Review: Photocatalytic effects of titanium dioxide nanoparticles on aquatic organisms – current knowledge and suggestions for future research

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Nanoparticles are entering natural systems through product usage, industrial waste and post-consumer material degradation. As the production of nanoparticles is expected to increase in the next decade, so too are predicted environmental loads. Engineered metal-oxide nanomaterials, such as titanium dioxide, are known for their photocatalytic capabilities. When these nanoparticles are exposed to ultraviolet radiation in the environment, however, they can produce radicals that are harmful to aquatic organisms. There have been a number of studies that have reported the toxicity of titanium dioxide nanoparticles in the absence of light. An increasing number of studies are assessing the interactive effects of nanoparticles and ultraviolet light. However, most of these studies neglect environmentally-relevant experimental conditions. For example, researchers are using nanoparticle concentrations and light intensities that are too high for natural systems, and are ignoring water constituents that can alter the light field. The purpose of this review is to summarize the current knowledge of the photocatalytic effects of TiO₂ nanoparticles on aquatic organisms, discuss the limitations of these studies, and outline environmentally-relevant factors that need to be considered in future experiments.

Keywords: nanoparticles; photocatalytic; aquatic organisms; UV; titanium dioxide

1. Introduction

Manufactured titanium dioxide (TiO₂) nanoparticles (NP; 1-100 nm) are extensively used in industrial and consumer goods, including sunscreens, personal care products, solar cells, and food (Galley & Fardell 1997; Canesi et al. 2010; Clemente et al. 2014; Weir et al. 2012). As the production of TiO₂ and its use in consumer products increases, so too does the likelihood of release into the environment as a result of spills, use of products, or post-consumer degradation of material. Predicted environmental concentrations of TiO₂ NP in water are on the order of ppb (Mueller & Nowack 2008; Gottschalk et al. 2011), with expected environmental loads as high as 2,000,000 to 6,000,000 tons in the next 10 years (Robichaud et al. 2009). The accumulation of NP in the environment is likely to be non-uniform (e.g., Gottschalk et al. 2011), with higher concentrations in near-shore waters which are more impacted by run-off, wastewater discharge, and proximity to human populations. TiO₂ NP concentrations of 1-100 mg/L are known to have deleterious effects on fish and invertebrates (Blaise et al. 2008). Thus, there is a need to understand the environmental impact of these NP.

The photocatalytic properties of metal oxide NP (e.g., TiO₂, CeO₂ and ZnO) are well known (Wold 1993; Matsuo et al. 2001; Ma et al. 2013), and TiO₂ NP have been exploited in a wide range of photocatalytic applications including coated nanocomposites in sunscreens for ultraviolet (UV) protection and in 'green' technology cements that actively purify the air of pollutants (Galley & Fardell 1997; Torrey 2014). In aqueous media, TiO₂ NP can produce radicals when activated by UV light (see below). The radicals, in turn, can damage microbes and provide an antimicrobial property that has been utilized by medical, food and water-treatment industries (Rai & Bai 2011). In fact, most previous reports regarding the effects of photoactive TiO₂ nanoparticles on bacteria have focused on methods to eliminate microbes from drinking

water (e.g., Maness et al. 1999, Cho et al. 2004, Rincón & Pulgarin 2003), a topic that will not be covered in this review.

Although the beneficial application of TiO₂ is clear, such photoactivity can have ecotoxicological implications when these NP enter natural systems. Understanding the combined effects of TiO₂ NP and UV light on aquatic organisms under environmentally relevant conditions is critical, but data demonstrating such effects are sparse. Many studies used TiO₂ concentrations above those deemed environmentally relevant (≥ 10 ppm to ppt, or coated surfaces), cultured bacteria, and/or UV exposure levels 2 to 10 times higher than those found in the photic zone of most near-shore waters (e.g., Rincón & Pulgarin 2003; Benabbou et al. 2007; Alrousan et al. 2009; Chorianopoulos et al. 2011; Dhandapani et al. 2012). For example, Ma et al. (2012) exposed water fleas to NP concentrations above those deemed environmentally relevant (2 g/l), and Reeves et al. (2008) exposed goldfish skin cells to UVA (340-420 nm; 0.007-0.03 mW/cm²) for 2 hours, a light duration and dose that highly underestimates natural conditions. Some potential photocatalytic effects have been demonstrated under environmentally relevant conditions. Faria et al. (2014) exposed zebrafish embryos to full-spectrum light (280-800 nm; 1.5-4.5 mW/cm²) and 1 mg/L NP, resulting in reactive oxygen species (ROS) production and oxidative stress.

Whereas only a few aquatic organisms have been investigated to date, and most studies have been performed on model organisms in order to streamline high-throughput toxicity tests, there is growing interest in the impact of photoactive NP in aquatic environments. Thus, in this review, we summarize current knowledge of the photocatalytic effects of TiO₂ on aquatic organisms, discuss the limitations of these studies, and outline the experimental and environmental factors that need to be considered in future experiments (e.g. organic matter, marine snow, light levels).

In exploring the photoactive effects of metal-oxide NP on aquatic organisms, it is crucial that experimental conditions are standardized and environmental parameters defined so that a more accurate understanding of potential impacts can be obtained.

