0% leachate concentration (control). All plates were maintained at 22 ± 2 °C, under a light intensity of 70 ± 10% μmol·m<sup>-2</sup>·s<sup>-1</sup> and with a daily rhythm of 14 h light and 10 h dark. pH was measured at the start and end of the test.

### 2.3.3. Acute toxicity assay with *Aliivibrio fischeri*

The toxicity of leachates was assessed using the bioluminescent bacteria *Aliivibrio fischeri*. Tests were performed on a series of dilutions: 50%; 33%; 25%; 10%; 3.33%; 1%; 0.33%; 0.1%; and 0%. Concentrations above 50% were not tested, as *A. fischeri* requires at least 50% of culture medium to grow. Leachate dilutions were carried out in Aged NSW (control) to avoid changing the culture conditions of *A. fischeri* between wells (salinity, carbon concentration, pH, etc.).

From cryostocks (−80 °C), *A. fischeri* was streaked on Marine Agar and incubated at 25 °C for 24 h. An isolated colony was inoculated into 5 mL Marine Broth and incubated horizontally at 25 °C, 150 rpm, for at least 5 h. When luminescence exceeded 1 × 10<sup>6</sup> RLU (measured on a spectrophotometer Victor NIVO, PerkinElmer®), the culture was used for assays. In transparent 96-well plates, 100 μL bacterial suspension was mixed with 100 μL of each test solution. Controls included NaCl (negative), Marine Broth (growth), Marine Broth (MB) only (contamination), and Aged NSW (leaching/seawater control). Triplicates were separated by empty wells to avoid optical cross-contamination. OD<sub>600nm</sub> and bioluminescence were recorded with a spectrophotometer every 15 min for 30 min.

### 2.3.4. Assessment of cellular stress using microbial biosensors

*E. coli* strains DPD2794, DPD2511, TV1061, and DPD2544 containing the fusion *recA::lux*, *katG::lux*, *grpE::lux* and *fabA::lux* respectively were used to record DNA (*recA*), oxidative (*katG*), protein (*grpE*) and membrane damage (*fabA*) (Bechor et al., 2002; Belkin et al., 1996; Van Dyk et al., 1995; Vollmer et al., 1997). Cultures from −80 °C stocks were streaked on Luria-Bertani (LB) agar + ampicillin (100 μg·mL<sup>-1</sup>) and incubated 24 h at 37 °C. A single colony was inoculated into LB + ampicillin and cultured overnight at 37 °C (150 rpm), then diluted into fresh medium and grown to exponential phase (OD<sub>600nm</sub> ≈ 0.5) to constitute the inoculum. For the assay, 100 μL of leachate or control was dispensed into white 96-well plates, followed by 100 μL of biosensor culture (OD<sub>600nm</sub> = 0.5). Controls included LB and Aged NSW (negative), LB only (contamination), and positive controls: phenol (1 g·L<sup>-1</sup>, *fabA*), EtOH (4%, *grpE*), H<sub>2</sub>O<sub>2</sub> (10 mg·L<sup>-1</sup>, *katG*), and nalidixic acid (10 mg·L<sup>-1</sup>, *recA*). All tests were run in triplicate, with empty wells interleaved to avoid optical interference. Luminescence and OD<sub>600nm</sub> were recorded on a Victor NIVO plate reader (PerkinElmer®) at 22 °C for 5 h in kinetic mode.

### 2.3.5. Impact on biofilm formation by environmental bacterial strains

To assess the impact of leachates on biofilm formation, four reference strains from the Microbial Biodiversity and Biotechnology Laboratory (LBBM, Oceanological Observatory of Banyuls-sur-Mer) were used: *Vibrio* sp. F5–F11, *Alteromonas* sp. BBCC2935, *Ruegeria* sp. BBCC1144 and *Labrenzia* sp. BBCC2184. Strains were cultured for 48 h in 48-well plates (500 μL per well) at 25 °C in MB supplemented with 500 μL of leachate (final volume 1 mL per well). Growth was monitored by OD<sub>630nm</sub> on a Victor 3 spectrofluorometer (PerkinElmer®). Plates were rinsed with phosphate-buffered saline (PBS), dried, stained with crystal violet (0.2% in 20% EtOH) for 15 min, rinsed three times, then decolorized with 33% glacial acetic acid to quantify biofilm by OD<sub>540nm</sub> (Adouane et al., 2024). Controls included 100% MB (growth) and Aged NSW (leachate reference).

### 2.3.6. Inhibition of quorum sensing mechanisms

To detect acyl-homoserine lactones (AHLs) and type 2 autoinducer (AI-2) inhibition by leachates, a set of biosensors was employed, considering the diversity of quorum sensing (QS) compounds. The experimental protocols used in this study were routine protocols previously published by the LBBM (Adouane et al., 2024; Blanchet et al., 2017; Romani et al., 2021; Tourneroché et al., 2019). *Pseudomonas putida* F117 (pRK–C12; Kmr; ppul::npt) was used to detect long-chain acyl homoserine lactone (Andersen et al., 2001), *Escherichia coli* MT102 (pJBA132) for medium-chain AHLs (Riedel et al., 2001), *Chromobacterium violaceum* CV026 for short-chain AHLs (McClellan et al., 1997) and *Vibrio harveyi* MM32 (luxN::Cm, luxS::Tn5Kan) for AI-2 detection (Bassler, 1999).

For AHLs assays, biosensors were supplemented with 3 μM AHLs (C6-HSL for CV026/MT102; 3-oxo-C10-HSL for F117/MT102) to allow inhibition detection. Negative controls included medium alone, biosensor + medium, and biosensor + AHLs, while positive controls consisted of biosensor + AHLs supplemented with the supernatant of the AHL-inhibiting strain BBCC116 (Doberva et al., 2017). For AI-2 assays, *V. harveyi* MM32 was tested with 1 μM 4,5-dihydroxy-2,3-pentanedione (DPD, AI-2 precursor), with controls being the biosensor with and without DPD, and the positive control being the fungal extract *Microsphaeropsis olivacea* AN329T (Tourneroché et al., 2019). In a 96-well microplate, 50 μL of each leachate or Aged NSW control was added to 150 μL of biosensors in its culture medium for 24 h. After incubation, growth (OD<sub>630nm</sub>) and QS phenotypes (fluorescence or bioluminescence) were then measured on a Victor 3 plate reader (PerkinElmer®).

### 2.3.7. Statistical analysis

To compare the effects of leachates across assays, raw optical density (OD) and luminescence/fluorescence signals were first corrected against medium-only blanks: ΔLUM = LUM<sub>test</sub> – LUM<sub>medium</sub> and ΔOD = OD<sub>test</sub> – OD<sub>medium</sub>. Normalized responses were then calculated as percentages of inhibition or induction using the general formula: %Effect =  $\frac{\text{Signal}_{\text{test}} - \text{Signal}_{\text{neg}}}{\text{Signal}_{\text{neg}}} \times 100$ , where Signal corresponds to an assay-specific ratio (e.g., ΔLUM/ΔOD<sub>600</sub>; ΔOD<sub>540</sub>/ΔOD<sub>630</sub>), Signal<sub>test</sub> represents the leachate condition, and Signal<sub>neg</sub> the negative control (aged NSW).

For *Aliivibrio fischeri*, acute toxicity was assessed using Signal = ΔLUM/ΔOD<sub>600</sub>, and induction of bioluminescence was expressed as % Inhibition =  $\frac{\text{Signal}_{\text{neg}} - \text{Signal}_{\text{test}}}{\text{Signal}_{\text{neg}}} \times 100$ . In quorum sensing assay, Signal = ΔLUM/ΔOD<sub>630</sub> and GFP (MT102, F117) or bioluminescence (MM32, AI-2) inhibition was calculated relative to AHL- or DPD-supplemented NSW controls using the same formula, while inhibition in CV026 was qualitatively assessed based on the absence of purple pigmentation (“+” for inhibition, “–” for no effect). In biofilm assays, Signal = ΔOD<sub>540</sub>/ΔOD<sub>630</sub>, and biofilm induction was expressed relative to NSW using the general formula. For *E. coli* stress biosensors, at time t\* corresponding to the maximal effect of the positive control, signals were scaled between negative (NSW) and positive controls (phenol, EtOH, H<sub>2</sub>O<sub>2</sub>, nalidixic

acid) according to the expression  $\%Induction = \frac{\Delta LUM_{test} - \Delta LUM_{neg}}{\Delta LUM_{pos} - \Delta LUM_{neg}} \times 100$ , where luminescence reflects stress-induced activation.

