

## Differential heterotrophic utilization of organic compounds by diatoms and bacteria under light and dark conditions

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### Abstract

The heterotrophic utilization of organic substrates by diatoms is likely an important survival strategy when light levels are too low for photosynthesis. The objectives of this study were: (1) to determine if heterotrophic utilization of a large array of organic compounds by eight common freshwater benthic diatom taxa was light-dependent, and (2) to determine if organic substrate utilization patterns differed between dark-grown diatoms and bacteria as a possible means of reducing competition by niche separation. Eight light- and dark-grown diatom taxa and five bacterial species were incubated in 96-well Biolog<sup>®</sup> Microtiter plates with each well containing 1 of 95 different organic substrates. Oxidation rates of each organic substrate were measured through time. There was a substantial increase in the number of organic substrates oxidized by diatoms grown in the dark compared to their light-grown counterparts, indicating that the transport systems for these molecules may be light activated. Therefore, diatoms likely only utilize these metabolically expensive uptake mechanisms when they are necessary for survival, or when substrates are plentiful. A principal components analysis indicated discernible differences in the types of organic-C substrates utilized by dark-grown diatoms and bacteria. Although bacteria were able to oxidize a more diverse array of organic substrates including carboxylic acids and large polymers, diatoms appeared to more readily utilize the complex carbohydrates. By oxidizing different organic substrates than bacteria, heterotrophically metabolizing diatoms may be reducing direct competition and enhancing coexistence with bacteria.

### Introduction

Photosynthesis is the primary means by which algae sequester carbon for cell maintenance and growth. However, algal cells often inhabit environments where levels of solar radiation are too low to support autotrophic metabolism. For example, algae are often located below polar ice sheets (Palmisano et al., 1985), buried in sediments (Wasmund, 1987), or shaded at the base of thick periphyton communities (Jorgenson et al., 1983; Burkholder et al., 1990; Liehr et al., 1990; Dodds, 1992; Tuchman, 1996; Johnson et al., 1997). If algae in irradiance-limited habitats are not able to

sequester light by other means such as stalk formation or motility (Johnson et al., 1997), they may resort to either entering a state of dormancy or utilizing heterotrophic metabolism (e.g., Stadelmann, 1962; Tuchman, 1996; Zhang et al., 2003). Under light limited conditions, many algae are capable of heterotrophically metabolizing a diverse range of organic carbon sources including pyruvates, acetate, lactate, ethanol, saturated fatty acids, glycollate, glycerol, hexoses, and amino acids (e.g., Parker et al., 1961; Neilson & Lewin, 1974). In nature, it has been demonstrated that in polar regions, for example, algae that encounter extended periods of low irradiance due to shading

by snow and ice can be metabolically active, incorporating amino acids, glucose, and other exogenous carbon sources (Palmisano et al., 1985; Rivkin & Putt, 1987).

Active transport appears to be the primary means by which algae acquire organic carbon substrates from the environment. Although passive diffusion of exogenous organic carbon is possible (Stadelmann, 1962), concentrations of organic sources are likely too low in most natural environments to sustain efficient passive uptake. Active transport of carbon sources has been documented in several species of diatoms (Hellebust & Lewin, 1977), and is likely the most common mechanism for sequestering these compounds.

These active transport mechanisms appear to be regulated by irradiance in many species of algae. For example, when irradiance is high enough to activate photosynthesis, the uptake of glucose by *Cyclotella cryptica* was minimized, while in the dark, glucose uptake was maximized (Hellebust, 1971). However, photoinhibition of heterotrophic metabolism does not always occur. Phototrophic (photoassimilating) cells have been shown to incorporate a selective range of organic carbon and nitrogenous compounds at low irradiance for storage products as a means of subsidizing their photosynthetic metabolism (Allison et al., 1953; Zotina et al., 2003). However, this 'luxury consumption' is generally less common and uptake rates are lower than by dark-grown algal cells. In certain species, nevertheless, it may contribute a significant portion of the carbon and nitrogen budgets of algae growing under low irradiance (e.g., Zotina et al., 2003).

