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Assessing the impact of UV-C exposure on pre-existing cultured marine diatom biofilms

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ABSTRACT

Ultraviolet-C (UV–C) has been considered as a potential tool in the maintenance and prevention of marine biofouling. However, it is unclear how irradiation disturbs biofouling, specifically biofilms, or the duration and frequency needed to reduce or eliminate their growth. The marine diatom, *Navicula incerta*, was used in a two-part lab study to determine the minimum time between UV-C duty cycling (also known as interval testing) that would result in the reduction and prevention of marine biofilms. The first study assessed the diatom's metabolic activity, morphology, behavior, and recovery to understand the influence of UV-C on the biofilm. Cell viability, biofilm matrixes, and chlorophyll *a* were significantly reduced immediately following UV-C exposure, yet when exposure was ceased biofilms began to recover. Based on these results, the second study investigated the duty cycle duration required to limit the growth of the cultured biofilm. Duty cycle testing was able to initially deplete cell viability and chlorophyll *a*; however, in between exposures, recovery was evident. These findings indicate that shorter (10 min; 1.86 J/cm^2) doses of UV-C exposures must be applied more frequently than more prolonged doses (60 min; 11.16 J/cm^2) to keep biofilm formation minimal. The duty cycles established here also provide a guideline of efficacy for future studies that can be applied to biofilms in both laboratory and the field.

1. Introduction

Marine biofilms, or microfouling, are intricate communities composed of bacteria, diatoms, and microalgae encased in a matrix of extrapolymeric substances (EPS), making them precursors for macrofouling. Limiting or preventing biofilms is important in the maritime industry, particularly for commercial and naval ships, because they alter the topography of the exposed surface, increasing turbulence and shear stress, which in turn raises fuel consumption [1–3]. Biofilms have also been suggested to contribute to an increase settlement of subsequent macrofouling organisms [4–6]. Biofouling is not just an external issues but also can cause internal problems. Production loss due to clogged pipes spike energy consumption, leading to costly unplanned maintenance [7]. Ultraviolet-C (UV–C) light is now emerging as a physical alternative to traditional biocidal/chemical methods typically employed to combat the effects of marine biofouling of ship hulls and other submerged surfaces.

UV-C irradiation has been used in the medical field for since the mid-1900s. It has become essential for the disinfection of medical-grade devices because it can kill microbes without causing them to become resistant over time [8,9]. UV-C was introduced for wastewater treatment in 1910 and showed great success. Unlike prior methods of disinfecting water such as chlorine, UV-C does not form any by-products, such as potentially harmful carcinogens [40,10]. UV-C generates DNA lesions and subsequent cyclobutane pyrimidine dimers (CPDs), which prevent DNA transcription, leading to cell death [11,12].

At least a dozen studies have been performed *in situ* to determine if UV-C is a reliable source to prevent or remove biofouling. While several studies have explored UV-C as an antifouling agent, they often focus on macrofouling organisms (e.g., barnacles, tubeworms, etc.) or broad microbial communities. For example, Whitworth et al. [13] demonstrated the long-term effectiveness of UV-emitting tiles in deterring macrofouling on marine surfaces *in situ*, while also observing shift in microbial communities. However, studies investigating the effects of UV-C on microfouling organisms—particularly benthic diatoms—remain limited.

Several studies have indicated biofilms are still present following UV-C treatment [14–16], which suggests some diatoms may have innately higher UV-C resistance than some bacteria. This relatively higher resistance to UV-C may be due to various types of DNA repair

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enzymes such as photolyase. Whitworth et al. [17] constantly pulsed a cultured biofilm of *Navicula incerta* with UV-C doses ranging from 0.01 to 18.70 J/cm^2 of UV-C. In this study, treated diatoms could not recover from higher UV-C doses (between 0.94 and 2.34 J/cm^2), resulting in detachment and reduced cell densities. Braga et al. [16] exposed both laboratory and field biofilms to various doses (1.62–9.75 J/cm^2) of UV-C irradiation and measured changes in biofilm viability (by indicating if cells are metabolically active). The laboratory results indicated that UV-C diminished viability significantly compared to controls, but viability did not significantly change when the field biofilms were exposed [16]. Furthermore, Martinez et al. [14] discovered that select biofilm-forming species (e.g., *Chlorella autotrophica*, *Chaetoceros calcitrans,* and *Phaeocystis globosa*) can recover from UV-C exposure, thus demonstrating that UV-C must fully penetrate an individual, for cell lysis and death to occur.

Unlike previous studies that aimed to eliminate established biofilms using continuous or high-frequency UV-C exposure, this study focuses on determining the minimum frequency at which a given UV-C dose can be applied to maintain biofilm biomass and activity below a recovery threshold. This preventive, maintenance-based approach supports the development of energy-efficient antifouling systems by identifying practical intervals for UV-C application. While the total dose of UV-C remains the same across treatments, this study shows how long a single dose remains effective before biofilms begin to recover—information that can be used to reduce power consumption and operational costs in future antifouling designs.

2. Materials and methods

2.1. Biofilm development

The diatom, *Navicula incerta* (Fig. 1), provided by North Dakota State University for Ocean Sciences, was used for both parts of this study due to it being a contemporary part in biofouling assemblages on marine vessels [3,18] and routine use in biofouling assays [17,19]. Upon receiving cultures, they were assessed and split using the method described in Braga et al. [16].

The experimental procedure was implemented in a laminar flow hood to limit sample contamination. 5 mL of the *N. incerta* culture was pipetted onto microscope slides held in a 4-well plate and allowed to settle for 48 h [16]. in ambient air under fluorescent lights (13:11 light cycle). As a precaution from contamination, lids were placed on top of the well plates. The lids were loose enough to allow air flow but prevent contaminates. Following the adherence period, a subset of the plated biofilms were selected to estimate the average cell density that were attached to the slides. In short this process was done by scraping the biofilms off the microscope slide and placing them in a centrifuge tube. 200 μL of the now suspended culture was placed on the hemocytometer, where cells were counted under a light microscope to determine the density. The rest of the samples were used for UV-C testing (described below).

