



National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file *Voire référence*

Our file *Notre référence*

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.



National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

Your file *Votre référence*

Our file *Notre référence*

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-82539-1

Canada



UNIVERSITÉ D'OTTAWA
UNIVERSITY OF OTTAWA

ACKNOWLEDGMENTS

I would like to thank the members of my supervisory committee, Dr. J.T. Arnason, and Dr. J. Sinclair. Special thanks to Frances R. Pick, my advisor, for her constant encouragement, interest and funding during my years as a graduate student. She delivered fresh field samples from Jack's Lake to Ottawa in less than 3 hours. I am particularly grateful to Dr. David Lean, Canada Center for Inland Waters, Burlington, ON, for the particulate organic carbon and nitrogen analysis; Dr. L. Pietrzak, Agriculture Canada, Ottawa, ON, tirelessly assisted during the microspectrofluorometric analyses.

Thanks to the many new friends made at the University of Ottawa, Christine Eberl, Leah Deforest, and Norman Thie, not excluding my lab-mates, Julie Chouinard, Marc Proulx, and Aspasia Tzaras. Thanks to Christopher Hauschild for his help in the lab with French Pressing; to Dr. L. Bendel Young who was always there for lively discussions; Ann Empey and anonymous reviewers for their helpful comments when reviewing different stages of this thesis.

I also thank the Ontario Graduate Scholarship Committee and the University of Ottawa for their financial support during 1989-91. This work is dedicated to Bryan, a true friend and special companion; my parents and sister. Thank you for all your support.

ABSTRACT

Picocyanobacteria (0.2 to 2.0 μm in diameter) of the genus *Synechococcus* and *Synechocystis* are often the most abundant photosynthetic organisms in aquatic systems and can contribute significantly to primary production. The description of natural populations has relied primarily on epi-fluorescence microscopy and the detection of their photosynthetic pigments. This type of enumeration and classification ignores the variation in intensity and spectral distribution of fluorescence, and hence, picocyanobacteria are considered as one large population. In freshwater systems, the diversity and physiological state of picocyanobacteria are largely unknown. The general objective of this thesis was to examine the effects of key environmental variables on fluorescence characteristics of picocyanobacteria using both culture and field experiments.

The first objective was to examine the effects of acclimation to light intensity and spectral quality in 2 marine strains of phycoerythrin (PE) containing *Synechococcus*, (phycoerthrobilin-phycourobilin containing WH7803, and phycoerthrobilin containing WH8018). Fluorescence excitation (of Chl *a* at 680 nm) and emission spectra were measured in strains grown in full spectrum light of 400 and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$, and in green (peak transmission-560 nm) irradiance of 28 $\mu\text{E m}^{-2}\text{s}^{-1}$. Photoacclimation was achieved mainly through changes in the peripheral light harvesting phycobiliproteins (PBP) relative to chlorophyll *a* (Chl *a*). Fluorescence ratios of PE to phycocyanin (PC), and PE to Chl *a*, were found to be good surrogates of pigment ratios.

Photoacclimation to low green light mimicked a greater photosynthetically usable irradiance (PUR) as calculated using the absorption of the extracted phycobiliproteins and the spectral distribution of green light. Chromatic acclimation to green light in WH7803 occurred through a decrease in the size of the photosynthetic unit (PSU) as indicated by the fluorescence excitation ratio, $F(PE/PC)$. This is unique in view of the hypothesized changes in only PSU size at low irradiance. Multiple correlations indicated significant positive relationships exist among cell parameters of fluorescence ratios (excitation or emission), PE/C, and growth. Growth and the fluorescence ratios were not found to be correlated with Chl *a*/C.

The second objective of this thesis was to examine the effects of nitrogen stress on fluorescence in 6 strains of picocyanobacteria; three PC strains (freshwater *Synechococcus leopoliensis*, and *Synechocystis sp.* and marine strain WH5701), one phycoerythrobilin strain (WH8018), and two phycoerythrobilin-phycourobilin strains (WH7803 and 48B66). The severity of nitrogen stress, as measured by the reduction in carbon to nitrogen ratios, PBP, and fluorescence ratios, appeared to be related to the pigment types of picocyanobacteria, in the order; Type III PC > Type II PE > Type I PE:PU. Nitrogen stress was difficult to achieve in PE strains and could only be detected in PE strains by a decrease in the PE/PC content and in the excitation ratio, $F(PE/PC)$.

The culture experiments provided three main interpretations for the fluorescence "signatures" of natural populations. The first was that

photoacclimation and pigment content are likely to be the main determinants of fluorescence characteristics. Secondly, only slight changes in fluorescence ratios can be expected between high full spectrum and low monochromatic growth irradiance (PSII light) as a result of the small differences in PUR between these two environments. Lastly, the fluorescence characteristics of low light acclimated picocyanobacteria can be significantly influenced by growth rate.

The final objective was to examine the response of freshwater picocyanobacteria assemblages to natural gradients of light and nitrogen using microspectrofluorometry. In Jack' Lake, 72 to 98 % of the assemblage of picocyanobacteria was comprised of a single PBP type, similar to the Type II PE marine strain WH8018. These observations lend support to empirical data that relate the distribution of major pigment groups to light penetration and/or spectral quality in lakes and oceans. Two populations of picocyanobacteria could be further distinguished by their fluorescence characteristics particularly from fluorescence emission as opposed to excitation spectra. One population showed enhanced photoinhibition as evidenced by higher fluorescence ratios at the surface and low fluorescence emission ratios deep in the water column. While growth irradiance was the major factor controlling the variation in fluorescence characteristics with depth, taxa differences in the fluorescence characteristics appeared to be related to nitrogen availability. Depth patterns in fluorescence ratios in response to light and nitrogen availability are consistent with differences in the seasonal and between-lake abundance of these two populations of PE-picocyanobacteria.

TABLE OF CONTENTS

ABSTRACT	iii
LIST OF FIGURES	ix
LIST OF TABLES	xi
LIST OF ABBREVIATIONS	xiv
INTRODUCTION	1
1.1 Picocyanobacteria and Their Role in Aquatic Systems	1
1.2 Picocyanobacteria: Pigments and Light Absorption Characteristics	3
1.3 Fluorescence from the Photosynthetic Apparatus.	7
1.4 Fluorescence in Algal Ecology.	12
1.5 Research Objectives and Hypothesis.	15
MATERIALS AND METHODS	16
2.1 Materials.	16
2.1.1 Picocyanobacteria Cultures.	16
2.1.2 Media Preparation	16
2.2 Culture Methods	18
2.2.1 Irradiance Experiments.	18
2.2.2 Nitrogen Limitation Experiments.	19
2.3 Cell Counts, Pigment, Carbon and Nitrogen Analyses.	20
2.4 Fluorescence Spectroscopy.	22
2.5 Effects of Excitation Wavelengths on Fluorescence Emission Intensity.	25
2.6 Effects of Preservation on Fluorescence.	26
2.7 Field Methods.	27
2.7.1 Site Description.	27
2.7.2 Sampling Protocol	28
2.7.3 Microspectrofluorometry.	29
2.8 Statistical Analysis of Data.	30
RESULTS	32
3.1 Photoacclimation Responses: Growth Rates, Carbon, Nitrogen and Pigment Content.	32
3.1.1 WH7803 - High vs Low White Irradiance.	32
3.1.2 WH7803 - Low White vs Green Irradiance.	32
3.1.3 WH8018 - High vs Low White Growth irradiance.	33
3.1.4 WH8018 - Low White vs Green Growth irradiance.	33
3.2 Photoacclimation Responses: Excitation Spectra of Chl <i>a</i>	38
3.2.1 WH7803.	38

3.2.2	WH8018	41
3.3	Photoacclimation Responses: Emission Spectra.	41
3.3.1	WH7803	41
3.3.2	WH8018	47
3.4	Effects of Growth irradiance on Fluorescence Yield.	51
3.5	Comparison of Photoacclimation Parameters.	53
3.6	Effects of Nitrogen Limitation: Phycocyanin Strains.	56
3.6.1	Carbon, Nitrogen and Pigment Content.	56
3.6.2	Excitation Spectra of Chl <i>a</i> .	59
3.6.3	Emission Spectra.	59
3.7	Effects of Nitrogen Limitation: Phycoerythrin Strains.	65
3.7.1	Growth Rates, Carbon, Nitrogen and Pigment Content: 48B66.	65
3.7.2	Excitation Spectra of Chl <i>a</i> Emission.	66
3.7.3	Emission Spectra.	66
3.7.4	Growth rates, Carbon, Nitrogen and Pigment Content: WH7803.	71
3.7.5	Excitation Spectra of Chl <i>a</i> .	77
3.7.6	Emission Spectra.	77
3.7.7	Growth Rates, Carbon, Nitrogen and Pigment Content: WH8018.	83
3.7.8	Excitation Spectra of Chl <i>a</i> .	83
3.7.9	Emission Spectra.	83
3.8	Effects of Nitrogen limitation on Fluorescence Yield.	91
3.9	Natural Populations of Picocyanobacteria.	94
3.9.1	Picocyanobacteria Abundance in Relation to Temperature and Irradiance in Jack's Lake.	94
3.9.2	Excitation Spectra of Chl <i>a</i> .	97
3.9.3	Depth Trends in Emission Spectra.	100
3.9.4	Effects of Nitrogen Addition on Emission Spectra.	103
DISCUSSION		106
4.1	Photoacclimation in Picocyanobacteria: fluorescence tracks pigment changes.	106
4.2	Effects of Light Quality: evidence for chromatic acclimation.	107
4.3	Effects of Nitrogen Deficiency.	113
4.4	Effects of Growth Rates on Fluorescence.	116
4.5	Fluorescence Yield in Relation to Growth irradiance and Nitrogen Stress.	118
4.6	Fluorescence as a Taxonomic Tool to Analyze Phytoplankton Communities.	128
4.7	Fluorescence Characteristics of Freshwater Picocyanobacteria.	131
SUMMARY AND CONCLUSIONS.		137

REFERENCES	138
APPENDIX	150
APPENDIX IA. Major peak excitation wavelengths (nm) for Chl <i>a</i> emission at 680 nm of the 6 study strains.	150
APPENDIX IB. Major peak emission wavelengths (nm) from the 6 study strains for FBP and Chl <i>a</i> emission, obtained using 470 nm and 420 nm excitation, respectively.	151
APPENDIX IIA. Raman scatter peak of seawater media using 420 nm, 440 nm, 460 nm, and 470 nm	152
APPENDIX IIB. Raman scatter peak of seawater media using 480 nm, 500 nm, 520 nm, and 540 nm excitation wavelengths.	153
APPENDIX IIC. Effect of excitation wavelength, 420 nm or 440 nm, on emission spectra of WH8018 grown at green, 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	154
APPENDIX IID. Effect of excitation wavelength, 460 nm or 470 nm, on emission spectra of WH8018 grown at green, 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	155
APPENDIX IIE. Effect of excitation wavelength, 480 nm or 500 nm, on emission spectra of WH8018 grown at green, 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	156
APPENDIX IIF. Effect of varying excitation wavelength, 520 nm or 540 nm, on emission spectra of WH8018 grown at green, 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	157
APPENDIX IIIA. Effects of glutaraldehyde preservation on the excitation spectra (for Chl <i>a</i> emission at 680 nm) of WH8018 grown in green 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	158
APPENDIX IIIB. Effects of glutaraldehyde preservation on the emission spectra (obtained violet excitation; 420 nm) of WH8018 grown in green 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	159
APPENDIX IIIC. Effects of glutaraldehyde preservation on the emission spectra (obtained blue excitation; 470 nm) of WH8018 grown in green 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.	160
APPENDIX IV. Multiple analysis of variance (MANOVA) partial correlation coefficients of combined PE picocyanobacteria data during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) growth irradiance.	161

LIST OF FIGURES

Figure 1. The absorption of light by different algal pigments in relation to pure water (represented by the curve below the hatched areas). The relative magnitude of the absorption spectra for the different pigments approximate those measured <i>in vivo</i>	8
Figure 2. Spectral distribution of growth irradiance from the two light quality regimes.	18a
Figure 3. Absorption spectra of extracted PBP of the experimental strains of picocyanobacteria illustrating the different PBP types.	23
Figure 4. Replicate (n=3) excitation spectra for Chl <i>a</i> emission at 680 nm of WH7803.	39
Figure 5. Effects of high white (HW), 400 $\mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized excitation spectra for Chl <i>a</i> emission at 680 nm, of exponentially growing WH7803.	40
Figure 6. Replicate (n=4) excitation spectra for Chl <i>a</i> emission at 680 nm of WH8018.	42
Figure 7. Effects of high white (HW), 400 $\mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized excitation spectra for Chl <i>a</i> emission at 680 nm, of exponentially growing WH8018.	43
Figure 8. Effects of high white (HW), 400 $\mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using blue excitation; 470 nm) of exponentially growing WH7803.	45
Figure 9. Effects of high white (HW), 400 $\mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using violet excitation; 420 nm) of exponentially growing WH7803.	46
Figure 10. Effects of high white (HW), 400 $\mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using blue excitation; 470 nm) of exponentially growing WH8018.	48
Figure 11. Effects of high white (HW), 400 $\mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using violet excitation; 420 nm) of exponentially growing WH8018.	49
Figure 12. Effects of nitrogen limitation on the normalized excitation spectra, for Chl <i>a</i> emission at 680 nm, of three PC strains.	60
Figure 13. Effects of nitrogen limitation on the normalized emission spectra using green excitation (545 nm), of three PC strains.	62
Figure 14. Effects of nitrogen limitation on the normalized emission spectra using violet excitation (420 nm), of three PC strains.	63
Figure 15. Effects of nitrogen limitation on the excitation spectra for Chl <i>a</i>	

emission at 680 nm, of strain 48B66.	69
Figure 16. Effects of nitrogen limitation on the emission spectra using blue excitation (470 nm), of strain 48B66.	72
Figure 17. Effects of nitrogen limitation on the emission spectra using violet excitation (420 nm), of strain 48B66.	73
Figure 18. Effects of nitrogen limitation on the excitation spectra for Chl <i>a</i> emission at 680 nm, of strain WH7803.	78
Figure 19. Effects of nitrogen limitation on the emission spectra, using blue excitation (470 nm), of strain WH7803.	80
Figure 20. Effects of nitrogen limitation on the emission spectra, using violet excitation (420 nm), of strain WH7803.	81
Figure 21. Effects of nitrogen limitation on the excitation spectra for Chl <i>a</i> emission at 680 nm, of strain WH8018.	86
Figure 22. Effects of nitrogen limitation on the emission spectra, using blue excitation (470 nm), of strain WH8018.	88
Figure 23. Effects of nitrogen limitation on the emission spectra, using violet excitation (420 nm), of strain WH8018.	89
Figure 24. Depth profiles of picocyanobacteria abundance, and temperature for the three sampling dates, Sharpe's Bay, Jack's Lake, Ontario, 1990.	95
Figure 25. Comparison of the depth (growth irradiance) response of the normalized excitation spectra for Chl <i>a</i> emission at 680 nm for PE-SYN among the 3 sampling dates.	98
Figure 26. Comparison of the depth (growth irradiance) response of the normalized excitation spectra for Chl <i>a</i> emission at 680 nm for PE-SYS among the 3 sampling dates.	99
Figure 27. Effect of depth (growth irradiance) on the normalized excitation spectra for Chl <i>a</i> emission at 680 nm for a centric diatom from the upper mixed zone (dotted line), and from the 1% light level (I_0) (solid line).	101
Figure 28. Examples of normalized emission spectra, using 470 nm excitation, for PE-SYN and PE-SYS from the upper mixed zone (dotted line), and the 1% light level (I_0) (solid line).	102
Figure 29. Depth trends in emission ratios and temperature for Aug. 14, 1990. a) using 470 nm ex; B(PE/Chl <i>a</i>), b) using 470 nm ex; B(PE/PC). PE-SYN; opened circles, PE-SYN plus nitrogen addition; closed circles, PE-SYS; open triangles, PE-SYS plus nitrogen addition; closed triangles.	104
Figure 30. Emission peak ratio, using 470 nm ex, for the upper mixed zone (open bars) and 10 m (closed bars) compared among sampling dates for the PE-SYN and PE-SYS.	105
Figure 31a. Graphical representation of the photosynthetic useable radiation as available to PBP absorption for strains WH7803 and WH8018 during exponential growth in low white growth irradiance.	111

Figure 31b. Graphical representation of the photosynthetic useable radiation as available to PBP absorption for strains WH7803 and WH8018 during exponential growth in low green growth irradiance.	112
Figure 32a. Fluorescence emission of Chl <i>a</i> (680 nm em:420 nm ex) from WH7803 vs Chl <i>a</i> content.	122
Figure 32b. Fluorescence emission of Chl <i>a</i> (680 nm em:420 nm ex) from WH7803 vs Chl <i>a</i> content.	123
Figure 33a. Fluorescence emission of Chl <i>a</i> (680 nm em:420 nm ex) from WH8018 vs Chl <i>a</i> content.	124
Figure 33b. Fluorescence emission of Chl <i>a</i> (680 nm em:420 nm ex) from WH8018 vs Chl <i>a</i> content.	125
Figure 34a. Fluorescence emission of PE (580 nm em:470 nm ex) from WH8018 vs PE content.	126
Figure 34b. Fluorescence emission of PE (580 nm em:470 nm ex) from WH8018 vs PE content. Individual cultures during exponential and nitrogen stressed growth conditions are plotted.	127

LIST OF TABLES

TABLE 1. Major pigment types of marine picocyanobacteria based on accessory pigment composition and fluorescence characteristics.	6
TABLE 2. Picocyanobacteria Strain Description.	17
TABLE 3. Growth rate (ln units d ⁻¹), and cellular carbon and nitrogen content (fg/Cell) of WH7803 during growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) irradiance.	34
TABLE 4. Pigment content per cell (fg/Cell) and per carbon (mg/g) of WH7803 during growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance.	35
TABLE 5. Growth rates (ln units d ⁻¹), and cellular carbon and nitrogen content (fg/Cell) of WH8018 during exponential growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance.	36
TABLE 6. Pigment content per cell (fg/Cell) and per carbon (mg/g) of WH8018 during exponential growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance.	37
TABLE 7. Pigment ratios (w/w) and peak excitation ratios, F(PE/Chl <i>a</i>) and F(PE/PC) for Chl <i>a</i> emission at 680 nm, of WH8018 and WH7803 during growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance.	44
TABLE 8. Pigment ratios (w/w), emission ratios obtained using 420 nm ex [B(PE/Chl <i>a</i>)], 470 nm ex [V(PE/Chl <i>a</i>)] and the composite 470/420 nm ex [BV(PE/Chl <i>a</i>)], of strains WH8018 and WH7803 during exponential growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance.	50
TABLE 9. Fluorescence emission normalized to pigment content for strains	

WH8018 and WH7803 during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) growth irradiance.	52
TABLE 10. Multiple analysis of variance (MANOVA) partial correlation coefficients from strain WH7803 during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) growth irradiance.	54
TABLE 11. Multiple analysis of variance (MANOVA) partial correlation coefficients of strain WH8018 during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) growth irradiance.	55
TABLE 12. Growth rates (ln units d^{-1}), and carbon and nitrogen content (fg/cell) of 3 PC strains at 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	57
TABLE 13. Pigment content per cell (fg/cell) and per carbon (mg/g) of 3 PC strains in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	58
TABLE 14. Excitation ratios, F(PC/Chla) of Chl <i>a</i> emission at 680 nm, of 3 PC strains in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	61
TABLE 15. Emission ratios using 420 nm ex, [V(PC/Chla)], 545 nm ex, [G(PC/Chla)], and the composite 545/420 nm ex, [GV(PC/Chla)] of 3 PC strains in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	64
TABLE 16. Growth rate (ln units d^{-1}), cellular carbon and nitrogen content (fg/cell) of strain 48B66 during growth in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	67
TABLE 17. Pigment content per cell (fg/cell) and per carbon (mg/g) of 48B66 during growth in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	68
TABLE 18. Pigment ratios (w/w) and excitation ratios, F(PE/Chla), F(PE/PC), and F(PE/PU), for Chl <i>a</i> emission of 680 nm, of strain 48B66 during growth in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	70
TABLE 19. Emission ratios using 470 nm ex, [B(PE/Chla)], 420 nm ex, [V(PE/Chla)], and the composite 470 nm/420 nm ex, [BV(PE/Chla)] of 48B66 during growth in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	74
TABLE 20. Cellular carbon and nitrogen content (fg/cell) of strain WH7803 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	75
TABLE 21. Pigment content per cell (fg/cell) and per carbon (mg/g) of strain WH7803 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	76
TABLE 22. Pigment ratios (w/w) and excitation ratios, F(PE/Chla), F(PE/PC), and F(PE/PU), for Chl <i>a</i> emission at 680 nm, of strain WH7803 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	79
TABLE 23. Peak emission ratios from 420 nm ex, [V(PE/Chla)], 470 nm ex, [B(PE/Chla)], and the composite 470/420 nm ex, [BV(PE/Chla)] of strain WH7803 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	82

TABLE 24. Cellular carbon and nitrogen content (fg/cell) of strain WH8018 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	84
TABLE 25. Pigment content per cell (fg/cell) and per carbon (mg/g) of strain WH8018 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	85
TABLE 26. Pigment ratios (w/w) and excitation ratios, F(PE/Chl <i>a</i>), F(PE/PC), and F(PE/PU), for Chl <i>a</i> emission at 680 nm, of strain WH8018 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. . .	87
TABLE 27. Emission ratios from 420 nm ex, [V(PE/Chl <i>a</i>)], 470 nm ex, [B(PE/Chl <i>a</i>)], and the composite 470/420 nm ex, [BV(PE/Chl <i>a</i>)] of strain WH8018 in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance.	90
TABLE 28. Effects of nitrogen limitation on Chl <i>a</i> fluorescence emission (em 680 nm, ex 420 nm) normalized for Chl <i>a</i> concentration (.	92
TABLE 29. Effect of nitrogen limitation of PE fluorescence emission normalized for PE concentration (F_p/PE) for	93
TABLE 30. Daily growth irradiance levels 4 days before and on the sampling dates.	96

LIST OF ABBREVIATIONS

C/N	carbon to nitrogen ratio (w/w).
Chl <i>a</i>	Chlorophyll <i>a</i> .
Chl <i>a/C</i>	Chlorophyll <i>a</i> /carbon (w/w).
PBP	Phycobiliprotein.
PE	Phycoerythrin.
PE/C	Phycoerythrin/carbon (w/w).
PC	Phycocyanin.
APC	Allophycocyanin.
PSI	photosystem I.
PSII	photosystem II.
PSU	photosynthetic unit.
PAR	photosynthetic available radiation.
PUR	photosynthetic useable radiation.
Type I	strain type based on phycoerythrin pigment which contains phycourobilin and phycoerythrobin (Wood et al. 1985).
Type II	pigment type containing the phycoerythrobin.
Type III	pigment type containing the phycocyanobilin.
em	Emission window
ex	Excitation window
F(PE/Chl)	ratio of PE to the blue Soret Chl <i>a</i> excitation peak (680 nm em).
F(PE/PC)	ratio of PE to PC excitation peaks (680 nm em)

- $F(\text{PC}/\text{Chl}a)$ ratio of PC to the blue Soret Chl a excitation peaks (680 nm em)
- $B(\text{PE}/\text{Chl}a)$ ratio of PE to Chl a emission peaks using blue excitation (470 nm ex).
- $V(\text{PE}/\text{Chl}a)$ ratio of PE to Chl a emission peaks using violet excitation (420 nm ex).
- $BV(\text{PE}/\text{Chl}a)$ ratio of PE emission (470 nm ex) to Chl a emission (420 nm ex).
- $G(\text{PC}/\text{Chl}a)$ ratio of PC to Chl a emission peaks using green excitation (545 nm ex).
- $GV(\text{PC}/\text{Chl}a)$ ratio of PC emission (545 nm ex) to Chl a emission (420 nm ex).
- $F_e/\text{Chl}a$ relative fluorescence intensity at 680 nm (420 nm ex) normalized to Chl a (mg/L).
- F_p/PE relative fluorescence intensity at 580 nm for strain WH8018 or 570 nm for strain WH7803 (470 nm ex) normalized to PE (mg/L).
- R.F.I. , relative fluorescence intensity

INTRODUCTION

Over the last decade, it has become evident that a significant portion of biomass and primary production in aquatic systems is associated with organisms less than 2.0 μm in diameter (Stockner and Antia 1986). This group of organisms is collectively referred to as the picoplankton in reference to their cellular mass (0.25-1.0 pg carbon/cell). Many of the photosynthetic picoplankton cells (0.2 - 2.0 μm) approach the theoretical minimum size of oxygenic photolithotrophes (0.5 μm in diameter, Raven 1986). The majority are cyanobacteria, and, in marine systems are thought to belong to the genus *Synechococcus* (Glover 1985, Waterbury et al. 1985). Picoplankton can comprise 20-80% of the total phytoplankton biomass and a similarly significant portion of primary production in aquatic systems (Sherr and Sherr 1991). While there is now considerable information on the ecology and physiology of marine picocyanobacteria, much less is known about the composition and physiological ecology of freshwater assemblages.

1.1 Picocyanobacteria and Their Role in Aquatic Systems.

Picocyanobacteria are found in a wide range of aquatic habitats from the open ocean (Waterbury, et al. 1979, Johnson and Sieburth 1979, Li et al. 1983) to coastal waters (Krempin and Sullivan 1981, Olson et al. 1988), coral reefs (Legendre et al. 1988), estuaries (Takahashi et al. 1989), rivers (Vaulot and Xiuren 1988) and freshwater lakes (Caron et al. 1985, Weisse 1988, Pick 1991). They are often the most abundant photosynthetic organisms, particularly in oligotrophic to mesotrophic systems where densities of $1-5 \times 10^5$ cell mL^{-1} have been reported

(Olson et al. 1990, Pick 1991). In these systems they account for a significant fraction of the photosynthetic assimilation of CO₂, (Caron et al. 1985, Glover et al. 1985a,b, 1986a, Fahnenstiel and Carrick 1991). Current estimates of carbon biomass indicate that picocyanobacteria also account for a significant proportion (20%) of the total planktonic biomass available for transfer to consumers in the food web (Krempin and Sullivan 1981, Pick and Caron 1987).

Two main explanations for the dominance of picocyanobacteria in oligotrophic waters have been proposed. The first explanation relates to their ability to utilize the low average irradiance typically experienced by cells in deeply stratified waters. Based on size alone picoplankton have a greater potential for photon absorption than larger cells. They are not subject to the "package effect". The "package effect" is a result of the confinement of pigments into discrete packages and can be significant in highly pigmented and/or large cells where a portion of the pigment is unavailable for energy absorption (Kirk 1986). Calculations of the photon efficiency of photosynthesis (from the initial slope of photosynthesis vs irradiance curves) have shown that picocyanobacteria have the ability to capture and utilize light with higher efficiency than larger cells (Glover et al. 1985a, 1985b, 1987). In the oceans, picocyanobacteria can account for a significant fraction of the light attenuation with depth despite their relatively low biomass (Sathyendranath 1986). They attenuate most of the light not only because they effectively scatter light but also because of their high absorption capabilities.

The second explanation relates to their ability to take up nutrients from dilute solutions. Small cells in general are less likely to be nutrient limited, than large cells, by virtue of their large surface area to volume ratio (A/V). This large A/V ratio enable proportionately higher nutrient uptake rates compared to larger cells (Raven 1986).

These physiological traits may also serve to explain the depth distribution of picocyanobacteria in lakes and oceans. Although picocyanobacteria can grow at high irradiance (Kana and Glibert 1987a,b), they often reach their maximum abundance at low light intensities deep in the water column, where they can effectively compete for light and nutrients (Prezelin et al. 1986, Pick and Agbeti 1991).

In all ecological studies, picocyanobacteria must be identified and enumerated using epi-fluorescence microscopy (fluorescence emission) (Glover 1985). Other microscopy techniques for identification are not suitable; picocyanobacteria ($< 2.0 \mu\text{m}$ dia) approach the minimum resolution of the light microscope. Using epi-fluorescence microscopy, the fluorescence emission provides the necessary contrast which can reveal both cell morphology and specific pigment groups (see below). An assumption of this technique is that fluorescence characteristics are not under environmental control.

1.2 Picocyanobacteria: Pigments and Light Absorption Characteristics.

Cyanobacteria are like other oxygenic phototrophs in having chlorophyll *a* (Chl *a*) contained in the Chl-protein complexes of photosystem I and photosystem

II. However, the major light-harvesting pigments are the water-soluble phycobiliproteins (PBP) (reviewed by MacColl and Guard-Friar (1987) and not the accessory chlorophylls. The phycobiliproteins (PBP) are also found in the Cryptophyta (cryptomonads) and Rhodophyta (red algae). In cyanobacteria, they can account for up to 50% of the cellular protein and up to 10% of cell dry weight (Yamanaka and Glazer, 1980, Kana and Glibert 1987a).

Wood et al. (1985) classified the different pigment groups of marine strains of *Synechococcus* according to the complement of accessory PBP using fluorescence excitation and emission characteristics (Table 1) . The PBP include three main types: allophycocyanin, phycocyanin and phycoerythrin. Each is comprised of protein subunits where each has one or more molecules of covalently bonded phycobilin chromophores. The phycobilins are open tetrapyrroles which vary only in the placement and number of double bonds. The three main types of phycobilins include the phycocyanobilin, phycoerythrobilin, and phycourobilin (Table 1). These latter two bilins are prosthetic groups attached to phycoerythrin in varying amounts and their relative proportions distinguish Type I from Type II strains. While this classification was developed for marine *Synechococcus* it is likely to apply to freshwater strains as well.

All cyanobacteria contain phycocyanobilins. The blue-green phycocyanin (PC) is composed of phycocyanobilin which produce one absorption maximum between 615 and 635 nm. Allophycocyanin (APC), like PC, has the same phycocyanobilin but the protein environment leads to an absorption maximum

centred at 650 nm.

Most cyanobacterial phycoerythrins (PE) absorb light energy between 500 and 570 nm and have only one major absorption maximum, reflecting a chromophore comprised solely of phycoerythrobilin. However, Alberte et al. (1984), found novel chromophores which, like red algal PE, contained two distinct phycobilin pigments; phycoerythrobilin and phycourobilin. Phycourobilin accounted for an additional blue-green absorption maximum around 498 nm. PE excitation of Chl *a* at 680 nm occurs at slightly shorter wavelengths if PE contains phycourobilin chromophores (Type I PE) (Table 1). The phycobiliproteins are found in supramolecular complexes called phycobilisomes which are located on the surface of the thylakoid membranes. The light energy transfer within the phycobilisomes occurs from the peripheral PE chromophores (fluorescing orange-yellow between 570-580 nm) to intermediately-placed PC (fluorescing light energy between 635 and 660 nm) then to the internal allophycocyanin (APC) (fluorescing red with an emission maximum between 660 nm and 680 nm) located on the thylakoid surface (MacColl and Guard-Friar, 1987). It is from APC that light energy absorbed by the phycobilisomes is transferred to photosystem II (PSII) particles integrally placed in the thylakoid membranes below the phycobilisomes. Most of the phycobiliproteins (90%) are associated with PSII with the remainder associated with photosystem I (PSI).

TABLE 1. Major pigment types of marine picocyanobacteria based on accessory pigment composition and fluorescence characteristics. All types contain phycocyanin. Variation and proportions of bilins in phycoerythrin form another 3 groups. (PEB; phycoerythrobilin, PUB; phycourobilin, and PBC; phycocyanobilin). Excitation (Ex max.) for Chl *a* emission at 680 nm and emission (Em max.) at phycolibprotein excitation maximum. Pigment type is based on the composition of phycobiliproteins (*) as modified from Wood et al. (1985).

Chromophores in

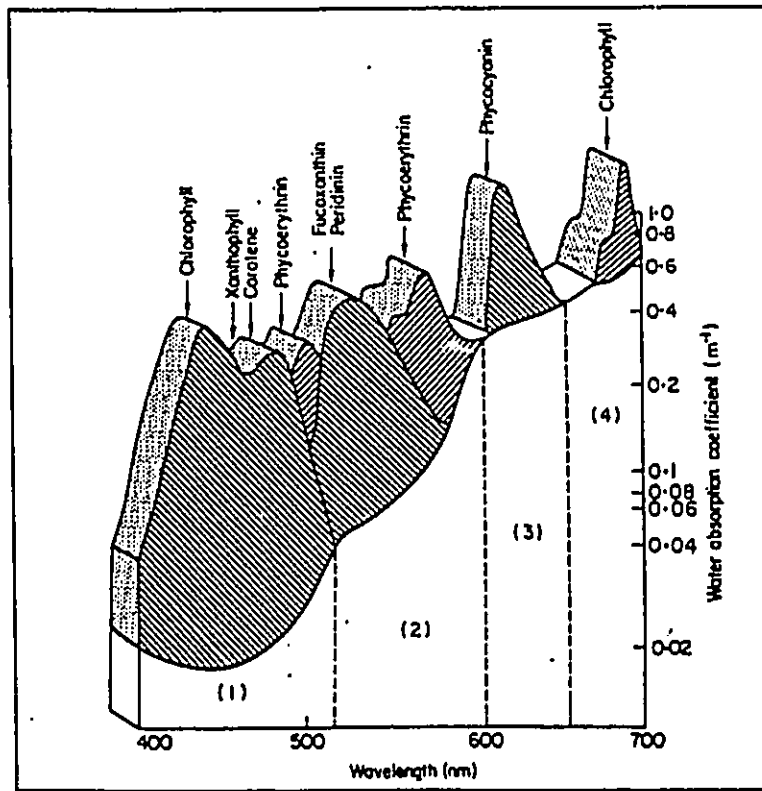
accessory pigment:		PBC	PEB	PUB	Ratio of PEB:PUB
Type Ia	Ex max.	*	556 ± 3nm	500 ± 1nm	1:1
	Em max.		550 ± 5nm		
Type Ib		*	"	"	1:5
Type II	Ex max.	*	576 ± 1nm		
	Em max.	*	570 ± 3nm		
Type III	Ex max.		653 ± 8nm		
	Em max.		636 ± 8nm		

The large variety of accessory bilins enhances the light absorption of cyanobacteria over a region of the visible spectrum not utilized by the accessory pigments of other taxa (Fig.1). The water absorption coefficient scales the energy absorption of water over the photosynthetic available spectrum. Here water absorbs most of the red end of the spectrum while other spectral wavelengths are absorbed by the major algal pigments. The major "clear window" of the water column is filled with the Soret absorption of the chlorophylls and carotenoids of all algae. The phycobilins of the blue green algae fill in a number of other "clear windows" and exploit a number of different wavelengths of PAR from the penetrating light of a water body. The dominance of PE (Type I) *Synechococcus* in the open ocean has been attributed not only to their efficiency of light and nutrient utilization but to their ability to trap the ambient blue-green wavelengths (470-530 nm) typically found deep in the ocean (Wood 1985, Glover et al. 1987).

1.3 Fluorescence from the Photosynthetic Apparatus.

Light striking an algal surface may be either scattered off the cell surface, transmitted through the cell or absorbed by the cell. Algal size, shape, pre-absorption by internal non-photosynthetic components, chloroplast arrangement, pigment content and pigment "packaging" into discrete thylakoid components all contribute to the spectral quality and irradiance finally absorbed by the photosynthetic pigments (Kirk 1983). In its simplest conception, absorbed light is further partitioned and is either 1) funnelled into photochemical events located on

Figure 1. The absorption of light by different algal pigments in relation to pure water (represented by the curve below the hatched areas). The relative magnitude of the absorption spectra for the different pigments approximate those measured *in vivo*. The bilins, along with Chl *a*, provide cyanobacteria with broad spectral absorption capabilities (as modified from Yentch and Yentch 1979).



the thylakoid membrane, 2) dissipated as heat, or 3) re-emitted as luminescence (mostly fluorescence). The light reactions of photosynthesis are responsible for most of the light absorption and fluorescent features of plant cells (Lichtenthaler 1988).

Fluorescence emission represents a loss of excess energy not funnelled through other processes. The *in vivo* fluorescence at room temperature arises from back reactions of the primary photochemical events occurring in the reaction centres and antennae pigments of PSII (Prezelin and Bocxar 1986, Krauss and Weis 1991). As a first approximation fluorescence and PSII photochemical reactions can be considered to be in competition for excitation energy within the antennae Chl *a* of PSII reaction centers. *In vitro*, the amount of fluorescence intensity is directly related to the content of extracted pigment; however, *in vivo* the fluorescence intensity per Chl *a* ($F_c/Chl a$) can be expressed as the product of two terms (Kiefer 1973b);

$$F_c/Chl a = ab \quad (1)$$

where: a = quanta absorption rate/Chl *a*, and

b = quanta emission rate/quanta absorption rate.

The first term (a) is related to quanta absorption efficiency. Besides the content of Chl *a*, the relative content of accessory pigments will increase the

quanta absorbed per unit Chl *a*. Differences in pigment composition result in variations in absorption efficiency for a given excitation wavelength. As mentioned previously, cell size is also a factor in the absorption of light. The second term (b) refers to fluorescence yield. Fluorescence yield is influenced by physiological factors that control the way light is utilized and balanced between the two photosystems. Nutrient stress and photoinhibition are two conditions that have been shown to cause large variations in (b) (Kiefer 1973a, b, Vincent 1979, 1980).

Processes that affect photosynthesis can affect fluorescence by modulating both the absorption efficiency (a) and the fluorescence yield (b). Efficient photosynthesis requires a balanced distribution of absorbed light between PSI and PSII. A number of mechanisms exist to keep a balanced energy distribution between the two phototransfer traps. Allen et al. (1989) have summarized these mechanisms on a series of time scales. Constant light conditions that favour the excitation of one photosystem over the other result in a stable difference in the amount or composition of light harvesting pigments. These differences in pigment composition can directly influence the absorption efficiency, (a), of a given spectral distribution of light. The changes in photosystem stoichiometry, induced over the long term (days), are typically referred to as photoacclimation (Falkowski and LaRoch 1991). As a special case, some PE containing cyanobacteria can also change the relative proportion of the PE to PC within PSII and this has been termed chromatic acclimation (Tandeau de Marsac 1977, Tandeau de Marsac and Houmard 1988, Hauschild, McMurter and Pick 1991).

Two other mechanisms involve small physiological changes in the way light is utilized. These changes are quick responses of the photosynthetic apparatus to short term fluctuations in light and will affect Chl *a* fluorescence yield, (b). The first mechanism is referred to as non-photochemical (energy dependent) quenching and occurs over a time period that can be measured in hours. Non-photochemical quenching can be due to photoinhibition, light-induced proton gradients across the thylakoid membrane, and/or phosphorylation of the light harvesting complexes of PSII. All quenching mechanisms do not occur simultaneously but each serves to dissipate excess energy as heat. The net result of non-photochemical quenching is a decrease in Chl *a* fluorescence.

For shorter time scales (minutes and seconds), regulation of light utilization by PSI and PSII can be achieved by small adjustments in the way light is transferred and shared between the two systems. These are often referred to as State 1 - State 2 transitions and occur primarily when shade adapted cells are suddenly exposed to brighter light. During State 1 transitions, PSII re-emits unused excitation energy which becomes partially trapped by PSI. The State 2 condition involves a reduction in the absorption cross section of PSII, by effectively closing phototaps to the input of excess excitation energy. State 2 may occur when electron carriers between PSII and PSI become reduced. The net result of a State 2 transition is an increase in Chl *a* fluorescence. State transitions have been proposed to occur during mixing events that bring deep dwelling, low light acclimated, algae up to the higher light of the surface (Vincent 1979, Therriault et

al. 1990).

Fluorescence emission of pigments can also be controlled by the spectral range of the exciting light. Excitation spectra are generated by monitoring the emission maximum for Chl *a* as a function of monochromatic excitation wavelengths. Excitation spectra reveal the pigments involved in absorption and transfer of light energy driving the light reactions of PSII. Typically Chl *a* excitation spectra are similar to the action spectra for oxygen evolution (Neori et al. 1984).

Most of the studies on plant fluorescence have specifically examined chlorophyll fluorescence. However, in cyanobacteria there is significant fluorescence emission from the phycobiliproteins. Although there is considerable information on the factors which affect Chl *a* fluorescence, both from laboratory and field work, very little is known about the factors that affect the fluorescence properties of phycobiliproteins.

1.4 Fluorescence in Algal Ecology.

Since the early work of Yentch and Menzel (1963), *in vivo* chlorophyll fluorescence has been routinely used to estimate *in situ* content of Chl *a* and hence algal biomass in oceans and lakes. More recently, fluorescence has been used as a taxonomic tool. Yentch and coworkers (Yentch and Yentch 1979, Yentch and Phinney 1984) were the first to characterize phytoplankton communities in different parts of the ocean using taxonomic differences in the excitation spectra for Chl *a* emission. This process was accomplished by calculating the excitation ratio of the accessory pigments (530 nm) to that of Chl *a* (450 nm). The choice of

the accessory pigment excitation wavelength enabled a qualitative distinction between diatom/dinoflagellate assemblages and other taxa. These fluorescence "signatures" are still being explored in different classes of algae (Oldam et al. 1985). Flow cytometry is now used to characterize natural populations in a similar fashion but is based on the analysis of fluorescence emission peaks rather than excitation peaks (Olson et al. 1988, 1990). As in Yentstch's work, characterization using flow cytometry, is based on only 1 or 2 wavelengths.

Algal fluorescence can also be detected via air borne sensors as a way of estimating global patterns in Chl *a* and primary production (Smith et al. 1982, Eppely et al. 1985). The presence of Chl *a*, and very recently phycoerythrin can be discerned using the LIDAR (light detection and ranging) fluorensor. This system uses a garnet and /or excimer dye laser to pulse the sea surface with 532 nm or 427 nm light (Prezelin and Bockxar 1986). The first wavelength primarily excites the accessory carotenoids (fucoxanthin, and peridinin) and PE, and the second preferentially excites Chl *a*. Hoge and Swift (1983) used LIDAR dual laser excitation to map Chl *a* and PE fluorescence and thereby estimated the distribution of these two pigments in the Gulf Stream. However, the fluorescence characteristics of Chl *a* and PE may vary in response to environmental conditions; photoacclimation effects may confound the predictions of biomass or phytoplankton composition obtained from fluorescence data

In summary, picocyanobacteria are an important group of organisms that have yet to be fully characterized, especially in freshwater ecosystems. In

particular the extent to which accessory pigment expression is under environmental control is not clear. This is paramount because our detection and grouping of picocyanobacteria is based on fluorescence from the accessory pigments.

