GRI-84/0182

MARINE BIOMASS PROGRAM

FINAL REPORT

APRIL 1981 – APRIL 1984

Gas Research Institute
8600 West Bryn Mawr Avenue
Chicago, Illinois 60631

GRI-81/0096

MARINE BIOMASS PROGRAM

FINAL REPORT

(April 1981-April 1984)

Prepared by

A.N. Tompkins and A.J. Bryce

General Electric Company
Advanced Energy Programs Department
501 Allendale Road
King of Prussia, Pennsylvania 19406

For

GAS RESEARCH INSTITUTE

Contract No. 5081-323-0452

GRI Project Manager
Dr. Kimon Bird
Biomass Department

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16. Abstract (Limit: 200 words)

This constitutes the Final Report of work performed on the Marine Biomass Program by the General Electric Company and its associates for the Gas Research Institute.

An overview of work performed from 1976 through 1982 is provided as background for a detailed discussion of the research and development tasks performed during 1983. The background section discusses previous major program activities such as the Offshore Test Platform, Nearshore Test Farm project at Goleta, and the Hemidome experiment. Also discussed in this section are the anaerobic process development and anaerobic microbiological research tasks. A list of all significant program publications is given.

Also provided in this Final Report are detailed progress reports on the 1983 projects which include the development of a comprehensive Kelp Farm Model. This work was performed by the California Institute of Technology and Scripps Institution of Oceanography under the direction of the General Electric Company. This section discusses research tasks in kelp physiology that were performed in order to provide detailed inputs for the model as well as discussing the rationale for construction of the comprehensive model.

17. Document Analysis a. Descriptors

Marine Biomass, Kelp Farming, Methane from Marine Biomass, Nearshore Test Farm, Hemidome, Anaerobic Digestion, *Macrocystis pyrifera*

b. Identifiers/Open-Ended Terms

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1. RESEARCH SUMMARY

1. RESEARCH SUMMARY

Title	Marine Biomass Program
Contractor	General Electric Company Advanced Energy Programs Department GRI Contract Number: 5081-323-0452
Principal Investigator	A.N. Tompkins Marine Biomass Program Manager
Report Period	April 1981-April 1984 Final Report
Objective	To obtain measurements of harvestable yield from adult kelp plants under natural as well as artificially induced environmental conditions and to utilize this data to determine the commercial feasibility of producing methane from a nearshore kelp farm.
Technical Perspective	Kelp yield data from nearshore test facilities, together with associated data for economic analysis, would allow GRI to assess the potential of methane from marine biomass against other biomass projects and subsequently to determine whether there was merit in continuing with the investigation of the overall marine biomass concept.
Results	Through analysis of detailed scientific and engineering data, using conservative projections, the results of the Marine Biomass Program indicate that production of methane from *Macrocystis pyrifera* on commercial sized farms is technically feasible. Projections of product cost using state-of-the-art production technology ($13.50/MMBTU) as well as using reasonably optimistic extrapolations of current technology ($6.00/MMBTU) indicate that the cost of gas produced on the commercial farm conceptualized would be higher than that produced from current conventional production sources but potentially competitive with other sources of substitute natural gas or unconventional natural gas. System and product cost can be reduced by utilization of improved methods of feedstock, production (genetic selection) and methane conversion (digestion system design). In a business and financing sense, methane from kelp systems may also be more rapidly commercialized if by-product and co-product recovery are incorporated into the system concept. Such systems may have an important role in commercialization of first of a kind facilities

through reducing cost risks. Improvements in such a facility can then lead to second generation facilities dedicated solely to pipeline gas production.

A major unknown in all projections is caused by the fact that all studies have been performed with wild plants. This factor has forced excessive conservatism on the entire concept. For example, improvement of crop yield as functions of planting and harvesting strategy, various nutrient management techniques, and the positive effects of hybridization and genetic selection could not be reasonably assessed. Any one of the above parameters could have major upward impact on yield with consequent reduction in cost. Additionally, the impact of genetic selection on plant morphologies better suited from our engineering point of view could lower farm costs dramatically. The combined result of optimization of all of these parameters may well yield a cost competitive system.

Technical Approach

The 1983 Marine Biomass Program had the major objective of completing the specifications which were used as the basis for the Economic and Systems Assessment of the Concept for Nearshore Kelp Farming for Methane (GRI, May 1983). The above named study was performed by The Ralph M. Parsons Co. under direct contract to GRI. Supporting data for the study was prepared and submitted to R.M. Parsons by the General Electric Company and its sub-contractors. The GE work in this area included:

- System concept development
- Preparation of specifications
- Integration of Parsons' activities with other Marine Biomass Program participants and
- Review and follow-up of the System Study

In addition to direct support of the System Study, GE provided technical direction to the California Institute of Technology (CIT) and Neushul Mariculture Incorporated (NMI) in support of kelp yield studies. (NMI was under direct contract to GE through April 1983 after which time NMI contracted directly with GRI). Anaerobic digestion research was conducted at a minimal level by GE. Other anaerobic digestion work during 1983 was performed by the Institute of Gas Technology under direct contract to GRI.

Project Implications

The Marine Biomass Program, focused originally on the kelp, Macrocystis, was the first major biomass to methane project for the Gas Research Institute. It was initiated during a period of uncertainty about gas supplies, and

was managed with an engineering perspective of producing near-term gas supplies. During this time, it became increasingly clear that unconventional geological sources of gas could fill the void between depletion of known conventional reserves and a need for substitute natural gas. Concurrent with this growing awareness was a better understanding of the tremendous need for a biological approach to kelp biomass production and conversion, rather than strictly an engineering approach.

Although early experiments encountered engineering problems, a tremendous amount of information was developed for kelp. This information has subsequently been published in some of the most prestigous international scientific and engineering journals, after approval by rigorous peer review.

The major accomplishment of the kelp program, from an industry point of view has been a better understanding of systems feasibility. Actual successful yield and farm experiments, as well as anaerobic digestion and engineering process designs have been used to estimate pipeline quality gas costs from kelp. These studies now indicate that price competitive methane from kelp may be achievable with realistic improvements in kelp cultivation and bioconversion. Such systems may be possible with kelp yields ranging from 15-23 dry ash free tons/acre/yr and methane yields of 5.6-6.5 SCF/pound of volatile solids added. Some of these goals have already been accomplished in small experimental studies.

The GRI Biomass Program has shifted to a focus on land based crops such as sorghum or napier grass, as a strong agricultural infrastructure already exists for their commercialization. Analyses of the marine systems studies have indicated a strong potential of algal breeding and selection for increasing yields and reducing farm engineering costs. Accordingly, the kelp biomass program has been redirected, reduced in scope, and transitioned with GRI's long term biomass goals, where the emphasis will be on applications of biotechnologies for kelp to methane system improvements.

1.1 Overall Project Objective

The overall objective of the GRI Marine Biomass Project is to define integrated processes, including feedstock production, harvesting, and conversion, to produce methane from seaweed in nearshore systems that are cost-competitive on a commercial basis with other alternative sources of energy. The technical, economic and energy requirements of a prototype commercial production system are to be determined so that the feasibility of producing cost-competitive methane from nearshore marine biomass farms can be fully established.

1.2 List of 1983 Publications

California Institute of Technology

1. Manley, S.L., 1983. Composition of Sieve Tube Sap from *Macrocystis pyrifera* (Phaeophyta) with Emphasis on the Inorganic Constituents. *J. Phycol*. 19, 118-121

2. Manley, S.L., Micronutrient Uptake and Translocation by *Macrocystis pyrifera* (Phaeophyta). In press *J. Phycol*. 20(2), 192-201

3. Manley, S.L., Physiological Basis of Kelp Growth. In press *Proc. Symp. on Effects of Waste Disposal on Kelp Communities*. SIO 1-83.

4. North, W.J., The Sea Urchin Problem. In Press *Proc. Symp. on Effects of Waste Disposal on Kelp Communities*. SIO 1-83

5. North, W.J., Separating the Effects of Temperature and Nutrients. In Press *Proc. Symp. on Effects of Waste Disposal on Kelp Communities*. SIO 1-83

6. North, W.J., Culturing and Utilization of Large Brown Seaweeds (Kelps). Submitted to *Proc. Oceans '83 Conf*. 8-83

7. North W.J., and Zimmerman, R.C., Influences of Macronutrients and Water Temperatures on Summertime Survival of *Macrocystis* Canopies. Submitted to *Proc. Inter. Seaweed Symp*. 11

8. Wilson, K.D., and North, W.J., Restoration and Management of Kelp Beds in Southern California. In press *Proc. World Mariculture Soc*.

General Electric Company

1. A Technical and Economic Evaluation of Production of Chemicals as By-Products/Co-Products of Methane Production from Kelp - GE-BIO-1868, April 1983

2. "A System Concept Evaluation of Commercial Scale Methane Production from Coastal California Kelp" by R. Hoppmann, K. Jain, W. Kugler, J. Wrobel, ASME Paper 83-Pet-3, Presented at 6th Annual Energy - Sources Technology Conference and Exhibition, Houston, Texas, February 1983.