The proximal relationship between algae and bacteria, particularly in benthic assemblages, may lead to competition between heterotrophically metabolizing algae and bacteria for organic substrates. In situations where heterotrophic algae and bacteria are competing for organic carbon, it has been assumed that the more numerically abundant bacteria with higher metabolism and a greater surface area to volume ratio would out-compete algae for most organic carbon substrates (Stewart, 1974). It is therefore possible that heterotrophically metabolizing algae utilize different substrates than bacteria in an attempt to reduce the effects of competition.

Although there are numerous examples of algal heterotrophy, the heterotrophic utilization of a large number of different organic carbon sources by algae has not been investigated under a single set of controlled conditions for an array of species. The purpose of this study was to survey the ability of freshwater benthic diatoms to sequester and utilize 95 naturally occurring organic carbon compounds. Uptake of these substrates was compared between diatoms grown in the light and those grown in total darkness to determine if uptake mechanisms were light dependent. In addition, diatom substrate utilization was also compared to that of five species of aquatic bacteria to determine if substrate use differed between these two groups of organisms, which could indicate niche separation as a mechanism by which they avoid competition.

## Materials and methods

### *Maintaining cultures*

Eight species of freshwater benthic diatoms were selected for study based on their diverse autecologies. *Achnanthydium minutissimum* (Kutz.) Czar. and *Achnanthydium rostratum* Ostr. are adnate, monoraphid, non-motile benthic diatoms that are commonly found at the base of periphyton mats in streams (Steinman et al., 1987; Tuchman & Stevenson, 1991). *Encyonema minutum* (Hilse in Rabh.) D. G. Mann, *Encyonema minutum* var. *pseudogracilis* (Choln.) Czar., and *Gomphonema accuminatum* Ehrenb. are biraphid, stalked benthic diatoms (Hudon & Bourget, 1981; McCormick & Stevenson, 1991). *Navicula trivialis* L.-Bert, *Nitzschia linearis* (Ag. Ex W. Sm.) W. Sm., and *Nitzschia palea* (Kutz) W. Sm., are biraphid and capable of motility (Bertrand, 1992).

Cultures of diatoms were obtained from the live diatom herbarium of Loras College (Dubuque, IA). Even though the algal cultures were not axenic, bacterial content of these cultures prior to treatment was determined to be negligible using an assay that incorporated an antibiotic protocol (Schollett, 1998). In addition, airborne bacterial contamination was also accounted for (see below) so that heterotrophy could be assumed to be of algal origin. Each culture was divided into eight

125 ml Erlenmeyer flasks containing 75 ml of sterile Alga-Gro inorganic nutrient media (Carolina Biological Supply Co., Burlington, NC). Flasks were stopped with sterile gauze plugs and placed into a Percival environmental chamber on a shaker table and maintained at 15 °C for 10 days under one of two illumination conditions: (1) 8 h of 250  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  and 14 h darkness (light cultures) or, (2) 24 h of 3  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (dark cultures).

Five aquatic bacterial species, *Aeromonas sobria* Popoff and Veron, *Aquaspirillum itersonii* (Giesberger) Hyleman, *Aquaspirillum serpens* (Muller) Hylemon et al., *Aquaspirillum sinuosum* (Williams and Rittenberg) Hylemon et al., and *Bacillus cereus* Frankland and Frankland (Carolina Biological Supply Co., Burlington, NC), were selected for study based on their abundance in natural aquatic habitats. Individual strains were received on agar plates and transferred to 75 ml of sterile, BBL inorganic nutrient media (Benton Dickinson Microbiology Systems, Cockeysville, MD) in autoclaved 125 ml Erlenmeyer flasks (eight replicates), stopped with sterile gauze and placed in a Fisher Science Isotemp Chamber at 23 °C with illumination levels of 3  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  to allow for cell growth. Cultures were manually shaken every 12 h for 10 days to circulate media and redistribute cells. Every 48 h, 50% of the culture media in each flask was pipetted from the surface and exchanged with fresh media to facilitate nutrient replenishment and waste removal.

