The Effect of Ultraviolet Light (UV-C) on Marine Phytoplankton Fluorescence

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Martin, R.B., Markus, D.D.R., and Sutherland, T.F. 2018. The effect of ultraviolet light (UV-C) on marine phytoplankton fluorescence. Can. Tech. Rep. Fish. Aquat. Sci. 3289: vii + 17 p.

The effect of ultraviolet radiation (UV-C) on the photosynthetic activity of a natural marine phytoplankton community was measured through fluorescence outputs. Phytoplankton samples, with and without UV-C treatment, were incubated in both light and dark conditions to mimic oceanic and ballast-tank settings. The control phytoplankton samples that were not exposed to UV-C light showed traditional growth and mortality curves associated with light and dark incubation conditions, respectively. Treated phytoplankton samples showed a sharp decrease (~63%) in fluorescence values within minutes of being exposed to UV-C light. A notable increase in fluorescence during the early stages of the dark incubation, relative to that of light incubation, suggests that some form of dark DNA repair may have taken place. Alternately, the fluorescence outputs under light incubation conditions remained relatively stable after treatment and initial decrease in fluorescent values. Phytoplankton incubation experiments should be carried out for long periods of time in order to test phytoplankton survivability and regrowth potential.

RÉSUMÉ

Martin, R.B., Markus, D.D.R., et Sutherland, T.F. 2018. Effet de la lumière ultraviolette (UV-C) sur la fluorescence du phytoplancton marin. Rapp. tech. can. sci. halieut. aquat. 3289: vii + 17 p.

L'effet du rayonnement ultraviolet (UV-C) sur l'activité photosynthétique d'une communauté de phytoplancton marin naturel a été mesuré par les sorties de fluorescence. Des échantillons de phytoplancton, avec et sans traitement UV-C, ont été incubés à la lumière et à l'obscurité pour imiter les paramètres de l'océan et des réservoirs de ballast. Les échantillons de phytoplancton de contrôle qui n'étaient pas exposés à la lumière UV-C montraient les courbes de croissance et de mortalité traditionnelles associées aux conditions d'incubation à la lumière et à l'obscurité, respectivement. Les échantillons de phytoplancton traités ont montré une forte diminution (~ 63%) des valeurs de fluorescence quelques minutes après avoir été exposées à la lumière UV-C. Une augmentation notable de la fluorescence pendant les premiers stades de l'incubation à l'obscurité, par rapport à celle de l'incubation à la lumière, suggère qu'une certaine forme de réparation de l'ADN sombre aurait pu avoir lieu. Alternativement, les valeurs de fluorescence dans des conditions d'incubation à la lumière sont restées relativement stables après traitement et diminution initiale des valeurs de fluorescence. Les expériences d'incubation de phytoplancton doivent être effectuées pendant de longues périodes afin de tester la capacité de survie et le potentiel de repousse du phytoplancton.

INTRODUCTION

Water and sediment transported by ballast tanks have been demonstrated to be an important vector of aquatic invasive species (AIS) (Hallegraeff 1998; Kelly 1993; Ruiz et al. 2000, 2011; Molnar et al. 2008; Sutherland and Levings 2013). While ballast water can support a planktonic population (e.g., bacteria, phytoplankton, zooplankton, fish and invertebrate larvae), ballast sediments may serve as a reservoir for phytoplankton resting spores, zooplankton eggs, and an invertebrate population (Hallegraeff and Bolch 1992; Cordell et al. 2008; Gregg et al. 2009; Klein et al. 2010; DiBacco et al. 2011). Once transported and released at a destination port beyond their natural and historic habitat range, taxa that survive colonization and establish a thriving population are considered aquatic invasive species (Molnar et al. 2008}. For example, seasonal summer "monocultures" of an invasive calanoid copepod (e.g. *Pseudodiaptomus forbesi*) that developed in the lower Columbia River (Cordell et al. 2008) may negatively influence ecosystem biodiversity through inter-taxa competition and food-wed interactions (prey availability). In addition, zebra mussels (*Dreissena polymorpha*) in the Great Lakes clogged water intakes impacting commercial activities, resulting in millions of dollars in annual maintenance and monitoring programs (Fernald and Watson 2013; Chakraborti et al. 2013).