Furthermore in natural populations, variations in fluorescence intensity are ignored and the entire assemblage of picocyanobacteria is considered as a single ecological and taxonomic unit. It is important to examine how key environmental variables such as light and nutrients, affect fluorescence characteristics. Fluorescence measurements are a potentially powerful tool because information on picocyanobacteria diversity and physiological status can be gained simultaneously.

1.5 Research Objectives and Hypothesis.

Picocyanobacteria are unique in that fluorescence emission is directly related to fluorescence yield, without the confounding influence of the "package effect". Differences in the composition of accessory pigments among different strains of picocyanobacteria provide the opportunity to assess the importance of the various photosynthetic pigments in the interpretation of fluorescence "signatures" (Yenstch and Yenstch 1979).

Specific questions addressed by this work include:

- 1) What are the relative contributions of irradiance (quantity and quality), and nitrogen availability to the fluorescence characteristics of picocyanobacteria?
- 2) What are the fluorescence signatures of picocyanobacteria in lakes? and,
- 3) How do natural gradients of light and nitrogen affect the fluorescent characteristics of natural population of picocyanobacteria?

The following general hypotheses were put forward:

- H_{o1} . Acclimation to irradiance, by changes in pigment content, are (not) directly observable in the fluorescence characteristics of picocyanobacteria.
- H_{o2} . The effects of nitrogen limitation on pigment content are (not) observable in the fluorescence characteristics of picocyanobacteria.

MATERIALS AND METHODS

2.1 Materials.

2.1.1 Picocyanobacteria Cultures.

A total of seven picocyanobacteria strains were used; four marine and three freshwater isolates representing the main pigment groups (Table 2). Marine *Synechococcus* strains WH7803, WH8018, WH5701 (SYN), and 48B66 were obtained from the Provasoli and Guillard Center for Marine Culture Collection, Bigelow, Maine (Anderson et al. 1991). Strains WH8018, WH7803, and 48B66, contain phycoerythrobilin. Both WH7803 and 48B66 contain phycourobilin along with phycoerythrobilin chromophores as the main accessory phycobilins. Freshwater *Synechococcus leopoliensis* (LB 2434) and *Synechocystis* sp. (LB 2470), were obtained from UTEX (University of Texas Culture Collection, Star and Zeikus 1987). The phycocyanobilin strains are represented by WH5701, *S. leopoliensis*, and *Synechocystis* sp.

2.1.2 Media Preparation.

Marine strains were grown and maintained in defined SNTR media which refers to an nutrient enriched seawater medium buffered by tris buffer (Anderson et al. 1991). This media was supplemented with vitamins consisting of B₁₂ (1 μ/l), Thiamine (2 μg/l), Biotin (1 μg/l), Folic acid (2μg/l), *p*-amino benzoic acid (10μg/l), Niacin (0.1mg/l), Inositol (1mg/l), Ca pantothenate (0.2 mg/l), Pyridoxine (0.1mg/l) [Guillard, Bigelow, Maine pers. commun.]. Seawater was obtained from the Sargasso Sea through the Woods Hole Marine Laboratory, Woods Hole,

TABLE 2. Picocyanobacteria Strain Description.

Isolate	Othername	Pigment type	Origin
48B66		Type I	coastal
WH7803	DC2	Type I	coastal
WH8018	L1604	Type II	coastal
WH5701	SYN	Type III	coastal
<i>Synechococcus leopoliensis</i>	<i>Anacystis nidulans</i>	Type II	freshwater
<i>Synechocystis</i> spp.		Type III	freshwater

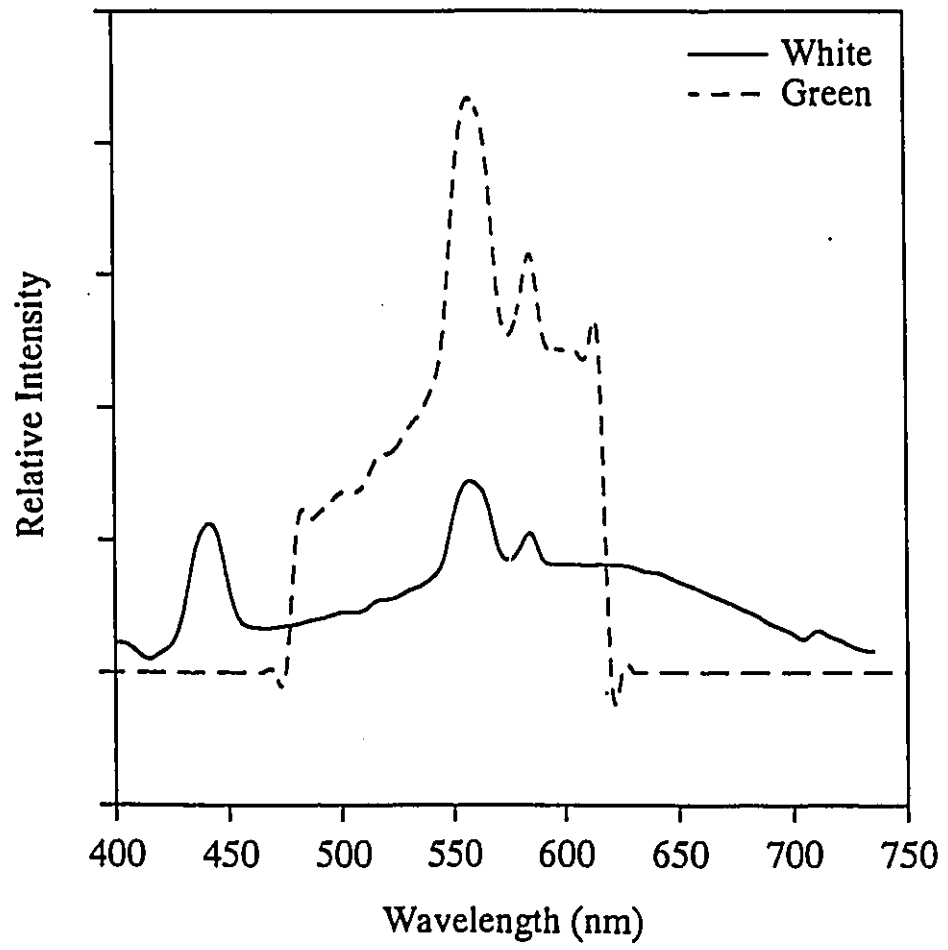
Massachusetts. Seawater was filtered through GF/F filters, autoclaved separately, and cooled rapidly to avoid crystallization. Individual nutrients were also autoclaved separately and added aseptically. SNTR media consists of 8 μM of NaNO_3 , 0.1 μM $\text{H}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 7 nM MnCl_2 , 0.7 nM ZnSO_4 , 0.08 nM CoCl_2 , 1 nM Na_2MoO_4 , 0.06 nM NH_4Cl , 30 nM FeNH_3 citrate, and 2 mM Tris buffer. The freshwater strains were grown in 50% BG-11 (Rippka et al. 1981).

2.2 Culture Methods.

2.2.1 Irradiance Experiments.

Cultures were grown in 500 mL or 2 L Pyrex erlenmeyer flasks in a temperature controlled incubator (Conviron S10h) at 20°C under continuous illumination. Cultures were grown in 3 different light regimes: high ($400 \mu\text{Em}^{-2}\text{s}^{-1}$) and low ($28 \mu\text{Em}^{-2}\text{s}^{-1}$) full spectrum white light provided by solar simulating Vita^R-lights, and a low ($28 \mu\text{Em}^{-2}\text{s}^{-1}$) green light. Green light (peak transmittance at 560 nm) was produced by filtering Vita^R - lights through Moss Green #89 Lee Colortran International filter (Fig.2). At this irradiance, this filter simulates very closely to the underwater light regime typically found at depth in oligotrophic lakes (Kirk 1983, Pick and Cuhel 1986). A five sided box constructed with this filter was placed over the culture flasks. The culture flasks and filter box were kept on a platform that was gently rotated. Photon flux density was measured with a Licor 4-185B quantum/radiometer/photometer equipped with a 4π (SDH-Quantum) sensor. Irradiance was adjusted to equal the prescribed intensity at the top of the culture flasks. The green photon flux density was made equal to the low white irradiance

Figure 2. Spectral distribution of growth irradiance from the two light quality regimes. Vita-lites^R (white) and a green filter (Moss green #89, Lee Colortran Int.) were chosen to simulate light quality at the top and bottom respectively of the euphotic zone in an oligotrophic lake.



by decreasing the distance between the light source and the filter box.

Cultures were grown at each spectral quality in batch culture for 1 week prior to the start of each experiment (representing 5-10 generations). Following this acclimation, growth rates were measured in 2 to 4 replicate batch cultures. Growth was measured by changes in optical density (O.D) at 750 nm using a Pye-Unicam SP-100 UV-spectrophotometer every 4 hours (Wyman and Fay 1986a, b). This O.D was chosen because it represents the absorbance minimum. Growth was calculated as the rate of change during the exponential phase of growth (ln units d^{-1} ; Stein 1973).

Fresh cultures, of all strains, were then maintained in semicontinuous growth at an O.D. of approximately 0.05 (close to 10^9 cells mL^{-1}) such that the growth was maintained between 75 and 80 % of the maximum rate for that growth irradiance. At this O.D. self-shading of cultures was minimal. After 5 to 10 generation times, subsamples of the cultures were then harvested for *in vivo* fluorescence spectroscopy, pigment, carbon and nitrogen analyses and cell abundance.

2.2.2 Nitrogen Limitation Experiments.

Nitrogen limitation experiments were proposed for two specific reasons. Firstly a significant proportion of cell nitrogen in cyanobacteria is tied up in the photosynthetic apparatus, specifically in the phycobiliproteins. Secondly, nitrogen is frequently limiting in marine and freshwater systems.

Batch cultures were grown up in low nitrogen media so that the stationary

phase was brought on by nitrogen limitation. Three consecutive such batch cultures were grown to ensure nitrogen limitation prior to the experimental run. The nitrogen concentration was 0.5% of the total nitrogen in 100% BG-11 and corresponded to a NaNO_3 content of $170 \mu\text{M}$ for the all strains except WH8018 which was grown in $265 \mu\text{M}$. Under these conditions the maximum O.D obtained was always less than 0.075 so as not to induce light limitation. The same parameters that were measured during the irradiance experiments were collected at three time periods. The first sampling period was in mid-exponential growth at an optical density of 0.040 to 0.050, the second at 24 h after the onset of stationary phase (nitrogen limitation) and again at 72 h after the onset of stationary phase. All strains were grown in nitrogen depleted batch cultures at low white irradiance ($28 \text{ m}^2\text{s}^{-1}$). The effect of light intensity ($400 \mu\text{E m}^2\text{s}^{-1}$ white irradiance) and nitrogen limitation was further examined in PE strains, WH7803 and WH8018.

Fluorescence analysis was not available during the initial work on nitrogen limitation with PC Strains. In this case cultures were re-grown for fluorescence analysis and found to have the same growth rate as in previous experiments.

2.3 Cell Counts, Pigment, Carbon and Nitrogen Analyses.

Cells were preserved with 10% glutaraldehyde buffered with Na-cacodylate (Caron et al. 1985) and enumerated with a Jena-Zeiss epifluorescence microscope at X 1250. For each enumeration, 1000 cells were counted in 20-25 random fields under either a broad blue excitation (450-490 nm, LP 510 nm) for PE-rich strains, or under green excitation (510-560 nm, LP 590 nm), for PC-rich strains (Pick

1991).

The DMSO-acetone method of Burnison (1980) was used to extract chlorophyll *a* from a 20 mL aliquot of culture filtered onto a Whatman GF/F glass fiber filter. The content of Chl *a* was calculated as:

$$\mu\text{g Chl } a \text{ L}^{-1} = 11.85 \cdot A_{664} - 1.54 \cdot A_{647} - 0.08 \cdot A_{630},$$

(Jeffrey and Humphrey 1975, and Burnison 1980).

A_x refers to the O.D. at that wavelength.

For extraction and assay of phycobiliproteins, an aliquot of 40 to 250 mL (irradiance experiments) or 500 mL (nitrogen experiments) of culture was centrifuged at 27,000 X *g* for 30 min. The supernatant was removed and the pellets were kept at approximately - 20°C until analysis. The pellet was then resuspended to 5 mL in 0.1 M sodium phosphate buffer, pH 7.0. Because increased irradiance or nitrogen limitation severely reduced cellular PBP, two of the replicates had their pellets combined. An aliquot of 0.5 mL was removed from the pellet suspension for cell counts. The suspension for pigment analysis was then passed three times through a French pressure cell at 20,000 psi. After each passage, the cells were frozen in liquid nitrogen (Kursar and Alberte 1983). This procedure resulted in the disruption of over 95% of the cells. The suspension was then centrifuged at 100,000 X *g* for 60 min to remove cellular debris (Tandeau de Marsac 1977). Phycobiliprotein absorption spectra were obtained with a Cary 2200 spectrophotometer for all strains (Fig. 3). Phycobiliprotein content for phycourobilin containing strains, WH7803 and 48B66, were estimated according

to the equations: $PC \mu g L^{-1} = 169A_{620} - 108A_{650}$, and

$$PE \mu g L^{-1} = 169A_{498} - 8.64A_{615} - 1.76A_{650},$$

(Kusar and Alberte 1983, Alberte et al. 1984). Using this method PE could not be detected below $6 \mu g mL^{-1}$. Phycobiliprotein content for WH8018 and PC-rich strains were estimated using the equations:

$$PC mg L^{-1} = A_{620} - 0.7A_{615} / 7.88,$$

$$APC mg L^{-1} = A_{650} - 0.19A_{620} / 5.65, \text{ and}$$

$$PE mg L^{-1} = A_{565} - (2.8PC) - (1.34APC) / 12.7$$

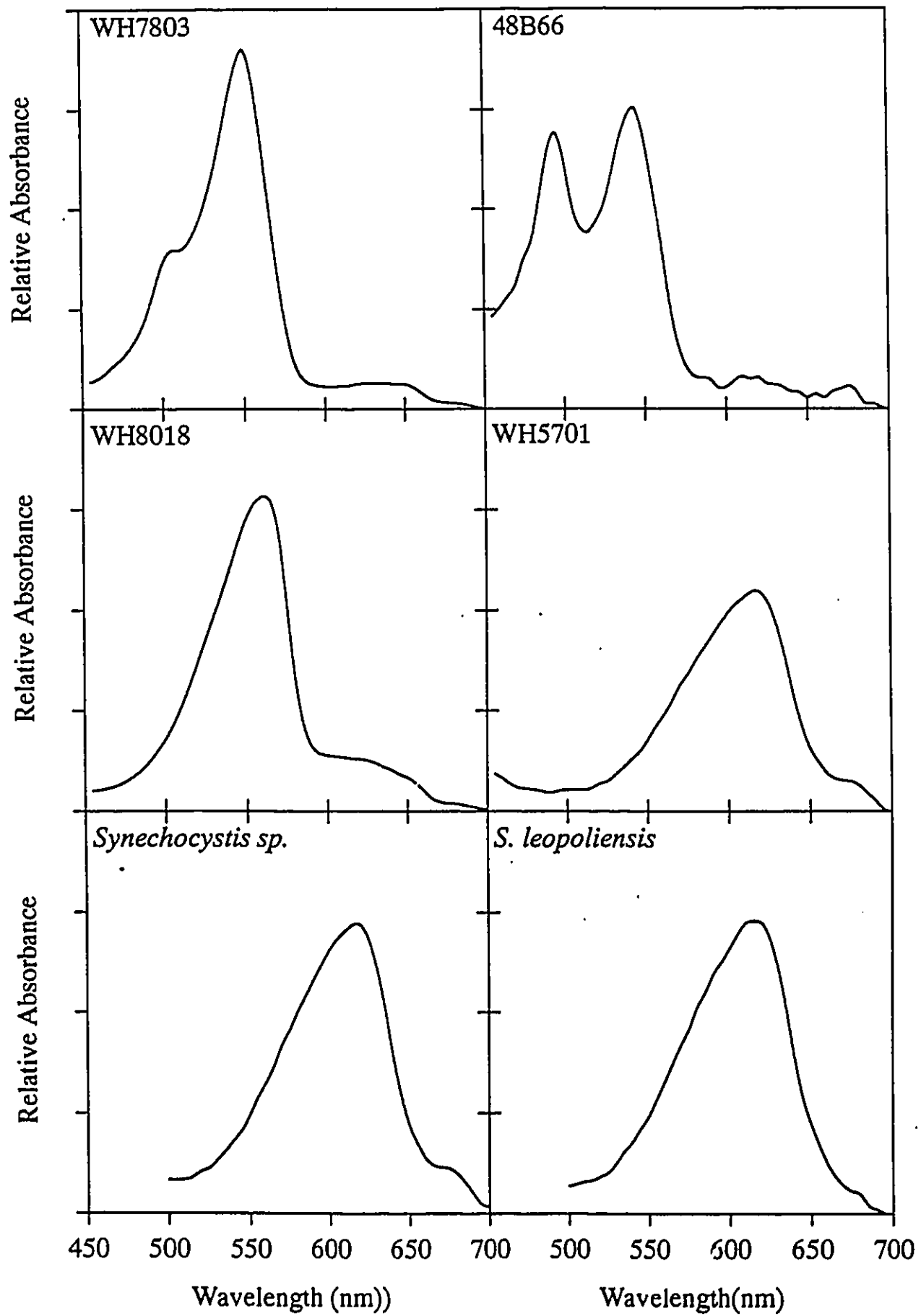
(Tandeau de Marsac 1977). Using this method PE could not be detected below $1 \mu g mL^{-1}$.

Particulate carbon and nitrogen determinations were obtained from a 10 mL aliquot of sample filtered through pre-ignited Whatman GF/F filters. The filters were then stored frozen until analysis with a Hewlett-Packard CHN analyzer. Analysis was courtesy of Dr. David Lean, Canada Center for Inland Waters, Burlington, ON.

2.4 Fluorescence Spectroscopy.

A 10 mL aliquot of the live culture was placed in glass vials in the dark for 20 min at room temperature until analysis. The sample was handled in dim light while transferring to a 1 cm quartz cuvette and then placed in the fluorometer. All cuvetts were matched (Fisher Brand - Gold Type). The fluorometer was a Perkin Elmer, model LS 50 Luminescence scanning spectrometer. Excitation light was a Xenon flash tube, which produces an intense, short duration pulse of radiation. The excitation beam is incident 90° from the emission beam. The emission from

Figure 3. Absorption spectra of extracted PBP of the experimental strains of picocyanobacteria illustrating the different PBP types. WH7803 and 48B66 are a Type I and contain phycourobilin and phycoerythrobilin, WH8018 is a Type II and contains phycoerythrobilin. WH5701, *S.leopoliensis*, and *Synechocystis* are Type III and contain only phycocyanobilin.



the xenon source has a width at half peak height of less than 10 microseconds, the integrators are opened for 80 microseconds to collect all the signal. The majority of the excitation beam is transmitted to the sample area via the focusing mirror, a small proportion is reflected by the beam splitter onto the reference photomultiplier. The Perkin Elmer has an internal Rhodamine dye calibration, which operates to correct for both the spectral response of the main photomultiplier tube and the transmission response of the beam splitter. When the instrument is operating two integrations for both emission and excitation signals are collected for every data collection cycle.

Excitation spectra for Chl *a* (680 nm emission) were obtained using scanning grating monochromators with excitation and emission bandwidths of 10 nm. Data collection occurred every 0.5 nm at a speed of 250 nm min⁻¹, thus duration of the full excitation was approximately a minute.

Two emission spectra were obtained, for PE strains, by excitation through scanning monochromators set at a violet (420 nm) or a blue (470 nm), respectively. Fluorescence emission data were collected every 0.5 nm with a band width of 10 nm. Similarly two emission spectra were obtained for PC strains, by excitation using 420 nm or, green, 545 nm wavelengths. Both the excitation and emission spectra were normalized by dividing the fluorescence intensity at each wavelength by the mean intensity of the spectrum. This normalization was utilized so that no specific emphasis was placed on any one spectral component (Mitchell and Kiefer, 1988). Normalized replicate spectra were then averaged at each data

point to yield the final mean normalized spectrum.

Corrections for background fluorescence (non cellular bound pigments in culture or background Raman scatter of light) were made by subtracting the spectra obtained with 0.2 μm filtered culture media. A check on the linearity of the fluorometer was performed using the marine PE strain (WH8018) grown semicontinuously in green light. The harvested culture had an O.D. of 0.05. WH8018 emission peaks of PE (575 nm) and Chl *a* (682 nm) were linearly related to dilution. Pigment concentrations were not found to be self quenching for emission.

2.5 Effects of Excitation Wavelengths on Fluorescence Emission Intensity.

The next two sections detail preliminary experiments were necessary to assess the protocol used in the fluorescence measurements on natural populations of picocyanobacteria.

For a number of reasons, sometimes only one excitation wavelength is available to a researcher; as is the case for Turner field fluorometers and flow cytometers. In our case only 2 filter sets (420 nm and 470 nm excitation) were available during examination of natural populations by the microspectrophotometry (see below). Certainly changes in excitation wavelengths will change emission peak wavelengths based purely on the pigment of maximum excitation. This affects the photomultiplier tube sensitivity setting as it is based on the maximum emission signal. For instance, the photomultiplier setting for PE emission from 470 nm excitation would not be suitable for the dominant Chl *a* emission using 420 nm ex.

Thus the microspectrophotometry setup can be a long and tedious procedure, particularly because of vibrations during filter changes (Campbell and Iturriaga 1988). If only one wavelength is to be chosen it must maximize excitation sensitivity for both pigments. Thus to look at the effects of environmental conditions on fluorescence from these two photosynthetic pigments one excitation source is necessary. This experiment was conducted to examine the sensitivity of the excitation wavelengths in producing phycoerythrin and Chl *a* emission.

Samples of cultures of WH8018, harvested from the green irradiance experiments, were subjected to the usual emission setup (see above) except that excitation wavelengths were varied from 420 nm to 540 in 20 nm increments.

While the excitation wavelength of 470 nm was not ideal for PE excitation, it did produce detectable signals for both PE and Chl *a* (Appendix IIB); which were adequately above the Raman scatter peak of water, 560 nm, at this excitation wavelength (Appendix IIA). This was not the case for the 420 nm excitation wavelength. In this case, variations about a low PE emission peak could be masked by the large raman scatter; effectively interfering with the PE signal.

2.6 Effects of Preservation on Fluorescence.

Often because of the distance between the sampling site and the laboratory a preservation technique may be necessary. For this reason a preservation technique was explored using the same method as that for cell counts. An aliquot of WH8018, from the low green light harvest was preserved in 0.5% glutaraldehyde overnight at 4°C. Background fluorescence was corrected by

subtraction of the preserved filtrate. Fluorescence spectra were compared to fresh samples from the same harvest.

Preservation did markedly change the fluorescence characteristics of WH8018 (Appendix III). Predominant effects were that the contribution of PE to Chl *a* emission was effectively reduced. Increases in the blue Soret band at 437 nm and the PC peak at 625 nm were also evident. Similarly, emission spectra showed enhanced PE emission relative to Chl *a*. Preservation appeared to effectively uncouple PE from the energy flow to Chl *a*.

2.7 Field Methods.

2.7.1 Site Description.

Jack's Lake, Ontario (Lat. 44°41' Long.78°04') was chosen to study the fluorescence properties of natural populations of picocyanobacteria, because its plankton is dominated in summer by a large population of PE-containing picocyanobacteria ($1-5 \times 10^5 \text{ mL}^{-1}$) (Pick and Agbeti 1991). Water samples were collected at the deepest station (44 m) in Sharpe's Bay. In this bay, summer stratification begins in early June and by the end of July the epilimnion is 7 m thick. The depth of the 1 % light level at this time is 10 m (Pick and Agbeti 1991). The spring total phosphorus content ranges from 12.5 to 18 $\mu\text{g l}^{-1}$ and the total nitrogen to phosphorus ratio is approximately 29. In summer the inorganic nitrate and ammonium content reach the detection limits of 10 $\mu\text{g.l}^{-1}$ and 5 $\mu\text{g L}^{-1}$ (Environment Canada 1979), respectively, from the surface down to 12 m. The C/N ratio of the plankton increases progressively through the summer in the

surface waters (6-15) (Pick et al. 1984) and indicates nitrogen deficiency(Healey 1975. C/N ratios are lower at 12 m and below.

2.7.2 Sampling Protocol.

Sampling was performed during early and late stratification of 1990 on July 31 and August 14, respectively, and during fall mixing on October 8. To study the effects of growth irradiance on single cell fluorescence, the two dominant morphotypes of picocyanobacteria (a *Synechococcus* sp. and a *Synechocystis* sp.) were compared between the epilimnion (2 and 4 m), and the metalimnion (10 m) on the 3 sample dates. The depth distribution of cellular fluorescence was examined in more detail on August 14 and October 8. On these dates samples were collected every 2 m to below the euphotic zone (12 m). On August 14, the effects of nitrogen were examined on surface (2 m) and metalimnetic populations (10 m), by comparing a nitrogen enriched sample (100 μM NH_4NO_3) with a non enriched sample.

All samples were subjected to the same procedure. The samples were collected at dusk using a 2.5 L Van Dorn bottle and maintained in large carboys (12 L) in the dark at *in situ* temperatures to mimic the day-night cycle. Measurements of temperature with depth were taken with a Yellow Springs Oxygen Temperature Probe (Model # 54). Incident irradiance (both global and photosynthetically available radiation (400-700 nm, PAR) was continuously recorded on site every half hour with an Eppley Pyrheliometer. Slide preparations of the samples took place the next morning and slides were kept in the dark and

examined for fluorescence within 2 hours of filtration. For enumeration, whole lake water aliquots were preserved with 1% glutaraldehyde in 0.1 M sodium cacodylate and counted at 1250 X with a Jena Zeiss epifluorescence microscope using blue excitation (490-510 nm band width) and green excitation (520-570 nm).

2.7.3 Microspectrofluorometry.

Preliminary experiments indicate that glutaraldehyde significantly alters the wavelength of peak excitation and reduce the Chl *a* signal (Appendix III). Hence fluorescence determinations were performed on unpreserved samples. Cells were obtained by filtering under low pressure (75 mm Hg) onto 0.2 μ m unstained Nuclepore filters and kept in the dark until analysis. Individual slides from different depths were randomly selected for the measurements. At least 3 randomly selected fields of each of the morphologically distinct groups of picocyanobacteria were scanned for fluorescence. The measuring spot was restricted to 4 μ m in diameter and each field contained 4 to 6 cells.

The microspectrofluorometer used was a Zeiss microscope (UMSP80) with epi-illumination through a 100 X Neofluor lens for a total magnification of 1600 X. Epi-illumination was chosen to minimize scattering and absorption difference that might occur between different cell morphotypes. The excitation source was a 75 W Xenon arc lamp. The modular system was driven by a Hewlett-Packard microcomputer using the Lambda Scan software package. The red sensitive photomultiplier tube was set to high voltage using bright deep-dwelling cells.

Excitation spectra for Chl *a* (680 nm emission) were obtained using scanning

grating monochromators as measured at 5 nm intervals from 400 to 650 nm with excitation and emission bandwidth of 5 and 20 nm, respectively. Each spectrum obtained per cell was an average of 5 readings per interval. Spectra were uncorrected with respect to the spectral differences in excitation. Lamp output was monitored as zero order scans and did not change significantly over 30 hrs of use. The duration of excitation was approximately one minute.

Preliminary experiment indicated that the wavelength of excitation does not significantly alter the emission ratio (Appendix II). Using violet (420 nm) or blue excitation (470 nm) on WH8018, grown in green irradiance, ranged from 0.45 to 0.55. The emission spectra for natural populations were obtained using blue excitation a BP 450-490, (full width half maximum 460-480 nm) FT 510, LP 515 nm filter. This wavelength appeared to adequately sensitize both PE and Chl *a*. Fluorescence emission was scanned every 5 nm with a band width of 10 nm.

All fluorescence spectra were corrected for background fluorescence by subtracting spectra obtained from adjacent sections of the supporting filter.

As a check on this instrument, microspectrophotometer spectra of the cultured picocyanobacteria cells were obtained and compared to those spectra obtained with bulk samples using the Perkin Elmer. Both excitation spectra and emission spectra were similar in shape.

2.8 Statistical Analysis of Data.

Statistically significant differences ($p < 0.05$) in the response parameters (growth rates, pigments, carbon, nitrogen, and fluorescence) to irradiance were

analyzed using a one way general linear model (GLM) as determined using Tukey's pairwise comparisons (SAS 1988). Normality and homoscedasticity of the data were examined using the absolute value of the data residuals. SAS provides a test of normality, while homoscedasticity was examined by performing an ANOVA on the residuals. Data found to be heteroscedastic were power transformed and retested for both normality and homoscedasticity. Similarly emission ratios obtained from natural populations were also compared using a two way GLM (depth and species). A multiple analysis of variance (MANOVA) using GLM was performed to examine the correlation between all the response variables to each other during exponential growth in the different light regimes. The nitrogen limitation data were inherently dependent because of repeated sampling over time from a batch culture. Thus their statistical analysis was by GLM using a repeated measure analysis (RMA).

RESULTS

3.1 Photoacclimation Responses: Growth Rates, Carbon, Nitrogen and Pigment Content.

3.1.1 WH7803 - High vs Low White Irradiance.

Table 3 shows changes in growth rate, carbon and nitrogen content for WH7803 among the 3 growth irradiances. Increasing the growth irradiance did not increase significantly the growth rate for strain WH7803. Carbon per cell, and to a lesser extent nitrogen per cell, were elevated during growth in low white growth irradiance. Increasing growth irradiance decreased pigment content (Table 4). Chl *a*/cell was reduced by 13 fold during growth at high growth irradiance but this change was not as pronounced when Chl *a* was expressed on a per carbon basis (3 fold). PE/Cell was reduced approximately 2.6 times in high growth irradiance, while PE/C was only 1.5 times less in high growth irradiance.

3.1.2 WH7803 - Low White vs Green Irradiance.

Growth rate was only 14% lower in green growth irradiance when compared to low white growth irradiance (Table 3). Carbon and nitrogen content per cell were not significantly different between low white and low green growth irradiance.

Pigment differences between high white and low green growth irradiances involved changes in both Chl *a* and PE. Chlorophyll *a* content on a per cell basis decreased by 20 fold while PE content decreased by 5.5 fold in high white relative to green growth irradiance. Reductions in Chl *a* and PE on a per carbon basis were

less pronounced (Chl *a*/C 5 fold, PE/C 4 fold). In general, growth in low green growth irradiance increased both Chl *a* and PE content the most when compared to high and low white growth irradiance (Table 4).

3.1.3 WH8018 - High vs Low White Growth irradiance.

Table 5 shows changes in growth rate, carbon and nitrogen content for WH8018 among the three growth irradiances. WH8018 achieved its highest growth rate at $400 \mu\text{Em}^{-2}\text{s}^{-1}$. Like WH7803, nitrogen and carbon content per cell were significantly reduced during growth at high white irradiance. As expected, pigments were also reduced at high white growth irradiance but the magnitude of the reduction depended on how the pigments were normalized (Table 6). Both PE and Chl *a* were 4 to 5 fold lower during growth in high white irradiance when expressed on a per cell basis. When expressed per carbon (w/w), the reduction in Chl *a* was not as pronounced (only a 2 fold) while the reduction in PE was similar.

3.1.4 WH8018 - Low White vs Green Growth irradiance.

Growth was not significantly different in green irradiance as compared to growth in the same intensity of white irradiance (Table 5). However, both carbon and nitrogen content per cell were greater during growth in white irradiance. Unlike WH7803, PE content per cell was greater in white as compared to the same intensity of green growth irradiance (Table 6). This was also true of PE/C content. Carbon and nitrogen were not significantly different during growth in green irradiance as compared to a more intense white irradiance. Similarly PE content per cell did not significantly differ between low green and the more intense white

TABLE 3. Growth rate (In units d^{-1}), and cellular carbon and nitrogen content (fg/Cell) of WH7803 during growth in $400 \mu E m^{-2}s^{-1}$ white (HW), $28 \mu E m^{-2}s^{-1}$ white (LW), and $28 \mu E m^{-2}s^{-1}$ green (LG) irradiance. (± 1 std. dev.). Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukeys pairwise comparison. ($n = 4$, $p < 0.05$).

	Growth	C/Cell	N/Cell	C/N
HW	0.70(0.06)a	120(20)a	35(30)a	6.2(0.6)a
LW	0.75(0.28)a	440(110)b	80(25)b	5.4(0.7)a
LG	0.65(0.28)a	490(40)b	75(5)ab	6.2(0.1)a

TABLE 4. Pigment content per cell (fg/Cell) and per carbon (mg/g) of WH7803 during growth in $400 \mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), $28 \mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and $28 \mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance. (± 1 std.dev.). Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukeys pair wise comparison. ($n=4$, $p \leq 0.05$). Missing std. dev. indicates $n=1$.

	Chl <i>a</i> /Cell	Chl <i>a</i> /C	PE/Cell	PE/C
HW	0.3(0.1)a	2.5(1.2)a	4.0(3.3)a	12
LW	3.7(1.5)b	8.3(1.0)b	10.5	18(5)a
LG	6.0(1.4)c	12.3(1.0)c	23(3.2)b	47(7)b

TABLE 5. Growth rates (In units d^{-1}), and cellular carbon and nitrogen content (fg/Cell) of WH8018 during exponential growth in $400 \mu E m^{-2}s^{-1}$ white (HW), $28 \mu E m^{-2}s^{-1}$ white (LW), and $28 \mu E m^{-2}s^{-1}$ green (LG) growth irradiance. (± 1 std.dev.). Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukeys pairwise comparison. ($n=4$, $p \leq 0.05$).

	Growth	C/Cell	N/Cell	C/N
HW	0.56(0.02)b	380(203)a	56(35)ab	6.4(0.4)a
LW	0.48(0.02)a	700(240)b	120(48)ab	6.6(0.5)a
LG	0.35(0.01)a	340(25)a	55(3)a	6.2(0.4)a

TABLE 6. Pigment content per cell (fg/Cell) and per carbon (mg/g) of WH8018 during exponential growth in $400 \mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), $28 \mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and $28 \mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance. (± 1 std.dev.). Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukey's pairwise comparison. ($n=4$, $p \leq 0.05$).

	Chl <i>a</i> /Cell	Chl <i>a</i> /C	PE/Cell	PE/C
HW	1.1(0.4)a	2.6(1.2)a	2.5(0.2)a	3.4(0.8)a
LW	4.8(0.6)b	5.0(0.5)b	13.2(9.6)b	24.0(13.0)a
LG	6.4(1.6)b	18.6(3.9)c	2.8(0.7)a	8.0(2.0)c

growth irradiance. In general, growth in low green irradiance produced greater Chl *a* but lower PE content when compared to that present during growth at low white irradiance.

3.2 Photoacclimation Responses: Excitation Spectra of Chl *a*.

3.2.1 WH7803.

Excitation spectra of Chl *a* (680 nm) were obtained at each growth irradiance. Figure 4 shows examples of uncorrected spectra from replicate cultures grown in green light. Differences in the peak heights are due to differences in cell abundance. In order to compare spectral "shapes", without emphasis on any one peak, the spectra were normalized to the mean fluorescence intensity.

Figure 5 shows the changes in WH7803 excitation spectra under the 3 growth irradiances studied. In addition to the PE peak at 543 nm, 3 other peaks contributed to Chl *a* excitation. These were the blue Soret band of Chl *a* at 437 nm, and a PU peak at 493 nm, and a PC peak at 633 nm (summarized in Appendix IA). These three latter peaks were quite distinct during growth conditions which reduced the PE component; ie at $400 \mu\text{Em}^{-2}\text{s}^{-1}$.

These peaks can be simply compared among the growth irradiances as the ratios of one another. The PE/PC and PE/Chl *a* excitation ratios and are given in Table 7. WH7803's pigment ratios followed closely the excitation ratio changes in the three growth irradiances. Both excitation ratios were significantly reduced during growth at high white growth irradiance (Table 7). Growth in low white and green growth irradiance gave similar, high ratios. Differences occurred between

Figure 4. Replicate (n = 3) excitation spectra for Chl *a* emission at 680 nm of WH7803. Semi-continuous cultures in green growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$).

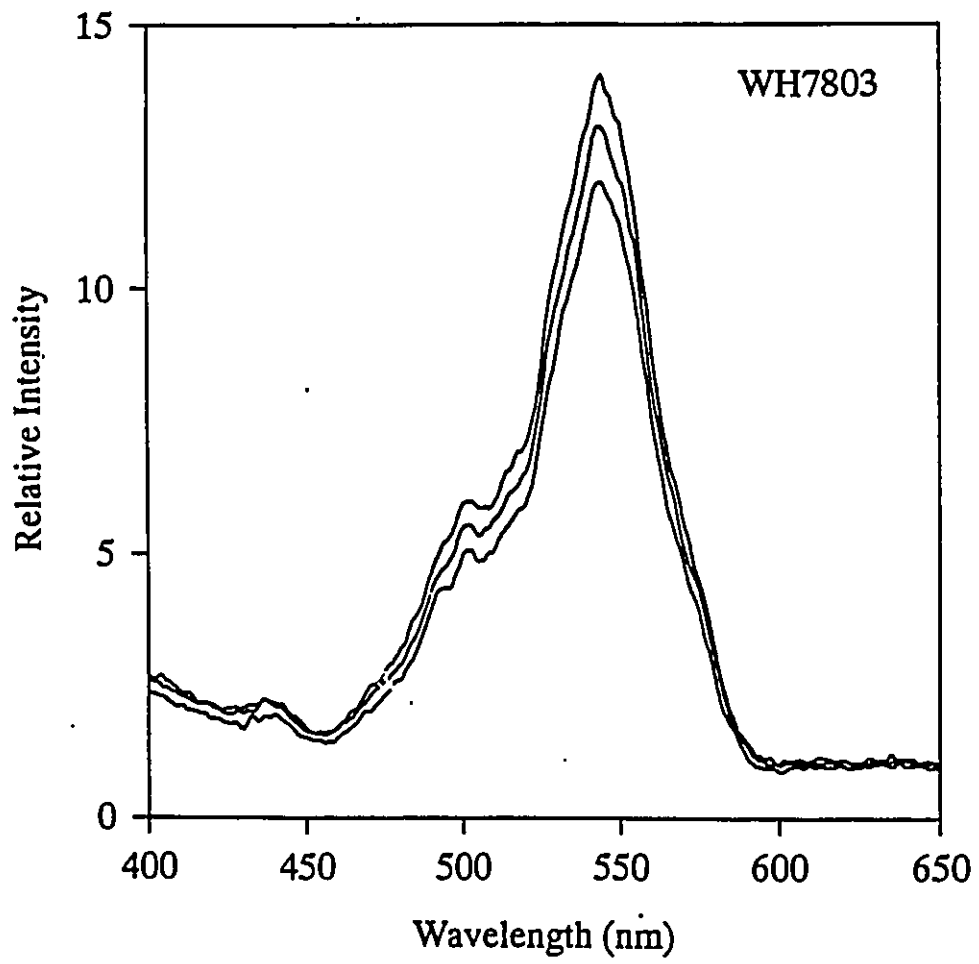
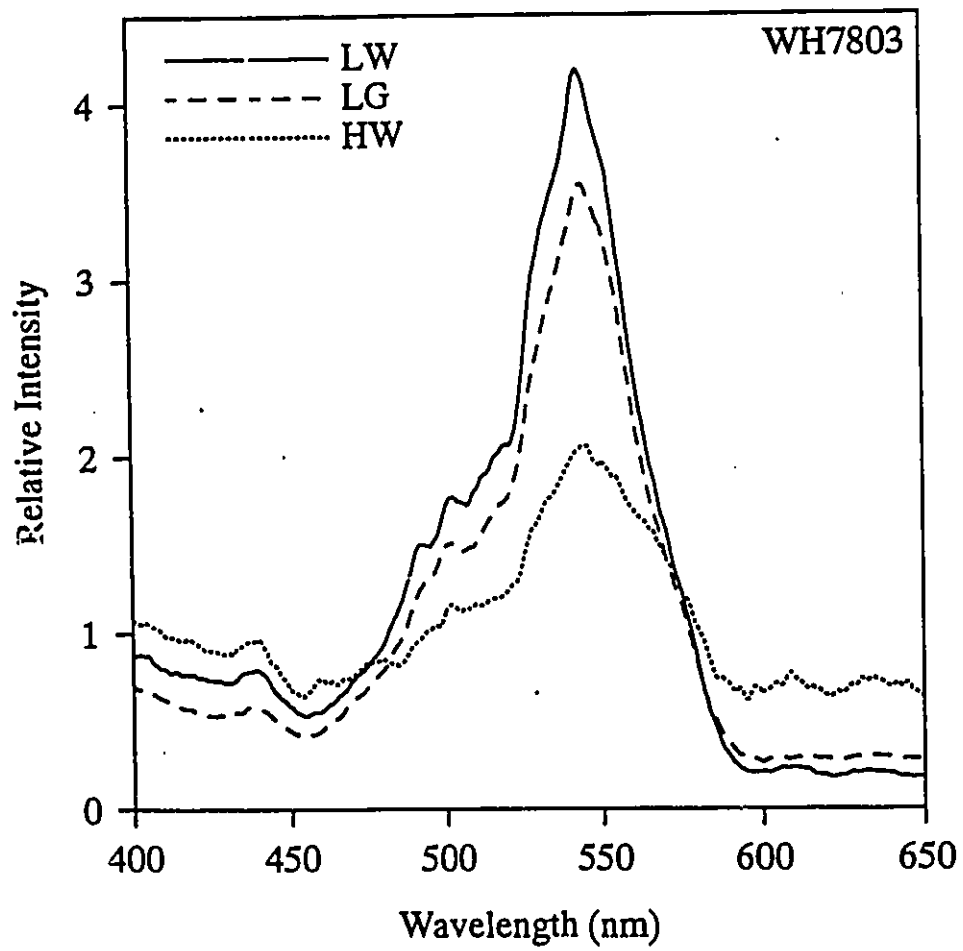


Figure 5. Effects of high white (HW), $400 \mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized excitation spectra for Chl *a* emission at 680 nm, of exponentially growing WH7803. Each line is the average of 4 cultures.



these two irradiances in that growth in green irradiance produced the greatest PE/Chl *a* ratio while growth in low white irradiance produced the greatest PE/PC excitation ratio.

