1998

Adaptation of the Freshwater Benthic Diatom Achanthidium Rostratum to Resource Limitation

Patrick Daniel Donovan

Loyola University Chicago

LOYOLA UNIVERSITY CHICAGO

ADAPTATION OF THE FRESHWATER BENTHIC DIATOM ACHNANTHIDIUM ROSTRATUM TO RESOURCE LIMITATION

A THESIS SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL IN CANDIDACY FOR THE DEGREE OF MASTER OF SCIENCE

DEPARTMENT OF BIOLOGY

BY

PATRICK DANIEL DONOVAN

CHICAGO, ILLINOIS

MAY 1998

ACKNOWLEDGEMENTS

I would like to thank my committee members, Dr. Nancy Tuchman, Dr. John Smarrelli and Dr. Christopher Peterson for their enthusiastic support and guidance, as well as there friendship. I would also like to acknowledge Dr. Howard Laten, Phyllis Paulo, John Quinn, Joe Schleup and the rest of my many friends who have made life at Loyola an enjoyable experience that I will always cherish.

This thesis is dedicated to my mother and father whose love and guidance have always been my inspiration

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ABSTRACT

In aquatic ecosystems, colonization of a substratum by periphyton and other benthic organisms often leads to development of a multi-tiered algal assemblage (or mat). Resource gradients within these assemblages frequently result in nutrient and/or light limitation for lower-tier cells. Survival of species at the base of well-developed algal mats may involve dormancy (spore formation), acclimation to reduced resources, or reliance on alternative energy sources including heterotrophic metabolism. In this study, we investigated the physiological condition of *Achnanthidium rostratum* (Østrup), a freshwater benthic diatom whose genus is commonly found at the base of developed algal mats, under reduced light and nutrient levels. Cell survival for up to 25 days in total darkness was confirmed through microscopic examination of cultures. A resumption of photosynthetic activity (measured as $NaH^{14}CO_3$ incorporation) following reintroduction to high illumination also reveals that these cells maintained photosynthetic capacity throughout the experiment. Cell-specific ^{14}C-glucose uptake appears to be up-regulated in the dark, indicating utilization of organics as an alternative energy source in the dark. Neutral lipid levels declined over time presumably due to a dilution effect in rapidly dividing cells, however, in resource limited cells, lipid oxidation may also account for declines observed. Similar cell growth among low- and high-light grown cultures indicates this species is also shade adapted. Increased chlorophyll *a* concentrations

observed under low light conditions may be one way cells adapt to reduced light intensities.

CHAPTER I

LITERATURE REVIEW

Benthic algae are an integral constituent of aquatic food webs due, in part, to their high biomass and ubiquity in aquatic systems. This, in turn, supports their roles in primary production and nutrient-cycling. Because of their ability to attach to essentially any submerged surface, benthic algae tend to be the major primary producers in many lotic systems (Lamberti 1996). In a relatively dynamic system, such as a swiftly flowing stream, attached benthic algae can be a consistent food source for consumers, as opposed to more transient sources such as and allochthonous organic material. However, benthic algal assemblages are not immune to loss of biomass by frequent external disturbances, often induced by grazers and spates, or by internal sloughing of senescent cells. As a result, the biomass and taxonomic structure of these assemblages can vary over space and time (Peterson 1996a). Algal cells that can withstand disturbances via adherence to the substratum may have selective advantages over detached cells, allowing them to recolonize the substratum unhindered by spatial and/or other resource constraints that commonly arise in highly developed mats.

In an aquatic environment, colonization of a solid substratum by microorganisms may

progress toward the development of a multi-layered periphyton mat. In lentic systems, microbial colonization frequently involves an initial accumulation of organic molecules followed soon after by the attachment of bacteria and fungi (Hoagland et al. 1982, Hudon and Bourget 1981, Korte and Blinn 1983). Following bacterial colonization, low profile opportunistic diatoms typically attach to the substratum via mucilage (Hoagland et al. 1982, Hoagland 1983, Hudon and Bourget 1981, Roemer et al. 1984). A relatively dense film of diatoms can develop on new substrata within one week (Tuchman and Blinn 1979). After initial proliferation of mostly adnate species over the mat surface, a new vertical dimension arises, characterized by apically arranged diatoms which include rosette/mucilagenous-pad forming *Fragilaria vaucheriae* and *Synedra acus* and stalk formers such as *Achnanthidium minutissimum,* (observed by Oppenheim and Paterson (1990) in both apical and adnate attachment), *Gomphonema olivaceum*, and *Cymbella affinis* (Hoagland et al. 1982, Roemer et al. 1984). In later successional stages of mat development, green and bluegreen filamentous algae often dominate the canopy (Tuchman and Stevenson 1991, Johnson 1996). This seemingly predictable order of mat development has been referred to as "microsuccession" (Hoagland et al. 1982) based on parallels to terrestrial plant succession.

In dynamic systems such as lotic environments, however, the distinct successional stages described above may be obscured. The "obligatory" organic/bacterial stage may not always be present (Hamilton and Duthie 1984). Also, non-mucilage producing algal species have been observed as early colonists (lightly-silicified *Nitzschia* spp.)of substrata within 1-2 days following substratum-mobilizing spates (Peterson 1996b). Moreover,

Steinman and McIntire (1986) observed early colonization by rosette-forming *Synedra* spp. followed by development of an understory of *Achnanthidium* spp. in a laboratory stream. In short, succession in benthic-epilithic periphyton may lead to a "climax" stage, but the successional pathways leading to the "climax" stage may vary depending on both chemical and physical traits, as well as species composition of the pool of available colonists in an environment (Steinman and McIntire 1986).

Pianka (1970) described two theoretical competitive strategies (r- and K- selection, terms coined by MacArthur and Wilson 1967) adopted by species that proliferate during different stages of community development. R-strategists are opportunistic species that quickly colonize sparsely populated habitats, expending most of their energy on reproduction as a means of competing for resources (hence reproductive competition as a means of sustainability). In contrast, K-strategists prevail in high-density habitats and expend more energy on resource competition and growth than reproduction. K-strategists are typically associated with the later stages of community development (Cambridge Dictionary of Biology 1989).

The model of r- and K- selection may be applied to benthic algal microsuccession. Adnate diatoms (r-strategists) that have the ability to detach from densely populated substrata may do so in order to escape resource limitations; and, as early colonizers of downstream substrata, would be the recipients of abundant resources (Stevenson 1990). This may be a likely strategy employed by various species of *Nitzschia* and *Synedra* which tend to proliferate rapidly when cell densities are low (Peterson et al. 1990, Stevenson et al. 1991). An alternative to this strategy may be one where cells unable to

detach from the base of substrata are capable of surviving resource limitations until a disturbance removes the canopy layer, hence, returning these cells to resource abundant conditions. This may be the case for certain nonmotile-adnate diatom taxa (Johnson et al. 1997, Steinman and McIntire 1986). K-strategists ("late-succession species" e.g. apically attached, rosette, stalk, and filament formers), associated with the canopy layers are better competitors for limited resources due to their physical characteristics. Yodzis (1978) referred to dominance-controlled shifts in population growth strategies when certain algae (i.e. apically attaching) use their capacity for vertical growth as a means of out-competing colonizer species for resources.

The progression of a periphyton community towards the development of an overstory (canopy) often leads to establishment of vertical resource gradients (i.e. light, nutrients, O_2, and CO_2) producing stratification within the mat (Burkholder et al. 1990, Hoagland 1983, Hudon and Bourget 1981, Jørgensen and Revsbech 1983, Karlström 1978, Nicholson et al. 1987, Stevenson and Glover 1993, Tuchman 1996, Yodzis 1978). An *in-situ* study by Hoagland (1983) of diatom mats grown on glass slides in a reservoir, demonstrated a decrease of approximately 45% of maximum light transmittance from the canopy to the basal cells after 8 days of community development. Johnson (1996) measured greater than 92% reduction in ambient light at the base of algal mats in an artificial stream system after 48 days of periphyton development. Similarly, Stock and Ward (1991) observed algal cells at the base of a lotic blue-green algal mat consisting primarily of *Oscillatoria submembranacea* were light limited and that >98% of the photosynthetic activity in these mats occurred in the top 1/3 of the mat.

The physical positioning of a species within an algal assemblage is often associated with nutrient availability and hence a cells' nutrient uptake capacity. Lower uptake rates of $^{33}PO_4$ were observed in adnate microalgae (diatoms, blue-greens, and green algae) growing at the base of developed epiphyton assemblages compared to loosely or apically attached algae in the canopy of these assemblages (Burkholder et al. 1990). Riber and Wetzel (1987) found that diffusion of nutrients from the water column into periphyton mats decreases as mats become thicker and that the delivery of nutrients to basal cells is further diminished in oligotrophic systems (see also Stevenson and Glover 1993).

The development of vertical resource gradients as benthic algal communities grow can create a limiting environment for those cells located at the base of these communities. Stresses involving minimal diffusion of nutrients to the lower tiers of an algal mat can be alleviated to an extent by internal recycling of nutrients, although exponential growth may still be nutrient limited (Mulholland 1996). Some species avoid resource-limiting conditions by apical attachment on substrata and subsequent formation of mucilagenous stalks or by true filamentous growth that elevates these cells into the resource-rich canopy (Hoagland et al. 1982). Alternatively, some species are capable of detachment and emigration out of an overcrowded mat. For example, the diatoms *Hannaea arcus* and *Diatoma tenue* can avoid prolonged exposure to darkness by internal regulation of bouyancy, and subsequently, detach from substrata under adverse conditions (Bothwell et al. 1989). Many pennate species of diatoms are motile, relying on mucilagenous secretions through slits (raphes) along their frustules, which may enable these species to escape from light limitation and other inclement chemical conditions found at the base of

algal mats (Round and Palmer 1966, Cohn and Disparti 1994).

Cells incapable of physical "escape" from resource-limiting conditions at the base of a well developed mat must rely on physiological means for survival. Survival during prolonged exposure to aphotic or anoxic conditions have been documented in both benthic and planktonic algal species (Moss 1977, Poulickova 1987, Wasmund 1989). Many diatom taxa form resting spores to survive prolonged exposure to resource-poor conditions. This phenomenon has been documented in the marine diatom, *Leptocylindrus danicus* under conditions of nitrogen depletion (Davis et al. 1980), and in freshwater planktonic diatom genera such as *Achnanthidium, Navicula, Nitzschia, Fragilaria, Aulacosira*, and *Stephanodiscus* in Great Lakes sediments under conditions of low temperature and darkness (Sicko-Goad et al. 1989).

As an alternative to "resting states", certain algal species employ facultative heterotrophy to procure energy (Lewin and Hellebust 1970, 1978, Hellebust and Lewin 1972, Rippka 1972, White 1974, Berman et al. 1977, Darley et al. 1979, Saks 1983, Bollman and Robinson 1985, Rivkin and Putt 1987). A facultative heterotroph (chemoorganotroph) has the ability to obtain energy from exogenous pre-formed organic compounds, or through normal photoautotrophic fixation of atmospheric CO_2 (see Tuchman 1996). Facultative heterotrophic ability is typically associated with benthic pennate diatoms such as *Amphora sp.* and *Nitzschia sp.*, but has also been noted in some planktonic centric species (Hellebust and Lewin 1977). Among facultatively heterotrophic species, chemoorganotrophy appears to be a mode of nutrition secondary to photoautotrophy, and used mainly as a survival mechanism, with the more metabolically

efficient photoautotrophy preferred (Lewitus and Kana 1994, Tuchman 1996).

Hellebust and Lewin (1977) identified numerous centric and pennate diatom species that possess transport mechanisms for the uptake of several different exogenous organic compounds. Schollett (1998) demonstrated that up to 96 different organic compounds, including carbohydrates, amino acids, nucleic acids, esters, and carboxylic acids can be metabolized by 8 benthic diatom species including *Nitzschia palea*, *Achnanthidium minutissimum*, and *A. rostratum*. While some algal populations may sustain their densities in darkness (Panella 1994), many are incapable of cell division in such conditions (Hellebust and Lewin 1977). Species capable of sustenance and/or growth in the dark have a distinct advantage under conditions of prolonged exposure to darkness (e.g. positioned at the base of a thick periphyton mat or prolonged burial in sediments). Low profile species able to survive the limiting conditions found in mature benthic algal mats have initial access to resources made available upon disturbance and removal of the overlying canopy (see Johnson et al. 1997).

^{14}C-labeled organic carbon compounds (i.e. glucose, glutamate, lactate, etc.) have often been used to test the ability of cells to grow in the dark on organic supplements (White 1974, Lewin and Hellebust 1975, Lewin and Hellebust 1978). Uptake of radio-labeled organics can infer the presence of an organic transport system in the cells, as well as delineate which populations can grow and which merely sustain their numbers through heterotrophic mechanisms. With this technique, one can determine those species that can supplement their nutrient requirements with an alternative energy source in conditions of lower light or inorganic nutrient supply. Hellebust and Lewin (1977) reported the

presence of organic transport systems in many diatom species, although few of these species were actually able to grow on organic molecules in the dark.

The facility for photosynthetic activity within periphyton mats is often gauged by measuring uptake of labeled ^{14}C-sodium bicarbonate ($NaH^{14}CO_3$) (Hellebust 1971, Mouget et al. 1993, Saks 1983) to determine if decreased CO_2 uptake rates are associated with decreased light levels. Measures of chlorophyll *a* pigment concentrations are also used to document algal response to reduced light, with per-cell increases in chlorophyll *a* levels often associated with reductions in light availability (Falkowski and Owens 1980, Geider et al. 1986, Neale and Melis 1986). By increasing chlorophyll *a* concentrations, cells in the lower tiers of a benthic mat may be able to sequester more light. Examining chlorophyll *a* levels along with CO_2 uptake in cells may, therefore, be useful in determining critical light thresholds in algal cultures.

Microalgae have been observed to accumulate neutral lipids under stressful conditions, such as nitrogen or phosphorous limitation (Ben-Amotz et al. 1985, Fogg 1956, Livne and Sukenik 1992, Reitan et al. 1994), silicate deficiency (Taguchi et al. 1987) and high pH (Guckert and Cooksey 1990). Unlike polar lipids, such as phospho- and glycolipids which are structural components of cell membranes and pigments, neutral or nonpolar lipids, composed of esterified saturated or mono-unsaturated fatty acids, are used as energy stores (Guckert and Cooksey 1990, Napolitano 1994). These substances, therefore, may be tapped by cells under stressful conditions to increase their probability of survival. Polar lipids tend to remain stable under varying environmental conditions. Levels of neutral lipids, in contrast, often fluctuate and, therefore, may be used as an

indication of the physiological condition of the cell. Cells depleted of essential nutrients or stressed by other environmental conditions often cannot divide because certain "building block" compounds are lacking, which may lead to inhibition of the cell cycle. Under such conditions, however, production of photosynthates continues and this material is used to synthesize neutral lipids (triglycerides) (Guckert and Cooksey 1990, Sicko-Goad et al. 1988). In the diatom *Cyclotella cryptica*, for example, the onset of silicate starvation induces cessation of cell division and a doubling of neutral lipids (Shifrin and Chisholm, 1981). Shifrin and Chisholm (1981) also revealed that the increase in the lipid fraction was not due to cell synchrony (i.e. cell growth stopped at lipid-rich stage of cell cycle) since, under optimal growth conditions, lipid fractions (of total cell mass) remained stable and independent of cell division. Upon resumption of favorable growth conditions (i.e. sufficient nutrient supply, etc.) neutral lipid levels declined proportionally with a rise in cell numbers, indicating a dilution or depletion in stored lipid quantities during cell division. Neutral lipid levels appear to be highest just prior to cellular division under ambient conditions, suggesting their use as an energy source for this process (Sicko-Goad et al. 1988). In addition, neutral lipid levels in algal cells appear to follow a 24-hour cycle, with high neutral lipid levels present at the onset of darkness and the lowest levels measured just prior to reillumination. This may indicate use of neutral lipids in place of photosynthetic energy for cellular activity in the dark.

Diatoms can oxidize a substantial volume of neutral storage lipids during cell division or during periods of darkness (Fisher and Schwarzenbach 1978, Otsuka and Morimura 1966). Two clones of the marine centric diatom *Thalassiosira pseudonana*, grown under

constant light, maintained growth rates when transferred to dark and incubated for 24 hours, but showed a marked decrease of C16:0 and C16:1 fatty acids (long chain saturated- and monounsaturated fatty acids respectively, stored as neutral lipid) during dark incubation (Fisher and Schwarzenbach 1978). However, in senescent (non-dividing) cells grown in the light, C16:0 and C16:1 fatty acids increased significantly, indicating synthesis of these fatty acids in the light (under optimal conditions) and, subsequently, utilization under sub-optimal conditions (i.e. in the dark) (Brown et al. 1996, Fisher and Swarzenbach 1978). The diatom *Nitzschia closterium* also has been shown to produce significantly higher concentrations of 16:0 and 16:1 fatty acids under high-light versus low-light conditions; presumably as a high-energy storage product (Orcutt and Patterson 1974).

In summary, adaptations of benthic algae to resource-limiting conditions that often exist in dense periphyton mats are complex and not well understood. Survival at the base of a dense periphyton assemblage could involve physically altering cell position through stalk or filament formation or by motility. Those cells unable to relocate from the base of the mat may employ physiological adaptations such as increasing chlorophyll *a* concentration, use of neutral lipid stores, or use exogenous organic compounds via heterotrophic metabolism. To assess the physiological status of those cells found at the base of an algal mat, various molecular techniques need to be employed to determine the survivability and overall fitness of the cells.