2. Photocatalytic reactions in aqueous media

An understanding of the photophysics and photochemistry of TiO_2 in water is essential in predicting not only the effects of TiO_2 on aquatic systems, but also how aqueous constituents affect the photocatalytic processes. TiO_2 is a semiconductor, which implies two critical properties (Mills & Le Hunte 1997). First, the wave-functions of the constituent atoms interact with each other over long distances. This means that instead of atomic orbitals combining to form discrete molecular orbitals, as when atoms combine to form molecules, atomic orbitals combine throughout an entire crystal to form bands (see **Figure 1**). Second, as the bands are populated from low to high energy with the electrons provided by the Ti and O atoms, the last electrons added exactly fill one band. This highest-energy filled band, the valence band, is akin to the highest occupied molecular orbital (HOMO) of a molecule. A bandgap (E_g) separates the top of the valence band from the bottom of the conduction band, the lowest unfilled band, which is akin to the lowest unoccupied molecular orbital (LUMO) of a molecule. In a perfect semiconductor at 0 K, the valence band would be completely filled and the conduction band completely empty. But TiO_2 forms with a slight oxygen deficiency, which leaves a small permanent electron population in the conduction band.

A photon whose energy ($h\nu$) is greater than or equal to E_g can promote an electron from the valence band to the conduction band. This produces the charge separation that drives all subsequent chemistry, since the conduction band electron (e_{CB}^-) is at a very negative potential, making it strongly reducing, and the ‘hole’ left behind in the valence band (h_{VB}^+) is at a very

positive potential, making it strongly oxidizing. Indeed, in principle they are capable of reducing water to H_2 and oxidizing it to O_2 , although this is kinetically slow (Fujishima & Honda 1972). The absorption-band cut-off for TiO_2 varies with the specific crystalline form. For example, the rutile and anatase forms have band gaps of ca. 3.0 and 3.2 eV, corresponding to absorption-band cut-offs of 413 and 388 nm, respectively (Yang et al. 2004; Nakano et al. 2009). Doping with certain elements can change the band-gap width, causing a shift in the absorption edge wavelength (Asahi et al. 2001; Shirley et al. 2010).

Once an electron-hole pair has been formed by photoexcitation, there are three main possibilities for their immediate fate— (1) reaction: one or both charges react with molecules adsorbed to the surface; (2) recombination: the electron returns to the hole, generally dissipating the absorbed photon energy as heat; (3) trapping: one or both charges migrate to defect states in the material. By far the most frequent outcome is recombination: about 90% of electron-hole pairs formed by photoexcitation quickly recombine with no chemical effect (Serpone et al. 1995). Trapping reduces the energy available to the trapped charge, making it a somewhat weaker reductant or oxidant, but it also serves to spatially separate the two charges from each other, making recombination less likely (Hoffmann et al. 1995). A trapped charge can still be lost ultimately to recombination, but the trap state gives it more time to engage in a chemical reaction. Ultimately, the fraction of photo-generated charges resulting in chemical reactions with other molecules is low, frequently in the region of 1 to 2% (Stafford et al. 1997).

Reaction with molecules of interest can occur either directly or indirectly. In a direct reaction, an organic molecule on the TiO_2 surface is reduced by e_{CB}^- , or is oxidized by having an electron stripped by h_{VB}^+ . In an indirect reaction, the direct reaction is with water molecules, dissolved oxygen, or other intermediates. Importantly, direct and indirect reactions both produce

radicals, since each reaction involves only one electron and therefore upsets the even number of valence electrons found in most molecules, leaving an electron unpaired. A direct reaction with an organic molecule therefore produces an unstable organic radical, which will undergo further reactions in solution that are likely to result in one or more bond cleavages. Indirect reactions are important because the initial products are radicals that can then attack organic molecules in aqueous solution. Radicals participate in a variety of reactions with organic and inorganic molecules in solution, spawning a wide set of radical species (Hoffmann et al. 1995).

Thus, UV irradiation of TiO_2 in water quickly results in a suite of radicals with complex chemistry (see **Figure 2**). Two indirect reactions will be singled out as having particular importance. First, the hole can oxidize water (or hydroxide ions) to form the hydroxyl radical, $\text{OH}^\bullet$. This is a very strong and indiscriminant radical with rapid kinetics for reaction with almost any molecule it encounters. There has been a long debate in photocatalysis as to whether the hole is a strong enough oxidant to generate a true free hydroxyl radical, or only a bound species. A free hydroxyl radical can diffuse through the surrounding water to attack various dissolved molecules, but because of its reactivity it does not travel more than a few nanometers from the particle surface. On the other hand, a bound hydroxyl radical is simply the chemical speciation of a trapped hole. It has been demonstrated that an irradiated TiO_2 film can degrade an organic film across an air gap (Tatsuma et al. 1999; 2001), indicating mobility of some active species. Hydroxyl radicals in particular have been detected in the gas phase over irradiated TiO_2 powder, indicating that a free $\text{OH}^\bullet$ is generated (Murakami et al. 2007). Second, the electron can be taken by molecular oxygen to form the superoxide radical $\text{O}_2^{\bullet-}$. This radical product engages in a series of reactions with water, oxygen, and additional e_{CB}^- , generating a suite of activated oxygen species including $\text{HO}_2^\bullet$, $\text{HO}_2^{\bullet-}$, H_2O_2 , and $\text{OH}^\bullet$. All of these are reactive and can oxidize organic