3.2.2 WH8018

Figure 6 shows examples of uncorrected spectra from replicate cultures grown in green light. Figure 7 shows changes in WH8018 excitation spectra under the 3 growth irradiances studied. In addition to the PE peak at 568 nm, 2 other peaks contributed to Chl *a* excitation. These were the blue Soret band of Chl *a* at 437 nm, and a PC peak at 635 nm (summarized in Appendix IA). The contribution of these 2 latter peaks, like WH7803, become accentuated at high growth irradiance when the PE component is reduced.

WH8018's excitation of Chl *a* by PE in relation to PC closely matched the PE/PC ratios (Table 7). This ratio was significantly reduced in the more intense white and less intense green growth irradiances. This was also the case for the proportion of PE to the blue Soret of Chl *a* peak excitation of Chl *a* (680 nm em).

3.3 Photoacclimation Responses: Emission Spectra.

3.3.1 WH7803.

WH7803 had PE and Chl *a* emission located at 570 and 680 nm, respectively (Fig.8 and Fig.9). Two other peaks, visible as shoulders of the Chl *a* emission, were at 620 and 645 nm (Appendix IB).

For comparative purposes the emission peaks were also summarized as ratios (Table 8). PE/Chl *a* emission ratios can be obtained in 3 ways: by blue

Figure 6. Replicate (n = 4) excitation spectra for Chl *a* emission at 680 nm of WH8018. Semi-continuous cultures in green growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$).

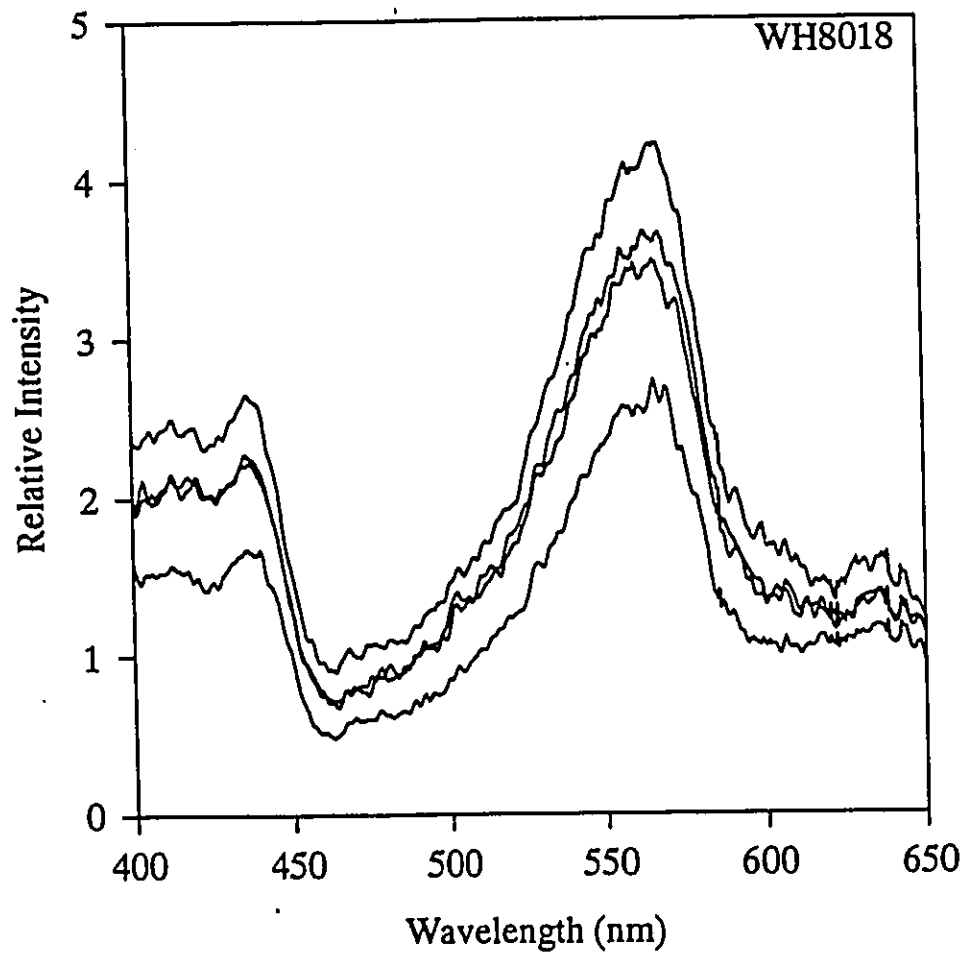


Figure 7. Effects of high white (HW), $400 \mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized excitation spectra for Chl *a* emission at 680 nm, of exponentially growing WH8018. Each line is the average of 4 cultures.

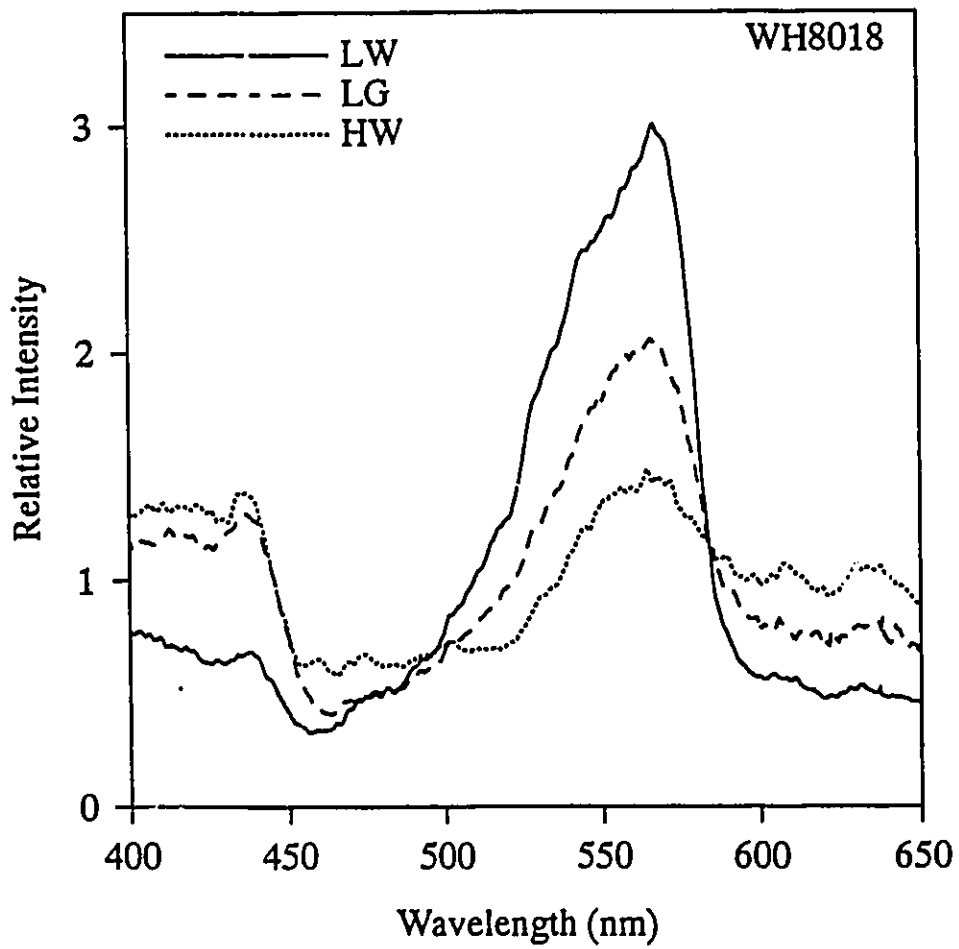


TABLE 7. Pigment ratios (w/w) and peak excitation ratios, F(PE/Chla) and F(PE/PC) for Chl *a* emission at 680 nm, of WH8018 and WH7803 during growth in 400 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ green (LG) growth irradiance. (\pm 1 std.dev.). Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukey's pairwise comparison ($n=4$, $p \leq 0.05$). <D.L.: below the detection limit. Missing std. dev. indicates $n=1$.

	PE/Chl <i>a</i> (w/w)	F(PE/Chla)	PE/PC(w/w)	F(PE/PC)
WH8018				
HW	1.0(0.4)a	1.11(0.1)a	1.4(0.2)a	1.4(0.4)a
LW	3.7(1.2)b	4.07(1.0)b	3.7(1.3)b	6.4(1.7)b
LG	0.5(0.2)c	1.86(0.6)c	2.1(0.5)c	2.5(0.2)c
WH7803				
HW	<D.L.	2.4(0.5)a	4.2	3.8(0.9)a
LW	5.8(0.9)a	5.7(0.4)b	16.7(0.6)a	19.0(2.4)b
LG	4.0(1.0)a	6.4(0.2)b	14.6(0.1)b	12.2(0.3)c

Figure 8. Effects of high white (HW), $400 \mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using blue excitation; 470 nm) of exponentially growing WH7803. Each line is the average of 4 cultures.

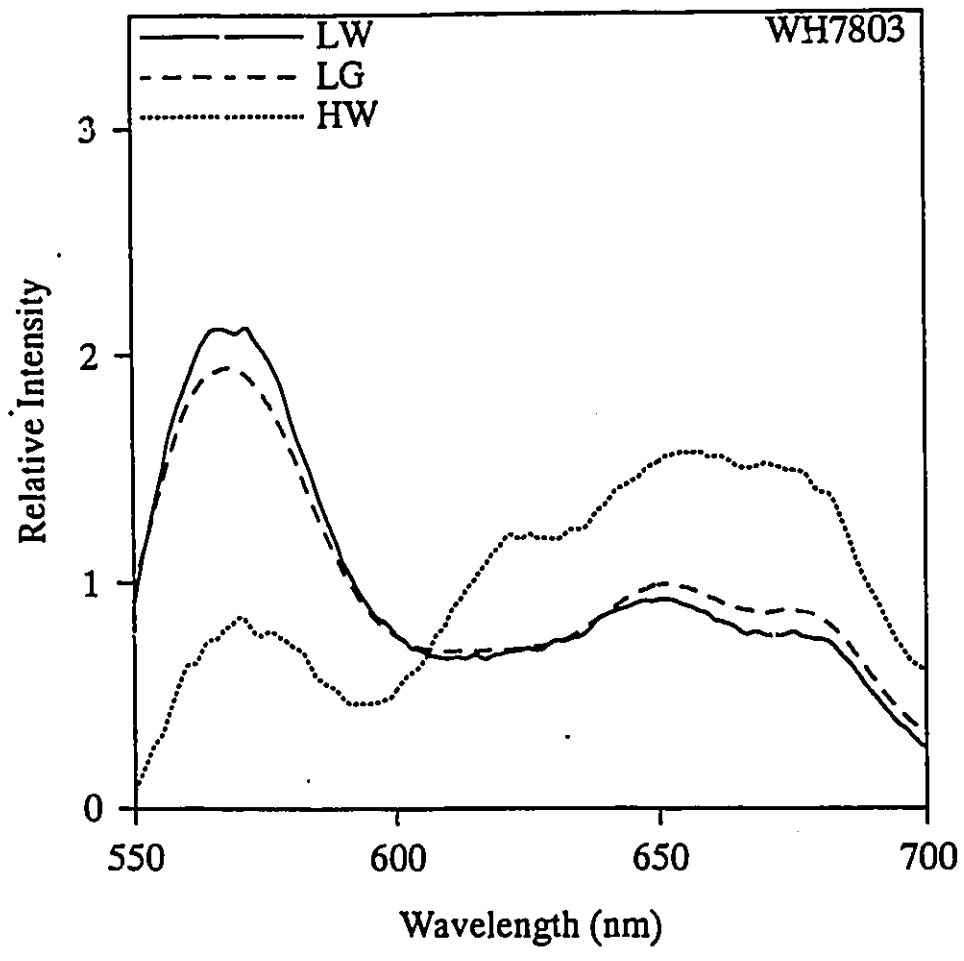
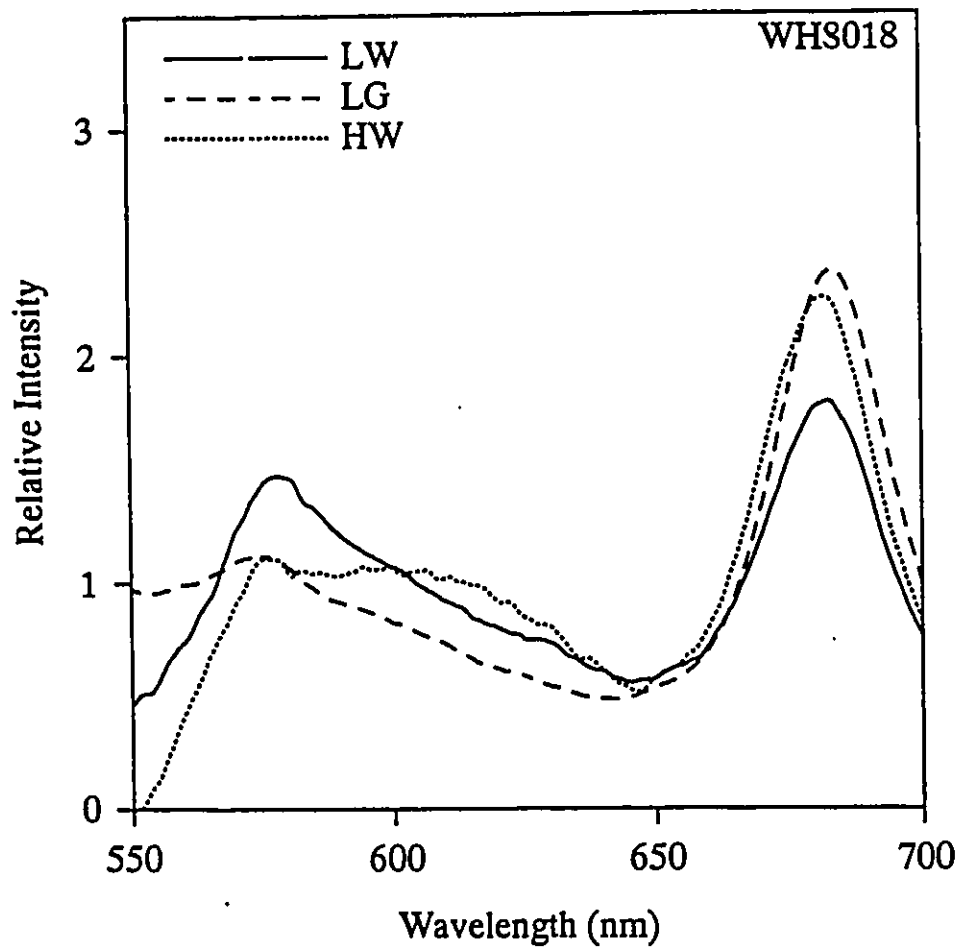


Figure 9. Effects of high white (HW), $400 \mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using violet excitation; 420 nm) of exponentially growing WH7803. Each line is the average of 4 cultures.



excitation at 470 nm, termed $B(PE/Chl a)$, by violet excitation at 420 nm, $V(PE/Chl a)$, or by a composite ratio of the PE emission from the 470 nm excitation divided by the Chl *a* emission from the 420 nm excitation, ($BV(PE/Chl a)$). The latter ratio was used by Barlow and Alberte (1985) in their study of these same strains. The composite ratio was calculated here because it incorporates the maximum fluorescence emission from each pigment.

WH7803 had the highest emission ratios during growth in high white and green growth irradiance if the violet excitation was considered, while the highest $B(PE/Chl a)$ ratio was obtained in low white and green growth irradiance. The composite, $BV(PE/Chl a)$ emission ratio was similar to $B(PE/Chl a)$.

3.3.2 WH8018.

Figure 10 show the changes in WH8018 emission spectra using the blue 470 nm excitation at three growth irradiances. Two main emission peaks were the PE peak at 580 nm and the Chl *a* at 680 nm. Two other peaks, visible as shoulders of the Chl *a* peak emission, were at 625 and 650 nm (summarized in Appendix IB). As with the excitation fluorescence spectra, emission spectra revealed the PC peaks best during growth at high white and/or green growth irradiances.

Figure 11 shows the changes in WH8018 emission spectra using the 420 nm excitation, respectively. Emission ratios for WH8018 are shown in Table 8. The emission ratios, like the excitation and pigment ratios all indicate the greatest proportion of PE relative to the other pigments occurs during growth in low white irradiance.

Figure 10. Effects of high white (HW), $400 \mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using blue excitation; 470 nm) of exponentially growing WH8018. Each line is the average of 4 cultures.

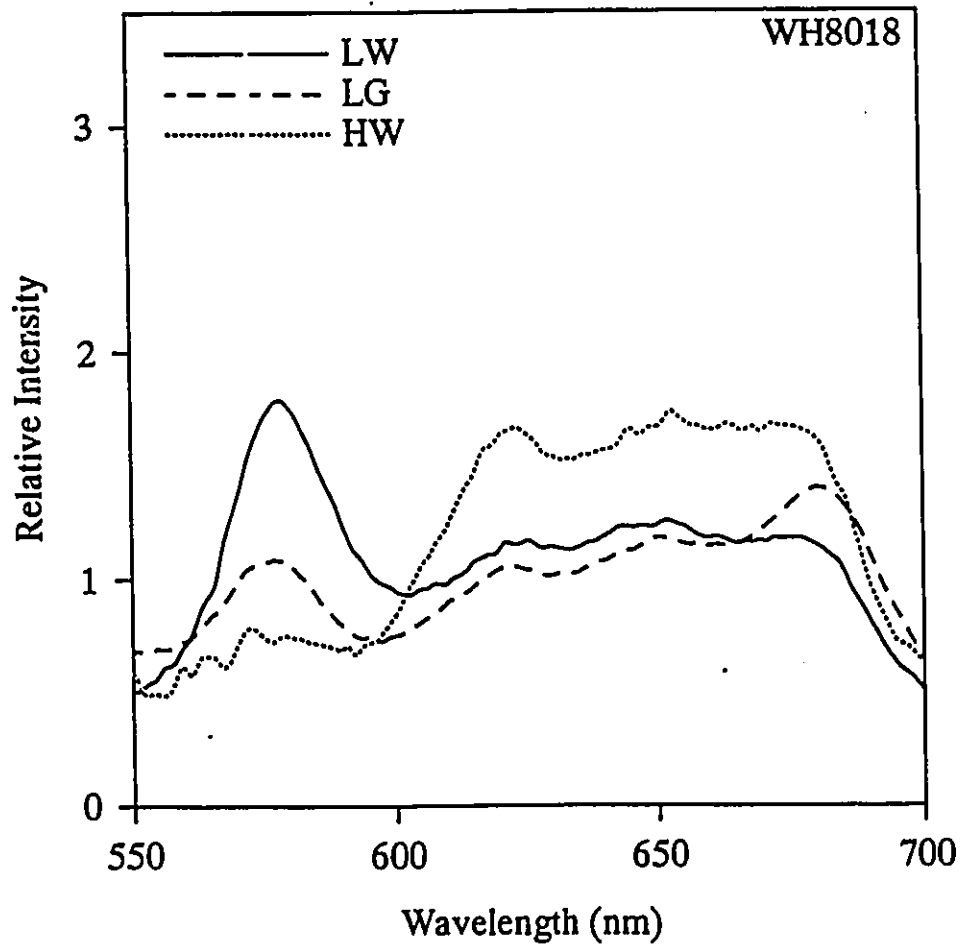


Figure 11. Effects of high white (HW), $400 \mu\text{E m}^{-2} \text{s}^{-1}$, low white (LW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$, and low green (LG), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ growth irradiance on the normalized emission spectra (using violet excitation; 420 nm) of exponentially growing WH8018. Each line is the average of 4 cultures.

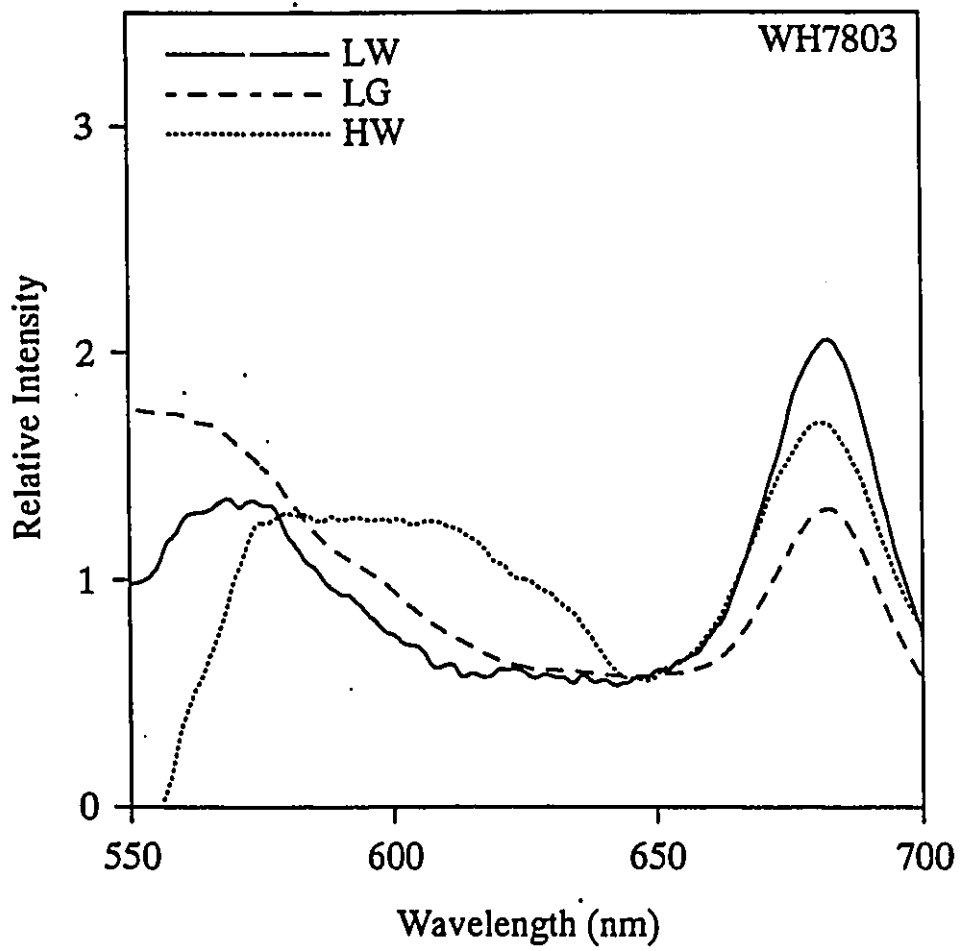


TABLE 8. Pigment ratios (w/w), emission ratios obtained using 420 nm ex [B(PE/Chla)], 470 nm ex [V(PE/Chla)] and the composite 470/420 nm ex [BV(PE/Chla)], of strains WH8018 and WH7803 during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) growth irradiance. B(PE/Chla) represents 470 nm ex:580 nm em for WH8018 and B(PE/Chla) represents 470 nm ex:570 nm em for WH7803. Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukey's pairwise comparison ($n=4$, $p \leq 0.05$). When $n=1$, PBP content was close to detection limits.

	B(PE/Chla)	V(PE/Chla)	BV(PE/Chla)	PE/Chl a
WH8018				
HW	0.35(0.14)a	0.43(0.07)a	0.18(0.06)a	1.0(0.4)a
LW	1.49(0.39)b	0.82(0.23)b	1.07(0.22)b	3.7(1.2)b
LG	0.76(0.04)c	0.47(0.03)a	0.30(0.02)c	0.5(0.2)a
WH7803				
HW	0.51(0.08)a	0.61(0.10)a	0.42(0.11)a	<D.L.
LW	2.83(0.07)b	0.74(0.13)a	2.52(0.40)a	5.8(0.9)a
LG	2.30(0.26)b	1.25(0.01)b	2.57(0.07)b	4.0(1.0)a

3.4 Effects of Growth irradiance on Fluorescence Yield.

There were differences in the fluorescence emission normalized per chl *a* or PE (ie. fluorescence yield) among the different light regimes (Table 9). The two emission peaks which were considered for comparison were the 470 nm excited PE emission at 580 nm (570 nm emission for WH7803), and the 420 nm excited Chl *a* emission at 680 nm.

The fluorescence yield of Chl *a* ($F_c/Chla$) for WH7803 did not significantly change between high white and green growth irradiance. The fluorescence yield of PE (F_p/PE) of WH7803 was significantly different among all the growth irradiances, being lowest in high white growth irradiance.

The $F_c/Chla$ for WH8018 was similar between high and low white growth irradiance, and between low white and green growth irradiance. $F_c/Chla$ was significantly depressed during growth in green growth irradiance as compared to high white growth irradiance. The F_p/PE of WH8018 was significantly different among all the growth irradiances, being lowest in high white growth irradiance.

TABLE 9. Fluorescence emission normalized to pigment content for strains WH8018 and WH7803 during exponential growth in $400 \mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and $28 \mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) growth irradiance. (± 1 std. dev.). $F_c/\text{Chl}a$ represents 420 nm ex ($680 \text{ nm em}/\mu\text{g Chl } a \text{ L}^{-1}$). F_p/PE represents 470 nm ex ($580 \text{ nm em}/\text{mg PE L}^{-1}$) for WH8018 and F_p/PE represents 470 nm ex ($570 \text{ nm em}/\text{mg PE L}^{-1}$) for WH7803. Values within a column with the same letters are not significantly different from each other as determined by a one way ANOVA, Tukey's pairwise comparison ($n = 4$, $p \leq 0.05$). N.A.: pigment and fluorescence determinations not performed at the same time.

	$F_c/\text{Chl}a$	F_p/PE
WH8018		
HW	92.3(42.8)a	12.3(5.2)a
LW	50.6(9.0)ab	47.7(23.2)b
LG	37.8(6.2)b	25.6(9.3)c
WH7803		
HW	51.2(9.6)a	8.7(4)a
LW	N.A.	N.A.
LG	40.2(5.7)a	26.8(3.8)b

3.5 Comparison of Photoacclimation Parameters.

A multiple analysis of variance of the 3 growth irradiances summarizes how the parameters co-varied during photoacclimation. In general excitation and emission ratios were significantly ($p < 0.05$) and positively correlated for both strains (Table 10 and 11). While photoacclimation processes were examined using all three emission ratios, each ratio alone adequately described photoacclimation in both strains. Thus while excitation closer to the absorption maximum gave greater emission of that pigment (Appendix IV), the fluorescence of both PE and Chl *a* was adequately determined using an intermediate excitation wavelength (470 nm). In general, PE/C was significantly and positively related to all the fluorescence ratios. This was not the case for Chl *a*/C, which was negatively (but not significantly) related to the fluorescence ratios.

Partial correlation coefficients for WH7803 are shown in Table 10. PE/C data were not available for the low white growth irradiance treatments and could not be included in the analysis. The F(PE/Chl*a*) excitation ratio was not significantly related to the composite BV(PE/Chl*a*) emission ratio. Furthermore, the F(PE/Chl*a*) excitation ratio was inversely related to the F(PE/PC) excitation ratio. WH7803 growth rate was positively related to the composite emission ratio, BV(PE/Chl*a*), and the excitation ratio F(PE/PC). Chl *a*/C was negatively related to growth but this relationship was not significant. For strain WH8018 the growth rate was positively related to all the parameters studied (Table 11.). The most significant relationship was with the F(PE/Chl*a*) excitation ratio.

TABLE 10. Multiple analysis of variance (MANOVA) partial correlation coefficients from strain WH7803 during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) irradiance. (numbers in brackets refer to the level of α).

DF=8	V(PE/Chia)	B(PE/Chia)	BV(PE/Chia)	F(PE/Chia)	F(PE/PC)	Chla/C	Growth
V(PE/Chia)	0.8700 (0.0009)	0.5020	0.1320	0.4600	-0.2130	0.5510	0.0990
B(PE/Chia)		0.3330	0.4390	0.1200	-0.0530	0.2630	0.4620
BV(PE/Chia)		0.3470	0.2040	0.7420	0.8850	0.7550	0.0116
F(PE/Chia)			-0.0790	0.7960	-0.3700	0.7550	0.0116
F(PE/PC)			0.8270	0.0060	0.2900	0.7550	0.0116
F(PE/PC)				-0.5750	0.2890	-0.4500	0.1900
Chla/C				0.0820	0.4200	0.1900	0.1900
Growth					-0.5300	0.9630	0.0001
					0.1130	0.9630	0.0001
					-0.5600	0.9630	0.0001
					0.0920	0.9630	0.0001

TABLE 11. Multiple analysis of variance (MANOVA) partial correlation coefficients of strain WH8018 during exponential growth in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) irradiance. (numbers in brackets refer to the level of α).

DF=7	V(PE/Chla)	B(PE/Chla)	BV(PE/Chla)	F(PE/Chla)	F(PE/PC)	Chla/C	PE/C	Growth
V(PE/Chla)	0.82	0.90	0.82	0.710	-0.28	0.9C	0.45	
	0.00	0.00	0.006	0.032	0.47	0.0008	0.23	
B(PE/Chla)	0.96	0.98	0.58	-0.100	0.89	0.65		
	0.0001	0.0001	0.098	0.800	0.001	0.057		
BV(PE/Chla)	0.97	0.610	-0.13	0.97	0.62			
	0.0001	0.082	0.740	0.0001	0.07			
F(PE/Chla)	0.64	-0.07	0.91	0.74				
	0.06	0.85	0.0006	0.02				
F(PE/PC)	0.028	0.53	0.60					
	0.94	0.1400	0.09					
Chla/C	-0.11	0.23						
	0.7800	0.55						
PE/C	0.53							
	0.14							
Growth								

3.6 Effects of Nitrogen Limitation: Phycocyanin Strains.

The onset of nitrogen limitation was considered to occur at the beginning of the stationary phase. The cultures were harvested approximately half way through the exponential phase and then at 24 h and 72 h after the beginning of the stationary phase. The same measurements made during the photoacclimation experiments were conducted as previously described.

3.6.1 Carbon, Nitrogen and Pigment Content.

The three phycocyanin (PC) strains all showed low C/N ratios, typical of nitrogen sufficient cyanobacteria, during exponential growth (Table 12). A significant increase in the C/N ratio (70-80%) occurred during nitrogen limitation, primarily as a result of an increase in cellular carbon. Pigment content generally declined during nitrogen limitation (Table 13), particularly when expressed per unit carbon. The Chl *a*/cell in WH5701 and in *Synechococcus leopoliensis* did not change significantly during nitrogen limitation. As expected the most pronounced effect of nitrogen limitation was on the phycobiliproteins. In *S. leopoliensis* and *Synechocystis*, PC content declined by at least 80%. Because of its small cell size, WH5701 had PC levels too low to measure when using the usual extraction volume (40 mL). Under extreme nitrogen deficiency, PC content in general was close to the detection limit. As with the Chl *a* data, the reduction in PC content with nitrogen limitation was most pronounced when content was expressed on a per carbon basis.

TABLE 12. Growth rates (ln units d⁻¹), and carbon and nitrogen content (fg/cell) of 3 PC strains at 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. PC strains were grown in nitrogen depleted batch cultures. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA (n = 4, p \leq 0.05).

	Growth	C/cell	N/cell	C/N
WH5701				
exp	0.65(0.10)	774(187)*	148(38)	5.06(0.76)*
24 h		680(102)	83.4(13)	8.18(0.64)
72 h		1258(168)	142(223)	8.96(1.37)
<i>S.teopollensis</i>				
exp	1.56(0.12)	1840(274)*	349(90.2)	5.48(1.23)*
24 h		2520(996)	268(80.2)	9.25(0.94)
72 h		2960(318)	316(76.8)	9.67(2.0)
<i>Synechocystis</i>				
exp	1.07(0.05)	3200(560)*	510(130)	6.38(0.52)*
24 h		3600(867)	412(155)	9.08(1.8)
72 h		4550(860)	420(113)	11.05(1.84)

TABLE 13. Pigment content per cell (fg/cell) and per carbon (mg/g) of 3 PC strains in $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. PC strains were grown in nitrogen depleted batch cultures. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). < D.L.; PC content below detection limit. * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	Chl <i>a</i> /Cell	Chl <i>a</i> /C	PC/cell	PC/C
<i>WH5701</i>				
exp	10.0(1.6)	13.2(2.8)	<D.L.	<D.L.
24 h	5.9(1.2)	8.6(0.6)		
72 h	11.4(1.6)	9.0(0.6)		
<i>S.teopoiensis</i>				
exp	43.0(8.5)	23.3((1.3)*	249(79)*	134(30)*
24 h	34.4(13.5)	13.6(0.8)	151(43)	62(9)
72 h	27.0(3.6)	9.1(0.4)	35.5(11)	12(4)
<i>Synechocystis</i>				
exp	59.4(10.9)*	18.6(0.8)*	258(87)	80(19)*
24 h	42.9(13.5)	11.8(1.2)	68(11)	21(8)
72 h	35.8(10.7)	8.1(1.7)	80(43)	17(6)

3.6.2 Excitation Spectra of Chl *a*.

Fluorescence spectra were obtained for each replicate culture during nitrogen limited batch growth in low white light. Examples of excitation (Chl *a* emission at 680 nm) spectra, for one of the replicate cultures during all phases of nitrogen limitation, are shown in Figure 12. The blue Soret and PC excitation peaks occurred at 437 nm and 628 nm respectively, essentially in all three PC strains (summarized in Appendix IA). Figure 11 shows that the Soret peak becomes more pronounced relative to the PC peak during nitrogen limited growth, particularly in *Synechocystis*.

Changes in the PC to chlorophyll *a* excitation ratio ($F(\text{PC}/\text{Chl}a)$) did not correspond well with the PC/Chl *a* ratios, particularly in *S. leopoliensis* (Table 14), and may be explained in part by the fact that these two parameters were not performed simultaneously. *Synechococcus leopoliensis* showed the least change in $F(\text{PC}/\text{Chl}a)$ during nitrogen limitation.

3.6.3 Emission Spectra.

Figure 13 shows the changes in emission spectra obtained from green excitation (545 nm) for one culture during nitrogen limited batch growth. Using this excitation one main excitation peak at 655 nm can be attributed to PC, while the Chl *a* emission at 683 nm was only discernable in the later stages of nitrogen limitation (Appendix IB). All three PC strains showed a decline in the proportion of PC emission relative to Chl *a* emission. The freshwater strains, *S. leopoliensis* and *Synechocystis* showed a red shift in the PC emission which can be attributed to

Figure 12. Effects of nitrogen limitation on the normalized excitation spectra, for Chl *a* emission at 680 nm, of three PC strains. PC strains were grown in nitrogen depleted batch cultures in low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (*exp*), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one cultures for each of the strains.

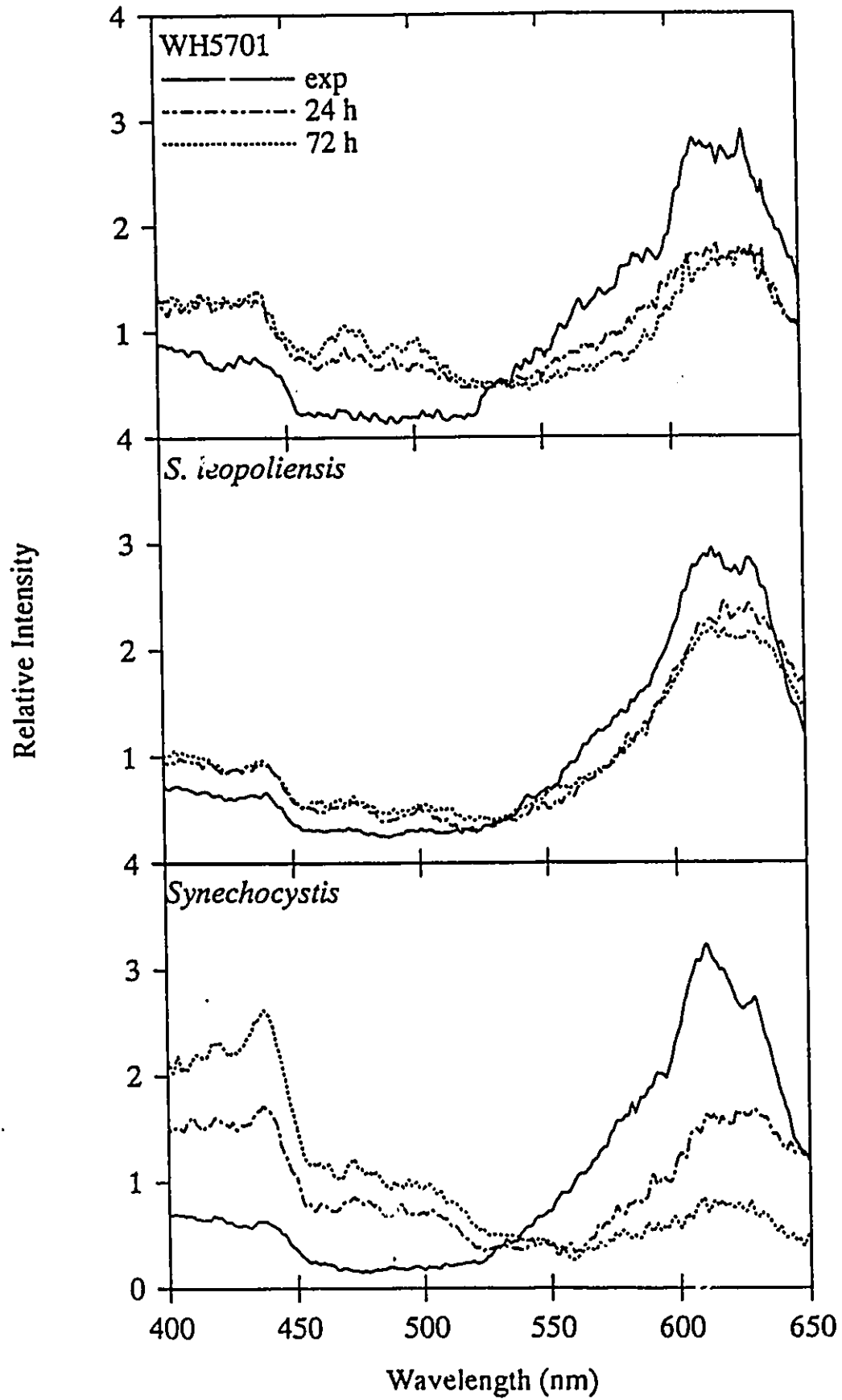


TABLE 14. Excitation ratios, F(PC/Chl a) of Chl a emission at 680 nm, of 3 PC strains in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. PC strains were grown in nitrogen depleted batch cultures. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (\pm 1 std. dev.). < D.L.; PC content below detection limit. * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	PC/Chl a (w/w)	F(PC/Chl a)
WH5701		
exp	<D.L.	3.90(0.44)*
24 h		2.08(1.45)
72 h		2.16(1.59)
<i>S.leopoliensis</i>		
exp	10.7(4.1)*	1.07(0.03)
24 h	7.3(1.2)	1.22(0.29)
72 h	1.4(0.4)	1.02(0.02)
<i>Synechocystis</i>		
exp	4.3(1.0)*	4.35(0.91)*
24 h	1.8(0.9)	1.08(0.16)
72 h	2.2(0.5)	0.34(0.11)

Figure 13. Effects of nitrogen limitation on the normalized emission spectra using green excitation (545 nm), of three PC strains. PC strains were grown in nitrogen depleted batch cultures in low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one cultures for each of the strains.

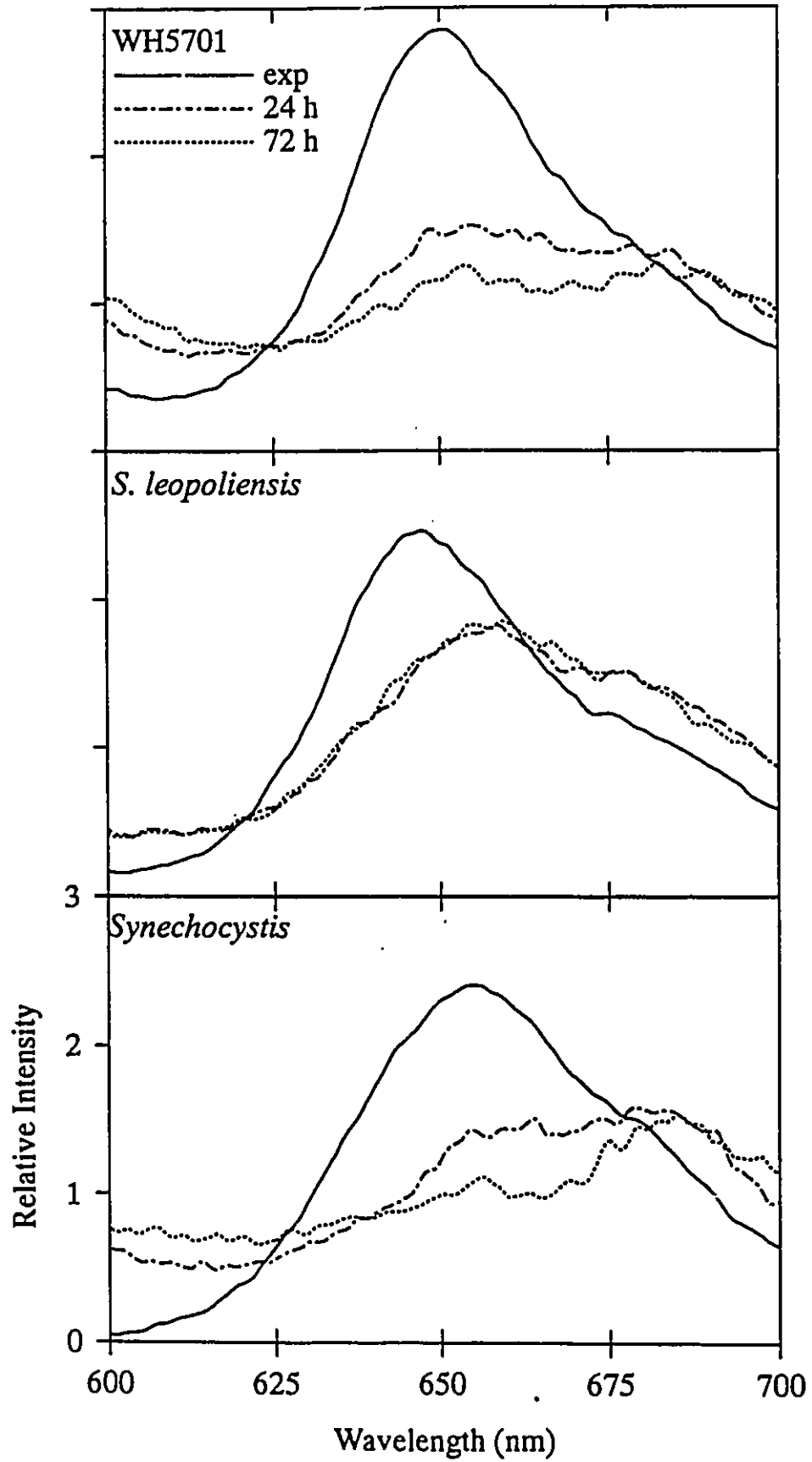


Figure 14. Effects of nitrogen limitation on the normalized emission spectra using violet excitation (420 nm), of three PC strains. PC strains were grown in nitrogen depleted batch cultures in low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture for each of the strains.