3. Catalina Test Farm Operating and Maintenance Manual, No. GE-BIO-1851, January 14, 1983

1.3 List of Annual Reports

1. Marine Biomass Program - Annual Report for the Period October 1976 through December 1977. GE-BIO-197. March 21, 1978

2. Energy from Marine Biomass Final Report for the Period January 1978 through December 1978 - Prepared for U.S. Department of Energy Fuels from Biomass Branch - Solar Technology and the Gas Research Institute under Cooperative Agreement ET-78-F03-2165, General Electric Company, May 18, 1979

3. Marine Biomass Program - Annual Report for 1979. GRI-79/0079, Gas Research Institute, September 1980

4. Marine Biomass Program - Annual Report for 1980. GRI-80-0080, Gas Research Institute, January 27, 1981

5. Marine Biomass Program - Annual Report for the Period September 1980 through December 1981, GRI 81-0118, Gas Research Institute, Solar Energy Research Institute

6. Marine Biomass Program - Annual Report for the Period January through December 1982, GRI-81-0182, Gas Research Institute, March 1983

7. Ghosh, S. et al, Research Study to Determine the Feasibility of Producing Methane Gas from Kelp, Final Report Project IU-4-22 for the Period August 3, 1976 to December 31, 1976

8. Chynoweth, D.P. et al, Research Study to Determine the Feasibility of Producing Methane Gas from Sea Kelp - Annual Report - IGT Project 6118, January 1 through November 22, 1977, Institute of Gas Technology, Chicago, Illinois, February 1978

9. Chynoweth, D.P. et al, Research Summary to Determine the Feasibility of Producing Methane Gas from Sea Kelp, Project 30502 - Annual Report for the Period December 1, 1977 - January 31, 1979, Institute of Gas Technology, Chicago, Illinois, March 1979

10. Chynoweth, D.P. et al, Research Summary to Determine the Feasibility of Producing Methane Gas from Sea Kelp, Project 30502 - Annual Report for the Period January 1 - December 31, 1979, Institute of Gas Technology, Chicago, Illinois, April 1980

11. Fannin, K.F. et al, Research Summary to Determine the Feasibility of Producing Methane Gas from Sea Kelp, Project 30502 - Annual Report for the Period January 1 - August 31, 1980, Institute of Gas Technology, Chicago, Illinois, February 1981

12. Fannin, K.F. et al, Marine Biomass Program: Anaerobic Digester System Development, Project 65044 - Annual Report for the Period September 1, 1980 - March 31, 1981, Institute of Gas Technology, Chicago, Illinois, October 1981

13. Gerard, V.A. et al, Marine Biomass Program Yield and Biological Studies - Annual Report for 1980, California Institute of Technology, Pasadena, California, January 7, 1981

14. North, W.J., List of Cal-Tech Marine Farm Activities, July to December 31, 1979, California Institute of Technology, Pasadena, California, January 1980

15. Barcelona, M.J. et al, Marine Biomass Program Kelp Production Studies, Final Report for September 1980 to December 1981, California Institute of Technology, Pasadena, California, January 1981

16. Arnold, K.E. et al, Marine Biomass Program Kelp Production and Yield Studies, Annual Report for January 1 to December 31, 1982, California Institute of Technology, Pasadena, California, 15 February 1983 (Also: Appendices A, B & C

17. Global Marine Development Inc., Marine Biomass Program Annual Report - # 4050-003 - 1977, Global Marine Development Inc., Newport Beach, California 92660, 12 February 1978

18. Oceanographic Services Inc., Oceanographic Site Selection and Final Characterization Final Report # 04589, Oceanographic Services Inc., Santa Barbara, California, January 1978

19. Global Marine Development Inc., Marine Biomass Program - Annual Report - 1978, Global Marine Development Inc., Newport Beach, California, February 1979

20. Global Marine Development Inc., Marine Biomass Program - Annual Report - 1979, Global Marine Development Inc., Newport Beach, California, February 1980

21. Global Marine Development Inc., Marine Biomass Program - Annual Report - 1980, Global Marine Development Inc., Newport Beach, California, February 1981

22. Global Marine Development Inc., Marine Biomass Program - Annual Report - 1981, Global Marine Development Inc., Newport Beach, California, February 1982

23. Global Marine Development Inc., Marine Biomass Program - Final Report - Global Marine Development Inc., Newport Beach, California, July 1982

24. Forro, J.R. et al, Marine Biomass Program Inoculum Development Final Report - 1977, Doc. No. 78SDR2122, General Electric Company, January 30, 1978

25. Hart, M.R. et al, Final Report - Ocean Food and Energy Farm Kelp Pretreatment and Separation Processes, July 1975 - July 1976, Western Regional Research Center, U.S. Department of Agriculture, Berkeley, California

26. Hart, M.R. et al, Annual Report - Kelp Pretreatment and Separation Processes for the Energy from Marine Biomass Project, April 1977 - June 1978, Western Regional Research Center, U.S. Department of Agriculture, Berkeley, California

27. Hart, M.R., Pre and Posttreatment Processing Studies for the Energy from Marine Biomass Program, Final Report - Western Regional Research Center, U.S. Department of Agriculture, Berkeley, California, June 15, 1981

28. Hart, M.R., Pre and Posttreatment Processing Studies for the Energy from Marine Biomass Program, Final Report for the Period of April 1977 through December 31, 1981, - April 2, 1982 - Western Regional Research Center, U.S. Department of Agriculture, Berkeley, California

29. Neushul, M. et al, Kelp Cultivation, Characterization and Genetics Program - Annual Report, May through August 1980, Neushul Mariculture Inc., Santa Barbara, California, October 1980

30. Neushul, M. et al, Kelp Biomass Production Coastal Test Farm Program Annual Report for 1982, Neushul Mariculture Inc., Santa Barbara, California, February 1983

31. Neushul, M. et al, Kelp Biomass Production Coastal Test Farm Program Annual Report September 1980 - December 1981 - Neushul Mariculture Inc., Santa Barbara, California, February 1982

1.4 List of Topical Reports

1. Anderson, N.B., Ocean Food and Energy Farm; Development of the Kelp Growth Model, Integrated Sciences Corporation, Santa Monica, California, August 1976. Prepared for the Naval Undersea Center, San Diego, California - Code 4006.

2. Aquaculture Associates Inc., Energy from Marine Biomass Program Review - Final Report - Prepared for the Gas Research Institute, Chicago, Illinois, Aquaculture Associates, Inc., Honolulu, Hawaii, December 1, 1982

3. Beleau, M.H. and D. Van Dyke, Ocean Food & Energy Farm Food Subsystem Final Report - September 1976. Performed by Naval Undersea Center under contract to the American Gas Association

4. Bird, K.T, Historical Perspective on Economic and System Cost of Kelp Cultivation for Bioconversion to Methane, Gas Research Institute, No Date

5. Bryce, A. - A Research & Development Program to Assess the Technical and Economic Feasibility of Methane Production from Kelp. Presented at the Ninth Synthetic Pipeline Gas Symposium, Chicago, Illinois, 10/31/77

6. Budhraja, V. et al. Systems Analysis of the Ocean Food and Energy Farm Draft Final Report. 30 June 1976 - Integrated Sciences Inc., Santa Monica, California. Prepared under ERDA/NUC Interagency Agreement No. E(49-26)-1027.

Volume I	Overall Economic Analysis of the Baseline Design
Volume II	Cultivation Subsystem
Volume III	Harvesting Subsystem
Volume IV	Processing Subsystem
Volume V	Support Subsystems
Volume VI	Mariculture Subsystem
Volume VII	Appendix

7. Charters, A.C., Neushul, M. and Coan, D. The Effects of Water Motion on Algal Spore Adhesion. Limnology and Oceanography, Volume 18, No. 6, November 1973, pp. 884-896

8. Dilley, J.F., Dispersion Predictions for the Channel Off Fisherman's Cove/Santa Catalina Island. General Electric Company, Phila., Pa. GE-BIO-1141, May 1981

9.. Druehl, L.D., The Cylinder Experiments: Studies into Various Aspects of Macrocystis Nutrient Fertilization - Topical Report- January 1982 - March 1983 - Canadian Kelp Resources Ltd., Vancouver, B.C., Canada

10. Fannin, K. et al, Marine Biomass Program: Anaerobic Digester Process Development - Final Report, Project 65044 & 30547. September 1, 1980 - December 31, 1981, Institute of Gas Technology, December 1982

11. Fannin, K. et al, Marine Biomass Program: Anaerobic System Development and Stabilization Study. Final Report for the Period February-December 31, 1982. Institute of Gas Technology, Chicago, Illinois, June 1983

12. Feldman, H., Pipeline Gas from Solid Waste by the Syngas Recycling Process. Battelle Columbus Laboratories, prepared by Syngas Recycling Company, Toronto, Ontario, December 17, 1976

13. Flowers, A. et al, Survey of Commercial Seaweed Production for the Marine Biomass Program. Gas Research Institute, Chicago, Illinois, Solar Energy Research Institute, Golden, Colorado, December 1980

14. Forro, John H., Alginate Utilization of Marine Anaerobes - Presented at the 78th Annual Meeting American Society for Microbiology, Las Vegas, Nevada - 1978, General Electric Company, Philadelphia, Pa.