2.2. Part 1: single UV-C exposure

Single UV-C exposures were used to observe how cultured biofilms reacted to irradiation and track their recovery for duty cycle testing. UV-C light was applied using a 25 W Aqua UV-C (254 nm) lamp [16]. While LEDs are commonly used as a UV-C source in studies, these types of light sources are costly and hard to waterproof. Although the difference in lighting source may be concerning, Giggie et al. [39] compared the efficiency of UV-C LEDs and the Aqua lamp against biofouling and found that the lamp performed the same and better over time than the LED. Additionally, the Aqua lamp has been utilized in various published studies, Hunsucker et al. [20], Braga et al. [21], Richard et al. [15], and Braga et al. [16] providing valuable results to the biofilm and UV-C community.

Two 3-D printed stands (Fig. 2) were fabricated to allow the lamp to be 25 mm from the microscope slides containing the biofilm, and a PRIME-digital lighting timer was used to control UV-C exposure to the microscope slides [15,16,20]. The plated biofilms were in water and the UVC lamp source was in the air above the water. The intensity of the light through this air/water interface, at 25 mm from the surface, was measured with a Solar Light sensor (PMA2180) at 0.0031 mW/cm. This intensity was used to calculate the dose of the exposure [22]. Biofilms were subjected to a single UV-C exposure time of 10 (1.86 J/cm^2), 30 (5.58 J/cm^2), or 60 (11.16 J/cm^2) minutes based on results from previous field experiments [16]. After UV-C treatment, biofilms were analyzed for responses in cell viability (XTT assay), biofilm density (crystal violet assay), biofilm recovery (chlorophyll *a*), and a microscope analysis (observations of morphological changes). Four controls (no UV-C) and 4 UV-C treated slides were used for each of the biofilm analyses listed above. Each test is explained in more detail below.

2.3. XTT assay

The XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay is a colorimetric test used to measure cellular metabolic activity. Following UV-C exposure, an XTT assay was performed on four replicates to determine the concentration of cell viability per slide (methods are modified from the Biotium XTT cell viability kit PI-30007) [16]. Radiated and control (non-UV-C exposed) biofilms were scraped off the microscope slide, placed in centrifuge tubes, and then transferred to a 96-well plate. 50 μL of XTT reagent was added to each well, including the blank (which contained only F/2 medium). The wells with biofilms (both treated and untreated) were incubated in the dark for 48 h, allowing for proper absorption by the live cells. Following the 48-h incubation period, the plate was analyzed in a SpectraMax® i3 plate reader for absorbance at 475 nm and 660 nm [16]. The absorbance values were then placed in the equation below (1) to get the specific absorbance. Averages and standard deviations were taken

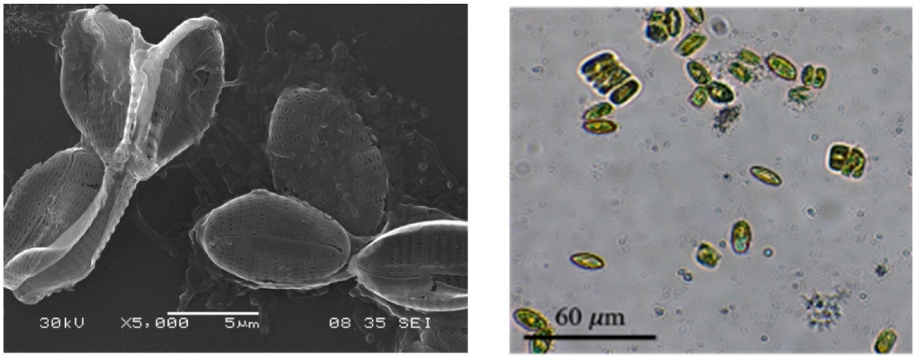

Fig. 1. *Navicula incerta* under scanning electron microscopy (left) and light microscopy (right).

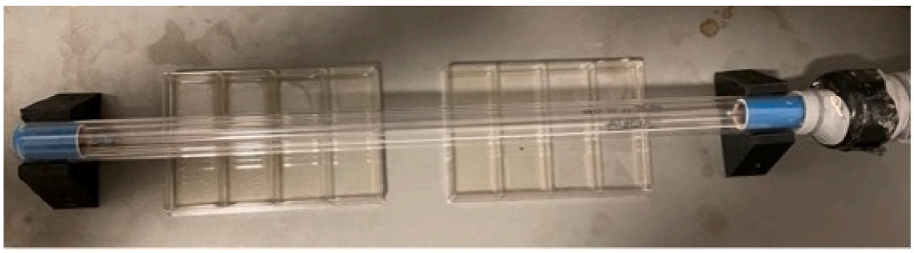

Fig. 2. The UV-C exposure was set up for single-dose and duty cycling testing. Microscope slides with 5 mL of *N. incerta* lie within the 4-well plate. 3-D printed stands hold the lamp 25 mm above the microscope slides.

from each time frequency and individually graphed.

Specific absorbance (nm) = A_{475nm} (test) - A_{475nm} (blank)- A_{660nm} (test) (1)

2.4. Crystal violet assay

The Crystal Violet Assay Kit (Abcam, ab232855) was used to indicate biofilm development. As the dead cells were washed away, the remaining cells were stained with crystal violet, providing a visual representation of biofilm quality. After UV-C exposure, biofilms (radiated and controls) were scrapped off the microscope slide and placed in centrifuge tubes, then transferred to three wells in a 96-well plate where the cells were allowed to settle for 24 h. Biofilms were then washed with 200 μL of washing solution. 50 μL of crystal staining was added to each well and allowed to stain for 20 min, then washed again with the washing solution several times. After washing, biofilms were allowed to dry out for 1 h. Once dry, 100 μL of stabilizing solution was added to each well and placed on a plate shaker for 20 min, then placed in a SpectraMax® i3 plate reader and analyzed for absorption at 570 nm. Averages and standard deviations were taken from each time frequency and individually graphed.