CHAPTER II

INTRODUCTION

Primary production in lotic systems may be supplied in part by macrophytic and allochthonous sources as well as by periphyton (benthic algae). Contributions of periphyton to primary production in lotic systems, once thought to be minimal, now are deemed significant, particularly in streams with relatively open canopies (Lamberti 1996, Minshall 1988). Energy input to midorder streams is thought to be supplied heavily from benthic algae (Vannote et al. 1980). Further studies of benthic algal survival strategies will help us better understand the ubiquitous nature of periphyton and their role as primary producers.

Development of a benthic algal assemblage may progress from an initial 2-dimensional framework to one of 3-dimensional physical stature (Hoagland et al. 1982). Initially, exposed substrata may provide abundant space and access to resources for colonizing species, permitting rapid reproduction and the eventual creation of a dense low-profile community. Once available substratum is covered, the accompanying spatial constraints create an environment conducive to species capable of apical (or vertical) growth, such as alga that form stalks or filaments. Over time a thick canopy can form creating vertical resource gradients within the mat where light and nutrient levels decrease from the upper story to the base of the mat (Hudon and Bourget 1981,

Burkholder et al. 1990, Stevenson and Glover 1993, Johnson et al. 1997).

The ability of cells in the lower tiers of an epilithic periphyton mat to survive resource-limiting conditions may depend on physical and or physiological adaptations. To alter their position within a mat to "escape" detrimental conditions, algal cells may employ stalk formation, detachment and emigration, or motility. Those low-profile cells unable to alter their position within a mat may employ physiological mechanisms for survival such as: entering a state of dormancy (resting state, spore formation) by reduction of metabolic rates, or by using alternative energy sources such as nutrient recycling, lipid oxidation, or heterotrophic metabolism. Under conditions of very low light, algal cells may elevate their chlorophyll *a* concentrations to sequester additional photons needed to maintain metabolism.

In this study, I investigated the physiological condition under different nutrient and light regimes of *Achnanthidium rostratum*, an adnate, monoraphid, non-motile diatom species within a genus that typically occurs in the lower tiers of natural freshwater benthic algal mats (Steinman et al. 1987, Tuchman and Stevenson 1991). Specifically, I wanted to determine if viability and metabolic-activity levels of *A. rostratum* changed with variations in light availability (light and dark) and nutrient regime (nutrient-replenished and non-replenished media). My specific objectives were to determine: 1) the ability of *A. rostratum* to survive and/or reproduce in the dark, 2) whether *A. rostratum* cells grown in the dark compensate for lack of light by depleting their energy stores of neutral lipids and/or increasing chlorophyll *a* concentrations, and 3) the ability of *A. rostratum* cells under light-limiting conditions to resume photosynthetic activity within 30 hours

after reintroduction to high light levels.

CHAPTER III

MATERIALS AND METHODS

Maintaining Culture Conditions

Cultures of *Achnanthidium rostratum* (strain L588, Loras College live diatom herbarium, Dubuque, Iowa) in a soil-extract medium (CR1) consisting of one ml soil to 10 ml deionized water containing 0.5 mg/ml $MgCO_3$ (Nichols 1973) were obtained twice from the collection over a one and a half year period. Initial cultivation of the two stock cultures was identical; cultures were first transferred to fifteen 125-ml erlenmeyer flasks (plugged with sterilized soft foam stoppers allowing for air exchange) containing 75 ml Bold's Basal Medium (Nichols 1973) made with bottled spring water with the addition of $1.25x10^{-5}$ M silicon. Cell cultures were grown without media replenishment in a greenhouse (light levels varied depending on cloud cover from 100-1000 + µmol quanta• m^{-2}•sec^{-1}) on a shaker table (100 rpm) to simulate a moderately turbulent system. After approximately 3-4 weeks, diatoms within each flask were detached with a rubber policeman, transferred to a single beaker to form an initial stock culture, and homogenized with a hand-held mixer (Braun® Drink Master). Triplicate aliquots of each stock culture were permanently mounted in Taft's syrup medium (Stevenson 1984) for initial live and dead cell enumeration (Nomarski optics-1000x magnification). The

homogenous stock cultures were then divided evenly into two sets of seventy-two 50-ml erlenmeyer flasks and each was diluted to 40 ml with fresh, axenically prepared Bold's medium (Bold's media in nutrient-replenished cultures contained organic supplements as described below). Cultures within each nutrient treatment were exposed to three light levels: High Light (HL ≅ 221-445 μmol quanta•m^{-2}•s^{-1}), Low Light (LL ≅ 12-25 μmol quanta•m^{-2}•s^{-1}) and Dark (D = 0 μmol quanta•m^{-2}•s^{-1}). The first set of flasks were grown in Bold's media without replenishment of media (Nutrient-Deplete), and were grown in a greenhouse (Loyola University of Chicago, Damen Hall, during July, Temp. ≅ 17±5^{0}C). The second set of flasks containing cultures obtained at a later date (Nutrient-Replete) were replenished 5 days prior to the start of the experiment with 30ml Bold's media supplemented with one mM concentrations of glucose, sodium acetate, and casamino acids (see Panella 1994) and were grown in an environmental chamber (Percival, Temp. ≅ 20±3^{0}C). Media in both the 72 nutrient-depleted and 72 nutrient-replenished flasks were initially diluted approximately 1.55:1 and 1.75:1 respectively with fresh Bold's medium to bring each flask to 40 ml total volume. Subsequent replenishment of nutrient-replete cultures with 15 ml aliquots of organic nutrient-enriched Bold's media were made on days 8, 11, 15, 19, and 23 to maintain high nutrient levels throughout the experiment. Flasks exposed to the low light (LL) treatment were covered with a wooden framed box wrapped in shading cloth to decrease light penetration (Panella 1994). Flasks comprising the dark (D) treatment were covered with aluminum foil to block all light penetration. Illumination intensity was quantified at a level parallel with the top of the shaker table with a Licor Quantum/Radiometer/Photometer. Fitness and viability of cells under each

set of conditions were compared using parameters outlined in Figure 1. Cultures were sampled at the same time each sampling day to avoid cellular variations affected by diurnal rhythms. Upon collection, cells from each treatment (3 replicates taken for each analysis) were transferred to test tubes (under minimal light for LL and D samples), and a subsample from each flask was centrifuged at setting # 8 for 20 min in a Centrific® centrifuge (Fisher Scientific, Pittsburgh, PA). The resulting pellet of cells was frozen with liquid N_2, and placed immediately in a freezer at -80°C for later analyses for neutral lipids, chlorophyll *a*, and total protein. An aliquot (1 ml) was also removed for cell counts and general microscopic examination of cultures. The remaining cells in each flask were incubated with ^{14}C at their respective light levels to quantify photosynthetic activity via $^{14}CO_2$ uptake and fixation, and heterotrophic activity via ^{14}C-labeled glucose incorporation into cellular carbohydrates.

Analyses performed on cultures in both experiments were the same except where noted (Figure 1). Additional analyses in the second experiment (nutrient-replenished cultures) were not intentionally left out of the first experiment, but were added to help gain a better understanding of *A. rostratum* survival strategies.

Biochemical and Physiological Analyses

Total Photosynthetic Activity

Photosynthetic activity of diatoms at different light and nutrient levels was monitored by measuring cell incorporation of ^{14}C-labeled sodium bicarbonate ($NaH^{14}CO_3$) (NEN Research Products) to determine the effects of diminished light on CO_2 uptake. For

Figure 1. Experimental design outlining procedures used for analysis of *Achnanthidium rostratum* cultures grown in nutrient-depleted and nutrient-replenished media conducive to both photoautotrophic and heterotrophic growth conditions.

Experimental Design

Nutrient-Deplete Experiment: July, 1992	**Nutrient-Replete Experiment:** February, 1994
(72)40ml flasks of *A. rostratum* were grown in Bold's media without replenishment in greenhouse at 17 ± 5°C for 25 days.	(72)40ml flasks of *A. rostratum* were grown in organic/inorganic nutrient supplemented Bold's media with replenishment on days 8,11,15,19,23 in an environmental chamber at 20 ± 3°C for 25 days.
Treatments:	**Treatments:**
Photoperiod follows summer hours 1) High Light (HL): 333 ± 112 *u*mol quanta m^{-2} s^{1} 2) Low Light (LL): 20 ± 5 *u*mol quanta m^{-2} s^{-1} 3) Dark (D): 0 *u*mol quanta m^{-2} s^{-1}	12:12 photoperiod 1) HL: 255 ± 10 *u*mol quanta m^{-2} s^{-1} 2) LL: 12 *u*mol quanta m^{-2} s^{-1} 3) D: 0 *u*mol quanta m^{-2} s^{-1}
Analyses:	**Analyses:**
Triplicate flasks from each light treatment were sampled for testing on days 2,4,6,8,10,15,20, and 25 except for analyses involving ^{14}C incorporation which were sampled every other day.	Triplicate flasks from each light treatment were sampled for testing on days 2,4,6,8,10,15,20, and 25 except for analyses involving ^{14}C incorporation which were sampled every other day.
1) Photosynthetic Activity: Na $^{14}CO_3$ incorporation 2) ^{14}C-glucose incorporation 3)Neutral lipid levels 4) Live/dead cell enumeration (by presence/absence of protoplast)	1) Photosynthetic Activity: Na $^{14}CO_3$ incorporation 2) $Na^{14}CO_3$ incorporation of LL and D cells after reillumination to HL for 30 h 3) ^{14}C-glucose incorporation 4) Chlorophyll *a* levels 5) Neutral lipid levels 6) Total protein levels 7) Live/dead cell enumeration (by presence/absence of protoplast)

nutrient-depleted cultures, triplicate 10-ml aliquots from each flask were transferred to test tubes on sample days 2, 6, 10, 15, 20, and 25, and incubated with 1 µl of 1.0 µCi $NaH^{14}CO_3$ for 30 min on a shaker table (100rpm) under their respective light levels, and then pelleted and washed with 2 ml of unlabeled 1M glucose ("cold" glucose used on day 2 only; "cold" $NaHCO_3$ was used on remaining days) to remove exogenous and non-specifically bound ^{14}C. The samples were again spun down, frozen with liquid N_2, and placed in a -80°C freezer until analyzed. Upon analysis, each pellet was resuspended in 10 ml Aquasol scintillation cocktail and the amount of ^{14}C incorporated into photosynthate was measured using a Beckman LS Scintillation counter. Results of cell enumeration were then used to calculate live-cell specific relative photosynthetic activity by dividing relative cpm per ml by cells per ml. For nutrient-replenished cultures, 5 ml portions from each flask (3 replicates per treatment) was sampled and processed as described above.

Reillumination Experiment

In nutrient-replenished cultures, cells grown under dark, low, and high (used as control) illumination for 2, 6, 10, 15, 20, and 25 days were reintroduced to high light levels for 30 hours to assess the ability of *A. rostratum* to "recover" from prolonged exposures to darkness or low light intensity. Following 30 h of reillumination, triplicate-samples were incubated with one µl of 1.0 µCi $NaH^{14}CO_3$ for an additional 30 min under identical conditions, pelleted, washed, and analyzed as described above to quantify rates of photosynthetic carbon fixation.

Glucose Uptake

Triplicate ten-ml samples obtained from each light treatment on days 2, 6, 10, 15, 20, and 25 were incubated with one µl (0.1 µCi) ^{14}C-glucose (NEN Research Products) for 30 min on a shaker table (100rpm) under experimental light levels to assess the ability of *A. rostratum* to incorporate glucose under different light and nutrient regimes. After incubation, the cells were pelleted, washed once with 2 ml of 1M unlabeled glucose to remove exogenous non-specifically bound ^{14}C, then repelleted and frozen as previously described. Upon analysis, the cells were resuspended in 10 ml Aquasol scintillation cocktail and radioactive incorporation was determined using a Beckman LS 7000 Scintillation counter, yielding live-cell specific relative incorporation of glucose.

Chlorophyll a

Chlorophyll *a* levels were measured in nutrient-replenished cultures to determine whether cells grown under low illumination compensated by increasing chlorophyll *a* concentrations. In nutrient-replete cultures, triplicate-frozen cell pellets (covered in foil to prevent chlorophyll *a* degradation) from each light treatment on days 2, 4, 6, 8, 10, 15, 20, and 25 were resuspended in 5 ml of 90% ethanol and boiled at 78°C for 5 min in a water bath (Sartory and Grobbelaar 1984). The samples were held in a refrigerator (5°C) overnight, then centrifuged (6,000 rpm) for 10 minutes. The supernatant poured into a 1-cm cuvette, and the absorbance was read at 665 and 750 nm on a Beckman DU-64 Spectrophotometer. Triplicate-samples were then acidified to convert chlorophyll *a* to phaeophytin *a* using 100µl of 0.3 M HCl, and allowed to incubate at room temperature in darkness for 30 minutes prior to reading absorbance again at 665 and 750 nm.

Chlorophyll *a* values were calculated using the method of Lorenzen (1967) with a chlorophyll *a* coefficient by Sartory (1982), and expressed as μg chlorophyll *a* per cell.

Neutral Lipid Analysis

Analysis of intracellular lipid stores of *Achnanthidium rostratum* cultures were assessed by spectrofluorometry according to Cooksey et al. (1987) to determine the effect of light and nutrient limitations on lipid levels. Previously frozen cell pellets were resuspended in 5 ml Bold's medium, homogenized (vortex), and divided into two 2.5 ml samples. The fluorophore dye, Nile Red (9-diethylamino-5H-benzo(a)phenoxazine-5-one) dissolved in acetone (250 μg/ml), was used to quantify the lipids. Ten μl of Nile Red solution was added to each sample and mixed for 30 seconds on a vortex prior to measurement on a Turner Model 430 Spectro Fluorometer with excitation and emission wavelengths set at 525 and 580, respectively. Lipid quantity was expressed as relative fluorescence per cell.

Protein Concentrations

Total protein quantities were measured on nutrient-replenished cultures to determine whether cellular protein levels changed under different light regimes. Protein concentration was quantified in an aliquot (5-10 ml) of *A. rostratum* cells from each light treatment using a modified Lowry Assay (Markwell et al. 1981, Bensadoun and Weinstein 1976). Cell pellets were resuspended in 100 μl of 1:1 ratio ddH_2O to 2x treatment buffer [0.125 M Tris-base pH 6.8, 4% (w/v) sodium dodecyl sulfate (SDS), 20% (v/v) glycerol, 10% (v/v) 2-mercaptoethanol, and 0.1% (v/v) bromophenol blue]. Proteins extracted in this manner were transferred to 1.5 ml Eppendorf microfuge tubes

and denatured in a water bath for 5 min at 100°C. At this stage samples could be frozen at -20°C until further analysis was performed. Ten µl aliquots were mixed with 50 µl of 1.3% sodium deoxycholate and incubated at room temperature for 5 min. Samples were diluted to 750 µl by the addition of dH_2O and the proteins precipitated by the addition of 25% trichloro-acetic acid (TCA) and incubated on ice for 10 min. The samples were then pelleted in a microcentrifuge for 10 minutes, the supernatant discarded, and the pellet resuspended in 250 µl 0.1 N NaOH. Standards were prepared using Bovine serum albumen (BSA) at concentrations of 0 µg/ml to 120 µg/ml in 0.1 N NaOH. Protein samples and standards were diluted with 750 µl of copper reagent (2% Na_2CO_3, 0.4% NaOH, 0.16% sodium potassium tartrate, 1.0% SDS, mixed 100:1 with 4% $CuSO_4$, 96% dH_2O), and incubated at room temperature for 10 minutes. After incubation, 75 µl (diluted 1:1 in dH_2O) of Folin - Ciocalteu's Phenol Reagent was added, mixed immediately, and incubated 45 minutes to allow for color development. A Bausch and Lomb spectrophotometer 21 was used to measure the absorbance of the samples and standards at 710 nm. Simple linear regression generated by the BSA standards was used to estimate protein concentrations of the unknown samples based on their respective absorbances at 710 nm. Protein concentrations were expressed as µg total protein per cell.

Statistical Analyses

Statistical analyses of the data were performed using Quattro Pro 6.0 for Windows (1994 Novell, Inc.) and Excel 7.0 for Windows 95 (1985-1995 Microsoft Corporation). Cell-density data were natural-log transformed to standardize variance. Net rates of cell

accrual and neutral-lipid depletion were determined by calculating the slope of the line (defining change in cell densities/neutral lipid over time) via simple linear regression. Data for analyses (chlorophyll *a*, protein, ^{14}C glucose uptake, $^{14}CO_2$ incorporation, and neutral lipid content) were standardized on a per cell basis. Two-way ANOVAs (cell condition x time) were performed to compare mean live-cell densities versus mean dead-cell densities within the same treatment over time. Two-way ANOVAs were also performed to compare light effects on cell-density within each experiment and experimental effects within each light level over time. One-way ANOVAs were performed to test differences in light level (and live- vs. dead-cell densities within the same light treatment) within each experiment on individual sample dates. Correlation Coefficients were run on cell densities versus relative neutral lipid levels over time and p-values were also calculated (t-tests: (Rosner 1990)).

CHAPTER IV

RESULTS

Nutrient and Light Effects on Growth Rates and Cell Densities

Conditions between experimental cultures (Nutrient-Replenished and Nutrient-Depleted) varied sufficiently to reveal distinct culture responses. Nutrient-replenished cultures of *Achnanthidium rostratum* had significantly higher mean-cell densities than nutrient-depleted cultures under all light regimes throughout the experiment (except on days 6 and 8 in dark-grown cultures; one-way ANOVA; $p>0.05$), (two-way ANOVA; nutrient $p<0.05$, time $p<0.05$; Table 1, Figure 2). The differences observed may result from nutrient levels employed, however, other factors may be influential, such as variations in light intensity and temperature between experimental location (greenhouse vs. environmental chamber), as well as potential differences in bacterial contamination from initial stock cultures. Mean cell-accrual rates were also consistently higher in nutrient-replenished cultures, although, due to a high degree of variability in cell counts, were not significantly different from those in nutrient-depleted cultures for any light regime (slope of log-transformed linear regression ± S.E.; Table 2).