15. Forro, J., Chynoweth, D., and Hart, M., Kelp Processing and Biomethanation Technology, AIAA-80-9170-CP. 15th Intersociety Energy Conversion Engineering Conf., Seattle, Washington, August 18-22, 1980

16. Fuel Gas Production from Solid Waste Final Report. Dynatech R&D Company, Cambridge, Massachusetts. NSF RANN No. C-827. Dynatech Report 1258, January 31, 1975

17. Gas Research Institute, Ralph M. Parsons. Economic and Systems Assessment of the Concept for Nearshore Kelp Farming for Methane. Gas Research Institute, Chicago, Illinois, May 1983

18. Gas Research Institute, An Overview of GRI Research on Methane from Biomass, Gas Research Institute, Chicago, Illinois, May 1980

19. Gas Research Institute, General Electric Company, Energy from Marine Biomass Program Presentation, presented at Bio-Energy '80 World Congress and Exposition, Atlanta, Georgia, April 23, 1980

20. Gilmartin, W.G., Ocean Energy Farm Project Microbiology Subsystem Annual Report. 1 July 1975 - 30 June 1976. Biomedical Group Code 402, NUC, San Diego, California

21. Goldman, J.C. and Ryther, J.H., Mass Production of Algae: Bioengineering Aspects. Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. Presented at Conference on "Biological Solar Energy Conversion", 15-18 November 1976, Miami, Florida

22. Goldman, J. et al, Topical Report on Sources and Systems for Aquatic Plant Biomass as an Energy Resource, Dynatech R&D Co., Cambridge, Mass., March 27, 1978, NTIS-DST-4000-77/1

23. Hart, M.R. et al., Ocean Food and Energy Farm, Kelp Pretreatment and Separation Processes. Final Report, Western Regional Research Center, Agricultural Research Services, U.S.D.A., Berkeley, California, Naval Weapons Center Contract No. 40201-16-75/ARS Agreement No. 12-14-5001-6402, July 1975 - July 1976

24. Hoppmann, R.F., Measurement of Polyethylene Pipe Parameters During an Ocean Deployment - Final Report, November 15, 1978, General Electric Company, Phila., Pa.

25. Jackson, G.A. & Winant, C., Effect of a Kelp Forest on Coastal Current. Continental Shelf Research, Vol. II, pp. 75-80. 1983

26. Kirchoff, R.H., Upwelling Nutrient Plume Path, Size and Concentration for the Ocean Farm Project. Final Report for Naval Undersea Center under Contract No. N66001-76-M-1C127. February 27, 1976

27. Lindner, E. et al., Ocean Food & Energy Farm Project - Chemical Variation of Chemical Constituents in Macrocystis pyrifera. January 1977. Naval Undersea Center, San Diego, California

28. Murphy, D.W., et al and Hoffman, R.T., et al., Preliminary Design Studies of Substrate & Upwelling Systems Final Report. Ocean Technology Department, Ocean Systems Division, NUC, Hawaii Laboratory, September 1976. ERDA/NUC Interagency Agreement No. E (49-26)-1027.

Volume I	Kelp Support Substrate Structures
Volume II	Artificial Upwelling

29. National Ocean Policy Study. Energy from Open Ocean Kelp Farms. Committee on Commerce, Science and Transportation and National Policy Study, United States Senate, U.S. Government Printing Office, Washington, D.C., September 1980

30. Neushul, M. et al., An In-Situ Study of Recruitment, Growth and Survival of Sudtidal Marine Algae: Techniques and Preliminary Results, University of California at Santa Barbara

31. Neushul Mariculture Inc., Kelp Plants and Kelp Beds in Southern California, Neushul Mariculture Inc., Goleta, Ca., May 6, 1982

32. Ninety-sixth U.S. Congress Ocean Biomass Conversion Oversight in Hearings before the Sub-Committee on Oceanography - Committee of Merchant Marine and Fisheries, House of Representatives, September 26, 1979

33. North, Wheeler J. and Jackson, G.A., Concerning the Selection of Seaweeds Suitable for Mass Cultivation in a Number of Large, Open Ocean, Solar Energy Facilities ("Marine Farms"). Final Report, Contract No. N60530-73-M-U176, U.S. Naval Weapons Center, China Lake, Ca., October 15, 1973

34. North, W.J., Observations on the Dispersion & Sinking of Artificially Upwelled Water in the Pacific Ocean, Crystal Cove, California, November 1975. California Institute of Technology conducted under Naval Undersea Center Contract No. N66001-6-000013-04.

35. North, W.J. and Staff, Ocean Food & Energy Farm Project; Biological Studies of M. pyrifera Growth in Upwelled Oceanic Water, Final Report November 1976. California Institute of Technology, W.M. Keck Engineering Laboratories, Pasadena, California. Prepared for ERDA under Interagency Agreement No. E(49-26)-1027, ERDA/NUC Navy Contract No. N00123-76-C-0313

36. North, W.J., Preliminary Report on the St Croix Experiments Studying the Effects of Upwelled Atlantic Water on Juvenile Macrocystis Sporophytes. California Institute of Technology, August 17, 1976

37. Roels, O.A. et al, Organic Production Potential of Artificial Upwelling Marine Culture, University of Texas Marine Science Institute, Port Aransas, Texas, October 1976

38. Rosenberg, Joseph, Cost Estimation for Methance from Biomass. Presented at Biomass Project Advisors Meeting, Gas Research Institute, Chicago, Illinois, March 9, 1982

39. Ryther, John H., Cultivation of Macroscopic Marine Algae and Freshwater Aquatic Weeds, Progress Report - May - December 15, 1976, Woods Hole Oceanographic Institute, January 1980. Prepared for Solar Energy Research Institute, Golden, Colorado

40. Seligman, P.F., Study of the Fate of Upwelled Water, Naval Undersea Center, San Diego, California, April 2, 1976

41. Seligman, P.F., Survey of Oceanographic & Meteorological Parameters of Importance to the Site Selection of an Ocean Food & Energy Farm in the Eastern Pacific. Final Report. Undersea Sciences Dept., Chemistry and Environmental Division, December 15, 1976. Performed for ERDA under Interagency Agreement No. E(49-26)-1027. ERDA/U.S. Naval Undersea Center, San Diego, California

42. Sullivan, R.J., A Study of Gas Cost Variations for Systems Producing SNG from Marine Biomass in New York State. General Electric Company, Philadelphia, Pa., November 13, 1981

43. Walsh, D., Method for Calculating Ocean Food & Energy Farm Costs. Final Report - Integrated Sciences Corporation, Santa Monica, California, February 1976. Prepared for Code 4006, Naval Undersea Center, San Diego, California

44. Wilcox, H.A., A Preliminary Drag Model for the Ocean Food & Energy Farm, Naval Undersea Center, San Diego, California, February 1976, Revised September 1976.

45. Wilcox, H.A., Artificial Oceanic Upwelling, Naval Undersea Center, San Diego, California, July 1975

46. Wilcox, H.A., Preliminary Design and Cost Models for Spherical and Cylindrical Buoys for the Ocean Farm Project, U.S. Naval Undersea Center, San Diego, California, February 1976

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2. SUMMARY OF ALL WORK PREVIOUSLY PERFORMED

2. SUMMARY OF ALL WORK PREVIOUSLY PERFORMED

The objective of the Marine Biomass Program since its inception has been to determine the technical and economical feasibility of commercial production of methane from marine biomass. A key element in developing the necessary data base for credible cost estimation was the ability to determine the sustained yield from a managed kelp crop. This data was not available on large scale kelp stands. Previous efforts had been confined to the measurement of single plant growth. It was not certain that single plant growth data could be unquestionably extrapolated to predict yields from large, managed stands. In addition, other quantitative data had to be developed in the areas of nutrition, (chemical and photosynthetic requirements), harvesting, planting, other elements of crop management and in conversion of feedstock into methane. As all of the above data is interrelated, it was felt by GRI and the General Electric Company that a systems analysis approach was mandatory in order to integrate the many lines of research; to provide orderly acquisition and utilization of the data and to provide effective management of budgets and schedules. A preliminary systems study was performed early in the program in order to meet the above objectives.

The initial thrust of the program was to determine kelp yield and nutritional requirements on an offshore test platform (OSTP) which was moored approximately 5 miles off the California coast. Nutrients were provided by artificial upwelling from a 1500 foot depth via a flexible pipe. The test platform is shown in Figure 2-1.

In parallel to the yield and nutrition study, work was being performed on anaerobic digestion. This work addressed the critical information needed for optimum design and operation of the basic digestion system as well as those elements associated with feedstock processing, both pre and post digestion. The output from these tasks included data on nutrient, temperature and rate requirements for best digestion as well as an examination of the mechanical