2.5. Biofilm recovery

Biofilm recovery was assessed using chlorophyll *a* (chl *a*) as a quantitative way to track how well the biofilm holds up and bounces back after UV-C exposure. It reflects pigment loss, biomass, and overall resilience under stress. Biofilms were sampled at selected time points following the initial exposure—specifically at 24, 48, 72, 120, and 144 h—to evaluate recovery over time. After initial UV-C treatment, "recovery" samples were kept under fluorescent lights (13:11 light cycle) until their respective sampling time (i.e., 24 h, 48 h, 72 h, 120 h, and 144 h). Chl *a* was extracted using a modified methodology from Ritchie [44]. Radiated biofilms were scraped off the microscope slide and placed in centrifuge tubes. These tubes were then centrifuged, the supernatant removed and washed three times with 1 mL of non-sterile deionized water. After the final wash, 100 % ethanol was added to the biofilms for chl *a* extraction. Biofilms were exposed to the ethanol for 30 min; then, the supernatant was removed and placed into three wells in a 96-well plate. The plate was run within a SpectraMax® i3 plate reader for 665 nm and 650 nm absorption. The readings were then placed in the equation below (2) to calculate chl *a* content [45]. Averages and standard deviations were taken from each day and graphed.

$$\text{Chlorophyll } a = 13.70 \times A_{665} - 5.76 \times A_{650} \quad (2)$$

2.6. Biofilm community composition & morphological changes

Biofilms were analyzed by light microscopy and scanning electron microscopy (SEM) to determine if UV-C exposure resulted in any morphological changes (i.e., modified size, valve outline, shape, and modification in raphe). Using the free application by Motic® (Moti-Connect), images were taken of the individuals on the microscope slides. All images were taken at 40x magnification. From these images, 10 images were selected at random. From these 10 images, 3 random individual diatoms were then measured to determine any size changes due to UV-C. The analysis included pre-exposure and post-exposure samples.

2.7. Part 2: UV-C duty cycling

Based on the recovery results from the single exposures and the single-dose treatments, *N. incerta* biofilms were subjected to UV-C duty cycles. The exact duration of exposure listed above was used for duty cycle testing: 10, 30, or 60 min. However, the time between irradiation was determined based on the biofilm recovery results from the single exposure treatments. Based on single-dose results in Part 1, the duty cycles were created to be 10:24, 30:72 and 60:48 (minutes on: hours off). The cycles were conducted 3 times. A subsection of the samples were tested for cell viability (XTT) and biofilm recovery (chl *a*), Additionally, biofilms were tested for cell viability (XTT) and biofilm recovery (chl *a*) assay following each cycle. Recovery analysis was only conducted following the third (final) UV-C exposure.

2.8. Statistical analysis

Each single dose and duty cycle were performed at different times resulting in variability in starting values. Thus, XTT and chl *a* values for both the single dose and duty cycles were standardized in order to properly compare to one another. Original chl *a* values were transformed by subtracting each data point from the mean value (of each dose) and then divided by the standard deviation [42].

For the single-dose testing, a nested multivariate analysis of variance (ANOVA) was performed on the XTT and crystal violet results. A correlation analysis between the XTT and crystal violet values was done following the ANOVA. Chl *a* measurements were compared using a factorial ANOVA. A *t*-test was used to determine any differences in the measurement of the *N. incerta* cells before and after UV-C irradiation. Normalized data was used to conduct an ANOVA coupled with a simple linear regression to compare the rate of recovery (slope of chl *a*) amongst the single doses. A factorial ANOVA was used to assess the XTT and chl *a* results for duty cycle testing. In addition, the percent change in chlorophyll and XTT values between radiated and non-radiated samples were also calculated to show how the application of UV-C altered the biofilm. Unfortunately, the data from 168 h of the 60 min duty cycle test was lost, so this data point was not considered in the data analysis. Normalized chl *a* values were used to compare duty cycles. All data was analyzed using R statistical software [41].

3. Results

3.1. Comparison of biofilm recovery rates across UV-C doses

The rate of recovery (slope) between each single dose treatment did not significantly differ from another. This was also true, when each

interval was compared to another. However, for both single doses and duty cycles there was variation, which are discussed below in more detail.

3.2. Short UV-C Exposures

Single dose: When biofilms were exposed to a single dose of 1.86 J/cm^2 (10 min), viability and chl *a* were significantly reduced compared to the controls; however, biofilm formation was not ($p > 0.05$). The viability of the control biofilms had a specific absorbance of 0.92 ± 0.36 AU, and the exposed biofilms were reduced by 83 %, with a specific absorbance of 0.15 ± 0.07 AU (Fig. 3b). While there was no significance among biofilm formation (Fig. 3a), a correlation analysis indicated a negative correlation between metabolic activity and biofilm formation ($r = -0.12$, $p = 0.58$). Similar to the viability results, initial chl *a* production was 58 % higher (0.45 μg/m^2) before the 1.86 J/cm^2 dose of UV-C exposure (0.19 μg/m^2). Nonetheless, chlorophyll increased after 24 h of recovery (Fig. 4a). Six days after a single dose of 1.86 J/cm^2, chl *a* noticeably increased nearly 3-fold, going from 0.19 μg/m^2 to 0.67 μg/m^2 ($p < 0.001$). Microscope analysis of the biofilms supported the loss of chlorophyll. Control images of *N. incerta* cells were very green, indicating they were full of chlorophyll, while the radiated cells were less pigmented, appearing slightly bleached (Fig. 5). Additionally, a large portion of diatoms appeared to be in girdle view after UV-C treatment, compared to the controls which remained in valve view. Individuals were also measured along the raphe to determine if there was any change in size, but this did not vary ($p = 0.47$).

