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Bacterial mechanisms for coping with the population and molecular level affects of ultraviolet radiation

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Bacterial Mechanisms for Coping with the Population and Molecular Level Affects of Ultraviolet Radiation.

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Abstract:

Solar ultraviolet radiation can have beneficial as well as detrimental effects on freshwater bacteria. Furthermore, bacteria are affected by ultraviolet radiation at both the community and metabolic level. Reported here are the results of a series of field and lab experiments exploring the damage and recovery of bacteria exposed to ultraviolet radiation (UVR). In the lab experiments, three isolated strains of bacteria were exposed to UV-B in a standardized UV lamp phototron. Cultures were exposed to damaging (UV-B) and repairing (UV-A and white light) radiation, with colony forming units counted as an indicator of survival. By denying or allowing repair radiation we explored the degree to which each strain uses light dependent repair mechanisms. UV-B dose rate was varied to explore the degree to which reciprocity holds in relation to UV damage and bacteria. Strains were found to be variable both in their tolerance of UV-B as well as in their usage of PRR. Dose rate was also found to be an important determinant of survival, with lower survival when radiation dose rate was higher.

With field experiments I explored accumulation and repair of DNA damage (cyclobutyl pyrimidine dimers) in UV transparent bags during a diel cycle. Organisms were held at lake surface and allowed to accumulate damage until midday, after which they were divided into treatments. Some bags received full sun while others were covered with UV-B blocking acrylic or opaque plastic to block all light. Repair rates were always faster when allowed to receive repair radiation, indicating
usage of photorepair. Damage in bags was always higher than lake levels, indicating that lake mixing reduced damage under natural circumstances. Damage was higher in the bacterial size fraction than larger organisms, indicating overall sensitivity of bacteria as a group. Experiments were repeated in April and July to look for seasonal effects. Overall damage was greater in the April experiments despite lower levels of UV-B, indicating that other factors such as water temperature may affect damage and repair dynamics in this system.
Introduction:

Ultraviolet radiation (UVR) is divided into three types: UV-C (100-290nm), UV-B (290-320nm), UV-A (320-400nm). The vast majority of UV-C is blocked by stratospheric ozone before it reaches earth’s surface. Ozone also blocks incoming UV-B, but enough penetrates that both UV-B and UV-A are environmentally significant. Anthropogenic ozone loss has led to a rise in the UV-B at earth’s surface over the past few decades (Madronich 1995). Although this has been most dramatic at high latitudes where ozone loss is prevalent, UVR rise has been detected in temperate zones as well and is predicted to persist for decades (Taalas et al. 2000). The interest generated by ozone loss has also generated questions about UVR dynamics in general, especially its influence upon ecological processes since many areas receive high levels of UVR even without ozone loss.

Much of the study of UVR has focused on aquatic systems. Exposure to UVR in aquatic environments is governed by several factors. UVR first must penetrate the atmosphere and reach the water surface, and will then be further attenuated in the water column. UVR attenuation in the water column varies with wavelength and water properties. Longer UVR wavelengths tend to penetrate deeper, with UV-A attenuated slower than UV-B. The UVR transparency of the water column will depend primarily on levels of particulates and dissolved organic matter (DOM), with
DOM being especially effective (Hargreaves 2003, Morris et. al. 1995). Low attenuation levels are observed in many aquatic systems, allowing UVR to remain at significant levels deep into the water column. Crater Lake Oregon for example, a very clear lake system, has a 1% 320 attenuation depth of 64m (Hargreaves et al 2006). By comparison, the 1% 320 nm depth at Lake Giles, where the field component of this project was carried out, is approximately 6 meters (http://www_orgs.muohio.edu/uvlakes/info.html).

While both UV-A and UV-B are environmentally significant, UV-B has numerous documented negative effects on aquatic organisms which span many trophic levels (Hader 1998, Williamson 1996). On the molecular level, UV-B has been observed to damage a variety of cellular components, but studies have focused on DNA damage, which is the most common and detrimental impact of UV-B exposure (Buma et al. 2003). High energy UV-B is absorbed by DNA, causing dimerizations between adjacent nucleotide bases in the genetic structure. The most common form of this damage is the formation of dimers in adjacent pyrimidine bases. The two major photoproducts caused by this reaction are the pyrimidine(6-4)pyrimidinone, [(6-4)PD], and the cyclobutyl pyrimidinone dimer, (CPD). T<>T CPDs are the most commonly induced photoproducts and are caused when a cyclobutyl ring forms between the 5 and 6 carbons of adjacent bases.

UV-B damage has influenced organisms throughout time and three mechanisms have developed for avoiding the negative effects of UV-B damage: 1)
Physical avoidance; 2) Use of natural sunscreens or shading pigments; and 3) Using repair mechanisms to correct incurred photodamage.

Bacteria in the water column (bacterioplankton) are thought to be the most UV-sensitive aquatic trophic level because they lack two of these protective mechanisms. Because of their small size, bacterial motility is limited and they cannot avoid UV damage by migration (Purcell 1976), their small size also makes them theoretically incapable of using shading by pigments or sunscreens (Garcia-Pichel 1994). As such bacteria are primarily reliant on mechanisms of damage repair to fix UV damage.

There are two main pathways in which bacteria repair DNA damage: Nucleotide excision repair (NER) and photoenzymatic repair (PER). NER is an enzymatic process which uses enzymes driven by cellular energy to replace damaged DNA. The enzyme system responsible is called excinuclease. In the excinuclease system, enzymes bind to the lesion, hydrolyze the bonds on either side of it, and carry the damaged nucleotide away. A copy of the intact nucleotide sequence of the duplicate strand is used to fill in the gap (Sancar 1994a). This system is regulated by the recA gene (Miller. 2000). In response to DNA damage preventing replication, recA is activated and initiates the SOS system. The SOS system activates a series of genes which cause the replication and replacement of damaged DNA as described above (Walker 1984). PER uses higher wavelength radiation (UVA and PAR) to repair DNA damage without the expenditure of cellular energy. This radiation is called photorepair radiation (PRR). The photorepair system uses a single enzyme.
DNA photolyase, which binds to damaged sites (such as a CPD), absorbs a photon of PRR, and uses this energy to split the cyclobutane ring and repair component pyrimidine bases (Sancar 1994b).