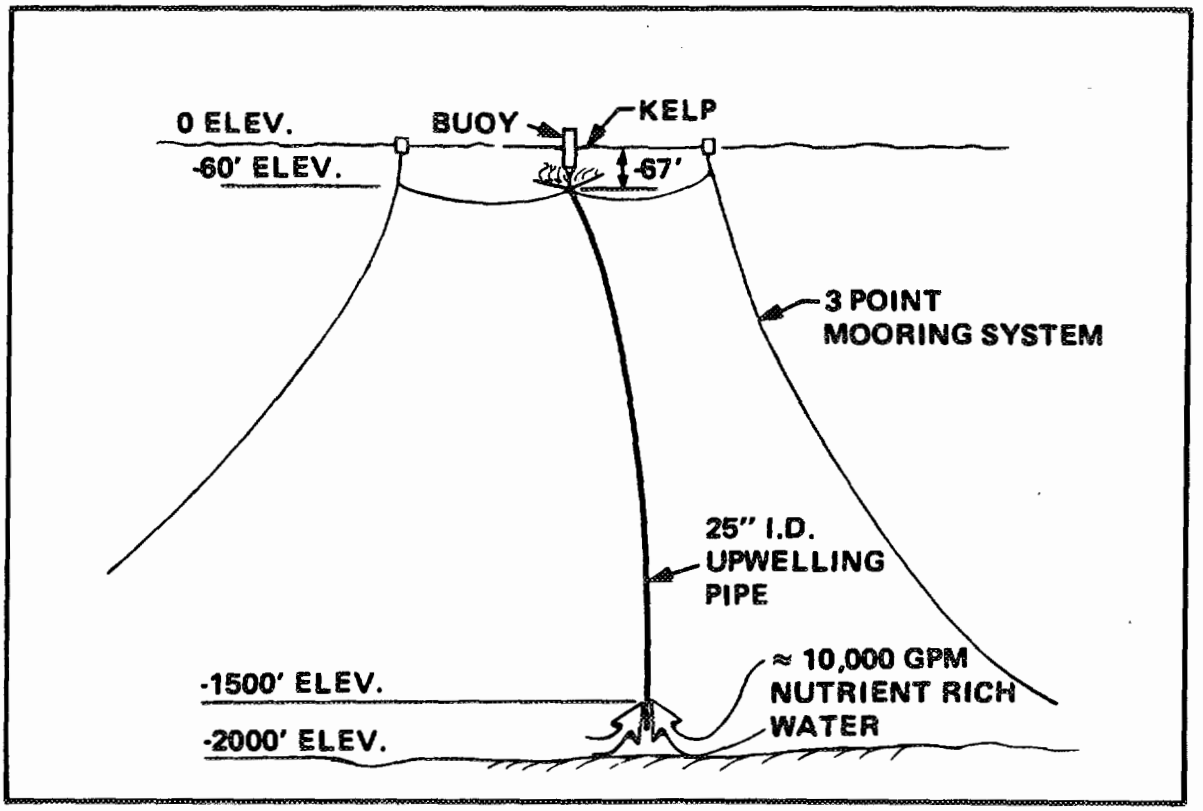


Figure 2-1. Offshore Test Platform

and energy requirements for feedstock preparation and post digestion treatment and residue disposal. No effort was expended on development of methane clean-up and compression techniques as these have been extensively studied by others.

In light of the data from the OSTP, it was decided to pursue the yield information via two parallel studies. One study investigated yield under controlled nutrition conditions. This was conducted in a kelp containment device referred to as the Hemidome (Figure 2-2). The Hemidome was used to monitor the growth and yield of 50 kelp plants. The plants were isolated from the ambient environment by the test apparatus. Use of the Hemidome allowed close monitoring of the input and output of nutrients and photosynthetic activity as well as allowing partial control of the temperature environment. The Hemidome was installed and operated at Catalina Island.

A second yield experiment had the objective of determining yield from a large number of plants (700), maintained in a nearshore environment, under saturated nutrient conditions (Figure 2-3). This group of plants was not enclosed. Associated supporting experiments on kelp physiology and cultivation were conducted in the laboratory. Also during this period, the program proceeded to develop anaerobic digestion process design data. The process design activity was an extension of the previous digestion research and process development projects.