Light-level effects were most evident between dark-grown and illuminated cultures, but ambiguous when comparing between high-light and low-light cultures. Regardless of

Table 1. Two-way ANOVA results on log-transformed *A. rostratum* cell densities over time; between high light (HL), low light (LL), and dark (D) treatments, and between experiments (Exp.): ND = nutrient depleted (no nutrient supplementation), and NR = nutrient replenished (replenishment with 1*m*M glucose, sodium acetate, and caseine amino acids in a modified Bold's medium). (N.S.) indicates no significant difference, (*) indicates a significant difference ($p<0.05$), with (↑) designating culture with higher cell density. Shaded regions indicate irrelevant comparisons. (n=3)

Table 1.

TREATMENT	Time	Exp.	Light level	Light vs. Time	Exp. vs. Time
ND (HL vs. LL)	*		N.S.	*	
ND (↑HL vs. D)	*		*	*	
ND (↑LL vs. D)	*		*	*	
ND (HL) vs. ↑NR (HL)	*	*			*
ND (LL) vs. ↑NR (LL)	*	*			*
ND (D) vs. ↑NR (D)	N.S.	*			N.S.
NR (HL vs. LL)	*		N.S.	N.S.	
NR (↑HL vs. D)	*		*	*	
NR (↑LL vs. D)	*		*	*	

Figure 2. Growth curves of log transformed mean cell densities (± S.E.) of *A. rostratum* cultures between experiments: nutrient-replenished (NR) and nutrient-depleted (ND) cultures under high light (HL), low light (LL), and dark (D) illumination. (n = 3)

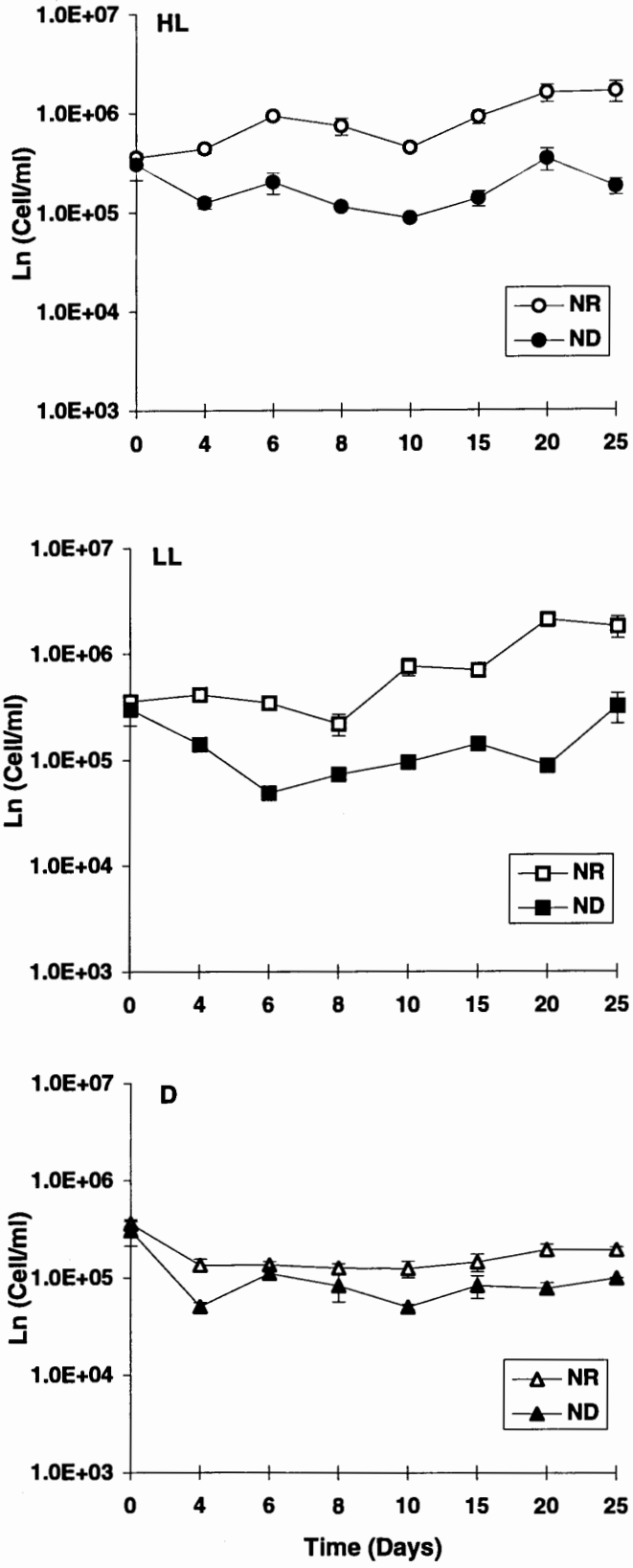

HL
LL
D
1.0E+07
1.0E+06
1.0E+05
1.0E+04
1.0E+03
Ln (Cell/ml)
NR
ND
0
4
6
8
10
15
20
25
Time (Days)

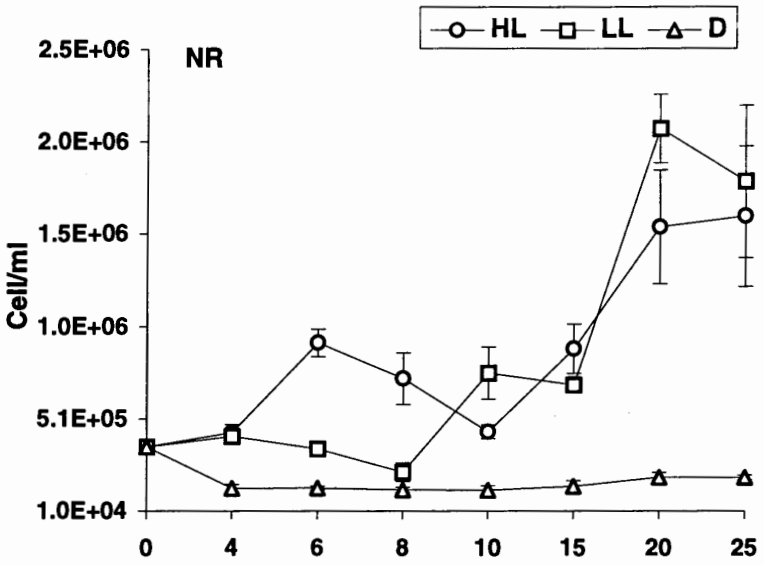
Table 2. Growth rates of *A. rostratum* over time based on the slope of log transformed linear regression ± S.E. Comparisons of both nutrient-replenished and nutrient-depleted cultures under high light, low light and dark conditions. (n=3)

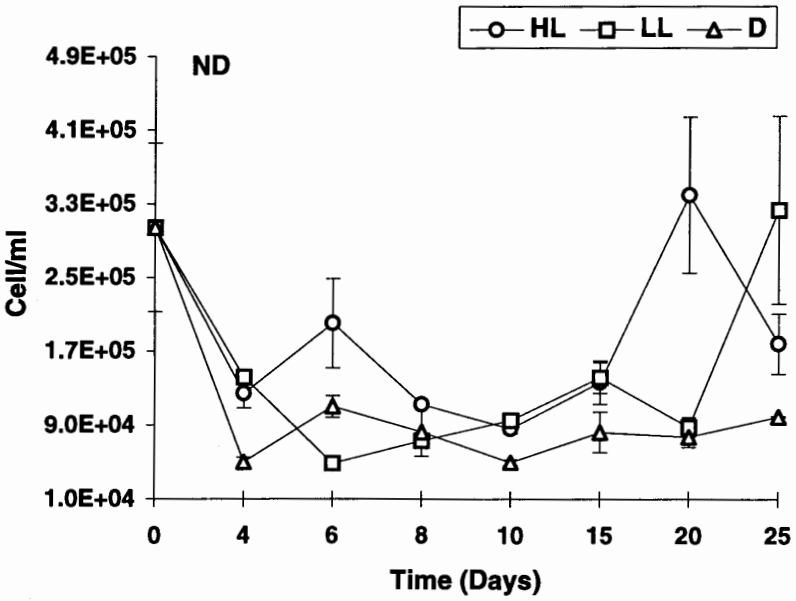
Treatment	**Nutrient-Replenished**	**± S.E.**	**Nutrient-Depleted**	**± S.E.**
High Light	0.024	0.008	0.013	0.010
Low Light	0.041	0.010	0.021	0.012
Dark	0.009	0.002	0.007	0.007

nutrient level, cell cultures incubated in the dark had significantly lower cell densities than illuminated cultures (two-way ANOVA; light $p<0.05$, time $p<0.05$; Table 1, Figure 3). However, differences in growth rate between illuminated and dark-grown cultures were magnified under nutrient supplementation. Nutrient-replenished cultures grown in the dark had significantly lower cell-accrual rates than their illuminated counterparts, although in nutrient-depleted cultures, rates did not significantly differ among light treatments (slope of log-transformed linear regression ± S.E.; Table 2). This suggests a divergence in the primary "limiting" resource between experiments; with light primarily limiting growth in nutrient-replenished cultures and nutrient levels controlling growth in nutrient-depleted cultures. Growth rates and mean cell densities did not differ between low and high-light levels, regardless of nutrient regime, indicating *A. rostratum* may be adapted to low light conditions (slope of log-transformed linear regression ± S.E.; two-way ANOVA; light $p<0.05$, time $p<0.05$; Table 2, Figure 3).

Growth rates within cultures incubated in darkness, while very low, were significantly greater than zero in nutrient-replenished cultures, whereas nutrient-depleted cultures maintained stable cell densities, but accrual rates were not greater than zero (slope of log-transformed linear regression ± S.E.; Table 2). Mean live-cell densities exceeded dead-cell densities in nutrient-replenished cultures and on the majority of days in nutrient-depleted cultures (except days 4, 10, and 15: one-way ANOVA; $p>0.05$), (two-way ANOVA; viability $p<0.05$, time $p>0.05$). Live cells accounted for approximately 67% of all cells enumerated throughout the experiment (mean percentage over 25 days: nutrient-replenished (NR), mean = 67.28 ± 1.13 S.E.; nutrient-depleted (ND), mean = 67.19 ±

Figure 3. Growth curves of mean cell densities (± S.E.) of *A. rostratum* cultures over time, grown under high light (HL), low light (LL), and dark (D) conditions in: nutrient-replenished (NR) and nutrient-depleted media (ND). (n = 3)


HL
LL
D
NR
2.5E+06
2.0E+06
1.5E+06
1.0E+06
5.1E+05
1.0E+04
Cell/ml
0
4
6
8
10
15
20
25


HL
LL
D
ND
4.9E+05
4.1E+05
3.3E+05
2.5E+05
1.7E+05
9.0E+04
1.0E+04
Cell/ml
0
4
6
8
10
15
20
25
Time (Days)

2.50 S.E.; Figure 4).

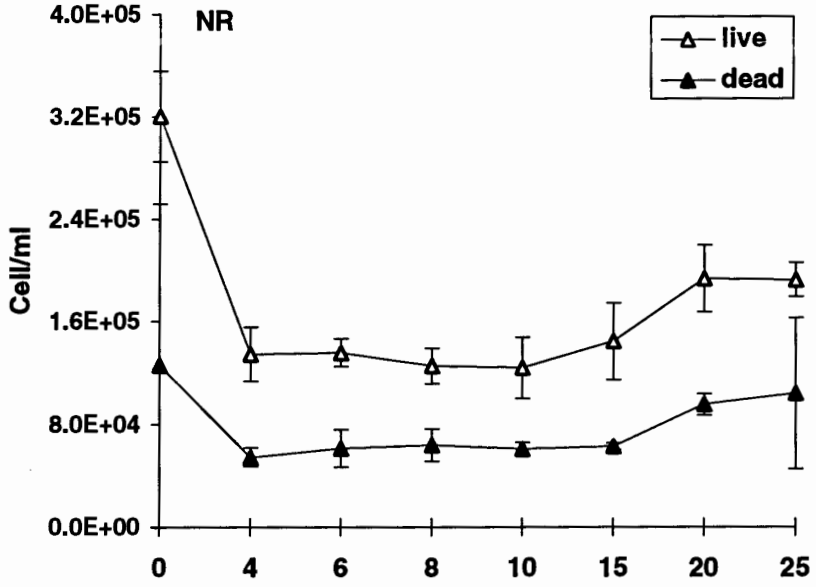
Cell Condition

Microscopic examination (1000x mag.) of cultures revealed differences in cell condition between experimental settings, most notably between experiments (Figures 5, 6 and 7). Cells within nutrient-depleted cultures contained large vacuoles under all light levels, indicating potential build-up of cellular storage products. In contrast, cells in nutrient-replenished cultures contained few vacuoles, and subjectively appeared less transparent and darker green in color than nutrient-depleted cultures. The condensed protoplasm observed in nutrient-replenished dark-grown cultures may be indicative of cells in a maintenance or resting state.

Photosynthetic Activity

Photosynthetic activity, measured as uptake of ^{14}C-labelled sodium bicarbonate, was higher in illuminated *A. rostratum* cultures than in the dark. Cell-specific uptake of $^{14}CO_2$ in the dark was significantly lower than that of cultures exposed to high light for all sample days in nutrient-replenished cultures (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 8) and for all but days 20 and 25 in nutrient-depleted cultures (one-way ANOVA; $p>0.05$), (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 8). Low-light cultures also had significantly higher uptake rates than dark grown cultures on all sample days except day 25 in nutrient-replenished media (one-way ANOVA; $p>0.05$), (two-way ANOVA; light $p<0.05$, time $p<0.05$). In nutrient-depleted cultures, photosynthetic activity was significantly lower in high-light treatment than under low-light for all but the last sample day (one-way ANOVA; $p>0.05$), (two-way ANOVA; light $p<0.05$, time

Figure 4. Mean live and dead cell densities of cultures incubated over 25 days in the dark under both replenished (NR) and depleted (ND) nutrient regimes. (n=3)


NR
live
dead
Cell/ml
4.0E+05
3.2E+05
2.4E+05
1.6E+05
8.0E+04
0.0E+00
0
4
6
8
10
15
20
25

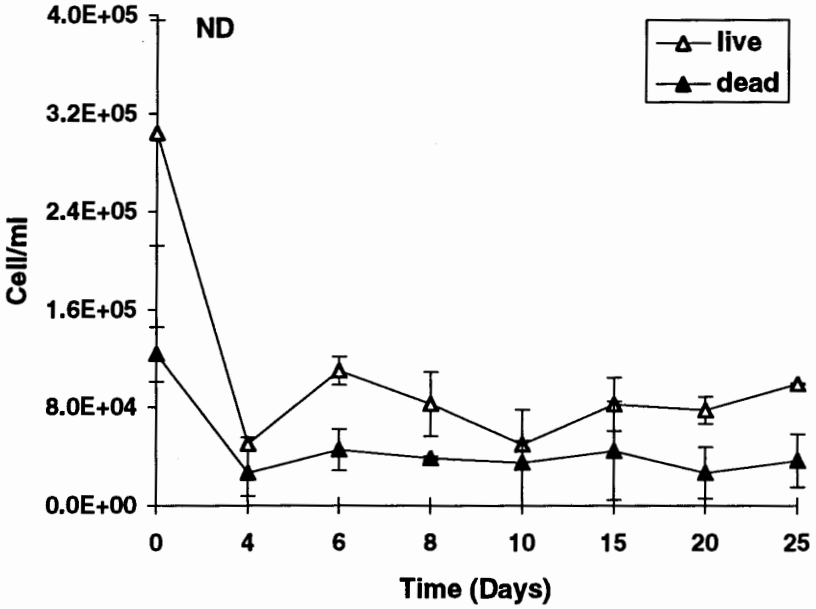
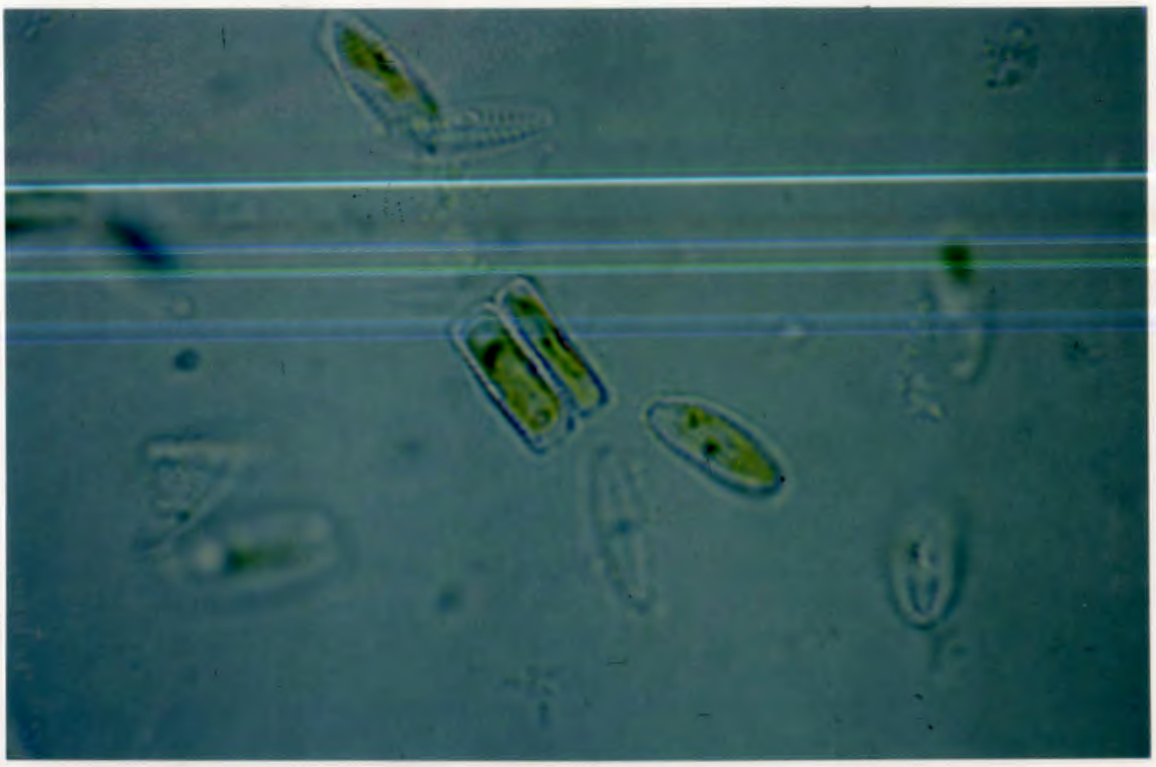

ND
live
dead
Cell/ml
4.0E+05
3.2E+05
2.4E+05
1.6E+05
8.0E+04
0.0E+00
0
4
6
8
10
15
20
25
Time (Days)

Figure 5. *Achnanthidium rostratum* cultures (x1000) grown under high light in nutrient-replenished (top) and nutrient-depleted (bottom) media.