Duty cycle Dose: The single dose recovery results suggested that a dose of 1.86 J/cm^2 (10 min) of UV-C should be applied every 24 h. Overall, cell viability varied ($p < 0.001$) throughout the 1.86 J/cm^2 duty cycle period, while chl *a* concentrations showed no differences between the initial culture to day six of recovery ($p > 0.05$). Cell viability was considerably reduced ($p < 0.001$) initially by 67 %, but after 24 h, they began to recover under natural light conditions (Fig. 6a). This trend was observed for the second and third duty cycles, suggesting that some cells still contained chl a even though they are not alive. By the third UV-C exposure, viability was decreased by 60 %. Chl *a* content was also reduced initially (0.52 μg/m^2) but could start recovering within 24 h, only to be depleted again (0.03 μg/m^2) with each exposure. By day three of recovery, there was no variance in chl *a* content compared to those immediately following exposure three, but chl *a* had dropped close to 50 % from the initial concentration (Fig. 7a). By day six of recovery, biofilms significantly recovered roughly 90 % with a final chl *a* concentration of 0.29 μg/m^2.

3.3. Moderate UV-C Exposures

Single Dose: Biofilms exposed to a dose of 5.58 J/cm^2 (30 min) UV-C

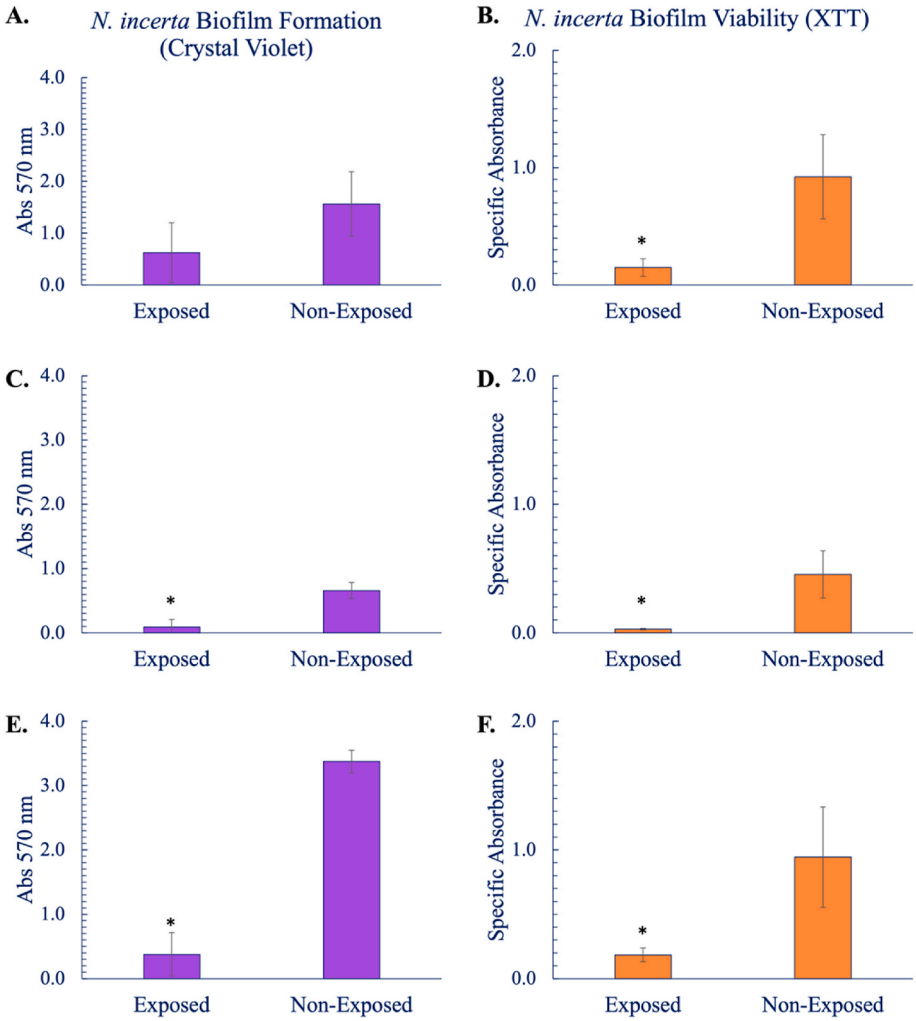

Fig. 3. Bar graphs depict two measurements, (A, C, E) *N. incerta* biofilm formation via a crystal violet stain and (B, D, F) viability, of UV-C Exposed and Non-Exposed *N. incerta* cultures. Biofilms were treated with a single dose of 1.86 J/cm^2 (Top), 5.58 J/cm^2 (Middle), or 11.16 J/cm^2 (Bottom). Vertical bars indicate standard deviation. Stars above the bars denote a significant difference among p-values.