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4 154 molecules. Any of the uncharged species could be involved in the above mentioned reaction
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6 155 across an air gap; while in water, all of the species can travel in solution to interact with organics.
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9 156 It is important to note that a reaction with e_{CB}^- or h_{VB}^+ is consequential not just for the
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11 157 radicals it produces, but because it deprives the opposite charge in TiO_2 of a partner for
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13 158 recombination, ensuring that both charges will ultimately react. In particular, it is thought that
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15 159 the reaction of O_2 with e_{CB}^- is important mainly because it is relatively rapid and allows h_{VB}^+ time
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17 160 to perform an oxidation reaction. Molecular oxygen therefore plays the role of an electron
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19 161 scavenger, providing time for h_{VB}^+ to react.
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23 162 **3. Light intensity and attenuation in aquatic environments - a critical consideration**

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26 163 An understanding of the interactive effects of UV light and TiO_2 NP on aquatic organisms
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28 164 requires an appreciation and comprehension of the light fields experienced by the organisms in
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30 165 the natural environment. Latitude, season, time of day, and atmospheric conditions all act to
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32 166 modulate the amount of light energy that reaches the surface of lakes, rivers and marine waters.
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34 167 After transmission across the surface, light is attenuated with depth through interaction with
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36 168 water molecules and the optically active constituents of the water column. While the literature of
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38 169 in-water optics is extensive, a basic explanation is provided here. For more information, the
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40 170 reader is directed to the references cited, particularly Kirk (1994) and Mobley (1994).
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45 171 Natural waters contain a variety of organic and inorganic substances which absorb and
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47 172 scatter light differentially by wavelength (Kirk 1994), including colored dissolved organic matter
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49 173 (CDOM), phytoplankton, and non-algal particles (NAP) such as mineral and organic sediments,
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51 174 bacteria, and zooplankton (Belzile et al. 2004). The combination of these substances, therefore,
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53 175 determines the overall intensity (total photon or energy flux) and spectral distribution (the
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55 176 relative amount of photons or energy at a given wavelength) of the local light field.
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4 177 Attenuation, the total decrease in light with distance through the water column, is the sum
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6 178 of absorption and scattering (Dierssen & Randolph 2013; Kirk 1994; Mobley 1994). Absorption
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9 179 removes photons from the light field, while scattering redirects them (Mobley 1994). Scattering
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12 180 increases the chances that a photon will be absorbed by increasing its path length. Some scattered
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14 181 photons will be transmitted back up and across the surface, leaving the water column entirely.
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16 182 This includes those reflected off of the bottom in shallow environments. These upwardly
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19 183 scattered photons represent only a tiny fraction of the light initially entering the water column,
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21 184 with the vast majority being eventually absorbed (Dierssen & Randolph 2013).

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24 185 Pure water absorption is significant in the UV, weak in the blue, and increases
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26 186 exponentially with wavelength (Lu 2006; Morel et al. 2007; Pope & Fry 1997; Smith & Baker
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29 187 1981). As a result, clear oceanic waters without significant photoactive constituents (like CDOM
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31 188 or phytoplankton) transmit blue and green light to depth, while red wavelengths are depleted
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34 189 near the surface (Kirk 1994; Morel 1988). Phytoplankton will absorb blue and red wavelengths
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36 190 while transmitting green, according to the absorption spectra of chlorophyll *a* (Kirk 1994) and
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39 191 other pigments. Different groups of phytoplankton contain a variety of photosynthetic pigments,
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41 192 so the exact absorption spectrum will depend on the identity and concentration of the
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43 193 phytoplankton (Morel 1988; Dierssen et al. 2006; Roy et al. 2011). Produced from the decay of
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46 194 plant matter and abundant in terrestrial and coastal waters, CDOM is operationally defined as
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48 195 those substances which pass through a 0.2 μm filter (Dierssen & Randolph 2013) and absorbs
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51 196 strongly in the UV and blue wavelengths, but absorption decreases exponentially towards the red
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53 197 (Belzile et al. 2002; Kirk 1994; Twardoski et al. 2004). The absorption of organic and mineral
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56 198 particles is typically greatest at short wavelengths, i.e. the UV and blue, but the exact shape of
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58 199 the absorption spectrum is determined by the properties of the particles themselves (Morel &
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Ahn 1990; Belzile et al. 2004; Kirk 1994), such as mineral content and organic coating. These particles are the optically active component most responsible for scattering (Aurin & Dierssen 2012; Babin et al. 2003a; Kirk 1994; Mobley 1994; Vincent et al. 2001).