#### *Determining metabolic activity of diatoms and bacteria*

To quantify the metabolism of exogenous organic carbon sources by bacteria, dark-grown diatoms, and light-grown diatoms, Biolog<sup>®</sup> Microtiter plates (Biolog Inc., Haywood, CA) were inoculated with bacteria or diatom samples from each illumination treatment. Biolog<sup>®</sup> Microtiter plates were used to test the ability of the microorganisms to utilize a wide range of different carbon sources that occur naturally in aquatic ecosystems. A plate consisted of 96 wells; one well contained water and served as a control for airborne bacterial contamination and the remaining 95 were pre-filled with equal molar amounts of

different organic carbon substrates (Table 1). Each well was inoculated with 150  $\mu\text{l}$  of microorganism culture in saline suspension. The culture suspensions were prepared by centrifuging the cultures, decanting the inorganic nutrient media, so as not to add additional substrates into each well, and resuspending cells in 0.85% saline solution. The density of cells in these suspensions was standardized among samples by diluting with 0.85% saline solution or concentrating by centrifugation until a 55% transmittance level at 680 nm was obtained on a Spectronic<sup>®</sup> Genesys<sup>™</sup> 2 spectrophotometer.

To compare biomass-specific oxidation rates among diatoms and bacteria, each individual aliquot of sample was adjusted to a uniform biovolume. In each Microtiter well, density of each species of bacteria and algae used to inoculate the well was quantified and standardized to an equivalent biovolume. Algal cell densities were determined for each species by preparing microscopic slides of cultures (densities that were spectrophotometrically quantified to 55% transmittance at 680 nm) and enumerating at least 300 cells at 1000X magnification. Biovolume of each algal species was estimated by measuring 10 random cells per slide and applying the appropriate geometric equation. Bacterial biovolumes were obtained from the literature (Pelczar et al., 1977). Observed oxidation slopes were then divided by biovolume per well to produce biovolume-adjusted oxidation rates.

Following inoculation, plates were incubated for 12 days under respective light and temperature conditions in which the cultures were originally maintained. If the microorganisms oxidized the carbon source, an associated tetrazolium dye produced a color change that was quantified by a Titertek Multiskan Plus turbidimeter. Readings of each plate were made at 4, 12 and then every 24 h for 12 days, after which no further increase in oxidation was observed in any well. Oxidation rates reported here were measured at day 12.

#### *Data analysis*

Oxidation rates for each carbon source were calculated from the slope of each oxidation curve and corrected for values obtained in the control wells.

Table 1. Substrates tested in Biolog GN Microtiter Plates. Bold headings represent chemical families

<b>Carbohydrates</b>	<b>Carboxylic acids</b>	<b>Amino acids</b>	<b>Aromatics</b>
Adonitol	Acetic acid	D,L-carnitine	Inosine
Alpha-D-glucose	Alpha-hydroxybutyric acid	D-alanine	Thymidine
Alpha-D-lactose	Alpha-keto butyric acid	D-serine	Uridine
Beta-methyl D-glucoside	Alpha-keto glutaric acid	Gamma-amino butyric acid	Urocanic acid
Cellobiose	Alpha-keto valeric acid	Glycyl-L-aspartic acid	
D-arabitol	Beta-hydroxybutyric acid	Glycyl-L-glutamic acid	<b>Alcohols</b>
D-fructose	Bromo succinic acid	Hydroxy L-proline	2,3-butanediol
D-galactose	cis-aconitic acid	L-alanine	2-amino ethanol
D-mannitol	Citric acid	L-alanyl-glycine	Glycerol
D-mannose	D,L-lactic acid	L-aspartic acid	
D-melibiose	D-galactonic acid lactone	L-asparagine	<b>Amines</b>
D-psiocose	D-galacturonic acid	L-glutamic acid	Phenyl ethylamine
D-raffinose	D-gluconic acid	L-histidine	Putrescine
D-sorbitol	D-glucosaminic acid	L-leucine	
D-trehalose	D-glucuronic acid	L-ornithine	<b>Esters</b>
Gentiobiose	D-saccharic acid	L-phenylalanine	Methyl pyruvate
<i>i</i> -erythritol	Formic acid	L-proline	Mono-methyl succinate
L-arabinose	Gamma-hydroxybutyric acid	L-pyroglutamic acid	
L-fucose	Itaconic acid	L-serine	<b>Phosphorylated hydrocarbons</b>
L-rhamnose	Malonic acid	L-threonine	Glucose-1-phosphate
Lactulose	<i>p</i> -hydroxy phenylacetic acid		Glucose-6-phosphate
<i>m</i> -inositol	Propionic acid	<b>Amides</b>	D,L-Alpha glycerol phosphate
Maltose	Quinic acid	Alaninamide	
<i>N</i> -acetyl-D-galactosamine	Sebacic acid	Glucuronamide	<b>Polymers</b>
<i>N</i> -acetyl-D-glucosamine	Succinic acid	Succinamic acid	Alpha-cyclodextrin
Sucrose			Glycogen
Turanose			Tween 40
Xylitol			Tween 80

This correction eliminated any plate-wide contamination effects from airborne bacteria.