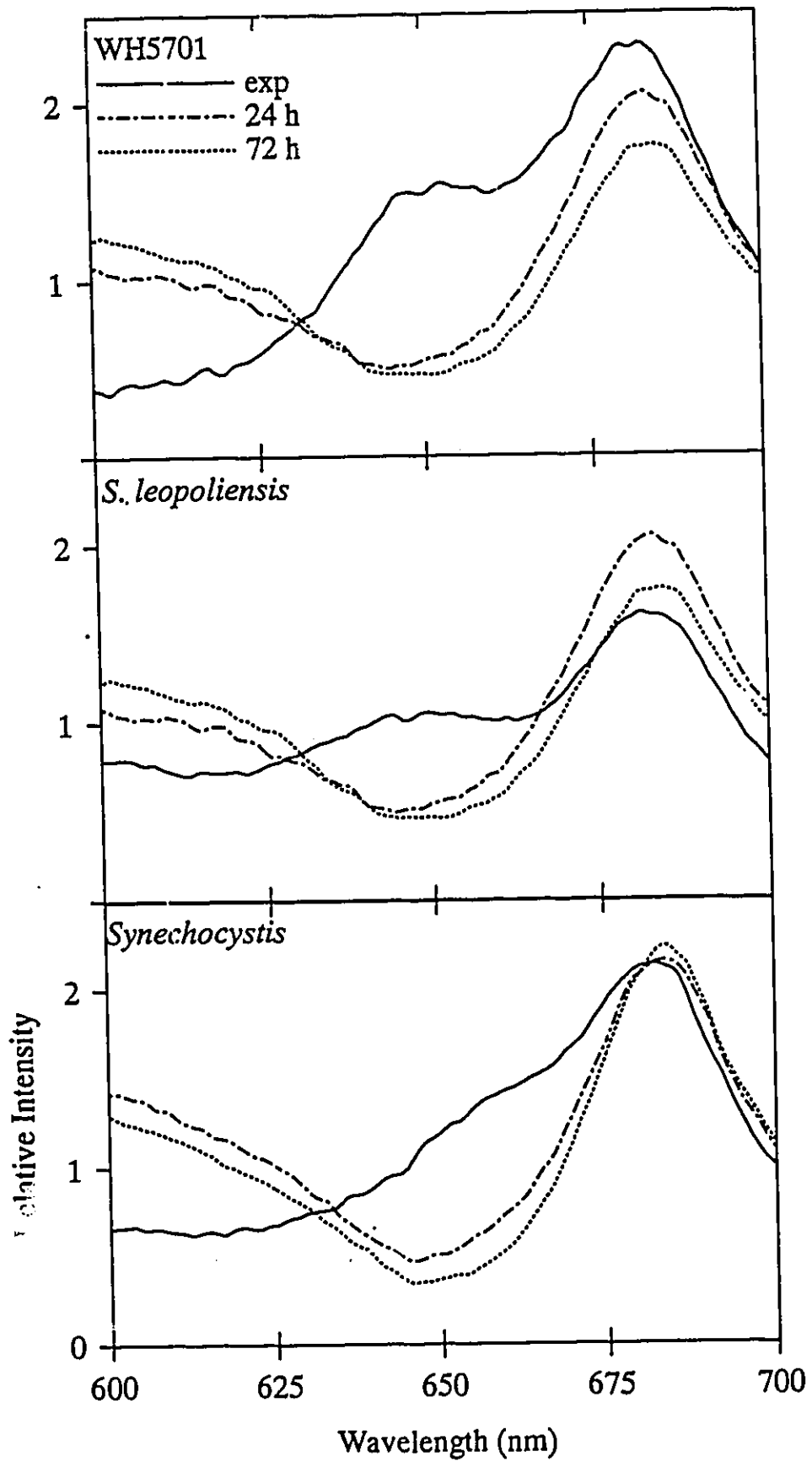


TABLE 15. Emission ratios using 420 nm ex, [V(PC/Chla)], 545 nm ex, [G(PC/Chla)], and the composite 545/420 nm ex, [GV(PC/Chla)] of 3 PC strains in $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. PC strains were grown in nitrogen depleted batch cultures. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n = 4$, $p \leq 0.05$).

	G(PC/Chla)	V(PC/Chla)	GV(PC/Chla)
<u>WH5701</u>			
exp	2.00(0.17)*	0.60(0.05)	1.72(0.17)*
24 h	1.28(0.15)	0.45(0.17)	0.89(0.52)
72 h	1.07(0.19)	0.42(0.12)	0.94(0.46)
<u><i>S. leopoliensis</i></u>			
exp	2.16(0.10)*	0.68(0.02)*	1.92(0.16)*
24 h	1.18(0.08)	0.35(0.02)	0.74(0.18)
72 h	1.22(0.05)	0.38(0.01)	0.64(0.07)
<u><i>Synechocystis</i></u>			
exp	1.61(0.04)*	0.56(0.01)*	1.45(0.39)*
24 h	0.86(0.11)	0.25(0.01)	0.23(0.04)
72 h	0.67(0.02)	0.17(0.01)	0.14(<0.01)

the detection of allophycocyanin, as phycocyanin declines in response to nitrogen limitation. Figure 14 shows the changes in emission spectra obtained from violet excitation (420 nm) for one culture during nitrogen limited batch growth. The changes in the PC and Chl *a* emission peaks were better discerned here.

The emission ratios were statistically compared in three types of calculations (Table 15). These were the green (G(PC/Chl*a*)) and violet (V(PC/Chl*a*)) excitation ratios. An additional calculation of a composite ratio of the PC emission from the 545 nm excitation divided by the Chl *a* emission from the 420 nm excitation (GV(PC/Chl*a*)) was used to obtain the maximum fluorescence emission from each pigment. All ratios declined with nitrogen limitation. The decline in emission ratios was more severe than the decline in excitation ratios and was clearly evident in all strains including *S. leopoliensis*.

3.7 Effects of Nitrogen Limitation: Phycoerythrin Strains.

The effects of nitrogen limitation were examined in the three phycoerythrin-containing (PE) strains. Both WH7803 and WH8018 were examined at high white ($400 \mu\text{E m}^{-2}\text{s}^{-1}$) and low white ($28 \mu\text{E m}^{-2}\text{s}^{-1}$) growth irradiance. Nitrogen limitation was examined in strain 48B66 at low white growth irradiance only.

3.7.1 Growth Rates, Carbon, Nitrogen and Pigment Content:

48B66.

Carbon and nitrogen per cell increased during nitrogen limitation but the C/N values did not increase significantly above the expected ratio (Table 16). During nitrogen limitation all pigments declined whether expressed on a per carbon or on a

per cell basis (Table 17).

3.7.2 Excitation Spectra of Chl *a* Emission.

Figure 15 shows the changes in excitation spectra (Chl *a* at 680 nm) for one culture during nitrogen limited batch growth. In addition to the PE peak at 543 nm, 2 other peaks contributed to Chl *a* excitation. These were the blue Soret band of Chl *a* at 439 nm and the high PU peak at 493 nm (Appendix IA). 48B66 exemplifies the Type I PE picocyanobacteria in having a larger ratio of PE/PU as compared to other Type I's (e.g. WH7803). Unlike strain WH7803, excitation by a PC component was not evident in the excitation spectra of strain 48B66 (Fig. 14). Nitrogen limitation did not appear to affect the relative importance of the wavelengths contributing to the excitation of Chl *a* (Table 18). This includes the excitation ratio of PE to PU (492 nm), not shown in Table 18.

3.7.3 Emission Spectra.

Figure 16 shows the changes in emission spectra obtained from blue excitation (470 nm) for one replicate during nitrogen limited batch growth. The dominant emission using this excitation was a PE peak located at 566 nm. Two other smaller peaks occurred at 651 nm and 682 nm which relate to PC of allophycocyanin, and to Chl *a*, respectively (Appendix IB). In low white light, a PC component at around 625 nm was not apparent for strain 48B66. The emission spectra from blue excitation, like the C/N ratios, and excitation spectra, did not show any significant change during nitrogen limitation (Table 19). However, nitrogen limitation appeared to increase the proportion of PE to Chl *a* emission,

TABLE 16. Growth rate (ln units d⁻¹), cellular carbon and nitrogen content (fg/cell) of strain 48B66 during growth in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. 48B66 was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential (exp), 24 h and 72 h past the onset of stationary phase. (\pm 1 std dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	Growth	C/cell	N/cell	C/N
exp	1.22(0.1)	1535(136)	275(12.4)	5.57(0.30)
24 h		595(37.2)	100(9.6)	5.88(0.07)
72 h		1742(304)	302(40.7)	5.75(0.39)

TABLE 17. Pigment content per cell (fg/cell) and per carbon (mg/g) of 48B66 during growth in $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. Strain 48B66 was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). Missing std. dev. indicates only one PC content value available. * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	Chl <i>a</i> /cell	Chl <i>a</i> /C	PE/cell	PE/C
exp	11.0(0.9)	7.2(0.5)	8.6(5.7)	5.28(3.0)
24 h	3.4(0.7)	5.6(0.9)	1.0(0.5)	1.68(0.8)
72 h	7.2(1.0)	4.2(0.8)	3.8(1.5)	2.1(1.0)

Figure 15. Effects of nitrogen limitation on the excitation spectra for Chl *a* emission at 680 nm, of strain 48B66. 48B66 was grown in nitrogen depleted batch culture in low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

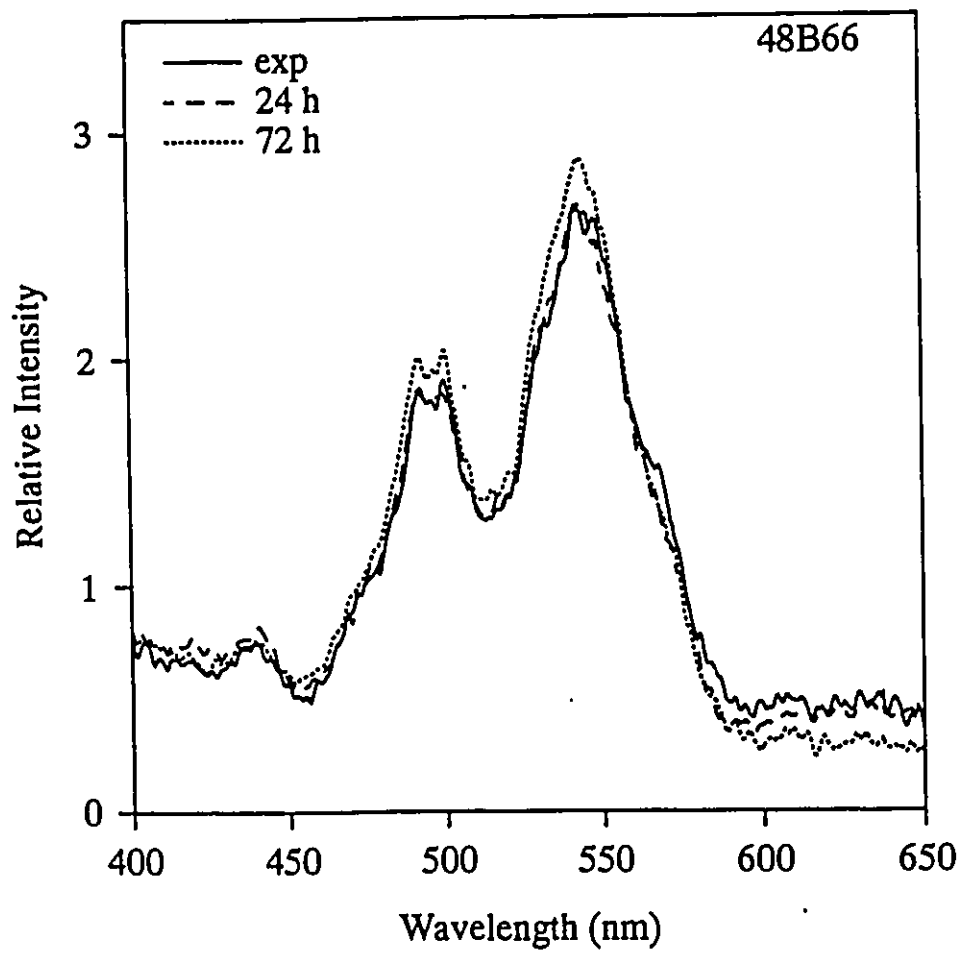


TABLE 18. Pigment ratios (w/w) and excitation ratios, F(PE/Chl a), F(PE/PC), and F(PE/PU), for Chl a emission of 680 nm, of strain 48B66 during growth in 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. Strain 48B66 was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential (exp), 24 h and 72 h past the onset of stationary phase. (\pm 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$). <D.L.: PC content below detection limit. Missing std. dev indicates a sample size of $n=1$.

	PE/Chl a	F(PE/Chl a)	PE/PC	F(PE/PC)
exp	0.53(0.06)	3.41(0.15)	120.4	4.98(1.24)
24 h	0.31(0.16)	3.61(0.17)	<D.L.	6.37(1.02)
72 h	0.55(0.27)	3.36(0.52)		6.26(2.90)

obtained from a violet excitation (V(PE/Chla, Fig. 17, Table 19). This effect may be entirely due to the background noise resulting from a large raman scatter peak of the media which occurs at the PE emission wavelength (Appendix IIA).

3.7.4 Growth rates, Carbon, Nitrogen and Pigment Content:

WH7803.

Table 20 shows changes in carbon, nitrogen and C/N ratios for WH7803 during nitrogen limitation in both high and low white growth irradiance. While cellular carbon and nitrogen increased during nitrogen limitation, this did not affect the proportion of C/N. Significant increases in Chla/Cell and PE/Cell also occurred during nitrogen limitation in both high and low light (Table 21). Chl *a* on a per carbon basis decreased or remained the same during nitrogen limitation in high and low light, respectively. Therefore relative increases in C/cell, N/cell and pigments/cell indicate cell volume increases due to stationary growth but not a nitrogen limitation response as C/N's remained constant.

Figure 16. Effects of nitrogen limitation on the emission spectra using blue excitation (470 nm), of strain 48B66. 48B66 was grown in nitrogen depleted batch culture in low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

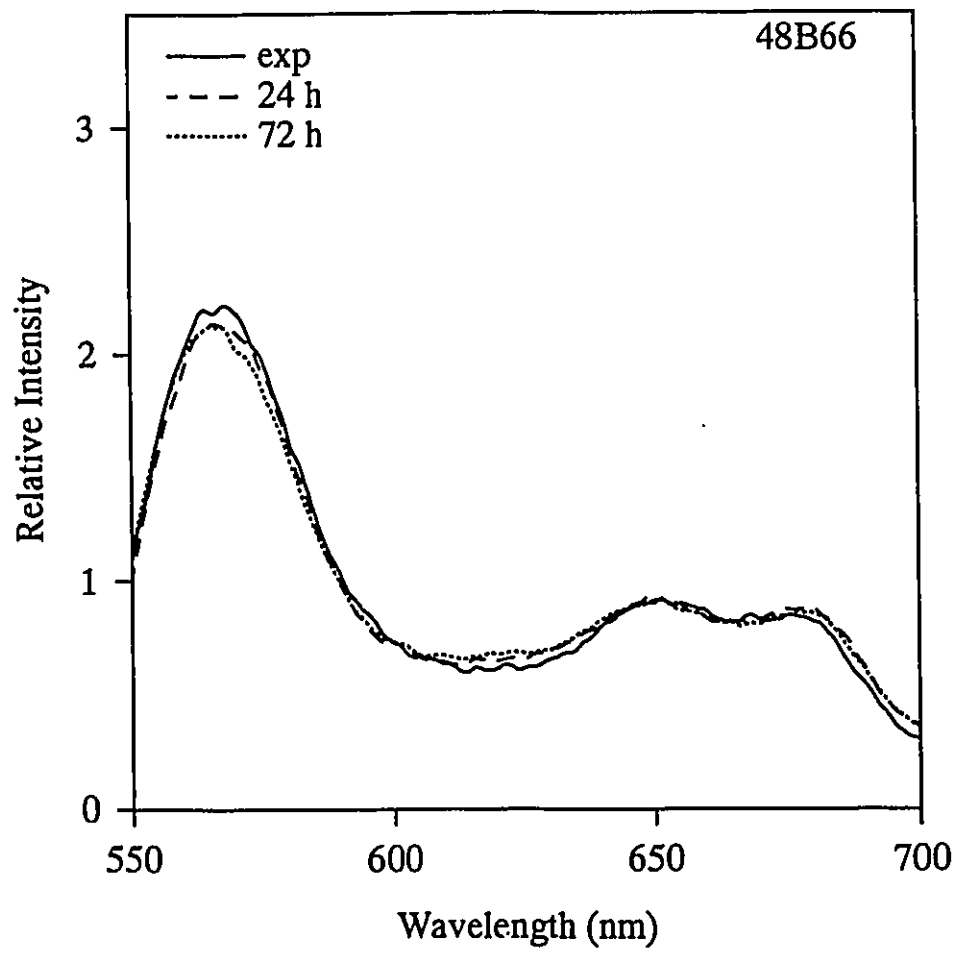


Figure 17. Effects of nitrogen limitation on the emission spectra using violet excitation (420 nm), of strain 48B66. 48B66 was grown in nitrogen depleted batch culture in low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

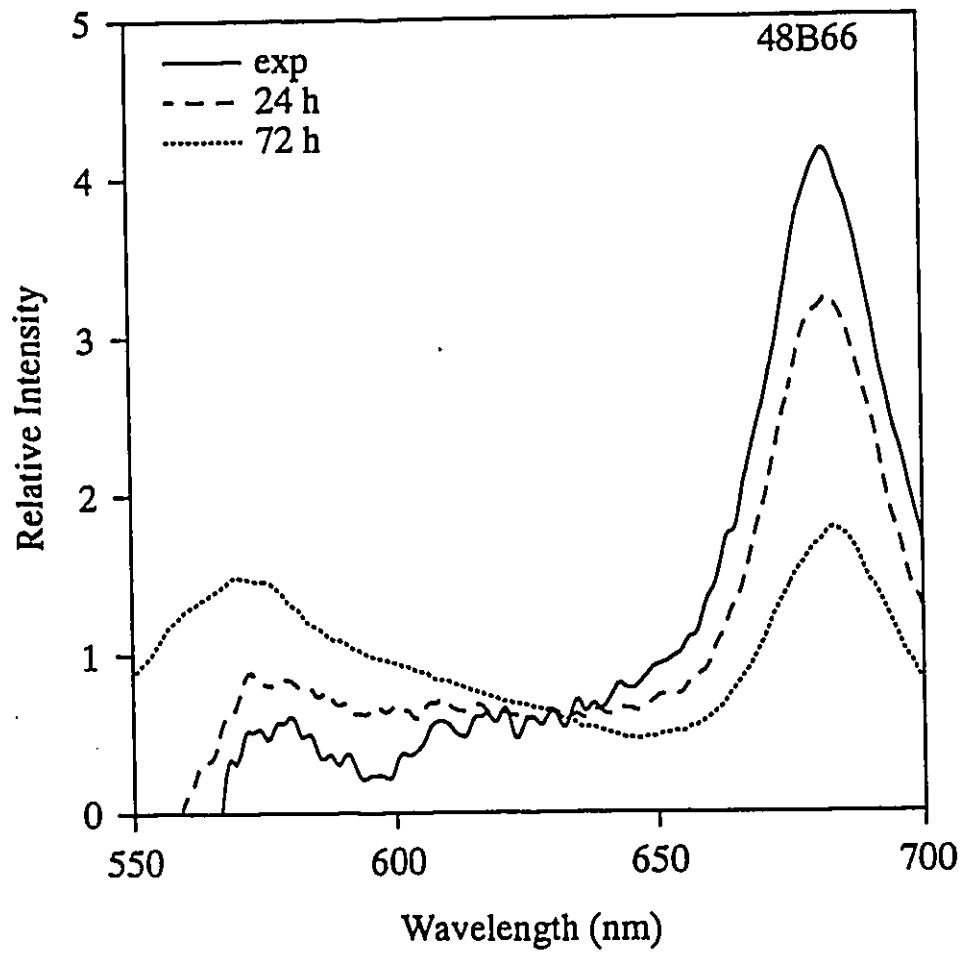


TABLE 19. Emission ratios using 470 nm ex, [B(PE/Chla)], 420 nm ex, [V(PE/Chla)], and the composite 470 nm/420 nm ex, [BV(PE/Chla)] of 48B66 during growth in $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. 48B66 was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	B(PE/Chla)	V(PE/Chla)	BV(PE/Chla)
exp	2.58(0.21)	0.11(0.08)*	3.23(0.34)
24 h	2.42(0.05)	0.35(0.18)	3.33(0.25)
72 h	2.82(0.62)	0.94(0.24)	3.65(0.95)

TABLE 20. Cellular carbon and nitrogen content (fg/cell) of strain WH7803 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH7803 strain was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	C/cell	N/cell	C/N
HW			
exp	120(18.6)*	19.4(1.1)*	6.18(0.65)
24 h	555.8(234)	72.4(6.2)	7.70(0.4)
72 h	597(63)	77.3(7.0)	7.73(0.54)
LW			
exp	437(112)	81.7(24.8)	5.41(0.73)
24 h	169(81.4)	28.7(14.6)	5.92(0.39)
72 h	674(130)	109(18.7)	6.16(0.24)

TABLE 21. Pigment content per cell (fg/cell) and per carbon (mg/g) of strain WH7803 in $400 \mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH7803 strain was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	Chl a/Cell	Chl a/C	PE/cell	PE/C
HW				
exp	0.39(0.1)	2.5(1.0)*	0.53(0.01)*	12.4(3.4)*
24 h	1.8(0.4)	3.2(0.6)	1.88(0.52)	1.26(0.1)
72 h	0.8(0.4)	1.4(0.7)	2.24(0.94)	5.5(0.3)
LW				
exp	3.72(2.10)*	8.3(1.7)	8.3(2.8)*	20.7(3.8)*
24 h	4.0(0.9)	25.6(10)	11.2(2.2)	75.9(28)
72 h	5.9(1.1)	8.9(1.9)	20.9(3.5)	31.7(6.9)

3.7.5 Excitation Spectra of Chl *a*.

Figure 18 shows the changes in excitation spectra (Chl *a* em 680 nm) during nitrogen limitation in high and low white growth irradiance for strain WH7803. In high light, nitrogen limitation significantly decreased the proportion of PE (543 nm) relative to the PC excitation peaks (633 nm), and the proportion of PE to the PU excitation peak (493 nm) but not the proportion of PE to the Soret band excitation (437 nm) (Table 22). In low light the F(PE/PC) declined even more (90% decline vs 33% in high light) while the F(PE/Chl*a*) increased and the F(PE/PU) remained unchanged. Pigment ratios (w/w) did not differ significantly during nitrogen limitation, but the trends corresponded with those observed with the excitation ratios.

3.7.6 Emission Spectra.

Changes in emission spectra obtained from blue excitation (470 nm) and violet excitation (420 nm) for one culture during nitrogen limited batch growth are presented in Fig. 19 and 20, respectively. The emission from PE at 566 nm appeared to increase in proportion to the PC (624 nm) and Chl *a* (682 nm) emission peaks for both high and low light treatments (Fig. 19). The effect of nitrogen limitation on the emission ratio of WH7803 was most pronounced at high

Figure 18. Effects of nitrogen limitation on the excitation spectra for Chl *a* emission at 680 nm, of strain WH7803. WH7803 was grown in nitrogen depleted batch cultures in high white ($400 \mu\text{E m}^{-2} \text{s}^{-1}$) and low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

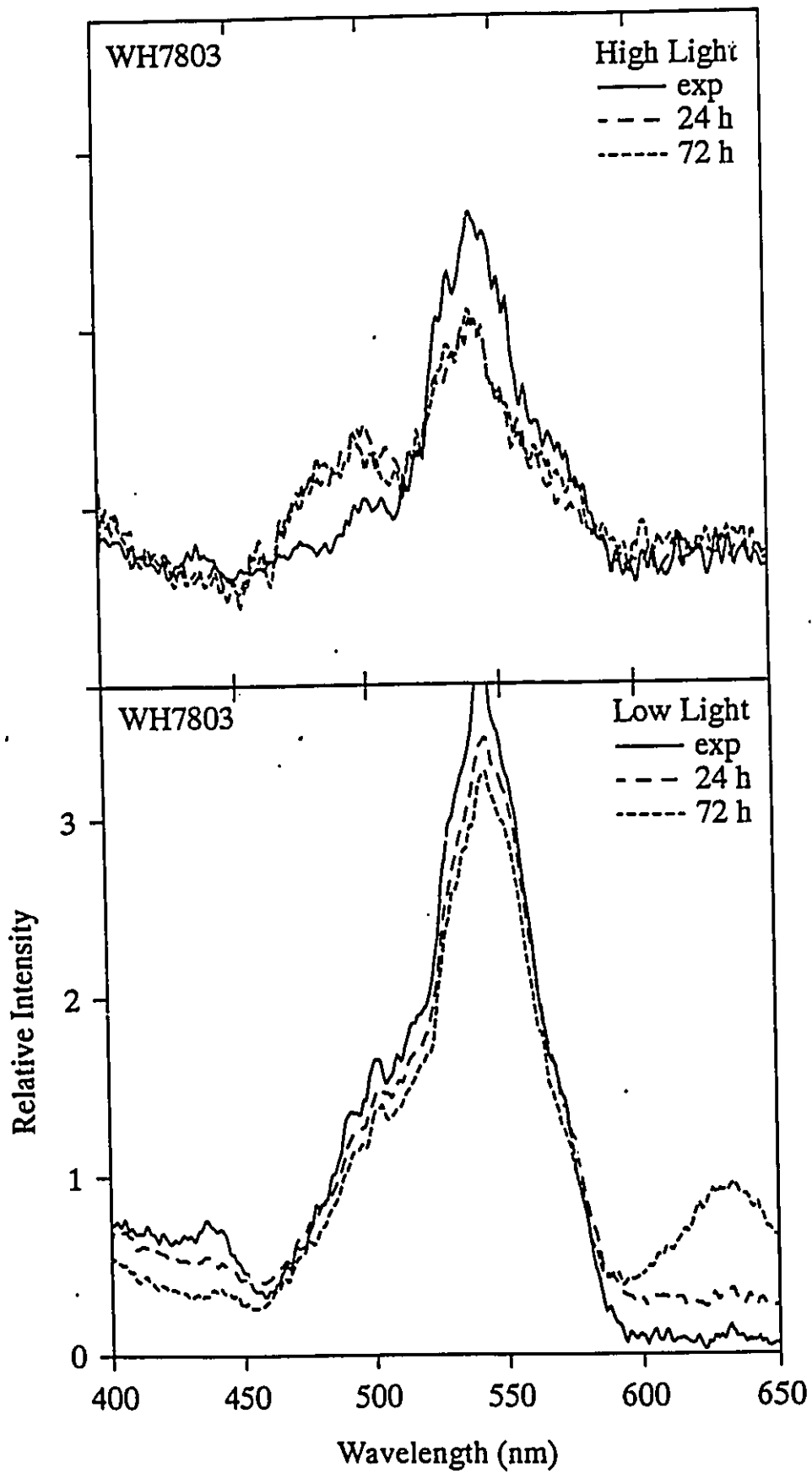


TABLE 22. Pigment ratios (w/w) and excitation ratios, F(PE/Chl a), F(PE/PC), and F(PE/PU), for Chl a emission at 680 nm, of strain WH7803 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH7803 strain was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase.(\pm 1 std. dev.). Missing std. dev. indicates only one PC content value available. * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	PE/Chl a	F(PE/Chl a)	PE/PC	F(PE/PC)	F(PE/PU)
HW					
exp	13.0(6.1)*	3.55(0.26)*	4.2	4.25(0.48)*	2.50(0.15)*
24 h	1.8(0.4)	3.58(0.33)	3.8(0.4)	2.91(0.52)	1.72(0.2)
72 h	5.1(3.5)	3.25(0.17)	5.0	2.85(0.51)	1.56(0.12)
LW					
exp	2.3(0.2)*	5.61(0.32)*	30*	36.0(18.7)*	2.48(0.22)
24 h	3.1(0.6)	6.31(0.28)	21(3)	11.0(0.4)	2.85(0.50)
72 h	3.7(1.3)	8.87(0.16)	22(3)	3.47(1.13)	2.47(0.20)

Figure 19. Effects of nitrogen limitation on the emission spectra, using blue excitation (470 nm), of strain WH7803. WH7803 was grown in nitrogen depleted batch cultures in high white ($400 \mu\text{E m}^{-2} \text{s}^{-1}$) and low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

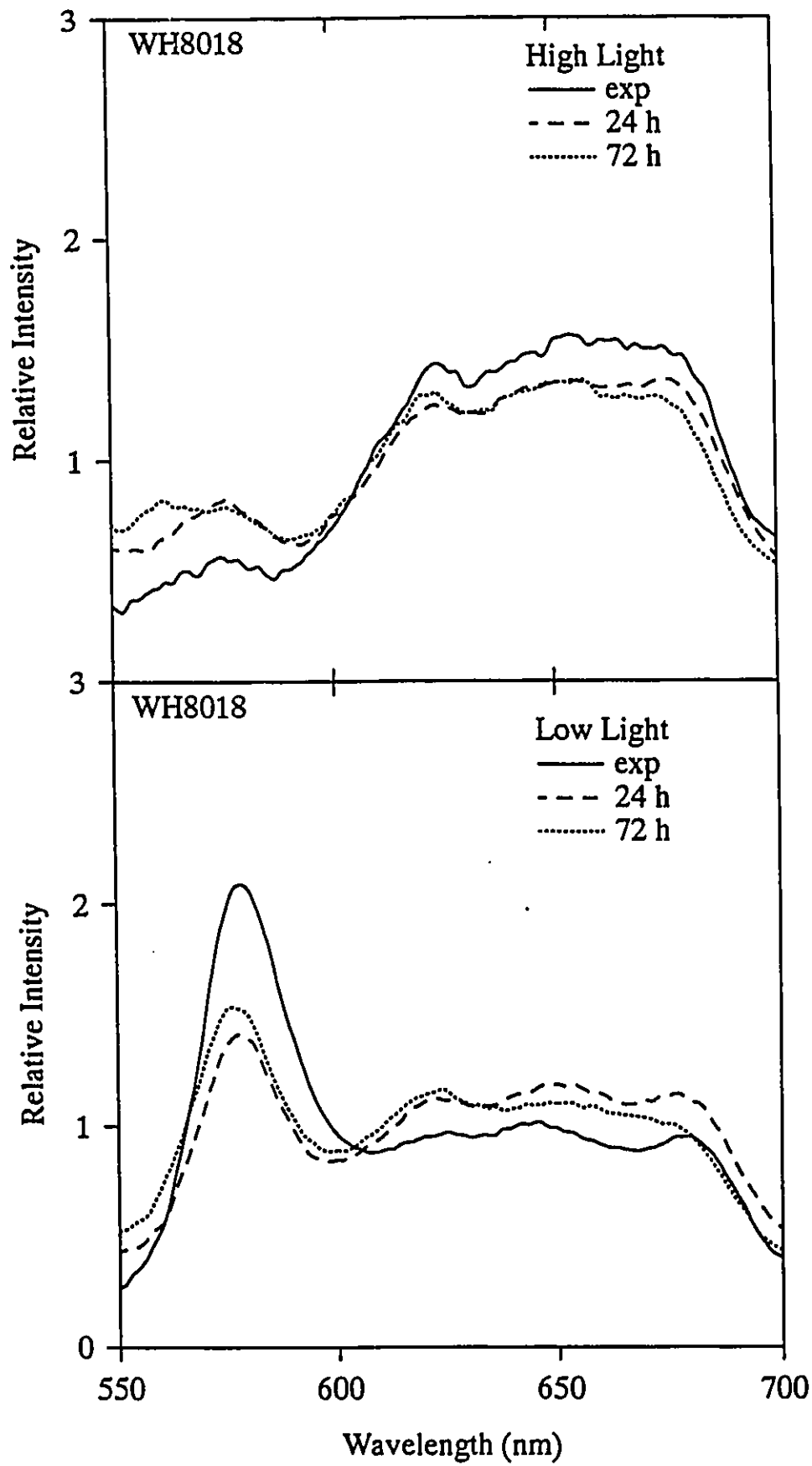


Figure 20. Effects of nitrogen limitation on the emission spectra, using violet excitation (420 nm), of strain WH7803. WH7803 was grown in nitrogen depleted batch cultures in high white ($400 \mu\text{E m}^{-2} \text{s}^{-1}$) and low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

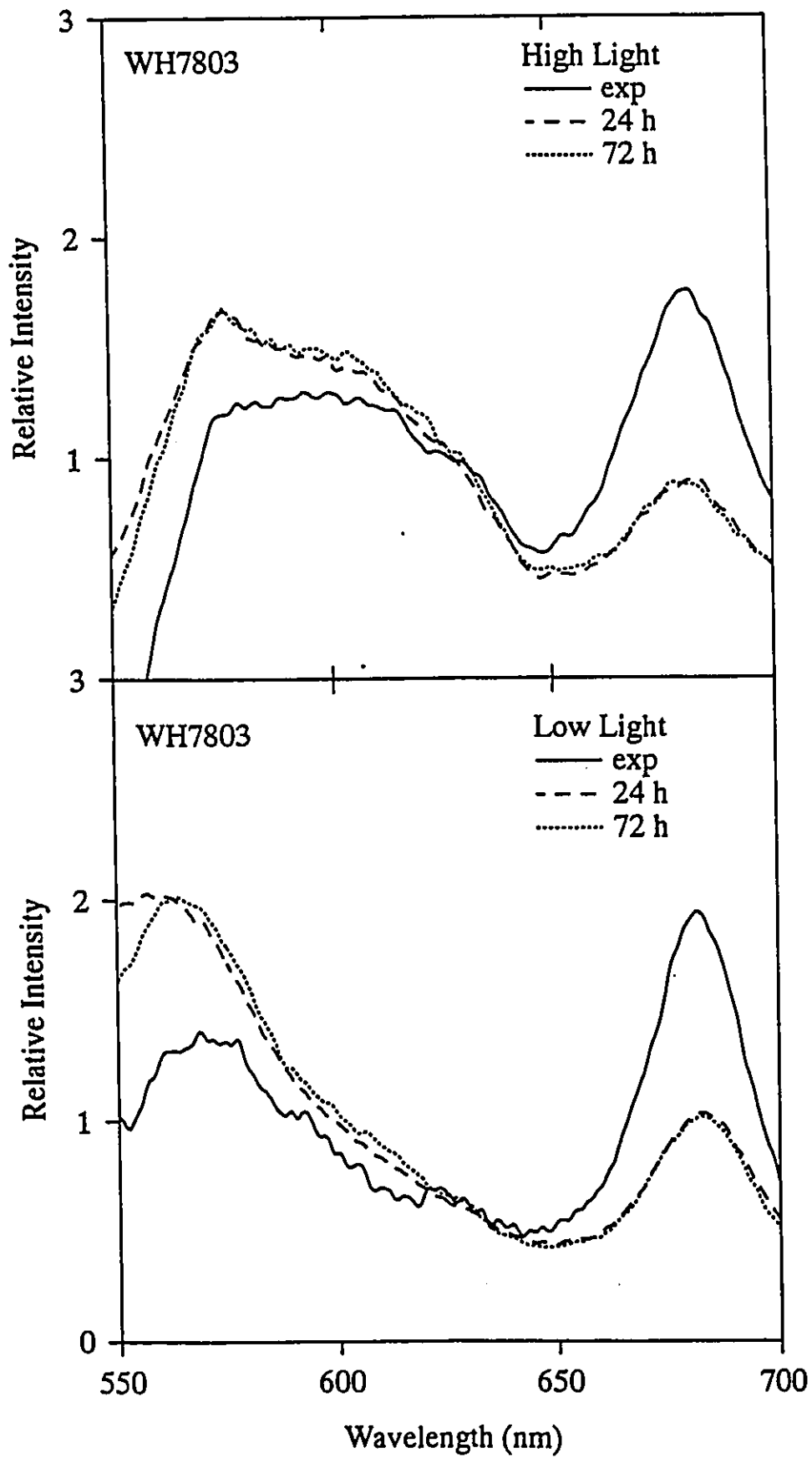


TABLE 23. Peak emission ratios from 420 nm ex, [V(PE/Chla)], 470 nm ex, [B(PE/Chla)], and the composite 470/420 nm ex, [BV(PE/Chla)] of strain WH7803 in $400 \mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH7803 strain was grown in nitrogen depleted batch culture. Sampling was performed at mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	B(PE/Chla)	V(PE/Chla)	BV(PE/Chla)
HW			
exp	0.57(0.14)*	0.61(0.08)*	0.45(0.09)*
24 h	0.88(0.05)	1.62(0.08)	1.06(0.05)
72 h	0.97(0.04)	1.67(0.05)	1.15(0.04)
LW			
exp	2.83(0.07)*	0.66(0.05)*	2.52(0.41)*
24 h	4.12(0.25)	1.87(0.02)	4.16(0.24)
72 h	4.36(0.24)	2.04(0.06)	3.51(0.19)

light for the composite ratio $BV(PE/Chla)$ was calculated (Table 23).

3.7.7 Growth Rates, Carbon, Nitrogen and Pigment

Content: WH8018.

Table 24 shows changes in carbon, nitrogen and C/N ratios for WH8018 during nitrogen limitation in both low and high white growth irradiance. Nitrogen limitation appeared to increase both carbon and nitrogen per cell during both light treatments but did not significantly increase the C/N ratio. While PE/C remained unchanged at high light, significant increases occurred for Chla/cell, Chl *a*/C and PE/cell. These trends were reversed in low light, with declines in pigment content occurring particularly for PE (Table 25).