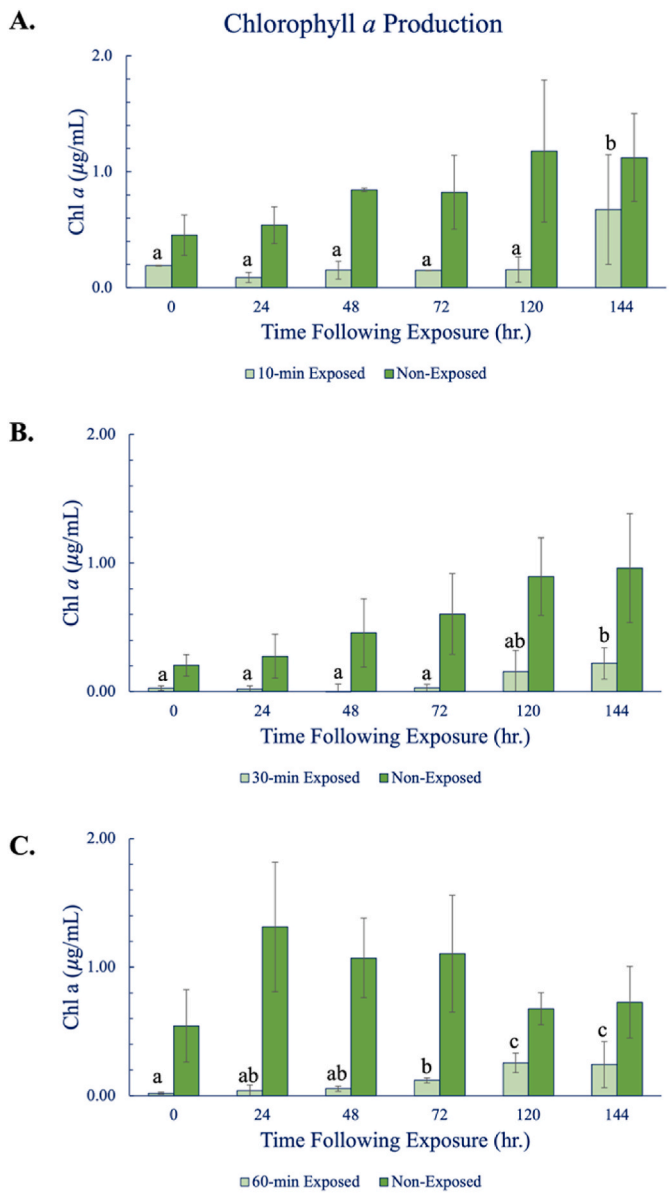

Fig. 4. Chlorophyll *a* production of the culture before and following UV-C irradiation. Four replicates of *N. incerta* biofilm-covered slides were exposed to doses of (A) 1.86 J/cm^2, (B) 5.58 J/cm^2, or (C) 11.16 J/cm^2. The dark green bars represent non-exposed chl *a* production, while the lighter green bars are chl *a* production for the UV-C exposed biofilms. Vertical bars indicate standard deviation. Letters above the bars indicate significance. Letters above the bars of the treated films were used to indicate significance for only those treated with UV-C. The same letter specifies a similarity, while different letters indicate a significant difference ($p < 0.05$).

had a significant decrease in biofilm development, cell viability, and chl *a*. Biofilm formation was dramatically reduced by 86 % ($p < 0.001$; Fig. 3c) due to UV-C from biofilms with low metabolic activity ($r = -0.11$, $p = 0.63$). Within this reduced biofilm density, viability was 0.03 ± 0.01 A, 94 % less than controls (0.45 ± 0.18 A; $p < 0.001$) (Fig. 3d). This weaker biofilm also reduced chl *a* production by 87 % after 5.58 J/cm^2 of UV-C irradiation ($p < 0.001$). *N. incerta* radiated with 5.58 J/cm^2 of UV-C indicated a loss of chlorophyll, reoriented themselves, and had no change in size ($p = 0.47$). Most of the 5.58 J/cm^2 radiated culture appeared like glass sheaths, completely bleached and rotated similar to those of the 1.86 J/cm^2 (Fig. 5).Like the 1.86 J/cm^2 exposed films, biofilms could recover, although it took 72 h, whereas the 1.86 J/cm^2 treated films took only 24 h (Fig. 4b). Recovery increased tremendously from 0.03 µg/m^2 to 0.22 µg/m^2 by day 6, just over the initial chlorophyll production (0.20 µg/m^2).

Duty cycle Dose: Based on the 5.58 J/cm^2 (30 min) single dose results, biofilms were exposed to 30 min of UV-C every 72 h. Viability from start to finish for the 5.58 J/cm^2 duty cycle testing significantly differed, whereas chl *a* concentrations did not. Throughout the 5.58 J/cm^2 cycle, *N. incerta* viability was initially depleted but showed no variance until the subsequent two UV-C exposures occurred (Fig. 6b). Between exposures, viability recovered roughly 40 % but still noticeably reduced from 0.73 ± 0.29 AU to 0.21 ± 0.05 AU compared to the initial concentration ($p < 0.001$). This drop accounted for an approximate total decrease of 70 % in cell viability. Chlorophyll *a* concentrations did not substantially differ from the start to end, which may be due to variation among the replicates ($p > 0.05$). While there were variations between exposures ($p < 0.001$), with cha *a* depleting by 20 % (0.006 µg/m^2; Fig. 7b), by day six (144 h) of their recovery, *N. incerta* was able to increase their chl *a* concentration well over 100 % (0.15 µg/m^2).

3.4. Prolonged UV-C Exposures

Single Dose: A dose of 11.16 J/cm^2 (60 min) biofilms resulted in a meaningful decline in biofilm density, cell viability, and chl *a*. Biofilm density was significantly reduced by over nine-fold ($p < 0.001$; Fig. 3e), which substantially reduced metabolic activity ($r = -0.12$, $p = 0.79$). The viability of the biofilms was diminished by almost 80 % (0.18 ± 0.05 AU; $p < 0.001$) (Fig. 3f). Chlorophyll *a* also degraded immediately comparable to exposure one, compared to the control, which had a concentration of 0.54 µg/m^2 ($p < 0.001$; Fig. 4c). When a microscope analysis was conducted, most of the culture was bleached except for a few individuals and experiences similar behaviors as those of the other doses, turning from valve to girdle view (Fig. 5), similar to those treated with 5.58 J/cm^2. Size did not significantly differ between exposed and control *N. incerta*. Nonetheless, chl *a* could start recovering but only needed 48 h, showing no variation. Chlorophyll recovery by the 48 h mark was approximately 0.05 µg/m^2 ultimately reaching 0.24 µg/m^2 by the final day (144 h), although this was still lower than the initial chl *a* production of 0.54 µg/m^2 (Fig. 4c).

Duty cycle Dose: Biofilms radiated with doses of 11.16 J/cm^2 of UV-C began to recover within 48 h; therefore, the 11.16 J/cm^2 duty cycles were done every 48 h. By the conclusion of the 11.16 J/cm^2 duty cycle testing, both cell viability ($p < 0.001$) and chl *a* ($p = 0.02$) considerably differed. Following 11.16 J/cm^2 UV-C duty cycles, the viability of the biofilm was significantly reduced ($p > 0.05$) by 86 % (Fig. 6c). Similar to the other two duty cycles (1.86 J/cm^2 and 5.58 J/cm^2), *N. incerta* viability recovered between exposures. Chlorophyll *a* was initially reduced by 97 % and went from 0.33 µg/m^2 to 0.01 µg/m^2 following the first exposure. Within two days, *N. incerta* biofilms could recover roughly 100 % since the previous exposure (Fig. 7c). While biofilms were recovering between exposure duty cycles, chl a was still approximately 90 % lower than the initial concentration; however, variance among replicates resulted in no profound difference. Unfortunately, the chl *a* data at 48 h was lost, so this data point was not present in the data analysis, but this did not affect the results where chl *a* concentrations were 0.10 µg/m^2, which was a 69 % difference from the original concentration (Fig. 7c).