DNA repair has been cited as a major reason for the failure of reciprocity (Grad et al. 2001, Trocine et al. 1981). The theory of reciprocity states that if the cumulative dose (dose rate x duration) of two exposures is equal, then the effect should be the same regardless of how the cumulative dose is attained. Experimental results have been mixed, and reciprocity is suspected to be dependant on the presence of repair mechanisms and the magnitude of difference between exposure regimes (Zagarese and Williamson 2000). Despite mixed results, reciprocity is often assumed or not addressed in UV-B exposure experiments, and for bacteria the concept is largely unexplored.

The dynamics of UV-B damage to bacterioplankton is of concern not only because of the potential loss of environmental function from direct bacterial damage, but also from the possibility of shifts in community composition due to varying tolerances of organisms to UV-B damage. Bacteria are suspected to be the most sensitive trophic group and they also hold a key role in the nutrient cycling and structure determination of aquatic systems (Azam et al. 1983, Pomeroy et al. 1974). As such, knowledge about the effects of UVR on bacterioplankton is essential for our determination of the effects of UVR on the entire system.

Isolated bacteria strains have been studied to determine bacterial tolerance of UV and to look for evidence of repair processes that may induce this tolerance.
Nucleotide excision repair is universal (Sancar 1994a) and is not separated in these studies from general “UV tolerance” which integrates all mechanisms of protection from UV damage as well as excision repair process. Photoreactivation is not found in all species (Sancar 1994a) and has been shown to vary from strong to absent in different bacterial strains. Joux et al (1999) showed that in absence of photorepair, there is strong variability in the tolerance of marine bacterial strains to UV-B exposure, but they also found varying usages of photorepair which led to less distinct differences in tolerances when photorepair radiation was given. Nicholson (1995) also found varying dependency on photorepair, but did not see dependency to differ based on sensitivity. This indicates that even with available repair mechanisms some bacterial strains will be more sensitive than others to UV damage based on varying efficiencies of PER and NER processes. Variability of bacterial sensitivity has also been shown to be independent of environment (Agogue et al. 2005, Arrage et al. 1993). Sensitive and tolerant strains have been found regardless of UV environment from which they are isolated.

The hypothesized inability of bacteria to use photoprotective compounds has also been supported by work with isolated strains. Agogue et al. (2005) isolated and exposed over 90 bacteria strains and found no evidence for increased tolerance among pigmented cultures. Gascon et al (1995) performed experiments on pigment deficient mutants, and again saw no indication that pigment conferred increased UV tolerance. Work with isolated strains has also demonstrated bacterial variability on other measures of bacterial activity such as productivity. Arrieta et al. (2000) exposed
marine isolates to UV-B and observed variation of leucine and thymidine incorporation during exposure.

Field studies have found the potential for photodamage in the environmental as well as the sensitivity of bacteria as a group compared to other trophic levels. Jeffrey et al. (1996a,b) were the first to demonstrate evidence of UV-B photodamage in aquatic environments, and observed this damage accumulation to a substantial depth in the water column (>10m). This study also showed the importance of wind driven water column mixing on patterns of damage accumulation in the natural environment. Under calm seas damage accumulation was observed, but when seas were moderate damage accumulation disappeared. These studies also showed a higher level of photodamage in the bacterial size fraction as compared to larger size fractions from the same environments.

The majority of studies of bacteria and UV dynamics have come from marine environments and isolates, and while these studies give good clues as to what may be happening in lake systems, the results cannot be assumed universal. Lakes vary greatly in their concentrations of DOM and as such will have highly variable UV environments from lake to lake, and between lakes and oceanic systems (Morris et al. 1995). Lakes are also found at varying altitudes which can alter the intensity of radiation by altering atmospheric absorption: UV-B increases by about 6% for every 1000m gain in altitude (Diffey 1991). Direct comparisons are also complicated because UV-B intensity is also reliant upon ozone concentration, season, latitude, and time of day.
There has been a demonstrated effect of temperature on the degree to which UVR impacts a system (Roos and Vincent 1998). DNA repair processes are enzymatic driven and as such are temperature dependent (Pakker et al. 2000, Dorrell et al. 1995). UV-B intensity varies in aquatic systems with UV-B in a given area being maximal approaching the summer solstice. In aquatic systems water temperatures will also vary, but these effects operate on different time scales. This disparity may become especially important during times of high UV-B before lake temperatures have warmed, and in high UV/low temperature systems such as high altitude alpine lakes. Oceanic systems are more thermally stable than lake systems, so temperature UV interactions may be more pronounced and fundamentally different in lake systems. Climate change predictions are also expected to alter the temperature and UVR in a system. Rae and Vincent (1998), for example, found that metabolically active bacteria could become a larger component of lake systems under increased UVR and higher temperatures.

The few studies of bacterial-UV interactions which have been performed in freshwater have not directly examined damage and repair mechanisms. Maranger et al. (2002) demonstrated the potential for UV-B to induce damage to membranes and cause cell death in the epilimnion of a Canadian high DOC lake (7-13 mg/L). Cell death due to direct damage was not separated from cell decreases due to trophic interactions, however, and damage to membranes was not directly correlated to DNA damage. Zenoff et al. (2006) examined bacterial from high altitude alpine lakes. This study found freshwater bacterioplankton to be tolerant of high UV environments, and
observed survival at high levels of artificial UV-B exposure. This study did not, however, examine repair mechanisms or damage at the cellular level. The findings of Zenoff et al. (2006) when compared to the findings of Agogue et al. (2005) and Arrage et al. (1993) indicate that a better understanding is needed to the degree to which the environment determines UV sensitivity. The dynamics of bacterial damage accumulation and repair remain largely unexplored in freshwater systems.