Trans-oceanic ballast-water exchange (BWE) has been either practiced or implemented as regulation in certain countries in order to control transport and widespread dispersal of aquatic invasive species (Galil and Hussmann 2002). Two common BWE processes consist of the 1) empty-refill method where tanks were not refilled with water until the entire ballast water volume is discharged; and 2) flow-through method where water is pump through the tanks continuously until the water volume has been exchanged. However, many studies have shown that it is difficult to achieve 100% efficiency in BWE and that the efficiency of BWE varies between exchange method, ship type, and tank specifications (Dickman and Zhang, 1999; Gregg et al., 2009). In addition, BWE efficiency does not always reflect the associated ballast taxa exchange efficiency, which would warrant a costly monitoring program to assess the risk of taxa invasion (Zhang and Dickman, 1999; Drake et al. 2002; Mimura et al.

2005; McCollin et al. 2007). Finally, conducting BWE at a mid-ocean location (trans-oceanic voyage) or a specified regulatory distance from shore (coastal voyage) is not always possible or safe in stormy conditions when empty ballast tanks cannot trim and stabilize cargo-free ships (Hutchings, 1992; Rigby and Taylor, 2001; IMO, 2004).

Although BWE serves as an interim option to reduce the transport of aquatic invasive species to foreign waters, ballast water treatment (BWT) systems can provide a means of deactivating organisms upon uptake or discharge of ballast water at port (Mamlook et al. 2008; Taylor and Rigby 2001; Gregg et al. 2009). BWT systems were developed to help meet evolving and stringent regulatory standards and also avoid BWE requirements under unsafe weather conditions. BWT systems may consist of both mechanical removal and inactivation treatment phases, where the latter may include chemical, ultraviolet radiation, heat, and/or ultrasound exposures (Mamlook et al. 2008; Gregg et al. 2009). Two-staged BWT systems consisting of consecutive filtration and ultraviolet light treatments have been developed and their efficacy assessed (Sutherland et al. 2001, 2003; Waite et al. 2003; Stehouwer et al. 2013, 2105; Castro et al. 2018). Ultraviolet radiation (UVR) may have advantages over other inactivation treatment technologies as it 1) is a proven water quality germicidal technology (waste, aquaculture, drinking water applications); 2) does not affect ship infrastructure through tank corrosion; and 3) does not leave behind a chemical residue (Chang et al. 1985; Gregg et al. 2009).

UVR is made up of several spectral bands that include UV-A (400-320 nm), UV-B (320-280 nm), and UV-C (280-200 nm), with UV-A and UV-B existing in earth's atmosphere and UV-C band being absorbed by the earth's ozone layer. Given this context, research has focused mainly on the effects of UV-B on phytoplankton in natural settings (Buma et al. 1996a,1996b; Neale et al. 1998; Barbieri et al. 2002; Rastogi et al. 2010; Wu et al. 2010; Li and Gao 2012). Both UV-B and UV-C induced damage occurs when phytoplankton nucleic acids and proteins absorb UVR resulting in cytotoxic and/or genotoxic effects (Buma et al. 1996a; Sinha and Hader 2002). Differences in DNA effects between UV-B and UV-C are based on relative proportions of cytosine- and thymine-containing photoproducts (Cleaver, 2006). UV-C lamps have been developed

to deliver high dose rates at 254 nm, which coincide with DNA maximum absorption capacity.

The objective of this study was to determine the effect of UV-C light on phytoplankton fluorescence and subsequent growth incubations under both light and dark conditions. The light incubation conditions can simulate natural oceanic settings following port-side ballast water discharge from tanks with or without UV-C treatment. The dark incubation conditions can simulate the ballast tank storage environment, following the uptake of oceanic water with or without UV-C treatment. Thus, the following treatment and control categories were established:

- 1) UV-C exposure and light incubation (UVC/light-incubation);
- 2) UV-C exposure and dark incubation (UVC/dark-incubation);
- 3) No UV-C exposure and light incubation (No-UVC/light-incubation); and
- 4) No UV-C exposure and dark incubation (No-UVC/dark-incubation).

MATERIALS AND METHODS

Sample collection: A marine phytoplankton sample was collected from the dock of the Pacific Science Enterprise Centre, West Vancouver, British Columbia, on August 8, 2017. Seawater was collected from the chlorophyll maximum in the water column using a 5-L Niskin bottle. The seawater was transferred from the Niskin bottle to an acid-washed Nalgene bottle and stored at 16°C in an environmental chamber for 1 hour.