4. Discussion

The present study used multiple assays to understand how marine biofilms react to UV-C treatment and observe changes in reproduction rates, providing clear feedback and suggestions for future testing. Recovery results highlighted that duty cycling was more effective at maintaining biofilms at minimal levels rather than fully eliminating them. Based on exposure times, lower doses require more frequent treatments to maintain control. This study does not aim to completely eliminate biofilms, but instead to determine the duration of suppression

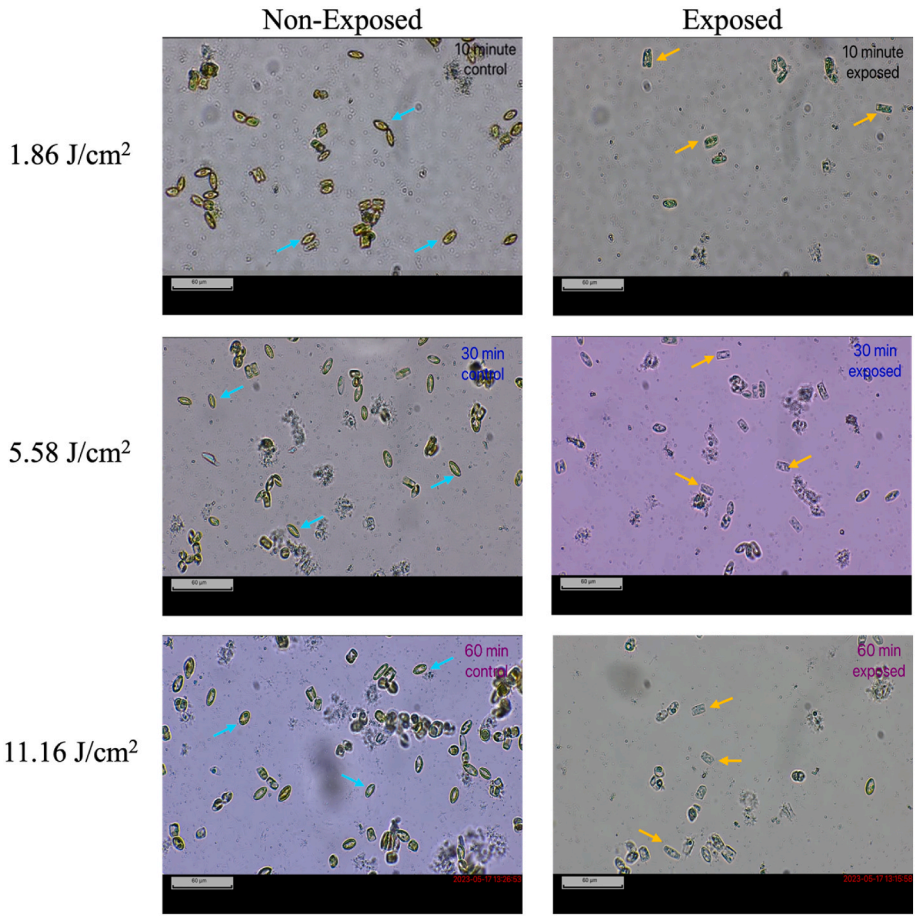

Fig. 5. Visual representation of Non-Exposed (left) and Exposed (right) *N. incerta* cells. Non-exposed cells appear rich in chlorophyll and are primarily oriented in valve view, indicated by blue arrows. In contrast, UV-C treated cells show reduced pigmentation—indicating chlorophyll loss—and a noticeable shift in orientation, with many appearing in girdle view, highlighted by orange arrows.

that can be achieved with a single UV-C dose. By focusing on how long biofilms remain at low levels following treatment, the findings support a maintenance-based approach—more aligned with realistic antifouling needs than total eradication.

Biofilm viability remained high in the days following initial exposure, with XTT assay readings increasing by 35 % between 24 and 72 h, indicating biofilm affinity to recover and reproduce, even under higher doses. The combination of the XTT and chl a, provided an insight as to the processes affected by UV-C. The XTT assay measured overall metabolic activity and showed a decline after UV-C exposure, aligning with the observed reduction in chlorophyll *a* and suggesting impaired photosynthetic function. While it is unclear why some diatoms remained metabolically active, previous studies suggest that UV exposure can enhance the activity of enzymes such as carbonic anhydrase [23] and ammonium chloride ($^{15}NH_4C1$) [24]. Nevertheless, further testing would need to be conducted to establish the full effect of UV-C on these systems.

Biofilm densities in this study were ≈$4x10^5$ to $7x10^5$ cells/mL, consistent with natural systems, densities that can have upwards of $1x10^5$ cells/mL to $1x10^6$ cells/mL. With high cell densities many individuals are compacted together, forming clusters of cells [25]. These clusters can protect the underlying individuals from damage, a phenomenon known as bioflocculation, which can aid biofilm resilience following UV-C dosing. Bioflocculation in bacteria reduces colony inactivation by lowering the fluency rate through floc formation [26–29]. Clustering was present in the current study and could have aided in the succession of the biofilm following irradiation.

Marine algae also possess several DNA repair mechanisms that allow for recovery under stress. Repair mechanisms include photoreactivation and excision repair [30,31]. While these were not directly observed, it is assumed that these mechanisms are utilized based on the viability results. In addition, *N. incerta* rapidly reproduces, reaching their logarithmic growth within 4 days [32]. This quick proliferation could largely explain the sudden increase in XTT and chlorophyll *a* analyses even after treatment. *N. incerta* diatoms also demonstrated behavioral alterations, suspected to reduce photodamage, previously recorded by Braga et al. [16]. However, when UV-C radiation is halted it suspected that these adaptations allowed the biofilms to essentially recover. This trend was continually observed during duty cycle testing.