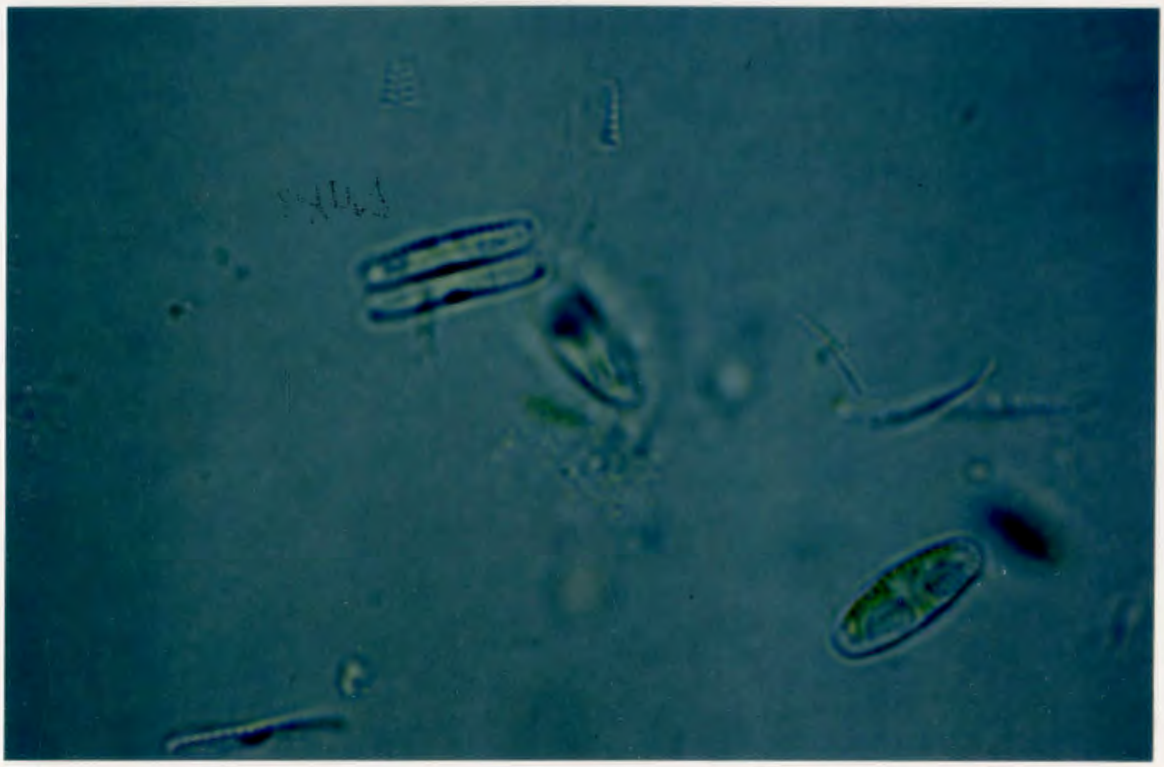
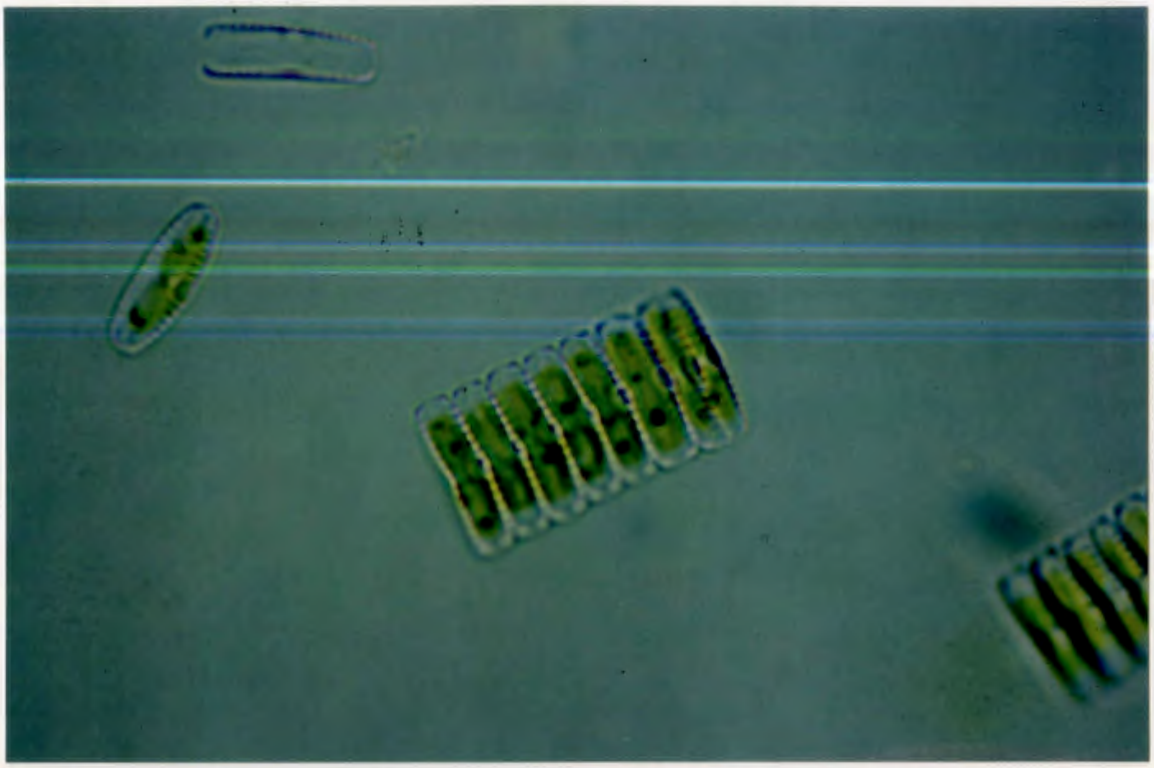


Figure 6. *Achnanthidium rostratum* cultures (x1000) grown under low light in nutrient-replenished (top) and nutrient-depleted (bottom) media.



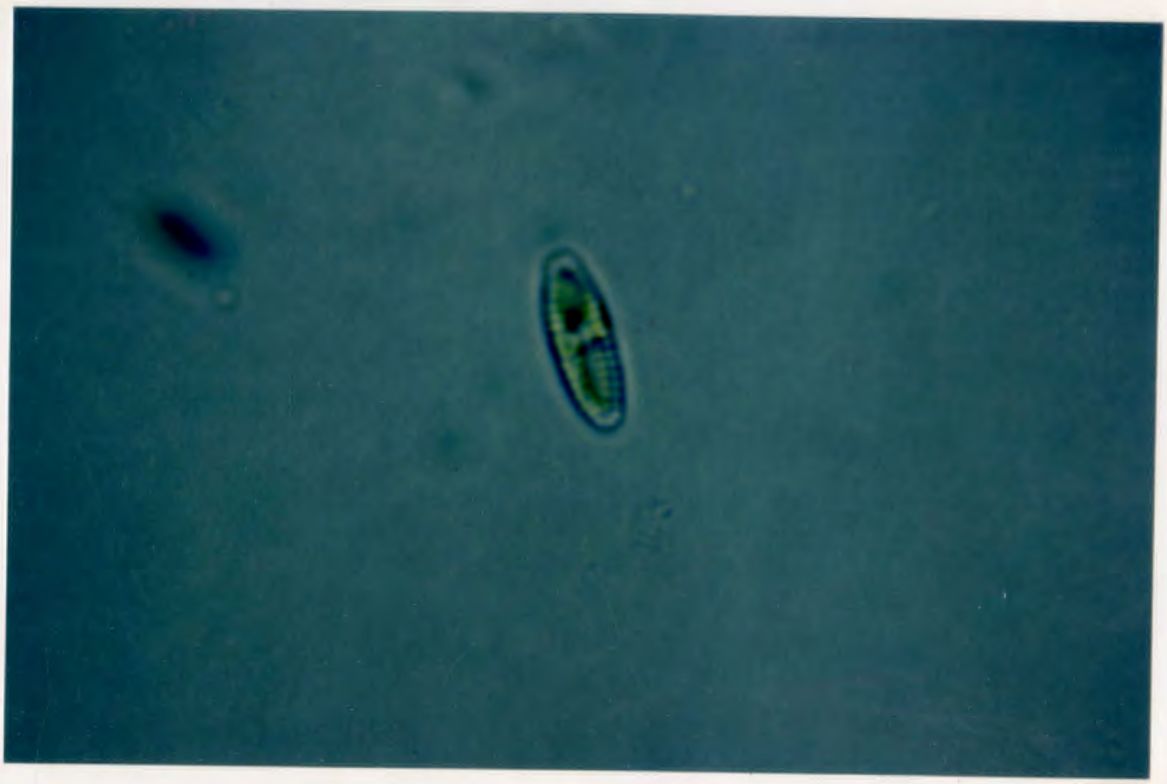
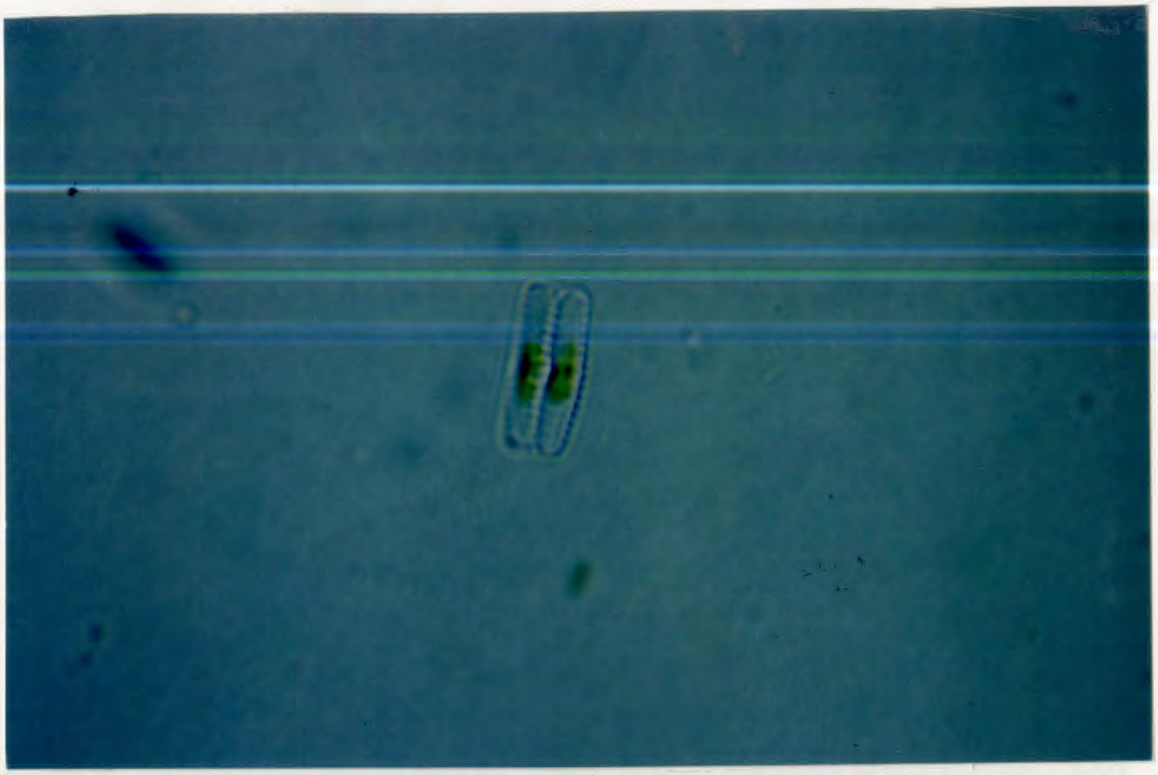


Figure 7. *Achnanthidium rostratum* cultures (x1000) grown in the dark in nutrient-replenished (top) and nutrient-depleted (bottom) media.



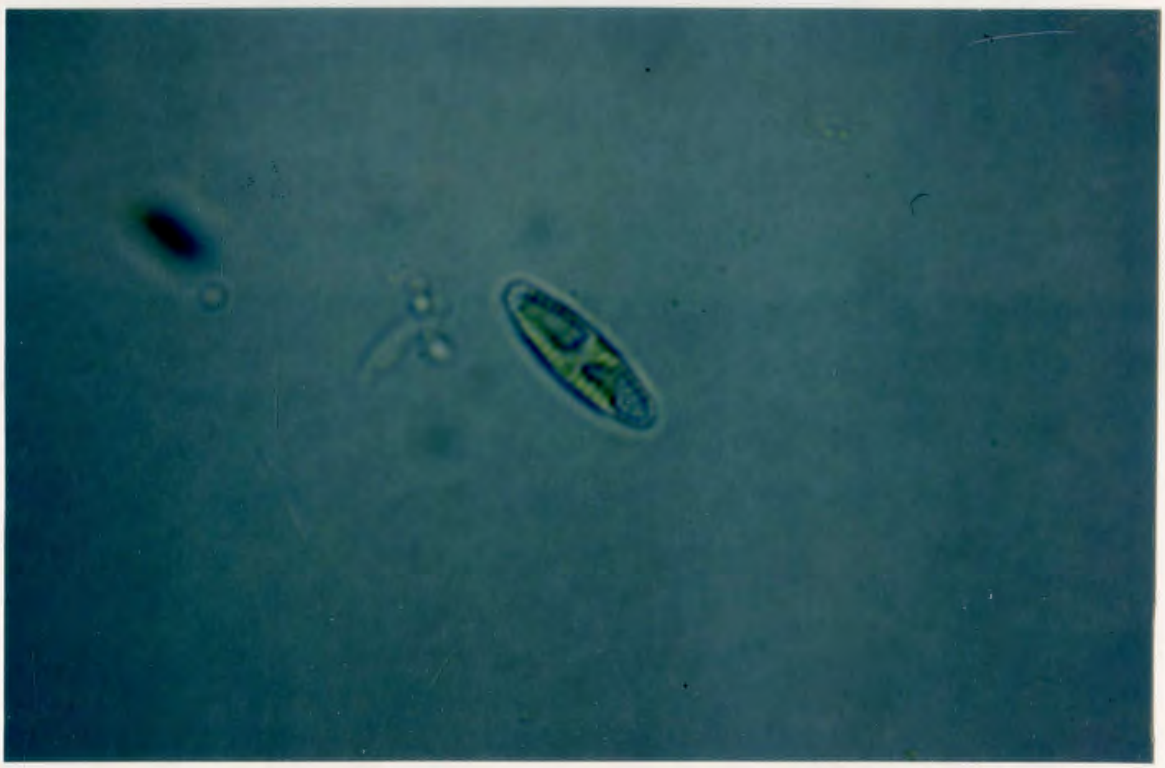
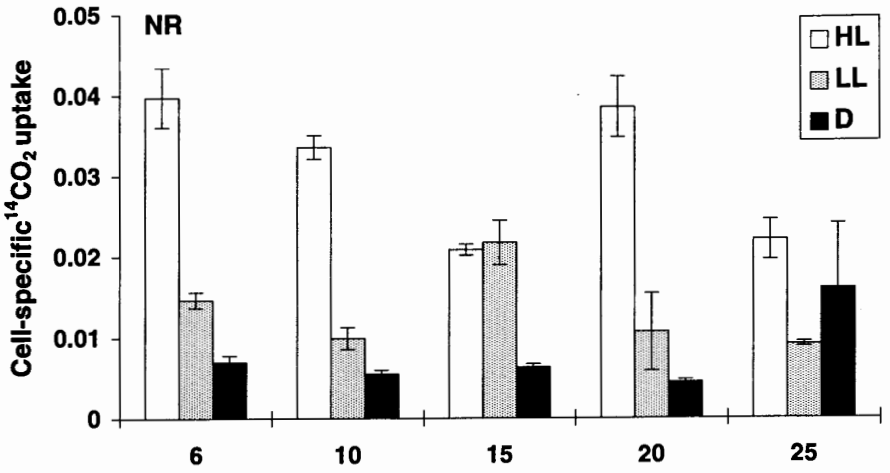
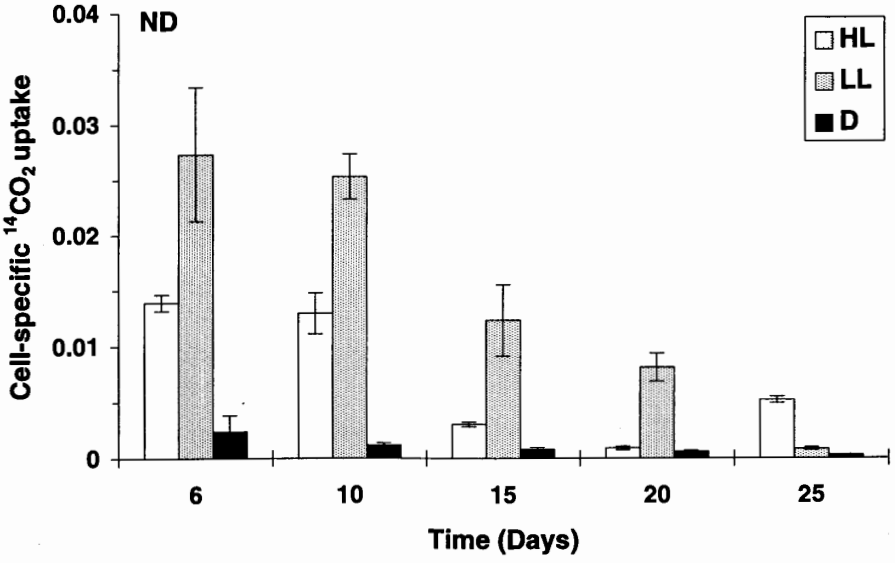


Figure 8. Mean photosynthetic activity of *A. rostratum* measured as cell-specific $^{14}CO_2$ uptake (± S.E.) over a 25 day period under high light (HL), low light (LL), and dark (D) illumination in: nutrient replenished media (NR) and nutrient depleted media (ND). (n=3)


NR
HL
LL
D
Cell-specific $^{14}CO_2$ uptake
0.05
0.04
0.03
0.02
0.01
0
6
10
15
20
25


ND
HL
LL
D
Cell-specific $^{14}CO_2$ uptake
0.04
0.03
0.02
0.01
0
6
10
15
20
25
Time (Days)

$p<0.05$). In contrast, photosynthetic activity in nutrient-replenished media was significantly greater under high illumination than low illumination (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 8).