All statistical analyses (including photos analysis) were performed in R (R 4.4.0 via RStudio, 2024-04-24). Data wrangling and visualization used dplyr (1.1.4), ggplot2 (3.5.2), ggpubr (0.6.1), patchwork (1.3.1), and tidyverse (2.0.0); statistical utilities used rstatix (0.7.2); and data export used openxlsx (4.2.8) (Kassambara, 2025, Kassambara, 2023; Pedersen, 2025; Schauburger et al., 2025; Wickham, 2016; Wickham et al., 2019, 2023). For *Ceramium tenuicorne* growth inhibition assay, specific growth rates were calculated from initial and final algal length measurements, and concentration–response analyses were used to estimate EC<sub>10</sub>, EC<sub>20</sub>, and EC<sub>50</sub> values in R. NOEC and LOEC were determined using non-parametric comparisons between each exposure concentration and the control (0% leachate). For all biotests, treatment effects were assessed using non-parametric statistical tests including Mann–Whitney U and Kruskal–Wallis tests, followed by Dunn's post-hoc (package rstatix). Figures were generated using ggplot2 and assembled with patchwork.

### 3. Results and discussion

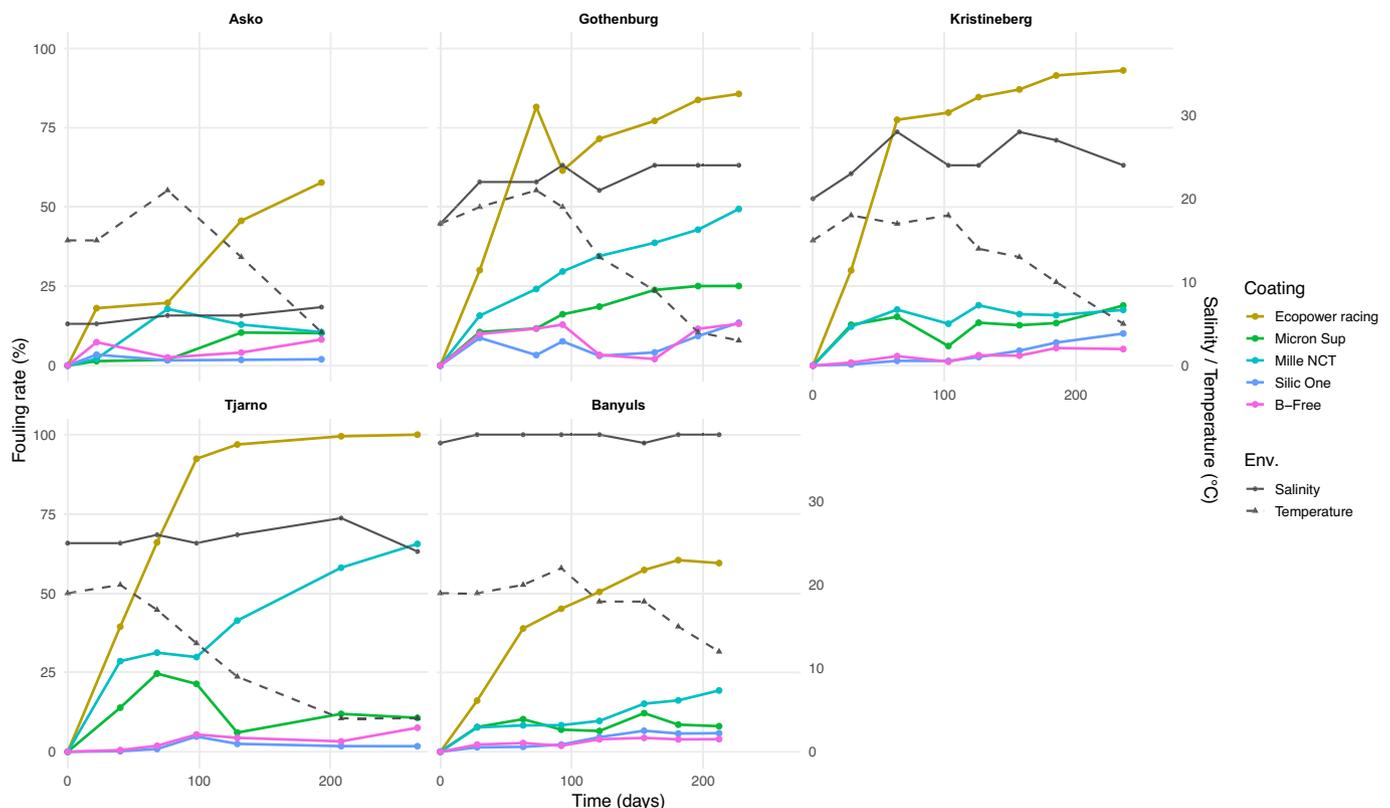
#### 3.1. Field evaluation of antifouling coating performance

Panels exposed under contrasting environmental conditions exhibited significantly different biofouling development among sites and coating types (Fig. 2 and Fig. A.1, Kruskal–Wallis,  $p < 0.001$ ). At most locations, fouling rates increased rapidly during the first one to three months of exposure—corresponding to the summer period of high biological activity—before stabilizing or increasing more slowly thereafter as temperatures declined. This pattern likely reflects a balance between settlement and interspecific competition on submerged surfaces, consistent with previous observations on seasonal biofouling dynamics

(Apolinario and Coutinho, 2009; Brown et al., 2018; Lagerström et al., 2020b; Lejars et al., 2012; Uzun et al., 2019; Wrangle et al., 2020; Yebra et al., 2004). Regular monitoring of biofouling development is therefore essential, as its intensity and composition can vary markedly from year to year depending on local environmental conditions such as temperature, salinity, and nutrient availability (Bai and Leow, 2002; Briand et al., 2017; Chiu et al., 2008).

The inert coating Ecopower Racing, used as a reference to evaluate relative fouling pressure, clearly illustrated these ecological gradients. In the absence of biocidal activity, it supported dense and taxonomically diverse fouling communities whose composition varied strongly between sites (statistics in Table A.2 and Fig. A.1). At Askö (5–7 PSU), panels were colonized by thick brown biofilm, filamentous algae, scattered barnacles, and encrusting bryozoans (FR = 57.8). At Gothenburg (17–24 PSU), fouling consisted mainly of stacked barnacles, green algae, and occasional mussels (FR = 85.6), whereas Kristineberg (20–28 PSU) hosted a more complex assemblage including barnacles, abundant orange encrusting bryozoans, red macroalgae, and calcareous tubeworms overgrowing the surface (FR = 93.1). At Tjärnö (24–28 PSU), fouling pressure was highest (FR = 100), with large blue mussels, oysters, barnacles attached to both the panel and the mussels, ascidians, calcareous tubeworms, and bryozoans. At the high-salinity Mediterranean site of Banyuls (37–38 PSU), panels accumulated very thick biofilm, green and red algae, spirorbid worms, and small calcareous sponges (FR = 60.4). Together with fouling rate values, this qualitative assemblage confirms that intermediate-salinity, nutrient-rich temperate sites (Skagerrak–Kattegat) support the most intense and structurally complex fouling, while low-salinity (Askö) and high-salinity (Banyuls) sites showed comparatively reduced but compositionally distinct fouling communities (Kim et al., 2025; Lagerström et al., 2020b; Wrangle et al., 2020; Wrangle et al., 2014).

More broadly, biocidal antifouling coatings encompass a wide range



**Fig. 2.** Temporal dynamics of fouling rates and environmental parameters across five European exposure sites. Fouling rate (%) of antifouling coatings was monitored over approximately 7 months at Tjärnö, Kristineberg, Gothenburg, Askö, and Banyuls. Temperature and salinity are shown on the secondary y-axis. Differences in fouling rates among coatings were assessed using Kruskal–Wallis tests, followed by Dunn's post hoc comparisons (Table A.2).