The crystal violet showed reduced biofilm biomass after UV-C exposure, which is consistent with diminished metabolic function. Interestingly, UV-C may also affect the extracellular matrix (EPS) itself. While few studies have addressed this, mostly focusing on bacterial systems, UV exposure is known to deform the EPS matrix and reduce its production [46]. Chen et al. [33] found that total cellular carbohydrates and sucrose significantly decrease following UV-B radiation, contributing to matrix breakdown. This would explain why the crystal violet stain was reduced considerably compared to the control. Furthermore, they also observed a post-treatment increase in reducing sugars, suggesting that organisms may reabsorb them for heterotrophic metabolism, and survive after treatments. Although crystal violet was not used between exposures in the present study, this mechanism could help explain rising viability between doses. Future studies are needed to test this hypothesis.

In natural systems, biofilms are constantly replenished by diatom settlement and reproduction, which would influence the required dosing

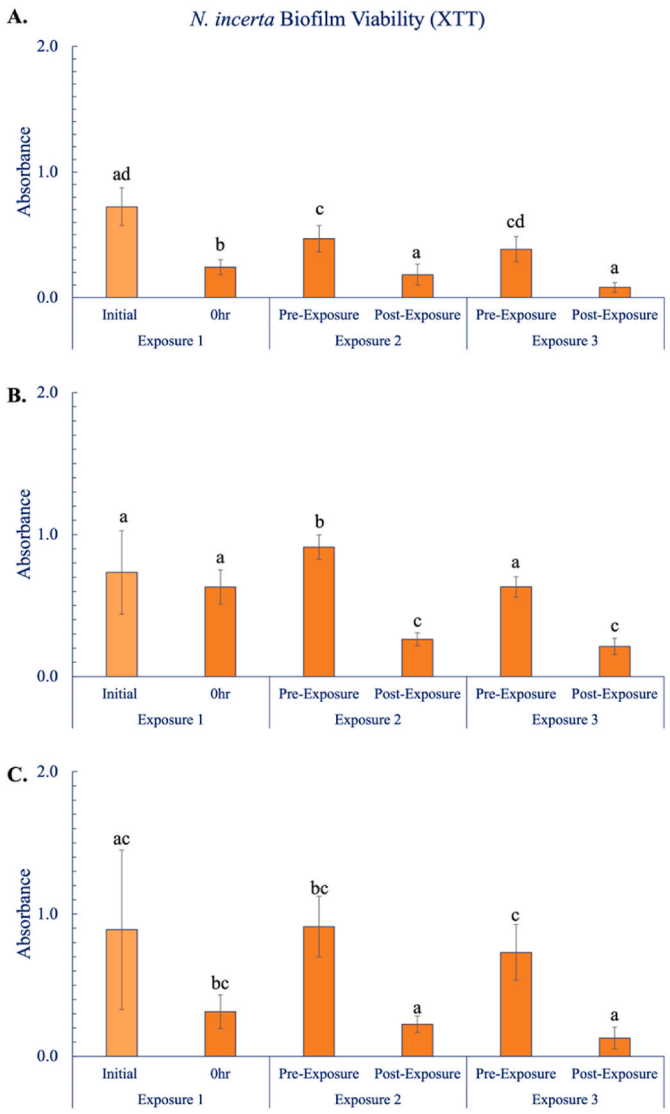

Fig. 6. Viability of the cultured biofilm before and throughout UV-C duty cycles. Biofilms were exposed to duty cycles of (A) 1.86 J/cm^2, (B) 5.58 J/cm^2, or (C) 11.16 J/cm^2. Each exposure bar represents the average specific absorbance of four samples. Vertical bars indicate standard deviation. Letters above the bars indicate significance. The same letter specifies no statistical difference, while different letters indicate a significant difference.

frequency. Due to the controlled aspect of this study, the effect of new, non-irradiated individual introductions was not considered. Nevertheless, such inputs could enhance recovery or replace damaged cells, complicating antifouling strategies. Natural biofilms exposed to UV-C tend to resemble newer communities, based on density and biofilm structure, regardless of the actual age of the biofilms [34]. Similarly, biofilms in this study did not return to pre-treatment levels, suggesting UV-C exposure altered their structure to resemble that of a younger biofilm. This shift in the overall community can ultimately alter macrofouling recruitment and settlement [35].

Field biofilms are more complex than lab-grown ones, and results may not translate directly due to increased heterogeneity [36]. Braga et al. [16] observed discrepancies in cell viability testing between laboratory and field-grown biofilms exposed to UV-C. Their results indicated no difference among treatments in the field-grown films due to the difficulty of the cell viability test penetrating the biofilm. However, only one assay was used to assess the biofilms, while the present study used multiple metrics, including viability ad chl *a*. Here, viability and chl a showed high mortality and slightly reduces pigment levels in treated samples. Therefore, the testing protocols used here would need to be replicated using natural field biofilms to fully determine the dose and frequency needed to prevent or minimize biofilm production based on coupling analyses.

Our findings may support future designs of UV-C antifouling systems that require lower energy input and targeted scheduling. While Whitworth et al. [13] successfully demonstrated long-term field-scale UV treatment for macrofouling, integrating optimized duty cycles—such as those tested in the present study—could enhance efficiency in managing both micro- and macrofouling communities. Rather than aiming to eliminate biofilms entirely, the focus on maintaining suppressed levels using minimal UV-C input aligns with practical deployment scenarios where energy efficiency and long-term operation are critical. These findings help refine duty cycle parameters for real-world applications, where consistent prevention may be more feasible than full eradication. Longer UV-C exposures resulted in more prolonged suppression of biofilm recovery compared to shorter exposures, suggesting they could be applied less frequently while maintaining effectiveness. This indicates that, from a cost-efficiency standpoint, investing in slightly longer UV-C doses may reduce the total number of treatment events required—an important consideration for energy-limited or autonomous marine systems.