Aqueous environments are a complex combination of water, CDOM, particles, and plankton. As a result, the local light field can vary dramatically with time, weather, region, and depth, particularly in coastal ocean areas and in-land water bodies (Babin et al. 2003b; Belzile et al. 2004; Etheridge & Roesler 2004; Kirk 1994; McPherson et al. 2011; Vincent et al. 2001). A common, straightforward metric of light intensity is Photosynthetically Available Radiation (PAR), the integrated photon flux from 400 – 700 nm (Dierssen & Randolph 2013; Kirk 1994). However, PAR provides only a relative indicator of UV levels. For the study of photocatalysis in natural waters, it is imperative that the intensity and spectral distribution of the light at relevant wavelengths be understood. While these can be measured *in situ* with spectrally resolved irradiance sensors, it is possible to accurately model light through the use of software such as Hydrolight (Sequoia Scientific) which can account for a variety of latitudes, times, atmospheric conditions, and water column compositions. For example, optical simulations of Long Island Sound (LIS) irradiances just below the surface are more than two times higher in the summer than in the winter (**Fig. 3**; integrated PAR values: 16.9 – 40.4 mW/cm²). Additionally, LIS irradiance changes drastically with depth, dropping to only 2.04 mW/cm² at 5 m depth (**Fig. 4**). Comparing the irradiance in LIS (41 °N) to three other latitudes at the same depth and season shows that latitude has a significant impact (see **Fig. 5**). A latitude of 1 °N has an irradiance of 43.3 mW/cm² just below the surface, which is twice the irradiance at 48 °N. The parameters used in these simulations are described in the figure legends.

In addition to its spectral and intensity components, the directionality of underwater light

must be considered for accurate characterization. Mobley (1994) provides a thorough characterization, elements of which are discussed here. The downward planar irradiance (E_d) is the downward directed light from the entirety of the upper hemisphere at a given depth (Dierssen & Randolph 2013; Mobley 1994). That is, the photons directed downwards within the water column through a horizontal plane. Light decreases exponentially according to the downward diffuse attenuation coefficient (K_d), as described by Beer's Law (Kirk 1994; Dierssen & Randolph 2013; Russell & Dierssen 2015):

$$E_d(z) = E_d(0^-)e^{-K_d z} \quad (1)$$

where $E_d(z)$ is downward irradiance at depth z , and $E_d(0^-)$ is irradiance just below the surface. Both $E_d(0^-)$ and K_d are functions of the wavelength (λ), and therefore $E_d(z)$ has different values for every wavelength within the region considered (**Fig. 3**). This relationship is the basis for the differential penetration to depth of regions of the light spectrum.

In particular, UVA/B is rapidly attenuated by water, CDOM, and sediments (Babin et al. 2003b; Belzile et al. 2002; Vincent et al. 2001), with values of UVA often $< 1 \text{ W/m}^2/\text{nm}$ at depths of $> 1 \text{ m}$ (Wozniak & Dera 2007). In many coastal environments, a downward-transmission maximum occurs between 500 and 550 nm, decreasing rapidly above and below these wavelengths (Kirk 1994). For example, just below the surface of the ocean the downward irradiance, $E_d(0^-)$, at 400 nm can be 3 to 5 times higher than the downward irradiance at 320 nm (UVA/UVB delineation; EUR 22217 EN 2006). Incident power of sunlight drops off around 450 nm in air as well, however, the change with depth is more drastic in water compared to air. Nonetheless, UV radiation does penetrate to depths that are ecologically significant (Vincent et al. 2001), and many aquatic species have mechanisms for photoprotection (Häder et al. 2007). The optical attributes of natural waters have not been fully considered in most previous studies

with photoactive NP, and research that has reported phototoxicity to aquatic organisms using ‘terrestrial’ irradiance levels should be interpreted with caution (e.g., Matsuo et al. 2001; Hund-Rinke & Simon 2006).

4. Interactions with the biological and physicochemical elements of aquatic environments

The environmental fate (transport & retention) of NP is influenced by properties of the surrounding media, including pH, ionic strength, and the concentration of organic material (Guzman et al. 2006; Domingos et al. 2009; French et al. 2009; Petosa et al. 2010).

Physicochemical interactions between NP and aqueous constituents have been well described, and have been demonstrated to affect agglomeration potential (Sillanpää et al. 2011; Shih et al. 2012; Doyle et al. 2014) and formation of homo- and hetero-aggregations (Brant et al. 2005; Navarro et al. 2008; Xie et al. 2008; Domingos et al. 2009; Johnson et al. 2009; Sharma 2009). Organic constituents of aqueous environments can also affect the photoreactivity of TiO₂ NP. For example, dissolved organic matter (DOM) can accelerate degradation of surfactant coatings of some NP, increasing their reactivity (e.g., glycerine & aluminum coating on UV-Titan, a type of TiO₂ NP; Aiken et al. 2011). Therefore, as nanomaterials ‘age’ in the environment their potential for risk can increase. On the other hand, DOM and natural organic matter (NOM) can influence the bioavailability of nanomaterials through NP association with other particles; the degree by which bioavailability is affected, however, depends on the organism and route of exposure (Aiken et al. 2011; Yang 2009). There is also evidence that NOM, comprised mainly of humic and fulvic substances, is a photosensitizer and ROS quencher (Golanoski et al. 2012; Nurmi & Tratnyek 2002), able to reduce the photoactivity of NP. Natural organic matter has been shown to decrease the toxicity of TiO₂ NP to *Daphnia magna* under natural light conditions (4-hr SSR) (Li et al. 2016). Similarly, Yang et al. (2013) found that humic acids (HA) coating

