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Rates and possible mechanism of light-dependent degradation of pigments in detritus derived from phytoplankton

by James R. Nelson

ABSTRACT

Rates of degradation of phytoplankton chlorophylls and carotenoids under visible light exposure were determined in laboratory experiments. Killed phytoplankton cells and copepod fecal pellets were used as sources of pigments. Chlorophylls, pheopigments and carotenoids were analyzed by HPLC. Experiments included aerated and N₂ bubbled treatments (light and dark), and in one experiment, killed phytoplankton cells were aged in darkness for 5 days in the presence of an active bacterial population prior to exposure to light. Pigment bleaching in solution was also examined.

Pigment degradation was light- and oxygen-dependent and showed apparent first-order kinetics with respect to light exposure. The chlorophylls, pheopigments and major carotenoids bleached at similar rates. Pigments in killed cells bleached faster than the pigments contained in copepod fecal pellets, perhaps due to a higher effective light dose for the smaller particles and to differences in the structural organization of pigments in killed cells versus fecal pellets. Light-dependent degradation of pigments in aerated organic solutions varied with solvent composition. In pure acetone, carotenoids and chlorophylls bleached at similar rates. In acetone:water mixtures, carotenoids were considerably more stable than chlorophylls.

A sensitized mechanism of pigment photooxidation could account for the apparent coupling of light-dependent degradation of different pigment types in detrital particles and in acetone solution. In detrital particles such as fecal pellets, sensitized photooxidations may be enhanced by the localized concentration of detrital pigments and other lipophilic material within hydrophobic microenvironments.

1. Introduction

Since the introduction of a standardized method by Harvey (1934), the measurement of phytoplankton pigments has provided biological oceanographers with their fundamental index of phytoplankton biomass. Subsequent development of analytical approaches for phytoplankton pigments has followed a general trend towards increased sensitivity (e.g., the introduction of the fluorometric method by Yentsch and Menzel, 1963) and increased specificity. Increased specificity in pigment measurements has been achieved through the application of chromatographic techniques, most recently through the use of high performance liquid chromatography, or HPLC.
(e.g., Mantoura and Llewellyn, 1983; Wright and Shearer, 1984). Several indirect optical methods have also been developed for estimating phytoplankton pigment concentrations (generally as the sum of chlorophylls plus pheopigments). Parameters measured by such optical approaches include stimulated fluorescence (e.g., Lorenzen, 1966), “natural” (solar-stimulated) fluorescence (Chamberlin et al., 1990), and remotely sensed ocean color (e.g., Gordon and Morel, 1983).

The accuracy of pigment-based estimates of phytoplankton biomass, whether pigment concentrations were determined directly or derived from optical measurements, will be influenced to varying degrees by the presence of detrital pigments; that is, the pigments associated with non-living particulate organic matter. The presence of detrital pigments is of particular concern for studies which estimate light absorption by phytoplankton from pigment concentrations (e.g., Bidigare et al., 1987), or use taxonomically diagnostic accessory pigments (accessory chlorophylls, certain carotenoids) as indicators of the abundances of major phytoplankton taxa (e.g., Gieskes et al., 1988; Everitt et al., 1990). Sources of pigment-containing detritus within the water column could include dead cells (such as from declining blooms), the egested particulate wastes of microzooplankton, fecal material of macrozooplankton grazers, and, in coastal waters, resuspended sediments.

Certain pigment alteration products produced by zooplankton grazing are chromatographically detectable. Fecal pigments can include pheopigment derivatives of chlorophyll a (e.g., Hallegraeff, 1981; Vernet and Lorenzen, 1987) and, in some cases, hydrolysis products of ester-substituted carotenoids (Repeta and Gagosian, 1984; Nelson, 1989). However, the guts and feces of macrozooplankton often contain unaltered chlorophylls and carotenoids (e.g., Hallegraeff, 1981; Kleppel and Piper, 1984; Nelson, 1989), and sedimenting material from declining phytoplankton blooms may contain a mixture of altered and unaltered pigments (e.g., Gowen et al., 1983). Thus for many field samples, chromatographic techniques do not provide an unequivocal indication of whether pigments are associated with living phytoplankton or with detrital particles.

The extent to which detrital pigments accumulate in the upper water column will be determined by particle dynamics (production and removal of detrital particles), by the extent to which pigments are conserved in the guts or digestive vacuoles of grazers, and by the stability of the pigments within detrital particles. In addition to removal of phytodetritus from the euphotic zone by particle sinking, pigments could be biologically or photochemically degraded within the euphotic zone. Previous studies have shown that the light-dependent degradation of pheopigments is rapid in well-illuminated near-surface waters (Lorenzen, 1967; SooHoo and Kiefer, 1982; Welshmeyer and Lorenzen, 1985). Dark degradation of detrital chlorophylls and pheopigments (presumably mediated by bacteria) is considerably slower (e.g., Daley, 1973; Daley and Brown, 1973; Welshmeyer and Lorenzen, 1985) and may be strongly temperature-dependent (Roy and Poulet, 1990).
This study examines the rates, pattern and possible mechanism of light-dependent degradation of chlorophylls, pheopigments and carotenoids in laboratory experiments. The central questions considered are: (1) whether differences in photooxidative rates could lead to a relative enrichment of more stable pigments (or their simple alteration products) in detrital particles; (2) the possible role of chlorophylls and pheopigments in the heterogeneous photochemistry of phytodetritus. The effects of light-dependent degradation of pigments on the spectral absorption of phytodetritus, and how this relates to particulate absorption spectra measured in the field are considered in a separate report (Nelson and Robertson, 1993).

2. Methods

2a. Biological material. The chlorophyte Dunaliella tertiolecta and the diatom Skeletonema costatum were grown in 12 liter batch cultures under a constant illumination of 47 μEin m⁻² s⁻¹. Cultures were harvested by centrifugation (in 250 mL bottles, X1000G at 4°C) and the packed cells were stored frozen (−20°C). Prior to experiments the concentrated cells were taken through several freeze-thaw cycles to provide some disruption of cellular structure. Compared to fresh extracts of cultured phytoplankton, the pigment composition of the frozen and thawed cells contained additional minor components (very small peaks in pigment chromatograms) that apparently resulted from either storage or thawing and resuspension of cells.