5. Conclusion

The application of UV-C irradiation offers a promising and environmentally friendly method for biofouling control. However, biofilms have demonstrated resilience, suggesting UV-C alone may not provide the complete solution to biofouling control. Traditional antifouling coatings are primarily used for biofouling control, however the increase in their use is creating some unintended environmental concerns [37, 38]. Combining marine coatings with UV-C can provide a more effective and sustainable approach to biofouling prevention. To date, only Hunsucker et al. [20] and Whitworth et al. [43] have begun to pair antifouling coatings with UV-C. Based on Hunsucker et al. [20] results, UV-C was able to limit fouling using lower doses than the current study. Although, this study indicated that longer UV-C exposures would be more cost-effective, pairing the UV-C with marine coatings may add additionally cost benefits, by further lowering the frequency at which a single dose is applied. Furthermore, the lower frequency of exposure could aid in preventing biofilm adaptions or tolerance.

This study used a monoculture of *Navicula incerta* to better understand of how UV-C impacts diatom biofilms and to establish a framework for future duty cycle testing. While prior studies have shown that UV-C alone can reduce biofilms, this work highlights the importance of using multiple analytical metrics to accurately assess UV-C effectiveness and recovery potential. Importantly, the results demonstrate that low-dose UV-C irradiation, when timed strategically, can effectively suppress biofilm growth without continuous application. This maintenance-based approach offers a more sustainable path forward for antifouling systems by balancing efficacy with operational cost.

CRediT authorship contribution statement

Kailey N. Richard: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrew Palmer:** Writing – review & editing, Supervision. **Geoffrey Swain:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Kelli Z. Hunsucker:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of interests

The authors declare that they have no known competing financial

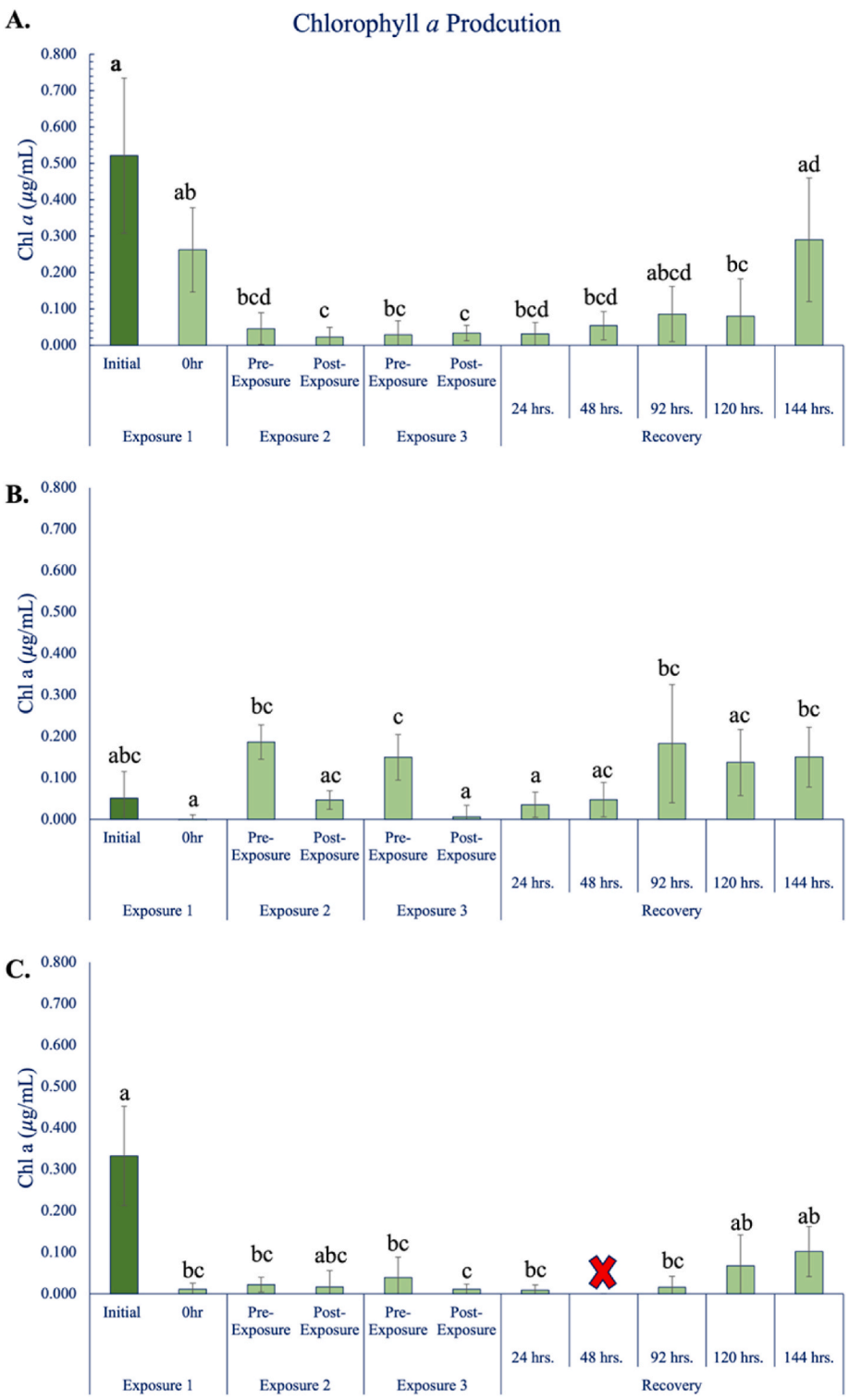

Fig. 7. Chlorophyll *a* production of the culture throughout UV-C duty cycles and up to 6 days following exposure. Four replicates of biofilm-covered slides were exposed to doses of (A) 1.86 J/cm^2, (B) 5.58 J/cm^2, or (C) 11.16 J/cm^2. The 48 h data was lost for the (C) 11.16 J/cm^2 minute treated slides, so this point is represented by the red X. Vertical bars indicate standard deviation. Letters above the bars indicate significance. The same letter specifies no statistical difference, while different letters indicate a significant difference.

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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