UV chamber and treatment: The UV chamber consisted of a wooden box (45 cm in height, 65 cm in width, and 48 cm in depth). A UV-C lamp (USHIO G15T8 15W Germicidal UVR; length: 43 cm) with a spectral output of 253.7 nm was mounted to the underside of the roof of the box. A black garbage bag, secured at the front of the open-faced box, provided a sealed curtain to prevent 1) natural light from entering the box and 2) UV-C light from exiting the chamber. Three labelled, square petri dishes (9 cm x 9 cm) were placed side-by-side on a bench surface located inside the UV chamber, creating a vertical distance of 19 cm between the petri dishes and the UV-C lamp. Petri dishes were restricted to the central portion of the UV-R lamp which provided relatively consistent UV-C readings across the dishes using the BLAK-RAY® Ultraviolet Meter (Model No. 1225). The UV-C dosages for each petri dish were: Petri-dish$^{\#}$1: 880 µW

cm^{-2}; petri-dish#2: 980 µW cm^{-2}; petri-dish#3 = 880 µW cm^{-2}. The mean UV-C dosage was 913 +/- 47 µW cm^{-2}.

UV-C treatment and incubation: Twelve 50-mL screw-top test tubes were sorted into 4 categories representing UV treatment and light/dark incubation conditions: 1) UVC/light-incubation; 2) UVC/dark-incubation; 3) No-UVC/light-incubation; and 4) No-UVC/dark-incubation. The test-tubes were acid-washed and rinsed several times with distilled water. The lighting system in the lab was reduced to avoid exposure of the phytoplankton to bright lights (light shock). A 1-L seawater sample was gently mixed to avoid disruption of phytoplankton cells and ensure that each test tube received a similar phytoplankton concentration. Each 50-mL test tube received 45 mL of the phytoplankton sample.

Phytoplankton fluorescence was measured using a Turner Design 10AU™ Fluorometer. Each test tube sample was gently mixed prior to being inserted into the fluorometer and recording a time-zero fluorescent reading. In terms of the UV-treatment-light conditions, each of the three phytoplankton samples was transferred to a labelled petri dish. The UV-C lamp was turned on for 20 minutes and the samples were transferred back to their respective test tubes following treatment. This procedure was repeated for the three UV-treatment-dark samples. The samples belonging to the dark condition categories (UV-treatment-dark; No-UV-treatment-dark) were sealed in 2 Rubbermaid tubs immediately following the fluorescence readings. All samples were placed in an environmental chamber set to a temperature of 16˚C and a light:dark cycle of 12:12 hours. The fluorescence readings were collected at the same time each day to avoid interactions with varying phytoplankton growth rates.

RESULTS

No statistical difference was observed between the mean fluorescence values on day 1 of the experiment (Table 1; $p = 0.510$), suggesting that phytoplankton abundance was similar prior to UV-C light treatment and/or light/dark incubation conditions. A 3-factor ANOVA revealed a significant interaction ($p = 0.028$) between UV-C treatment, light-dark incubation conditions, and incubation time (growth/mortality). Statistical

significant interactions were observed for the following 2-Factor ANOVAS: 1) UV-Treatment and light-dark conditions ($p < 0.001$), and 2) UV-Treatment and incubation Time ($p = 0.001$). In contrast, a significant interaction between light-dark conditions and incubation time was not observed ($p = 0.200$). In terms of single factor-ANOVAS, the mean fluorescence values were significantly different within the UV-Treatment ($p < 0.001$) and the light-dark conditions ($p < 0.001$). The time factor did not show significant differences between mean phytoplankton fluorescence ($p=0.446$).

Tukey test results revealed the following: 1) mean fluorescence values derived from No-UVC/light-incubation conditions were statistically different from those of all other treatment/incubation scenarios ($p < 0.001$); 2) mean fluorescence values derived from UVC/light-incubation and No-UVC/light-incubation conditions were statistically different ($p = 0.004$); 3) mean fluorescence values derived from UVC/dark-incubation and No-UVC/dark-incubation conditions were statistically similar ($p = 0.107$); and 4) mean fluorescence values exposed to UVC/light-incubation and UVC/dark-incubation conditions were also statistically similar ($p = 0.626$).

The fluorescent readings of the seawater samples exposed to UV-C light showed immediate decreases (~ 63%) in fluorescence on day one of the experiment, prior to incubation (Figure 1). In terms of the No-UVC/light-incubation conditions, while a positive growth curve was observed during light incubation treatment, a decline in fluorescence was observed during dark incubation conditions. In terms of UVC/light-incubation treatment, a sharp decrease in fluorescence was observed immediately following exposure, followed by a stabilization of fluorescence readings for the remaining 7 days.