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4 268 TiO₂ NP increased stability and reduced NP exposure to zebrafish. However, when the solutions
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6 269 were exposed to simulated sunlight, the photoactive NP degraded the HA coating, and oxidative
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9 270 deoxyribonucleic acid (DNA) damage and mortality increased in the zebrafish. Lastly, it has
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11 271 been found that lake water with high dissolved organic carbon (DOC) concentrations make NP
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14 272 more stable and therefore more toxic to bacteria (Farkas et al. 2015). These findings highlight the
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16 273 complexity of NP interactions with aqueous constituents and the importance of considering
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19 274 environmental factors when assessing risk of nanomaterials. In addition to chemical properties of
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21 275 water, there are also physical and biological processes that can affect NP interactions.

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24 276 Physical and biological processes of aquatic systems operate to aggregate smaller
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26 277 particles into larger masses (100s of μm) referred to as marine, lake, or river snow (aka,
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29 278 macroaggregates, flocs; Alldredge & Silver 1998; Jackson 1990; see fig. in Azam & Malfatti
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31 279 2007). In the marine environment, large proportions (> 70%) of natural particulates are
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33 280 incorporated in marine snow at certain times of the year (Alldredge et al. 1993; Crocker &
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36 281 Passow 1995). In the laboratory, TiO₂ NP can be incorporated into marine snow at efficiencies
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38 282 that exceed ca. 70% after 24 hrs. If such incorporation occurs in the natural environment it would
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41 283 expose the diverse community of microorganisms that reside in marine snow (Azam & Long
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43 284 2001; Simon et al. 2002; Kiørboe et al. 2003; Lyons et al. 2007) to a high concentration of NP
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46 285 relative to the water. Although aggregates tend to sink out of the photic zone faster than
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48 286 individual particles (Stokes Law; Hill 1998; Waite et al. 2000), tidal forces and storm events can
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51 287 re-suspend settled aggregates and re-expose them to light (e.g., Lyons 2008). Within an
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53 288 aggregate, some NP could be shielded from light and be less reactive, or brought into contact
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56 289 with microorganisms producing more deleterious effects. Given the importance of organic
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4 291 aquatic systems (e.g., Alldredge & Jackson 1995; Alldredge 2000; Kiørboe 2001; Simon et al.
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6 292 2002), understanding the effects of NP and light on the microbial communities that inhabit
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9 293 aggregates is a fertile area for future research.

11 294 **5. Nanoparticulate and photocatalytic effects of TiO₂ NP on aquatic organisms**

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14 295 Similar to other nanomaterials, TiO₂ NP have the potential to be toxic to cells and tissues,
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16 296 likely attributed to their infinitesimal size, high surface area and reactivity (Johnston et al. 2009;
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19 297 Blaise et al. 2008; Schultz et al. 2014; Scown et al. 2010). The infinitesimal size of
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21 298 nanomaterials facilitates their movement across cellular membranes and into organelles where
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24 299 they can generate ROS that can damage DNA and proteins (Simic & Jovanovic 1986; Deng &
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26 300 Fridovich 1989), and interfere with the structural integrity of the cell membrane (Moore 2006).
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29 301 Some metal-oxide NP also are photocatalysts. When activated by sufficient light energy, they
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31 302 form radicals that can produce oxidative stress and damage cells in contact with the NP (see
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33 303 section 2 above; Fujishima et al. 2000; Mills & Lee 2002; Sharma 2009). Although the
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36 304 absorption-band cut-off of various forms of TiO₂ are different, most absorb strongly in the UVA
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38 305 (320-400 nm) and UVB (290-320 nm) range (ca. 80-90% absorption; Li & Ishigaki 2003). Thus,
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41 306 NP interacting with UV light in the natural environment can have deleterious effects on
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43 307 organisms that are in addition to those effects caused by NP in the absence of light.

45 308 Studies on several species of fish and invertebrates demonstrate that TiO₂ NP can be ingested
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48 309 or otherwise taken up by aquatic organisms (e.g., Griffitt et al. 2008; Galloway et al. 2010),
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51 310 including by several species of bivalve molluscs (e.g., Canesi et al. 2010; Couleau et al. 2012;
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53 311 Doyle et al. 2015). Bioaccumulation of TiO₂ NP has also been reported in viscera and gills of
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55 312 carp, intestine and gills of goldfish and tissues of rainbow trout (Sun et al. 2007; Zhang et al.
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58 313 2007; Mehmet et al. 2013; Federici et al. 2007; see also review by Nam et al. 2014). The level of
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bioaccumulation and the amount of time required for elimination of nanoscale titania from tissues, however, has been little studied. For some types of TiO₂ NP, oysters, *Crassostrea virginica*, and mussels, *Mytilus edulis*, can rapidly eliminate > ca. 90% of the material from gill, mantle, and visceral mass tissues 6 to 12 hrs after acute exposure (Doyle et al. 2015; 2016).