of formulations, and their antifouling performance is strongly influenced by biocide content, binder chemistry, and environmental conditions (Finnie and Williams, 2009; Lagerström et al., 2018; Paz-Villarraga et al., 2022; Yebra et al., 2004). Within this framework, Micron Superior generally outperformed Mille NCT, particularly at Gothenburg, Tjärnö, and Banyuls (FR +24, +54, +11;  $p < 0.001$ ). These differences are likely linked to its copper content being about double that of Mille NCT (31.93 vs 17.1%) but also differences in the binder system: the rosin-based self-polishing matrix of Micron Superior promotes more regular erosion and sustained  $\text{Cu}^+$  release, whereas the acrylic–rosin hybrid of Mille NCT may erode more slowly and may harden under low-flow conditions (Table A.1) (Kiil et al., 2003, Kiil et al., 2002; Yebra et al., 2005; Yebra and Weinell, 2009). The difference in zinc oxide composition may also contribute, although only approximate percentages are available (Micron superior 10–15%, Mille NCT 5–10%). However, both copper-based paints were consistently colonized by a thick microbial biofilm (bacteria and fungi), regardless of site (Fig. A.1), suggesting the persistence of copper-tolerant communities—a well-documented limitation of Cu antifouling coatings (Barranguet et al., 2000; Cassé and Swain, 2006; Chen et al., 2013; Dobretsov et al., 2021; Finnie and Williams, 2009; Zargiel et al., 2011). Differences between the two formulations were mainly associated with the presence of algae and larger macrofoulers: at medium-salinity sites (Gothenburg, Tjärnö), the lower-copper paint (Mille NCT) supported the development of filamentous green algae, whereas Micron Superior maintained algal growth at minimal levels. At the high-salinity Mediterranean site (Banyuls), part of the fouling—on both copper-based and FRC coatings—was also removed by grazing fish, contributing locally to reduced macrofouling compared with temperate North Sea sites, whereas the inert coating did not show evidence of grazing. In contrast, at Askö (6 PSU) and Kristineberg (20–28 PSU), no algal colonization occurred on either copper-based paint, which explains the similar fouling rates observed at these sites ( $p = 0.999$  and  $p = 0.861$ ). This highlights that environmental conditions—particularly salinity, temperature, and the structure of the local fouling community—strongly modulate both the antifouling performance of Cu/Zn coatings and the composition of organisms developing on their surfaces.

In contrast to biocidal systems, both FRC coatings, Silic One and B-Free Explore, exhibited excellent and consistent antifouling performance across all sites. Fouling rarely exceeded 10%, and performance differences between the two formulations were not significant ( $p \approx 1.00$  at Gothenburg;  $p = 0.447$  at Tjärnö;  $p = 0.954$  at Banyuls). At the organism level, FRC panels supported only light, non-attached fouling, consisting mainly of thin biofilms, occasional filamentous green algae, or external colonizers such as loose bryozoans or barnacles. B-Free Explore performed slightly better at Kristineberg (FR -4.9 compared to Silic One,  $p = 0.023$ ), whereas Silic One showed higher efficiency at Askö (FR -6.25,  $p = 0.0017$ ). Their low surface energy minimizes organism adhesion and facilitates detachment, making them more efficient than copper-based coatings even under static conditions (Kim et al., 2025; Lagerström et al., 2022). The thin and transient biofilms occasionally observed on some panels likely reflect a dynamic colonization–detachment process over time, rather than true fouling establishment, possibly influenced by weak hydrodynamic forces or intermittent water movement. As these coatings are specifically designed for dynamic conditions, an even higher antifouling efficiency can be expected under operational circumstances where water flow enhances self-cleaning and detachment processes (Hu et al., 2020; Lejars et al., 2012). Extending field evaluations over longer timescales and under controlled hydrodynamic conditions would be valuable to better assess long-term antifouling performance and benchmark fouling-release coatings against manufacturer-reported service lifetimes. While the present study focused on biocide-free silicone-based FRCs, other non-biocidal technologies, including fluorinated or hybrid formulations, may exhibit different performance and environmental interactions and warrant comparative investigation in future studies.

Overall, the integrated analysis of field data reveals a clear

performance gradient among the coatings: Silic One  $\lesssim$  B-Free Explore  $<$  Micron Superior  $<$  Mille NCT  $\ll$  Ecopower Racing. This ranking reflects both the intrinsic physicochemical properties of the coatings and the environmental variability among exposure sites. The consistently high performance of biocide-free FRCs, even under static exposure, underscores their strong potential as effective alternatives to conventional biocidal paints, while avoiding the release of copper and zinc that contribute to marine environmental contamination.

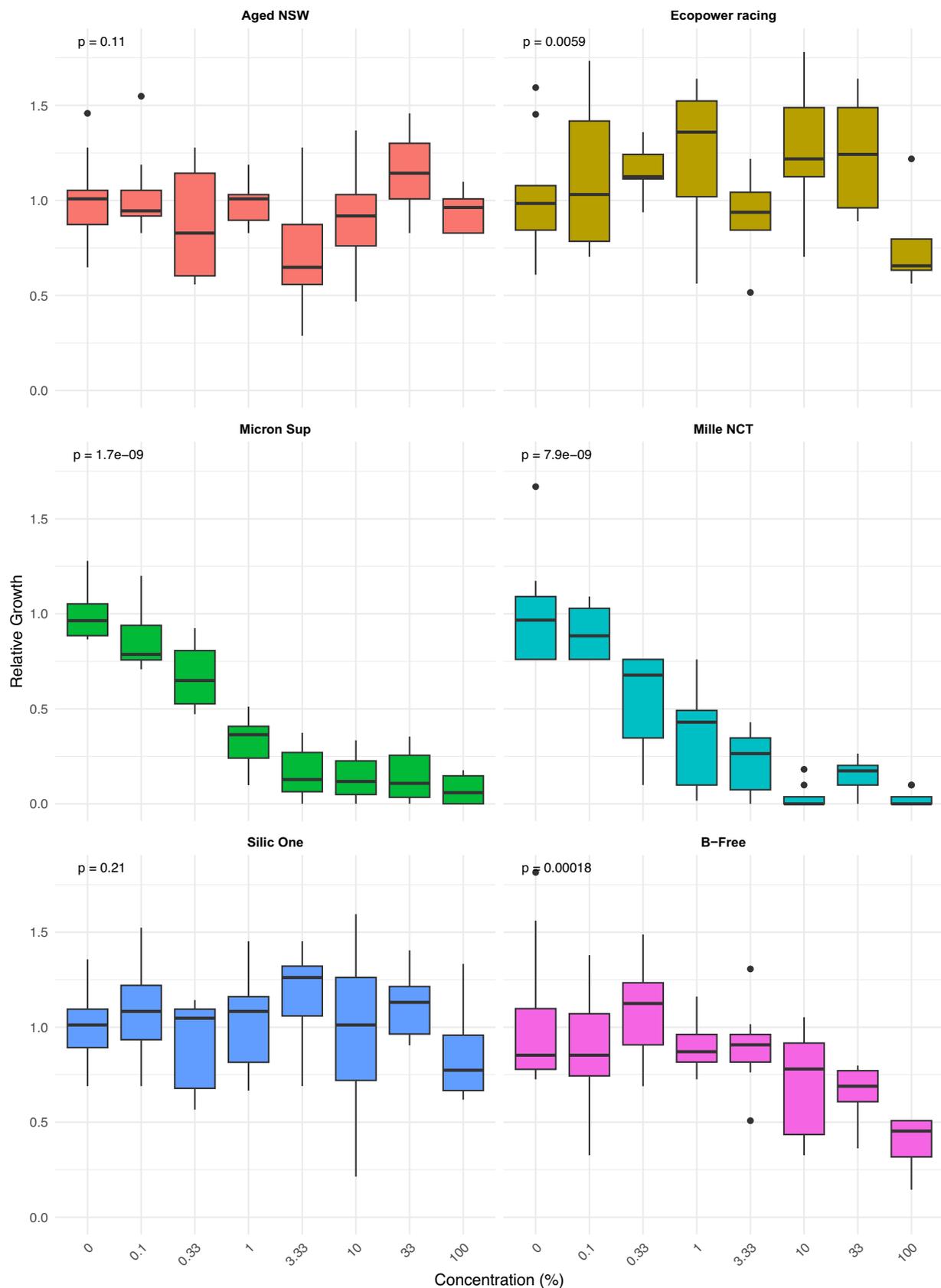
### 3.2. Environmental impact of antifouling coating leachates

#### 3.2.1. Growth inhibition of *Ceramium tenuicorne*

To evaluate the environmental impact of antifouling coating leachates, we first conducted a growth inhibition assay with the red macroalga *C. tenuicorne*, a well-established marine bioindicator of toxicity. This test integrates algal dose-responses to dissolved contaminants, reflecting both physiological stress and growth inhibition (Eklund, 2005; Karlsson et al., 2010; Karlsson and Eklund, 2004).

The dose–response patterns (Fig. 3) differed markedly among coatings. The negative control (Aged NSW) showed no significant dose–response trend (ns) across the tested concentration range. Accordingly, no inhibitory effect was detected, and EC values were not estimated. Consistently, the NOEC was 100% and no LOEC could be identified (Table 3). The inert Ecopower Racing coating showed a moderate response ( $p = 0.0059$ ), with  $\text{EC}_{50} = 53.3\% \pm 10.0$  (95% CI: 33.7–73.0), consistent with low overall toxicity. Since the leachates were aerated before testing, volatile solvents can be ruled out. The observed effect likely originates from trace additives such as 2,5-di-tert-butylhydroquinone (TBHQ) and long-chain aliphatic diamines (Table A.1), both classified as Aquatic Acute 1 with algal  $\text{EC}_{50}$  values below  $0.05 \text{ mg}\cdot\text{L}^{-1}$ . TBHQ may induce oxidative stress by disrupting photosynthetic redox balance, while cationic diamines can damage algal membranes (Badmus et al., 2021; Imhoff and Hansen, 2010; Qv and Jiang, 2013; Zhao et al., 2020). Nevertheless, no significant effect was detected below the highest tested concentration (NOEC = 100%, LOEC not detected), confirming the low ecological risk associated with this coating.