Reported here are the results of a series of lab and field based experiments exploring the dynamics of damage and repair in freshwater bacterioplankton. First discussed is a series of standardized lab experiments performed to examine the UV-B tolerance of freshwater isolates and determine the degree to which each strain has the potential for photorepair. We also looked for evidence of reciprocity in bacterial UV-B exposures. Also reported are the findings of a series field experiments examining diel cycles of DNA damage and repair in bacteria vs. larger organisms. These experiments were run in two different seasons to examine the potential for seasonal effects on the ability of organisms to repair DNA damage.
Chapter 1: UV tolerance and photoreactivation in bacterioplankton: Examining bacterial coping at the population level.

Introduction:

Atmospheric ultraviolet radiation (UVR) has increased globally over the last several decades, most noticeably in areas located under depleted ozone (Madronich 1995). This trend of increasing UVR exposure has generated concern about the potential effects of UVR on biological systems, especially the damaging effects of UV-B (290-320nm). In aquatic environments particularly, UV-B has been observed to have a broad range of biological impacts spanning multiple trophic levels (Hader 1998, Williamson 1996).

Bacterioplankton are very important to nutrient and energy cycling in aquatic environments and play a key role in structuring aquatic ecosystems (Azam et al. 1983, Pomeroy et al. 1974). Since bacteria are generally considered more sensitive to the damaging effects of UV-B (i.e. formation of DNA adducts) than other trophic levels (Jeffrey et al. 1996a,b), it seems likely that increased exposure of this important trophic level to UVR could have important implications for susceptible aquatic environments. Therefore, studying the mechanisms by which bacteria overcome UV-B damage will elucidate the importance of UVR in structuring bacterial communities in freshwater environments.

Three main mechanisms for overcoming UVR effects have been observed: 1) avoidance; 2) protection including the use of sunscreen compounds or pigments to
absorb damaging UVR; and 3) mechanisms to repair molecular damage caused by
UVR (i.e. DNA adducts). Limited motility (Purcell 1977) does not allow effective
UV avoidance in bacteria and their small size makes them theoretically incapable of
using pigments or sunscreen-compounds for UVR absorption (Garcia-Pichel 1994).
In absence of these mechanisms of damage avoidance, bacteria are considered
primarily reliant upon repair processes for alleviating damage induced by UVR.
When repairing damaged DNA two main strategies are nucleotide excision repair
(“NER”) and photoenzymatic repair (“light repair”). Nucleotide excision repair
requires energetic output from the cell to remove and replace photoproduct mutations
(Sancar 1994a). Photoenzymatic repair uses longer wavelength energy, UVA (340-
400nm) and near UV visible light, (blue, 420-490nm) to repair damage without
cellular energetic cost (Sancar 1994b). This radiation is referred to as photorepair
radiation (PRR).

Studies examining bacterial sensitivity have supported the lack of pigment
influence on cellular UV-B tolerance (Gascon et al. 1995, Agogue et al. 2005).
General sensitivity of bacterioplankton has also been observed environmentally, and
UV-B adequate to cause detectable damage has been observed at substantial depths in
the ocean (Jeffrey et al. 1996a,b). Thus the potential for significant UV-B damage to
aquatic bacteria exists and survival of damaged populations depends upon the
availability and efficiency of damage repair mechanisms. Bacteria, however, are not
homogenous in their sensitivity to UV-B, nor in their usage of molecular repair
Most knowledge of UV sensitivity and repair in environmental bacteria comes from work in marine systems. Since effects of UVR are prevalent in lake systems, the dynamics of the effects of UVR on lake bacterioplankton warrant further examination. Lakes vary substantially in their UVR environment and, as such, oceanic results cannot be assumed to apply to lake systems. Dissolved organic matter (DOM), for example, is one of the primary determinants of UV attenuation in natural waters (Morris et al. 1995), and levels and quality of DOM are more variable in lakes than in typical marine systems. Lakes are also found at a variety of elevations and high elevation lakes are often exposed to higher levels of UVR than low elevation lakes due to less atmospheric absorption (Diffey 1991).

The few studies of freshwater bacteria and UVR tolerance have found them to be potentially damaged under ambient UVR (Maranger et al. 2002), and potentially tolerant under extreme UV conditions (Zenoff et al. 2006). The survival and repair dynamics of individual strains has not been adequately explored for freshwater bacteria, especially for freshwater bacteria in a moderate UV temperate environment. Furthermore, there is a lack in the understanding of bacteria and UVR as to the degree to which reciprocity holds true, i.e. is dose rate an important determinant of observed UVR effects. DNA repair has been cited as a major reason for the failure of reciprocity in other trophic levels (Grad et al. 2001, Trocine et al. 1981), and despite...
mixed experimental results, reciprocity is often assumed or not addressed in the literature.

Here we examine the UV-B sensitivity and photorepair usage of three cultured strains of heterotrophic planktonic lake bacteria. We also explore whether dose rate is important in determining UV-B sensitivity and repair. The goal of this study is to expand the understanding of how bacteria cope with UV-B damage at population levels, and to widen the range of environments within which this question has been explored.