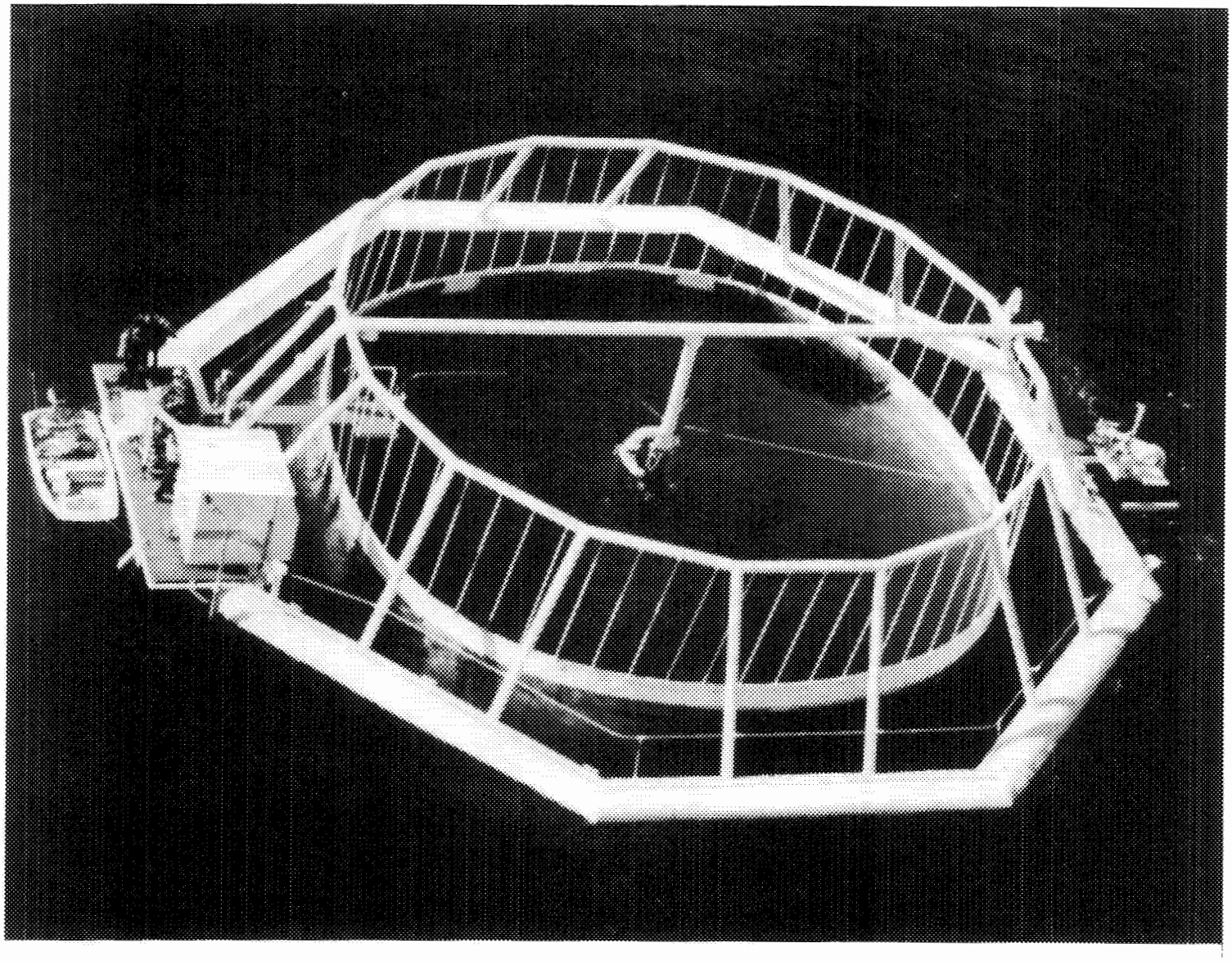


Figure 2-2. The Hemidome

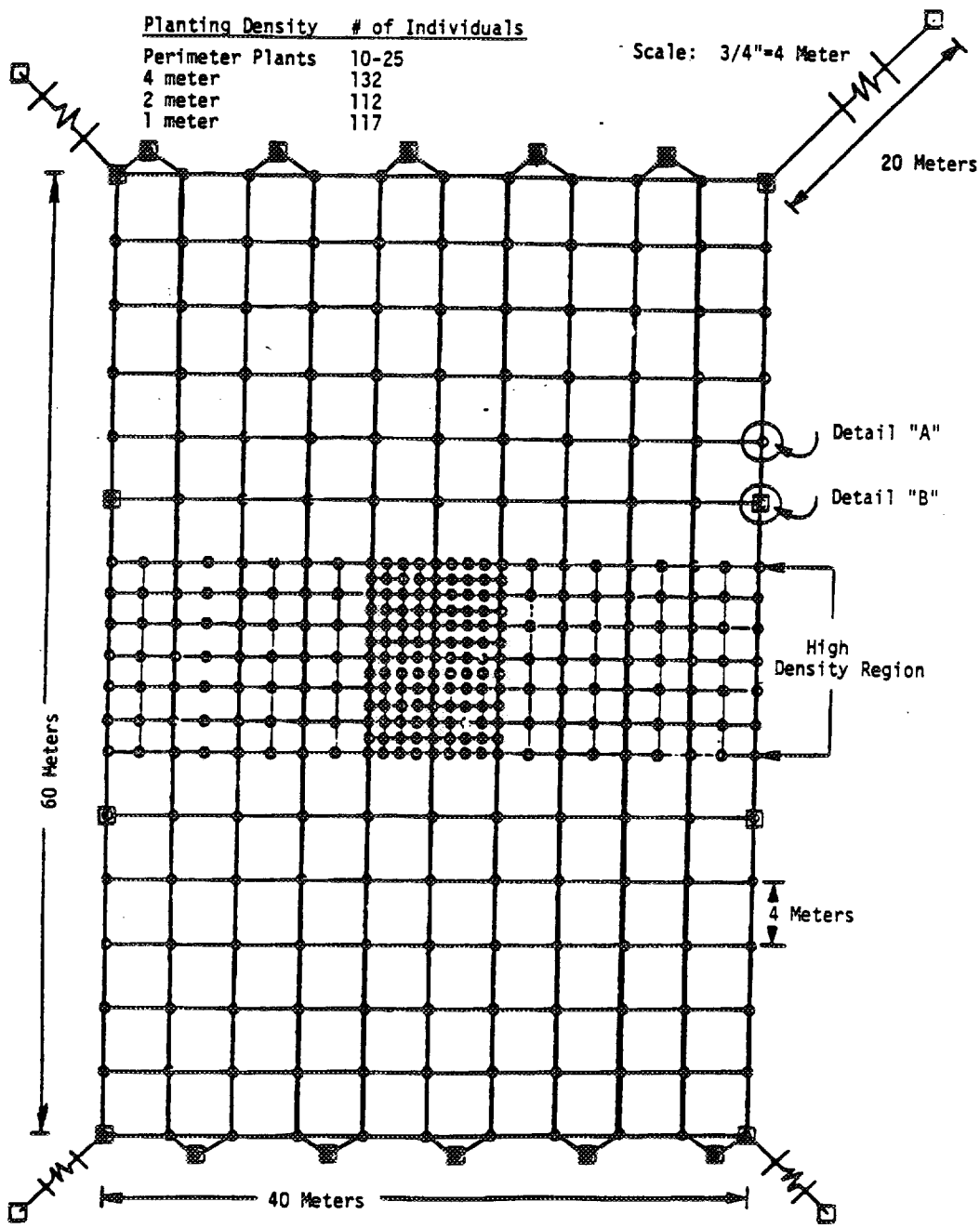


Figure 2-3. Goleta Test Farm Layout and Substrate

2.1 Highlights of Key Results Obtained During the 1976-1982 Programs

A. Kelp Growth and Nutrition

1. OSTP Studies

Data provided by the OSTP showed that the requisite yield information could only be obtained offshore if a test platform of very large dimensions was used, probably on the order of one or more acres. (The OSTP was less than 0.2 acres in area.) The large-size requirement was forced by the interaction of offshore currents and light with the nutritional needs of the plants. These negative environmental impacts on the OSTP were magnified by the small size of the farm. Nutrients could not be dispersed evenly, and ambient currents pulled the plants down, thus significantly reducing ambient light. In a much larger farm, these interactions would have represented only edge effects and affected only a small portion of the planted area. In the case of the small, unenclosed platform, however, the entire planted area represented an edge. Enlargement or enclosure of the offshore test platform was not feasible due to cost constraints. Therefore, it was decided to pursue alternate methods of developing the critical yield data.

Significant results from the OSTP experiment included the observations that:

Kelp can grow and reproduce on an offshore structure given an adequate nutrient environment and that survivability of juvenile kelp on the OSTP exceeded that of juveniles in natural beds. Oceanographic studies that were associated with the OSTP experiment led to significant cost reductions in the projected system costs. The oceanographic data showed that upwelling depth could be raised from 1500' to 300' for a commercial offshore farm.

2. Kelp Physiology Studies

Significant physiological findings acquired during this period included determination of the nitrogen budget of Macrocystis. This information allowed the systems analysts to more accurately predict the minimum nitrogen levels that were needed for active plant growth. The data had major impact on reducing cost requirements for future experimental studies as well as for reducing the projected product costs in commercial farms.

It was also found, during this period, that nutrient uptake rates in the plants were significantly decreased as a function of depth. Plant tissue at 30 ft depth has 40% of the nitrogen uptake rate of plants at the surface. This information was the first obtained that provided a rationale for selection of permissible nutrient and current environments in a managed farm.

3. Kelp Engineering Studies

In support of preparation of the systems analysis specification, a number of tests were performed in order to determine various physical or engineering parameters of kelp plants. These studies included measurements of buoyancy and drag of individual kelp plants as well as the horizontal forces that would be required to dislodge plants of various sizes. These values were determined as functions of plant density, length and planting depth.

Buoyancy was related to cumulative submerged frond length (calculated from the frond size distribution) at six planting depths for typical plants ($\sim$6-10 fronds). The relationship between buoyancy and submerged frond length was found to be $Y = 0.3 + 0.005\ X$ (where Y = buoyancy and X = frond length) i.e. each foot of Macrocystis frond length has approximately 0.005 lb of buoyant force. Drag of whole plants was determined by means of tow testing at sea. The relationship between drag and current velocity is shown graphically in Figure 2-4. Subsequent buoyancy and drag measurements were made on instrumented plants, in place, at the Offshore Test Platform. The measurement techniques were

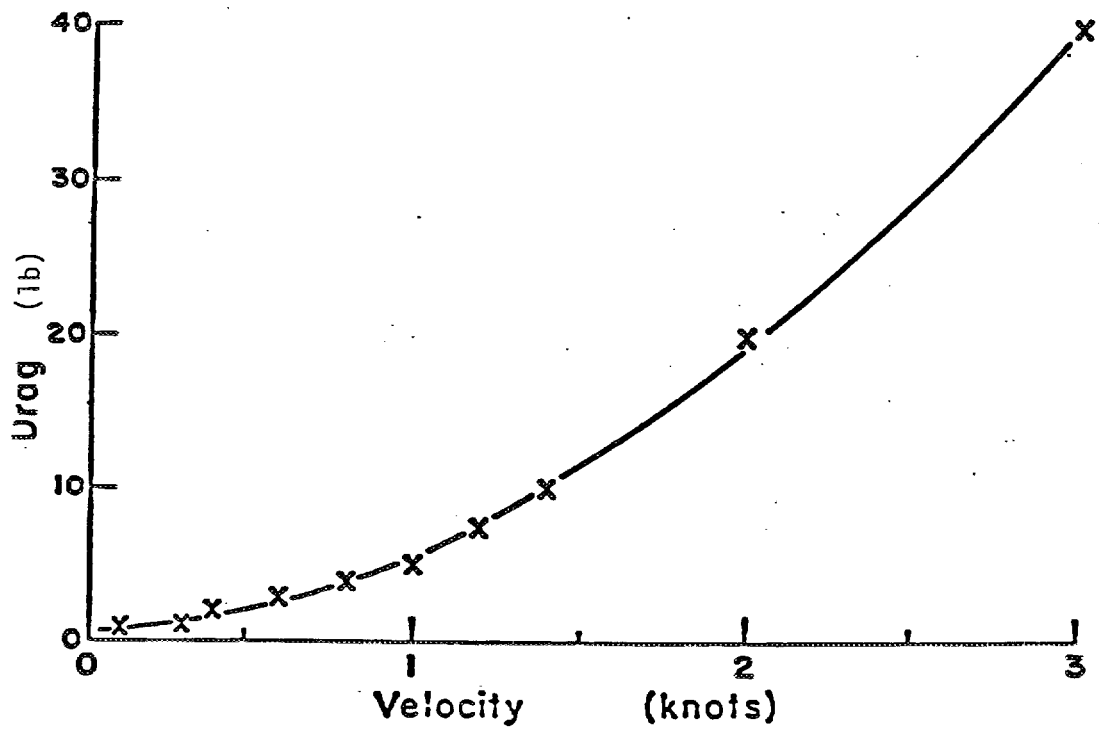


Figure 2-4. Drag of the Experimental Kelp Plant Towed at Various Velocities at the Surface

more refined than those described above, however, the results were in substantial agreement. Detailed descriptions of these activities can be found in the General Electric Program Information Releases; U-1K8-81-91-661, "Buoyancy Measurements on Macrocystis pyrifera" by John McGinn, 10/8/81; and U-F03-80-91-635, "Experiments with Elastic Tethers and Damping Plate" by John McGinn, 5/20/80; and in "System Functional Requirements and Specification for a Nearshore Kelp to SNG Production Facility" - Revision A, that the General Electric Company prepared for the Gas Research Institute, October 8, 1982.

The output of these tasks was used in providing design parameters for the Parsons/GRI Nearshore System Analysis and was extremely valuable in defining critical system design requirements for the planting subsystem. Additional studies determined the net longshore current ($\sim$1 Km/day) in an average density (1-5 fronds/m^2) kelp forest, ibid., and the holding power of Macrocystis holdfasts in sandy bottom. In the latter study, it was found that the force required to dislodge the holdfast from the bottom ranged from a low of 10 lbs for a 7 frond plant to more than 220 lbs for 36 frond plants. Figure 2-5 shows the relationship between plant size and the horizontal force required to dislodge the holdfast. Also shown is the maximum hydrodynamic drag projected for the systems analysis design conditions. This data was extremely valuable in that it allowed significant reduction in projected system costs by shifting the anchoring requirement for adult plants from the system hardware to the plant. The estimated requirement for plant anchor size used in the form was therefore reduced from 230 lbs/plant to $\sim$10 lb/plant due to the acquisition of this data. Note that on Figure 2-5, the maximum hydrodynamic drag estimated to be experienced by a 200 frond plant during 20 ft seas at a 20 second period is $\sim$20 lbs, while a horizontal force of $\sim$50 lbs is required to dislodge the holdfast. A detailed discussion of this work can be found in "Measurement of Holdfast Tearout Resistance of Macrocystis angustifolia by R. Hoppmann and R. Berthold, General Electric Company, October 29, 1982.