In contrast, both copper-based coatings induced strong algal growth inhibition even at low leachate concentrations. Micron Superior and Mille NCT exhibited nearly identical  $\text{EC}_{50}$  values ( $0.46\% \pm 0.12$ , 95% CI: 0.23–0.69; and  $0.46\% \pm 0.15$ , 95% CI: 0.17–0.75, respectively; Table 3), revealing comparable toxicity despite substantial differences in binder formulation (Table A.1). For both coatings, the NOEC was 0.1%, while growth inhibition became significant at 0.33%, defining a shared LOEC and revealing a narrow transition between no-effect and adverse-effect concentrations. The similarity in  $\text{EC}_{50}$ , LOEC, and NOEC values, despite different copper contents (32% for Micron Superior and 17% for Mille NCT), may reflect differences in zinc content or binder chemistry influencing the release kinetics of metal ions (Kiil et al., 2003, Kiil et al., 2002; Yebra et al., 2005; Yebra and Weinell, 2009). At lower thresholds, Micron Superior showed  $\text{EC}_{10} = 0.092\% \pm 0.051$  and  $\text{EC}_{20} = 0.166\% \pm 0.065$ , while Mille NCT had  $\text{EC}_{10} = 0.057\% \pm 0.034$  and  $\text{EC}_{20} = 0.124\% \pm 0.049$ . This strong sensitivity of *C. tenuicorne* to metal-rich leachates was confirmed by mortality data: Micron Superior caused 100% mortality between 10 and 100% leachate ( $\approx 50\%$  at 3.33%), and Mille NCT reached 100% mortality above 10% ( $\approx 90\%$  at 3.33%;  $\approx 62\%$  at 1%) (Fig. A.2). Similar toxicity of commercial copper- and zinc-based antifouling has been widely reported in both artificial seawater (ASW) and NSW (Karlsson et al., 2010; Karlsson and Eklund, 2004; Ytreberg et al., 2010). The toxicity of copper is well documented for many marine organisms (Fokina et al., 2013; Malhotra et al., 2020; Manzo et al., 2008; Wang and Zheng, 2008), but macroalgae, particularly red algae, are among the most sensitive groups (Babu et al., 2014; Eklund and Kautsky, 2003). Dissolved  $\text{Cu}^{2+}$  rapidly disrupts photosystem II, inhibits pigment synthesis, alters cell-wall integrity, and induces oxidative stress (Eklund and Kautsky, 2003; Gouveia et al., 2013; Pinto et al., 2003). Red



**Fig. 3.** Concentration-dependent effects of antifouling paint leachates on the growth of *Ceramium tenuicorne*. Relative growth of the red macroalga *Ceramium tenuicorne* exposed to serial dilutions (0, 0.1, 0.33, 1, 3.33, 10, 33, 100%) of leachates from five antifouling coatings (Ecopower Racing, Micron Superior, Mille NCT, Silic One, and B-Free). Aged natural seawater (NSW) from uncoated Petri dishes served as the negative control. Statistical significance was tested using Kruskal–Wallis tests ( $p$ -values shown above panels). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 3**

Inhibitory concentrations (EC<sub>50</sub>, EC<sub>20</sub>, EC<sub>10</sub>) of antifouling paint leachates on the growth of *Ceramium tenuicorne*. Values represent the mean concentration ( $\pm$  SE) of paint leachate required to inhibit algal growth by 50%, 20% or 10%. NOEC and LOEC correspond to the highest concentration without, and the lowest concentration with, a significant effect compared to the control (Aged NSW, 0% leachate), respectively. NA: no effect detected within the tested range; NS: not significant.

Coating	EC <sub>50</sub>	EC <sub>20</sub>	EC <sub>10</sub>	NOEC (%)	LOEC (%)	Dose-response interpretation
Aged NSW (no coating)	NS	NS	NS	100	NA	Not significant
Ecopower racing	53.34 $\pm$ 9.07	49.12 $\pm$ 8.10	46.81 $\pm$ 7.83	100	NA	Significant (not monotonic/low)
Micron Superior	0.46 $\pm$ 0.11	0.17 $\pm$ 0.06	0.09 $\pm$ 0.05	0.1	0.33	Significant
Mille NCT	0.46 $\pm$ 0.14	0.12 $\pm$ 0.04	0.05 $\pm$ 0.03	0.1	0.33	Significant
Silic One B-Free Explore	NS >100%	NS 13.66 $\pm$ 6.95	NS 3.55 $\pm$ 2.85	100 10	NA 33	Not significant Significant

macroalgae such as *Ceramium*, *Gracilaria*, *Porphyra*, and *Chondrus* often display EC<sub>50</sub> values in the low  $\mu\text{g}\cdot\text{L}^{-1}$  range (Eklund, 2005; Eklund and Kautsky, 2003; Gouveia et al., 2013; Moenne et al., 2016; Ytreberg et al., 2010; Zhu et al., 2017), matching the strong sensitivity observed here. The steep concentration-response curve obtained in this study therefore reflects the well-established vulnerability of red algae to Cu exposure and underscores the ecological risk posed by metal-rich antifouling leachates.

The silicone-based FRC coatings exhibited notably low toxicity toward *C. tenuicorne* within the tested range. B-Free Explore caused measurable inhibition only at unrealistically extrapolated high concentrations (EC<sub>50</sub> > 100%) while Silic One showed no detectable effect ( $p = 0.21$ ), with off-scale modeled EC values (EC<sub>10</sub>  $\approx 2.1 \times 10^5\%$ , EC<sub>20</sub>  $\approx 3.2 \times 10^5\%$ ), confirming the absence of measurable inhibition. At lower levels, B-Free Explore displayed a weak but quantifiable effect (EC<sub>20</sub> = 13.66  $\pm$  6.95%), indicating minimal toxicity. This slight response may reflect formulation differences between B-Free Explore and Silic One — notably the absence of silica filler in B-Free Explore (Table A.1). The lack of filler could slightly increase the diffusion of trace compounds such as silicone-derived fragments, the curing agent 2-pentanone O,O',O''-(ethenylsilylidyne)trioxime, or the cyclic siloxane octamethylcyclotetrasiloxane (D4). According to the Danish EPA (2024), non-biocidal coatings are generally safe, although some contain cyclic siloxanes such as octamethylcyclotetrasiloxane D4 (here 0.1–0.3%), classified as PBT (Persistent, Bioaccumulative, Toxic) or vPvB (very Persistent, very Bioaccumulative) substances (The Danish Environmental Protection Agency, 2024). These occur only as minor impurities and are expected to evaporate during curing, limiting their environmental persistence.

Overall, NOEC and LOEC values corroborate EC-based estimates and clearly discriminate copper-based coatings from inert and silicone-based formulations. At sublethal thresholds, FRC leachates are more than 100 times less toxic than copper-based paints (EC<sub>20</sub> 13.66% vs. 0.124–0.166%), and the difference exceeds 300 $\times$  when considering EC<sub>50</sub>, highlighting their substantially lower ecological risk. Notably, Silic One showed no detectable toxicity even at 100% leachate, indicating that the actual safety margin between FRCs and Cu/Zn coatings is even larger than these estimates suggest.

### 3.2.2. Cellular stress response (*E. coli* biosensors)

To assess potential sublethal cellular effects of paint leachates, we

exposed *Escherichia coli* biosensors targeting four stress pathways—DNA damage (*recA*) (Vollmer et al., 1997), membrane integrity (*fabA*) (Bechor et al., 2002), oxidative stress (*katG*) (Belkin et al., 1996), and protein damage (*grpE*) (Van Dyk et al., 1995)—to 50% leachates from the tested coatings (Fig. 4). In this bioassay, increased luminescence reflects stress induction and was expressed relative to the Aged NSW control. No significant induction of DNA damage (*recA*) or oxidative stress (*katG*) was detected, although a slight background effect compared with LB medium was noted ( $p = 0.0334$ ). Membrane integrity (*fabA*) was unaffected by most leachates, except for a small luminescence inhibition (5–16%) in response to the Silic One leachate ( $p = 0.0101$ ).