3.7.8 Excitation Spectra of Chl *a*.

In high light, nitrogen limitation led to a slight increase in the proportion of PE excitation relative to the blue Soret of Chl *a* and relative to the PC excitation peaks in WH8018 (Fig. 21). In low light there was a significant decrease in both the $F(PE/Chla)$ and the $F(PE/PC)$ ratios (Table 26). While the relative content of pigments did not change significantly during nitrogen limitation, they did appear to track the concomitant changes in excitation ratios

3.7.9 Emission Spectra.

Changes in emission spectra obtained from blue excitation (470 nm) and violet excitation (420 nm) for one replicate culture during nitrogen limited batch growth are presented in Fig. 22 and 23, respectively. Emission from PE decreased in proportion to the PC and Chl *a* emission peaks for only low light while the

TABLE 24. Cellular carbon and nitrogen content (fg/cell) of strain WH8018 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH8018 strain was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	C/cell	N/cell	C/N
HW			
exp	650(49)	63.9(69)	5.60(1.4)
24 h	732(396)	112(43)	6.70(0.6)
72 h	708(74)	103(5)	6.84(0.4)
LW			
exp	234(66)	30.1(11)	7.67(0.84)
24 h	302(170)	46.8(26)	6.45(0.31)
72 h	380(69)	60.0(16)	6.41(0.52)

TABLE 25. Pigment content per cell (fg/cell) and per carbon (mg/g) of strain WH8018 in $400 \mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH8018 strain was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	Chl <i>a</i> /Cell	Chl <i>a</i> /C	PE/cell	PE/C
HW				
exp	0.84(0.22)*	2.9(0.3)*	0.7(0.2)*	3.4(1.0)*
24 h	1.99(1.60)	6.3(2.0)	1.9(0.2)	4.6(0.6)
72 h	2.56(0.50)	6.7(0.7)	1.9(0.2)	4.7(0.6)
LW				
exp	7.20(0.77)*	11.1(2.0)*	22.8(6.5)*	34.8(7.3)*
24 h	7.55(3.78)	10.4(0.5)	22.6(16.9)	28.8(7.5)
72 h	3.97(0.67)	5.6(0.4)	9.2(3.0)	12.8(2.9)

Figure 21. Effects of nitrogen limitation on the excitation spectra for Chl *a* emission at 680 nm, of strain WH8018. WH8018 was grown in nitrogen depleted batch cultures in high white ($400 \mu\text{E m}^{-2} \text{s}^{-1}$) and low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

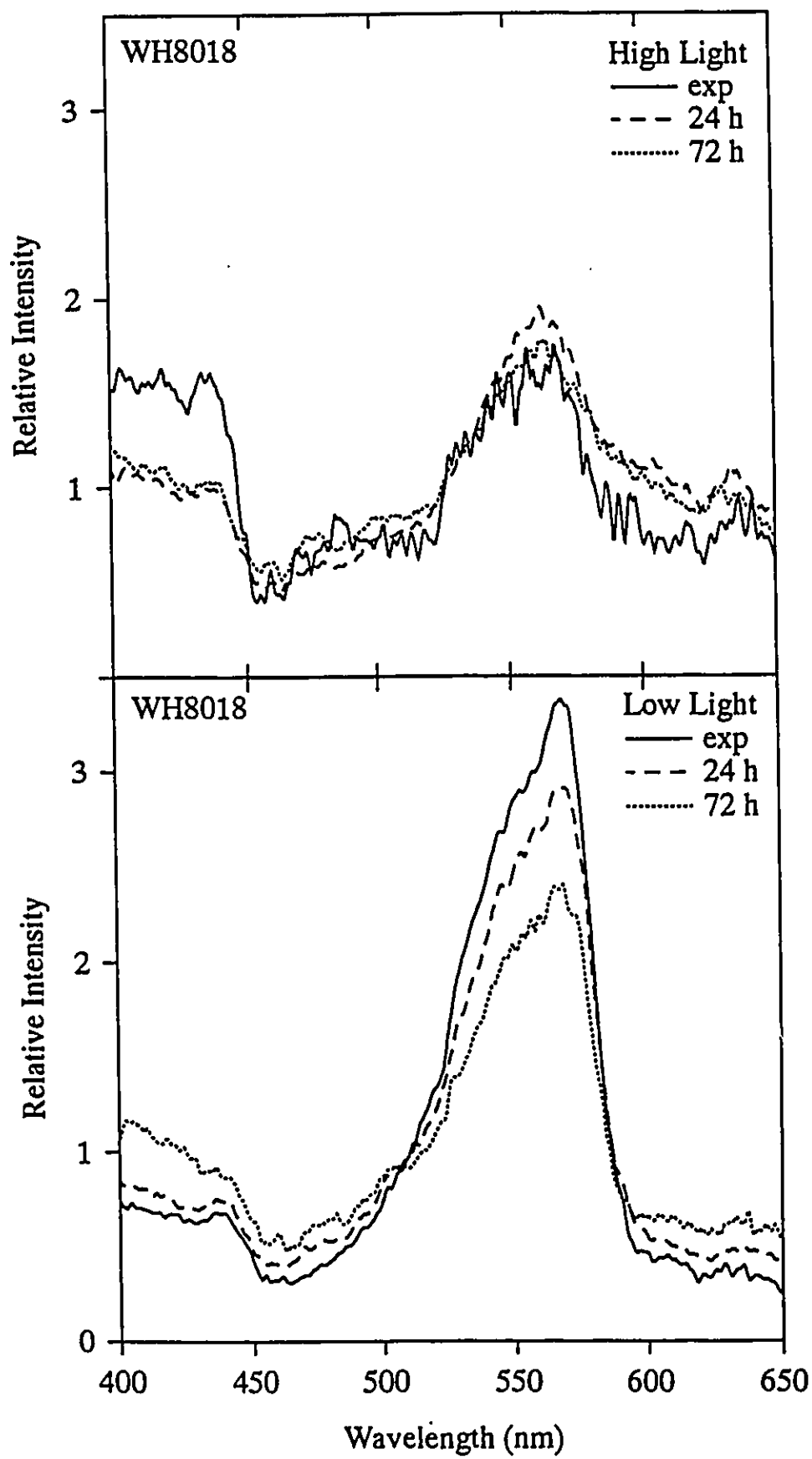


TABLE 26. Pigment ratios (w/w) and excitation ratios, F(PE/Chl a), F(PE/PC), and F(PE/PU), for Chl a emission at 680 nm, of strain WH8018 in 400 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (HW) and 28 $\mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH8018 strain was grown in nitrogen depleted batch culture. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (\pm 1 std. dev.). Missing std. dev. indicates only one PC content value available. * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	PE/Chl a	F(PE/Chl a)	PE/PC	F(PE/PC)
HW				
exp	1.2(0.4)	0.8(0.2)	1.11	1.1(0.5)
24 h	0.8(0.3)	1.7(0.2)	1.3(0.8)	1.58(0.13)
72 h	0.7(0.1)	1.8(0.2)	2.4(1.1)	1.70(0.08)
LW				
exp	3.2(1.2)	4.9(0.1)*	5.9(0.1)	8.2(0.23)*
24 h	2.8(0.8)	3.6(0.4)	4.9(0.7)	5.56(0.9)
72 h	2.3(0.4)	2.8(0.2)	5.0(0.9)	4.15(0.4)

Figure 22. Effects of nitrogen limitation on the emission spectra, using blue excitation (470 nm), of strain WH8018. WH8018 was grown in nitrogen depleted batch cultures in high white ($400 \mu\text{E m}^{-2} \text{s}^{-1}$) and low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

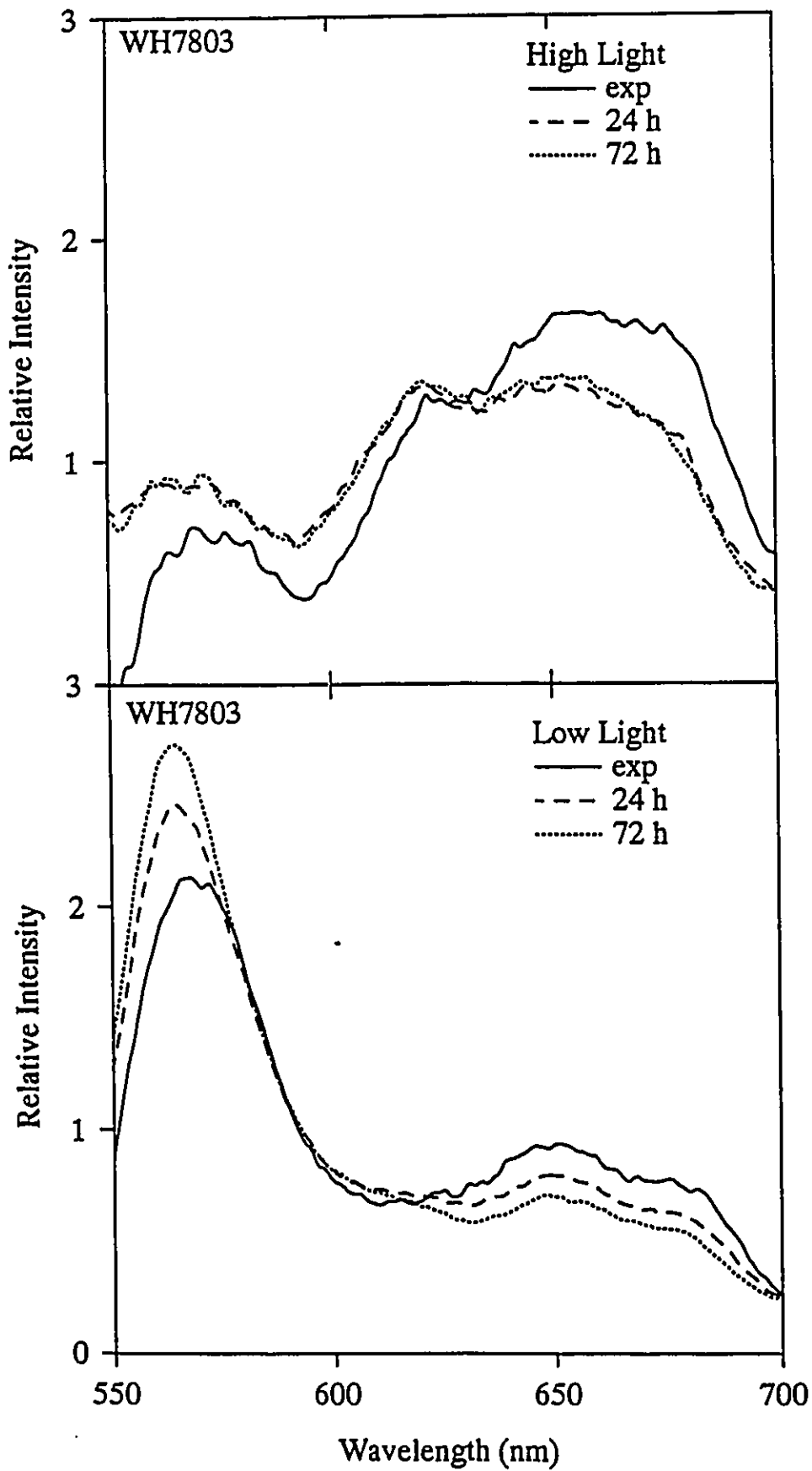


Figure 23. Effects of nitrogen limitation on the emission spectra, using violet excitation (420 nm), of strain WH8018. WH8018 was grown in nitrogen depleted batch cultures in high white ($400 \mu\text{E m}^{-2} \text{s}^{-1}$) and low white growth irradiance ($28 \mu\text{E m}^{-2} \text{s}^{-1}$). Sampling was performed during mid-exponential growth phase (exp), 24 h and 72 h past the onset of stationary phase. Shown are the changes in one culture.

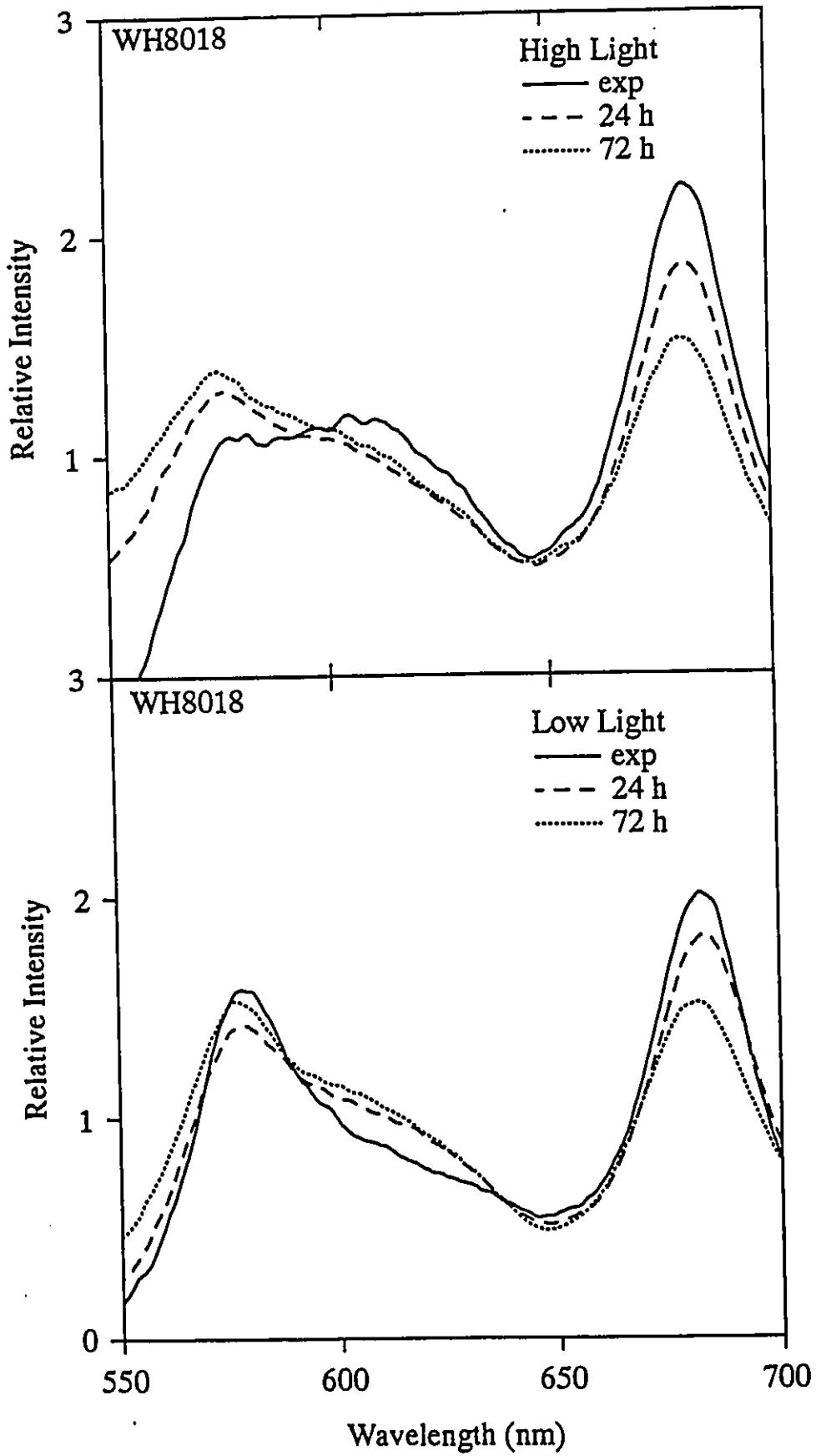


TABLE 27. Emission ratios from 420 nm ex, [V(PE/Chla)], 470 nm ex, [B(PE/Chla)], and the composite 470/420 nm ex, [BV(PE/Chla)] of strain WH8018 in $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW) growth irradiance. WH8018 strain was grown in nitrogen depleted batch culture. Sampling was performed at mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	B(PE/Chla)	V(PE/Chla)	BV(PE/Chla)
HW			
exp	0.24(0.1)*	0.39(0.08)*	0.61(0.08)*
24 h	0.53(0.04)	0.63(0.04)	1.62(0.08)
72 h	0.61(0.02)	0.86(0.11)	1.68(0.05)
LW			
exp	2.18(0.01)*	0.81(0.16)*	1.24(0.14)*
24 h	1.22(0.05)	0.79(0.02)	0.73(0.03)
72 h	1.59(0.03)	0.99(0.01)	0.88(<0.01)

opposite was true for the high light treatments. Changes in emission ratios were most pronounced when the composite ratio (BV (PE/Chl a)) was examined (Table 27)

3.8 Effects of Nitrogen limitation on Fluorescence Yield.

The effect of nitrogen limitation on fluorescence emission normalized per unit Chl a ($F_p/Chl a$) was compared for 3 different pigment types of picocyanobacteria (Table 28). Fluorescence yields remained unchanged for WH8018 or decreased for WH7803 in high light. In low light, nitrogen limitation did not affect $F_p/Chl a$ for strain WH5701 but increased the yield for WH8018. The PE yield (F_p/PE) while it did appear to increase in both PE strains in high light and also increase in low light for WH8018 this was not statistically significant. (Table 29).

TABLE 28. Effects of nitrogen limitation on Chl *a* fluorescence emission (em 680 nm, ex 420 nm) normalized for Chl *a* concentration ($F_c/Chla$) for PC strain WH5701 and PE strains WH7803 and WH8018. Growth was in $400 \mu E m^{-2} s^{-1}$ white (HW) and $28 \mu E m^{-2} s^{-1}$ white (LW) growth irradiance, in nitrogen depleted batch cultures. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$).

	WH7803	WH8018
	$F_c/Chla$	$F_c/Chla$
HW		
exp	42.6(4.0)*	94.2(40.7)
24 h	21.9(3.0)	56.4(16.8)
72 h	13.4(2.7)	39.2(3.2)
LW	WH5701	WH8018
exp	47.8(6.7)	44.3(0.01)*
24 h	35.5(10.1)	35.6(0.5)
72 h	25.25(14.0)	48.6(4.2)

TABLE 29. Effect of nitrogen limitation of PE fluorescence emission normalized for PE concentration (F_p/PE) for WH8018 and WH7803. Growth was in $400 \mu E m^{-2} s^{-1}$ white (HW) and $28 \mu E m^{-2} s^{-1}$ white (LW) growth irradiance, in nitrogen depleted batch cultures. Sampling was performed during mid-exponential growth (exp), 24 h and 72 h past the onset of stationary phase. (± 1 std. dev.). F_p/PE represents 470 nm ex (580 nm em) for WH8018 and F_p/PE represents 470 nm ex (570 nm em) for WH7803. * indicates a significant effect over time as determined by a one way Repeated Measures ANOVA ($n=4$, $p \leq 0.05$). N.A. not available.

	WH7803	WH8018
	F_p/PE	F_p/PE
HW		
exp	8.8(4.0)	13.8(5.2)
24 h	17.7(4.7)	10.6(1.4)
72 h	18.4(1.8)	17.8(4.1)
LW		
exp	N.A.	17.9(4.7)
24 h		9.8(2.5)
72 h		19.4(4.8)

3.9 Natural Populations of Picocyanobacteria.

3.9.1 Picocyanobacteria Abundance in Relation to Temperature and Irradiance in Jack's Lake.

Epi-fluorescence observations revealed that two morphologically different PE-containing populations dominated the picocyanobacteria assemblage of Jack's Lake and represented 70-98% of the total assemblage. One population consisted of a pronounced rod shaped "*Synechococcus* - like" member ($0.5 \times 1.5 \mu\text{m}$) dividing in one plane (PE-Syn) and the other consisted of an ovoid coccoid "*Synechocystis* - like" member ($1 \times 1.5 \mu\text{m}$) dividing in two planes and forming distinct tetrads (PE-Sys). This distinction between *Synechococcus* and *Synechocystis* follows the classification of Rippka et al. (1979).

Depth profiles of total PE-picocyanobacteria abundance and temperature are presented in Figure 24. The maximum abundance of cells, $1.2 \times 10^5 \text{ cells}\cdot\text{mL}^{-1}$, occurred during the Aug. 14 sampling. A small peak in abundance ($8.0 \times 10^4 \text{ cells}\cdot\text{mL}^{-1}$) occurred within the thermocline, between 8 and 10 m (1% of incident light), during the summer. Changes in cell abundance with depth were not as evident on October 8.

Table 30 shows the light history during, and 4 days before the 3 sampling dates. Both July and August sampling days had an increase in total radiation as compared to the average radiation from the preceding 4 days of 18 and 50 %, respectively. Cells in August had previously received a lower light level, and then experienced a large increase in radiation on Aug. 14. During the October 8

Figure 24. Depth profiles of picocyanobacteria abundance, and temperature for the three sampling dates, Sharpe's Bay, Jack's Lake, Ontario, 1990.

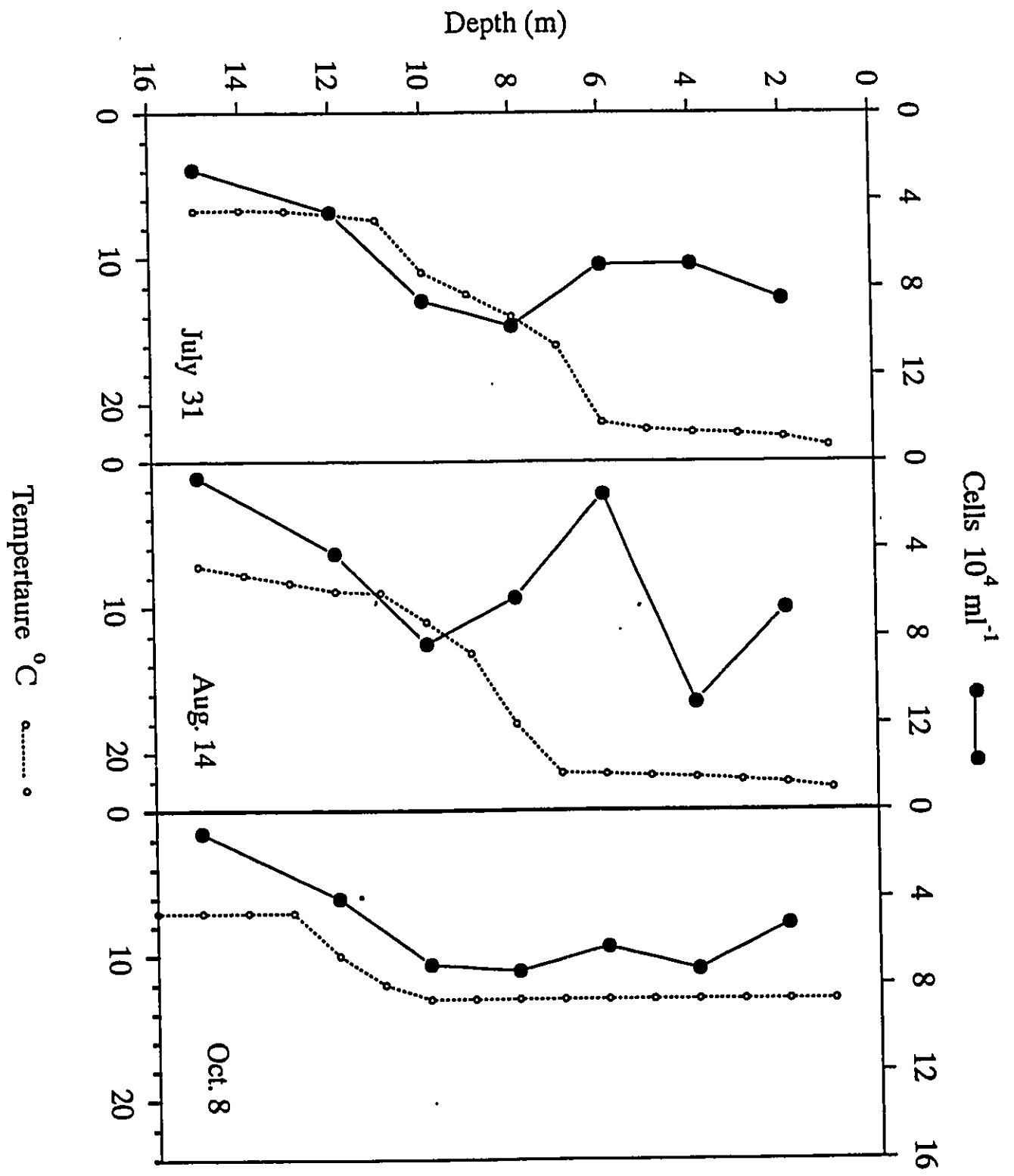


TABLE 30. Daily growth irradiance levels 4 days before and on the sampling dates. The % difference represents the change in radiation for the sampling day as compared to the mean of previous 4 days.

Date	Langely d^{-1}	previous 4 day mean	% difference
July 27	173		
July 28	340		
July 29	243		
July 30	231		
July 31	334	272	+18
Aug. 10	137		
Aug. 11	99		
Aug. 12	105		
Aug. 13	29		
Aug. 14	198	92	+53
Oct. 04	78		
Oct. 05	186		
Oct. 06	169		
Oct. 07	124		
Oct. 08	39	140	-72

sampling picocyanobacteria experienced a drop in total radiation of 72 % as compared to the preceding days.

3.9.2 Excitation Spectra of Chl *a*.

Fluorescence excitation spectra of Chl *a* emission (680 nm) determined on picocyanobacteria cells consistently showed a Type II PE peak at 565 nm \pm 3 nm (Fig. 25 and 26). Excitation spectra for both morphological types were almost identical to those measured from marine strain WH8018. While at times it did appear that PE provided a greater contribution to the excitation spectra from surface cells, this increase was only slight. Blue and red excitation components, from Chl *a* and PC, respectively, were only obvious during the July 31 sampling. Since the other component peaks, besides PE, were not well defined it was not justified to calculate an excitation ratios for the field populations. Comparison of the relative contribution of the PE peak in relation to the blue and red components of Chl *a* excitation for any one day can only be qualitatively assessed. Mean normalized excitation spectra of Chl *a* for the PE-SYS and PE-SYN morphotypes indicate that the contribution of PE was only slightly greater in deep-dwelling cells. Small increases in the contribution of PE at low growth irradiance were consistent with photoacclimation patterns of WH8018 excitation spectra which also showed only slight increases in this ratio between high white and low green growth irradiance.

Figure 25. Comparison of the depth (growth irradiance) response of the normalized excitation spectra for Chl *a* emission at 680 nm for PE-SYN among the 3 sampling dates. Samples from the upper mixed zone are represented by the dotted line and from the 1% light level (I_0) are represented by the solid line, ($n = 4$).

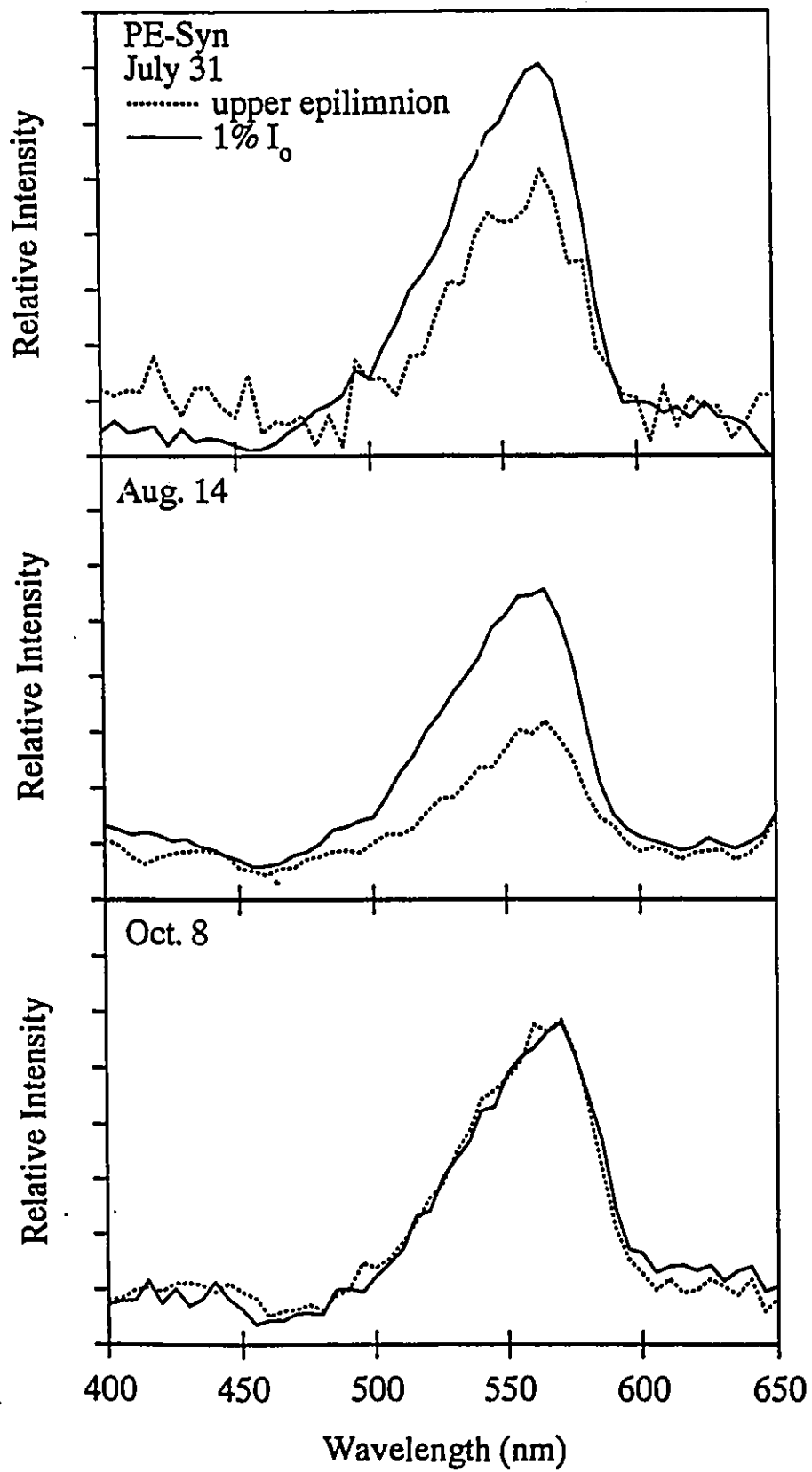
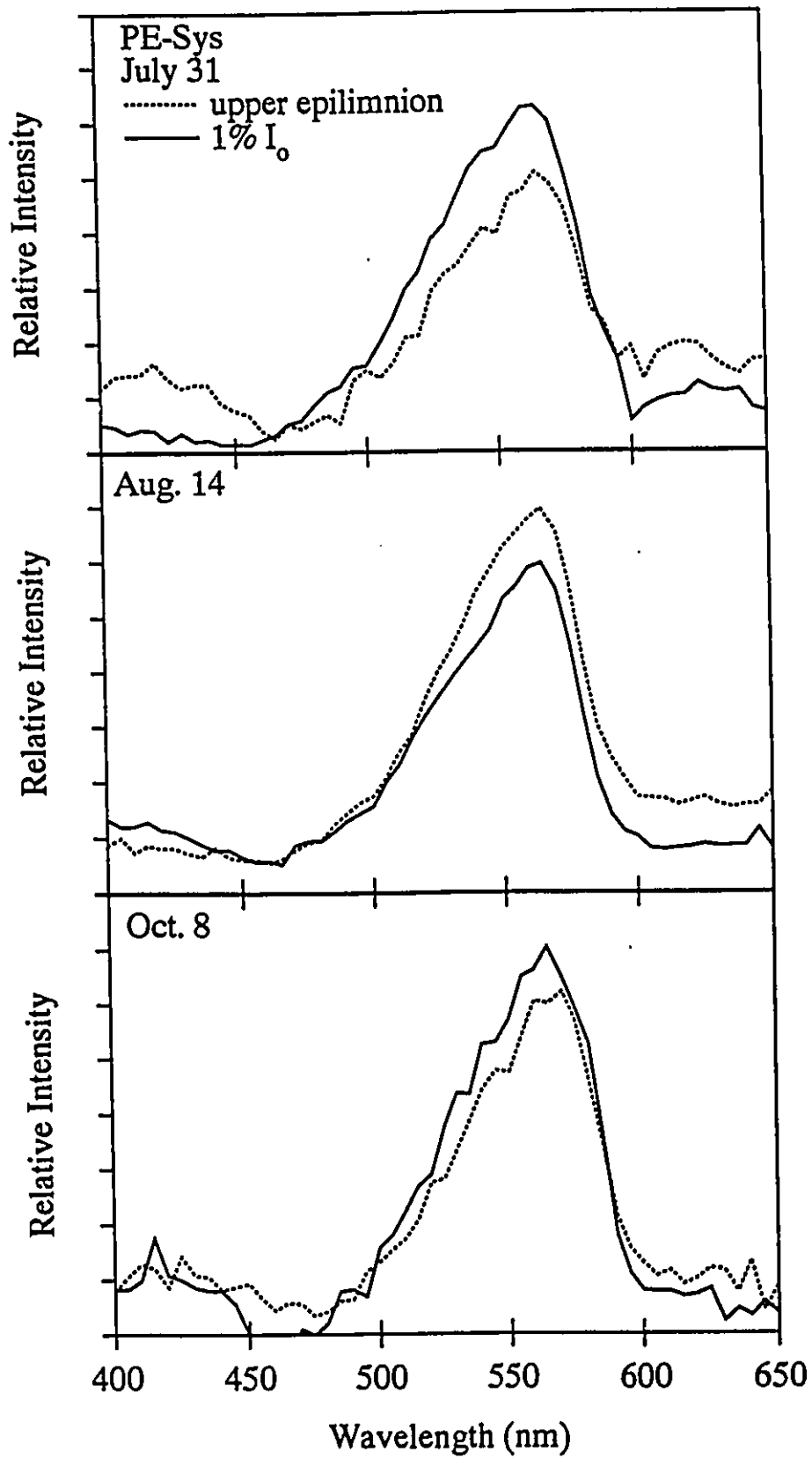


Figure 26. Comparison of the depth (growth irradiance) response of the normalized excitation spectra for Chl *a* emission at 680 nm for PE-SYS among the 3 sampling dates. Samples from the upper mixed zone are represented by the dotted line and from the 1% light level (I_0) are represented by the solid line, ($n = 4$).



Distinctly different excitation spectra were obtained for a centric diatom (*Cyclotella sp.*). This alga exhibited marked changes in the wavelength of peak excitation between epilimnetic (the upper epilimnion, 2 and 4 m) and metalimnetic (1% of incident light, 10 m) cells (Fig. 27); *Cyclotella* cells in the upper epilimnion showed a maximum excitation between 400-450 nm while deeper dwelling cells had a peak excitation between 430-480 nm.

3.9.3 Depth Trends in Emission Spectra.

Figure 28 shows mean normalized emission spectra obtained using blue excitation for the two morphotypes during August 14. Cells growing at low irradiance (1% light level) showed a greater PE (580 nm) relative to Chl *a* (680 nm) emission. While the two morphological types could not be distinguished using excitation spectra, emission spectra showed distinct difference in the PC peak at 665 nm. This peak, attributed to phycocyanin of APC, was always more pronounced in PE-Sys, and appeared mainly as a shoulder of the Chl *a* emission peak for PE-Syn.

The two morphotypes not only differed in their relative emission peaks, but also differed in the emission response to photoacclimation (Fig.29a). The ratio of blue excited PE to Chl *a*, summarized as $B(PE/Chl a)$, was always lowest for the PE-Syn morphotype. In addition, this ratio did not change with depth for PE-Syn, but increased 2 fold, from 1.5 to 3.75, for PE-SYS at 12 m (0.5 % light level), ($p < 0.002$). This was analogous to the response of the Type II strain. WH8018 also doubled $B(PE/Chl a)$, from 0.23 to 0.76, between high white and low

Figure 27. Effect of depth (growth irradiance) on the normalized excitation spectra for Chl *a* emission at 680 nm for a centric diatom from the upper mixed zone (dotted line), and from the 1% light level (I_0) (solid line). Sample was taken on July 31, (n = 2).

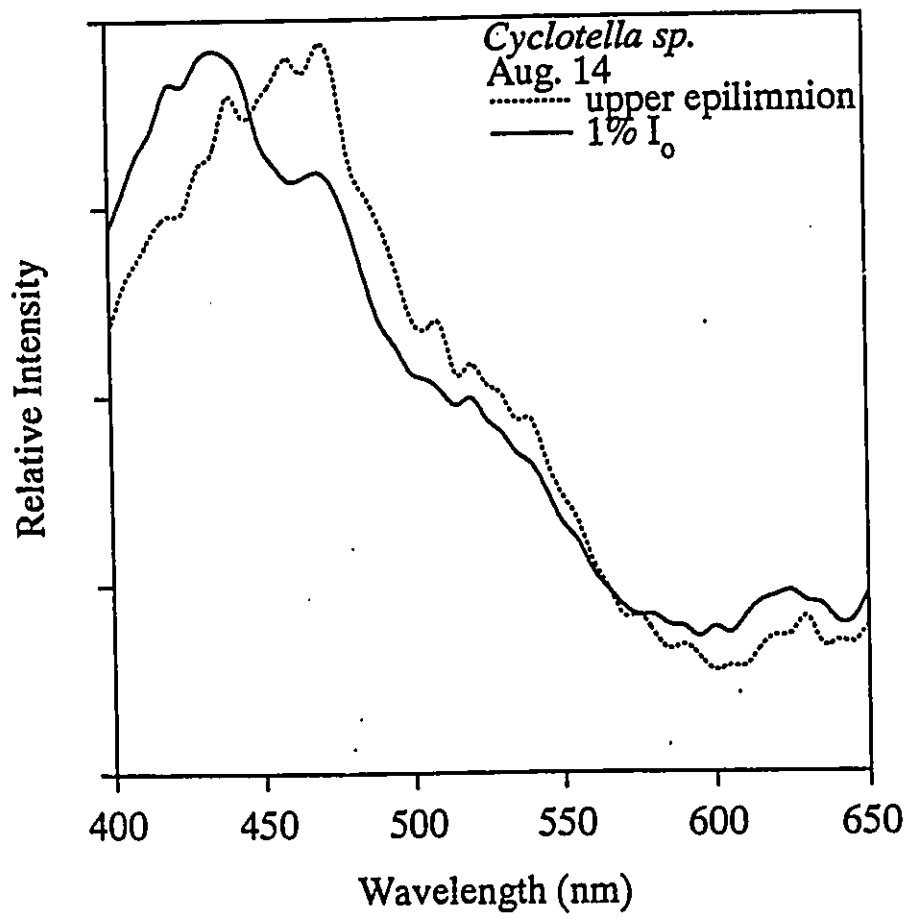
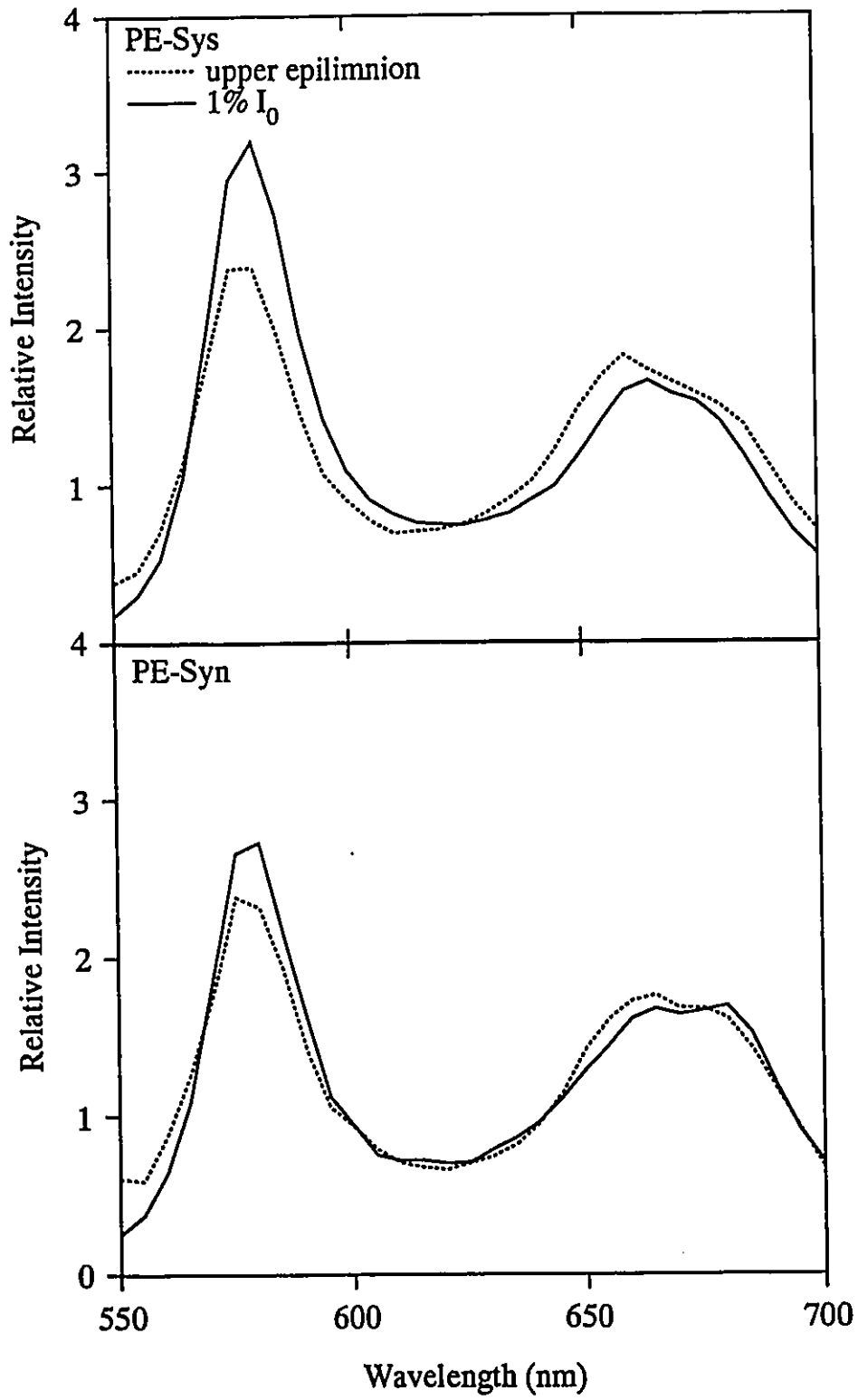


Figure 28. Examples of normalized emission spectra, using 470 nm excitation, for PE-SYN and PE-SYS from the upper mixed zone (dotted line), and the 1% light level (I_0) (solid line). Samples were taken on Aug. 14, 1990, ($n=4$).



green growth irradiance, respectively. In general the ratio of the PE to PC emission peaks, summarized as $B(PE/PC)$, increased with depth ($p < 0.022$) (Fig. 29b).

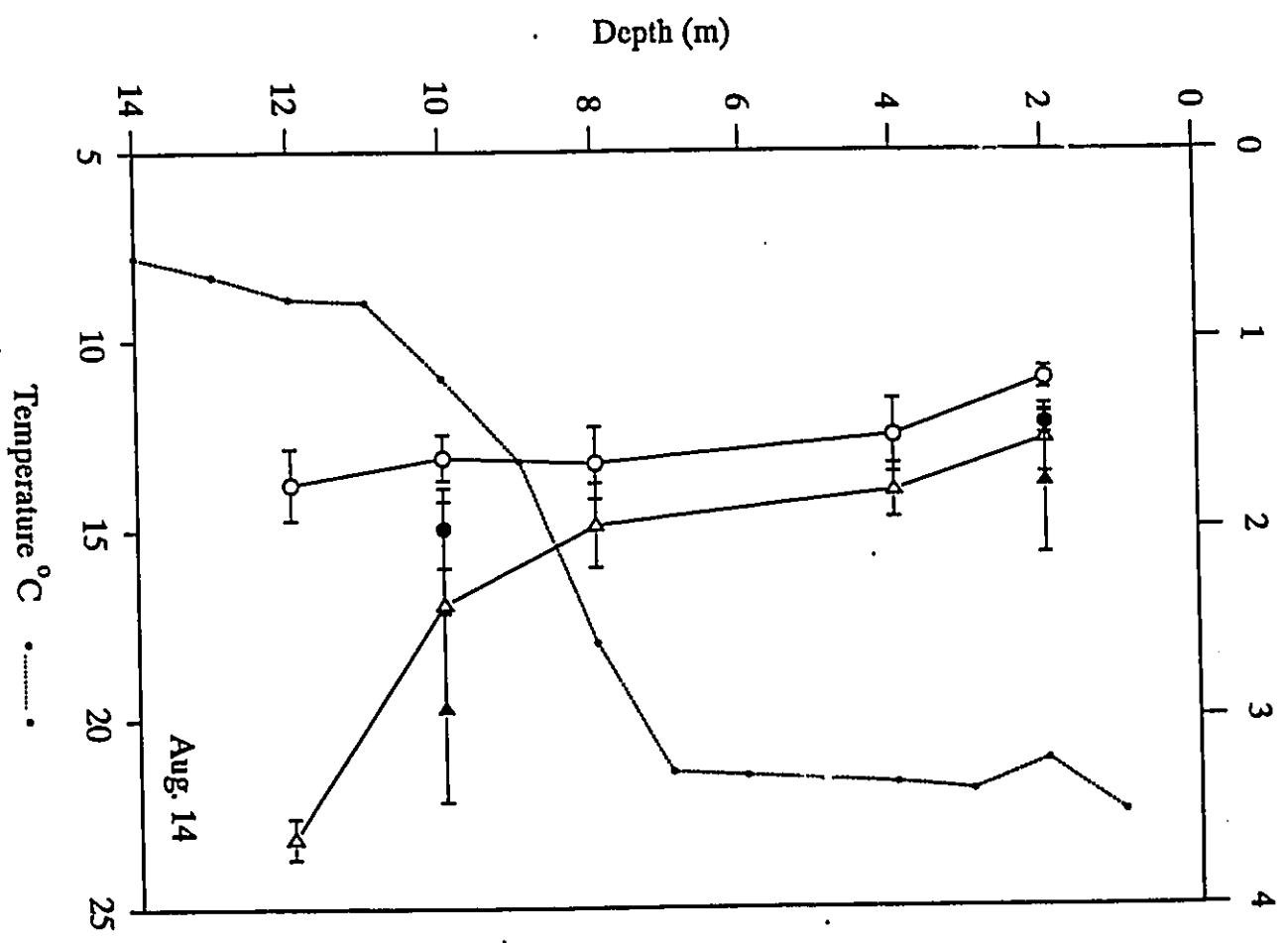
Figure 30 shows the variation in $B(PE/Chla)$ between high and low growth irradiance cells for both morphological types of picocyanobacteria on the three sampling dates. The expected increase in the emission ratio during growth at low growth irradiance (10 m) was not always evident. On July 31, the emission ratio of PE-Syn was significantly higher at depth ($p < 0.003$) while that of PE-Sys remained relatively unchanged. On August 14, the opposite pattern was observed: the emission ratio of PE-Syn remained relatively unchanged with depth while that of PE-Sys was significantly higher at depth ($p < 0.050$). During the Oct. 8 sampling, both PE-Syn and PE-Sys had similar mean fluorescence peak ratios (with large standard errors) for cells from the upper epilimnion and the 1 % light level. Data from the Oct. 8 sampling may be simply the result of recent mixing of the water column, which resulted in a combination of cells that had both high and low light histories (Ganff et al. 1990) (Table 30).