As a source of biologically processed pigments, fecal pellets of a fairly large oceanic copepod were collected. Adult female Eucalanus hyalinus (about 5.3–6.3 mm in length, collected on the outer shelf of the South Atlantic Bight), were maintained in 6 liter beakers containing filtered seawater and fed cultured diatoms (S. costatum or Thalassiosira weissflogii). E. hyalinus feeds in waters of higher algal concentrations, but slows its activity and maintains itself on lipid reserves in waters of lower algal concentrations (Price and Paffenhofer, 1986). Following addition of diatom cultures, fecal pellets produced by the copepods were siphoned from the bottoms of beakers, settled, rinsed in 0.45 μm filtered seawater, then frozen in small vials. The time between egestion of pellets and their collection ranged from several hours to about one day. To provide sufficient material for experiments, fecal pellets collected over a two month period were pooled.

2b. Experiments. Incubations were carried out in seawater which had been filtered (Gelman AE), autoclaved, then sterile-filtered (0.45 μm sterile cartridge filter) into autoclaved glass incubation bottles (4 liter capacity for light treatments, 2 liter capacity for dark controls). The bottle contents were mixed with a magnetic stir bar and by bubbling with sterile-filtered air or N₂. Illumination was provided by a bank of four 40 W fluorescent lamps in a constant temperature room. A fairly broad spectral distribution of visible light was provided by using a mix of Cool-White and Daylight lamps (2 of each). Temperatures differed slightly between experiments, but all fell
within the range of 19.8–22.2°C, with about a 1°C range of variation during an individual experiment. Prior to each experiment, light exposure (as PAR) was measured within water-filled incubation bottles using a Biospherical Instruments QSL-100 quantum scalar irradiance meter. Due to their curved walls, illumination was not even within the bottles. Measurements from the bottle centers (noted in figure captions) were used to estimate light exposure during the incubations (the product of measured photon flux and the time length of exposure).

c. Treatments. In Experiment 1, killed *D. tertiolecta* cells were incubated as aerated light and dark treatments. In Experiment 2, killed *S. costatum* cells were incubated under aerated and anaerobic (*N₂* bubbled) conditions in light and dark bottles. In Experiment 3, the effect of an active bacteria population on the pigment content and spectral characteristics of phytodetritus was assessed. 50 ml of 1.0 μm filtrate from a 200 liter algal culture that was contaminated with free-living bacteria was added to the incubation bottle containing freeze-thawed *S. costatum* cells. The bottle was kept in darkness (mixed and aerated) for 4 days at 20.5–21.5°C prior to exposure to light. In Experiment 4, copepod fecal pellets were incubated in aerated light and dark treatments.

d. Sampling. Duplicate or triplicate samples for pigment analyses were filtered (Whatman GF/F) for each time point (0.75–1.5 h intervals for the incubations exposed to light and at longer intervals for dark controls). Filtered samples were stored frozen until analyzed (within several days). During the 5 day dark incubation in Experiment 3, samples for pigments, particulate spectral absorption, particulate carbon and nitrogen, and bacteria counts were taken daily, then hourly once exposed to light. Pigment analyses only are reported here, other results are presented in Nelson and Robertson (1993).

e. Pigment analyses. Chlorophylls, pheopigments, and carotenoids were analyzed by high performance liquid chromatography (HPLC) using a binary gradient system (Shimadzu LC-6A). A C-18 cartridge column was employed (Brownlee RP-18, 5 μm spherical particles, columns of 220 × 4.6 mm and 30 × 4.6 mm directly coupled within the cartridge holder). Absorbance and fluorescence detectors were employed in series with visible absorbance measured at 440 nm (Shimadzu SPD-6AV) and fluorescence (SpectroVision FD100) in filter-selected wavebands (Corning 5–60 for excitation, No. 23A for emission). Detector signals were recorded and peaks integrated with a dual-channel recording integrator (Shimadzu CR-5A). A segmented gradient program was run between solvent mixture A (80:20 MeOH:125 mM aqueous ammonium acetate buffer, pH 7.1) and solvent mixture B (70:30 MeOH: acetone). The gradient profile following sample injection was: 0 to 1 min 100% A, 1 to 5 min 0% B to 45% B; 5 to 10 min 45% to 85% B; 10 to 15 min 85% to 100% B; 25
to 55 min held at 100% B. Calibration factors for the major chlorophylls and carotenoids were determined using pigment standards isolated from cultured phytoplankton by semi-preparative HPLC (Nelson and Wakeham, 1989).

f. Kinetic analyses. Apparent first-order rate constants for the light-dependent degradation of pigments were calculated by linear regression analysis (natural logarithm of the ratio of concentration at the time of sampling to the initial concentration versus cumulative light exposure). Calibration factors were not available for all pigments (including several fluorescent chlorophyll derivatives). In these cases, a linear relationship between integrated peak area and the sample mass injected was assumed and changes in concentration were estimated from peak areas normalized to the initial peak area (weighted by factors accounting for extraction and injection volumes and the sample volume filtered).

g. Pigment solution experiments. Rates and patterns of pigment bleaching in organic solutions were determined using extracted pigments of D. tertiolecta in 100% acetone and 90% acetone. These experiments had two basic objectives: (1) to determine whether the rates and pattern of pigment photooxidation differed between detrital particles and pigment solutions; (2) to investigate the effects of solvent composition on pigment photooxidation in solutions containing mixtures of pigments. Pigment concentrations in solutions were kept comparable to those in the detrital pigment experiments ( < 0.3 µg ml⁻¹ initial concentration). For HPLC analyses, the pigments in solution were concentrated onto solid phase extraction cartridges (Hamilton PRP) and eluted with acetone. Cartridge conditioning and concentration procedures are described in Nelson and Wakeham (1989).