Mean oxidation rates and the percentage of all 95 substrates that were utilized were calculated for each chemical group and compared between diatoms (each species used as a replicate,  $n=8$ ) grown in the dark and light using a series of paired *t*-tests (1-tailed,  $H_0$ : dark  $\leq$  light). Mean oxidation rates for each chemical family by dark-grown diatoms ( $n=8$ ) were compared to bacteria (each species used as a replicate,  $n=5$ ) using *t*-tests (Systat version 9, SPSS Inc., Chicago, IL).

Patterns in bacterial substrate utilization were compared to dark grown diatom substrate utilization using principal components analysis (Systat version 9, SPSS Inc., Chicago, IL). All data were

first  $\text{LOG}_{10}(x+1)$  transformed. The analysis was performed on the correlation matrix with no rotations. An ordination plot was created using the first two principal component axes, and *t*-tests were performed between bacteria and algae using component scores from each axis.

## Results

### *Light- and dark-grown diatoms*

Greater numbers of substrates were utilized by each diatom species when grown in the dark than the light (Fig. 1, Appendix), with an average of 68% utilized in the light to an average of 94% in

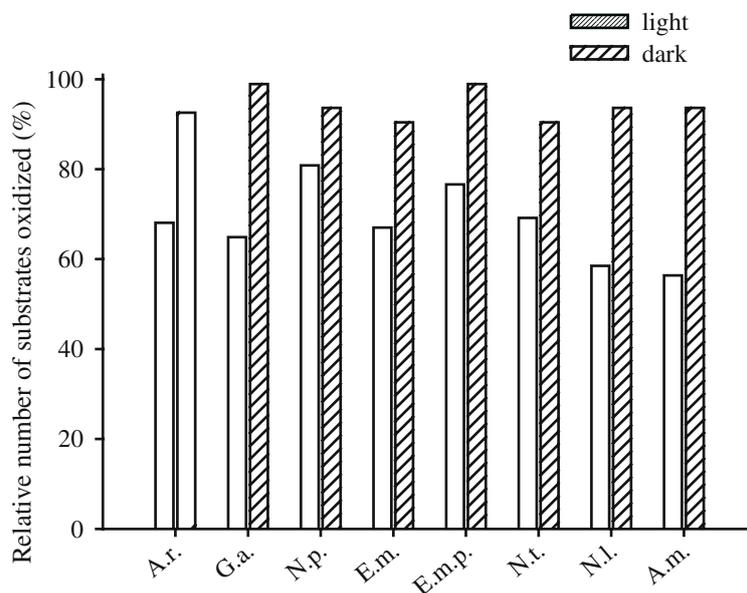


Figure 1. Relative number (%) of 95 substrates utilized by 8 diatom species grown under both light and dark conditions. A.r. = *Achnantheidium rostratum*, G.a. = *Gomphonema accuminatum*, N.p. = *Nitzschia palea*, E.m. = *Encyonema minutum*, E.m.p. = *Encyonema minutum* var. *pseudogracilis*, N.t. = *Navicula trivialis*, N.l. = *Nitzschia linearis*, A.m. = *Achnantheidium minutissimum*.

the dark (paired *t*-test,  $p < 0.001$ ). The number of substrates used in the light ranged from 81% by *Nitzschia palea* to 56% by *Achnantheidium minutissimum*. In the dark, the number of substrates utilized ranged from 99% by *Encyonema minutum* var. *pseudogracilis* and *Gomphonema accuminatum* to 90% used by *Encyonema minutum* and *Navicula trivialis*. The percentage of total substrates utilized increased significantly (paired *t*-test,  $p < 0.05$ ) in the dark vs. the light treatments for each individual chemical family (Fig. 2). This increase was most pronounced in the amides, where the number of substrates oxidized increased from 0 to 100%.