DISCUSSION

Both shipboard and lab-bench BWT studies have shown that UV-C irradiation can influence phytoplankton viability (Sutherland et al. 2001; Waite et al. 2003; Oemcke et al. 2004; Sassi et al. 2005; Halac et al. 2010; Heibling et al. 2011; Olsen et al. 2015, 2016). The results of this study show that the UV-C dosage had an immediate impact represented by a sharp decrease in the phytoplankton community fluorescence

following treatment. Given that the fluorescence values were recorded within 1 minute of UV-C exposure, UV-C damage likely takes place instantaneously. This observation follows that of Sutherland et al. (2001) where the abundances of *Skeletonema costatum* and *Chaetoceros gracile* were reduced by a factor of 7 and 2, respectively, immediately following UV-C exposure. Potential UV-C impacts may include 1) bleaching of photosynthetic pigments and break down of important biomolecules such as proteins and lipids; 2) chromophore formation of highly reactive oxygen species (ROS) which break down proteins, pigments and other vital cellular biomolecules; and 3) DNA absorption of UV-C and subsequent thymine dimers formation (DNA lesions) disrupting DNA transcription and replication (Buma et al. 1996a,1996b; Sinha and Hader, 2002).

Phytoplankton have protective mechanisms for UV-C exposure which minimize damage and may account for the observed above-zero fluorescence values maintained during the incubation period. These protection mechanisms vary with phytoplankton taxa based on cell size, structure, and physiology (Karentz, 1991). For example, small diatoms with relatively high surface-area:volume ratios are more susceptible to UV-B damage per DNA unit relative to large diatoms with a lower surface-area:volume ratios where UV-B radiation may not reach central structures (Karentz et al. 1991). Further, the reflective “glass” exoskeleton (frustule) of diatoms may provide extra protection against UV light relative to that of susceptible naked flagellates that lack extra cell-wall armour (Elleguard et al. 2018). Carotenoid “accessory” pigments, that vary in composition and amount across phytoplankton guilds, absorb excess energy to alleviate damage to the photosynthetic apparatus. Mycosporine-like amino acids (MAA) generated in the outer cytoplasmic layer absorb in the UV light range, preventing up to 7 out of 10 UV photons from reaching central targets (e.g. DNA in nucleus) (Singh and Sinha 2011). Larger phytoplankton cells have higher MAA concentration levels. Photo-inhibition typically takes place in coastal surface waters to reduce the yield of photons from excessive radiation (PAR and UVR) thereby lowering photosynthesis activity and preventing damage (Huner et al. 2002). Resting spores or cysts vary in design ranging from simple temporary cysts or elaborate dormant cysts under unfavourable conditions (McQuoid and Hobson 1996; McQuoid et al. 2002). When analyzed on a diverse population level, the interaction of these protection mechanisms may have a cumulative

effect in insulating individuals or certain taxa. For example, microbial mats in top layer absorb detrimental UV radiation protecting the lower layers. In this manner, active movement within the mat allows a fine tuned adjustment of the light level that each individual receives.

Light and dark DNA repair systems may account for differences in daily mean fluorescent values between light and dark incubation conditions following UV-C exposure. UV-induced DNA damage consists of structural alteration (cross-linking of two bases) preventing cellular processes (e.g. DNA replication) from occurring (Cleaver, 2006). These DNA repair pathways are based on enzymes that rely on either light or dark conditions to perform their actions. Light repair or photo-reactivation is a direct pathway that reverses DNA damage using an enzyme (phytolase) that relies on light (330–459 nm). The DNA pyrimidine dimers are repaired by breaking the cyclobutane ring joining the pyrimidines (Sancar and Sancar 1988). Buma et al (1996a) showed that all thymine dimers removed from UV-B damaged *Cyclotella* DNA took place after 8 hours of photosynthetically active radiation (PAR) exposure. Consequently, *Cyclotella* extended the S-phase of DNA replication process until dimers were removed. Dark repair takes place in several ways: 1) An enzyme (N-glydosylase), that cleaves DNA cross-links; 2) Recombination repair that skips over cross-linked DNA bases whose gap are filled with the opposite chromosome after replication; and 3) Excision repair where a protein complex removes bases before and after a DNA cross-link which is replaced with a non-distorted replicate template.

In this study, the increase in fluorescence on day 3 under dark incubation conditions suggests that some form of dark repair took place. In terms of the light incubation conditions, a very slight fluorescence increase took place over the last 5 days, following a no-net light repair in the initial 2 days of incubation. It is possible that the photosynthetic apparatus was either impaired beyond repair or underwent additional damage after being placed in high-light incubation conditions. Other BWT UV-C studies have suggested that incubation experiments should be carried out for long periods of time in order to test phytoplankton survivability and regrowth potential (Sutherland et al. 2001; Waite et al. 2003; Hess-Erga et al. 2010; Liebich et al. 2012; Martinez et al. 2013). The results of this study specifically suggest that phytoplankton, single celled

organisms which rely on light and photosynthesis to live, may survive temporarily in dark conditions in ballast tanks following UV-C exposure.