3. Results

a. Killed cell experiments. The visible light-dependent degradation of chlorophylls, pheopigments, and carotenoids showed a good fit to first-order kinetics for the pigments contained in the detrital chlorophyte (Fig. 1) and diatom cells (Fig. 2). Table 1 lists first-order rate constants for pigment degradation with respect to cumulative light exposure (units of m² Ein⁻¹) calculated for the killed cell experiments. Since the rate constants represent the overall kinetics for multicomponent reactions in which light was present in excess, these should be considered apparent or pseudo first-order. Little or no loss of pigments occurred in dark controls in short term experiments ( < 10% over 5–6 h) or in Experiment 3, where freeze-thawed S. costatum cells were aged 5 days in darkness in the presence of an active bacteria population (Fig. 3, Table 1). A pronounced oxygen-dependence for pigment photodegradation was demonstrated in Experiment 2 (Figure 2, Table 1). Apparent first-order rate constants for photodegradation of the major S. costatum pigments in the N₂ bubbled treatment were 18% of those in the aerated treatment.
Figure 1. The fit to apparent first-order kinetics for the light-dependent degradation of *Dunaliella tertiolecta* pigments (aerated conditions). Filled symbols represent values for dark control samples shown at the equivalent time points as samples from the parallel illuminated treatment. Irradiance for the light treatment was 299 µEin m⁻² s⁻¹ (PAR).

Apparent first-order rate constants determined for the light-dependent degradation of chlorophylls, pheophorbide *a* and the major carotenoids (fucoxanthin, diadinoxanthin, and lutein) ranged from 0.15–0.32 m² Ein⁻¹ in the killed cell experiments (Table 1). Somewhat higher photodegradation rates were observed for chlorophylls *a* and *c* in the diatom cells than for chlorophylls *a* and *b* in the chlorophyte cells. In each killed cell experiment, several minor carotenoids bleached at considerably higher rates than the chlorophylls, pheophorbide *a*, and the major carotenoids. Among *D. tertiolecta* pigments these were β-carotene, neoxanthin and violaxanthin, and among *S. costatum* pigments these were β-carotene, diadinoxan-
Figure 2. The fit to apparent first-order kinetics for the light-dependent degradation of *Skeletonema costatum* pigments: aerated and illuminated (open symbols, solid lines); N\textsubscript{2} bubbled and illuminated (open symbols, dashed lines); aerated and dark (filled symbols, as in Fig. 1). Irradiance for the light treatments was 261 µEin m\textsuperscript{-2} s\textsuperscript{-1} (PAR).

thin and diatoxanthin (Table 1). Higher rates of bleaching for these pigments were evident in both aerated and N\textsubscript{2} bubbled treatments (Experiment 2).

b. *Copepod* fecal pigments. Fecal pellets produced by diatom-fed *E. hyalinus* contained the major pigments of diatoms as well as a number of alteration products. A greater number of unidentified small peaks were present in the fecal pigment chromatograms than in the killed cell samples, including what were apparently minor alteration products of both chlorophylls and carotenoids (Fig. 4). It is possible that some alteration products were formed after egestion of the pellets, either before collection or during storage of the frozen pellets prior to the experiment.

The pheopigments in *E. hyalinus* fecal pellets included pheophorbide *a*, phycophtin *a* and what are tentatively identified as pyropheophorbide *a* and pyropheophytin *a* (Fig. 4). The latter pigment (peak 14 in Fig. 4) was isolated by semi-preparative HPLC from pooled and concentrated sample extracts (as described in Nelson and Wakeham, 1989). Its visible absorption spectrum in acetone (absorbance maxima at 410 nm and 666 nm, with a ratio of absorbances at these wavelengths of 2.41) and relatively nonpolar chromatographic position are consistent with pyropheophytin *a*
Table 1. Calculated first-order rate constants ($k_i$) for light-dependent degradation of pigments in killed cell experiments. The rate constants represent the opposite of slopes of regression lines determined as $\ln \left(\frac{c}{c_0}\right) = -k_i \cdot D$, where $c$ = the concentration at the time of sampling, $c_0$ = the initial concentration, and $D$ = the cumulative light exposure (Ein $\cdot$ m$^{-2}$, PAR).

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dunaliella</em> pigments</td>
<td><em>Skeletonema</em> pigments</td>
<td>“<em>aged</em>” <em>Skeletonema</em> pigments</td>
</tr>
<tr>
<td>Pigment$^{(t)}$</td>
<td>Aerated</td>
<td>Aerated</td>
</tr>
<tr>
<td>Pigment (m$^2$ Ein$^{-1}$)</td>
<td>Regression $k_i$ ($r^2$)</td>
<td>Pigment (m$^2$ Ein$^{-1}$)</td>
</tr>
<tr>
<td>Chl $a$</td>
<td>0.19</td>
<td>0.97</td>
</tr>
<tr>
<td>Chl $b$</td>
<td>0.12</td>
<td>0.92</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.24</td>
<td>0.95</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neox</td>
<td>0.53</td>
<td>0.99</td>
</tr>
<tr>
<td>Violax</td>
<td>0.37</td>
<td>0.98</td>
</tr>
<tr>
<td>$\beta$-car</td>
<td>0.37</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Mean = 0.42</td>
<td>s.d. = 0.03</td>
</tr>
</tbody>
</table>

$^{(t)}$Pigments: Chl $a$, $b$, $c$ = Chlorophyll $a$, $b$, $c$; Neox = neoxanthin; Violax = violaxanthin; $\beta$-car = $\beta$-carotene; Fucox = Fucoxanthin; Pheoph $a$ = pheophorbide $a$; Diadinox = diadinoxanthin; Diatox = diatoxanthin.
Figure 3. Relative changes in concentrations for pigments contained in freeze-thawed *Skeletone
nema costatum* cells aged in darkness for 117.5 hours in the presence of bacteria, then
exposed to light. Note the scale interval for time incubated changes at 115 hours. Light
exposure of 143 µEin m⁻² s⁻¹ (PAR) began at 117.5 hours (arrows). Regression $r^2$ values for
the fit to apparent first-order kinetics of light-dependent degradation are reported in Table
1. Filled symbols are values for dark controls at the end of the light incubation period.

(Schoch et al., 1981). Pyropheopigments are formed from pheopigments through the
loss of a carboxymethyl group (Pennington et al., 1964), which results in a chromatograph-ically less polar molecule. Pyropheopigments have been reported to be prod-
ucts of chlorophyll catabolism in dark-grown mixotrophic algae (Schoch et al., 1981;
Ziegler et al., 1988) and in maturing citrus fruit (Shimokawa et al., 1990), and are
digestive products of chlorophyll in mammalian guts (Pennington et al., 1964 and
references therein).