The rate of oxidation of most substrates by diatoms also increased in the dark (Fig. 3). Oxidation rates were significantly higher in the dark than in the light for amides ( $p = 0.005$ ), amines ( $p = 0.011$ ), amino acids ( $p = 0.002$ ) aromatics ( $p = 0.002$ ), carboxylic acids ( $p = 0.048$ ), esters ( $p = 0.001$ ), phosphorylated hydrocarbons ( $p = 0.003$ ) and polymers ( $p = 0.006$ ). They did not differ significantly between light and dark for alcohols ( $p = 0.438$ ) or carbohydrates ( $p = 0.447$ ).

#### Differences in bacterial and dark-grown diatom substrate utilization

Bacteria and diatoms appeared to differ in their use of the different organic substrates. Unlike diatoms, each bacterial species utilized all 95 of the organic substrates. Differences between diatoms and bacteria were primarily due to oxidation rates of different compounds. At the chemical family level, only polymers were significantly different ( $p = 0.012$ ) between dark-grown diatoms and bacteria (Fig. 3). Bacteria oxidized polymers at twice the rate of diatoms. The results of the principal components analysis indicate that 36% of the variance in the data could be explained by the first component (PC1), which was heavily weighted by the utilization of amino acids. The second axis explained 22% of the variance. The ordination plot indicated that bacteria and diatoms were separated distinctly along PC2, but not along PC1 (Fig. 4). Principal component scores did not differ significantly between bacteria and algae with respect to PC1 ( $p = 0.500$ ), but did differ significantly with respect to PC2 ( $p < 0.001$ ).

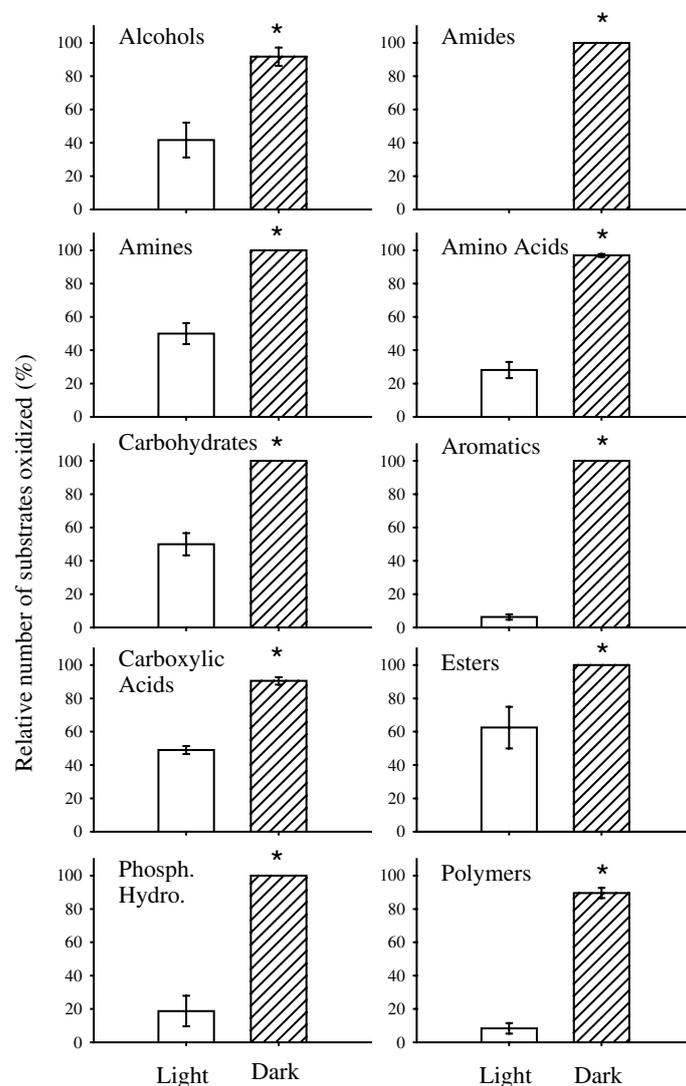


Figure 2. Number of substrates (mean %  $\pm$  1 SE) in each chemical family oxidized by diatoms ( $n=8$ ) grown in the light and dark measured over 12 days. Asterisks represent pairs of means that are significantly different ( $p < 0.05$ ).