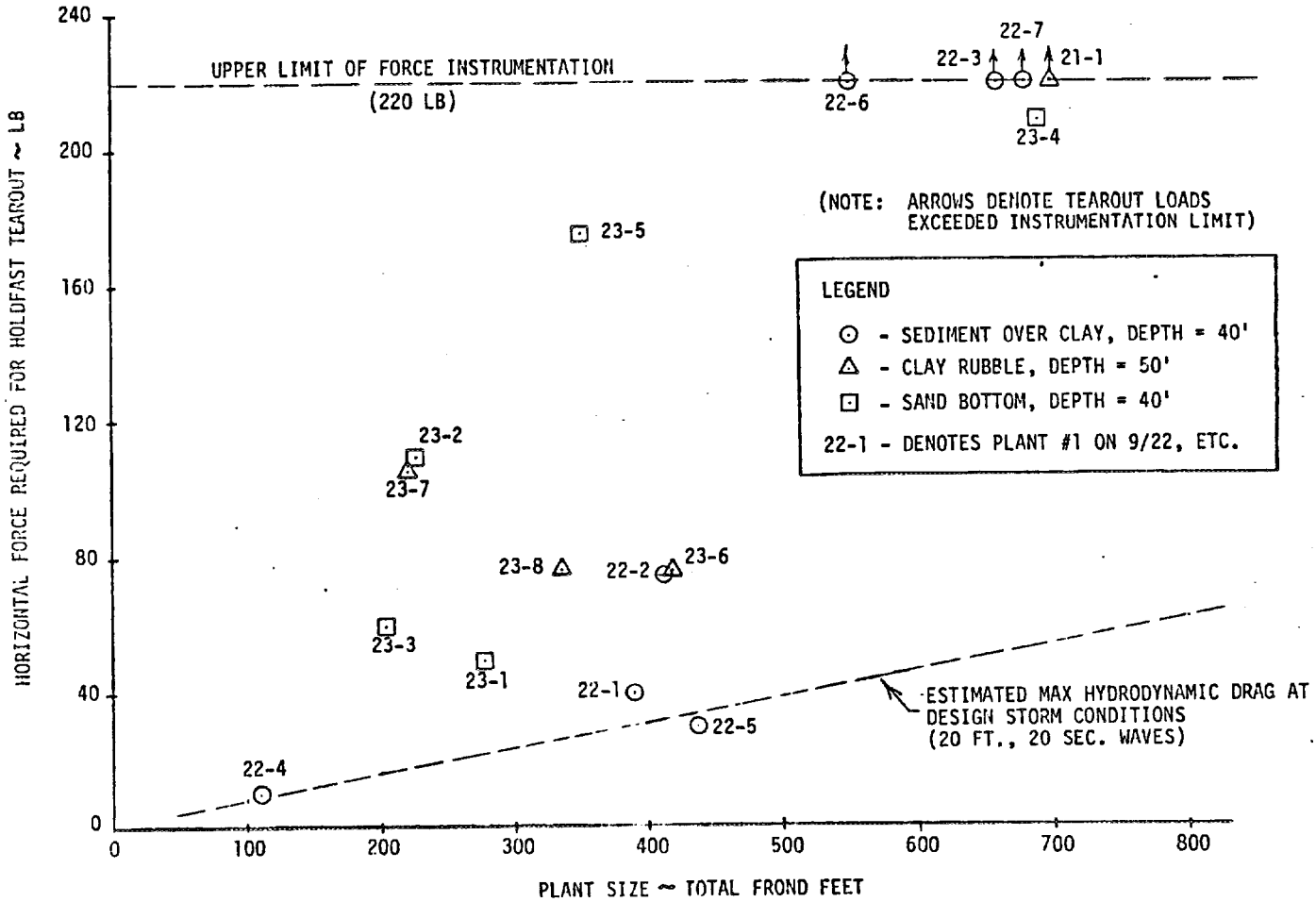


Figure 2-5. Resistance of Macrocystis angustifolia Holdfasts to Tearout by Horizontal Forces

The data from the above tests allowed the estimation of realistic design requirements for the conceptual system. Once the physical characteristics of the plants were determined in-situ, former overly conservative design requirements could be adjusted to more realistic levels. Determination of actual buoyancy and drag values allowed a better estimate of the degrees to which photosynthesizing canopies would be pulled down by ambient currents and the length of time to which canopies would be exposed to added nutrients as a function of intra-bed currents.

4. Near Shore Test Farm

The Near Shore Test Farm provided the first harvest yield values from a large number of plants that have ever been obtained.

The results from this experiment provided the basis for valid estimation of sustainable yield as a function of density in a commercial farm. Such estimates were critical to performance of a credible systems analysis. The experiment also yielded insight into strategies that may be used for optimizing single plant production rates. In addition, to the yield data, the study determined that certain individual plants within the general population were capable of consistently producing 3-4 times the amount of biomass, per measuring period, as the population average. These plants were isolated with the objective of producing a uniform population of high producing plants.

Further insight was gained into strategies that may be used for increasing crop yield through various planting, or "crop arrangement" configurations. For example, a series of densely planted rows at widely spaced intervals may be more productive than a uniformly planted crop. Also, variation in harvesting intervals may be required during different growth cycles of the crop.

Overall, the output of this work was critical to production of the system/economic analysis and was also vital in providing data which could be used for projecting credible cost reduction scenarios.

5. Hemidome

The Hemidome experiment failed to satisfactorily meet its objective of determining yield as a function of ambient nutrient level and planting density. Enclosure of the plants in the test apparatus (Figure 2-2) apparently caused intensification of local biological and chemical effects within the system. These effects led to degradation of the plants within the system in relatively short periods of time (6-8 weeks). The planned experimental interval was one year. Although some useful biological information was obtained, it was felt that the cost of maintaining the hardware was not justified by the results. All operations in the Hemidome were discontinued during the first quarter of 1983. A revised experimental program was defined for the balance of 1983. This program will be discussed in detail in Section 4 of this report.

6. Research Matrix

During April 1981, a comprehensive review of all current program needs was held by the Gas Research Institute and the Solar Energy Research Institute. All program technical participants contributed. The objective was to re-examine all of the data needs that would be required for preparation of the comprehensive system/economic analysis considering timing, value, research budget, project schedule and project activities in progress. As a result of this review, a research matrix was developed which listed and prioritized all data requirements, and the tasks that needed to be accomplished in order to produce the requisite information. The ranking system used for prioritization incorporated considerations of timing and technical value of the data. Timing considered the length of time required to produce

the information, and also, when in the analytical cycle, the data that would be needed; either for use in planning subsequent work or for providing direct inputs to the systems analysis. Technical Value ranking considered whether a given task was interdependent with other specified tasks, and also, the degree of criticality of a given data input to production of a credible systems study. In order to control the scope and to assist in judging value, the matrix participants were required to identify where in the planned program the specified data would be used. Further, an assessment of where the work would be performed was included in order to provide early identification of any facilities or technical skills that were not currently available to the program.

Table 2.1-1 shows the completed Research Matrix. The Comments section of the table provides a summary status of the given tasks and in most cases identifies a secondary reference for more detailed information. Primary references can generally be found in the sources indicated. The rankings indicated in the table were reached by consensus of all the technical participants in addition to GRI and SERI.

Task Ranking System

Timing

1. Start immediately in order to meet program time schedules.

2. Can be started later without impacting program schedule.

3. Timing will not impact program schedule:

   - Data is available or,
   - Data not essential to baseline system specification or analysis or,

- o Reasonable estimates can be calculated without impacting credibility of system analysis

Technical Value

A. Data does not exist; confident estimates cannot be made and are essential to production of credible systems analysis

B. Preliminary data is available and reasonably confident projections can be made, essential to systems analysis

C. Data is not essential to the baseline systems analysis

Note: Tasks B.3, B.4, and B.7 have double entries e.g.; 3/1 for Timing and B/B for Technical Value. In these cases, a group consensus could not be reached; therefore, rankings show a difference of opinion within the group.

All subsequent program planning, budget allocations and research activities were based on the Matrix rankings. Note that all tasks ranked 1 A were completed in time for the systems analysis. Exceptions are those involving operation of the Hemidome facility. These were implemented but could not be completed due to failure of the test system.

B. Anaerobic Digestion

The 1976-1982 program met all objectives of developing a data base for the processing and anaerobic digestion of kelp.

- Nutrients and level of nutrients critical to active digestion were determined.
- Feedstock pretreatment requirements and methods were defined.

- Pretreatment methods were tested at pilot plant scale.

- By the end of this test period, digestion stability had been obtained at rates 20% higher than those achieved in the beginning of the project.

- A data base was accumulated which served as the basis for later digestion process design, and subsequently, for cost estimation in the systems/economic study.

- In parallel with digestion process development, basic research was conducted in order to determine the microbiological and biochemical interactions occurring within the digester. The objective of this work was to understand and optimize the digestion process in order to provide cost reducing inputs to the systems analysis. These include; lower temperature digester operation, reduction in digester size, more stable process operation, and increase in operating reliability. This work was successful in characterizing the microorganisms and substrates critical to the methane production process. In addition, several potentially competing organisms were identified (those which digest kelp but do not produce methane). Overall, although no single development from this basic research impacted process development, the usefulness of its findings in assisting system development and analysis was material in regards to suggesting potential process controls and new reactor configurations.