The presence of relatively nonpolar pheophorbide $a$-like or pheophytin $a$-like
products has been reported in several studies of pigments in the feces of macro-
zooplankton grazers (Vernet and Lorenzen, 1987; Nelson, 1986; Downs, 1989; Roy
and Poulet, 1990) and in particulate samples from the field (Klein and Sournia, 1987;
Downs, 1989). The dominance of pyropheophytin $a$ among the pheopigments of *E.*

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Retention Time (min)

Figure 4. HPLC chromatograms for pigments in fecal pellets produced by *Eucalanus hyalinus*; the upper chromatogram from the absorbance detector (440 nm), the lower from the fluorescence detector. Peak identifications: (S) solvent peak from injection; (1) chlorophyll $c_1 + c_2$; (2) pheophorbide $a$; (3) fucoxanthin; (4) pheophorbide $a$-like, possibly pyropheophorbide $a$; (5) diadinoxanthin; (6) possibly diadinochrome (potentially an artifact of storage); (7) diatoxanthin; (8) unidentified chlorophyll $a$-like derivative; (9) unidentified chlorophyll $a$-like derivative; (10) chlorophyll $a$; (11) unidentified chlorophyll derivative; (12) $\beta$-carotene plus pheophytin $a$, pheophytin $a$ only in the lower (fluorescence) chromatogram; (13) unidentified pheopigment; (14) tentatively pyropheophytin $a$. The concentration of peak 9 increased during the initial period of light exposure and did not decrease below the initial concentration during the incubation.

*hyalinus* is somewhat unusual in that the principal pheopigments in copepod fecal pellets have typically been reported to be pheophorbides (e.g. Downs, 1989; Roy and Poulet, 1990). It is possible that this difference in the pattern of chlorophyll alteration reflects differences in the digestive physiology of *E. hyalinus* (a fairly large
As mentioned above, the post-egestion formation of some pigment alteration products in the present study cannot be discounted. However, no additional formation of pheopigments was noted in *Eucalanus hyalinus* feces in dark controls (Fig. 5), suggesting that the primary formation of various pheopigments occurred within the guts of the animals. Roy and Poulet (1990) also found no significant changes in the relative composition of chlorophylls and pheopigments in copepod fecal pellets after 2 weeks in darkness (at 5°C and 15°C).

Light-dependent degradation of pigments contained in *E. hyalinus* fecal pellets showed a reasonable fit to apparent first-order kinetics with respect to light exposure, although the variance around the first-order regression line was somewhat greater than that found for the killed cell suspensions (Fig. 5, Table 2). The higher sampling variability for the fecal pellet suspension may, at least in part, have been due to greater heterogeneity in pigment content among the fecal particles. As in the killed cell experiments, chlorophylls, pheopigments and the major carotenoid (fucoxanthin) in the fecal pellets bleached at similar rates. However, the values of apparent first-order rate constants for pigment bleaching in the fecal pellets (Table 2) were about half those determined for the major pigments contained in killed phytoplankton (Table 1). Furthermore, the minor carotenoids in the fecal pellets, diadinoxanthin and diatoxanthin, bleached at rates similar to those of the major pigments,
Table 2. Apparent first-order rate constants with respect to light exposure \((k_i)\) for degradation of copepod fecal pigments (aerated suspensions).

<table>
<thead>
<tr>
<th>Pigment</th>
<th>(k_i)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll (a)</td>
<td>0.13</td>
<td>0.95</td>
</tr>
<tr>
<td>Chlorophyll (c_1 + c_2)</td>
<td>0.10</td>
<td>0.94</td>
</tr>
<tr>
<td>Fucoxanthin</td>
<td>0.10</td>
<td>0.89</td>
</tr>
<tr>
<td>Pheophorbide (a)</td>
<td>0.14</td>
<td>0.96</td>
</tr>
<tr>
<td>Pheophytin (a)</td>
<td>0.13</td>
<td>0.93</td>
</tr>
<tr>
<td>Pyropheophytin (a^{(t)})</td>
<td>0.10</td>
<td>0.86</td>
</tr>
<tr>
<td>Diadinoxanthin</td>
<td>0.13</td>
<td>0.94</td>
</tr>
<tr>
<td>Diatoxanthin</td>
<td>0.14</td>
<td>0.92</td>
</tr>
</tbody>
</table>

\((^{(t)}\)For linear regression of \(\ln (c/c_o) = -k_i \cdot D\), where \(D = \) light dose \((\text{Ein} \text{ m}^{-2})\).

\((^{(t)}\)Tentative identification.

rather than showing the appreciably higher rates of light-dependent degradation found in the killed cell experiments. These results could reflect differences in both the effective light dose received by pigments in the two particle types (killed cells versus fecal pellets) and in their molecular environments; factors which could be expected to affect the rates and pattern of pigment photooxidation (discussed below).

c. Products of pigment photooxidation. For the most part, products resulting from light-dependent alterations of chlorophylls, their pheopigment derivatives, and carotenoids were not detected. Exceptions were small increases in what are apparently alteration products of chlorophyll \(a\). Several small peaks eluted at a slightly more polar (earlier) position than chlorophyll \(a\), as is expected for oxidized derivatives of chlorophyll \(a\). Isolation of these pigments by semi-preparative HPLC confirmed their chlorophyll \(a\)-like absorption, but not enough material was collected for detailed spectral characterization. If these pigments represent intermediates in the photooxidation of chlorophyll \(a\), the limited extent of their increase suggests that they were also being degraded.