Under conditions of low or no UV exposure, TiO₂ NP have been shown to cause mortality, reduced growth, and negative impacts on cells and DNA of aquatic organisms. For example, nanoscale titania can be toxic to *D. magna* (Adams et al. 2006; Clément et al. 2013), and cause oxidative stress in the rainbow trout and common carp (Federici et al. 2007; Lee et al. 2012). Adverse effects in some species of bivalves have also been reported including reduced phagocytosis capability of hemocytes after 2 to 24-hour exposure times (Chalew et al. 2012; Canesi et al. 2010; Ciacci et al. 2012; Couleau et al. 2012), and further impacts on the immune function including decreased hemocyte viability, reduced phagocytosis capability and an increase in ROS production after longer, 4 to 9-day exposure times (Barmo et al. 2013; Canesi et al. 2014; Wang et al. 2014). For a more detailed review on the impacts of NP on bivalves, we refer readers to the review by Canesi et al. (2012). Adhesion of TiO₂ NP to the exoskeleton of planktonic organisms can also produce physical effects such as loss of mobility (Baun et al. 2008; Miller et al. 2012), and NP incorporation into phytoplankton cells can limit growth (Li et al. 2015). In contrast, some studies have reported little or no toxic effects from TiO₂ NP on several aquatic species, including *D. magna* (48-hr), *Daphnia pulex* (48-hr), *Danio rerio* (48-hr), and *Oncorhynchus mykiss* (96-hr), (e.g., Warheit et al. 2007, 10-100 mg/l; Griffitt et al. 2008, up to 10 mg/l), despite using very high concentrations of TiO₂ NP. The reasons behind the minimal toxicity in these cases is uncertain, but are possibly due to variations in experimental design (e.g., sonication techniques). Although the above studies provide a good foundation, many of

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4 337 them neglect environmentally-relevant interactions, including acute exposure times (< 48 hours)
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6 338 and high NP concentrations (> 10 mg/L), and thus are difficult to extrapolate to natural
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9 339 ecosystems where animals likely experience chronic exposures of lower NP concentrations.
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11 Increasing numbers of studies have been designed to examine photocatalytic effects of TiO₂
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14 341 on aquatic organisms, in particular the interactive effects of UV radiation and TiO₂ NP on
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16 342 freshwater species. UV was most commonly delivered from benchtop lamps containing outputs
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19 343 in the 340-420 nm range. Under such exposure conditions, nanoscale TiO₂ (anatase) and UVA
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21 344 can produce low cell viability in goldfish skin cells after only 2 hours (340-420 nm; Reeves et al.
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24 345 2008), and suppress growth in both freshwater and marine phytoplankton (Lee & An 2013,
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26 346 Metzler et al. 2011, Miller et al. 2012). Similarly, Vevers and Jha (2008) found that the
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29 347 genotoxic effects of TiO₂ was enhanced when fish (*O. mykiss*) cells were exposed to NP (e.g., 5-
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31 348 50 µg/ml) and UV light (340-420 nm; 0.08-0.17 mW/m²) *in vitro*. P25 NP were significantly
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34 349 more toxic to *D. magna* when the animals were exposed to UVA light (340-420 nm, 0.56
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36 350 mW/cm²; Amiano et al. 2012). In contrast, neither anatase nor rutile forms of TiO₂ NP were
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39 351 toxic to *Daphnia similis* when exposed to UVA and visible light for 2 days (Marcone et al.
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41 352 2012). In a number of studies, a Simulated Solar Radiation (SSR) apparatus was used which
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43 353 included a temperature-controlled water bath and lamps that mimic solar radiation in the range of
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46 354 280 – 800 nm. The output of SSR can be adjusted using neutral density filters to lower or
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48 355 eliminate UV. Those studies that use SSR to achieve a 4-hour dose of UV during each
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51 356 experimental day have all shown negative impact of P25 NP, resulting in mortality in *D. magna*,
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53 357 Japanese medaka fish larvae, and freshwater benthic invertebrates (Ma et al. 2012, Li et al.
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55 358 2014a & b), and ROS production and oxidative stress in *D. rerio* embryos (Faria et al. 2014).
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A few studies have used natural light to examine the interaction between NP and environmentally-relevant irradiances. One marine study performed on hepatopancreas tissue of oysters (*C. virginica*) used controlled levels of natural sunlight to ‘activate’ NP solutions prior to adding them to the tissue samples, and found a decrease in lysosomal stability (Johnson et al. 2015). Unfortunately, the researchers only measured PAR (400-700 nm) so the amount of UV and the wavelengths to which the NP were exposed is unknown. Additionally, the kinetics of photoactive NP are rapid, with reactions stopping once NP are no longer exposed to light. Thus, the method of pre-activating NP prior to experiments in order to achieve photocatalytic effects does not align with known chemical reaction times and researchers need to demonstrate the lasting effects of pre-exposure using proxy assays. Mansfield et al. (2015) also used natural outdoor sunlight and filters to achieve 10, 50 and 100% ambient light levels and found significant mortality of *D. magna*, however, this study used high NP concentrations (20 and 200 ppm).