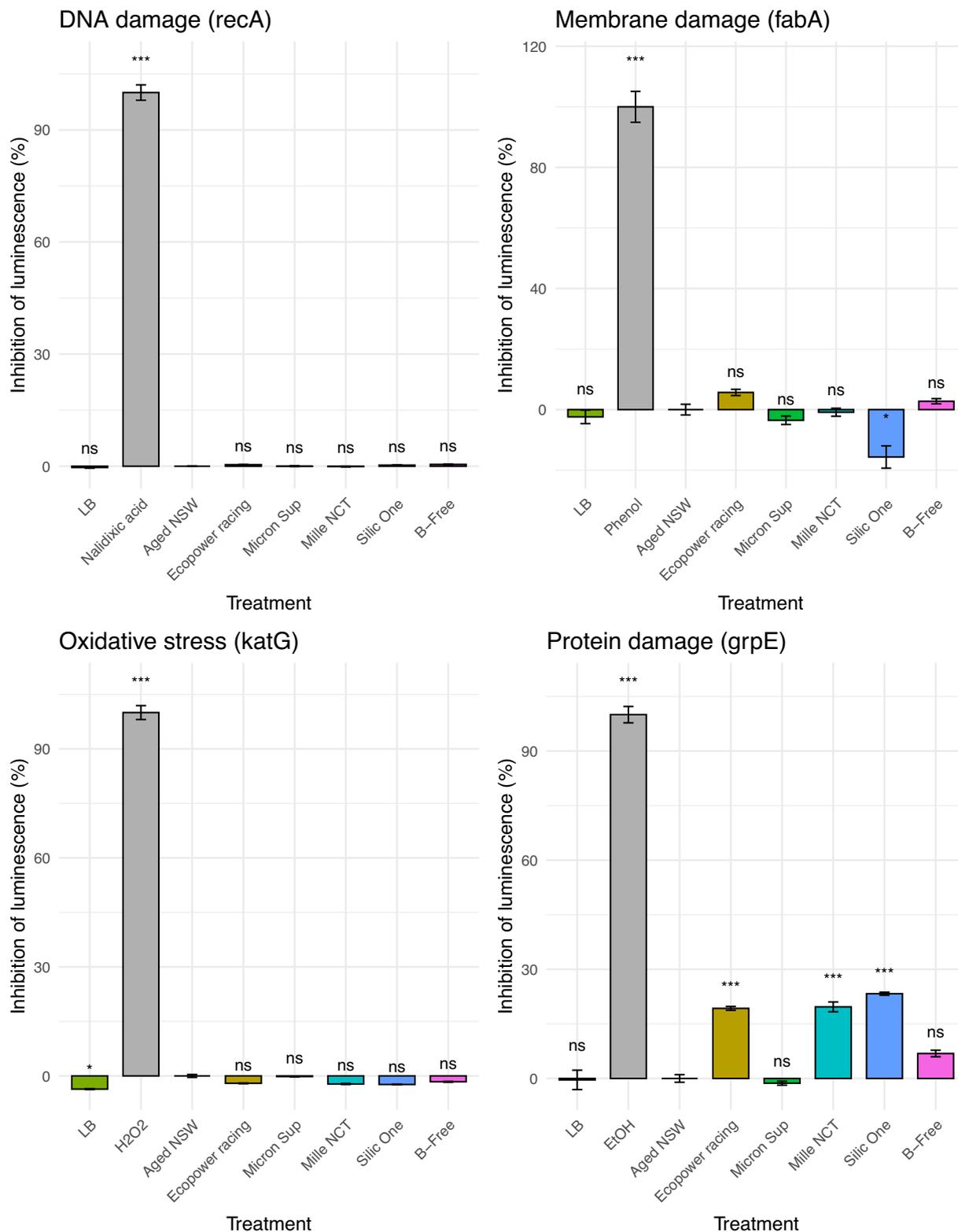
In contrast, significant toxic effects on proteins (*grpE*) were measured with the leachates from Ecopower Racing (19.2% luminescence induction,  $p < 0.0001$ ), Mille NCT (19.7%,  $p < 0.0001$ ), and Silic One (23.29%,  $p < 0.0001$ ), whereas coatings from another manufacturer did not elicit any *grpE* response. Although the antifouling topcoats from this manufacturer do not share an identical composition according to the SDSs (Table A.1), all *grpE*-inducing coatings were applied over the same light primer and curing system, thereby identifying this primer system as a common element across the formulations. This toxic effect on proteins could therefore originate from components associated with the primer/curing agent or from compounds that react with each other, producing toxic products. Based on SDS information (Table A.1), amine-based curing agents present in the primer system represent plausible contributors to the observed protein stress. Amines and polyamide-amine hardeners are known to be toxic (Finlay and Callow, 1996; Poste et al., 2014; Rudawska et al., 2022), but their toxicity specifically to proteins has not yet been demonstrated. The contributory role of aromatic solvents not completely evaporated in the primer formulations (e.g., xylene, ethylbenzene, toluene) cannot be excluded, particularly through synergistic effects at high exposure levels.

This highlights the need for chemical characterization of the leachates themselves with advanced analytical approaches such as pyrolysis-GC-MS/MS, LC-MS/MS non-target screening, or high-resolution mass spectrometry (HRMS). These methods may help identify trace compounds, polymer fragments, or microplastic-like debris potentially released from binders, primers, or silicone matrices (Bhardwaj et al., 2024; Bork et al., 2025; Özgenç, 2024). Given that protein homeostasis was consistently the most sensitive pathway, future work should incorporate proteomic analyses (e.g., differential expression, stress-response pathways, protein misfolding/chaperone activity) to elucidate the cellular processes affected by these leachates (Han et al., 2013; Kwon et al., 2025; Qian et al., 2010; Xu et al., 2024). Together, these results indicate that protein integrity is the primary cellular target disrupted by antifouling leachates, whereas other stress pathways remain largely unaffected under the conditions tested. These responses were measured under standardized high-exposure conditions designed to resolve mechanistic cellular stress pathways, with broader considerations of environmental relevance addressed in the biofilm response section.

### 3.2.3. *Aliivibrio fischeri* toxicity and quorum sensing modulation

A bioluminescence inhibition assay with *Aliivibrio fischeri* was used to evaluate the effects of antifouling paint leachates (Fig. 5). Leachates were tested from 0.01 to 50%, and the percentage of change in bioluminescence was calculated relative to the Aged NSW control, which remained statistically indistinguishable from the 2% NaCl condition (optimal growth). Positive values indicate an increase in luminescence (induction), whereas negative values indicate a decrease (inhibition).

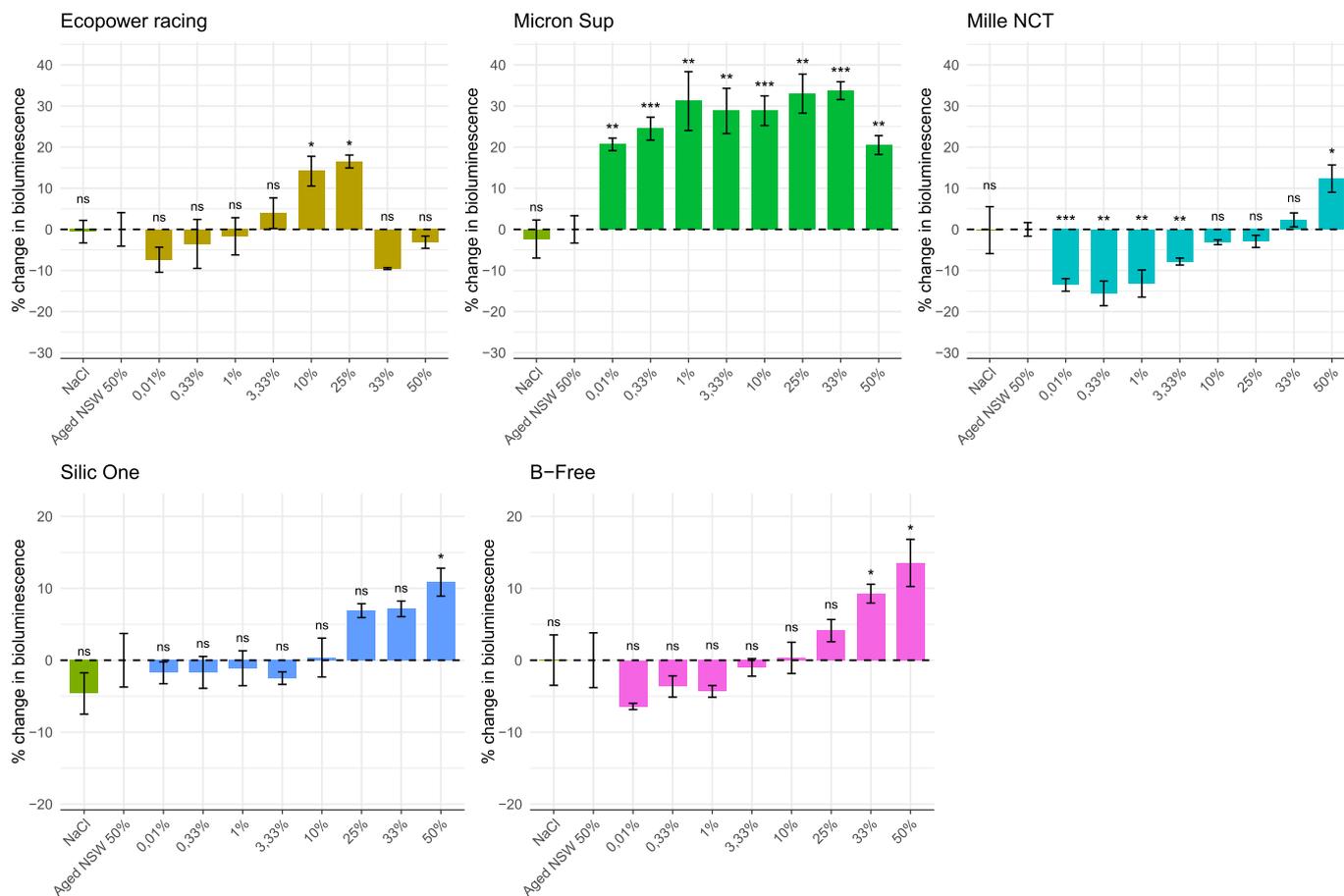
The inert coating Ecopower Racing showed no measurable toxicity across concentrations; however, a positive change in bioluminescence (~10–18%) was observed at 10–25% leachate, suggesting metabolic stimulation rather than inhibition. The two copper-based paints showed contrasting patterns: Micron Superior produced a consistent positive change in bioluminescence at all concentrations (~19–42%), whereas