**Methods:**

**Bacterial Strains:** Three strains of bacteria were isolated from lakes in the Pocono Mountains of Pennsylvania. Two strains were isolated from Lake Giles, a clear oligotrophic lake. One strain (*Pseudomonas* sp.) was isolated from the surface waters (~10 cm deep) and one (*Sphingomonas* sp.) was isolated from 17 m (below the thermocline). The third strain (*Bacillus* sp.) was taken from the surface of Lake Beaver, a nearby high DOM, low UV lake. Putative identification of the strains is based on sequencing of 16S rDNA genes by SeqWright DNA Technology Services.

**Isolation and Culture Conditions:** Strains were isolated by spread-plating lake water samples onto Difco R2A agar, a low nutrient growth medium. After growth, distinct colonies were collected and isolated using the streak dilution technique. Bacteria from
the agar plates were then inoculated into R2A broth and grown at 20°C until early stationary phase was reached. The cells were harvested by centrifugation for 10 min at 8000 rpm, washed twice and the cell pellet resuspended in an appropriate volume of filter sterilized (0.2μm) and autoclaved Lake Giles water.

**Phototron Setup:** Resuspended bacteria were exposed to UV radiation in a UV lamp phototron (Williamson et al. 2001). The phototron method exposes suspended cells held in quartz dishes on a rotating wheel (Figure 1). One to three UV-B lamps (Spectronics XX15B) are suspended above the wheel exposing the dishes to damaging UV-B. Lamp output varied slightly between lamps, but each had a dose rate of about 10-14 W m⁻² at full exposure, or 1-1.5 W m⁻² when covered with cellulose acetate film. Photorepair radiation bulbs (two 40-W cool white fluorescent, and two 40-W Q-Panel 340 bulbs) shine through the bottom of the wheel. The duration of the experiment and the total dose kJ m⁻² varied for different exposure regimes (see below).

The system is designed to minimize stray radiation, and the dishes are placed within opaque collars to prevent light except from directly above and below. Metal disks can be inlaid on the wheel to allow or block PRR in replicate dishes for +PRR and -PRR treatments. All experiments were run at 20°C in a walk-in incubator. After exposure, bacteria from dishes were serial diluted to countable concentrations and plated on R2A agar. They were then put into a 20°C incubator and allowed to grow in
the dark for 48-96 hours (depending on the strain). Colony forming units (CFU’s) were counted to estimate survival in each treatment.

**UV Exposure Conditions:** Three schemes of exposure were used in this study. Two were intended to examine UV-B tolerance with and without photorepair radiation (+PRR and -PRR). A third was intended to examine the rates of photorepair in each strain. The two damage protocols were a 12 hour chronic exposure, and an acute exposure lasting from ~30 seconds to several minutes. Treatments were compared to dark controls to eliminate dish effects. The difference between +PRR and -PRR treatments at the same exposure is assumed to be the result of PER activity. -PRR treatments represent a combination of background tolerance of damage and any light independent repair processes such as NER.

12 hour The 12 hour method exposed bacteria under UV-B for a duration of 12 hours. During exposure the +PRR treatments received simultaneous exposure to 4 cool white lights which served as the source of photorepair radiation. UV-B exposure level was altered by using neutral density filters that consisted of screening of various densities placed over the quartz dish tops. UV-B lamps were covered with cellulose acetate to block residual UV-C. *Psuedomonas* sp. and *Bacillus* sp. used only one lamp; dose rate ~1.34 W m\(^{-2}\). Screening varied the dose rate range between ~0.01-1.34 W m\(^{-2}\) depending on treatment, and cumulative doses ranged between ~0.6 and 40 kj m\(^{-2}\). *Sphingomonas* sp. used two lamps; dose rate ~2.78 W m\(^{-2}\). Screening
varied dose rate range between ~0.65-2.78 W m\(^{-2}\), and cumulative doses were between ~28 and 120 kJ m\(^{-2}\).

**Acute** The acute exposure scheme used a higher dose rate of UV-B over a short period of time, with no repair radiation given during exposure. +PRR treatments received a separate 1 hour regiment of 2 cool white and 2 UV-A lamps after exposure was complete. In this setup PRR lamps were covered by two sheets of Mylar to prevent possible damaging radiation from the UV-A lamps. UV-B lamps were not covered with cellulose acetate and the output of the lamps was estimated taking into account this change in damaging UV intensity. Exposure level was manipulated by time instead of screening, although one neutral density screen (~40% transmittance) was used in all experiments to prevent immediate overexposure. Three lamps were used in all experiments. When screening was accounted for total dose rate of this system is ~20 W m\(^{-2}\), and cumulative doses ranged from ~1.5 to 35 kJ m\(^{-2}\). In this acute setup ten levels of exposure were administered to each strain. At each exposure point survival data was taken for –PRR treatments. After exposure, 1 hour of PRR exposure was given to the +PRR treatments, and then survival data was again collected. Each experiment was run twice for replication.

**Results:**

12 Hour Exposures: UV-B tolerance varied between the three strains (Fig 2a-2c. Table 1.2). *Pseudomonas* sp. was the most sensitive culture showing a D\(_{37}\) of 4.49 kJ in –PRR conditions; *Bacillus* sp. was moderately tolerant (D\(_{37}\) of 10.03 kJ for –PRR):
and Sphingomonas sp. was very tolerant of UV showing a $D_{37}$ of 70.65 for -PRR. Of the three species only Psuedomonas sp. was highly reliant on PRR, with $D_{37}$ increasing 6 fold when PRR was given. Bacillus sp. did not show evidence of repair; +PRR and -PRR values falling within error of each other. Pseudomonas sp. showed some usage of PRR under 12 hour conditions (Table 2).

**Acute Exposures:** The three strains again exhibited a range of UV tolerance under the acute exposures (Table 1). Relative sensitivity of the strains was the same as in the 12 hour experiments, but overall tolerances were sharply lower than were seen in the 12 hour regime (Fig 2a-2c). Of the three species only Pseudomonas sp. showed evidence of PRR usage, and was again highly reliant on PRR. Bacillus again did not show any repair. Sphingomonas sp. did not show any evidence of PRR usage in acute exposure experiments (Table 2).