The ordination plot indicates that diatoms primarily used substrates that were negatively correlated to PC2, while those that were positively correlated were primarily utilized by bacteria. Substrates that were highly correlated with PC2 ( $r > |0.6|$ ) are listed in Table 2. Tween 80 (a polymer) had the highest positive correlation with PC2, while putrescine had the highest negative correlation. Substrates that were negatively correlated to PC2 and thereby more heavily utilized by diatoms were largely made up of carbohydrates (7 out of 11). Substrates that were

positively correlated to PC2 and thereby more widely used by bacteria consisted of a more even mixture of substrate families. However, polymers (1st and 3rd highest loadings) and carboxylic acids appeared to be more heavily utilized by bacteria.

## Discussion

The diatom species examined in this study all inhabit benthic habitats where they may often encounter light depletion due to frequent burial in

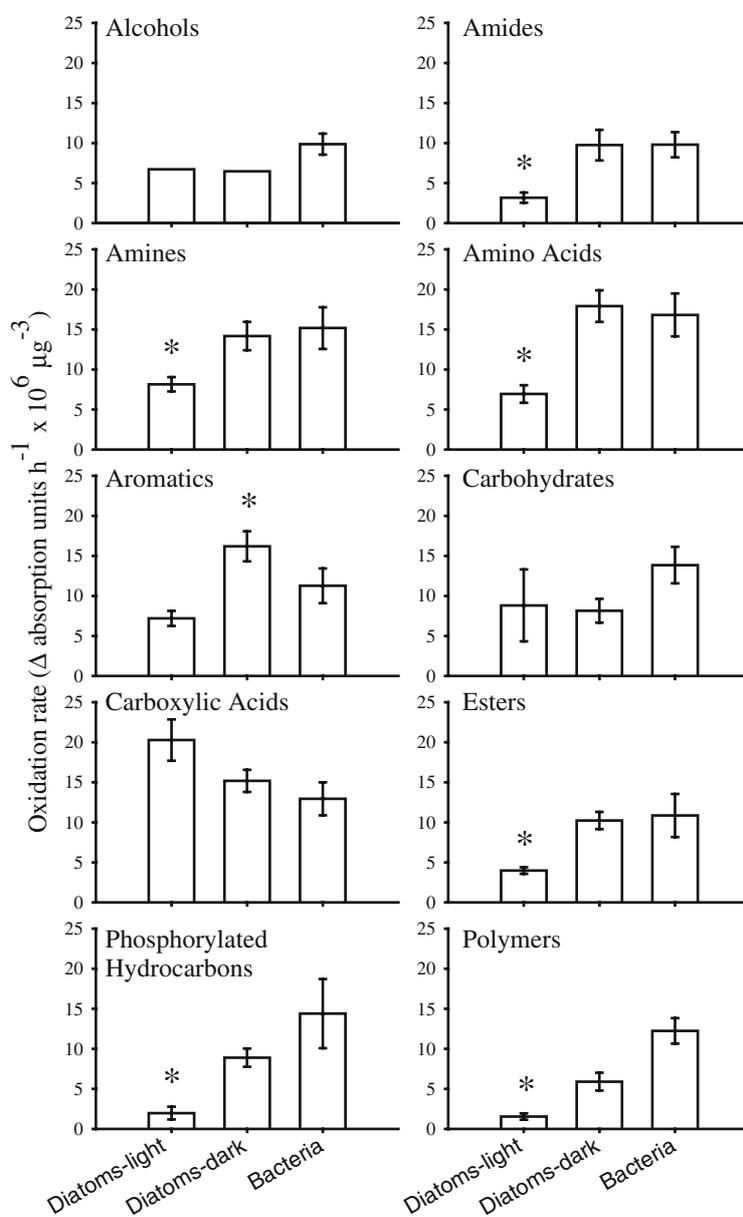


Figure 3. Biomass specific oxidation rates (mean  $\pm$  1 SE) for each chemical family by diatoms ( $n=8$ ) grown in the light and dark for 12 days vs. bacteria ( $n=5$ ), after 12 days. Asterisks represent pairs of means that are significantly different ( $p < 0.05$ ).