Reillumination

Nutrient-replenished cultures grown in the dark responded positively to reillumination when transferred to high-light intensities for 30 hours. Reilluminated dark-grown cultures incorporated $^{14}CO_2$ at significantly higher rates than under previous dark conditions throughout the 25 day experiment, exhibiting $^{14}CO_2$ assimilation rates similar to those of high-light grown cultures (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 9). As expected, high-light cultures did not respond to reillumination (except day 2 which showed an unexplained negative response to reillumination), and in low-light cultures, response to reillumination was transient, with elevated response to increased light noted within the first 6 days of reillumination only (one-way ANOVA $p>0.05$).

Glucose Uptake

Uptake of ^{14}C-glucose in *Achnanthidium rostratum* cultures was enhanced in the dark, perhaps indicating heterotrophic metabolism. Cells incubated in nutrient-replenished media had significantly higher glucose uptake rates when grown in the dark than under either low or high illumination (2 x two-way ANOVA comparing Dark vs. High light and Dark vs. Low light cultures; light $p<0.05$, time $p<0.05$; Figure 10). A similar trend was observed in nutrient-depleted cultures, but was not significant (two-way ANOVA; light $p>0.05$, time $p>0.05$; Figure 10). Cell-specific glucose uptake did not differ between high- and low-light cultures in either nutrient condition (two-way ANOVA; light $p>0.05$,

Figure 9. Effects of exposure to high-light on photosynthetic activity measured as $NaH^{14}CO_3$ incorporation in *A. rostratum* cells (± S.E.) grown in nutrient-replenished cultures (NR): A) CO_2 uptake for high light (HL) vs. reilluminated* HL (control), B) CO_2 uptake for low light (LL) vs. reilluminated* LL, and C) CO_2 uptake for dark (D) vs. reilluminated* D. (n = 3)

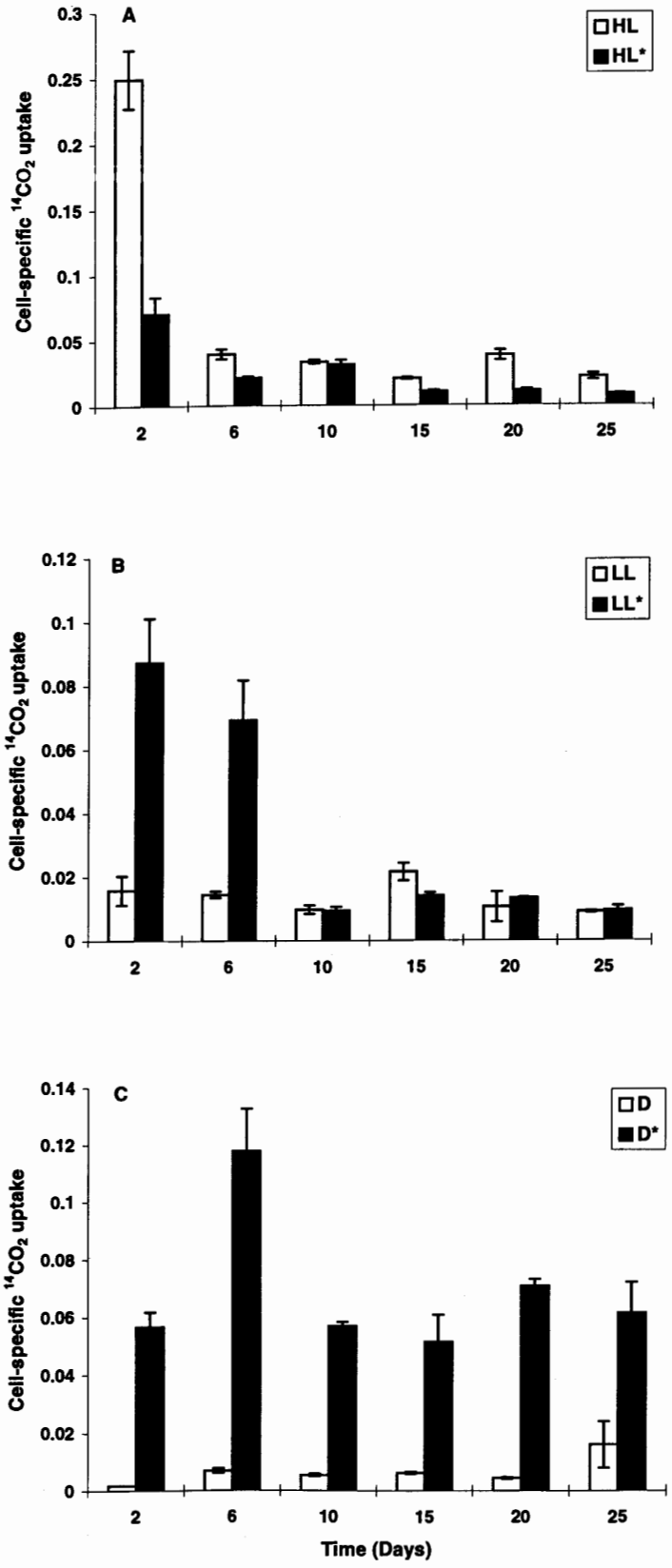
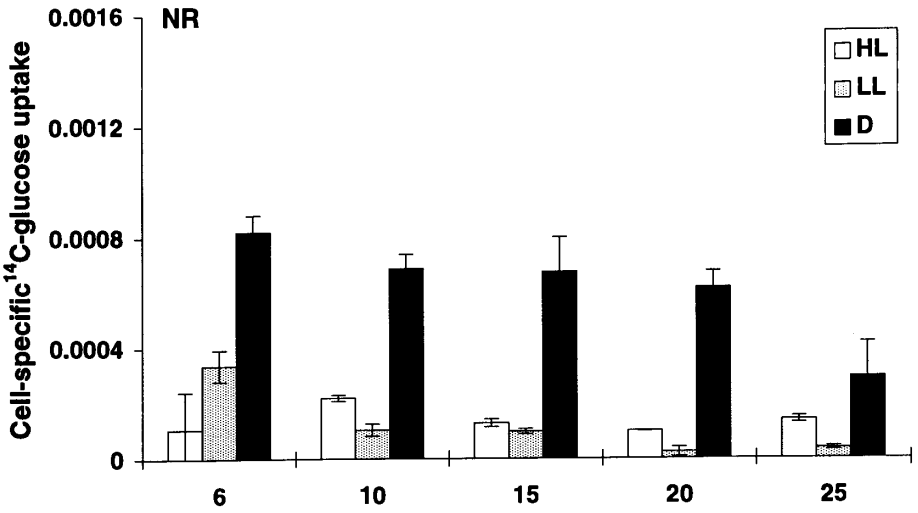
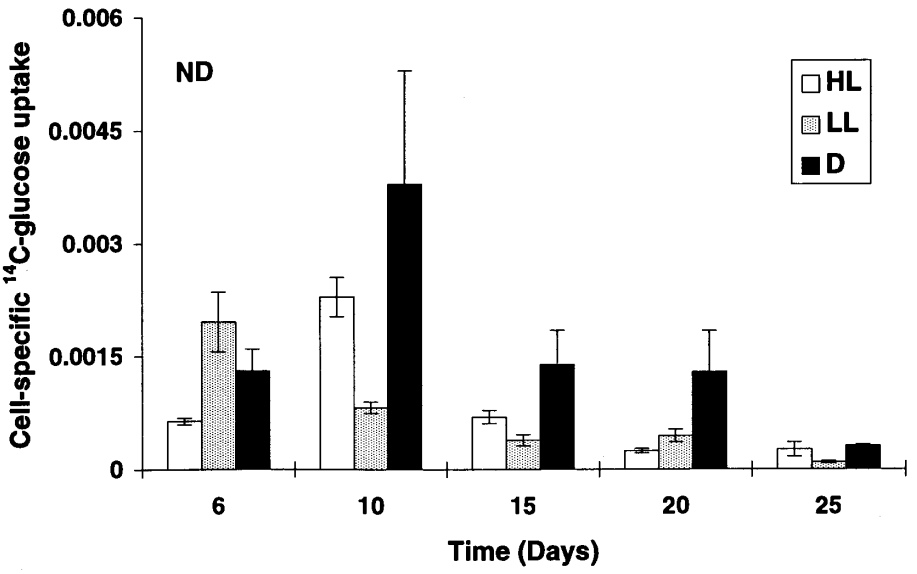

A
HL
HL*
Cell-specific $^{14}CO_2$ uptake
0.3
0.25
0.2
0.15
0.1
0.05
0
2
6
10
15
20
25
B
LL
LL*
Cell-specific $^{14}CO_2$ uptake
0.12
0.1
0.08
0.06
0.04
0.02
0
2
6
10
15
20
25
C
D
D*
Cell-specific $^{14}CO_2$ uptake
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
2
6
10
15
20
25
Time (Days)

Figure 10. Cell-specific glucose uptake (± S.E.) by *A. rostratum* over a 25 day period under high light (HL), low light (LL), and dark (D) conditions in: nutrient-replenished (NR), and nutrient-depleted media (ND). (n = 3)


NR
0.0016
0.0012
0.0008
0.0004
0
Cell-specific ^{14}C-glucose uptake
HL
LL
D
6
10
15
20
25


ND
0.006
0.0045
0.003
0.0015
0
Cell-specific ^{14}C-glucose uptake
HL
LL
D
6
10
15
20
25
Time (Days)

time $p>0.05$). Illuminated cultures grown in non-replenished media had significantly higher ^{14}C-glucose uptake than illuminated cultures in nutrient-replenished media, suggesting retention of a glucose-uptake mechanism in the absence of glucose, however, other factors may have influenced uptake rates, such as potential variations in bacteria levels (i.e. affecting glucose uptake) between experiments (two-way ANOVA; nutrient $p<0.05$, time $p<0.05$). Figure 11 depicts the relationship of photosynthetic activity to glucose uptake. As stated earlier, in nutrient-replenished cultures, $^{14}CO_2$ incorporation (a measure of photosynthetic activity) was significantly greater in high-light than dark-grown cultures, and the opposite was true with ^{14}C-glucose uptake, indicating up-regulation of glucose in the dark (two-way ANOVA; nutrient $p<0.05$, time $p<0.05$).

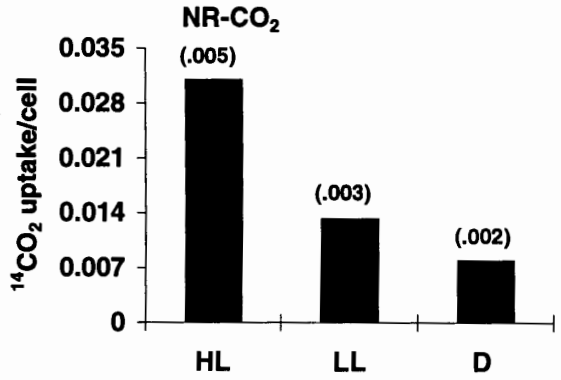
Chlorophyll *a*

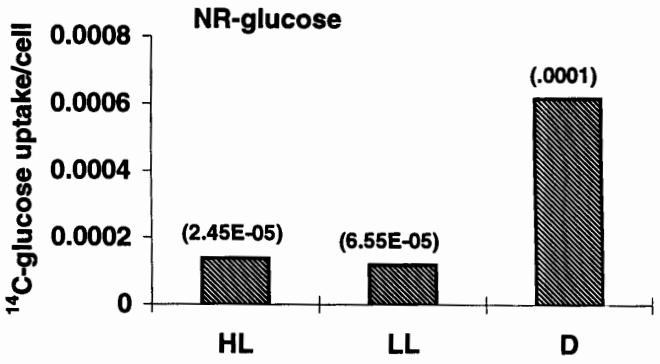
Chlorophyll *a* concentrations were measured in nutrient-replenished cultures to determine whether varied light intensity affects cell-specific chlorophyll *a* levels. Cells grown under reduced light levels may increase their chlorophyll *a* concentrations to sequester additional photons necessary to maintain metabolism. Cultures grown under low illumination exhibited significantly greater concentrations of chlorophyll *a* per cell than either high-light or dark-grown cultures on days 2, 6, 8, 15, and 25 (one-way ANOVA; $p<0.05$; Figure 12). Chlorophyll *a* levels per cell were not significantly different between high-light and dark-grown cultures (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 12).

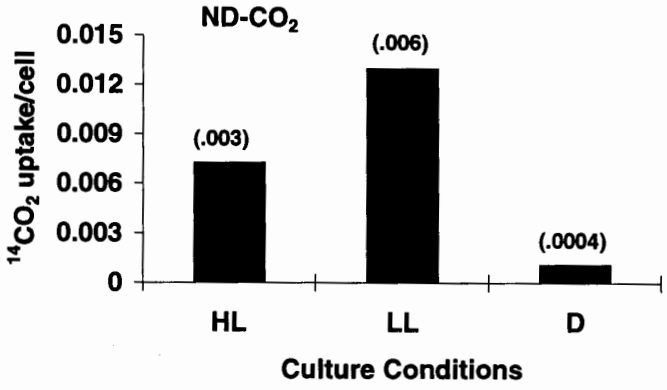
Neutral Lipid Analysis

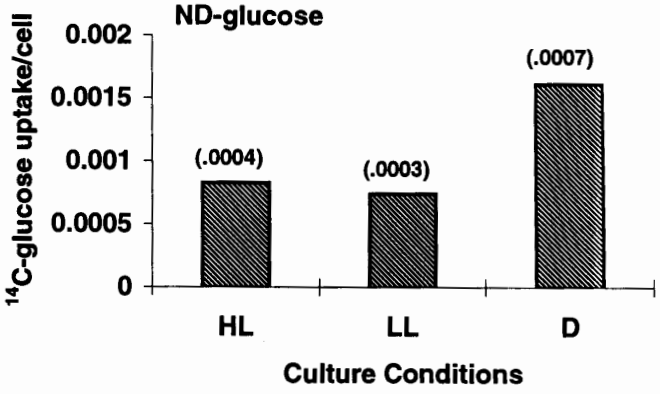
Relative cell-specific neutral lipid stores declined over time in all cultures. Depletion

Figure 11. Side by side comparison of $^{14}CO_2$ and ^{14}C-glucose uptake over 25 days in both nutrient-replenished (NR) and nutrient-depleted media (ND) under the following illumination: High Light (HL), Low Light (LL), and Dark (D). Values are the average cell-specific uptake of 5 sample days with the standard error designated in parentheses above each treatment bar. (n = 3)


NR-CO2
0.035
0.028
0.021
0.014
0.007
0
14CO2 uptake/cell
(.005)
(.003)
(.002)
HL
LL
D


NR-glucose
0.0008
0.0006
0.0004
0.0002
0
14C-glucose uptake/cell
(2.45E-05)
(6.55E-05)
(.0001)
HL
LL
D


ND-CO2
0.015
0.012
0.009
0.006
0.003
0
14CO2 uptake/cell
(.003)
(.006)
(.0004)
HL
LL
D
Culture Conditions


ND-glucose
0.002
0.0015
0.001
0.0005
0
14C-glucose uptake/cell
(.0004)
(.0003)
(.0007)
HL
LL
D
Culture Conditions

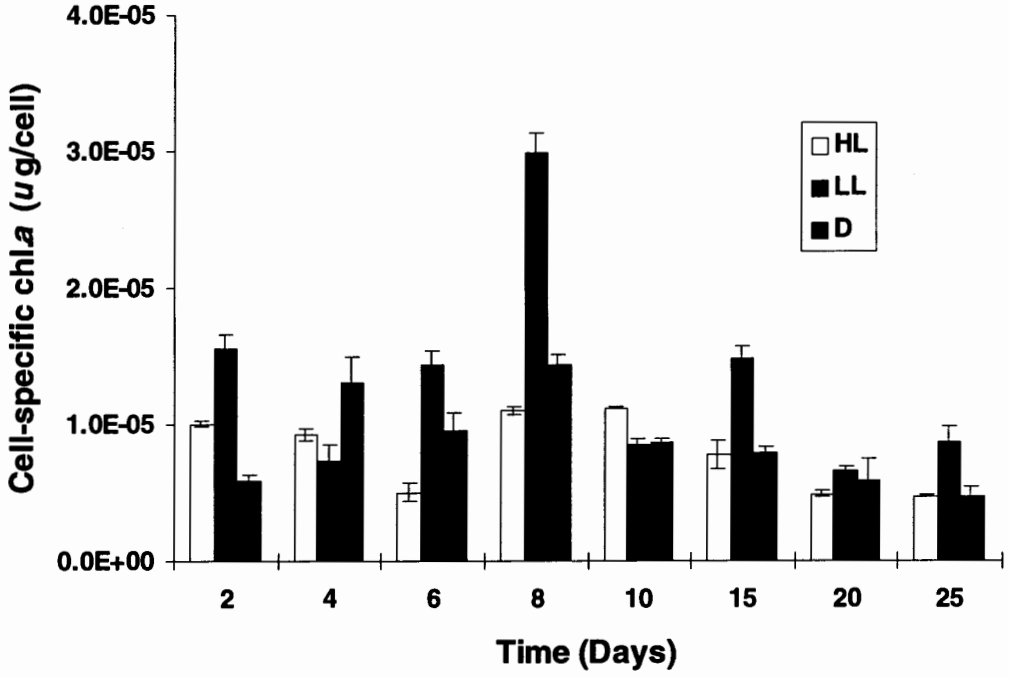


Figure 12. Cell-specific chlorophyll *a* concentrations (± S.E.) of *A. rostratum* over a 25 day period under high light (HL), low light (LL), and dark (D) illumination, grown in nutrient-replenished cultures (NR). (n = 3)

rates were more substantial in nutrient-depleted cultures, although only between dark-grown cultures was there a significant difference (log-transformed linear regression; 95% confidence interval; Table 3). Nutrient-replenished cultures grown in the dark had significantly higher relative-lipid levels per cell compared to their illuminated counterparts (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 13). Strong negative correlations were measured between cell density and relative-lipid level per cell in all treatment groups, although nutrient-replenished cultures grown in the dark exhibited positive lipid accumulation up to day 10, independent of the cell growth rates which were near zero during this same period. After day 10, however, a similar inverse trend was observed as cell growth began to rise with a corresponding decrease in lipid levels (Figures 14 and 15).