**Discussion:**

Our results show that the methods by which bacteria tolerate UVR are variable and that there is no set bacterial strategy for enduring UVR. Pseudomonas sp. is sensitive, so is likely to be damaged environmentally, but seems to have an effective and rapid PER system. Sphingomonas sp. shows weak PER, but is very tolerant of UVR damage so likely does not need to rely on PER. This was our only pigmented culture, but studies have found bacterial pigmentation to be ineffective
(Gascon et al. 1995, Agogue et al. 2005). This indicates that this strain is either very effective at NER processes such as nucleotide excision repair or else has some other unknown mechanism of preventing damage from UVR. *Bacillus* sp. showed no PER, but was moderately tolerant of UV-B. *Bacillus* sp.’s $D_{37}$ without PRR was about equal to that of *Pseudomonas* sp. with PRR usage. Since both of these bacterial strains persist in the environment this tolerance is sufficient, although attained by different means.

In 12 hour exposures strains were able to withstand high doses of cumulative UV-B. This is likely due to the low dose rate of his exposure regime (~0.01-1.34 W m$^{-2}$) and the simultaneous repair radiation given. Our acute exposures were substantially higher in dose rate (~48.8 W m$^{-2}$) and cumulative doses tolerated were substantially lower. All three cultures showed less survival at similar cumulative UV-B doses in acute exposures. This is an indication that dose/rate is an important determinant of a strain's ability to withstand UV-B damage. Reciprocity did not hold even when no PRR was given, indicating either that NER processes are sufficient to offset this relationship, or that bacterial survival under UV-B simply is not a reciprocity based process. Our result may be due to the magnitude of difference between our treatments, which is thought to be one of the factors which will determine if reciprocity will hold (Zagarese and Williamson 2000).

Joux et al. (1999) exposed cells to UV-B at a dose rate of 2.3 W m$^{-2}$. The maximum given doses in this study was ~3 kJ m$^{-2}$ and several cultures could not withstand it, again showing low survival when dose rate is high. Agogue et al. (2005)
examined growth curves after exposing cells to UV-B at a dose rate of 1.2 W m\(^{-2}\).
Sensitive cultures showed no growth after 30 minutes of exposure, while tolerant strains were able to grow after 30 kJ given in 7 hours. Despite our inability to directly compare these results, magnitude of UV-B tolerance observed in the Agogue study agrees with our 12 hour results, with strains tolerating several kJ of cumulative UV-B dose. General magnitude of effects was also similar in Hernandez et al. (2006) which used natural solar radiation. Our acute studies are comparable with high dose-rate exposure studies from the literature and show bacteria to endure lower cumulative doses of UV-B compared to those endured at lower dose rate exposures.

Previous studies have demonstrated a wide diversity of bacterial tolerance to UVR (Gascon et al. 1995, Joux et al. 1999, Agogue et al. 2005, Hernandez et al. 2002, Qui et al. 2004). Our damage experiments support this result, with both 12 hour and acute regimes showing diverse tolerance in examined strains. We also observed varying degrees of PER in our three cultures: *Pseudomonas* sp. used PER extensively; *Sphingomonas* sp. showed some usage of photorepair; and *Bacillus* sp. showed no usage of photorepair. Joux et al. (1999) observed varying photorepair in the strains they examined, also noting a tendency for PRR to equalize some of the differences in UV-B tolerance seen without PRR. Nicholson (1995) found variable use of photorepair with no apparent correlation with the tolerance of the organism in question. We do not have enough cultures to make a strong claim, but our most sensitive strain (in absence of PRR) did show the most effective photoenzymatic repair.
Differences in spectral quality of solar radiation and that of the lamps used prevent direct extrapolation of these tolerances to natural systems. We can, however, observe mechanism responses and thus despite the artificial nature of these experiments our results have real world implications. Our data shows that reciprocity will not hold under different exposure conditions for the strains tested. High dose rate lab studies may therefore underestimate the ability of bacteria to withstand UV-B damage in the environment. We also see that temperate lake bacteria, like those from marine environments, are variable in UV-B tolerance and usage of PRR. This supports the idea that changing light environments will affect different strains of bacteria differently, potentially leading to community structure and function changes.
Chapter 2: Environmental DNA Damage: Examining bacterial coping at the molecular level.

Introduction:

Atmospheric ultraviolet radiation (UVR) has increased globally (Madronich, 1995) and questions regarding the effects of UVR have come to be of immediate concern. Solar UVR is divided into three types, but only two are ecologically relevant; UV-B (290-320nm) and UV-A (340-400nm). UV-B has been observed to have a broad range of usually detrimental biological impacts spanning multiple trophic levels (Hader 1998, Williamson 1996).

In aquatic systems bacterioplankton are suspected to be especially vulnerable to UV-B damage (Jeffrey et al. 1996a,b). Since bacterioplankton have been found to be important to nutrient cycling and play a key role in structuring aquatic ecosystems (Azam et al. 1983, Pomeroy et al. 1974) it is of especial importance to understand bacterial vulnerability to UV-B and the methods by which they cope with UV-B in the environment.

UVR has been an environmental pressure throughout time, and organisms have developed several ways of coping with it. The three main mechanisms by which organisms do this are: 1) avoidance, 2) use of sunscreening compounds or pigments, and 3) mechanisms to repair UV induced molecular damage. Bacteria are considered
more vulnerable because they are suspected to lack two of these three main mechanisms. Bacteria’s limited mobility prevent them from avoiding UV-B (Purcell 1976), and their small size makes them theoretically incapable of using pigments or other shading compounds (Garcia-Pichel 1994). The ineffectiveness of bacterial pigments has been supported by several studies (Gascon et al. 1995, Agogue et al. 2005). The idea of limited motility is supported by Jeffrey et al. (1996a,b). In this study calm seas led to observed photodamage, but moderate seas eliminated this. Bacteria must rely on waves and other external forces to move them away from damaging UV-B. Higher amounts of photodamage were also observed in the bacterial size fraction, giving environmental evidence of bacterial sensitivity. There was also a demonstrated depth pattern of DNA damage, indicating attenuation of UV-B in the water column.