**Fig. 4.** Microbial stress responses induced by antifouling paint leachates. Percentage inhibition of luminescence in four microbial stress biosensors—DNA damage (recA), membrane damage (fabA), oxidative stress (katG), and protein damage (grpE)—after exposure to 50% leachates from the five coatings. Nalidixic acid, phenol, H<sub>2</sub>O<sub>2</sub>, and ethanol served as positive controls for recA, fabA, katG, and grpE, respectively. Negative controls were LB medium and aged NSW leachate from uncoated Petri dishes. Statistical significance: ns (not significant), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

Mille NCT caused a negative change in bioluminescence (~8–14% at 0.01–3.33%), followed by a shift toward positive change at higher concentrations of leachates ( $\geq 25\%$ ). The silicone-based FRCs (Silic One, B-Free Explore) had no significant effect up to 33%, then yielded a mild

positive change in bioluminescence at 33–50% (~10–16%). Altogether, all leachates—biocidal, inert, and silicone-based—elicited a measurable response in *A. fischeri*, with bioluminescence responses predominantly characterized by increases rather than decreases, indicating no evidence



**Fig. 5.** Concentration-dependent effects of antifouling-paint leachates on *Aliivibrio fischeri* bioluminescence. Percentage change in bioluminescence (%) of *A. fischeri* after exposure to serial dilutions of coating leachates (0.01, 0.33, 1, 3.33, 10, 25, 33, 50%; diluted in aged natural seawater). Panels correspond to coatings (Ecopower Racing, Micron Sup, Mille NCT, Silic One, B-Free). NaCl (optimal growth control) and Aged NSW 50% (experimental control) are included. Statistical significance: ns (not significant), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

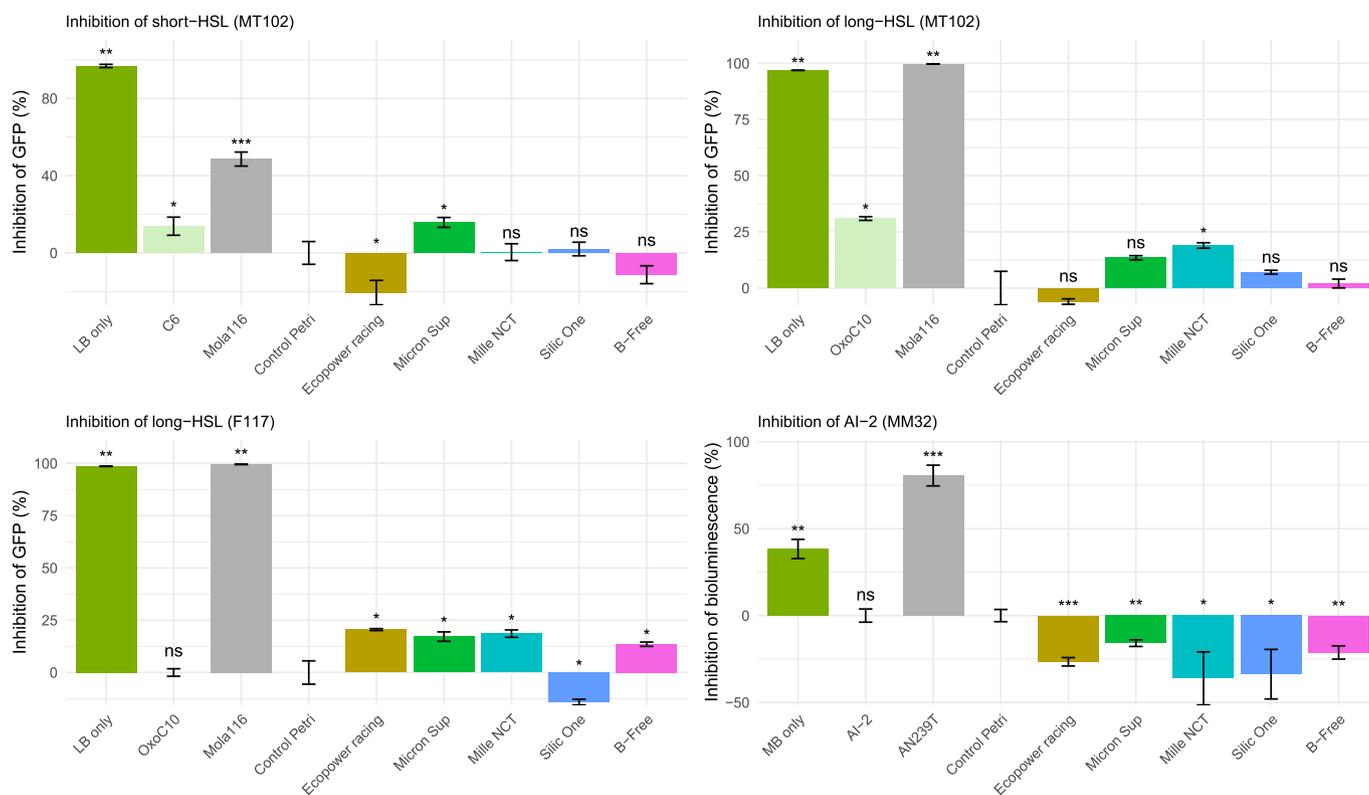
of acute toxicity under these conditions. Importantly, *A. fischeri* is one of the most widely used bacterial models in ecotoxicology, as the Microtox® assay provides a rapid, sensitive, and cost-effective measure of chemical toxicity (Abbas et al., 2018). However, its reliance on quorum-sensing (QS)-regulated bioluminescence represents a key limitation: substances that modulate QS—or alter membrane permeability—can increase or decrease light emission independently of true toxicity (Bulich et al., 1981; Lami et al., 2022; Schaefer et al., 1996). Therefore, the observed positive changes in bioluminescence are more consistent with QS signal modulation than with direct toxic stress, underscoring the need to complement this test with additional microbial assays when evaluating antifouling leachates.

To test this, QS inhibition/induction assays were run using a panel covering the main bacterial signaling systems (Fig. 6 and Table A.3): AI-2 via *Vibrio harveyi* MM32; short-chain AHLs (C6-HSL) via *Chromobacterium violaceum* CV026 and *Escherichia coli* MT102; medium-/long-chain AHLs (C10-HSL) via *E. coli* MT102 and *Pseudomonas putida* F117. At 25% leachate, Aged NSW had no effect. All coatings induced AI-2 (MM32) with ~16–50% induction, while AHL systems varied: Micron Superior and Mille NCT inhibited both short- and long-chain AHL signaling (MT102, F117); Ecopower Racing induced short-chain AHL biosensors (MT102, CV026); Silic One induced the long-chain AHL sensor (F117); and B-Free Explore inhibited F117 by ~13%.