It must be emphasized that for pigment photooxidation products to have been detected by the HPLC procedure used here, they must have been soluble in the extraction solution and shown either significant absorbance at 440 nm or red fluorescence under blue excitation. Pigment photooxidation products could have been produced that were not detected. For example, loliolides are reported to be products of sensitized photooxidation (involving singlet oxygen) of carotenoids (Isoe et al., 1972). Loliolides have been identified in small amounts in suspended particles and sediment trap material, and in larger amounts in sediments, from the continental shelf off Peru by HPLC analysis using uv absorbance detection (Repeta, 1989). The sedimentary loliolides are diagenetic products of carotenoids, apparently resulting
Table 3. Light-dependent rates of bleaching of *Dunaliella tertiolecta* pigments in solution (apparent first-order rate constants with respect to light exposure). Initial concentration of chlorophyll *a* = 288 ng/ml. Irradiance = 166 μEin · m⁻² s⁻¹.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>100% Acetone</th>
<th>90% Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>k</em> \textsuperscript{i} (m² Ein⁻¹)</td>
<td><em>r</em>²</td>
</tr>
<tr>
<td>Chl <em>a</em></td>
<td>0.62</td>
<td>0.96</td>
</tr>
<tr>
<td>Chl <em>b</em></td>
<td>0.52</td>
<td>0.95</td>
</tr>
<tr>
<td>Lutein</td>
<td>0.56</td>
<td>0.94</td>
</tr>
</tbody>
</table>

from microbially mediated reactions in anoxic sediments (Repeta, 1989), thus this class of compounds cannot be considered to be unequivocal indicators of photooxidative alterations.

d. Possible losses of detrital pigments to solution. The apparent first-order rate constants for photodegradation of pigments (Tables 1 and 2) were determined from the declining concentrations of pigments associated with particles. Significant losses of pigments from the disrupted cells to solution or to colloids that were not retained on glass fiber filters seems unlikely. Dark controls under a similar mixing regime showed little change in particulate pigment concentrations. Furthermore, in preliminary experiments, filtrate (GF/F) from killed cell incubations was passed through a reversed-phase sample preparation cartridge (Baker SPE, conditioned with sequential rinses of 100% MeOH, 50% aqueous MeOH, distilled water and filtered seawater). These cartridges can effectively retain “dissolved” chlorophylls, pheopigments and carotenoids, which may then be eluted in acetone or other suitable solvents (Nelson, 1986). Only very small quantities of *D. tertiolecta* and *S. costatum* pigments were detected in filtrates from killed cell suspensions. This is not the case when concentrated cells of a photoautotrophic dinoflagellate (*Heterocapsa pygmaea*) were disrupted by freeze-thaw cycles and resuspended in filtered seawater. With the dinoflagellate, a considerable amount of peridinin and chlorophyll *a* was found in the filtrate due to the water-soluble protein component of the peridinin-chlorophyll *a*-protein complex (see Haxo et al., 1976). Dinoflagellates were not utilized in the subsequent killed cell experiments.

e. Pigment bleaching in solution. The rates of light-dependent degradation of *D. tertiolecta* pigments in solution (Table 3) were considerably higher than those for the same pigments contained in killed cells (Table 2). The results of these experiments show that the rates and patterns of pigment bleaching in solution depend strongly upon the solvent composition (Table 3). In 100% acetone, chlorophylls *a* and *b* and lutein (the major carotenoid) bleached at similar rates. In 90% acetone, the
Table 4. Comparison of the photo-lability of detrital pigments at three levels within the euphotic zone. Estimates assume a daily surface irradiance (PAR) of 60 Ein m\(^{-2}\) d\(^{-1}\), no vertical mixing, and the mean \(k_t\) determined in (1) killed cell experiments \((n = 11)\), and (2) copepod fecal pellet experiment \((n = 7)\).

\[
\begin{array}{cccc}
\text{Light Level (% } I_o)\text{(b)} & \% \text{detrital pigments degraded after one day} & \\
\text{(for } I_o = 60 \text{ Ein m}^{-2} \text{ d}^{-1}) & 37\% I_o & 10\% I_o & 1\% I_o \\
\text{(m}^2 \cdot \text{Ein}\text{)}^{-1}) & 3.0 & 99.4\% & 75\% & 13\% \\
\text{(Ein} \cdot \text{m}^{-2}) & 5.8 & 93\% & 51\% & 7.2\% \\
\end{array}
\]

(\(^{(1)}\)Light exposure under which the pigments are reduced to half their initial concentrations. 
(\(^{(2)}\)Light levels are equivalent to 1, 2.3 and 4.6 optical attenuation depths.

chlorophylls bleached faster than in 100\% acetone, while the carotenoid was considerably more stable.

4. Discussion

a. Pattern and rates of light-dependent pigment degradation. The results of this study indicate that pigments contained in small particles will be rapidly degraded in the upper portion of the euphotic zone. Table 4 compares pigment bleaching for three levels within the euphotic zone under an assumed surface irradiance of 60 Ein m\(^{-2}\) d\(^{-1}\) (a value representative of mid-latitude waters under a clear summer sky). Based on the mean apparent first-order rate constants determined for copepod fecal pigments, it is predicted that detrital pigments will be reduced to half their initial concentrations after exposure to 5.8 Ein m\(^{-2}\) (PAR). Near the base of the euphotic zone or in turbid waters, detrital pigment lifetimes could be fairly long. Under a cumulative light exposure of 0.6 Ein m\(^{-2}\) (1\% of the assumed daily surface irradiance), it is predicted that photooxidation would reduce detrital pigments to half their initial concentrations only after 9.6 days. Thus, under low light conditions, particle sinking or biologically mediated degradation of pigments may have a greater impact on detrital pigment concentrations than pigment photooxidation.

The experimental results indicate that the photooxidative degradation of detrital pigments will not impart a bias to pigment ratios in the upper water column through the accumulation of more light-stable pigments. There was no relative enrichment of any major pigment in the detrital particles after exposure to visible light. The chlorophylls, pheopigments, and major accessory carotenoids bleached at similar rates within an experimental treatment. The rates of pigment bleaching did, however, vary between particle types. The experimentally determined rate constants were higher for the killed cell experiments (Table 1) than they were for the copepod fecal pellet experiment (Table 2). This could result from differences in the effective
light dose received by pigments or in the microenvironment of pigments within the
two particle types (killed cells versus fecal pellets). These factors are discussed
below.

Previous studies which considered the rates of photooxidation of various accessory
pigments relative to chlorophyll \(a\) and pheopigments reached conclusions that are
somewhat contradictory to those of the present study. Based on the greater stability
observed for chlorophyll \(c\) than chlorophyll \(a\) \textit{in vitro} (90\% acetone), Jeffrey (1974)
proposed that differences in photooxidation rates could account for high chlorophyll
\(c:a\) ratios observed in some field samples. However, \textit{in vitro} photooxidation of
pigments in solution may not be an appropriate model for detrital pigments. In the
present study, bleaching of mixed chlorophyte pigments in solution varied with
solvent composition (Table 3), and the pattern of pigment photooxidation in 90\%
acetone was quite different from that of killed cells, copepod fecal pellets, and the
same pigment mixture in 100\% acetone. High chlorophyll \(c:a\) ratios in field samples
could result from a greater stability of chlorophyll \(c\) than chlorophyll \(a\) in the guts of
zooplankton.