3.9.4 Effects of Nitrogen Addition on Emission Spectra.

During the August 14 sampling, $B(PE/Chla)$ appeared to increase for all cells with the addition of nitrogen (Fig. 29a). This effect was significant particularly for PE-Syn at 10 m ($p < 0.018$). In contrast there was no significant change in $B(PE/PC)$ to nitrogen addition for both PE-Syn and PE-Sys morphotypes (Fig. 29b).

Figure 29. Depth trends in emission ratios and temperature for Aug. 14, 1990. a) using 470 nm ex; B(PE/Chla), b) using 470 nm ex; B(PE/PC). PE-SYN; opened circles, PE-SYN plus nitrogen addition; closed circles, PE-SYS; open triangles, PE-SYS plus nitrogen addition; closed triangles. Error bars represent ± 1 SE, $n = 7$.

B(PE/Chia)



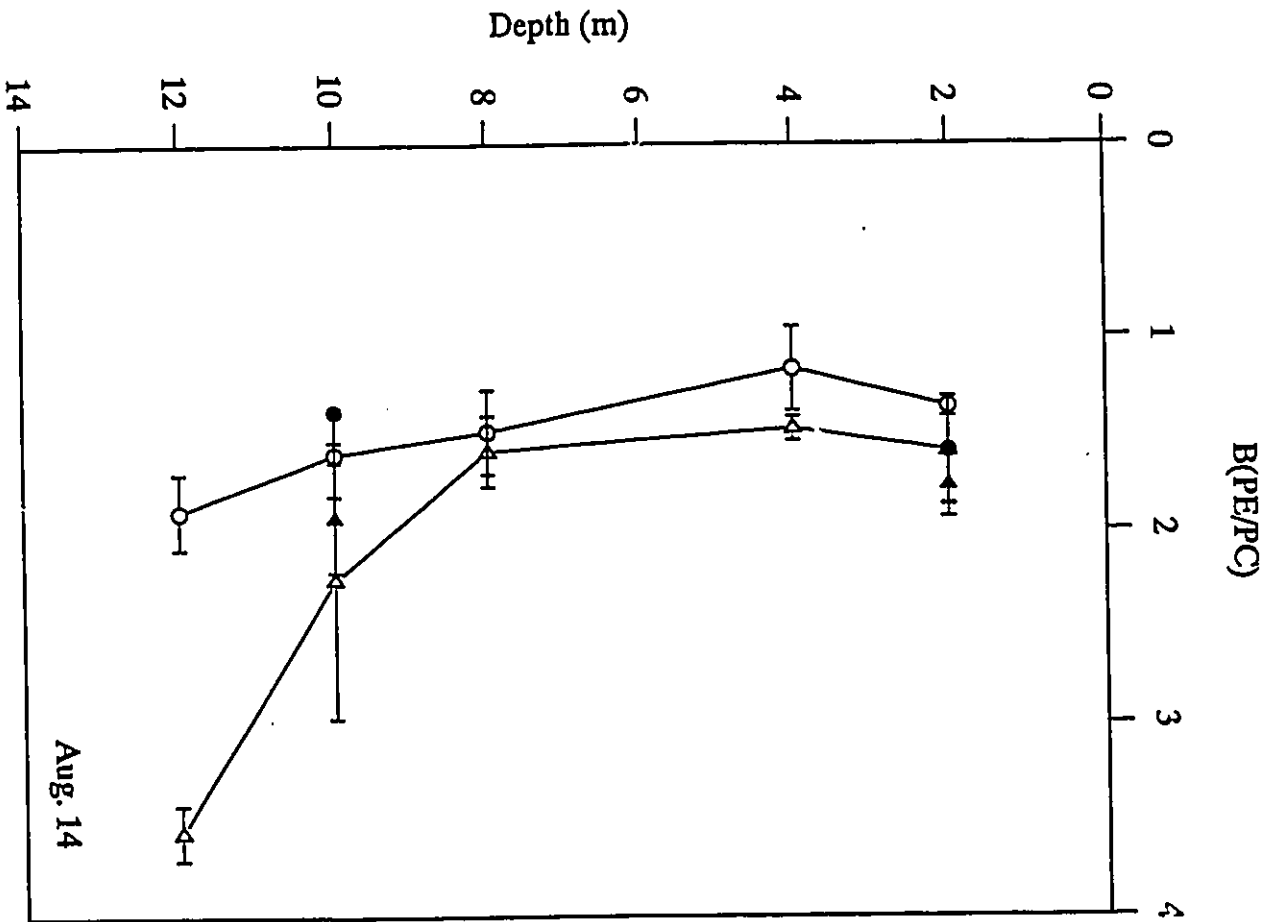
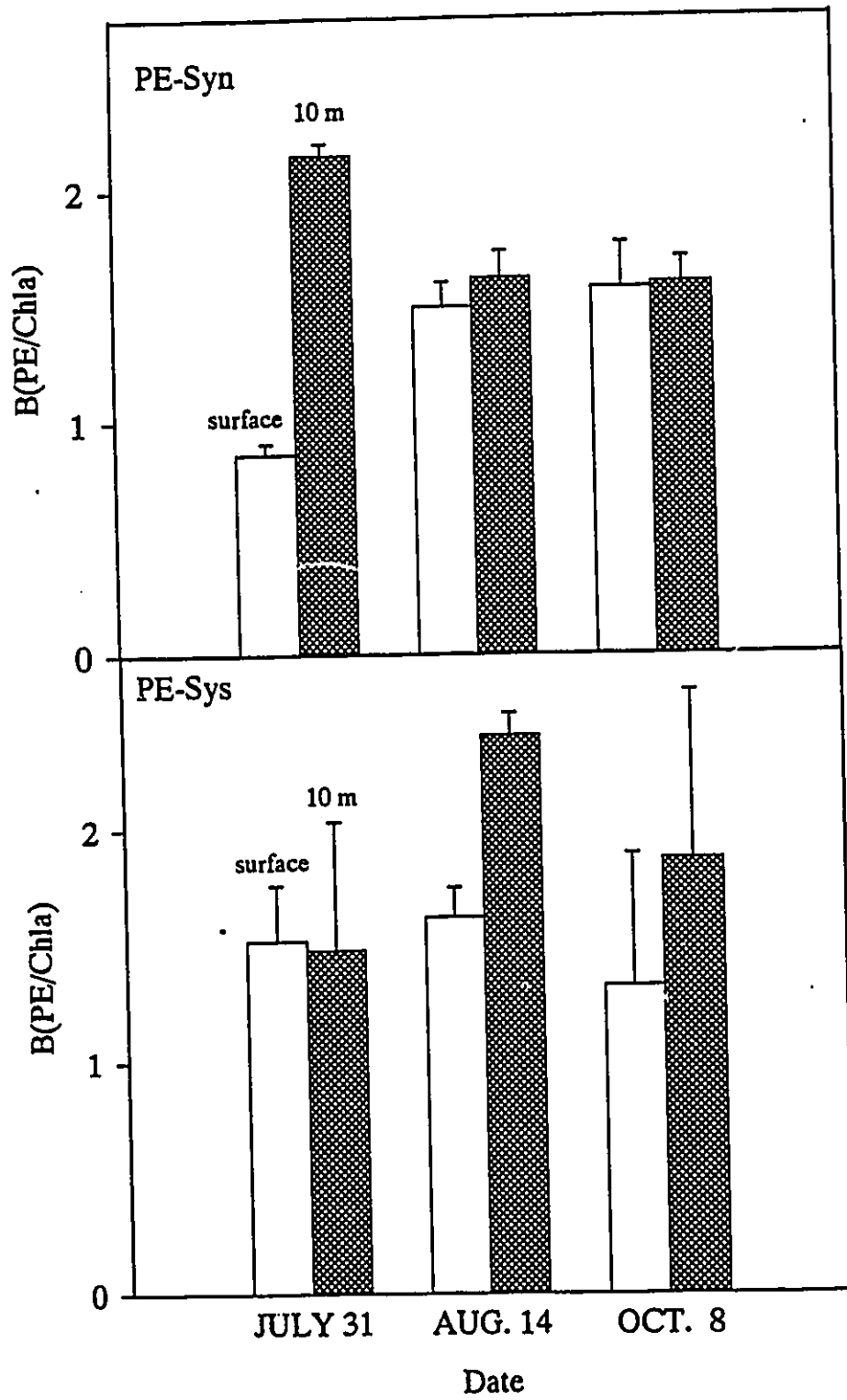


Figure 30. Emission peak ratio, using 470 nm ex, for the upper mixed zone (open bars) and 10 m (closed bars) compared among sampling dates for the PE-SYN and PE-SYS. Error bars represent ± 1 SE, $n=7$.



DISCUSSION

4.1 Photoacclimation in Picocyanobacteria: fluorescence tracks pigment changes.

This study has shown that photosynthetic pigments and in particular the accessory pigments increase in response to decreasing growth irradiance thus corroborating an extensive literature on "shade" adaptation in other algae (Richardson et al. 1983). In the PE-picocyanobacteria studied, phycoerythrin was the most plastic photosynthetic pigment (Table 4, 6, Kana and Glibert 1987a). However, the trends in pigment content between high and low growth irradiance differed slightly depending on how pigment content was expressed (per cell or per carbon).

Since carbon per cell was reduced by increased growth irradiance (Table 3, 5) some of the changes in pigment content may have been due to changes in cell size. It has been shown that at high growth irradiance or high growth rates cell size is reduced (Kana and Glibert 1987a, Glibert et al. 1986) and by volume constraints must therefore lead to reduced pigment content per cell. In WH7803 the observed reduction in pigments at high light was greater when pigments were expressed on a per cell basis (Table 4). In WH8018 this was also the case for chlorophyll but not phycoerythrin: the reduction in PE per unit carbon during growth at high growth irradiance was in fact more pronounced than if PE was expressed on a per cell basis (Table 6). Hence the changes in PE in WH8018 were mostly due to changes in growth irradiance.

The excitation spectra show clearly the contribution of the various pigments to Chl *a* excitation during photoacclimation (Fig. 5,7). Since the PE measurements at times approached detection limits, fluorescence provided a better index of photoacclimation processes at PSII than pigment content. The MANOVA (Table 10, 11) indicated that all of the fluorescence ratios of PE/Chl*a* (excitation or emission) were positively and significantly related to each other and to PE content.

Photoacclimation strategies, other than the short term State I and State II transitions, are related to changes in the reaction centres and light harvesting pigments (the photosynthetic unit, PSU) (Richardson et al. 1983). These strategies involve either changes in the size of the PSU or changes in the numbers of PSU. Kana and Glibert (1987b) have shown for WH7803 that during reductions in white light from 2000 to 700 $\mu\text{E m}^{-2}\text{s}^{-1}$ the change in Chl *a* reflected a change in the stoichiometry of PSI and PSII and/or an increase in the antenna size of PSI. Further reductions in light (but above light saturation 150 $\mu\text{E m}^{-2}\text{s}^{-1}$) caused an increase in phycobilisome size which was indicated by an increase in the PE/PC ratio. Below light saturation photoacclimation processes were dominated by changes in the number of PSU, because PE and PC increased proportionately to one another. In the present study, low growth irradiance led to increases in F(PE/Chl*a*) and F(PE/PC) implying only an increase in size of PSU. However, more than 2 growth irradiances are required to determine the photoacclimation strategy and the green growth irradiance experiments (below) provided this information.

4.2 Effects of Light Quality: evidence for chromatic acclimation.

Most studies of phytoplankton photoacclimation have examined responses of individual species confronted with an increase or decrease in white growth irradiance (Vita lights or cool fluorescent light), (e.g. Foy et al. 1976, Prezelin and Sweeny 1978, Raps et al. 1983, Kana and Glibert 1987a). Even in field experiments, natural populations are often incubated under white growth irradiance which is filtered through neutral density filters (e.g. Glover et al. 1985a, b, Prezelin et al. 1986). However, the natural light environment is not a gradient of white light, it is a gradient between high white growth irradiance to low growth irradiance of a restricted and specific spectral distribution.

Water acts like a red monochromatic filter. In natural waters the presence of varying amounts of dissolved yellow substances (gelbstoff or gelvin) tends to restrict the light spectrum to wavelengths between 480 nm to 650 nm (Jerlov, 1976). Often the spectral distribution of the penetrating wavelengths corresponds closely to the trophic state of the body of water: blue, green and red water are typical of oligotrophic, mesotrophic and eutrophic systems respectively.

Several studies have stressed the importance of light quality on algal growth (Wyman and Fay 1986b, Hauschild, McMurter and Pick 1991), photosynthesis (Glover et al. 1986b, 1987, Prezelin et al. 1989, Prezelin and Glover 1991, Takahashi et al. 1989) and community structure (Wall and Briand 1979, Vernet 1983, Bidigare et al. 1989, Ondrusek et al. 1991, Pick 1991). But the major review articles on photoacclimation do not or cannot reasonably conceive of chromatic adaptation as a useful ecological strategy (Richardson et al. 1983,

Falkowski and LaRoch 1991). This view has developed because studies have been conducted on algal classes which do not undergo classical chromatic adaptation (eg. Morel et al. 1987 for diatoms), and/or because changes in pigmentation due to light quality are thought simply to mimic an intensity effect (Saffo 1987). Kirk (1983) theorizes that during evolution the common association between low growth irradiance and the virtual absence of some wavelengths of light led to the development of a regulatory mechanism which responds automatically to low light as well as making the cell better suited to absorb the available wavelengths. However, pigment and fluorescence data from the present study indicate that a unique mechanism exists for acclimation to low green growth irradiance in some picocyanobacteria.

Chromatic acclimation, as mentioned previously, is the distinct synthesis of PE over PC in response to the spectral quality of light and has been shown to occur in a number of cyanobacteria strains (Bennett and Bogorad 1973, Tandeau de Marsac 1977). To distinguish a chromatic effect from an intensity effect one must first determine how green light is "perceived" by cells in terms of usable radiation. Theoretically, green light, because it excites specifically the phycobiliproteins of PSII, should be perceived as a higher growth irradiance (Saffo 1987).

The photosynthetic usable radiation (PUR) is the product of the spectral distribution of PAR and of the *in vivo* absorption (Morel et al. 1987). In low growth irradiance grown picocyanobacteria light absorption by whole cells is not affected

by the "package effect" and is largely due to PE (Bidigare et al. 1989, Kana et al. 1988). Thus in this case one can use the absorption spectrum of the extracted PBP rather than that of whole cells in the calculation PUR. Figures 31a and 31b show that green light provides a higher growth irradiance (25%) to WH8018 than to WH7803. Further, the total growth irradiance for WH8018 may be actually 50 % greater in green light than in white light for the same amount of PAR. Green light for strain WH7803 only represents a 16% increase over that of white light.

Figure 31a. Graphical representation of the photosynthetic useable radiation as available to PBP absorption for strains WH7803 and WH8018 during exponential growth in low white growth irradiance.

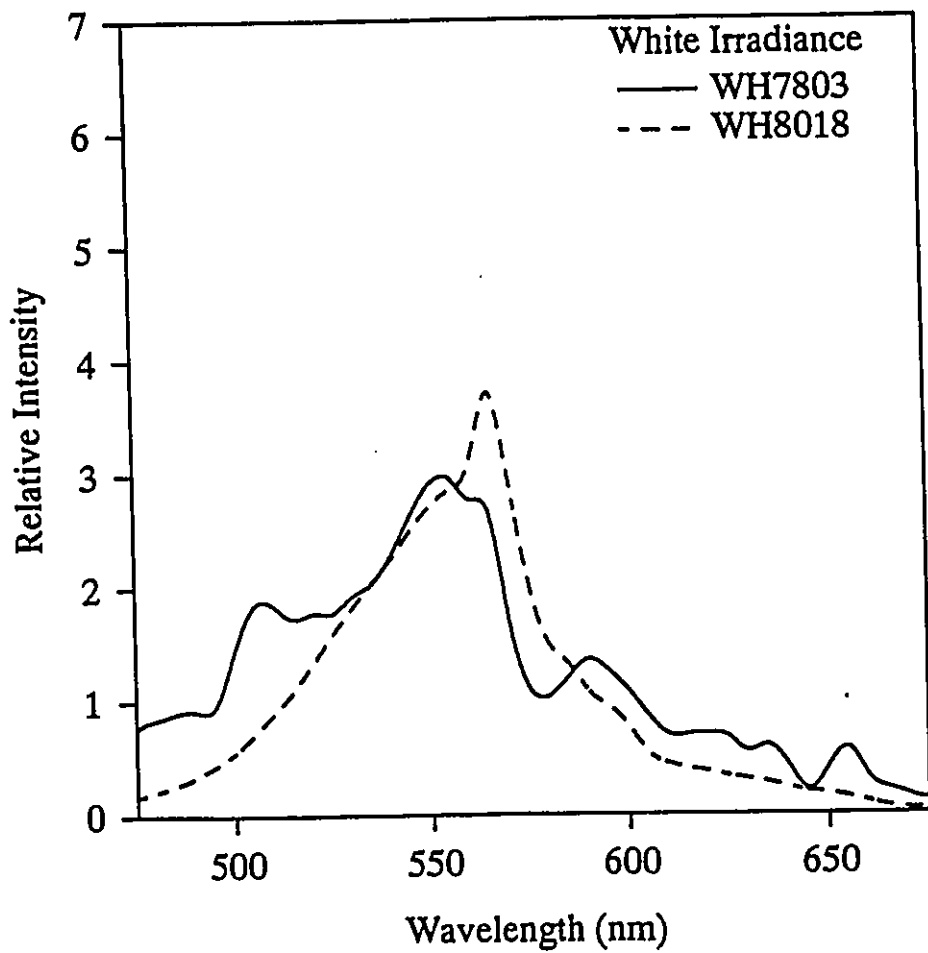
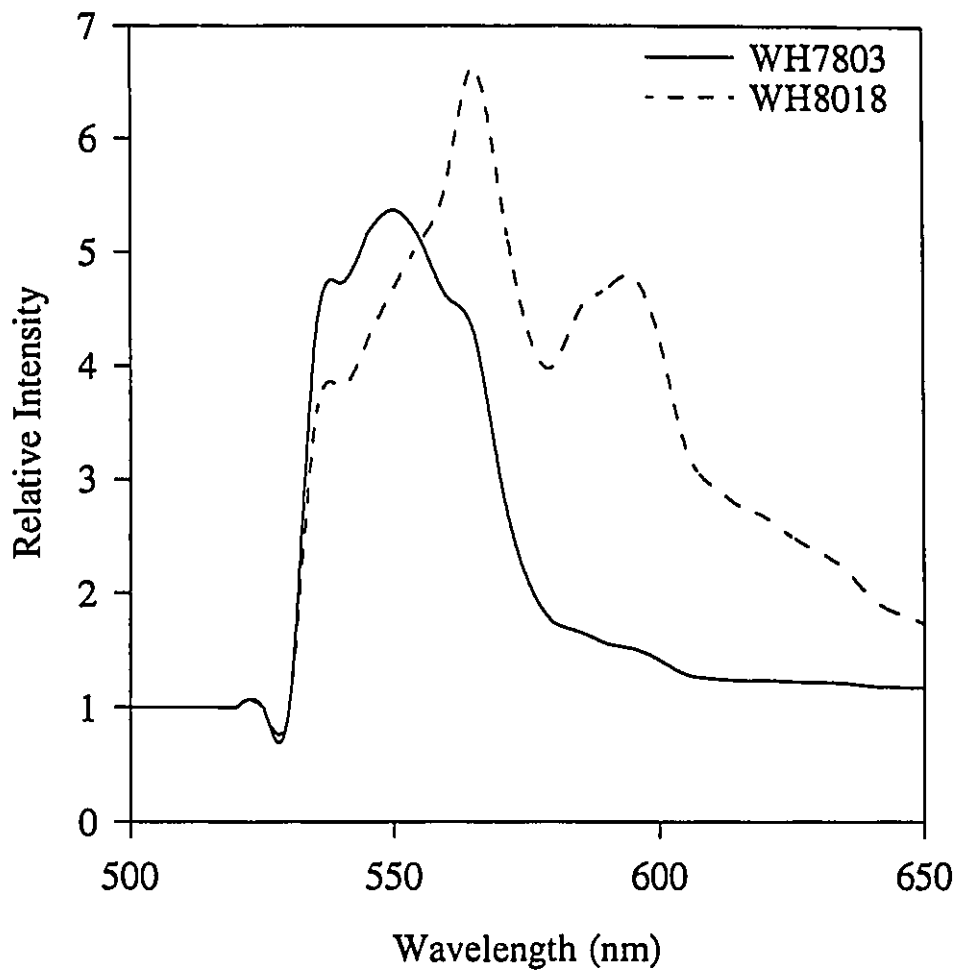


Figure 31b. Graphical representation of the photosynthetic useable radiation as available to PBP absorption for strains WH7803 and WH8018 during exponential growth in low green growth irradiance.



Acclimation from high white to low green growth irradiance for strain WH8018 was dominated by stoichiometric adjustments between PSII and PSI. Little change in PSII PSU size occurred during photoacclimation to low green light as indicated by the unchanging F(PE/Chl a) or F(PE/PC) ratios (Table 7). Therefore, green light in many respects mimicked a high intensity for WH8018. For strain WH7803, photoacclimation to low green growth irradiance was both by photosystem stoichiometric adjustments (increases in Chl a/C , Table 4) as well as increases in PSII PSU size (increases in F(PE/Chl a) and F(PE/PC) ratios, Table 7). Since Kana and Glibert (1987a) showed that the PE/PC ratio during light limiting conditions (up to 150 $\mu\text{E m}^{-2}\text{s}^{-1}$) remains constant, the decrease in PE/PC at 28 $\mu\text{E m}^{-2}\text{s}^{-1}$ of green light was not expected. Previous workers have stated that chromatic acclimation does not exist in the genus *Synechococcus* (Waterbury et al. 1986). If adjustments in numbers of PSU are the main acclimation strategy for WH7803 during light limiting conditions (Kana and Glibert 1987b), chromatic acclimation can be further distinguished as size adjustments in the photosynthetic unit of PSII by changes in the phycobilisome (PE and PC) and not between Chl a and PE. Chromatically induced decreases in PSII represent a distinct photoacclimation strategy, which can be only detected at low growth irradiance.

4.3 Effects of Nitrogen Deficiency.

There are various biochemical indices of light and nutrient limitation. High light adapted phytoplankton have low pigment content but high polysaccharide and lipid reserves because photoassimilated carbon is in excess of metabolic

requirements. The ratio of carbohydrate to protein or particulate organic carbon to nitrogen (C/N) can be used to identify which factor (light or nitrogen) potentially limits algal growth (Healey 1975).

Nitrogen requirements can be expected to be greater at low growth irradiance for two reasons. First, in cyanobacteria more protein is associated with the PBP than the chlorophyll-protein complexes (Raven 1984), thus reduced excitation of PSII at low growth irradiance would effectively increase the protein content. Secondly low growth irradiance leads to a greater incorporation of carbon into protein relative to carbohydrates and lipids (Pick and Cuhel 1986). One would predict then that under light limiting conditions C/N ratios may be somewhat lower. Table 3 and 5 show that carbon to nitrogen ratios were similar during the adapted growth in high and low white, as well as in low green growth irradiance. However, the ratios of C:N did change during transitions between one adapted state to the next (Table 20 and 24).

Stationary phase or the limitation of further growth by nitrogen stress was indicated by increases in C:N. The relative increases in C/N were greatest for Type III PC strains (WH5701, *S. leopoliensis*, and *Synechocystis*), followed by the PE strains WH7803 then WH8018 (Table 12). Only the PC strains exhibited the expected decline in the phycobiliproteins (Allen and Hutchinson 1980, Yamanaka and Glazer 1980, Stevens et al. 1981, Carr 1988): a mechanism for mobilizing nitrogen when the exogenous supply of nitrogen is depleted. Chlorophyll *a* was not so readily affected (Table 13).

Often nitrogen stress in cyanobacteria decreases the size of the PSII antennae in relation to the reaction centers, but PSU density is maintained (Chl *a* remains unaltered) (Yamanaka and Glazer 1980, Turpin 1991). Reduction in PSII antennae could be observed in this work by the reduction in peripheral bilins. This was most evident in excitation fluorescence spectra which showed the progressive loss of phycocyanin (not APC) in the PC strains (Fig. 12) and phycoerythrin (not PC) in the PE strains (Fig. 15, 18, 21). Among the PE-strains, 48B66 was less affected than WH7803 followed by WH8018 (Table 18, 22, 26): the more PUB present the less the strain was affected by nitrogen stress.

Glibert et al. (1986) explored nitrogen limitation of batch growth in strains WH7803 and WH8018. They also observed increases in size and density of PSII by increases in both PE and Chl *a*, but they attributed these results to decreasing light in more turbid cultures at peak biomass. Cultures in the present study may also be undergoing a decrease in light. Nitrogen and irradiance had opposite effects on the C/N ratio. The Type I PE strain, which had the greatest proportion of phycourobilin, 48B66, appeared to show no signs of nitrogen limitation from the C/N ratios but did show signs of light limitation by the greater fluorescence ratio $F(PE/PC)$. Glibert and workers (Glibert et al. 1986, Ray and Glibert 1990) have shown that, given the same nitrogen content, WH8018 became more protein starved than WH7803. Obviously there exists strain differences in response to low nitrogen or light availability (Ray and Glibert 1990, Cochlan et al. 1991).

Nitrogen limitation was studied in 2 PE strains at both high and low light. At

high white growth irradiance changes in light availability due to culture turbidity had a greater effect on pigment content and fluorescence ratios than nitrogen limitation. Nitrogen limitation was difficult to achieve in the Type I PE strains, 48B66 and WH7803.

4.4 Effects of Growth Rates on Fluorescence.

So far this thesis has found that changes in fluorescence ratios tracked the changes in pigment content during photoacclimation and or nitrogen stress. The MANOVAs indicated that growth significantly co-varied with the fluorescence ratio $BV(PE/Chla)$ (Table 10, 11). For strain WH8018 growth also co-varied with PE/C . The interpretation of this correlation is not straight forward but it does imply that differences in growth rate may account for some of the differences in fluorescence ratios. As the MANOVA for WH8018 was dominated with data from low light growth regimes (low white and low green), the amount of fluorescence may only co-vary with growth at low light.

These correlations can be interpreted in terms of current models of photosynthesis and growth in algae (Prezelin et al. 1991). The product of pigment/C and the absorption coefficient of the pigment is equal to the absorption cross-section of cellular carbon. In the case of picocyanobacteria the absorption cross-section of the cell is effectively proportional to the pigment content by virtue of the small size of the cell. The absorption cross-section can be used to predict growth and photosynthesis (Cullen 1990): thus one would expect that at adapted states changes in pigment/C and light explain most of the variation in growth for

picocyanobacteria. Conversely this implies that fluorescence ratios vary in proportion to not only to light intensity but also to growth rate.

The effect of growth on Chl *a*/C is better known than for PE/C. According to Langdon (1988), and Sosik and Mitchell (1991), Chl *a*/C increases linearly with growth in several algae when growth is manipulated by a limiting nutrient. However, when growth is manipulated by growth irradiance, Chl *a*/C is constant up to the light saturation level after which it declines asymptotically. These patterns may be somewhat similar for PE/C in cyanobacteria. Healey (1985) found that with a PE picocyanobacteria, Chl *a*/C did not vary significantly over light limiting conditions, but PE/C changed significantly with the growth rate. This was also found for WH7803 (Kana and Glibert 1987b): at light saturation, growth was maintained at a maximum, and PE/C was directly related to intensity and not growth. During light limitation PE/C did not vary with intensity implying that a minimum content of PE is required to maintain maximal growth rates.

WH8018, at any given growth irradiance, had lower growth rates when compared to WH7803 and in general had lower fluorescence ratios, $F(\text{PE}/\text{Chl}a)$, $B(\text{PE}/\text{Chl}a)$, $V(\text{PE}/\text{Chl}a)$ and $BV(\text{PE}/\text{Chl}a)$. If all parameters for both strains were pooled as if they were the same strain, stronger correlations between PE/C, fluorescence ratios and growth were found by MANOVA (Appendix IV). As Chl *a*/C was not significantly correlated with any fluorescence or growth parameter, it would appear to have little bearing on the fluorescence ratios of low light grown PE picocyanobacteria.

4.5 Fluorescence Yield in Relation to Growth irradiance and Nitrogen Stress.

The fact that all the fluorescence ratios tracked changes in PE does not necessarily imply that fluorescence intensity is a linear function of pigment content. According to equation 1 (section 1.3) fluorescence yield should remain constant if photosynthesis is at steady state. However, significant differences were found in acclimated picocyanobacteria fluorescence yield of PE (F_p/PE , Table 9) among the different light regimes. It is possible that the low yields at high growth irradiance are related to a unique quenching of PE (Manodori and Melis 1986). Contrarily, it could be simply an artifact of experimental design; spectrofluorometric response signals were not fully corrected to examine fluorescence yield. Certainly high variations in PE content, within a treatment, would contribute to low ANOVA power (Dayn and Quinn 1989, Eckblad 1991, Shrader-Frechett and McCoy 1992). Significant differences detected using ANOVA must be viewed with caution (Dayn and Quinn 1989).

In the case of Chl *a* fluorescence, an obvious exception to theory occurs if a significant proportion of Chl *a* is in the non-fluorescent PSI. While it has been observed that most of the photoacclimation process involves a modulation of pigments in PSII, certainly some modulation is specific to PSI. Barlow and Alberte (1985) report a greater reaction centre density in PSI for WH7803 and WH8018 than in PSII near saturating light intensities ($250 \mu\text{Em}^{-2}\text{s}^{-1}$). Even over relatively low energy fluxes (up to $250 \mu\text{Em}^{-2}\text{s}^{-1}$) not all of the response can be accounted for by

changes in photosystem size. Hence, in green light, the over excitation of PBP of PSII could be balanced by increases of Chl *a* in PSI. The net result is an apparent lowering of fluorescence yield ($F_v/Chl\ a$) in green light even though the cells are in steady state (Table 9). Even at steady state, Manodori and Melis (1986) report unique quenching in a PC *Synechococcus* when grown in yellow (PSII) as opposed to red (PSI) light. Certainly a repertoire of mechanisms exist to balance the distribution of quanta between the two photosystems, even in steady state cultures.

In a broader sense, when all experiments with exponentially growing culture are considered together, the fluorescence emission of Chl *a* (ex 420 nm, em 680 nm) was linearly related to Chl *a* content in both WH7803 and WH8018 (Fig. 32a, Fig. 33a). In addition, the slope (the relative yield) was not considered different between the two strains (26.6 for WH7803 vs 28.9 for WH8018). Similarly, the fluorescence of PE for exponentially growing WH8018 (ex 470 nm, em 580 nm) was linearly related to PE content (Fig. 34a).

While fluorescence emission in cultures acclimated to their growth irradiance did not appear to vary, cultures undergoing nitrogen stress showed marked changes in fluorescence yield. As observed before, batch cultures were not only experiencing nitrogen stress but perhaps a decrease in growth irradiance due to increasing culture turbidity. This latter fact may account for the absence of the typical nitrogen stress responses in Chl *a* fluorescence in this study. In only one circumstance did nitrogen stress appear to increase Chl *a* fluorescence; WH8018

grown in low white light. This may be the only growth condition in which slow growth rates did not substantially add to cell biomass in batch cultures.

Nitrogen stress induces a number of changes which potentially decrease photosynthetic energy conversion efficiency. In particular a reduction in the synthesis of specific proteins such as CP43 and CP47 (Falkowski et al. 1989). These Chl *a*-protein complexes, common to all oxygenic photoautotrophs, mediate energy transfer from the Chl *a* light harvesting complexes to PSII reaction centres. A reduction in these proteins relative to light harvesting proteins leads to a decreased energy transfer efficiency causing an increase in fluorescence. This phenomena has been reported in a number of classes of algae (Kiefer 1973, Shimura and Fijitia 1975, Cleveland and Perry 1987, Kolber et al. 1988, Herig and Falkowski 1989). Theoretically, although not documented in the literature, this phenomenon should occur for all antennae pigments including the phycobiliproteins. Indeed during low growth irradiance, when WH8018 did show increased Chl *a* fluorescence yield (Table 28), PE fluorescence yield also increased. Even in other nitrogen stress experiments, which did not show specific enhancement in Chl *a* fluorescence yield, PE fluorescence increased slightly. While nitrogen stress was indicated by a reduction in $F(PE/PC)$, other measurements (increasing C/N and decreasing $F(PE/Chla)$), did not indicate severe nitrogen stress. Increases in PE fluorescence during these conditions then cannot be specifically related to nitrogen stress. Glibert et al. (1986), using single cell flow cytometry, also report unchanging Chl *a* and PE fluorescence during nitrogen depletion in

WH7803 and WH8018. When nitrogen starved and exponentially growing cultures were considered together the fluorescence emission of Chl *a* (ex 420 nm, em 680 nm) was still linearly related to Chl *a* content in both WH7803 (Fig. 32b) and WH8018 (Fig. 33b). Including the nitrogen starved data in the PE regression did not appear to change the regression equation for WH8018 (Fig. 34b).

Figure 32a. Fluorescence emission of Chl *a* (680 nm em:420 nm ex) from WH7803 vs Chl *a* content. Individual cultures during exponential growth are plotted. Line is the result of linear regression with 95% confidence intervals and formulates as:
$$\text{R.F.I.} = 26.6 \text{ Chl } a \text{ mg}\cdot\text{L}^{-1} + 1.43. \quad r = 0.89.$$

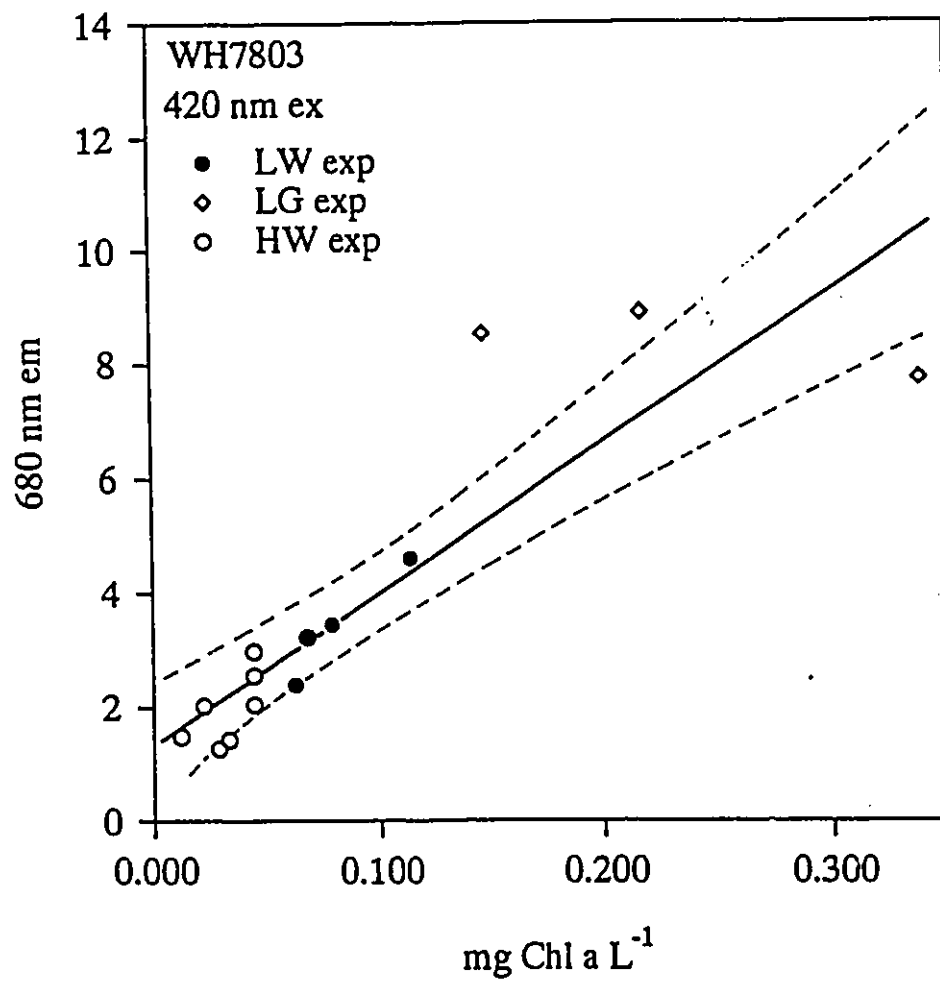


Figure 32b. Fluorescence emission of Chl *a* (680 nm em:420 nm ex) from WH7803 vs Chl *a* content. Individual cultures during exponential and nitrogen stressed growth conditions are plotted. Line is the result of linear regression with 95% confidence intervals and formulates as: Relative fluorescence intensity = $16.6 \text{ Chl } a \text{ mg}\cdot\text{L}^{-1} + 1.55$. $r=0.75$.

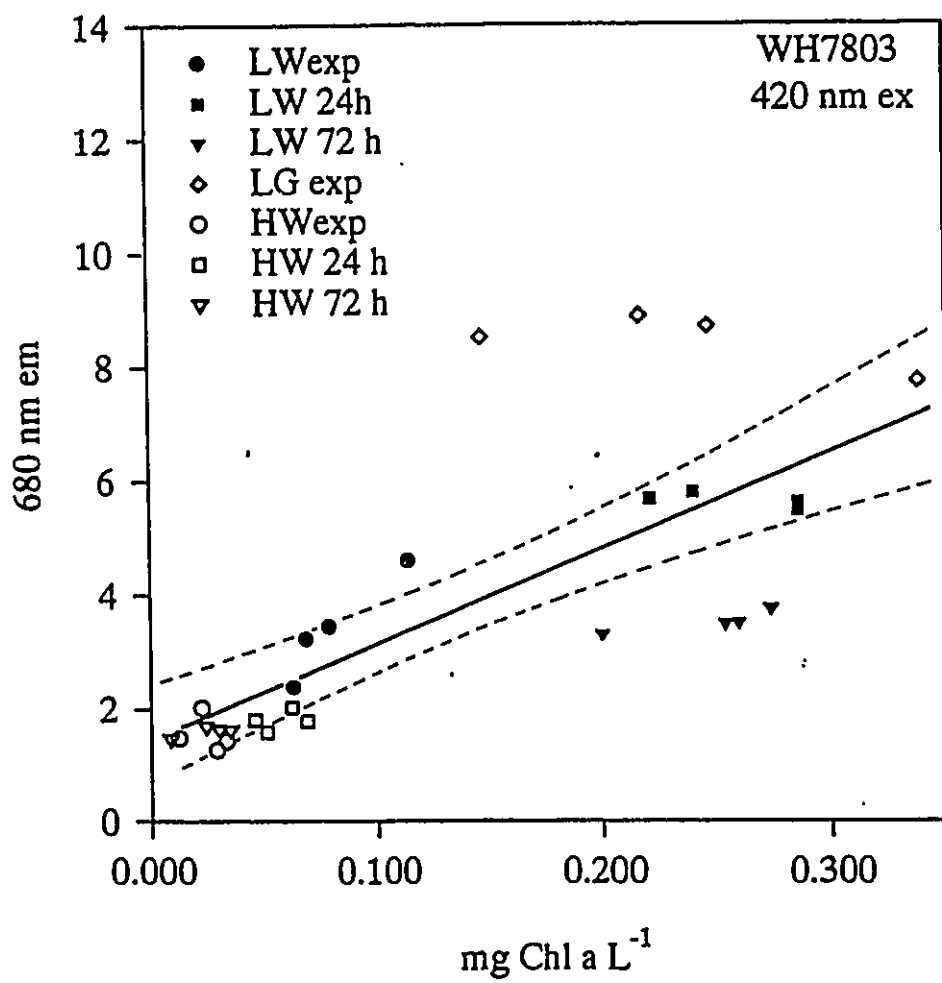


Figure 33a. Fluorescence emission of Chl *a* (680 nm em:420 nm ex) from WH8018 vs Chl *a* content. Individual cultures during exponential growth are plotted. Line is the result of linear regression with 95% confidence intervals and formulates as:
Relative fluorescence intensity = $28.9 \text{ Chl } a \text{ mg}\cdot\text{L}^{-1} + 1.93$. $r = 0.76$.

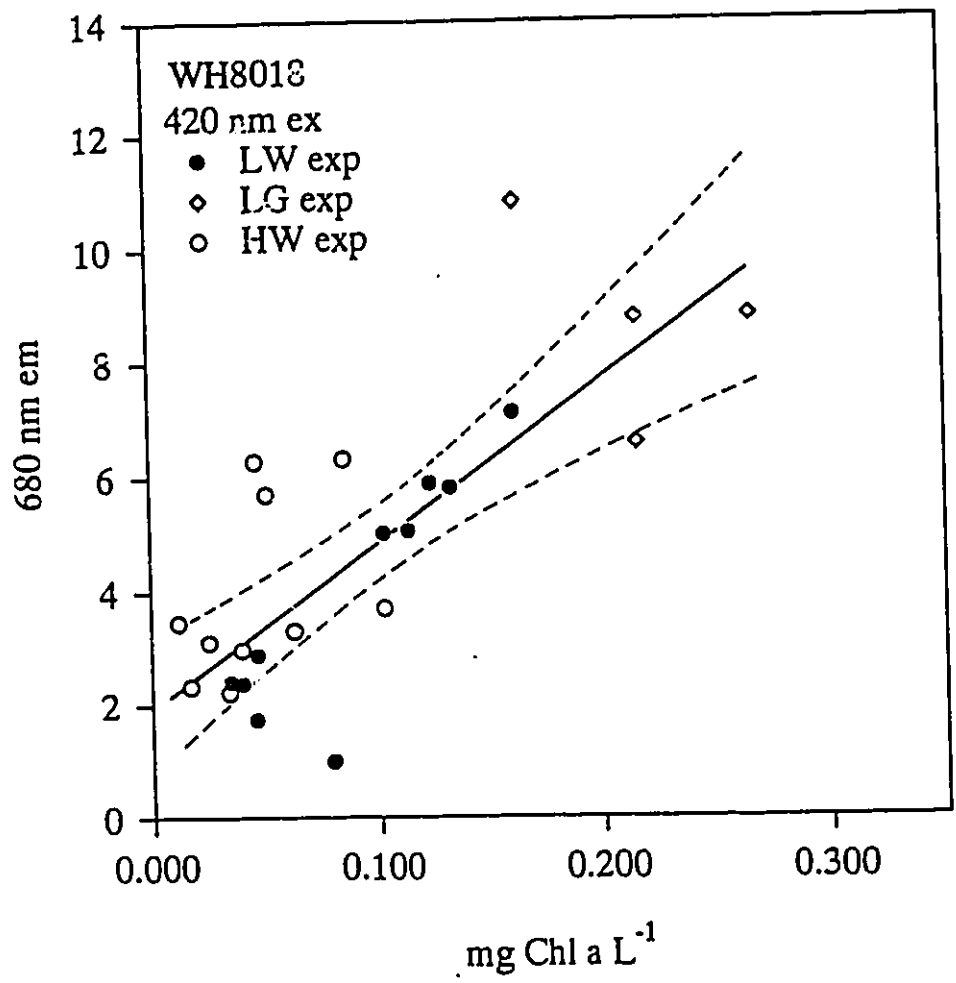


Figure 33b. Fluorescence emission of Chl *a* (680 nm em:420 nm ex) from WH8018 vs Chl *a* content. Individual cultures during exponential and nitrogen stressed growth conditions are plotted. Line is the result of linear regression with 95% confidence intervals and formulates as: Relative fluorescence intensity = $32.0 \text{ Chl } a \text{ mg}\cdot\text{L}^{-1} + 1.99$. $r = 0.75$.