Table 2.1-1. Research Matrix

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
A. PRODUCTION BIOLOGY							
1. MACROCYSTIS YIELD UNDER GROWTH-SATURATED NUTRIENT CONDITIONS (AT A SPECIFIED PLANTING DEPTH AND HARVEST STRATEGY)	MEASURE SUSTAINED YIELD (HAND HARVESTED) AND PRODUCTIVITY OF ADULT PLANTS IN NUTRIENT-ENRICHED SURFACE WATER AND RELATE TO MEASURED YIELDS, CONTROLLED ENCLOSED TEST ENVIRONMENT WITH MEASURED/MONITORED CONDITIONS. DRUEHL CYL. FOR CONTROL DATA.	BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT MODEL DEVELOPMENT	CTF	CIT/SMS	1	A	HEMIDOME EXPERIMENT PERFORMED. REF. GRI ANNUAL REPORTS; GRI 81-0118, 81-0182, & GRI 81-0096
2. MACROCYSTIS YIELD UNDER OPEN, NUTRIENT-SUPPLEMENTED, COASTAL CONDITIONS (AT A SPECIFIED PLANTING DEPTH AND HARVESTSTRATEGY).	MEASURE SUSTAINED YIELD (MACHINE AND/OR HAND HARVESTED) AND PRODUCTIVITY OF ADULT PLANTS IN OPEN, CULTIVATED, COASTAL FARM PLOTS. NATURAL NUTRIENTS WILL BE SUPPLEMENTED WITH ARTIFICAL NUTRIENTS. ENVIRONMENTAL CONDITIONS WILL BE MEASURED/MONITORED. UNFERTILIZED TEST PLOTS WILL BE USED FOR CONTROL DATA.	BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT MODEL DEVELOPMENT	GTF	NMI	1	A	GOLETA TEST FARM INSTALLED & OPERATED. REF. GRI ANNUAL REPORTS; GRI 81-0118, 81-0182 & GRI 81-0096
3. OPTIMAL FROND DENSITY, PLANT SIZE, AND PLANTING DENSITY EVALUATION	COMPARE YIELD AND PRODUCTIVITY WITH VARIOUS PLANT SIZES, FROND DENSITIES AND PLANTING DENSITIES UNDER CONTROLLED ENVIRONMENTAL CONDITIONS TO DETERMINE OPTIMUM PLANTING STRATEGY.	BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT MODEL DEVELOPMENT FARM CONCEPT DEVEL.	CTF	CIT	1	A	GOLETA TEST FARM IN PROGRESS. REF. OP. CIT AND WORK IN PROGRESS 1984
	COMPARE YIELD AND PRODUCTIVITY WITH VARIOUS PLANT SIZES AND PLANTING DENSITIES UNDER SIMILAR MEASURED/MONITORED ENVIRONMENTAL CONDITIONS IN OPEN COASTAL TEST PLOTS.		GTF	NMI	1	A	GOLETA TEST FARM IN PROGRESS. REF. OP. CIT AND WORK IN PROGRESS 1984
4. OPTIMAL PLANTING DEPTH	COMPARE PRODUCTIVITY OF ADULT PLANTS AT DIFFERENT HOLDFAST DEPTHS IN NATURAL BEDS AND IN THE CONTROLLED ENVIRONMENT OF THE CTF.	BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT MODEL DEVELOPMENT FARM CONCEPT DEVEL.	NATURAL BEDS, CTF	CIT	2	A	PLANNED BUT DELETED IN 1983 DUE TO BLDG. RESTRICTION.
	COMPARE YIELD FROM OPEN COASTAL FARMS PLANTED AT VARIOUS DEPTHS WITH SIMILAR MEASURED/MONITORED ENVIRONMENTAL CONDITIONS.		GTF	NMI	2	A	PLANNED BUT DELETED IN 1983 DUE TO BLDG. RESTRICTION
5. EFFECT OF ENVIRONMENTAL VARIATIONS	COMPARE PRODUCTIVITY/YIELD DATA FOR DIFFERENT ENVIR. CONDITIONS (E.G. DIFFERENT SEASONS) IN CLOSED/OPEN FARMS.	MODEL DEVELOPMENT SYSTEM MODEL INPUT	CTF/GTF	CIT/NMI	1	A	GOLETA TEST FARM OP. CIT & IN PROGRESS
6. COMBINED EFFECTS OF ENVIRONMENTAL AND BIOLOGICAL VARIABLES	DEVELOP BIOLOGICAL MODEL INCLUDING EFFECTS OF ENVIRONMENTAL VARIABLES (E.G. LIGHT, TEMPERATURE, NUTRIENTS) AS WELL AS BIOLOGICAL PARAMETERS (E.G., PHOTOSYNTHESIS, RESPIRATION, NUTRIENT STORAGE, ...) TO PREDICT PRODUCTIVITY AND YIELD OF PLANTS AND GROUPS OF PLANTS.	SYSTEM MODEL INPUT	SCRIPPS	JACKSON (?)	2	A	INITIATED 1983. IN PROGRESS 1984. REF. SECTION 4 OF THIS REPORT
7. OPTIMUM HARVESTING STRATEGY (DEPTH/FREQUENCY)	EXTEND BIOLOGICAL MODEL, BASED ON EXPERIMENTAL DATA, TO PREDICT SUSTAINED YIELDS UNDER VARIOUS HARVESTING CONDITIONS. TEST MODEL PREDICTIONS BY MEASURING YIELD UNDER CONTROLLED ENCLOSED CONDITIONS AT THE CTF. FURTHER TEST PREDICTIONS BY MEASURING YIELDS UNDER OPEN COASTAL CONDITIONS AT GTF. HARVEST DEPTH AND FREQUENCY WILL BE VARIED TO TEST MODEL OVER RANGE OF PARAMETERS.	SYSTEM MODEL INPUT MODEL DEVELOPMENT FARM CONCEPT DEVEL. HARVEST CONCEPT DEVEL.	CTF/GTF	CIT/NMI	2	A	PLANNED 1984, 1985 AT GOLETA TEST FARM
8. PRODUCTIVITY OF OTHER STRAINS OF MACROCYSTIS	MEASURE PRODUCTIVITY AND YIELD USING PLANTS IMPORTED FROM NEW ZEALAND (MULTI-APICES), MONTEREY OR KERGUELEN (UPWELLING-ADAPTED), AND NMI LABORATORY SELECTED/BRED PLANTS (HYBRID OR SELECTED PURE-BRED STRAINS), IN A CONTROLLED, CLOSED ENVIRONMENT. ALSO EVALUATE PRODUCTIVITY OF PLANTS, WHERE FEASIBLE, IN EARLY ON-SITE MEASUREMENTS OF GROWTH IN THEIR NATURAL ENVIRONMENT.	BASIC RESEARCH	NATURAL BEDS AT VARIOUS LOCATIONS AND CTF	CIT	3	B	NEW ZEALAND PLANT COLLECTED AND IN CULTURE AT NEUSHUL MARICULTURE INCORPORATED
9. EVALUATION OF OTHER SPECIES, AND GENERA FOR POTENTIAL PROGRAM UTILIZATION	CONTINUE TO EVALUATE POTENTIAL OF OTHER PLANTS FOR PROGRAM APPLICATION. CONTINUE EVALUATION OF GENETICS/HYBRIDS, ETC IN THE LABORATORY. MEASURE PRODUCTIVITY AND YIELD OF PROMISING CROSSES ON OPEN COASTAL FARM. PROVIDE PLANTS FOR TESTING AT CTF WHERE REQUIRED.	BASIC RESEARCH PLANTING SYSTEM CONCEPT DEVEL. IMPROVEMENT OF MACROCYSTIS	NMI LABS GTF	NMI	1	B	IN PROGRESS AT NEUSHUL MARICULTURE INC. (LOW LEVEL OF ACTIVITY)

Table 2.1-1. Research Matrix (cont'd.)

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
10. RELATION BETWEEN "CROP QUALITY" AND THE VARIOUS NUTRIENT, ENVIRONMENTAL AND HARVEST CONDITIONS.	MEASURE PERTINENT CHEMICAL COMPOSITION IN JUVENILE AND ADULT PLANTS HELD UNDER DIFFERENT NUTRIENT (NO_3 & PO_4^{-3}) AND HARVEST CONDITIONS.	SYSTEM MODEL INPUTS PROCESSING DEVEL. FARM CONCEPT DEVEL.	CIT LAB NMI LAB	CIT NMI	1	A	PLANNED FOR HEMIDOME: UNSUCCESSFUL DUE TO EXP. SYSTEM FAILURE
11. QUALITY OF CROP FOR GAS GENERATION	PART OF "CONVERSION BIOLOGY" TASKS. MEASURE METHANE YIELD, METHANE PRODUCTION RATE FOR BIOMASS HARVESTED FROM CTF/GTF EXPERIMENTS UNDER VARIOUS CONDITIONS.	SYSTEM MODEL INPUT PROCESSING DEVEL.	GE-IGT	GE IGT	1	A	INITIATED 1982. REF. GRI 81-0182; DELETED 1983 DUE TO BLDG. RESTRICTION
12. DEFINE MINIMUM NUTRIENT CONCENTRATIONS FOR SATURATED GROWTH	COMPARE GROWTH OF ADULT PLANTS HELD AT DIFFERENT NUTRIENT CONCENTRATIONS (NO_3^-, PO_4^{-3}, SELECTED MICRONUTRIENTS). TESTS IN DRUEHL CYLINDERS. TEST RESULTS FOR MAXIMIZING YIELD OF ADULT POPULATION IN CTF YIELD EXPERIMENT.	SYSTEM MODEL INPUT NUTRIENT REQTS. FARM CONCEPT DEVEL. BIOLOGICAL MODEL	CTF	CIT/BMS	1	A	COMPLETED 1983
13. UPWELLED WATER AS A SOURCE OF NUTRIENTS	COMPARE GROWTH OF ADULT PLANTS HELD IN UPWELLED WATER AND ENRICHED SURFACE WATER. USE DRUEHL CYLINDERS AT THE OSTP.	DATA CONFIDENCE	OSTP	CIT/BMS	2	C	DELETED DUE TO HEMIDOME EXPERIMENT DESIGN CHANGE
14. MORPHOLOGICAL SITES OF NUTRIENT UPTAKE BY ADULT PLANTS	MEASURE NUTRIENT UPTAKE RATES BY VARIOUS ADULT PLANT TISSUES IN SITU AND/OR IN LABORATORY (NO_3^-, PO_4^{-3}, SELECTED MICRONUTRIENTS).	BIOLOGICAL MODEL INPUT MODEL DEVELOPMENT SYSTEM MODEL INPUT NUTRIENT REQTS. FARM CONCEPT DEVEL.	NATURAL BEDS, CIT LABS.	CIT	1	B	COMPLETED 1983; REF. SECTION 4 OF THIS REPORT
15. TRANSLOCATION OF NUTRIENTS WITHIN AN ADULT PLANT	MEASURE MOVEMENT OF RADIOACTIVE (P^{32} AND SELECTED MICRONUTRIENTS) OR HEAVY (N^{15}) ISOTOPES WITHIN AND BETWEEN HARVESTED AND UN-HARVESTED FRONDS.	MODEL DEVELOPMENT BIOLOGICAL MODEL INPUT NUTRIENT REQTS. SYSTEM MODEL INPUT FARM CONCEPT DEVEL.	T&D	CIT	2	B	COMPLETED 1983; REF. OP. CIT.
16. N LOST IN UNHARVESTED FROND PARTS	MEASURE N IN REMAINING FROND PORTIONS OVER TIME AFTER A HARVEST. PART OF CTF YIELD EXPERIMENTS.	MODEL DEVELOPMENT BIOLOGICAL MODEL INPUT NUTRIENT REQTS. SYSTEM MODEL INPUT	CTF/GTF	CIT/NMI	1	B	PERFORMED AT CIT, 1983. DATA REDUCTION & REPORT IN PROGRESS BY DR. V.A. GERARD
17. STORAGE OF NUTRIENTS WITHIN AN ADULT PLANT	MEASURE CRITICAL TISSUE CONSTANTS FOR N, P, SELECTED MICRONUTRIENTS UNDER VARIOUS NUTRIENT CONDITIONS/ HISTORIES.	MODEL DEVELOPMENT BIOLOGICAL MODEL INPUT NUTRIENT REQTS. SYSTEM MODEL INPUT	NATURAL BEDS	CIT	1	B	COMPLETED 1983; REF. OP. CIT.
18. EFFICIENCY OF STRATIFIED FERTILIZING	MEASURE PLANT GROWTH AND N-BUDGET UNDER STRATIFIED NUTRIENT (NO_3^-) CONDITIONS TO VERIFY SPATIAL FERTILIZATION STRATEGIES BASED ON UPTAKE/TRANSLOCATION RESULTS.	BIOLOGICAL MODEL INPUT SYSTEMS MODEL INPUT NUTRIENT REQTS. FARM CONCEPT DEVEL.	SCRIPPS INST. DEEP TANK	CIT/SCRIPPS	3	C	NOT PERFORMED