These data support that copper-based paints tend to inhibit AHL-mediated quorum sensing (QS), consistent with previous reports showing that  $\text{Cu}^{2+}$  and ZnO can interfere with AHL signaling pathways (Alavi et al., 2023; Gagné, 2017; Khan et al., 2020; McGivney et al., 2018; Padaga et al., 2024). In contrast, the consistent induction of the

AI-2 system across all coatings, including FRCs, suggests a broader and more complex mechanism. For instance, Fitzgerald et al. (2018) demonstrated that PFAS compounds can enhance *A. fischeri* fluorescence by increasing membrane permeability to QS-related molecules, thereby amplifying QS compounds reception (Fitzgerald et al., 2018). Although no PFAS have been identified in the antifouling formulations tested here, some silicone-derived constituents—such as low-molecular-weight cyclic siloxanes—exhibit PFAS-like properties (high persistence, hydrophobicity) and could theoretically modulate QS in a comparable manner. In the present case, it remains difficult to identify the exact compounds responsible for this effect, as it was observed for all antifouling formulations. QS induction may therefore arise from silicone- or polymer-derived leachates, or from physicochemical interactions between NSW constituents and coating-derived molecules, which may promote AI-2-mediated interspecies communication (matrix effects).

Running parallel assays in ASW could help distinguish these effects; however, such tests deviate from environmentally realistic leaching conditions (Ytreberg et al., 2010). Notably, Ytreberg et al. (2010) reported that Cu release rates were 4–6 times lower in NSW than in ASW, likely due to higher dissolved organic carbon in NSW (~4.7 vs. 0.8  $\text{mg}\cdot\text{L}^{-1}$ ), rapid conditioning film/biofilm formation on coatings (Almeida et al., 2007; Yebrá et al., 2004), and biofilm-mediated reduction of Cu release (Valkirs et al., 2003). These matrix effects could similarly influence QS modulation and must be considered when comparing NSW and ASW results. Consequently, *Vibrio*-based luminescence assays should systematically be complemented by dedicated QS bioassays to distinguish genuine toxicity from signal-mediated luminescence modulation (Lami et al., 2022).



**Fig. 6.** Effects of antifouling paint leachates on bacterial quorum sensing. Inhibition of quorum sensing (QS) by antifouling paint leachates at 50% concentration, tested with biosensors responsive to type I autoinducers [short-chain HSL (MT102) and long-chain HSL (MT102, F117)] and type II autoinducers (AI-2, MM32). Positive control: BBCC116 (formerly MOLA116). Negative controls: absence of autoinducer (LB/MB only), presence of autoinducer (C6/OxoC10/AI-2), and aged natural seawater (Aged NSW). Results are expressed as percentage inhibition of GFP or bioluminescence (mean  $\pm$  SE). Statistical significance: ns (not significant), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

From an ecological perspective, disturbances in QS networks can have far-reaching consequences for the transition from initial microbial colonization to mature biofilms and, ultimately, to macroscopic fouling communities. Both AHL- and AI-2-mediated systems regulate adhesion, extracellular polymeric substance production, metabolic cooperation, and microbial community assembly on submerged surfaces, thereby structuring early successional stages that condition surfaces for subsequent colonization (Coolahan and Whalen, 2025; Dang and Lovell, 2015; Dobretsov et al., 2009; Lami et al., 2022; Urvoy et al., 2022). Importantly, modulation of QS by antifouling leachates should not be interpreted as a beneficial inhibition of microbial communication at the coating surface. Field observations clearly showed the presence of microbial biofilms on all coatings, indicating that QS-regulated processes remain active. The effects reported here therefore reflect the capacity of coating-derived compounds, once released into seawater, to interfere with bacterial signaling pathways under controlled exposure conditions rather than a suppression of QS at the surface itself.

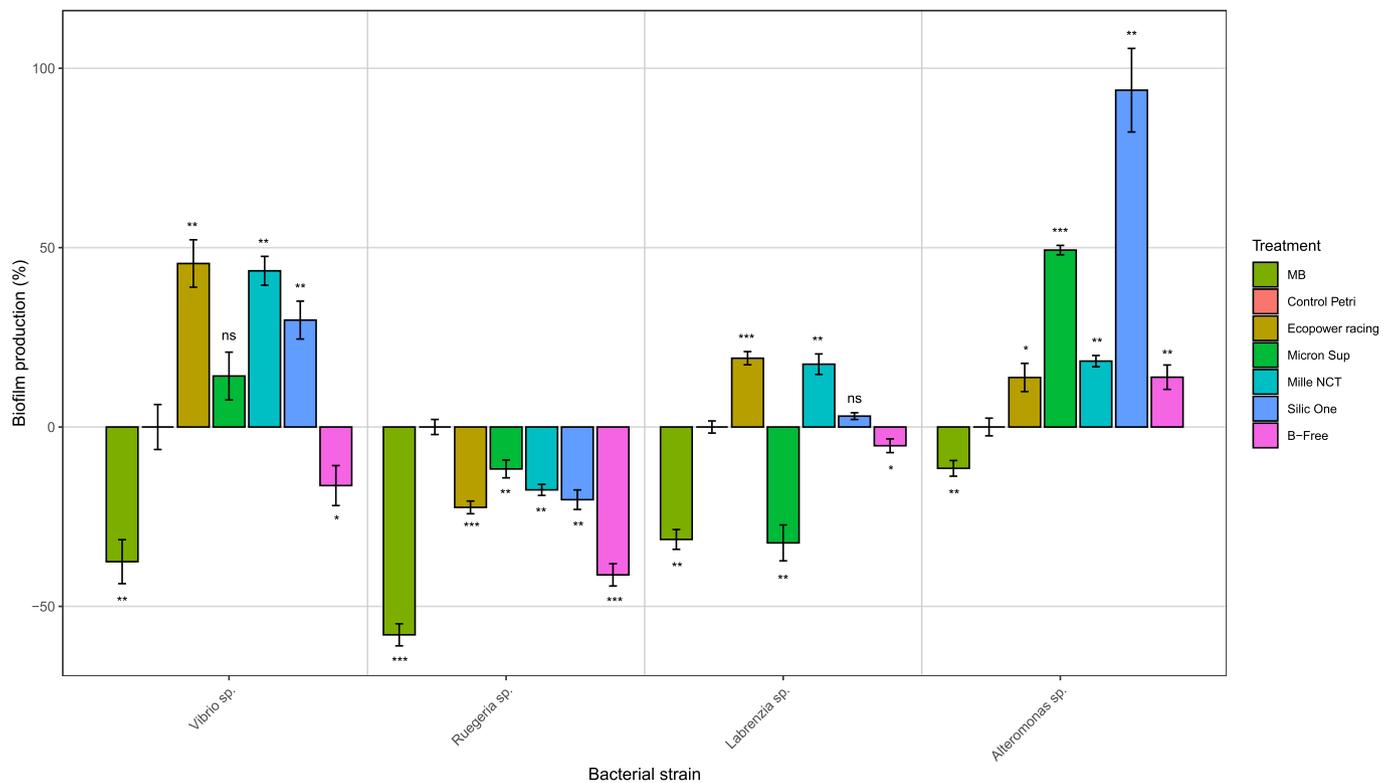
Such interference is more likely to result in desynchronization or alteration of microbial coordination than in inhibition of biofilm development. Even in the absence of acute toxicity, disturbance or deregulation of QS network can modify biofilm structure, succession dynamics, and stability, thereby influencing the chemical and biological cues that mediate larval settlement and macrofouling development in the marine environment (Dobretsov et al., 2009; Hadfield, 2011; Lami, 2019; Qian et al., 2022; Qian et al., 2007). These findings demonstrate that antifouling leachates, irrespective of their biocidal content, can interfere with bacterial communication systems, thereby influencing surface conditioning and microbial coordination. This hypothesis was further investigated by examining how the same leachates affected biofilm formation by representative marine bacterial isolates.

### 3.2.4. Effects on biofilm production by environmental bacterial strains

The influence of antifouling coating leachates on biofilm formation by marine environmental isolates was evaluated using a crystal-violet assay after 48 h exposure. Percent variations were calculated relative to the Aged NSW reference and statistical differences are reported in Fig. 7.