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Table 1: Descriptive statistics of mean phytoplankton fluorescence values at time zero prior to ultraviolet-C (UV-C) light exposure.

Parameter	No UV-C Exposure		UV-C Exposure		ANOVA
	Light Incubation	Dark Incubation	Light Incubation	Dark Incubation	p-value
Mean	1.393	1.340	1.393	1.430	
Standard Deviation	0.015	0.115	0.064	0.046	p = 0.510
Replicates	3	3	3	3	

Table 2: A comparison of mean phytoplankton fluorescence values during a 7-day incubation period following ultraviolet-C (UV-C) exposure. The lines signify that no significant difference exists between the connected mean fluorescent values. Mean UV-C dosage = 913 +/- 47 µW cm^{-2}.

Parameter	No UV-C Exposure		UV-C Exposure		ANOVA
	Light Incubation	Dark Incubation	Dark Incubation	Light Incubation	p-value
Mean	2.206	0.976	0.611	0.419	
Standard Deviation	0.937	0.287	0.325	0.390	$p < 0.001$
Replicates	3	3	3	3	

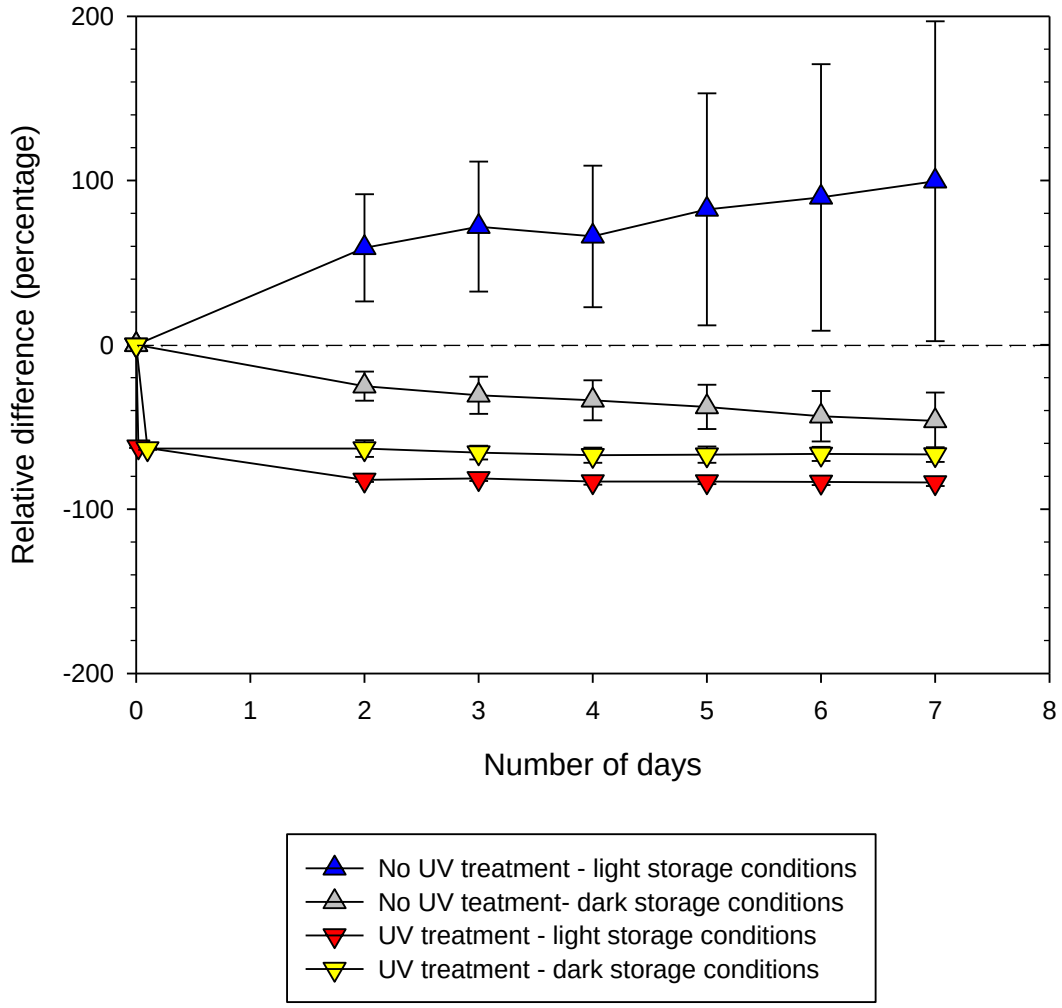

Figure 1: Relative mean percent difference between daily phytoplankton fluorescence and time-zero fluorescence values for various UV-C treatment, incubation, and control seawater cultures.