Although UV-B can cause a variety of damaging effects, DNA damage is the most common and often the most detrimental. There are several types DNA damage that can be induced by UV-B (Burna et al. 2003) but the most abundant are cyclobutyl pyrimidinone dimers (CPDs). CPDs are often measured as an estimate of DNA damage by UV-B since they are only induced by UV-B wavelengths. Without mechanisms to avoid DNA damage, bacteria are forced to use repair processes to remove CPDs.

There are two main repair processes by which bacteria repair cellular DNA damage, photoenzymatic repair (PER) and nucleotide excision repair (NER). Excision is a highly regulated molecular process involving numerous enzymes which use
cellular energy is used to remove DNA photoproducts (Sancar 1994a). Photoenzymatic repair uses radiation energy to remove photoproducts without energetic cost (Sancar 1994b). The types of radiation used by PER are longer wavelength UVA (340-400nm), and near UV visible light, (blue, 420-490nm). This radiation is referred to as photorepair radiation (PRR).

Determination of bacterial sensitivity to UVR and usage of repair mechanisms comes mostly from work in lab settings. These studies have found bacteria to be variable in their sensitivity to UV-B, and in their usage of PER and NER molecular repair (Gascon 1995, Joux 1999, Agogue 2005, Hernandez 2006, Qui 2004, Muella 1999). It is important, however to examine UV-B damage and repair dynamics in the field.

Jeffrey et al. (1996a,b) were the first to demonstrate evidence of UV-B photodamage in aquatic environments, and observed this damage accumulation to a substantial depth in the water column (>10m). While controlled lab study allows for determination of potential mechanisms, how they function in the environment is easier assessed in field study.

There has also been a demonstrated effect of temperature on the degree to which UVR impacts a system (Roos and Vincent 1998). DNA repair processes are enzymatic driven and as such are temperature dependent (Pakker et al. 2000, Dorrell et al. 1995). UV-B intensity varies in aquatic systems with UV-B in a given area being maximal approaching the summer solstice. In aquatic systems water temperatures will also vary, but these effects operate on different time scales. This
gives a potential seasonal shift in damage and repair dynamics. Climate change predictions are also expected to alter the temperature and UVR in a system (Rae and Vincent, 1998).

Here we report the results from an examination of diel patterns of DNA damage and repair in surface waters of Lake Giles in the Pocono Mts. of Pennsylvania. Experiments are designed to examine molecular level damage by UV-B and the patterns by which this damage is accumulated and repaired. Comparisons are made between different cell size fractions. Optical filters allow a first examination of the potential usage of NER vs. PER in lake microbial communities. Seasonal comparisons provide initial information on the role of seasonal shifts in UVR and temperature on relative damage accumulation and rates of repair kinetics.

**Methods:**

**Location:** Experiments were performed in Lake Giles, a clear oligotrophic lake in the Pocono Mountains of Pennsylvania: Latitude 41° 22′34″ N Longitude 75° 05′33″ (Morris and Hargreaves 1997). Experiments were clipped to anchored ropes in the deepest part of the lake each time. Filter apparatus was setup on shore and all filtration was done on sight. Optical data was collected from a nearby sight using a Smithsonian SR-18 (Neale et al. 2005).
Experimental Design: Diel experiments were conducted in April and July of 2004. Each session was run as two consecutive 24 hour experiments. During each experiment one gallon UV-transparent bags (Bitran s-series) were filled with water from 3m before 0700. Water was collected using a hand powered diaphragm pump. Filled bags were placed in floated PVC racks which held them at the lake surface. Racks were rectangular, holding 20 bags in a 2 x 10 arrangement. PVC racks were wrapped with Styrofoam on the short sides and the middle of the long sides for floatation. Bags were held in place by light density netting supported by elastic cord. These floating racks removed potential effects of water column mixing during exposure. Bags were exposed to surface solar radiation until the time of estimated max UVR (1330), at which point they were divided into treatments. A third of the bags were covered with acrylic (cutoff 365 nm) to block damage but allow photorepair wavelengths (PRR). Acrylic pieces were laid over entire sections of the rack and secured with zip ties through small drilled holes. The second group was covered with opaque black plastic to block out all light. These bags were covered individually with opaque plastic sleeves and placed back into the floating racks. The remaining bags were left in full sun.

At each sample time 10 of the one gallon bags from each treatment were pooled into two 20L cubitainers (5 per cubitainer). These were covered with opaque plastic and rowed to shore where they could be filtered. Each cubitainer was filtered sequentially through 8 μm, 0.8 μm, and 0.2 μm membrane filters (Supor, PALL membrane). At each time point two 20L cubitainers of lake surface water were also
collected using the diaphragm pump and sequentially filtered. Filters were immediately frozen on dry ice for later DNA damage analysis using the RIA method (Mitchell 1996).

**Sampling regime:** There were 5 sampling times for each of these experiments. The initial lake sample was collected as the bags were being filled (0700). Subsequent samples were collected from the lake and each treatment at 1330, 1600, 1900, and 0700 the following morning.