In another study, Klein \textit{et al.} (1986) followed the loss of pigments that resulted
from the grazing of a heterotrophic flagellate on a cryptophyte. Since their experi-}
mental system contained a mixture of living algae, feeding flagellates and the egested
particulate wastes of the flagellates, light-dependent degradation of detrital pig-
ments was inferred from the difference in pigment losses between light and dark
treatments. Klein \textit{et al.} (1986) concluded that chlorophyll \(c\) bleached faster than
chlorophyll \(a\), while pheophytin \(a\) and a carotenoid (alloxanthin) appeared to be
somewhat more light-stable than chlorophyll \(a\). Klein \textit{et al.} (1986) found dark losses
of pigments to be significant and proposed that degradation of pigments to colorless
products occurred in the digestive vacuoles of protozoan grazers. Their study
suggests that the pigment content of biogenic detritus produced by protozoan grazers
could differ significantly from that of macrozooplankton fecal material.

Table 5 compares estimates of first-order rate constants for pigment photooxida-
tion (with respect to cumulative light exposure) made in this and other studies. The
latter are either the published rates or rates calculated from the data presented in
those references. The types of detrital particles examined, light sources and analyti-
cal approaches are noted. SooHoo and Kiefer (1982) and Welshmeyer and Lorenzen
(1985) estimated rates of photooxidation for detrital pheopigments that are lower by
about a factor of 3–4 than those determined in the present study for killed cells and
about half those determined for the copepod fecal pellets. On the other hand, the
first-order rate constant for the photooxidation of pheopigments calculated from
data reported by Guerrero \textit{et al.} (1988) is comparable to those determined in the
present study for copepod feces. The rates and pattern of pigment photooxidation
estimated from data of Klein \textit{et al.} (1986) varied between the two experiments they
reported. Pheophytin \(a\) and alloxanthin bleached at comparable rates in one
Table 5. Comparison of estimates of apparent first-order rate constants for the light-dependent degradation of chlorophylls, pheopigments and carotenoids.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Pigment source</th>
<th>Light source</th>
<th>$k_1$ (m$^2$⋅Ein$^{-1}$)</th>
<th>Pigment analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophylls $a, b, c$</td>
<td>killed phytoplankton</td>
<td>fluorescent</td>
<td>0.25$^(*)$</td>
<td>HPLC</td>
<td>present study</td>
</tr>
<tr>
<td>Major carotenoids</td>
<td>killed phytoplankton</td>
<td>fluorescent</td>
<td>0.23$^(*)$</td>
<td>HPLC</td>
<td>present study</td>
</tr>
<tr>
<td>Chlorophylls, pheopigments, carotenoids</td>
<td>copepod feces (laboratory)</td>
<td>fluorescent</td>
<td>0.12$^(*)$</td>
<td>HPLC</td>
<td>present study</td>
</tr>
<tr>
<td>Pheopigments</td>
<td>natural detritus (&lt;10 $\mu$m)</td>
<td>fluorescent, and sunlight</td>
<td>0.060$^{(**)}$</td>
<td>fluorometric</td>
<td>SooHoo and Kiefer, 1982</td>
</tr>
<tr>
<td></td>
<td>salp feces (field)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheopigments</td>
<td>natural particles (&lt;10 $\mu$m)</td>
<td>sunlight</td>
<td>0.061</td>
<td>fluorometric</td>
<td>Welshmeyer and Lorenzen, 1985</td>
</tr>
<tr>
<td>Pheopigments</td>
<td>natural particles (&lt;20 $\mu$m)</td>
<td>artificial lighting (not specified)</td>
<td>0.14</td>
<td>spectrophotometric</td>
<td>Guerrero et al., 1988</td>
</tr>
<tr>
<td>Chl c</td>
<td>cultured <em>Rhodomonas</em></td>
<td>fluorescent</td>
<td>0.23, 0.87$^{(*)}$</td>
<td>HPLC</td>
<td>Klein et al., 1986</td>
</tr>
<tr>
<td>Pheophytin $a$</td>
<td></td>
<td></td>
<td>0.11, 0.44$^{(*)}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alloxanthin</td>
<td></td>
<td></td>
<td>0.10, 0.18$^{(*)}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorophyll $a$ and peridinin</td>
<td>crude preparation of dinoflagellate PCP complex in filtered seawater$^{(t)}$</td>
<td>sunlight (polycarbonate bottles)</td>
<td>0.055</td>
<td>spectrophotometric</td>
<td>Nelson, 1986</td>
</tr>
</tbody>
</table>

$^(*)$Mean values.
$^{(**)}$Calculated value for 20°C.
$^{(*)}$Results for two experiments. Calculated from data digitized from figures.
$^{(t)}$Filtered seawater extraction of the peridinin-chlorophyll $a$-protein complex (PCP) from lyophilized *Amphidinium carterae.*
experiment, but not in the second. Chlorophyll c appeared to bleach rapidly in their experiments, but with a good fit to apparent first-order kinetics for only the initial portion of the incubations.

Differences in the light sources used in different experiments and how light exposure was measured could account for some of the discrepancies between various estimates of pigment photooxidation rates. The estimated rates of pigment photooxidation could also be influenced by the nature of the particles used in the different experiments. The experiments in the current study were intended to model an excess light situation, such as might be present in a surface mixed layer. Particle concentrations were kept dilute and particles were kept in suspension by mixing. Hence, internal shading of pigments within individual particles, particularly within the small phytoplankton cells, would have been limited. The degree of structural organization of pigments within various detrital particles could also significantly affect the rates of pigment bleaching (discussed below).