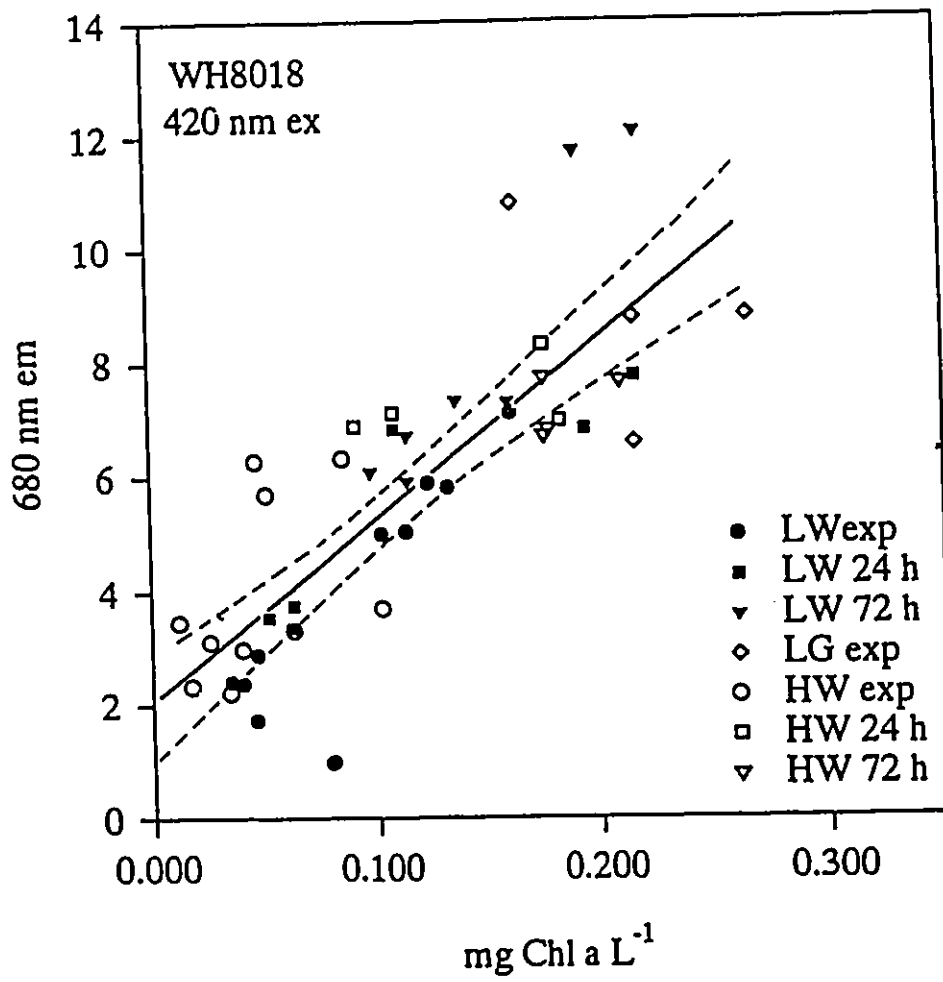


Figure 34a. Fluorescence emission of PE (580 nm em:470 nm ex) from WH8018 vs PE content. Individual cultures during exponential growth conditions are plotted. Line is the result of linear regression with 95% confidence intervals and formulates as: Relative fluorescence intensity = $16.6 \text{ PE mg}\cdot\text{L}^{-1} + 0.40$. $r = 0.92$.

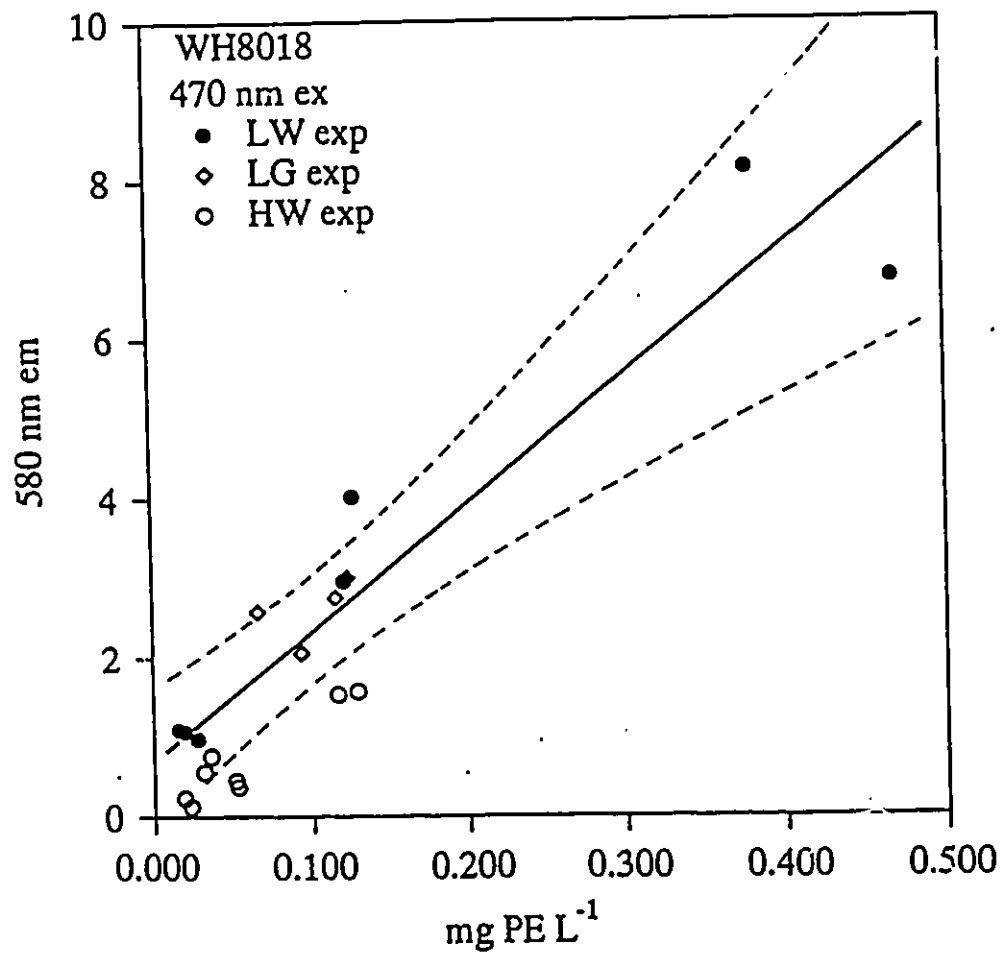
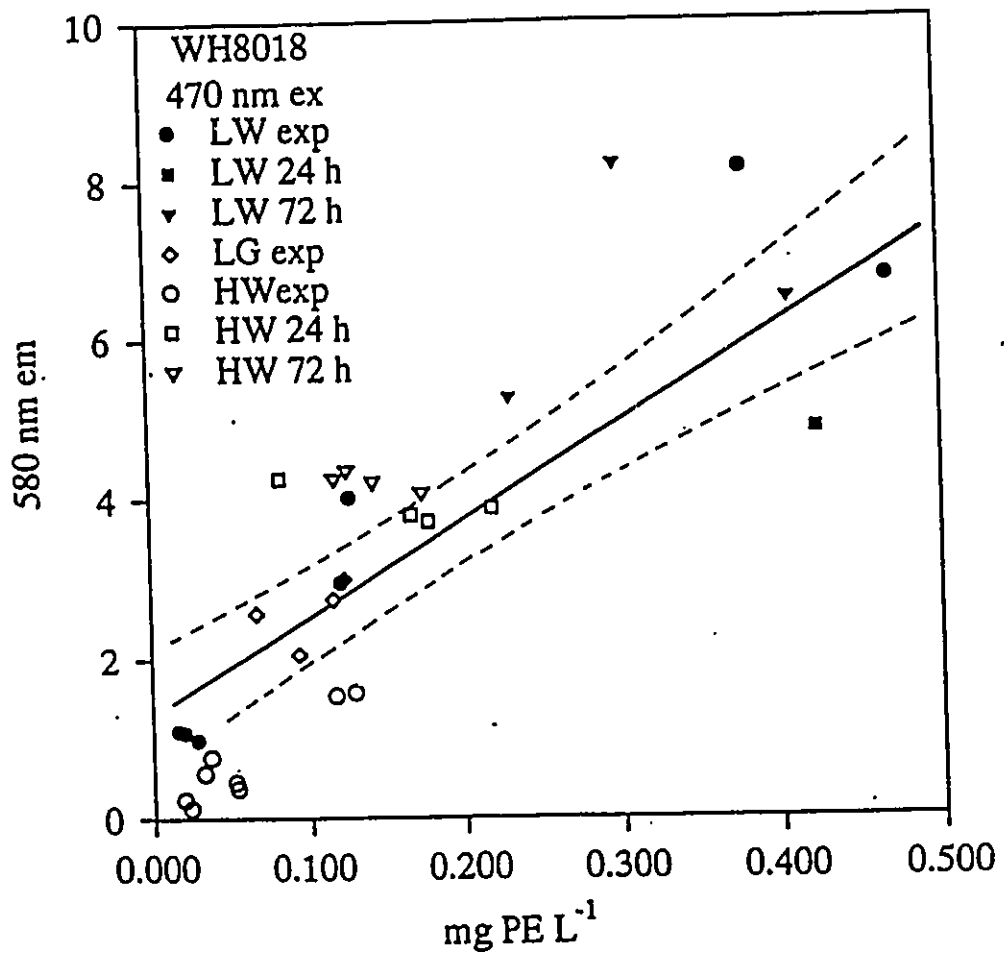


Figure 34b. Fluorescence emission of PE (580 nm em:470 nm ex) from WH8018 vs PE content. Individual cultures during exponential and nitrogen stressed growth conditions are plotted. Line is the result of linear regression with 95% confidence intervals and formulates as: $\text{Relative fluorescence intensity} = 12.2 \text{ PE mg}\cdot\text{L}^{-1} + 1.32$. $r = 0.81$.



4.6 Fluorescence as a Taxonomic Tool to Analyze Phytoplankton Communities.

Yenstch and Yenstch (1979) advocated the use of an excitation ratio from a 530 nm peak and a 450 nm peak, $F(530 \text{ nm} / 450 \text{ nm})$, to represent the contribution to Chl *a* excitation from the accessory pigments, and the blue Soret of Chl *a*, respectively. The fluorescence spectral signature inherent in the calculation of these ratios could then be used to characterize phytoplankton populations into major taxonomic groups. A ratio which averaged 0.85 was assigned to the diatoms and dinoflagellates, and a low ratio of < 0.35 was attributable to green algae. Similarly, excitation ratios have been further examined by Oldham et al. (1985) and Hilton et al. (1989). Ratios were similar in algal classes containing carotenoid pigments (diatoms, dinoflagellates, golden-browns). Green algae and the Xanthophytes gave a range of excitation ratios from 0.12 - 0.25 (Hilton 1989 see their Table 1). Cyanobacteria often had the highest excitation ratios (greater than 1) but these were not statistically different from Chrysophytes.

In this study, the analogous ratio is $F(543 \text{ nm}/440 \text{ nm})$ for strain WH7803, or $F(568 \text{ nm}/440 \text{ nm})$ for strain WH8018. This ratio was always above 1.0 and at times as high as 6.4. The effect of photoacclimation on the fluorescence excitation ratio of two strains of cyanobacteria can vary as much as 62 % for WH7803 and 40% for strain WH8018 (Table 7). The differences between the fluorescence ratios of picocyanobacteria strains were as great as the differences due to photoacclimation within a strain.

The use of the excitation ratio, $F(550\text{ nm}/450\text{ nm})$ of Yentstch and Yentstch (1979) is not an appropriated for PC picocyanobacteria (Fig. 12). However the maximum excitation ratio for PC strains using $F(550 /450)$ would be 1.0. In the present work this ratio could distinguish PC and PE pigment types of picocyanobacteria. When grown at the same irradiance Type III, Type II and Type I had ratios of approximately 1, 4, and above 5.7, respectively.

To date no one has reported excitation spectra (Chl *a* emission 680 nm) for natural mixed populations of phytoplankton which show evidence of PE containing picocyanobacteria. Yentstch and Yentstch (1979) noted on one occasion the presence of phycoerythrin, but only from emission spectra. In this study picocyanobacteria emission ratios ($B(580/680)$) were much more sensitive to photoacclimation processes than excitation ratios. For other algae emission ratios would be of little use since their accessory carotenoid pigment do not emit fluorescence (Prezelin and Bocxar 1986).

The variation in the emission of PE to that from Chl *a* from a blue excitation (470 nm) varied by 78% for strain WH7803 and 54 % for strain WH8018 (Table 8). Similar ratios for the two PE pigment types of picocyanobacteria were found during growth at high growth irradiance. This occurs even if the emission ratios were calculated using the same representative accessory pigment emission wavelength. This suggests that it may not be possible to further resolve the taxonomic composition from remote sensed fluorescence emission. This situation is further exacerbated at high irradiance where the fluorescence ratio of accessory

pigments to Chl *a* for all algae are usually at a minimum.

The accessory pigments which are used to "fingerprint" PE picocyanobacteria responded to alterations in growth irradiance by increasing or decreasing their relative and absolute cellular content. In adapted algal populations the fluorescence ratio will vary for a number of reasons. Besides the obvious differences in spectral composition of accessory pigments, variations can be expected due to photoacclimation - which is inextricably linked to growth rates. However the wavelength of maximum excitation or emission from the accessory pigments is constant in picocyanobacteria. Under a wide variety of growth conditions, including green light, variations about these wavelengths were less than 1% (Appendix IA and B).

4.7 Fluorescence Characteristics of Freshwater Picocyanobacteria.

The two dominant populations of Jack's Lake were of a single phycobiliprotein type similar to the Type II, WH8018. The excitation spectra for both (PE-Syn and PE-Sys) were similar for all depths and among the sampling dates (Fig. 25 and 26, respectively). Excitation by the blue Chl *a* Soret band was never a dominant feature in the excitation spectra of this picocyanobacteria assemblage. This is in contrast to the results for a centric diatom (Fig. 27), and to other published results from marine phytoplankton assemblages (Neori et al. 1984, Mitchell and Kiefer 1984, 1988) which show changes in the wavelength of maximum Chl *a* excitation with depth.

In natural populations of PE picocyanobacteria, PSII light reactions would appear to be solely driven by PE absorption and energy transfer. This reliance on PE would tend to restrict this pigment group (Type II) to waters having green-yellow penetrating light. In Jack's Lake, the wavelengths of maximum penetration are between 570 - 590 nm (Pick and Cuhel 1986) and closely match those that excite Chl *a* (680 nm) in the dominant picocyanobacteria. These results lend support to the hypothesis and empirical data that suggest that light quality controls the distribution of pigment types of picocyanobacteria (Wood 1985, Pick 1991). Since the quality of penetrating light for many oligotrophic to mesotrophic lakes range from 560 to 590 nm (Kirk 1983), all such lakes may contain only the Type II PE picocyanobacteria.

While the wavelength of peak Chl *a* excitation (680 nm) did provide a broad

taxonomic identification of the dominant picocyanobacteria, emission spectra revealed taxa differences within this pigment type; PE-Syn, as compared to the PE-Sys, always had a greater allophycocyanin peak. Sosik and Chisholm (1989) working on marine cultures also found that it was the differences in the relative abundances of Chl *a* and accessory pigments that were important in distinguishing populations. Here, it was the emission ratio response during photoacclimation to low growth irradiance that further emphasized the difference between these two populations.

The increases in emission ratio through the water column (growth irradiance gradient) imply that the size of PSII increases in response to lower growth irradiance. This trend was evident in the field populations, but it was both date and morphotype specific. Depth increases in the $B(PE/Chl a)$ were evident for PE-Syn only on July 31, and PE-Sys only on Aug. 14 (Fig. 30). The reverse was also true but on opposite dates during which no or very little difference was observed in $B(PE/Chl a)$. The culture work on defined strains indicated that small changes in emission ratios can be expected between high full spectrum and low monochromatic-PSII growth irradiance (Table 8 and 9). The primary reason relates to differences in PUR between these regimes. Furthermore, low emission ratios in low light conditions can also be expected when growth rates and PE synthesis are limited. As both morphotypes seem to have the same complement of phycobilliproteins, small differences in the emission ratio between surface and deep populations of picocyanobacteria may be explained by differences in nitrogen

requirements for PE synthesis and growth.

In marine systems, picocyanobacteria pigments have been shown to be significantly affected by nitrogen limitation (Vernet et al. 1990). It has also been shown that increases in biomass (growth) of picocyanobacteria can occur following sudden inputs of atmospheric nitrogen (Glover et al. 1988). Prezelin and workers (1989) indicated that nitrogen can be sufficiently limiting to reduce cellular PE. Thus it is not surprising that nitrogen limitation has also been reported to affect P_{max} (Prezelin et al. 1989) and the abundance of both marine (Olson et al. 1990) and freshwater (Wehr 1989) populations of picocyanobacteria.

Nitrogen stress appears to decrease PSII activity in most algae and so increase Chl *a* fluorescence (reviewed by Turpin 1991). The present work indicates that limitation of growth and PE synthesis maintained low PE fluorescence. The differences between these effects may be related to nitrogen stress (no growth) and nitrogen limitation (of growth). In light of the fact that nitrogen stress was not easily inducible in PE picocyanobacteria, populations in the field were probably nitrogen limited and not stressed.

The immediate response to the resupply of nitrogen is a decrease in Chl *a* fluorescence through a state transitions (Turpin and Bruce 1990). This quenching mechanism, a response to the metabolic demands of nitrogen assimilation, regulates the distribution of excitation energy. The induction of state transitions helps to direct more energy to PSI to increase the production of ATP (Turpin and Bruce 1990). On slightly longer times scales, cyanobacteria have been shown to

quickly increase PE (3 to 12 hr) upon the resupply of nitrogen (Yamanaka and Glazer 1980, Steven et al. 1981, and Evans 1988, Allen et al. 1990, Ray and Glibert 1990). In this study, picocyanobacteria incubated with nitrogen over their natural night cycle (16 hr) may have had sufficient time to synthesize PE and so increase the low light (10 m) emission ratio, B(PE/Chl a) (Figure 29a).

The assumption of this field work was that fluorescence was proportional to pigment content and that most of the changes in peak emission ratio arose predominately from a change in PE content. Since both high light and nitrogen limitation would reduce PE content, the increases of B(PE/Chl a) for both surface populations on Aug. 14 indicates that this ratio may not entirely reflect pigment content. While the culture work and that of other workers have found pigment emission to be proportional to cellular content (Glibert et al. 1986, Olson et al. 1990), increased fluorescence emission ratios, could also be the result of photoinhibitory quenching of Chl a and/or increased energy dissipation by PE. Both responses have been shown to occur following abrupt and significant increases in growth irradiance for a PE containing cryptomonad (Neale et al. 1989).

Photoinhibition occurs at very high growth irradiance and is promoted by various environmental stress factors that impair the normal utilization of photosynthetic energy in carbon metabolism (Krauss and Weis 1991). Nitrogen limitation may exacerbate photoinhibition which may explain the emission ratios for PE-Sys on July 31. At this time, nitrogen limitation for this population could explain both the enhanced surface emission ratio and the depressed emission ratios at

depth. As photoinhibition is reversible during dark incubation, the surface cells must have experienced severe photodamage. Recovery from photoinhibition is a function of both the prior exposure and the growth (acclimation) rate (Lewis and Cullen 1988). As observed in this work and by Healey (1985) nitrogen limitation was only detectable at low growth irradiance. At low growth irradiance the demand for light harvesting protein-pigment complexes can be expected to be at a maximum.

To differentiate the effects of these environmental variables on PE and Chl *a* emission, simultaneous quantification of pigment content or maximum (uncoupled) pigment fluorescence would have to be performed. As the separation and determination of pigment content for these two populations, is at present impossible, DCMU-enhanced fluorescence emission should be included in future research.

It is tempting to speculate that the detection of fluorescence differences in these natural populations have meaning in the context of ecological preferences for light and nitrogen. Because two distinct PE-morphotypes appeared to be dominant in Jack's Lake, I returned to the same sampling point during May 15 and Aug. 3 of 1991 to enumerate the depth distribution of these two picocyanobacteria. I found that during May 15 only PE-Sys was present, while PE-Syn was dominant on Aug. 3, but only above the thermocline. Examination of a more oligotrophic lake, Bay Lake (Pick and Agbetti 1991) having greater light penetration, showed only the presence of PE-Syn. PE-Syn appears then to be better adapted to high light and

low nitrogen. PE-Sys, on the other hand, may have a higher requirement for nitrogen and thus it is found in higher abundance below the thermocline and in lakes of higher available nitrogen; PE-Sys dominates in Lake Ontario (Pick unpublished) which has high levels of nitrate (Lean and Knowles 1987).

SUMMARY AND CONCLUSIONS.

Fluorescence from the photosynthetic apparatus is a complex signal. Many photophysiological components act in concert to maintain, quench or promote energy driven processes (fluorescence) at PSII. Some general conclusions though can be made concerning the fluorescence characteristics of picocyanobacteria.

1. In steady-state cultures, fluorescence yield, emission and excitation ratios (PE/Chl a or PE/PC) tracked the changes in pigment content and composition through photoacclimation.
2. Picocyanobacteria perceive light quality as a specific shift in light intensity. However, chromatic acclimation defined as a modification of PE to PC was evident in a Type I PE strain - WH7803.
3. Even though nitrogen stress was difficult to achieve in picocyanobacteria cultures, particularly in PUB strains, the fluorescence yield of PE increased slightly under nitrogen stress, particularly in low light.
4. The freshwater assemblage in the study lake was dominated by Type II picocyanobacteria. Excitation spectra of Chl a from these cells closely matched the underwater light field.
5. In natural assemblages of picocyanobacteria, growth irradiance appeared to be the most important factor modulating fluorescence. Two Type II organisms could be distinguished based on emission spectra. At low light, nitrogen limitation of growth rates could also account for some of these differences.

REFERENCES

- Alberte, R.S., Wood, A.M., Kursar, T.A. & Guillard, R.R.L. 1984 Novel phycoerythrins in marine *Synechococcus* spp.: characterization and evolutionary and ecological implications. *Plant Physiol.* 75: 732-9.
- Allen, J.F., Mullineaux, C.W., Sanders, C.E. & Melis, A. 1989. State transitions, photosystem stoichiometry adjustment and non-photochemical quenching in cyanobacterial cells acclimated to light absorbed by photosystem I or photosystem II. *Photosynth. Res.* 22: 157-66.
- Allen, M.M. & Hutchinson, F. 1980. Nitrogen limitation and recovery in the cyanobacterium *Aphanocapsa* 6308. *Arch. Microbiol.* 128: 1-7.
- Allen, M.M., Law, A. & Evans, E.H. 1990. Control of photosynthesis during nitrogen depletion and recovery in a non-nitrogen fixing cyanobacterium. *Arch. Microbiol.* 153: 428-31.
- Andersen, R.A., Jacobson, D.M. & Sexton, J.P. 1991. Catalog of Strains; Provasoli-Guillard Center for Culture of Marine Phytoplankton. West Boothbay Harbour, Maine, U.S.A. pp 98.
- Barlow, R.G. & Alberte, R.S. 1985. Photosynthetic characteristics of phycoerythrin-containing marine *Synechococcus* spp. *Mar. Biol.* 86 : 63-74.
- Bennett, A. & Bogorad, L. 1973. Complementary chromatic adaptation in a filamentous blue-green alga. *J. Cell. Biol.* 58: 419-35.
- Bidigare, R.R., Schofield, O. & Prezelin, B.B. 1989. Influence of zeaxanthin on quantum yield of photosynthesis of *Synechococcus* clone WH7803 (DC2). *Mar. Ecol. Prog. Ser.* 56: 177-89.
- Bidigare, R.R., Marra, J., Dickey, T.D., Iturriaga, R., Baker, S., Smith, R.C. & Pak, H. 1989. Evidence for phytoplankton succession and chromatic adaptation in the Sargasso Sea during spring 1985. *Mar. Ecol. Prog. Ser.* 60: 113-22.
- Burnison, B. K. 1980. Modified dimethylsulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. *Can. J. Fish. Aquat. Sci.* 37: 729-33.
- Campbell, L. & Iturriaga, R. 1988. Identification of *Synechococcus* spp. in the Sargasso Sea by immunofluorescence and fluorescence excitation spectroscopy performed on individual cells. *Limnol. Oceanogr.* 33: 196-201.

- Caron, D.A., Pick, F.R. & Lean, D.R.S. 1985. Chroococcoid cyanobacteria in Lake Ontario: vertical and seasonal distribution during 1982. *J. Phycol.* 21: 171-5.
- Carr, N.G. 1988. Nitrogen reserves and dynamic reservoirs in cyanobacteria. In: Rogers, L.J. & Gallon, J.R. [Eds]. *Biochemistry of the Algae and the Cyanobacteria*. Oxford Scientific Publishing Co. pp. 13-21.
- Cleaveland, J.S. & Perry, M.J. 1987. Quantum yield, relative specific absorption and fluorescence in nitrogen-limited *Chaetoceros gracilis*. *Mar. Bio.* 94: 489-97.
- Cochlan, W.P., Price, N.M. & Harrison, P.J. 1991. Effects of growth irradiance on nitrogen uptake by phytoplankton: comparison of frontal and stratified communities. *Mar. Ecol. Prog. Ser.* 69: 103-16.
- Cullen, J.J. 1990. On models of growth and photosynthesis in phytoplankton. *Deep-Sea. Res.* 37: 667-83.
- Cullen, J.J. & Lewis, M.R. 1988. The kinetics of algal photoadaptation in the context of vertical mixing. *J. Plank. Res.* 10: 1039-1063.
- Day, R.W. & Quinn, G.P. 1989. Comparisons of treatments after an analysis of variance in ecology. *Ecol. Monogr.* 59: 433-63.
- Eckblad, J. W. 1991. How many samples should be taken?. *BioScience.* 41: 346-8.
- Environment Canada. 1978. Analytical methods manual, v. 1. Inland Waters Directorate. Water Quality Branch, Ottawa.
- Eppley, R.W., Stewart, E., Abbott, M.R. & Heyman, U. 1985. Estimating ocean primary production from satellite chlorophyll. Introduction to regional differences and statistics for the southern California Bight. *J. Plankton. Res.* 7: 57-70.
- Evans, E.H. 1988. The effects of nitrogen depletion/repletion on photosynthesis in *Aphanocapsa* 6308. In: Rogers, L.J. and Gallon, J.R. [Eds]. *Biochemistry of the Algae and Cyanobacteria*. Clarendon Press, Oxford. pp.41-42.
- Fahnenstiel, G.L. & Carrick, H.J. 1991. Physiological characteristics and food-web dynamics of *Synechococcus* in Lake Huron and Michigan. *Limnol. Oceanogr.* 36: 219-34.

- Falkowski, P. & LaRoch, J. 1991. Acclimation to spectral growth irradiance in algae. *J. Phycol.* 27: 8-14.
- Falkowski, R.G., Sukenik, A. & Herzig, R. 1989. Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). II. Relative abundance of chloroplast proteins. *J. Phycol.* 25: 471-8.
- Foy, R.H., Gibson, C.E. & Smith, R.V. 1976. The influence of daylength, light intensity and temperature on the growth rates of blue-green algae. *British Phycol. Soc.* 11: 151-63.
- Ganff, G.G., Heaney, S.I. & Corry, J. 1990. Light absorption and pigment content in natural populations and cultures of a non-gas vacuolate cyanobacterium, *Oscillatoria bourrellyi* (= *Tychomema bourrellyi*). *J. Plank. Res.* 13: 1101-21.
- Glibert, P.M., Kana, T.M., Olson, R.J., Kirchman, D.L. & Alberte R.S. 1986. Clonal comparisons of growth and photosynthetic responses to nitrogen availability in marine *Synechococcus* spp.. *J. Exp. Mar. Biol. Ecol.* 101: 199-208.
- Glover, H.E. 1985. The physiology and ecology of the marine cyanobacterial genus *Synechococcus*. In: Jannasch, H.W. & Williams, P.J. [Eds]. *Advances in Aquatic Microbiology*. Vol 3. Academic Press, London. pp. 49-107.
- Glover H.E., Campbell, L. & Prezelin, B.B. 1986a. Contribution of *Synechococcus* to size-fractionated primary productivity in three water masses in the Northwest Atlantic Ocean. *Mar. Biol.* 51: 40-51.
- Glover, H.E., Keller, M.D. & Guillard, R.R.L. 1986b. Light quality and oceanic ultraphytoplankters. *Nature.* 319: 142-43.
- Glover, H.E., Keller, M.D. & Spinard, R.W. 1987. The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. *J. Exp. Mar. Biol. Ecol.* 105: 137-59.
- Glover, H.E., Prezelin, B.B., Campbell, L., Wyman, M. & Garside, C. 1988. A nitrate-dependent *Synechococcus* bloom in surface Sargasso Sea water. *Nature.* 331: 161-2.
- Glover, H. E., Phinney, D.A., & Yentsch, C.S. 1985a. Photosynthetic characteristics of picoplankton compared with those of larger phytoplankton population in various water masses in the Gulf of Maine. *Biol. Oceanogr.* 3: 223-248.
- Glover, H.E., Smith, A.E. & Murphy, L.S. 1985b. Diel variation in photosynthetic

- rates: comparisons of ultraphytoplankton with a larger phytoplankton size fraction. *J. Plankton Res.* 7: 519-535.
- Hauschild, C., McMurter, H.J.G. & Pick, F.R. 1991. Effects of spectral quality on growth and pigmentation in picocyanobacteria. *J. Phycol.* 27: 698-702.
- Healey, F.P. 1975. Physiological indicators of nutrient deficiency in algae. *Fish. Mar. Serv. Res. Dev. Tech. Rep.* Vol. 585. pp 30.
- Healey, F.P. 1985. Interacting effects of light and nutrient limitation on the growth rate of *Synechococcus linearis* (Cyanophyceae). *J. Phycol.* 21: 134-46.
- Herig, R. & Falkowski, P.G. 1989. Nitrogen limitation in *Isochrysis galbana* (Haptophyceae). I. Photosynthetic energy conversion and growth efficiencies. *J. Phycol.* 25: 462-71.
- Hilton, J., Rigg, E. & Jaworski, G. 1989. Algal identification using in vivo fluorescence spectra. *J. Plankton Res.* 11: 65-74.
- Hoge, F.E. & Swift, R.N. 1990. Photosynthetic accessory pigments: evidence for the influence of phycoerythrin on the submarine light field. *Remote Sens. Environ.* 34: 19-35.
- Hooks, C.E. 1987. The influence of light intensity and quality on the growth, size, and pigment composition of select ultraphytoplankters. Ph.D. dissertation, University of Florida. 87 pps.
- Jeffrey, S.W. and Humphrey, G.F. 1975. New spectrophotometric equations for determining chlorophylls a, b, c₁, and c₂ in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen (BPP)*, Bd. 167: 191-4.
- Jerlov, N.G. 1976. *Marine optics*. Elsevier. Amsterdam.
- Johnson, P.G. & Sieburth, J. McN. 1979. Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass. *Limnol. Oceanogr.* 24: 928-935.
- Kana. T.M. & Glibert, P.M. 1987a. Effect of growth irradiances up to 2000 $\mu\text{E m}^{-2}\text{s}^{-1}$ on marine *Synechococcus* WH7803 - I Growth, pigmentation and cell composition. *Deep-Sea Res.* 34: 479-95.
- 1987b. Effect of growth irradiances up to 2000 $\mu\text{E m}^{-2}\text{s}^{-1}$ on marine *Synechococcus* WH7803 - II. Photosynthetic responses and mechanisms. *Deep-Sea Res.* 34: 497-516.

- Kana, T.M., Glibert, P.M., Goericke, R. & Welschmeyer, N. A. 1988. Zeaxanthin and B-carotene in *Synechococcus* WH7803 respond differently to growth irradiance. *Limnol. Oceanogr.* 33: 1623-27.
- Kiefer, D.A. 1973a. Fluorescence properties of natural phytoplankton populations. *Mar. Biol.* 22: 263-269.
- Kiefer, D.A. 1973b. Chlorophyll *a* fluorescence in marine centric diatoms: responses of chloroplasts to light and nutrient stress. *Mar. Biol.* 23: 39-46.
- Kirk, J.T. 1983. Light and photosynthesis in aquatic ecosystems. Cambridge University Press. Cambridge.
- Kolber, Z., Wyman, K.D. & Falkowski, P.G. 1990. Natural variability in photosynthetic energy conversion efficiency: a field study in the Gulf of Maine. *Limnol. Oceanogr.* 33: 72-9.
- Kolber, Z., Zehr, J. & Falkowski, P. 1988. Effects of growth irradiance and nitrogen limitation of photosynthetic energy conversion in Photosystem II. *Plant Physiol.* 88: 923-929.
- Krauss, G.H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis; the basics. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42: 313-49.
- Krempin, D.W. & Sullivan, C.W. 1981. The seasonal abundance, vertical distribution and relative microbial biomass of chroococcoid cyanobacteria at a station in southern California coastal water. *Can. J. Microbiol.* 27: 1341-44.
- Kursar, T.A. and Alberte, R.S. 1983. Photosynthetic unit organization in red alga: relationships between light-harvesting pigments and reaction centres. *Plant Physiol.* 72: 409-14.
- Langdon, C. 1989. On the causes of interspecific differences in the growth-growth irradiance relationship for phytoplankton. *J. Plankton Res.* 9: 459-82.
- Lean, D.R.S. & Knowles, R. 1987. Nitrogen transformation in Lake Ontario. *Can. J. Fish. Aquat. Sci.* 44: 2133-43.
- Lean, D.R.S., Murphy, T.P. & Pick, R.R. 1982. Photosynthetic response of lake plankton to combined nitrogen enrichment. *J. Phycol.* 18: 509-21.
- Legendre, L., Demers, S., Delesalle, B. & Harnois, C. 1988. Biomass and photosynthetic activity of phototrophic picoplankton in coral reef waters

- (Moorea Island, French Polynesia). *Mar. Ecol. Prog. Ser.* 47: 153-160.
- Lewis, M.R. & Cullen, J.J. 1988. The kinetics of algal photoadaptation in the context of vertical mixing. *J. Plank. Res.* 10: 1039-63.
- Li, W.K.W., Subba-Rao, D.V., Harrison, W.G., Smith, J.C., Cullen, J.J., Irwin, B. & Platt, T. 1983. Autotrophic picoplankton in the tropical ocean. *Science.* 219: 292-295.
- Lichtenthaler, H.K. (ed.). 1988. *Applications of Chlorophyll Fluorescence.* Kluwer Academic Publishers, London. pp. 363.
- MacColl, R. & Guard-Friar, D. 1987. *Phycobiliproteins.* CRC Press Inc., Florida, pp. 218.
- Manodori, A. & Melis, A. 1986. Cyanobacterial acclimation to photosystem I or photosystem II. *Plant Physiol.* 82: 185-89.
- Mitchell, B.G. & Kiefer, D.A. 1984. Determination of absorption and fluorescence excitation spectra for phytoplankton. In: Holm-Hansen, O., Bolis, L. & Giles, R. [Eds.], *Marine phytoplankton and productivity.*, Springer-Verlag, Berlin. pp. 157-69.
- Mitchell, B.G. & Kiefer, D.A. 1988. Variability in pigment specific particulate fluorescence and absorption spectra in the northeastern Pacific Ocean. *Deep-Sea Res.* 35: 665-89.
- Morel, A., Lazzara, J. & Gostan, J. 1987. Growth rate and quantum yield time response for a diatom to changing growth irradiance (energy and color). *Limnol. Oceanogr.* 32: 1066-84.
- Neale, P.J., Cullen, J.J. & Yentsch, C.M. 1989. Bio-optical inferences from chlorophyll *a* fluorescence: What kind of fluorescence is measured in flow cytometry? *Limnol. Oceanogr.* 34: 1739-48.
- Neori, A., Holm-Hansen, O., Mitchell, B.G. & Kiefer, D.A. 1984. Photoadaptation in marine phytoplankton: changes in spectral absorption and excitation of chlorophyll *a* fluorescence. *Plant Physiol.* 76: 518-524.
- Neori, A., Vernet, M., Holm-Hansen, O. & Haxo, F.T. 1986. Relationship between action spectra for chlorophyll *a* fluorescence and photosynthetic O₂ evolution in algae. *J. Plank. Res.* 8: 537-48.
- Neori, A., Vernet, M., Holm-Hansen, O. & Haxo, F.T. 1988. Comparison of

chlorophyll far-red and red fluorescence excitation spectra with photosynthetic oxygen action spectra for photosystem II in algae. *Mar. Ecol. Prog. Ser.* 44: 297-302.

- Oldam, P.B., Zillioux, E.J. & Warner, I.M. 1985. Spectral "fingerprinting" of phytoplankton populations by two-dimensional fluorescence and fourier-transform-based pattern recognition. *J. Mar. Res.* 43: 893-906.
- Olson, R.J., Chisholm, S.W., Zettler, E.R. & Armbrust, E.V. 1990. Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. *Limnol. Oceanogr.* 35: 45-58.
- Olson, R.J., Chisholm, S.W., Zettler, E.R. & Armbrust, E.V. 1988. Analysis of *Synechococcus* pigment types in the sea using single and dual beam flow cytometry. *Deep-Sea Res.* 35: 425-440.
- Ondrusek, M.E., Bidigare, R.R., Sweet, S.T., Defreitas, D.A. & Brooks, J.M. 1991. Distribution of phytoplankton pigments in the North Pacific Ocean in relation to physical and optical variability. *Deep-Sea Research.* 38: 243-266.
- Pick, F.R. 1991. The abundance and composition of freshwater picocyanobacteria in relation to light penetration. *Limnol. Oceanogr.* 36: 1457-62.
- Pick, F.R. & Agbeti, M. 1991. The seasonal dynamics and composition of photosynthetic picoplankton communities in temperate lakes in Ontario, Canada. *Int. Rev. Ges. Hydrobiol.* 76: 000.000
- Pick, F.R. & Caron, D.A. 1987. Picoplankton and nanoplankton biomass in Lake Ontario: Relative contribution of phototrophic and heterotrophic communities. *Can. J. Fish. Aquat. Sci.* 44: 2164-72.
- Pick, F.R. & Cuhel, R.L. 1986. Light quality effects on carbon and sulfur uptake of a metalimnetic population of the colonial chrysophyte *Chrsospharella longispina*. In: Kristiansen, J. & Anerson, R.A. [Eds]. *Chrysophytes: aspects and problems*. Cambridge Univ. Press, Boston. pp. 197-206.
- Pick, F.R., Lean, D.R.S. & Nalewajko, C. 1984. Nutrient status of metalimnetic phytoplankton peaks. *Limnol. Oceanogr.* 29: 960-71.
- Prezelin, B.B. 1981. Light reactions in photosynthesis. *Can. Bull. fish. Aquat. Sci.* 210: 1-83.
- Prezelin, B.B. & Bocxar, B.A. 1986. Molecular bases of cell absorption and fluorescence in phytoplankton: potential applications to studies in optical

oceanography. Prog. Phycol. Res. 4: 350-464.

Prezelin, B.B., Putt, M. & Glover, H.E. 1986. Diurnal patterns in photosynthetic capacity and depth-dependent photosynthesis-growth irradiance relationships in *Synechococcus* spp. and larger phytoplankters in 3 water masses in the Northwest Atlantic Ocean. Mar. Biol. 51: 33-9.

Prezelin, B.B. & Glover, H.E. 1991. Variability in time/space estimates of phytoplankton, biomass and productivity in the Sargasso Sea. J. Plankton Res. 13: 45-67.

Prezelin, B.B., Tilzer, M.M., Schofield, O. & Haese, C. 1991. The control of the production process of phytoplankton by the physical structure of the aquatic environment with reference to its optical properties. Aquat. Sci. 53: 136-88.

Prezelin, B.B., Glover, H.E., van Hoven, B., Steinberg, D., Matlick, H.A., Schofield, O., Nelson, N., Wyman, M. and Campbell, L. 1989. Blue-green light effects on light-limited rates of photosynthesis: relationship to pigmentation and productivity estimates for *Synechococcus* populations for the Sargasso Sea. Mar. Ecol. Prog. Ser. 54: 121-36.

Raps, S., Wyman, K., Siegelman, H.W. & Falkowski, P.G. 1983. Adaptation of the cyanobacterium *Microcystis aeruginosa* to light intensity. Plant Physiol. 72: 829-832.

Raven, J.A. 1986. Physiological consequence of extremely small size for autotrophic organisms in the sea. In: Platt, T. & Li, W.K.W. [Eds]. Photosynthetic Picoplankton. Can. Bull. Fish. Aquat. Sci. 214: 71-120.

Ray, R.T. & Glibert, P.M. 1990. Different patterns of growth and nitrogen uptake in two clones of marine *Synechococcus* spp. Mar. Biol. 107: 273-80.

Ray, R.T., Haas, L.W. & Sieracki, M.E. 1989. Autotrophic picoplankton dynamics in a Chesapeake Bay sub-estuary. Mar. Ecol. Prog. Ser. 52: 273-85.