**Analysis of data:** Filters were sent frozen on dry ice to David Mitchell at the M.D. Anderson Cancer Center for CPD analysis by the RIA method (Mitchell 1996). Each time point produced 2 filters per treatment, and each was analyzed in duplicate. The largest size fraction (>8), zooplankton and larger phytoplankton, produced heterogeneous filters which led to highly variable data. The <8 >0.8um fraction likely contained mostly phytoplankton and protists. The <0.8 >0.2um size fraction is assumed to be predominantly bacterioplankton, and is the primary focus of our analysis.

**Results:**

Radiation data was collected using the SR-18 (Figure 3). April experiments were both clear days, and UV-B measurements were similar with cumulative 306 nm
doses of 684.7 and 804.2 J m\(^{-2}\) nm\(^{-1}\). July weather was slightly more variable, and both days had variable cloud coverage throughout the day. The first day had cumulative 306nm dose of 1084.3 J m\(^{-2}\) nm\(^{-1}\), while second day showed levels of 806.4 J m\(^{-2}\) nm\(^{-1}\).

In all diel experiments water column samples were variable but showed no apparent diel trend. Water column samples showed more damage overall in April than July. Experimental manipulations increased photodamage above lake levels, showing more CPDs in both size fractions by the 1330 sample in all bags vs. lake.

The April diels showed evidence of repair dynamics, with distinct differences between treatments (Figure 4a-4b). Bags left in full sun increased in damage until 1900, after which point CPDs decreased by the next morning. Acrylic treatments peaked in damage at 1600. After 1600, however, acrylic samples for both size fractions decreased quickly and by 1900 recovered to a level of damage which remained constant until 0700. While dark treatments stopped accumulating damage after they were covered at 1330, apparent NER did not start until after 1900. Rates gained from this data show a higher maximum photorepair rate vs. maximum NER rate in both size fractions (Table 3).

The larger size fraction (<S >0.8) recovered to water column damage levels by the following morning in all treatments except the full sun treatment which remained somewhat higher. The small size fraction all ended at nearly the same level of CPDs regardless of treatment, but no treatment fully returned to water column levels. The small size fraction (<0.8 >0.2um) had higher overall concentrations of photoproducts.
regardless of treatment. This pattern is true throughout all diel experiments performed here and is observed in lake samples as well.

July diel data for the acrylic and dark treatments was inconclusive (not shown). Data from full sun bags was used to calculate repair rates which could be compared to April diels. Rates are shown (Table 4).

**Discussion:**

**Lake Trends:** The lack of a diel pattern in the lake samples is an indication that there is significant mixing within the water column. In the water column samples, damage accumulation was observed, but no apparent diel pattern. The ability for damage to be observed the absence of a predictable pattern in the water column is likely due to mixing motion within the lake. This agrees with Jeffrey et. al. (1996) who found that during a calm period CPD accumulation could be seen in marine systems, accumulating most at the surface and decreasing with depth as predicted by light attenuation. When waves were moderate, however, this pattern of damage accumulation disappeared. Organisms within our diel experiment bags had no opportunity to mix and as such were shown to accumulate damage above the lake level. We also observed overall greater levels of net CPDs in the April experiments as compared to the July experiments. This result is not conclusive but may indicate
higher damage during colder lake temperatures due to slower repair kinetics (see below).

**April Repair Dynamics:** The April experiments indicate that both size fractions have the ability to use both DNA repair systems; photoenzymatic repair (PER), and excision (NER) repair. In both size fractions the maximum rate of PER was higher than the rate of maximum NER. The bacterioplankton size fraction, exhibited a faster maximum photorepair rate than the larger size fraction. Effective photorepair is one of the proposed ways that theoretically sensitive bacterioplankton are able to tolerate UV damage. We observed the bacterial fraction to exhibit a slower NER system than the larger fraction however (Table 3). This usage of repair mechanism under our experimental manipulations only concludes that these organisms have the ability to use them, not necessarily that they are used. PER, for example, was observed in the acrylic treatment of both size fractions, but we see no indication of its use in the full sun bags, which more closely resemble the natural conditions the organisms would experience during their daily cycle.

In both size fractions the acrylic treatments continued to gain damage even after damaging UV should have been blocked by addition of acrylic suggesting possible light leakage around the acrylic filters. From 1600 to 1900, however, both size fractions see a sharp decline in CPDs which is not seen in the other treatments. This seems to be evidence of effective photorepair induction. It is possible that between 1300 and 1700 the light leakage inhibited photorepair or that the rate of
repair was less than the induction rate. Once photorepair is observed the rates are rapid, especially in the bacterial fraction.

Dark treatments did not show a net CPD gain damage after bags were covered. This indicates that at least damaging radiation was sufficiently blocked. These bags did not show evidence of repair, however, until after the 1900 sample. There is a lag time again in both fractions between when damage stops and when repair begins. In natural conditions this may be the equivalent of waiting until night before instating NER mechanisms. This would allow the cells to maximize photorepair while adequate radiation is available, thus repairing without metabolic cost before expending energy on NER. Otherwise this might be an indication of careful timing of repair mechanisms by the cells.

The endpoints for repair treatments (acrylic and dark) are very close to each other in both size fractions. Both acrylic and NER treatments seem to reduce net CPD concentrations to the same level, but the slower rates of NER takes longer. In the larger size fraction the two repair treatments recover to about the same level as the lake surface, but the full sun bags are somewhat higher at the end of the experiment. This incomplete repair may be indicative that damage surpassed the maximum rate of repair possible under the conditions of the lake at that time such as temperature or nutrients. The full sun bags show no evidence of repair until 1900 at which point adequate photorepair radiation is not present. The rate of repair after 1900 also indicates that only NER is occurring. Measurement of net CPDs cannot determine whether these full sun bags used photorepair during daylight hours to slow damage
accumulation. It is also possible that photorepair system was either repressed by the cell or photoinhibited during high light exposure.