the sediments, highly turbid or stained overlying water, or from high density algal mats. Under such light-limited conditions these diatoms can activate mechanisms for uptake and metabolism of organic substrates as a survival strategy. In addition, they have the ability to turn off these metabolically costly uptake mechanisms when irradiance is adequate for photosynthesis. The capacity of a

microorganism to oxidize a compound is initially dependent on two conditions; first the organism must possess a transport system for that particular compound, and second, the environmental conditions must be appropriate for activation of the transport system (Amblard, 1991). The existence of transport systems for a few compounds has been well documented in various species of

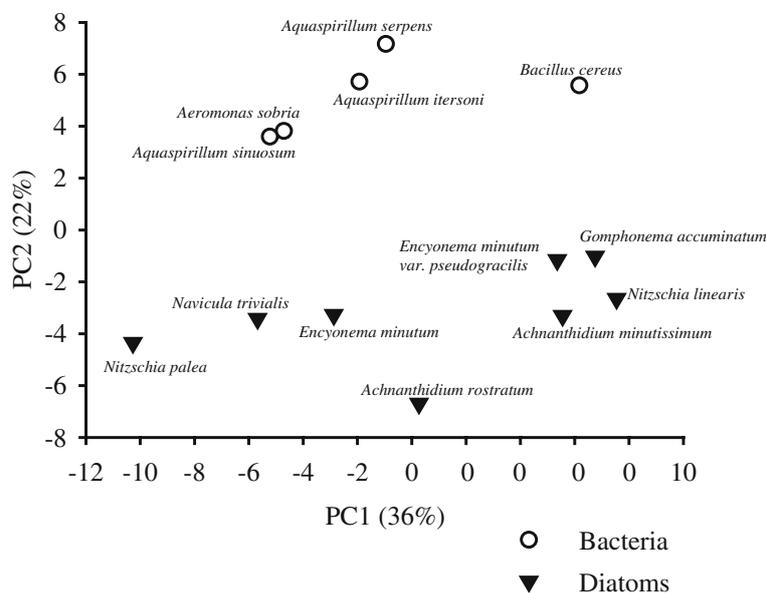


Figure 4. Ordination of 8 dark-grown diatom species and 5 bacterial species using the first two principal component axes (PC1 and PC2).

diatoms (Hellebust & Lewin, 1977). Hellebust & Lewin (1977) demonstrated the specificity of these transport systems for an individual compound when they observed the requirement for a least three transport systems for amino acid uptake: one for acidic, one for basic, and one for neutral amino acids. The high specificity of these transport systems suggests that to use a wide range of compounds, an equally diverse series of transport systems must be operational.

In addition to transport mechanisms, many environmental factors contribute to the regulation of an individual transport system, such as light, pH, temperature, and substrate concentration (Neilson & Lewin, 1974). The results of this study indicate that the eight benthic diatom species tested possess a diverse array of transport systems to allow utilization of a large number of natural substrates, and that many of these mechanisms are regulated in part by irradiance.

Since *Nitzschia palea* was capable of oxidizing 81% of the organic substrates in the light, it is possible that, in addition to low irradiance, substrate concentrations are important for activating uptake mechanisms in this species. *Nitzschia palea* often thrives in habitats that are organically enriched, such as downstream of sewage outfalls (e.g.,

Michels, 1998; Gurbuz & Kivrak, 2003). It is likely that *Nitzschia palea* is able to supplement carbon fixation with heterotrophy in high irradiances when organic substrate concentrations are high.

Although some of the compounds examined in this study do not likely occur in nature in the concentrations or chemical forms used in the experiments, the diversity of substrate oxidation demonstrated in this study suggests that diatoms under dark conditions can likely utilize similar forms of these compounds. Comparable forms of these compounds and their derivatives are present in aquatic ecosystems and may be introduced into the environment from a number of natural sources, including decomposition, allochthonous inputs, autochthonous inputs, animal or plant excretions, or UV-B photodegradation of DOC (Wetzel et al., 1995; De Lange et al., 2003).