Richardson, K., Beardall, J. & Raven, J.A. 1983. Adaptation of unicellular algae to growth irradiance: an analysis of strategies. New Phytologist. 93: 157-92.

Rippka, R., Waterbury, J.B. & Stainer, R.Y. 1981. Isolation and purifications of cyanobacteria: some general principles. In: Starr, M.P., Stolp, H., Trupper, H.G. & Balow, A. [Eds] The prokaryotes: a handbook on habitats, isolation, and identification of bacteria. Vol. 1. Springer-Verlag, New York. pp. 212-20.

- Saffo, M.B. 1987. New light on seaweeds. *BioScience*. 37: 654-64.
- Samuelsson, G., Lonneborg, A., Gustafsson, P. and Oquist, G. 1987. The susceptibility of photosynthesis to photoinhibition and capacity of recovery in high and low light grown cyanobacteria, *Anacystis nidulans*. *Plant Physiol.* 83: 438-41.
- Sathyendranath, S. 1986. Remote sensing of phytoplankton; a review, with special reference to picoplankton. In: Platt, T. & Li, W.K.W. [Eds]. *Photosynthetic Picoplankton*. *Can. Bull. Fish. Aquat. Sci.* 214: 71-120.
- SAS Institute Inc. 1988. *SAS/STAT/ User's Guide*, Release 6.03 Edition. SAS Institute Inc., Cary, NC. pp. 1028.
- Sherr, E.B. & Sherr, B.F. 1991. Planktonic microbes: tiny cells at the base of the Ocean's food webs. *TREE*. 6: 6-13.
- Shimura, S. & Fujita, Y. 1975. Changes in the activity of fucoxanthin-excited photosynthesis in the marine diatom *Phaeodactylum tricornutum* grown under different culture conditions. *Mar. Bio.* 33: 185-94.
- Shrader-Frechette, K.S. & McCoy, E.D. 1992. Statistics, Costs and Rationality in ecological inference. *TREE*. 7: 96-99.
- Smith, R.C., Eppely, R.W. & Baker, K.S. 1982. Correlation of primary production as measured aboard ship in southern California coastal waters and as estimated from satellite chlorophyll images. *Mar. Biol.* 66: 281-88.
- SooHoo, J.B., Kiefer, D.A., Collins, D.J. & McDermid, I.S. 1986. *In vivo* fluorescence excitation and absorption spectra of marine phytoplankton: 1. Taxonomic characteristics and responses to photoadaptation. *J. Plank. Res.* 8: 197-214.
- Sosik, H.M. & Chisholm, S.W. 1989. Chlorophyll fluorescence from single cells: Interpretation of flow cytometric signals. *Limnol Oceanogr.* 34: 1749-61.
- Sosik, H.M. & Mitchell. 1991. Absorption, fluorescence, and quantum yield for growth in nitrogen-limited *Dunaliella tertiolecta*. *Limnol. Oceanogr.* 36: 910-21.
- Starr, C.R. & Zeikus, J.A. 1987. UTEX - The culture collection of algae at the University of Texas at Austin. *J. Phycol. Suppl.* Vol. 23. pp. 44.

- Stein, J.R. (ed.). 1973. Handbook of phyecological methods. Culture methods and growth measurements. London, Cambridge Univ. Press. pp. 448.
- Stevens, Jr., S.D., Balkwill, D.L. & Paone, D.A.M. 1981. The effects of nitrogen limitation on the ultrastructure of the cyanobacterium *Agmenellum quadruplicatum*. Arch. Microbiol 130: 204-12.
- Stockner, J.G. & Antia, N.J. 1986. Algal picoplankton from marine and freshwater ecosystems: A multidisciplinary perspective. Can. J. Fish. Aquat. Sci. 43: 2473-503.
- Takahashi, M., Ichimura, S., Kishinom, M. & Okami, N. 1989. Shade and chromatic adaptation of phytoplankton photosynthesis in a thermally stratified sea. Mar. Biol. 100: 401-9.
- Tandeau de Marsac, N. 1977. Occurrence and nature of chromatic adaptation in cyanobacteria. J. Bacteriol. 130: 82-91.
- Tandeau de Marsac, N. and Houmard, J. 1988. Complementary chromatic adaptation: Physiological conditions and action spectra. In. Packer, L. & Glazer, A.N. [Eds]. Methods in Enzymology. Academic Press, Inc. San Diego. pp. 318-28.
- Therriault, J.C., Booth, D., Legendre, L. & Demers, S. 1990. Phytoplankton photoadaptation to vertical excursion as estimated by an in vivo fluorescence ratio. Mar. Ecol. Prog. Ser. 60: 97-111.
- Turpin, D.H. 1991. Effects of inorganic N availability on algal photosynthesis and carbon metabolism. J. Phycol. 27: 14-20.
- Turpin, D.H. & Bruce, B. 1990. Regulation of photosynthetic light harvesting by nitrogen assimilation in the green alga *Selenastrum minutum* FEBS. 263: 99-103.
- Vaulot, D. & Xiuren, N. 1988. Abundance and cellular characteristics of marine *Synechococcus* spp. in the dilution zone of the Changjiang (Yangtze River, China). Continental Shelf Research. 8: 1171-86.
- Vernet, M., Mitchell, B.G. & Holm-Hansen, O. 1990. Adaption of *Synechococcus* phycoerythrin-543 at a coastal station off the Southern California coast, USA. Mar. Ecol. Prog. Ser. 63: 9-16.
- Vincent, W.F. 1979. Mechanisms of rapid photosynthetic adaptation in natural phytoplankton communities. 1. Redistribution of excitation energy between

- photosystems I and II. *J. Phycol.* 15: 429-34.
- Vincent, W.F. 1980. Mechanisms of rapid photosynthetic adaptation in natural phytoplankton communities. II. Changes in photochemical capacity as measured by DCMU-induced chlorophyll fluorescence. *J. Phycol.* 16: 568-77.
- Wall, D. & Briand, F. 1979. Response of lake phytoplankton communities to in situ manipulations of light intensity and colour. *J. Plankton. Res.* 1: 103-12.
- Waterbury, J.B.S., Watson, S.W., Guillard, R.R. & Brand, L.E. 1979. Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. *Nature.* 277: 293-4.
- Waterbury, J.B.S., Watson, S.W., Valois, F.W. & Franks, D.G. 1986. Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. In: Platt, T. & Li, W.K.W. [Eds]. *Photosynthetic Picoplankton.* *Can. Bull. Fish. Aquat. Sci.* 214: 71-120.
- Wehr, J.D. 1989. Experimental tests of nutrient limitation in freshwater picoplankton. *Appl. Environ. Microbiol.* 55: 1605-11.
- Weisse, T. 1988. Dynamics of autotrophic picoplankton in Lake Constance. *J. Plankton Res.* 10: 1179-1188.
- Wood, A.M., Horan, P.K., Muirhead, K., Phinney, D.A., Yentsch, C.M. & Waterbury J.B. 1985. Discrimination between types of pigments in marine *Synechococcus* spp. by scanning spectroscopy, epifluorescence microscopy, and flow cytometry. *Limnol. Oceanogr.* 30: 1303-15.
- Wood, A.M. 1985. Adaptation of photosynthetic apparatus of marine ultraphytoplankton to natural light fields. *Nature, Lond.* 316: 253-55.
- Wyman, M. & Fay, P. 1986. Underwater light climate and the growth and pigmentation of planktonic blue-green algae (Cyanobacteria) II. The influence of light quality. *Proc. R. Soc. Lond.* 227: 381-93.
- Yamanaka, G. & Glazer, A.N. 1980. Dynamic aspects of phycobilisome structure; Phycobilisome turnover during nitrogen starvation in *Synechococcus* spp. *Arch. Microbiol.* 124: 39-47.
- Yentsch, Ch.S. 1980. Light attenuation and phytoplankton photosynthesis. In: Morris, I. [Ed]. *The physiological ecology of phytoplankton.* Blackwell scientific publications, Oxford. pp. 95-128.

- Yentsch, Ch.S & Menzel, D.W. 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea. Res.* 10: 443-448.
- Yentsch, Ch.S. & Phinney, A. 1984. Observed changes in spectral signatures of natural phytoplankton populations: The influence of nutrient availability. In: Holm-Hansen, O., Bolis, L. & Giles, R. [Eds]. *Lecture Notes on Coastal and Estuarine Studies; Marine Phytoplankton and Productivity*. Vol. 8. pp. 129-140.
- Yentsch, Ch.S. & Yentsch, C.M. 1979. Fluorescence spectral signatures: The characterization of phytoplankton populations by the use of excitation and emission spectra. *J. Mar. Res.* 37: 471-83.

APPENDIX

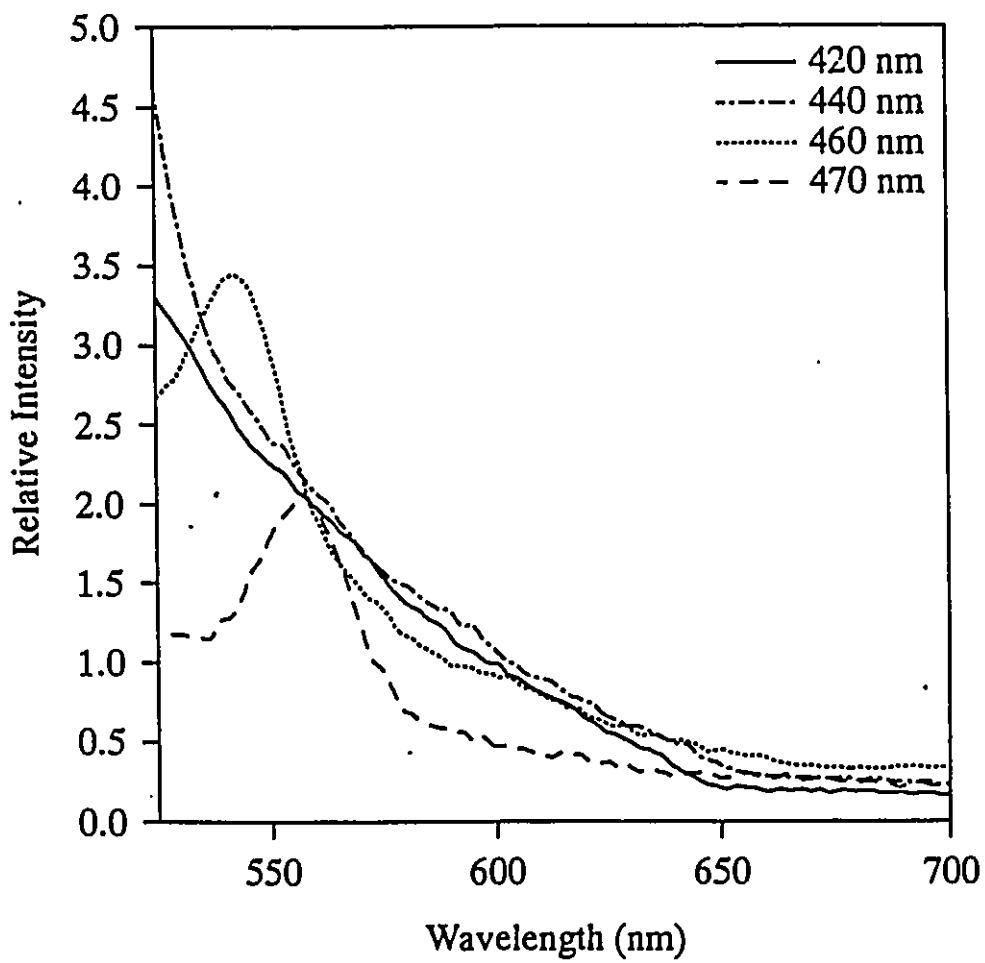
APPENDIX IA. Major peak excitation wavelengths (nm) for Chl *a* emission at 680 nm of the 6 study strains. Values are averaged over all experiments. (\pm 1 std. dev.). N.A. not present.

	Soret Chl <i>a</i>	PU	PE	PC
WH8018, n = 52	437(1.3)	N.A.	568(1.6)	635(2.8)
WH7803, n = 34	437(1.7)	493(2.2)	543(1.9)	633(3.3)
48B66, n = 13	439(0.9)	492(1.5)	543(0.7)	633(1.3)
	Soret Chl <i>a</i>	PC		
WH5701, n = 11	438(1.1)	629(1.2)		
<i>S.leopoliensis</i> , n = 13	436(1.6)	628(1.2)		
<i>Synechocystis</i> , n = 13	437(1.0)	628(1.4)		

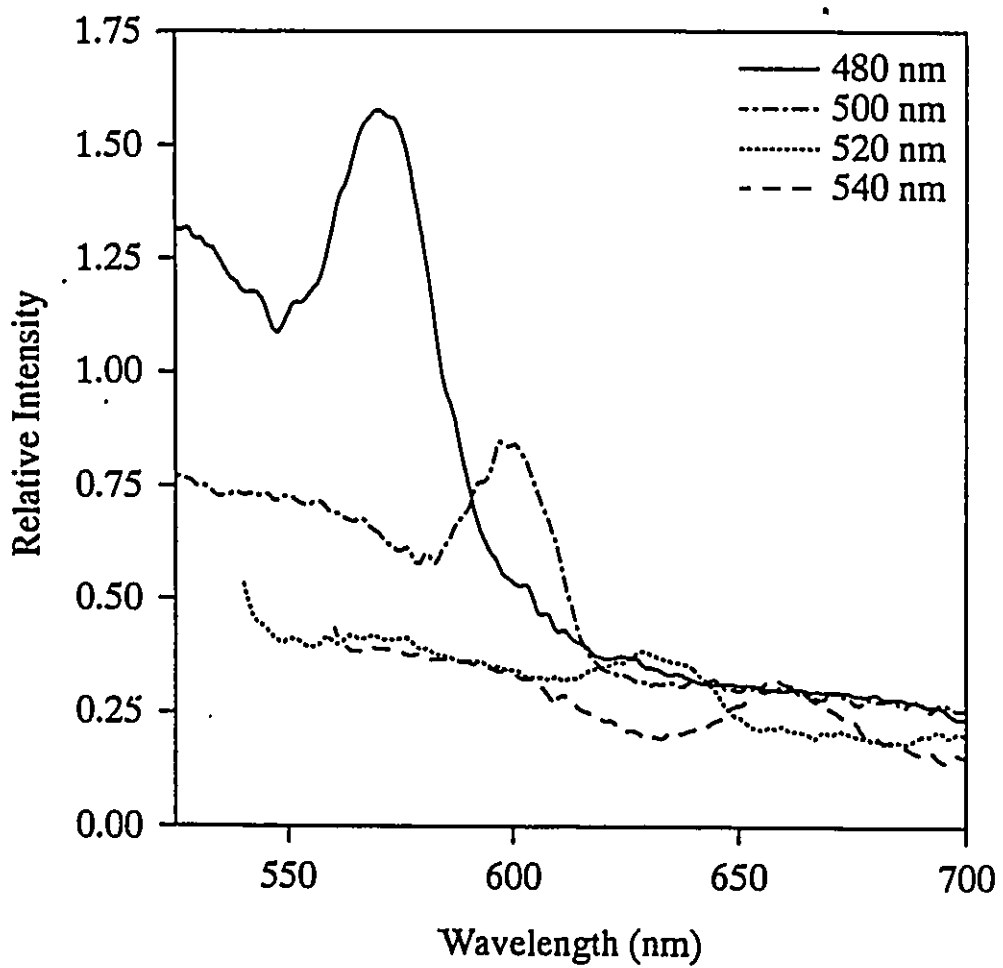
APPENDIX IB. Major peak emission wavelengths (nm) from the 6 study strains for PBP and Chl *a* emission, obtained using 470 nm and 420 nm excitation, respectively. Values are averaged over all experiments. (\pm 1 std. dev.). N.D. not detected.

	Chl <i>a</i>	PE	PC	APC
WH8018,n=50	682(1.3)	577(1.3)	624(1.5)	653(2.1)
WH7803,n=25	682(1.4)	566(2.6)	624(1.6)	652(2.9)
48B66,n=13	682(0.5)	566(1.5)	N.D.	651(0.5)
WH5701,n=11	682(1.3)	--	--	652(2.9)
<i>S.leopoliensis</i> ,n=13	683(1.2)	--	--	653(5.6)
<i>Synechocystis</i> ,n=13	683(0.8)	--	--	655(0.9)

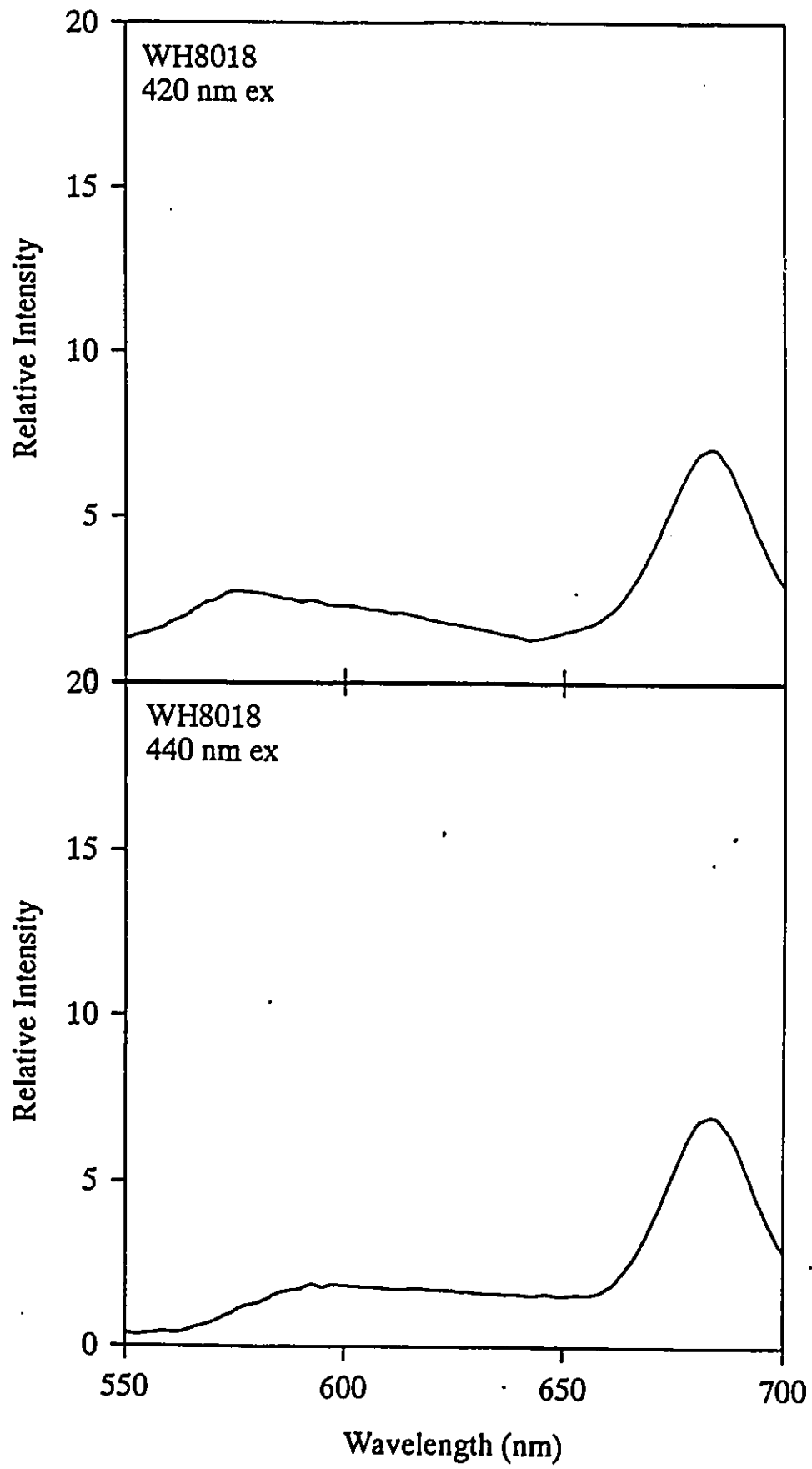
APPENDIX IIA. Raman scatter peak of seawater media using 420 nm, 440 nm, 460 nm, and 470 nm excitation wavelengths.



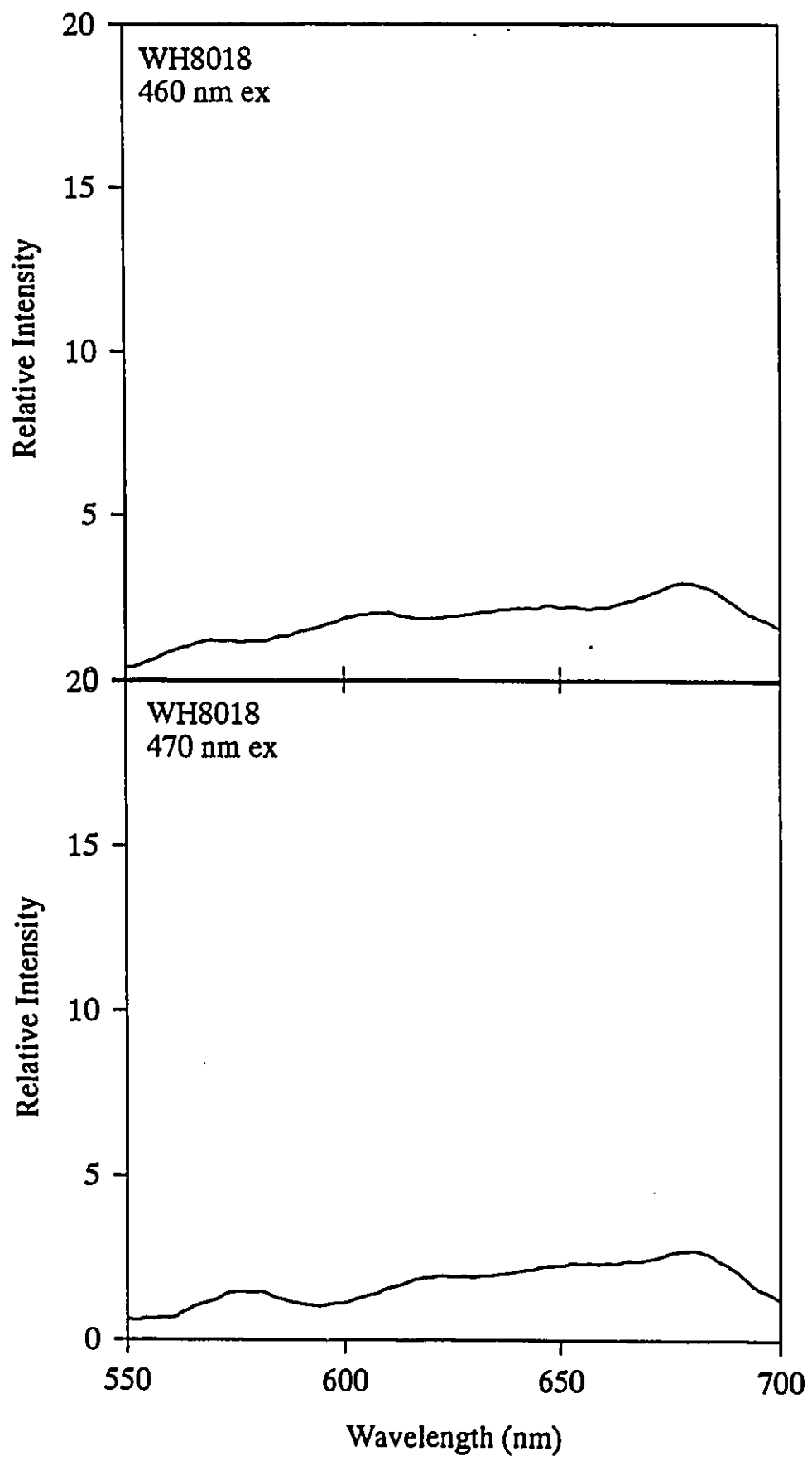
APPENDIX IIB. Raman scatter peak of seawater media using 480 nm, 500 nm, 520 nm, and 540 nm excitation wavelengths.



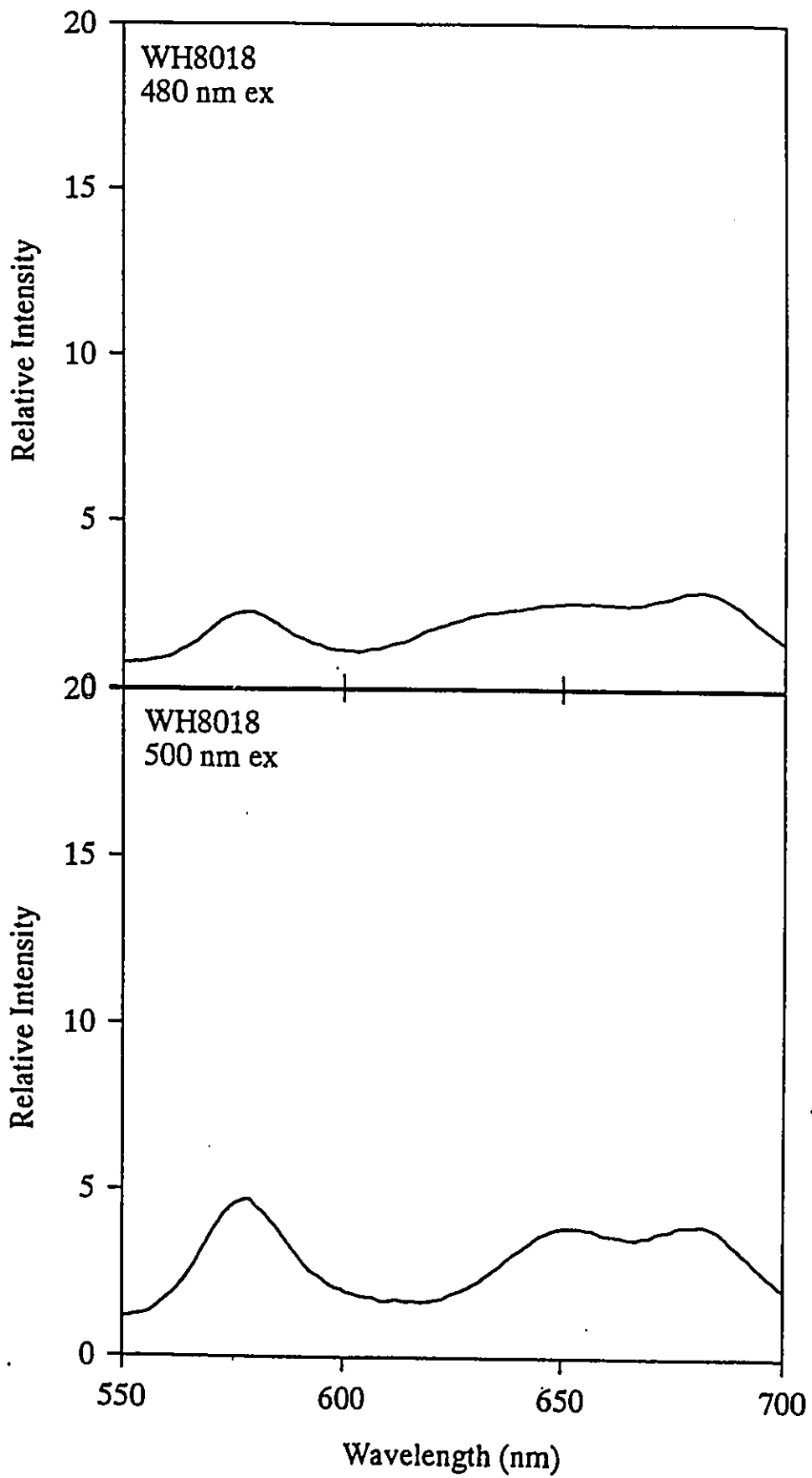
APPENDIX IIC. Effect of excitation wavelength, 420 nm or 440 nm, on emission spectra of WH8018 grown at green, $28 \mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



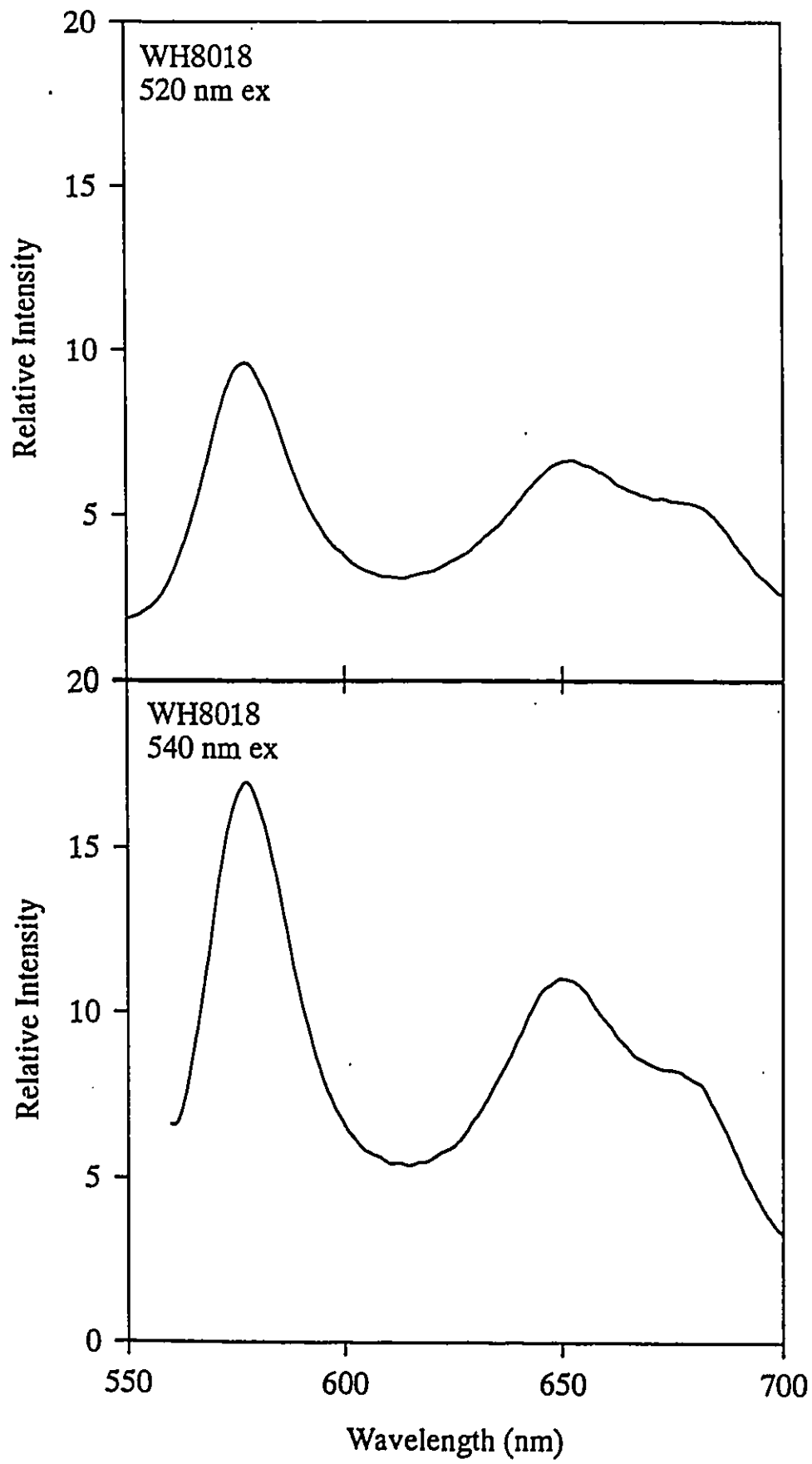
APPENDIX IID. Effect of excitation wavelength, 460 nm or 470 nm, on emission spectra of WH8018 grown at green, $28 \mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



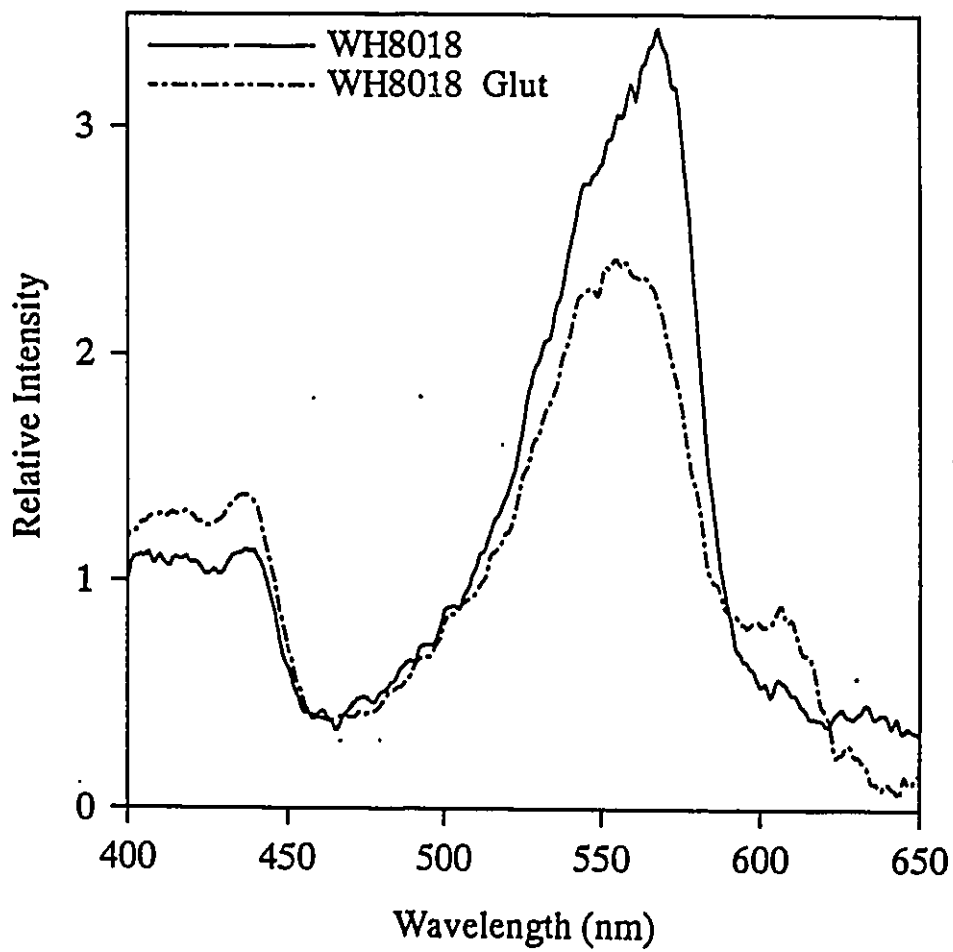
APPENDIX IIE. Effect of excitation wavelength, 480 nm or 500 nm, on emission spectra of WH8018 grown at green, $28 \mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



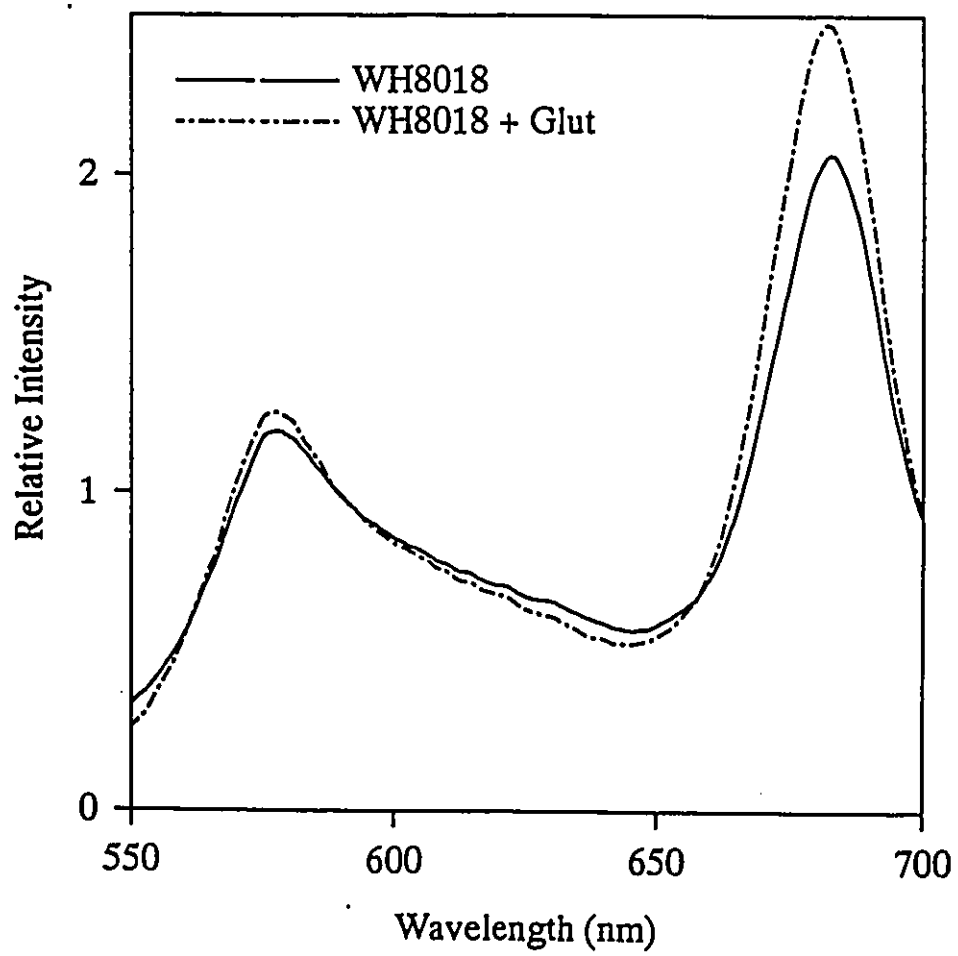
APPENDIX IIF. Effect of varying excitation wavelength, 520 nm or 540 nm, on emission spectra of WH8018 grown at green, $28 \mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



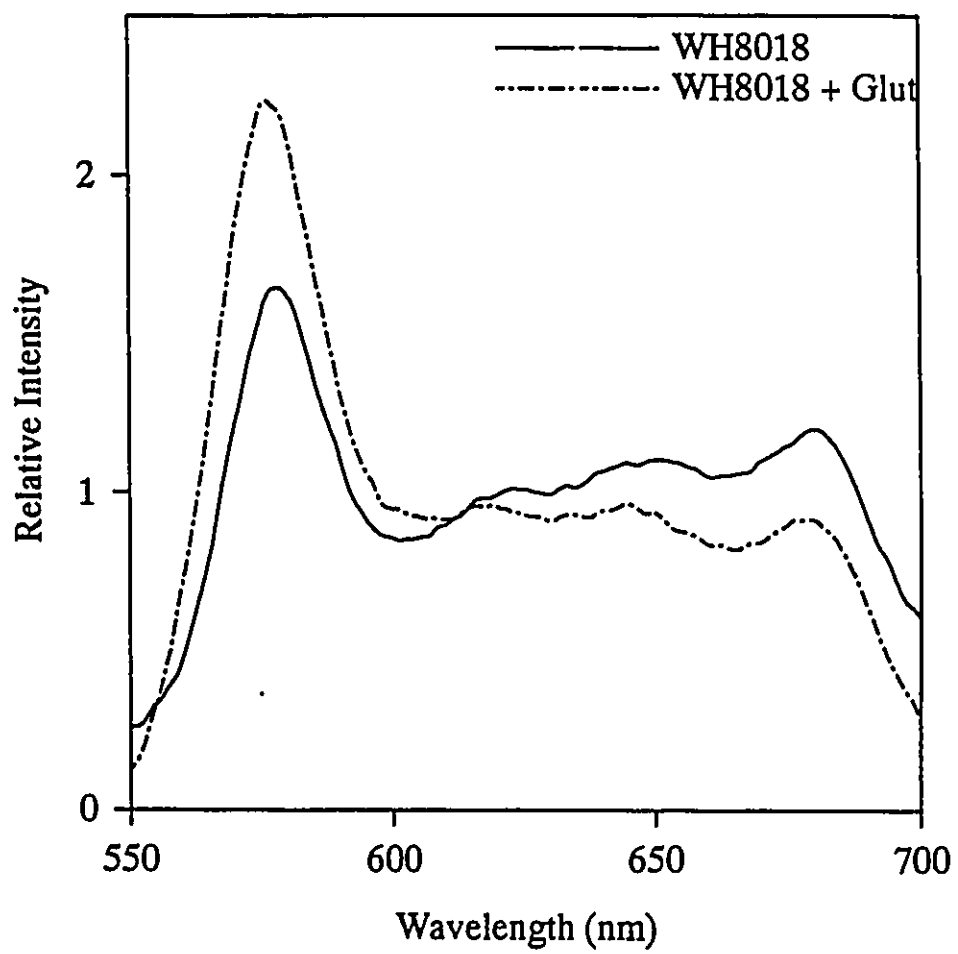
APPENDIX IIIA. Effects of glutaraldehyde preservation on the excitation spectra (for Chl *a* emission at 680 nm) of WH8018 grown in green $28 \mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



APPENDIX IIIB. Effects of glutaraldehyde preservation on the emission spectra (obtained violet excitation; 420 nm) of WH8018 grown in green 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



APPENDIX IIIC. Effects of glutaraldehyde preservation on the emission spectra (obtained blue excitation; 470 nm) of WH8018 grown in green 28 $\mu\text{E m}^{-2} \text{s}^{-1}$, growth irradiance.



APPENDIX IV. Multiple analysis of variance (MANOVA) partial correlation coefficients of combined PE picocyanobacteria data during exponential growth in $400 \mu\text{E m}^{-2} \text{s}^{-1}$ white (HW), $28 \mu\text{E m}^{-2} \text{s}^{-1}$ white (LW), and $28 \mu\text{E m}^{-2} \text{s}^{-1}$ green (LG) irradiance. (numbers in brackets refer to the level of α).

DF=23	V(PE/Chla)	B(PE/Chla)	BV(PE/Chla)	F(PE/Chla)	F(PE/PC)	Chla/C	Growth
V(PE/Chla)	0.6800 0.0002	0.7200 0.0001	0.9000 0.0001	0.1700 0.4100	-0.6700 0.0003	0.4800 0.0160	
B(PE/Chla)		0.8100 0.0001	0.6700 0.0002	-0.4500 0.0245	0.8500 0.0001		
BV(PE/Chla)			0.8400 0.0001	0.7200 0.0001	-0.5700 0.0030	0.8900 0.0001	
F(PE/Chla)				0.3500 0.0850	-0.6600 0.0003	0.6500 0.0004	
F(PE/PC)					-0.3000 0.1500	0.9000 0.0001	
Chla/C						-0.4700 0.0190	
Growth							