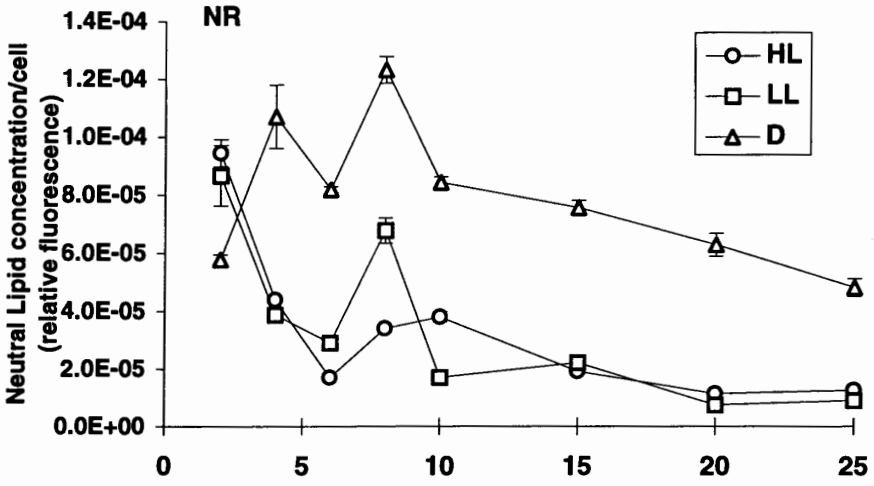
Protein Levels

Protein concentrations were measured to examine effects of reduced illumination on cultures. Nutrient-replenished cultures incubated in the dark for 25 days maintained significantly greater total protein concentrations per cell than illuminated cultures, both of which exhibited similar cell-specific protein concentrations (two-way ANOVA; light $p<0.05$, time $p<0.05$; Figure 16).

Table 3. Neutral lipid depletion rates of *A. rostratum* over time based on the slope of log transformed linear regression ± S.E. Comparisons of both nutrient-replenished and nutrient-depleted cultures under high light, low light and dark conditions. (n = 3)

Treatment	**Nutrient-Replenished**	**± S.E.**	**Nutrient-Depleted**	**± S.E.**
High Light	-2.32E-06	4.21E-08	-6.11E-06	1.86E-07
Low Light	-2.64E-06	8.82E-08	-1.18E-05	1.10E-06
Dark	-1.53E-06	5.68E-07	-8.69E-06	8.8E-07

Figure 13. Relative cell-specific neutral lipid levels (± S.E.) of *A. rostratum* cell cultures over a 25 day period under high light (HL), low light (LL), and dark (D) conditions in: nutrient-replenished (NR) and nutrient-depleted (ND) cultures. (n = 3)


NR
Neutral Lipid concentration/cell (relative fluorescence)
1.4E-04
1.2E-04
1.0E-04
8.0E-05
6.0E-05
4.0E-05
2.0E-05
0.0E+00
0
5
10
15
20
25
HL
LL
D

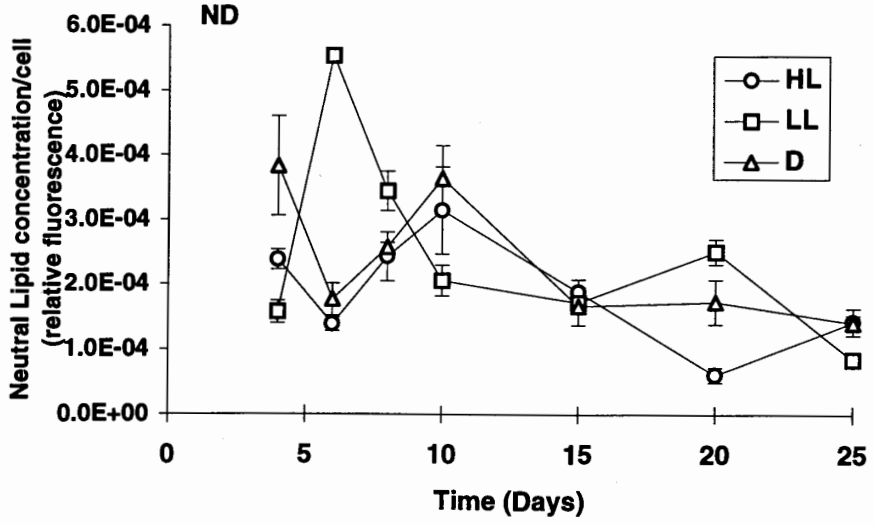

ND
Neutral Lipid concentration/cell (relative fluorescence)
6.0E-04
5.0E-04
4.0E-04
3.0E-04
2.0E-04
1.0E-04
0.0E+00
0
5
10
15
20
25
Time (Days)
HL
LL
D

Figure 14. Relationship between cell density and cell-specific relative neutral lipid levels over time. Cultures grown in nutrient-replenished media (NR) under High Light (HL), Low Light (LL), and Dark (D). Where CC = Correlation Coefficient and p-values were calculated from t-tests. (± S.E.). (n = 3)

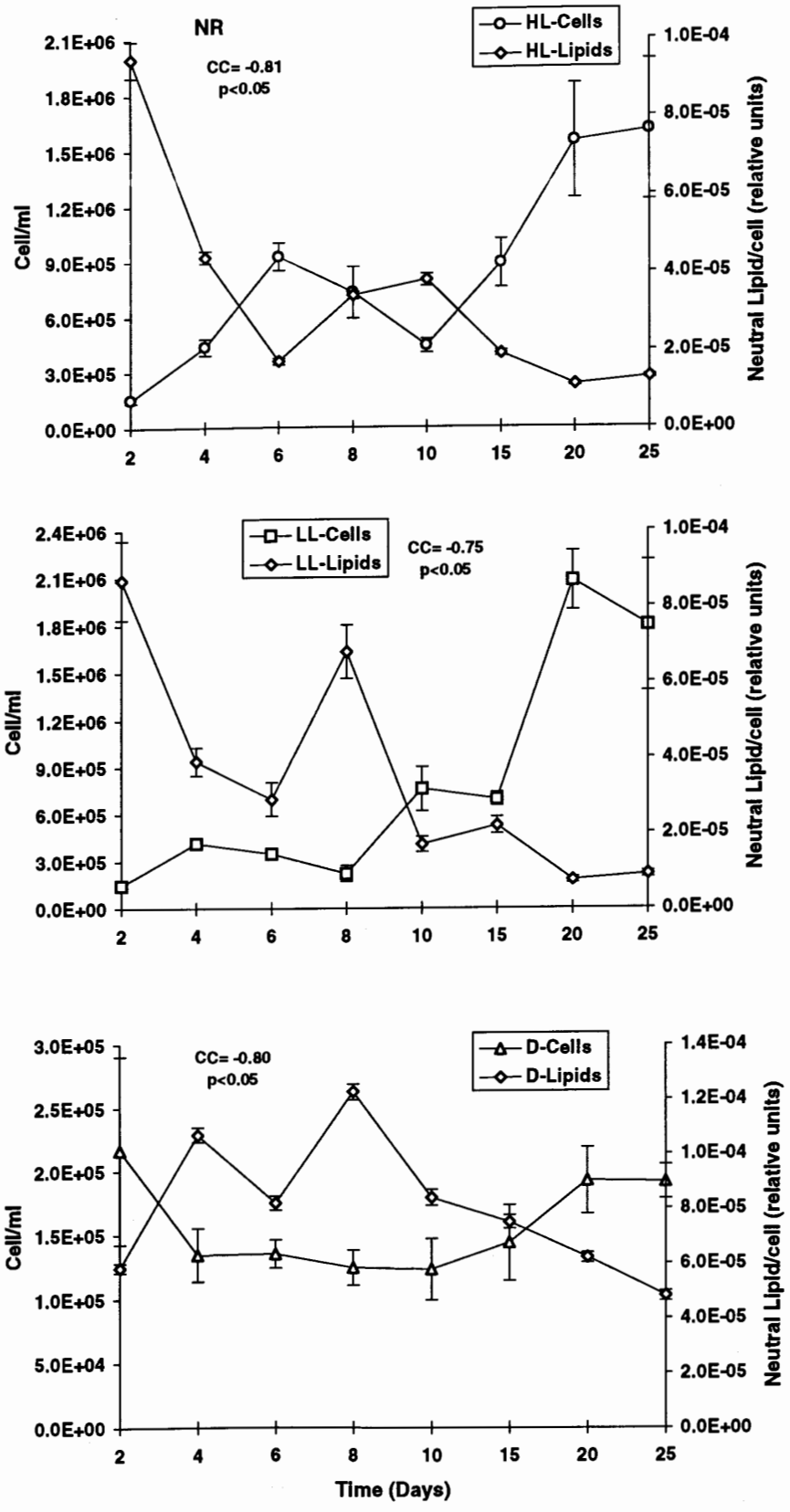
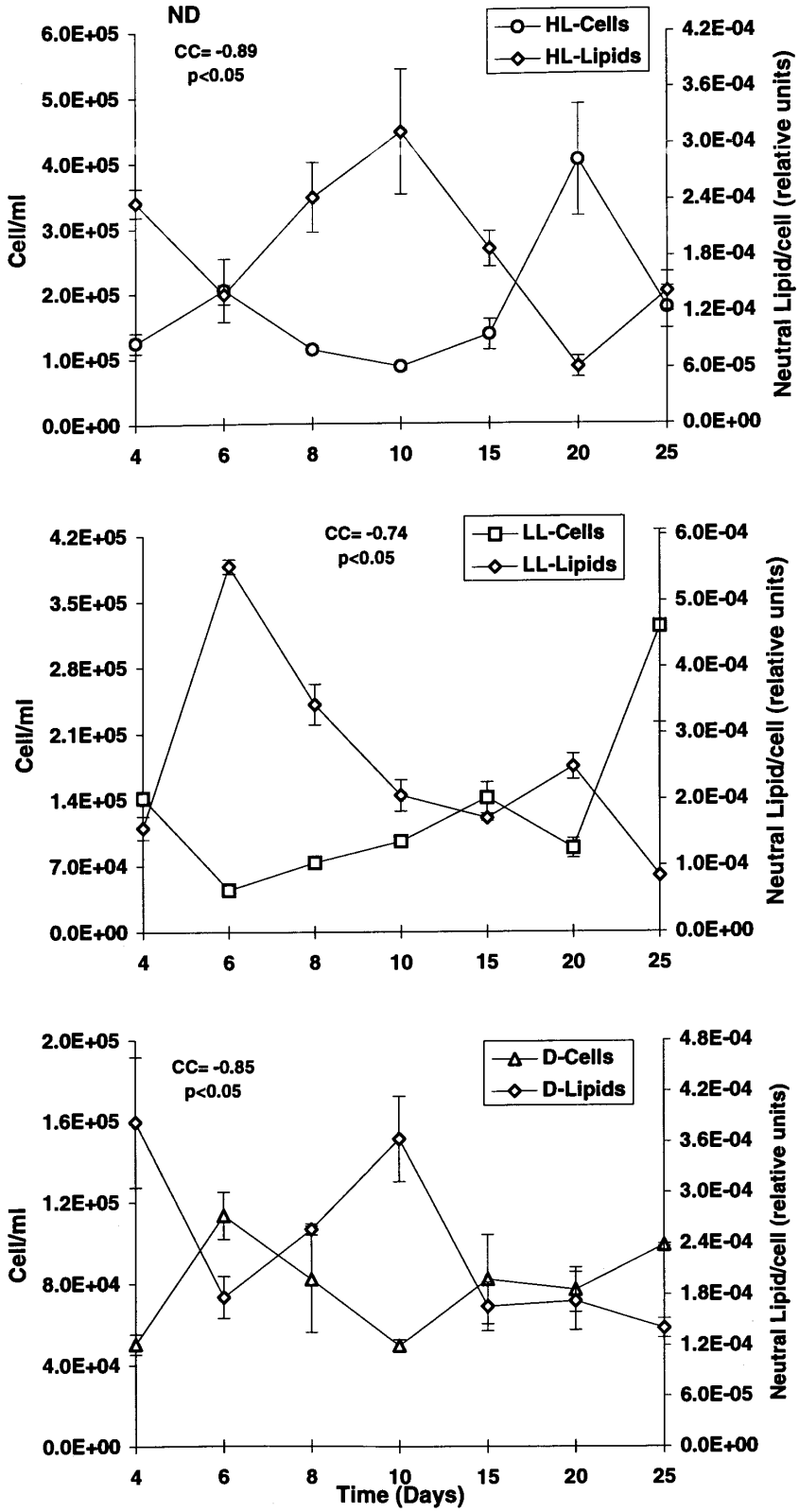

NR
HL-Cells
HL-Lipids
CC= -0.81
p<0.05
Cell/ml
Neutral Lipid/cell (relative units)
LL-Cells
LL-Lipids
CC= -0.75
p<0.05
D-Cells
D-Lipids
CC= -0.80
p<0.05
Time (Days)

Figure 15. Relationship between cell density and cell-specific relative neutral lipid levels over time. Cultures grown in nutrient-depleted media (ND) under High Light (HL), Low Light (LL), and Dark (D). Where CC = Correlation Coefficient and p-values were calculated from t-tests.(± S.E.). (n = 3)


ND
CC= -0.89
p<0.05
HL-Cells
HL-Lipids
Cell/ml
Neutral Lipid/cell (relative units)
6.0E+05
5.0E+05
4.0E+05
3.0E+05
2.0E+05
1.0E+05
0.0E+00
4.2E-04
3.6E-04
3.0E-04
2.4E-04
1.8E-04
1.2E-04
6.0E-05
0.0E+00
4
6
8
10
15
20
25
CC= -0.74
p<0.05
LL-Cells
LL-Lipids
Cell/ml
Neutral Lipid/cell (relative units)
4.2E+05
3.5E+05
2.8E+05
2.1E+05
1.4E+05
7.0E+04
0.0E+00
6.0E-04
5.0E-04
4.0E-04
3.0E-04
2.0E-04
1.0E-04
0.0E+00
4
6
8
10
15
20
25
CC= -0.85
p<0.05
D-Cells
D-Lipids
Cell/ml
Neutral Lipid/cell (relative units)
2.0E+05
1.6E+05
1.2E+05
8.0E+04
4.0E+04
0.0E+00
4.8E-04
4.2E-04
3.6E-04
3.0E-04
2.4E-04
1.8E-04
1.2E-04
6.0E-05
0.0E+00
4
6
8
10
15
20
25
Time (Days)

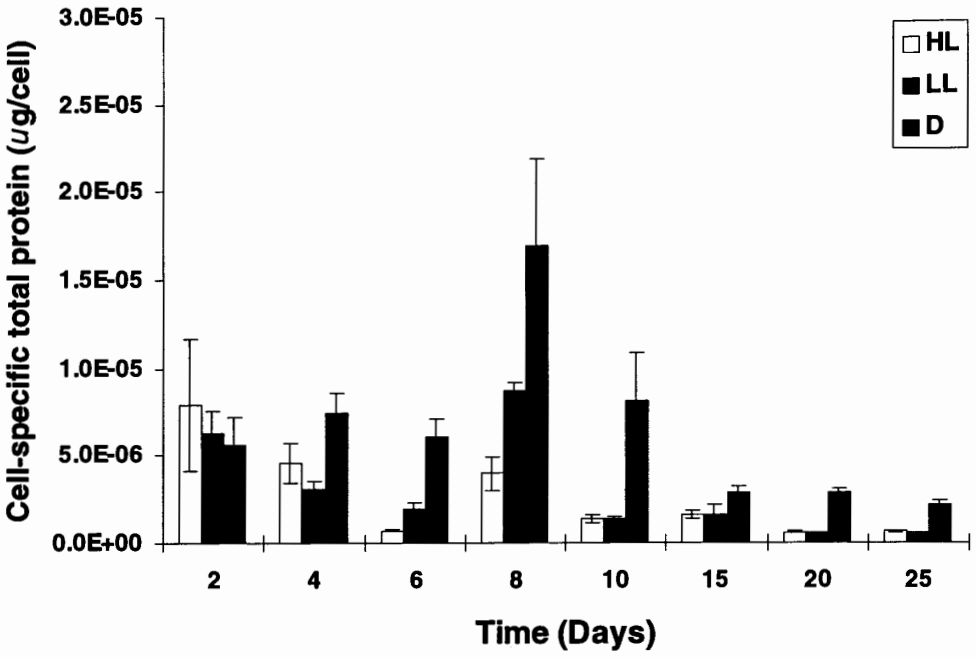


Figure 16. Cell-specific total protein concentrations (± S.E.) of *A. rostratum* over a 25 day period under high light (HL), low light (LL), and dark (D) illumination, grown in nutrient-replenished cultures (NR). (n = 3)

CHAPTER V

DISCUSSION

Freshwater periphyton are known to play an important role as primary producers in aquatic food webs (Lamberti 1996, Minshall 1988). In lotic systems, the ability of benthic algae to attach to different types of substrata and reproduce rapidly helps create a consistent energy source for consumers, as opposed to more transient input of allochthonous organic matter. Densely packed algal assemblages support the energy needs and nutrient cycling processes of their surroundings and also act as "seeds" for colonization down stream following their detachment (via disturbances) from substrata.