In the field, the light exposure received by detrital pigments will depend upon the vertical distribution of light, variations in the spectral composition of light (with depth and between water types), and particle trajectories in the upper water column. It must be emphasized that the experiments described here were conducted under fluorescent illumination. Although the light provided by the mixed fluorescent lamps did not match the solar spectrum, wavelength-dependent effects in the visible region would have been minimized since particle suspensions were fairly dilute. This experimental system did not, however, allow for the consideration of ultraviolet light effects. Most phytoplankton pigments show significant absorption in the near-ultraviolet. In the upper water column of oceanic waters, phytodetritus could receive considerable near-ultraviolet exposure (Smith and Baker, 1981). Rates of pheopigment photooxidation have also been demonstrated to be temperature-dependent (SooHoo and Kiefer, 1982), a factor which could give detrital pigments somewhat greater stability in polar seas (Letelier et al., 1987).

b. Possible mechanism of pigment photooxidation. Perhaps more significant than the magnitudes of the rate constants determined for the light-dependent degradation of pigments is the observation that these rates varied so little between chlorophylls, pheopigments and the major carotenoids. This pattern may result from a coupling of the light-dependent degradation of carotenoids to that of the chlorophylls and pheopigments through a sensitized photooxidation. As discussed below, such a mechanism would require the close association of different pigment types within detrital particles.

The arrangement of pigment-protein complexes in the photosynthetic membranes of living phytoplankton permits a number of interactions between different pigments. One example is the transfer of energy from antennae pigments to photosynthetic reaction centers. Another is the photoprotective function provided by carot-
Figure 6. Potential pathways for chlorophyll (or pheopigment) excitation energy in phytodetritus. The dark boxes indicate electronically excited molecules and the superscripts indicate singlet or triplet states. Based on a similar figure in Foote (1974).

Figure 6 depicts several possible fates for energy from the excited singlet state of chlorophyll a formed by the absorption of light. In healthy cells, the primary route for energy from the excited chlorophyll singlet is to the fast photochemical reactions of photosynthesis (Foote, 1974). In dead cells or phytodetritus, this pathway would not be functional, thus an accelerated rate of formation of the excited chlorophyll a triplet (and that of other accessory chlorophylls and pheopigments) would be expected. The rate of formation of chlorophyll/pheopigment triplets and formation of singlet oxygen might then exceed the quenching capacity of carotenoids. The similar rates of photooxidation noted for the various detrital pigments (Tables 1 and 2) could thus reflect the net effect of chlorophyll/pheopigment sensitization (which would tend to accelerate the rates of pigment bleaching) and carotenoid quenching.

enoid quenching of electronically excited states of chlorophyll and molecular oxygen (Krinsky, 1971; Foote et al., 1970). For oxygen-evolving photoautotrophs, this shunt for excess excitation energy is critical for preventing photodynamic damage to the photosynthetic membranes (Knox and Dodge, 1985). Chlorophyll a and pheopigments have been shown to be potent sensitizers of indirect photooxidation reactions (Endo et al., 1984; Kessel and Smith, 1989); what are classified as Type II photooxidations (Gollnick, 1968). By this mechanism, energy from the excited triplet state of chlorophyll a (formed via intersystem crossing from the short-lived excited singlet state) is dissipated through interaction with molecular oxygen to produce singlet oxygen. Singlet oxygen is highly reactive toward a number of electrophilic compounds, including certain amino acid constituents of proteins, unsaturated lipids, chlorophylls, and conjugated polyenes such as the carotenoids (Foote, 1974).
(which, to some extent, might retard the rates of pigment photooxidation in detrital particles). The bleaching of pigments in phytodetritus bears some resemblance to the light-dependent breakdown of chloroplast pigments in plants treated with herbicides which block photosynthetic electron transport (e.g., diuron or DCMU). The phototoxic effects of these herbicides have been attributed to the overloading of carotenoid photoprotective mechanisms (Pallett and Dodge, 1980).

The pronounced oxygen dependence of pigment photodegradation is consistent with a photosensitized mechanism. Light-dependent rates of pigment photooxidation in N₂ bubbled killed cell treatments were 18% of those in aerated treatments (Table 1, Fig. 2). Similar oxygen-dependence for bleaching of chlorophyll a and pheopigments was noted by Daley and Brown (1973) for broken cell preparations of a cyanobacterium. The results of the bleaching experiments for pigments in solution (Table 3) also suggest interaction between chlorophylls and carotenoids, and the possible involvement of singlet oxygen. Similar rates of photooxidation were observed for a mixture of chlorophyte pigments in 100% acetone, while in 90% acetone, lutein was considerably more stable than chlorophyll a and chlorophyll b. While differences in the degree of aggregation of pigments (and coextracted lipids) in the two solvents might influence pigment photooxidation, these results may, at least in part, be explained by the differences in singlet oxygen lifetimes in the two solutions. Water is an efficient quencher of singlet oxygen, and the lifetime of singlet oxygen in pure acetone is more than ten times that in water (Merkel and Kearns, 1972). In 100% acetone, the parallel bleaching of chlorophylls and lutein could result from a balance between the competing processes of sensitized production of singlet oxygen and carotenoid quenching.

The possible involvement of direct (Type I) photooxidation reactions in the bleaching of pigments cannot be ruled out, particularly for the killed cell experiments. Although taken through several freeze-thaw cycles, the photosynthetic membranes of the killed cells may have retained a considerable degree of their original organization. The presence of variety of electron donors and acceptors in relatively intact chloroplasts would enhance the likelihood of direct photooxidations, and the formation of reactive transients such as peroxides (e.g., Heath and Packer, 1968). Furthermore, the light-dependent formation of singlet oxygen by a chemical mechanism in illuminated chloroplasts (electron transfer from the superoxide anion) has also been proposed (Takahama and Nishimura, 1975). Direct photooxidation could have contributed to both the higher rates of pigment photooxidation in killed cells, and to the bleaching of diatom pigments observed in the N₂ bubbled treatment (Fig. 3, Table 1).