In addition to using these compounds as a source of carbon, diatoms might utilize amides, amines, and amino acids as a source of nitrogen as well. Diatoms readily take up dissolved organic nitrogen in both benthic (e.g., Nilsson & Sundbäck, 1996) and planktonic habitats (see reviews by Anitia et al., 1969; Flynn & Butler, 1986; Paerl, 1991). For example, Nilsson & Sundbäck (1996) demonstrated that a number of benthic algae,

Table 2. Correlations of individual substrates to the second principal component axis (PC2). Only substrates with correlations  $>|0.6|$  were included. Positive values represent substrates that were more intensively used by bacteria and negative values represent substrates that were more intensively used by diatoms

Substrate	Correlation with PC2	Chemical family
Tween 80	0.907	Polymers
L-histidine	0.870	Amino acids
Dextrin	0.840	Polymers
L-serine	0.825	Amino acids
Urocanic acid	0.783	Aromatics
D,L-lactic acid	0.774	Carboxylic acid
Mono-methyl succinate	0.772	Esters
Alpha-hydroxybutyric acid	0.710	Carboxylic acid
Glycyl-L-aspartic acid	0.688	Amino acids
Citric acid	0.676	Carboxylic acid
Alpha-D-glucose	0.666	Carbohydrates
L-threonine	0.645	Amino acids
D-mannose	0.637	Carbohydrates
cis-aconitic acid	0.634	Carboxylic acid
Sucrose	0.626	Carbohydrates
Bromo succinic acid	0.626	Carboxylic acid
D-glucosaminic acid	0.601	Carboxylic acid
Putrescine	-0.788	Amines
D,L-carnitine	-0.773	Amino acids
D-raffinose	-0.773	Carbohydrates
Gentiobiose	-0.693	Carbohydrates
L-fucose	-0.690	Carbohydrates
D-melibiose	-0.658	Carbohydrates
m-inositol	-0.653	Carbohydrates
D-glucuronic acid	-0.652	Carboxylic acid
Alpha-D-lactose	-0.625	Carbohydrates
D,L-Alpha glycerol phosphate	-0.613	Phosphorylated hydrocarbons
Lactulose	-0.611	Carbohydrates

including diatoms, could utilize free amino acids. Similarly, Liu & Hellebust (1973) reported that algal cells exhibit growth on glutamine and arginine at rates equal to that of nitrate, and ornithine, asparagine, glycine, alanine, and aspartate can also support growth, although at lower rates than that of inorganic nitrogen.

Although diatom and bacterial species were tested in monocultures under conditions which do not reflect the potential competitive environment that would be encountered in nature, discernible differences in substrate utilization patterns between diatoms and bacteria may reflect specializations that have evolved allowing these two groups of organisms to coexist. For example,

diatoms and bacteria most effectively utilized different organic compounds. The three carbohydrates that were utilized more by bacteria were simple, while diatoms appeared to use more complex carbohydrates. Of the 11 carbon sources that were utilized more by diatoms (high negative correlation to PC2), seven were complex carbohydrates. The two chemical families that appeared more highly utilized by bacteria were polymers and carboxylic acids.

Although a strong potential exists for competition between bacteria and heterotrophically metabolizing diatoms for organic substrates in nature, it is also possible that the interaction between these two groups of organisms is largely

mutualistic. Algae can exude organic compounds that can be assimilated by bacteria (e.g., Petit et al., 1999; Descy et al., 2002; Puddu et al., 2003), and bacteria can make both organic and inorganic carbon available to algae (e.g., Klug, 2005). Algae and bacteria may exchange trace organic substances such as vitamins (Cole, 1982), and bacteria may also release exoenzymes that degrade complex organic molecules (e.g., Sinsabaugh & Linkins, 1988) that are not transportable across cell membranes. Indeed, in this study we found that growing diatoms in cultures that contained bacteria was much more successful than growing axenic diatom cultures.

This study demonstrates several important points: (1) that eight species of benthic diatoms have the ability to sequester and utilize a wide range of organic substrates, (2) that uptake mechanisms are largely light activated, and (3) that diatoms likely sequester a different set of organic substrates than bacteria to possibly reduce competition. These ideas need to be expanded to include organic substrate utilization under natural conditions, which would include monitoring multi-species assemblages and a range of natural organic and inorganic substrate concentrations. However, the information gained here suggests that freshwater benthic diatoms may be much more metabolically active under light-limiting conditions than was previously acknowledged, which may begin to explain, in part, how diatoms that are 10 cm below the sediment/water interface can be metabolically active.

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