Accrual of algal biomass on substrata often progresses towards development of multi-tiered algal assemblages (or mats) (Hoagland et al. 1982). Numerous studies have shown that vertical growth of algal assemblages creates resource gradients within the mat (Burkholder et al. 1990, Hoagland 1983, Hudon and Bourget 1981, Jørgensen and Revsbech 1983, Karlström 1978, Nicholson et al. 1987, Stevenson and Glover 1993, Tuchman 1996, Yodzis 1978). Species inhabiting algal mats, therefore, may differ in autecological characteristics depending on their tendency for early or late colonization, or correlating to their position within the mat. Early colonizing species may benefit from

abundant resources initially, however, progression towards a mature algal mat tends to deplete essential nutrients and light reaching lower tier cells.

Both the persistence of algal assemblages and the speed with which they recover following sloughing events may be partly reliant on survival of basal species (see Peterson 1996a). Live cells at the base of mats help secure canopy layers to the substratum by means of mucilagenous secretions (Hoagland et al. 1982). Further, viable cells retained on the substratum following sloughing events may speed recovery rates through reproduction. Also, affixed cells and detrital material may also aid in the attachment of other species, thus accelerating recovery (Korte and Blinn 1983). The mechanisms responsible for lower-tier species survival under both light- and nutrient-limited conditions are not well understood. Early successional species that are unable to physically escape high density/low resource conditions may resort to various survival strategies including: dormancy (resting states, spore formation), heterotrophic metabolism, or maintenance via tolerance or acclimation to low resource conditions. My research reveals potential survival strategies for a particular species of diatom whose genus is associated with the base of developed algal mats.

Results from my study demonstrate that *Achnanthidium rostratum* can survive at least 25 days in complete darkness under both nutrient-depleted and nutrient-replenished conditions. Approximately 67% of all cells in dark-grown cultures in both nutrient regimes maintained viability (i.e. contained cellular material) over the 25-day period indicating that, although populations were not increasing at a significant rate, no increases in death rates occurred. Survival in the dark has been noted in other studies on planktonic

algal species (Sicko-Goad et al. 1989, Wasmund 1989) and polar sea-ice diatoms (Palmisano and Sullivan 1982). Adverse growth conditions often induce "resting" states in cells characterized by either physical modification of cells, including increased silicification of cell walls (to form spores), or by physiological changes, such as condensed cytoplasm or enlarged vacuoles (Palmisano and Sullivan 1983). Light-microscopic examination of my cultures revealed no evidence of spore formation. However, enlarged vacuoles were observed in all nutrient-depleted cultures, suggesting accumulation of a storage material such as neutral lipids. In nutrient-depleted cultures, production of neutral lipids may result from nitrogen and/or phosphorous limitation (Ben-Amotz et al. 1985, Livne and Sukenik 1992), or silicate deficiency (Taguchi et al.1987). Nutrient-replenished cultures grown in the dark exhibited condensed protoplasts, a possible indication of the onset of a resting state.

The ability of *Achnanthidium rostratum* cultures to survive extended periods of darkness, and to exhibit relatively high growth rates under low-light conditions was confirmed through various means. Algae typically rely on photoautotrophic metabolism for energy procurement. However, under light-limited conditions, photoautotrophic capability may be severely restricted or nonexistent and cells must rely on other means for survival, including utilization of alternative energy resources. Dark-grown cultures of *A. rostratum* in my study, not surprisingly, exhibited minimal photoautotrophic metabolism (measured as cell-specific $^{14}CO_2$ incorporation) when compared with cultures grown in the light. Consequently these cultures exhibited minimal growth rates. Upon reintroduction to high-light conditions, however, dark-grown cultures responded with a

sharp increase in $^{14}CO_2$ incorporation within 30 hours. These data are consistent with results of Panella (1994) where dark-grown cultures of *A. rostratum* resumed exponential growth after a 6 day lag period following reillumination. It appears that cells grown in the dark are not only alive, but also equipped or "primed" for rapid resumption of photoautotrophic activity even after extended periods of darkness.

Nutrient replenished cultures responded positively to illumination via elevated growth rates. The corresponding low growth rates observed in dark-grown cultures suggests light as the primary limiting factor in this experiment, and that these cells are not capable of fully utilizing heterotrophic metabolism for growth, but actually enter a "maintenance mode" when subjected to darkness. Nutrient-depleted cultures did not respond significantly to illumination indicating that additional factors (depleted nutrient supply or other experiment-specific affects) may be limiting growth.

Stable cell densities observed in dark-grown nutrient-replenished cultures over time may have resulted from increased glucose uptake rates, an indication of their ability to use exogenous organic carbon as a supplemental energy source. Glucose uptake has been shown to be up-regulated in the dark in the diatom *Cyclotella cryptica* (Hellebust 1971), possibly allowing cells to reallocate cellular resources from photosynthetic pathways to more functional alternative mechanisms such as organic-nutrient uptake. As observed by Schollett (1998), the benthic diatom species: *Achnanthidium rostratum*, *A. minutissimum*, *Encyonema minutum*, *E. minutum* var. *pseudogracilis*, *Gomphonema accuminatum*, *Navicula trivialis*, *Nitzshia linearis*, and *N. palea* increase incorporation rates of numerous exogenous organic compounds (including glucose in *A. rostratum*) in the dark.

However, many algal species capable of active uptake of organic molecules in the dark cannot reproduce under these conditions (Hellebust and Lewin 1977). Similar growth rates between dark-grown cultures grown in nutrient-replenished versus nutrient-depleted media in my study may imply that nutrient supplementation only aids in maintenance of cells, or possibly, that cells require a "lag" period longer than 25 days to activate an efficient metabolic pathway for use of exogenous organic molecules. Admiraal and Peletier (1979) observed heterotrophic growth in the dark in the estuarine benthic diatom *Stauroneis constricta* following a lag period of 20 days after transfer to organic-supplemented media. The positive growth trends observed in nutrient-replenished cultures suggests cells may require a longer lag period prior to achieving heterotrophic growth.

Despite evidence of glucose uptake in *A. rostratum* (Schollett 1998), glucose uptake rates measured in this study may be inaccurately estimated because of the potential unaccounted for effects of bacterial activity on uptake rates. However, assuming there is no light effect on bacterial activity, glucose uptake by bacteria should be the same in all light treatments. Therefore under this assumption, cell-specific glucose uptake observed in my experiments may be high but relative trends between light treatments should be the same as if tested axenically. A further issue may involve the effects of illumination on algal uptake of glucose. Potential up-regulation of glucose in the dark (Hellebust 1971) by algal species may lead to increased bacterial uptake of glucose at higher illumination as a result of less algal competition for the available organic supply. Schollett (1998) also showed higher uptake of glucose in the dark compared to high light in *A. rostratum*

cultures, suggesting glucose uptake in the light may be disproportionately high as a result of bacterial activity.

Algal species may need organic concentrations as high as 300µg/l (180-200µg/l glucose in present study) to compete effectively with bacteria for available organic material (Cavari and Hadas 1979). These concentrations are much higher than would typically be found *in situ* (Cavari and Hadas 1979). Therefore, active uptake of organics by algal species in nature may be limited by ambient concentrations. Bacteria, in contrast, are capable of fulfilling their metabolic needs at much lower organic concentrations (Wetzel 1983). Species capable of facultative heterotrophy in nature may therefore rely on uptake of organic molecules as a supplement to photoautotrophy.

The ability of *A. rostratum* cultures to assimilate glucose may be the result of a specific active-transport mechanism in the cell. Glucose uptake measured in both nutrient-replenished and nutrient-depleted experiments suggests this is an inherent mechanism that does not require previous exposure to glucose in the media (Hellebust 1971, Admiraal and Peletier 1979). Also, the higher glucose-uptake rates observed in dark-grown cultures implies increased activation of uptake mechanisms in the absence of light.

In nutrient-replenished cultures, total protein levels within dark-grown cells were higher than in either illuminated culture on all but the first sample day. Increased total protein under extended periods of darkness may result from activation of specific transport mechanisms responsible for organic (glucose) nutrient uptake, however, research corroborating this phenomenon has not been found. These results differ,

however, from those obtained by Panella (1994) for *A. rostratum* cultures, A discrepancy possibly attributable to the limited number of sample days used by Panella (2 vs. 8 test dates in my study).

Evidence indicates that the high glucose concentrations taken up by dark-grown cells were incorporated into a number of metabolic pathways. Cultures grown under darkness in nutrient-replenished media had significantly higher cell-specific neutral lipid levels (in the form of triglycerides) than their illuminated counterparts. Inhibition of cell division induced by resource limitation often leads to accumulation of neutral lipids in algal cultures (Guckert and Cooksey 1990, Larson and Rees 1996, Reitan et al. 1994). Production of neutral lipid relies on photoautotrophic metabolism (sunlight energy + inorganic carbon source). Hence, in theory, cultures grown in the dark would not have the energy needed to produce (neutral) lipids. However, my observations of elevated cell-specific lipid levels in conjunction with increased glucose uptake in the dark suggests conversion of exogenous organic carbon into lipids. In a resource-limited environment, it may be feasible to convert organic carbon into a less complex energy form (hence a more efficient use of energy), which would be the most practical metabolic mechanism to use until favorable growing conditions resume (Napolitano 1994, Orcutt and Patterson 1974). Potentially, lipids may be converted via the Glyoxylate Cycle back to glucose for the energy needed for survival. Use (via oxidation) of storage products, such as neutral lipids, as an energy source in algal cells during cell division in the dark has been documented by Fisher and Schwarzenbach (1978) and Otsuka and Morimura (1966).

Declines in neutral lipid levels for all cultures over time may have resulted from both

dilution effects and oxidation. It is plausible that higher neutral lipid levels at the start of all treatments may be the result of slow growth from the initial lag period following inoculation and transfer to individual test flasks. Rapid cell division (observed in illuminated nutrient-replenished cultures) most likely depleted neutral lipid levels through dilution and less from oxidation. Neutral lipids are high energy sources, though not readily metabolized, therefore, under optimal growth conditions cells may rely on photosynthates for rapid reproduction, leaving accumulated lipids to dilute out over time. In contrast, declines in lipid levels in nutrient-depleted cultures exhibiting minimal growth, likely result from lipid oxidation for cell maintenance.

Increased relative cell-specific neutral lipid levels observed from days 2 through 10 in dark-grown nutrient-replenished cultures suggest an exogenous-organic carbon source (glucose) was used to manufacture triglycerides in the dark. Following day 10, these cultures resumed trends similar to those observed in illuminated cultures (i.e. steady cell growth with corresponding lipid dilution), perhaps indicating the onset of heterotrophic growth.

Benthic periphyton trapped at the base of well-developed algal assemblages are likely subjected to extremely low irradiances (Johnson 1996, Stock and Ward 1991) rather than total darkness, and can be exposed to extreme light fluctuations if canopy layers are disrupted. Many algal species can adapt to such low-light conditions (Geider et al. 1986, Johnson 1996, Palmisano et al. 1985). Shade-tolerant species are able to maintain their viability and positioning within algal mats and, therefore, remain competitive upon eventual removal of the canopy layer. In my study, cultures grown under low-light

conditions in both nutrient regimes exhibited similar growth rates and cell densities as their high-light counterparts. This may be an indication that the maximum light levels employed were not sufficient to stimulate growth (i.e. light saturation point was not reached). More likely, these data suggest that *A. rostratum* is adapted (or can acclimate itself) to reduced light levels. The longer lag period prior to exponential growth observed under low light (8 days vs. 4 days in high-light cultures) may indicate cells require time to acclimate to reduced illumination. Algal species may adapt to reduced light availability by increasing cell-specific chlorophyll *a* levels to sequester more photons (Falkowski and Owens 1980, Geider et al. 1986, Neale and Melis 1986). Higher cell-specific chlorophyll *a* levels were measured in *A. rostratum* cultures under low light (12 µmol quanta $\bullet m^{-2} \bullet sec^{-1}$) for most sample days. This corresponds with subjective light-microscopic (x1000) observations which revealed characteristic dark green appearances for low-light cells, in contrast to brown coloration observed in high-light grown cultures. However, photosynthetic rates (measured as $^{14}CO_2$ uptake) increased from low to high-light levels, indicating that elevated chlorophyll *a* levels under low light did not assist in sequestering as many photons for photosynthesis as in high-light *A. rostratum* cultures. Also, all other parameters measured between illuminated cultures revealed no differences. It may, therefore, be feasible that *A. rostratum* is light saturated at 12 µmol quanta$\bullet m^{-2} \bullet sec^{-1}$, or that some other cellular mechanism not revealed in this study is compensating for low illumination. Light compensation points (light level at which respiration is just balanced by gross photosynthesis) less than 1 µmol quanta$\bullet m^{-2} \bullet sec^{-1}$ have been recorded for the

marine diatom *Phaeodactylum tricornutum* (Geider et al. 1986). Further, certain species of sea ice diatoms are light saturated at 11 μmol quanta•m^{-2}•sec^{-1} (Palmisano et al. 1985), indicating *A. rostratum* may very well be light saturated at 12 μmol quanta•m^{-2}•sec^{-1}.

The ability of dark-grown *Achnanthidium rostratum* cultures to subsist in a maintenance mode for at least 25 days provides further evidence that this species is adapted to potentially severe light-limited environments common at the base of benthic algal assemblages. Shaded cultures appeared to be illuminated at a level which does not limit growth, indicating potential low-light tolerance. Evidence of *Achnanthidium* spp. associated with the lower tiers of benthic algal assemblages (see Steinman and McIntire 1987, Tuchman and Stevenson 1991) indicates the likelihood of their shade adaptability, although the hypothesized mechanism (i.e. maintaining optimum photosynthetic activity via increased chlorophyll *a* levels) (Falkowski and Owens 1980, Geider et al. 1986, Neale and Melis 1986) responsible for their adaptability was not conclusive in this study.

Survival of periphyton in a fluctuating environment depends on individual species adaptability. Algal species rely on various survival strategies ranging from physically altering their position within a mat to physiologically altering cellular functions. The ability of some species to survive in resource-limited conditions encountered at the base of mature benthic-algal mats would convey individual advantages, which, in turn, enhances the overall fitness of the community. Eventual detachment of the canopy layer allows those remaining attached algae access to resources previously unavailable and, therefore, an opportunity to rapidly recolonize the available substratum. Further, it has

been hypothesized that viable cells capable of withstanding sloughing events will help accelerate the recovery of the substratum, thus renewing successional patterns (Peterson 1996a).

Survival of *Achnanthidium rostratum* cultures under various resource limiting conditions has been shown by both Panella (1994) and by my results. Relatively minimal growth rates in dark-grown cultures indicate cells may be in a maintenance mode, however, a longer-term study might reveal that cells require more than 25 days under dark conditions to implement heterotrophic growth. Increased cell growth in nutrient-replenished dark cultures after day 10, along with corresponding declines in neutral lipid levels, point in this direction. Likewise, steady levels of glucose uptake throughout the experiment indicate that these cells maintained a functional glucose-transport mechanism over an extended period of darkness. Study of axenically-grown cultures would provide a more accurate assessment of organic-carbon uptake rates in this diatom species, allowing for comparisons with other species that may compete for the same resources *in situ*. Also, by varying organic concentrations in the media we may be able to determine optimal levels necessary for survival and or growth as well as better understand their actual organic uptake abilities (i.e. active- or passive-uptake capacities).

Further research on the response of *A. rostratum* to varying illumination may help determine levels at which this species becomes light limited and light saturated. Growth rate similarities between both high-light and low-light cultures implies that these cells are shade adapted, however, elevated chlorophyll *a* levels did not correspond with increases in photosynthetic rates, leading one to believe that these cells may be light saturated at

levels close to 12 μmol quanta•m^{-2}•sec^{-1}.