The organization of pigments within the photosynthetic membranes could also have made several minor carotenoids more susceptible to bleaching in the killed cells (neoxanthin, violaxanthin, and β-carotene from D. tertiolecta; diatoxanthin and β-carotene from S. costatum). For example, in diatoms β-carotene is primarily
associated with photosynthetic reaction centers (Brown, 1988), the foci of energy transfer from antennae pigments. Bleaching of β-carotene has been found to precede that of chlorophyll $a$ in isolated chloroplasts treated with monuron, an inhibitor of electron transport (Pallett and Dodge, 1980).

c. The microenvironment of detrital pigments. The coupling of chlorophyll, pheopigment and carotenoid photooxidation in detrital particles through a photosensitized mechanism requires a close, molecular-scale association of the pigments. The nature of the pigments and the types of compounds with which they are likely to be associated could ensure this, even in highly modified phytodetritus. Chlorophylls, pheopigments and most phytoplankton carotenoids are not readily soluble in aqueous solutions. Unless complexed with water-soluble proteins or solubilized in detergent-like micelles, these pigments would tend to remain associated with other hydrophobic cellular material, such as membrane lipids, in phytodetritus.

Distinct pigmented inclusions, olive-green to yellow in color, have been described in several microscopic studies of oceanic detritus (e.g., Silver and Bruland, 1981 and references therein). Silver and Bruland (1981) concluded that what had been referred to as "olive-green cells" in the earlier literature were derived from algae. From transmittance electron micrographs of macrozooplankton fecal material they inferred a transitional sequence from ingested algal cells to pigmented inclusions lacking ultrastructural features. The vertical distribution of "olive-green cells" described by Fournier (1971) for the North Atlantic is consistent with a light-dependent degradation of the pigments; concentrations were quite low in the euphotic zone and increased at greater depths. The mid-water maximum in the concentration of "olive-green cells" (400 to 1000 m) has been proposed to result from the release of the pigmented inclusions from disintegrating fecal material (Silver and Bruland, 1981).

The microenvironment of pigmented inclusions within detrital particles could have important consequences for pigment photooxidation. First, detrital pigments would remain in a close, molecular-scale association at relatively high localized concentrations, even though the ordered structure of the thylakoid membrane has been disrupted. Thus, the likelihood of interactions between pigments (e.g., quenching of excited chlorophyll/pheopigment triplet states by carotenoids, and sensitized photooxidation of carotenoids) would be enhanced. Second, a hydrophobic microenvironment would tend to exclude water. As mentioned above, singlet oxygen is quenched far more rapidly in aqueous solutions than in organic solvents (Merkel and Kearns, 1972), and solubility of singlet oxygen in a lipid-rich hydrophobic microenvironment could be similar to that in organic solvents. Thus, the lifetime of singlet oxygen produced from sensitizers in a lipid-rich hydrophobic microenvironment could be longer, and its potential diffusive distance greater, than if produced by sensitizers in aqueous solution (see Suwa et al., 1977).
d. Some possible consequences of photosensitized oxidations in phytodetritus. The photochemistry of phytodetritus which contains the chlorophyll and pheopigment sensitizers could be considerably more complex than the simplified scheme illustrated in Figure 6. Previous studies have demonstrated the photoproduction of peroxides in isolated chloroplasts (Heath and Packer, 1968) and the hydroxyl radical in chlorophyll a-containing micelles (Harbour and Bolton, 1978). Where some degree of structural integrity of thylakoid membranes remains in phytodetritus, as TEM studies have shown can be the case in macrozooplankton feces (Silver and Bruland, 1981; Nott et al., 1985), the production of such reactive transients may be enhanced. Harbour and Bolton (1978) proposed that the hydroxyl radical could play an important role in the photooxidation of chlorophylls. Furthermore, singlet oxygen is known to initiate free radical chain peroxidation of unsaturated lipids (Rawls and van Santen, 1970). Unsaturated fatty acids generally predominate in algal lipids, particularly in the photosynthetic membranes (Wood, 1974). Thus, the photooxidative effects of chlorophyll/pheopigment sensitization might be amplified within a lipid-rich hydrophobic microenvironment.

One result of lipid peroxidation in chloroplasts is the production of low molecular weight organic volatiles such as ethane (e.g., Percival and Dodge, 1983). The heterogeneous photochemistry associated with phytodetritus could represent a significant source of low molecular weight organic compounds in near-surface waters. Light-dependent formation of organic volatiles has been noted in estuarine waters (R. F. Lee, personal communication), and over much of the North Atlantic there appears to be a net flux of ethane and other low molecular weight hydrocarbons from surface waters to the atmosphere (Rudolph and Ehhalt, 1981). Photooxidation of unsaturated fatty acids in surface waters has also been proposed as the source of ω-oxycarboxylic acids detected in marine aerosols (Kawamura and Gagosian, 1987).

Photooxidative reactions associated with phytodetritus may also play a role in the formation of higher molecular weight organic compounds. Harvey et al. (1983) proposed that the oxidative cross-linking of unsaturated fatty acids could play a role in the formation of marine humic acids, with a key step in the sequence being the light-dependent formation of reactive oxygen species (hydroxyl radical, peroxides). Again, the hydrophobic microenvironment of phytodetritus would provide high localized concentrations of unsaturated fatty acids (major components of phytoplankton membrane lipids) and visible light absorbing photosensitizers which would enhance the generation of the reactive species.

The presence of potent, visible light absorbing sensitizers in phytoplankton could also be of significance to grazers. Assimilation of chlorophylls and pheopigments could make animals susceptible to photodynamic damage in near-surface waters. For macrozooplankton, this might be minimized by egestion of chlorophylls and pheopigments in feces. For protozoan grazers, which incorporate ingested phytoplankton
into intracellular digestive vacuoles, the sensitizers would be in closer proximity to potentially vulnerable cellular components (e.g., membrane lipids). The digestive destruction of chlorophylls and pheopigments noted by Klein et al. (1986) for a flagellate grazer could thus provide a photoprotective as well as a digestive function.

The fairly rapid and parallel bleaching of detrital chlorophylls, pheopigments and carotenoids means that the detrital phytoplankton pigments are not likely to influence near-surface pigment ratios or optical properties in stratified oceanic waters. On the other hand, under low daily light exposures, such as in the lower portion of the euphotic zone or in turbid, mixed coastal waters, photodegradation of pigments would be slow. In these situations, detrital phytoplankton pigments could contribute significantly to the total pigment signal. Under both high and low light regimes, an important question relating to the biooptical characterization of different water types concerns what, if any, absorbance of visible light remains after bleaching of the detrital pigments. Visible light-dependent changes in phytodetritus are considered in a related study (Nelson and Robertson, 1993).

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