For *Vibrio* sp., biofilm production in the Aged NSW control was 37% higher than in Marine Broth (MB). It further increased in the presence of Ecopower Racing (+46%), Mille NCT (+44%), and Silic One (+30%) leachate. Micron Superior did not differ significantly from the control ( $p = 0.055$ ), while B-Free Explore reduced biofilm formation by -16%. For *Alteromonas* sp., biofilm production in Aged NSW was 12% higher than in MB, and all leachates stimulated additional formation (+14% to +94%), with Silic One producing the strongest effect. For *Ruegeria* sp., Aged NSW supported 58% more biofilm than MB, yet all leachates reduced production by 12–41% compared to the control. Finally, for *Labrenzia* sp., biofilm levels in Aged NSW exceeded MB by 31%; Ecopower Racing and Mille NCT increased biofilm formation (+18%), Micron Superior and B-Free Explore decreased it (-32% and -5%), and Silic One showed no significant difference ( $p = 0.069$ ). Overall, the responses were strongly species dependent. *Vibrio* sp. and *Alteromonas* sp. were stimulated by almost all leachates, *Ruegeria* sp. was consistently inhibited, and *Labrenzia* sp. displayed an intermediate pattern. Collectively, these contrasting behaviors reveal that antifouling leachates do not simply act as toxicants but as selective chemical cues shaping microbial strategies, highlighting a far more nuanced interaction between coating leachates and early biofilm formers than previously recognized.

Ecologically, these effects are significant as microbial biofilms are central to marine ecosystems, mediating nutrient cycling, organic matter retention, and larval settlement as the primary interface between substrates and seawater (Dang and Lovell, 2015). Alterations in biofilm



**Fig. 7.** Impact of antifouling paint leachates on biofilm formation by four environmental bacterial strains. Relative biofilm production (%) of *Vibrio sp.*, *Ruegeria sp.*, *Labrenzia sp.*, and *Alteromonas sp.* after exposure to leachates (50%) from antifouling coatings (Ecopower Racing, Micron Sup, Mille NCT, Silic One, and B-Free). Results are expressed relative to the negative control aged natural seawater (NSW, uncoated Petri dishes incubated for 14 days). An additional negative control was marine broth (MB). Data represent mean  $\pm$  SE. Statistical significance compared to aged NSW is indicated as: ns (not significant), \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

formation—whether stimulatory or inhibitory—can reshape microbial succession, disrupt biogeochemical processes, and modify macrofouling trajectories (Antunes et al., 2019; Dobretsov and Rittschof, 2023; Qian et al., 2022, Qian et al., 2007). The ability of all coatings, including non-biocidal ones, to modulate biofilm growth suggests that antifouling leachates influence microbial community structure and function even without overt toxicity. Since QS regulates many of the same processes, its disruption in *A. fischeri* likely contributes to these effects, desynchronizing microbial coordination and leading to less stable, functionally altered biofilms (Lami, 2019; Urvoy et al., 2022). Altogether, these findings indicate that antifouling leachates—even from environmentally “safe” coatings—act as subtle chemical cues reshaping microbial behavior, with potential cascading impacts on nutrient cycling, microbial competition, and the stability of fouling communities (Coolahan and Whalen, 2025; Urvoy et al., 2022).

Importantly, this study reveals for the first time that all coating types, including biocide-free formulations, can actively modulate microbial behaviors rather than simply inhibiting growth. This constitutes a major advance in understanding how antifouling leachates shape early-stage microbial processes. While our assays relied on cultured isolates, they provide clear mechanistic insight into species-specific responses and offer a robust foundation for extending these findings to complex communities. These species-dependent patterns are consistent with recent work showing that antifouling paint particles can restructure natural microbial assemblages (Tagg et al., 2024). In sediments, these particles suppress parts of the rare biosphere while favoring disturbance-tolerant or culturable taxa. Taken together, these observations indicate that antifouling-derived compounds act as subtle but persistent drivers of microbial community assembly. Nevertheless, these responses (cellular stress, quorum sensing modulation, and biofilm formation) were measured at a deliberately high leachate concentration (25–50%) to

reveal mechanistic effects. Environmental concentrations of antifouling leachates remain largely unknown and are expected to be substantially lower under open-water conditions. Further studies under environmentally realistic conditions—such as in ports, marinas, or in situ microcosms—will be essential to determine the extent to which these microbial shifts occur in natural settings, and to establish environmentally relevant exposure thresholds, representing an important area for future research. Exploring these effects at the community scale—via microcosm experiments, metabarcoding, metagenomics, or proteomics—would build on our results and help unravel how leachates influence microbial succession, functional pathways, and sediment or surface biogeochemical processes in situ.

### 3.3. Integrated interpretation

Together, the field and laboratory results provide an integrated understanding of antifouling performance and ecological impact. Field data revealed a clear performance hierarchy: silicone-based FRCs showed the most stable and efficient antifouling behavior under static conditions, followed by copper-based paints (Micron Superior, Mille NCT) and the inert reference (Ecopower Racing). These results confirm that FRCs can maintain strong antifouling efficiency even without biocides, due to their low surface energy and hydrophobicity, which limit adhesion and promote passive detachment (Lejars et al., 2012). Building on these findings, extending field deployments over longer periods and incorporating controlled hydrodynamic conditions or cleaning events would provide valuable additional insight into the long-term performance and operational behavior of antifouling coatings under realistic use scenarios.

Ecotoxicological assays, however, highlighted sharp contrasts between coating types. Copper- and zinc-based paints caused pronounced

toxicity at sub-percent concentrations, especially toward *C. tenuicorne*, consistent with their high metal content and biocide release (Karlsson et al., 2010; Ytreberg et al., 2010). In contrast, FRC leachates were approximately 100 times less toxic than Cu/Zn-based paints, confirming their classification as environmentally safer alternatives (Lagerström et al., 2022). Although they showed negligible acute toxicity, FRCs were not entirely inert: subtle cellular stress responses and impacts on microbial signaling were detected, indicating that “non-biocidal” does not necessarily mean “biologically neutral” (Feng et al., 2012; Piazza et al., 2018; Watermann et al., 2005).

At the microbial level, all coatings—biocidal and non-biocidal—modulated QS signaling and biofilm development. Copper-based paints tended to inhibit AHL-mediated communication, whereas silicone-based and inert coatings stimulated AI-2 signaling, suggesting that leachate–seawater interactions can alter microbial communication. Such QS disruptions may cascade through the biofilm community, affecting early colonization, succession, and ultimately macrofouling establishment. Since microbial biofilms regulate nutrient cycling, organic matter retention, and larval settlement, both inhibition and overstimulation can disturb key ecological processes (Coolahan and Whalen, 2025; Qian et al., 2022; Urvoy et al., 2022).

Methodologically, it is important to note that leachate preparation can strongly influence observed toxicity. Factors such as seawater type (ASW vs. NSW), agitation, immersion duration, coating geometry, and conditioning film formation can alter the release kinetics of metals, polymers, or additives (Piazza et al., 2018). As shown by Karlsson and Eklund (2004) and Ytreberg et al. (2010), leaching rates may vary by a factor of 4–6 depending on these parameters, underscoring the importance of standardized leaching protocols for interstudy comparability. Accordingly, the concentration ranges used here were selected for comparative and mechanistic purposes rather than direct environmental extrapolation. Furthermore, this study assessed acute responses, but chronic exposure often reveals delayed or cumulative effects—particularly for sublethal endpoints such as oxidative stress, reproduction, or developmental toxicity. FRCs, although far less toxic in the short term, should be systematically evaluated using chronic assays on multiple functional groups (microalgae, invertebrate larvae, echinoderms, crustaceans, and fish embryos) to provide a broader assessment of their long-term ecological safety. Such tests, coupled with chemical analysis, would be crucial to detect low-level effects of silicone fragments, curing by-products, or polymer degradation residues that may accumulate or interact with microbial communities over time.

From a sustainability perspective, these findings underscore the need to integrate performance and ecotoxicological criteria when evaluating antifouling strategies. Sustainable coatings should minimize metal leaching and maintain microbial community integrity while ensuring effective long-term protection. Overall, this study demonstrates that even biocide-free coatings can influence microbial signaling at the base of the fouling ecosystem. Nevertheless, silicone-based FRCs remain the most environmentally compatible option, combining high antifouling efficiency with minimal toxicity. Future developments should therefore focus on optimizing their formulation, assessing long-term and community-level effects, and validating microbial responses under realistic environmental conditions, particularly in marinas and ports where boating activity is highest.

#### CRediT authorship contribution statement

**Emilie Adouane:** Visualization, Validation, Software, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – review & editing, Writing – original draft. **Lena Granhag:** Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization, Writing – review & editing, Writing – original draft. **Camille Ferré:** Resources, Methodology, Investigation, Formal analysis, Writing – review & editing. **Raphaël Lami:** Validation, Resources, Methodology,

Investigation, Formal analysis, Writing – review & editing. **Carole Vecklerlé:** Methodology, Investigation. **Renaud Vuillemin:** Resources. **Erik Ytreberg:** Validation, Supervision, Resources, Project administration, Funding acquisition, Formal analysis, Conceptualization, Writing – review & editing, Writing – original draft.

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#### 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.marpolbul.2026.119303>.

#### Data availability

Data will be made available on request.

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