Although valuable data have been obtained on the interactive effects of UV light and nanoscale titania, it is difficult to compare and contrast results of previous studies because of the varied methodologies employed. For example, the light levels used in the aforementioned experiments ranged from UVA only (340-420 nm) to full spectrum (280-800 nm), and UV exposure durations ranged from minutes to days (see **Table 1**). Another inconsistency is using high UV intensities that correspond to irradiances above the water surface instead of a light field that matches the organism’s environment (see section 3 above). Additionally, none of the studies measured how much UV was absorbed by the experimental containers in which the animals were held, which included glass beakers, petri dishes and containers with and without lids. Lastly, there is a lack of consideration of the constituents of the aqueous media that could affect UV

intensity. Whether results of these studies can be applied to the natural environment, therefore, is uncertain.

Certainly, as a photoreactive material TiO_2 NP have the potential to produce deleterious effects on organisms at all levels of biological organization when activated by the correct wavelengths of UV at sufficient intensities. A main consideration, however, is whether the selected wavelengths and their intensities used in laboratory-based experiments are relevant to organisms that live within the natural environment. For example, UV penetration in water changes drastically with different water properties (e.g., oceanic vs. coastal water bodies, chlorophyll *a*, CDOM and turbidity), and latitude and season both act to alter the light spectrum in the natural environment (Kirk 1994; Mobley 1994). It has also been shown that 10% of the surface irradiance penetrates to a depth of 10 meters in some coastal waters, and UVB is still detectable at 1-meter depth in some locations (Booth et al. 1997). Farkas et al. (2015) found that ecologically significant UV-PAR levels (below surface and 1 m depth) did not increase toxicity of TiO_2 NP to freshwater bacteria compared to treatments without UV-PAR exposure. Another consideration is the interactions between NP and dissolved, colloidal, and particulate material of the system. As highlighted in section 4 above, it has been shown that the interactions of NP with water constituents such as DOM and DOC can complicate the behavior of NP in water, including the homo- and hetero-aggregation of NP. It is crucial that researchers understand how NP interact with the surrounding aqueous media, and how UV intensity can change with different water properties.

6. Recommendations for future studies

There have been a limited number of studies investigating the photocatalytic effects of TiO_2 NP on aquatic organisms. The few studies that have been conducted all differ in NP

concentration, light intensity, and duration, and highlight a lack of consensus in the field on how to perform these types of experiments. For future studies, it is paramount to carefully define the light level used in experimental exposures. Additionally, radicals produced by UV activation are short-lived, so effects on oxidative stress cannot be accurately measured without constant UV dosing. Researchers also need to be careful when using SSR, because while it is a good starting point, its output is not intended for aqueous environments. Filters can be used to adjust intensity and wavelength of exposure, but these simulated light levels must be appropriate for the depth, latitude, season, and water column parameters that the organism being studied would experience. Also, given UV-damage repair mechanisms, such as those well-studied in phytoplankton (see Sinha & Häder 2002), the question remains - is a high dose over 4 hours comparable to a lower dose over a longer period? Therefore, we suggest that the duration of UV exposure be relevant to natural light fields in order to maintain NP reactivity (e.g., 15 hour light exposure : 9 hour dark exposure for summer light levels). Additionally, UV intensity can change in response to physical and biological characteristics of the water (i.e., chlorophyll *a*, turbidity, CDOM, DOC). Specifically, for marine systems, it is important to consider the incorporation of NP into larger aggregations, and how certain physicochemical properties (i.e., mixing, DOC) can affect NP suspension in experimental matrices, both of which have the potential to alter the photoreactivity and exposure of nanomaterials. Therefore, it is necessary to use an optical model (i.e., Hydrolight) that incorporates water characteristics in order to select an appropriate light spectrum. Prior to experimentation, researchers should characterize the natural system in which the organisms they are studying live. The output from experimental solar lamps should then be matched to the modeled light spectrum that corresponds to the natural system using a spectroradiometer. It is also critical to use a light source that includes both UVA and UVB

wavelengths, as they have been shown to be ecologically significant, even in some coastal systems (Booth et al. 1997). Additionally, studies that used only UV light should be re-evaluated because visible light is required for photolyases which repair DNA caused by UV damage (Rupert et al. 1958). Visible light also is necessary in some cases to maintain the normal behavioral and physiological functions of some animals (e.g., phytoplankton and zooplankton). Finally, experiments that are being performed in a closed system should include reported measurements of UV-vis absorption by experimental bottles and containers with lids.

Although only a relatively small number of aquatic organisms have been investigated, there is increased interest in assessing the impacts on aquatic environments. Therefore, the recommendations we outline here are important for future studies to more accurately predict the effects of TiO₂ NP on aquatic organisms. We suggest that researchers in this field move away from a toxicological perspective (i.e., eliciting mortality with extreme doses) and towards an ecotoxicological approach where animal exposures are carried out under environmentally-relevant conditions. The behavior of NP in aqueous media suggests they have the ability to impact a wide range of organisms, from the smallest particle-attached bacterium to a filter-feeding bivalve. We also must consider that certain organisms could be more vulnerable to photoreactive NP depending on their natural history, e.g., translucent filter feeders that are frequently exposed to sunlight. It is necessary to carry out experiments considering natural conditions to better define the risk associated with photoreactive nanomaterial exposures.

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Declarations of interest

The author's affiliation is as shown on the cover page. The authors have sole responsibility for the writing and content of the paper.