LITERATURE CITED

Admiraal, W. and H. Peletier. 1979. Influence of organic compounds and light limitation on the growth rate of estuarine benthic diatoms. Br. Phycol. J. 14:197-206.

Ben-Amotz, A., T.G. Tornabene, and W.H. Thomas. 1985. Chemical profile of selected species of microalgae with emphasis on lipids. J. Phycol. 21:72-81.

Bensadoun, A. and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. Anal. Biochem. 70:241-250.

Berman,T., O. Hadas, and B. Kaplan. 1977. Uptake and respiration of organic compounds and heterotrophic growth in *Pediastrum duplex* (Meyen). Freshwat. Biol. 7:495-502.

Bollman, R.C. and G.G.C. Robinson. 1985. Heterotrophic potential of the green alga, *Ankistrodesmus braunii* (Naeg.). Can. J. Microbiol. 31:549-554.

Bothwell, M.L. 1989. Phosphorus-limited growth dynamics of lotic periphytic diatom communities: areal biomass and cellular growth rate responses. Can. J. Fish. Aquat. Sci. 46:1293-1301.

Brown, M.R., G.A. Dunstan, S.J. Norwood and K.A. Miller. 1996. Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudonana*. J. Phycol. 32:64-73.

Burkholder, J.M., R.G. Wetzel, and K.L. Klomparens. 1990. Direct comparison of phosphate uptake by adnate and loosely attached microalgae within an intake biofilm matrix. App. Env. Microbiol. 2882-2890.

Cambridge Dictionary of Biology, 1989 ed., s.v. "K-strategist." Cambridge University Press.

Cambridge Dictionary of Biology, 1989 ed., s.v. "r-strategist." Cambridge University Press.

Cavari, B.Z. and O. Hadas. 1979. Heterotrophic activity, glucose uptake and primary

productivity in Lake Kinneret. Freshwat. Biol. 9:329-338.

Cohn, S.A. and N.C. Disparti. 1994. Environmental factors influencing diatom cell motility. J. Phycol. 30:818-828.

Cooksey, K.E., J.B. Guckert, S.A. Williams, and P.R. Callis. 1987. Fluorometric determination of the neutral lipid content of microalgal cells using Nile Red. J. of Microbiol. Meth. 6:333-345.

Darley, W.M., C.T. Ohlman, and B.B. Wimpee. 1979. Utilization of dissolved organic carbon by natural populations of epibenthic salt marsh diatoms. J. Phycol. 15:1-5.

Davis, C.O., J.T. Hollibaugh, D.L.R. Siebert, W.H. Thomas, and P.J. Harrison. 1980. Formation of resting spores by *Leptocylindrus danicus* (Bacillariophyceae) in a controlled experimental ecosystem. J. Phycol. 16:296-302.

Falkowski, P.G. and T.G. Owens. 1980. Light-Shade Adaptation. Two strategies in marine phytoplankton. Plant Physiol. 66:592-595.

Fisher, N.S. and R.P. Schwarzenbach. 1978. Fatty acid dynamics in *Thalassiosira pseudonana* (Bacillariophyceae): Implications for physiological ecology. J. Phycol. 14(2):143-150.

Fogg, G.E. 1956. Photosynthesis and formation of fats in a diatom. Ann. Bot. 20:78.

Geider, R.J., B.A. Osborne, and J.A. Raven. 1986. Growth, photosynthesis and maintenance metabolic cost in the diatom *Phaeodactylum tricornutum* at very low light levels. J. Phycol. 22:39-48.

Guckert, J.B., and K.E. Cooksey. 1990. Triglyceride accumulation and fatty acid profile changes in *Chlorella* (Chlorophyta) during high pH - induced cell cycle inhibition. J. Phycol. 26:72-79.

Hamilton, P.B., and H.C. Duthie. 1984. Periphyton colonization of rock surfaces in a boreal forest stream studied by scanning electron microscopy and track autoradiography. J. Phycol. 20:525-532.

Hellebust, J.A. 1971. Glucose uptake by *Cyclotella cryptica*: Dark induction and light inactivation of transport system. J. Phycol. 7:345-349.

Hellebust, J.A. and J. Lewin. 1972. Transport systems for organic acids induced in the marine pennate diatom, *Cylindrotheca fusiformis*. Can. J. Microbiol. 18:225-233.

Hellebust, J.A., and J. Lewin. 1977. Heterotrophic nutrition. In The biology of Diatoms,

ed. D. Werner. University of California Press, Berkeley.

Hoagland, K.D., S.C. Roemer, and J.R. Rosowski. 1982. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). Amer. J. Bot. 69(2):188-213.

Hoagland, K.D. 1983. Short-term standing crop and diversity of periphytic diatoms in a eutrophic reservoir. J. Phycol. 19:30-38.

Hudon, C., and E. Bourget. 1981. Initial colonization of artificial substrate: community development and structure studied by scanning electron microscopy. Can. J. Fish. Aquat. Sci. 38:1371-1384.

Johnson, R.E. 1996. The effect of density dependent limitations on the vertical position of diatoms in a periphyton mat. M. Sc. Thesis. Loyola University Chicago, IL.

Johnson, R.E., N.C. Tuchman and C.G. Peterson. 1997. Changes in the vertical microdistribution of diatoms within a developing periphyton mat. J. N. Am. Benthol. Soc. 16(3):503-519.

Jorgensen, B.B., N.P. Revsbech, and Y. Cohen. 1983. Photosynthesis and structure of benthic microbial mats: Microelectrode and SEM studies of four cyanobacterial communities. Limnol. Oceanogr. 28(6):1075-1093.

Karlström, U. 1978. Role of the organic layer on stones in detrital metabolism in streams. Verh. Internat. Verein. Limnol. 20:1463-1470.

Korte, V.L., and D.W. Blinn. 1983. Diatom colonization on artificial substrata in pool and riffle zones studied by light and Scanning Electron Microscopy. J. Phycol. 19:332-341.

Lamberti, G.A. 1996. The role of periphyton in benthic food webs. In Algal Ecology, ed. R.J. Stevenson, M. Bothwell, and R. Lowe. Academic Press Inc., San Diego.

Larson, T.R. and T.A.V. Rees. 1996. Changes in cell composition and lipid metabolism mediated by sodium and nitrogen availability in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol. 32:388-393.

Lewin, J., and J.A. Hellebust. 1970. Heterotrophic nutrition of the pennate diatom, *Cylindrotheca fusiformis*. Can. J. Microbiol. 16:1123-1129.

Lewin, J., and J.A. Hellebust. 1975. Heterotrophic nutrition of the marine pennate

diatom *Navicula pavillardi* Hustedt. Can. J. Microbiol. 21:1335-1342.

Lewin, J., and J.A. Hellebust. 1978. Utilization of glutamate and glucose for heterotrophic growth by the marine pennate diatom *Nitzschia laevis*. Mar. Biol. 47:1-7.

Lewitus, A.J. and T.M. Kana. 1994. Responses of estuarine phytoplankton to exogenous glucose: Stimulation versus inhibition of photosynthesis and respiration. Notes. Limnol. Oceanogr. 39(1):182-189.

Livne, A., and A. Sukenik. 1992. Lipid synthesis and abundance of Acetyl CoA Carboxylase in *Isochrysis galbana* (Prymnesiophyceae) following nitrogen starvation. Plant Cell Physiol. 33(8):1175-1181.

Lorenzen, C.J. 1967 Determination of chlorophyll and pheopigments: spectrophotometric equations. Limnol. Oceanogr. 12:343-346.

MacArthur, R.H., and E.O.Wilson. 1967. The theory of island biogeography. Princeton Univ. Press, Princeton, N.J. 203 p.

Markwell, M.A.K., S.M. Haas, N.E. Tolbert and L.L. Bieber. 1981. Protein determination in membrane and lipoprotein samples: manual and automated procedures in: Methods in Enzymology. Academic Press, Inc. 72:296-303.

Minshall, G.W. 1988. Stream ecosystem theory: A global perspective. J. North Am. Benthol. Soc. 7:263-288.

Moss, B. 1977. Adaptations of epipelic and epipsammic freshwater algae. Oecologia (Berl.) 28:103-108.

Mouget, J-L, R.C. Beeson Jr., L. Legendre, and J. de la Noüe. 1993. Inadequacy of Rubisco initial and total activities to account for observed rates of photosynthetic carbon dioxide assimilation by *Scenedesmus ecornis*. Eur. J. Phycol. 28:99-106.

Mulholland, P.J. 1996. Role in nutrient cycling in streams. In Algal Ecology, ed. R.J. Stevenson, M. Bothwell, and R. Lowe. Academic Press Inc., San Diego.

Napolitano, G.E. 1994. The relationship of lipids with light and chlorophyll measurements in freshwater algae and periphyton. J. Phycol. 30:943-950.

Neale, P.J. and A. Melis. 1986. Algal photosynthetic membrane complexes and the photosynthesis-irradiance curve: A comparison of light-adaptation responses in *Chlamydomonas reinhardtii* (Chlorophyta). J. Phycol. 22:531-538.

Nichols, H.W. 1973. In Handbook of Phycological Methods. Vol. 1. Culture methods and growth measurements. Stein, J.R. ed. Cambridge University Press, p. 8-24.

Nicholson, J.A.M., J.F. Stolz, and B.K. Pierson. 1987. Structure of a microbial mat at Sippewissett Marsh, Cape Cod, Massachusetts. FEMS Microbiology Ecology. 45:343-364.

Oppenheim, D.R., D.M. Paterson. 1990. The fine structure of an algal mat from a freshwater maritime antarctic lake. Can. J. Bot. 68:174-183.

Orcutt, D.M. and G.W. Patterson. 1974. Effect of light intensity upon composition of *Nitzschia closterium* (*Cylindrotheca fusiformis*). Lipids. 9(12):1000-1003.

Otsuka, H. and Y. Morimura. 1966. Change of fatty acid composition of *Chlorella ellipsoidea* during its cell cycle. Plant & Cell Physiol. 7:663-670.

Palmisano, A.C., J.B. SooHoo, D.C. White, G.A. Smith, G.R. Stanton, and L.H. Burckle. 1985. Shade adapted benthic diatoms beneath antarctic sea ice. J. Phycol. 21:664-667.

Palmisano, A.C. and C.W. Sullivan. 1983. Physiology of sea ice diatoms. II. Dark survival of three polar diatoms. Can. J. Microbiol. 29:157-160.

Panella, J.R. 1994. Photoacclimation of *Achnanthidium rostratum* to reduced levels of illumination. M. Sc. Thesis. Loyola University Chicago, IL.

Peterson, C.G., K.D. Hoagland, and R.J. Stevenson. 1990. Timing of wave disturbance and the resistance and recovery of a freshwater epilithic microalgal community. J. N. Am. Benthol. Soc. 9(1):54-67.

Peterson, C.G. 1996a. Response of benthic algal communities to natural physical disturbance. In Algal Ecology, ed. R.J. Stevenson, M. Bothwell, and R. Lowe. Academic Press Inc., San Diego.

Peterson, C.G. 1996b. Mechanisms of lotic microalgal colonization following space-clearing disturbances acting at different spatial scales. Oikos 77:417-435.

Pianka, E.R. 1970. On r- and K-Selection. In The American Naturalist. 592-597.

Poulickova, A. 1987. Algae in ground waters below the active stream of a river. (Basin of the Morava River, Czechoslovakia). Arch. Hydrobiol. Suppl. 78.1 (Algological Studies 46):65-88.

Reitan, K.I., J.R. Rainuzzo, and Y. Olsen. 1994. Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. J. Phycol. 30:972-979.

Riber, H.H., and R.G. Wetzel. 1987. Boundary-layer and internal diffusion effects on phosphorus fluxes in lake periphyton. Limnol. Oceanogr. 32(6):1181-1194.

Rippka, R. 1972. Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. Arch. Mikrobiol. 87:93-98.

Rivkin, R.B., and M. Putt. 1987. Heterotrophy and photoheterotrophy by antarctic microalgae: light-dependent incorporation of amino acids and glucose. J. Phycol. 23:442-452.

Roemer, S.C., K.D. Hoagland, and J.R. Rosowski. 1984. Development of a freshwater periphyton community as influenced by diatom mucilages. Can. J. Bot. 62:1799-1813.

Rosner, B.A., ed. 1990. Fundamentals of Biostatistics. Boston: PWS-Kent Publishing Company.

Round, F.E., and J.D. Palmer. 1966. Persistent, vertical-migration rhythms in benthic microflora. II. Field and laboratory studies on diatoms from the banks of the River Avon. J. mar. biol. Ass. U.K. 46:191-214.

Saks, N.M. 1983. Primary production and heterotrophy of a pennate and a centric salt marsh diatom. Mar. Biol. 76:241-246.

Sartory, D.P. 1982. Spectrophotometric analysis of chlorophyll *a* in freshwater phytoplankton. Hydrological Research Institute, Dept. of Env. Affairs, Pretoria, South Africa, Technical Report. T.R. 115.

Sartory, D.P. and J.U. Grobbelaar. 1984. Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. Hydrobiol. 114:177-187.

Schollett, M.A. 1998. Organic nutrient preferences for benthic diatoms: an approach to quantifying heterotrophic metabolism. M. Sc. Thesis. Loyola University Chicago, IL.

Shifrin, N.S., and S.W. Chisholm. 1981. Phytoplankton Lipids: Interspecific differences and effects of nitrate, silicate, and light-dark cycles. J. Phycol. 17:374-384.

Sicko-Goad, L., and M.S. Simmons, D. Lazinsky, and J. Hall. 1988. Effect of light cycle on diatom fatty acid composition and quantitative morphology. J. Phycol. 24:1-7.

Sicko-Goad, L., E.F. Stoermer, and J.P. Kociolek. 1989. Diatom resting cell rejuvenation and formation: time course, species records and distribution. J. Plank. Res. 11:2:375-389.

Steinman, A.D. and C.D. McIntire. 1986. Effects of current velocity and light energy on the structure of periphyton assemblages in laboratory streams. J. Phycol. 22:352-361.

Steinman, A.D., C.D. McIntire, S.V. Gregory, G.A. Lamberti, and L.R. Ashkenas. 1987. Effects of herbivore type and density on taxonomic structure and physiognomy of algal assemblages in laboratory streams. J. N. Am. Benthol. Soc. 6(3):175-188.

Stevenson, R.J. 1984. Procedures for mounting algae in a syrup medium. Trans. Am. Micros. Soc. 103(3):320-321.

Stevenson, R.J. 1990. Benthic algal community dynamics in a stream during and after a spate. J.N. Am. Benthol. Soc. 9(3):277-288.

Stevenson, R.J., and R.Glover. 1993. Effects of algal density and current on ion transport through periphyton communities. Notes. Limnol. Oceanogr. 38(6):1276-1281.

Stevenson, R.J., C.G. Peterson, D.B. Kirschtel, C.C. King, and N.C. Tuchman. 1991. Density-dependent growth, ecological strategies, and effects of nutrients and shading on benthic diatom succession in streams. J. Phycol. 27:59-69.

Stock, M.S., and A.K. Ward. 1991. Blue-green algal mats in a small stream. J. Phycol. 27:692-698.

Taguchi, S., J.A. Hirata, and E.A. Laws. 1987. Silicate deficiency and lipid synthesis of marine diatoms. J. Phycol. 23:260-267.

Tuchman, M. and D.W. Blinn. 1979. Comparison of attached algal communities on natural and artificial substrata along a thermal gradient. Br. phycol. J. 14:243-254.

Tuchman, N.C. 1996. The role of heterotrophy in benthic algae. In Algal Ecology, ed. R.J. Stevenson, M. Bothwell, and R. Lowe. Academic Press Inc., San Diego.

Tuchman, N.C. and R.J. Stevenson. 1991. Effects of selective grazing by snails on benthic algal succession. J. N. Am. Benthol. Soc. 10(4):430-443.

Vannote, R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, and C.E. Cushing. 1980. The river continuum concept. Can. J. Aquat. Sci. 37:130-137.

Wasmund, N. 1989. Live algae in deep sediment layers. Int. Revue Ges. Hydrobiol. 74:589-597.

Wetzel, R.G. 1983. Limnology. 2d ed. Saunders College Publishing. Philadelphia.

White, A.W. 1974. Uptake of organic compounds by two facultatively heterotrophic marine centric diatoms. J. Phycol. 10:433-438.

Yodzis, P. 1978. Competion for space and the structure of ecological communities in: Lecture notes in biomathematics. Springer-Verlag, New York. 25:1-25.

VITA

The author, Patrick Daniel Donovan graduated from Augustana College in Rock Island, Illinois in 1989 receiving the degree of Bachelor of Arts in Biology.

In 1992, he entered Loyola University of Chicago to pursue a Master of Science degree. Mr. Donovan was awarded a partial Graduate Assistantship for the years of 1993-95, and completed the Degree of Master of Science in May of 1998. He is currently a Research Scientist at The Monsanto Company in Mt. Prospect, IL.

THESIS APPROVAL SHEET

The thesis submitted by Patrick Daniel Donovan has been read and approved by the following committee:

Nancy C. Tuchman, Ph.D., Co-Director
Associate Professor, Biology
Loyola University Chicago

John Smarrelli, Ph.D., Co-Director
Chair and Professor, Biology
Loyola University Chicago

Christopher G. Peterson, Ph.D.
Associate Professor, Natural Science
Loyola University Chicago

The final copies have been examined by the directors of the thesis and the signatures which appear below verify the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the committee with reference to content and form.

The thesis is therefore accepted in partial fulfillment of the requirements for the degree of Master of Science.

24 March 1998
Date

Nancy C. Tuchman
Co-Director's Signature

3/24/98
Date

Co-Director's Signature