In the bacterial fraction all three bag treatments end at nearly the same endpoint. This endpoint is well above the background lake level, however. Bacteria were shown to accumulate more damage overall, and this again may be an indication that repair mechanisms were overburdened. This is not unexpected as the diel bags eliminated mixing effects.

We again see the acrylic treatment using effective photorepair from 1600-1900, but it does not show evidence of NER afterwards, despite still having significant damage above water column levels. In addition, dark treatments do not seem to initiate until 1900, even when bags were covered at 1300.

Our data seems to conclude that both size fractions have the ability to use both methods of DNA repair, but it is apparent that the regulation of these repair mechanisms in the environment is complex. Bacterial photorepair may be somewhat more effective than that of larger organisms, but overall sensitivity seems to be higher in bacteria as indicated by more average CPDs as well as their inability to recover to background levels in all bag treatments.

Seasonal Comparison: Despite inconclusive data from acrylic and dark treatments in the July diels (data not shown) we can make some observations concerning the full sun bags. Full sun bags during both months showed no significant repair of CPDs until after 1900. Repair after this point can be assumed to be only NER, and thus we
can compare rates of NER during the two seasons. Examining the bacterial fraction, April diels showed NER rates of about 22 CPDs/hour and 1.73 CPDs/hour, the July diels showed rates of 51 and 18 CPDs/hour. Though these numbers are not statistically significant this may indicate faster rates of repair on average in July. We suspect this may be the result of warmer water temperatures leading to more efficient enzymes, which in turn will improve repair. Water temperatures in April were 5.5 C in the mixed layer, as compared to 22.5 C in July.

If we compare DNA damage to radiation dosage for the two experimental periods we see that the higher levels of damage seen in April and the (possibly) lower rates of repair cannot be explained simply by a disparity in UV-B dosage. The July UV-B dosages were higher than those of April, and if UV-B dose alone was causing this effect we would expect the reverse of the observed pattern. Overall, we cannot conclusively say that July rates were faster based on our full sun bag rate calculations. We do, however, see overall greater damage in both July experiments regardless of light treatment. This is an indication that some seasonal effect is present. This may be based in UV/temperature interactions, but also may indicate a shift in community structure, or change in water qualities such as nutrient levels or DOM concentration. Further study would be needed to eliminate these possibilities.
Tables and Figures:

<table>
<thead>
<tr>
<th>Culture</th>
<th>Exposure Regime</th>
<th>$R_2$</th>
<th>$D_{37}$</th>
<th>$D_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas sp.</td>
<td>12 Hour +PRR</td>
<td>0.9018</td>
<td>11.004</td>
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<td>12 Hour -PRR</td>
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Table 1: Summary of the $D_{37}$ and $D_{10}$ values for each phototron experiment. Data was fit to an exponential equation, $R_2$ values are given.

<table>
<thead>
<tr>
<th>Culture</th>
<th>Exposure Regime</th>
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<th>Sig</th>
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<td>12 Hour PRR</td>
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<td>.006</td>
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<td>.001</td>
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<tr>
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<td>Bacillus sp.</td>
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<tr>
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<td>Acute PRR</td>
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<td>.825</td>
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Table 2: Results of 2 way ANOVA analysis examining the effect of UVR and PRR on each strain.
**April Maximum Repair Rates**

<table>
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<th>&lt;.8 &gt;.2</th>
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<tr>
<td>Photorepair (CPD's/hour)</td>
<td>65.29</td>
<td>79.48</td>
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<tr>
<td>NER (CPD's/hour)</td>
<td>13.72</td>
<td>7.91</td>
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</table>

Table 3: Size fraction comparison of April repair rates. NER rates were calculated from overnight repair. Repair during daylight after UV-B was blocked is called photorepair.

**Seasonal Comparison Of NER Rates**

<table>
<thead>
<tr>
<th>Month</th>
<th>Repair Rate (CPD/hr)</th>
<th>Average Repair Rate</th>
<th>Temp (Celsius)</th>
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<tr>
<td>April</td>
<td>21.76</td>
<td>10.88</td>
<td>5.5</td>
</tr>
<tr>
<td>April</td>
<td>1.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>July</td>
<td>51.42</td>
<td>34.71</td>
<td>22.5</td>
</tr>
<tr>
<td>July</td>
<td>18.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Comparison of repair rates between seasons. There is overlap in the data so the rates are not statistically different, but data may suggest faster rates of repair on average in July. Surface water temperatures for each experiment period are given.
Figure 1: Diagram of the UV lamp phototron. The damaging UV-B source is suspended over quartz dishes which rotate on a motorized wheel. Repair radiation shines from below and can be blocked by covering the holes beneath each dish.
Figure 2a-c: Results of 12 Hour exposures (squares) showing variability in UV tolerance (slope of response) and usage of photorepair (difference between closed and open squares. Comparison of 12 hour to acute exposures (triangles) shows the importance of dose-rate and lack of reciprocity in determining survival
Figure 3: UV-B radiation for the experimental periods as collected by SR18. April (squares and circles) had less UV-B than July (diamonds). Cumulative 306nm dose was 684.7 and 804.2 J m$^{-2}$ nm$^{-1}$ for April and 1084.3 and 806.4 J m$^{-2}$ nm$^{-1}$.
Figure 4a-b: Results of April diel data for (a) <8 >.8 \mu m and (b) <8 >.2 \mu m size fractions. Experiment time spans one diel cycle. The dark blue is lake samples, while the other colors represent various experimental manipulations.
References:


**Vita**

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- Experience in using analytical instruments (i.e. spectrophotometer, fluorometer, elemental analyzer and use of ultraviolet lamps).
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- Administered recitation for Current Environmental Issues class

**Presentations:**

Guida, T.A. September 2006. Bacterial mechanisms for coping with population and molecular level affects of ultraviolet radiation exposure. Masters Presentation. Lehigh University, Bethlehem, PA