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Table 1. Summary of photocatalytic studies on aquatic organisms using TiO₂ NP.

Publication	NP Type	Light intensity	Light duration	Experimental duration	[NP]	Organism	Effect
Amiano et al. 2012	P25	UVA (0.56 mW/cm ²)	16 hours UVA : 8 hours dark	2 days	1 - 10.5 mg/L	<i>Daphnia magna</i>	increased immobilization; increased toxicity with UVA
Faria et al. 2014	P25	SSR; 1.5-4.5 mW/cm ²	4 hours	6 days	1 mg/mL	<i>Danio rerio</i> embryos	ROS production/oxidative stress
Farkas et al. 2015	Anatase/rutile	Sunny conditions at Lake Erken (Sweden)	summer day time hours	5 days	15 - 1000 µg/L	freshwater bacteria	UV/PAR did not enhance toxicity
Johnson et al. 2015	unreported	PAR; 386-694 uE/m ² /sec	4 hours	24 hours	50 µg/L	<i>Crassostrea virginica</i> hepatopancreas tissue	Decreased lysosomal stability
Lee & An 2013	P25	UVA (316-400 nm); UVB (281-315 nm)	20 min pre-irradiation	3 days	0.5 - 10 mg/L	green algae <i>Pseudokirchneriella subcapitata</i>	suppressed growth
Li et al. 2014(a)	P25	SSR; 0.94 mW/cm ²	4 hours	2 days	20 mg/L	benthics: <i>H. azteca</i> , <i>L. variegatus</i> & <i>C. dilutus</i>	Significant mortality
Li et al. 2014(b)	P25	SSR; 0.22 mW/cm ²	4 hours	2 - 4 days	100 mg/L	benthic amphipod, <i>Hyalella azteca</i>	21-fold increase in toxicity with SSR
Ma et al. 2012	P25	SSR; UV 1.7 mW/cm ²	4 hours	2 days	2 g/L	<i>Daphnia magna</i>	LC50 29.8 ug/l
-----	P25	SSR; UV 1.7 mW/cm ²	4 hours	4 days	2 g/L	Japanese medaka fish larvae	LC50 2.2 mg/l
Mansfield et al. 2015	Anatase	Natural sunlight (avg. 4.7 mW/cm ²)	summer day time hours	8 hours	20 & 200 mg/L	<i>Daphnia magna</i>	significant mortality
Metzler et al. 2011	P25	1764 lux	4 days	4 days	10 - 1000 mg/L	green algae <i>Pseudokirchneriella subcapitata</i>	suppressed growth; NP adhesion to surface
Marcone et al. 2012	Anatase & rutile	UV-vis fluorescent lamp	2 days	2 days	100 mg/L	<i>Daphnia similis</i>	Non-toxic
Miller et al. 2012	Anatase/rutile	UV-vis fluorescent lamp	14 hour light : 10 hour dark	4 days	1 - 7 mg/L	marine phytoplankton	suppressed growth; NP adhesion to surface
Reeves et al. 2008	Anatase	UVA (340-420 nm)	2 hours	2 hours	1000 µg/mL	goldfish skin cells	lowered cell viability
Vevers and Jha 2008	Rutile/anatase	0.15 - 0.3 mW/cm ² ; UVA (340-420 nm)	20 - 40 minutes	4 hours - 2 days	500 µg/mL	rainbow trout gonadal tissue	cytotoxicity; DNA strand breaks

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 1. Electronic structure of a semiconductor, with unfilled conduction band and filled valence band separated by the bandgap energy E_g .

Figure 2. Key reactions of irradiated TiO_2 including (top left) reduction of O_2 by a conduction band electron, giving rise to activated oxygen species, and (bottom right) oxidation of hydroxide by a valence band hole, forming a hydroxyl radical.

Figure 3. Seasonal variation of irradiance spectrum from Hydrolight simulations at ocean surface $E_d(0^-)$ in middle of Long Island Sound ($41^\circ\text{N}/72^\circ\text{W}$). Water parameters included chlorophyll a (0.1, 5, 1 and 7 mg/m^3 for winter – fall, respectively; data from NOAA CoastWatch 2016), CDOM (0.3 m^{-1} ; Kirk 1994), and total suspended solids (TSS; 3 g/L ; NOAA CoastWatch 2016). Color bar corresponds to PAR wavelengths and adjacent UV and infra-red (IR) boundaries are indicated.

Figure 4. Variation in irradiance spectrum from Hydrolight simulations at depths 0, 1, 5 and 10 m in Long Island Sound ($41^\circ\text{N}/72^\circ\text{W}$) during summer. Water parameters included chlorophyll a (1 mg/m^3 ; data from NOAA CoastWatch 2016), CDOM (0.3 m^{-1} ; Kirk 1994), and TSS (3 g/L ; NOAA CoastWatch 2016).

Figure 5. Irradiance spectrum from Hydrolight simulations at ocean surface $E_d(0^-)$ during fall at four different latitudes ($1, 26, 41$ and 48°N). Water parameters included chlorophyll a (7 mg/m^3 ; data from NOAA CoastWatch 2016), CDOM (0.3 m^{-1} ; Kirk 1994), and TSS (3 g/L ; NOAA CoastWatch 2016).