Table 2.1-1. Research Matrix (cont'd.)

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
19. EFFECT OF HARVESTING ON FERTILIZING EFFICIENCY	USE BIOLOGICAL MODEL TO EVALUATE FERTILIZING EFFICIENCY AS A FUNCTION OF HARVESTING PARAMETERS. TEST MODEL BY MEASURING GROWTH AND N-BUDGET OF HARVESTED AND CONTROL PLANTS. TESTS TO BE CONDUCTED IN ENCLOSED HIGH-NUTRIENT ENVIRONMENT, OPEN COASTAL FARM, AND NATURAL BEDS.	BIOLOGICAL MODEL INPUT MODEL DEVELOPMENT SYSTEM MODEL INPUT NUTRIENT REQTS FARM CONCEPT DEVEL.	NATURAL BEDS CTF GTF	CIT/NMI	3	B	NOT PERFORMED
20. VALUE OF DIGESTER EFFLUENT	INVESTIGATE VALUE OF DIGESTER EFFLUENT AS A SOURCE OF N, P AND TRACE METALS FOR KELP GROWTH	SYSTEM MODEL INPUT FARM CONCEPT DEVELOPMENT.	CIT LAB	CIT/NMRC	3	B	PRELIMINARY ANALYSIS PERFORMED. REF: GRI-81-0182
21. FEASIBILITY OF LOCALIZED FERTILIZATION TECHNIQUES	INVESTIGATE INJECTION OF NUTRIENTS DIRECTLY INTO BOUNDARY LAYER, LOCALIZED OR PULSE FEEDING TECHNIQUES TO EVALUATE FEASIBILITY OF REDUCING NUTRIENT REQUIREMENTS. CONDUCT TESTS ON MATURE PLANTS AT SELECTED COASTAL AND OCEAN LOCATIONS.	MODEL DEVELOPMENT SYSTEM MODEL INPUT NUTRIENT REQTS. FARM CONCEPT DEVEL.	GTF OSTF	NMI GE	3	B	NOT PERFORMED
22. DEFINITION OF UPTAKE-SATURATING WATER MOTION AND RELATION BETWEEN VELOCITY NUTRIENT CONCENTRATION AND UPTAKE RATE.	DETERMINE UPTAKE-SATURATING WATER MOTION AND COMPARE IN SITU WATER MOTION OVER KELP LAMINAE. MEASURE UPTAKE RATES AT OTHER LOWER VELOCITIES TO ESTABLISH THE RELATION BETWEEN VELOCITY (OR PLANT MOTION), NUTRIENT CONCENTRATION AND UPTAKE RATES.	MODEL DEVELOPMENT SYSTEM MODEL INPUT NUTRIENT REQTS. FARM CONCEPT DEVEL. BIOLOGICAL MODEL INPUT	NATURAL BEDS CIT LABORATORY GTF NMI LABORATORY	CIT/NMI	3	A	COMPLETED BY CIT 1982 (IN NATURALIZED BED AND LABORATORY.) REF, GRI-81-0018
23. RELATION BETWEEN ENCRUSTING EPIPHYTES AND PLANT NUTRITION	COMPARE EPIPHYTE ENCRUSTATION ON NUTRIENT-RICH VS STARVED PLANTS IN INSHORE KELP FORESTS AND COMPARE NUTRIENT UPTAKE BY TISSUES WITH DIFFERENT EPIPHYTE ENCRUSTATION	MODEL DEVELOPMENT BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT NUTRIENT REQTS. FARM CONCEPT DEVEL.	NATURAL KELP BEDS	CIT	3	C	NOT PERFORMED

Table 2.1-1. Research Matrix (cont'd.)

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
B. YIELD BIOLOGY							
1. MECHANISMS OF LOSS DUE TO WATER MOTION	DETERMINE TISSUE LOSS FROM PLANTS HELD UNDER VARYING WATER MOTION CONDITIONS. ESTABLISH ABILITY OF PLANTS TO SURVIVE VARIOUS LEVELS OF WATER MOTION AND WAVE ACTION.	BIOLOGICAL MODEL DEVELOPMENT SYSTEM MODEL INPUT FARM CONCEPT DEVEL FARM DES. REQTS ENVIRONMENTAL IMPACT	OSTP NATURAL BEDS GTF	CIT/GE NMI	3	A	TISSUE LOSS NOT DETERMINED. PRELIMINARY SURVIVAL EVAL. INITIATED IN 1983 (HOLDFAST TEAROUT STUDY) REF. SECT. 2 OF THIS REPORT
2. MECHANISMS OF LOSS DUE TO PLANT-SUBSTRATE INTERACTIONS	DETERMINE TISSUE LOSS FROM PLANTS ATTACHED TO VARIOUS SUBSTRATES IN DIFFERENT GROUP CONFIGURATIONS. ESTABLISH CRITERIA FOR ALLOWABLE RELATIVE MOTION, CONFIGURATIONS, ETC. BY OCEAN TEST INCORPORATING VARIOUS STRUCTURE CONCEPTS.	BIOLOGICAL MODEL DEVELOPMENT SYSTEM MODEL INPUT FARM DES. REQTS FARM CONCEPT DEVEL ENVIRONMENTAL IMPACT	OSTP NATURAL BEDS	CIT/GE	3	A	PRELIMINARY EVAL. COMPLETED 1982. REF. GRI 81-0118. (MYRTLE EXPERIMENTS)
3. INFLUENCE OF STORMS ON TISSUE LOSS	COMPARE TISSUE LOSS RATES BEFORE AND AFTER STORMS AT NATURALLY EXPOSED AND PROTECTED INSHORE KELP FORESTS.	BIOLOGICAL MODEL DEVELOPMENT SYSTEM MODEL INPUT ENVIRONMENTAL IMPACT DEFINE STORM LOSSES	NATURAL BEDS/GTF	CIT/NMI	3/1	B/B	NOT PERFORMED
4. EFFECTS OF HARVESTING ON TISSUE LOSS	COMPARE TISSUE LOSS RATES FROM HARVESTED AND CONTROL INSHORE KELP FORESTS.	BIOLOGICAL MODEL DEVELOPMENT SYSTEM MODEL INPUT HARVEST CONCEPT DEVEL ENVIRONMENTAL IMPACT	NATURAL BEDS/GTF	CIT/NMI	3/1	B/B	NOT PERFORMED
5. RELATION BETWEEN EXPOSURE, MORPHOLOGY, AND TISSUE LOSS	COMPARE MORPHOLOGY OF AND TISSUE LOSS RATES FROM ADULT PLANTS ADAPTED TO EXPOSED AND SHELTERED CONDITIONS. TESTS TO BE CONDUCTED EITHER IN NATURAL BEDS OR AT THE GTF.	BIOLOGICAL MODEL DEVELOPMENT SYSTEM MODEL INPUT PLANT SELECTION CRITERIA FARM CONCEPT DEVEL ENVIRONMENTAL IMPACT	NATURAL BEDS GTF	CIT/NMI	3	C	NOT PERFORMED
6. INFLUENCE OF EDGE EFFECTS ON TISSUE LOSS	COMPARE TISSUE LOSS RATES FROM PLANTS AT VARYING DISTANCES FROM THE EDGE AND CENTER OF LARGE INSHORE KELP FORESTS.	DEFINITION OF EDGE "SIZE"/LOSSES GTF SIZE REQT'S MODEL DEVELOPMENT SYSTEM MODEL INPUT FARM CONCEPT DEVEL	NATURAL BEDS	CIT/NMI	3	C	NOT PERFORMED
7. DETERMINE LOSSES BY EXUDATION/RESPIRATION BEFORE HARVEST	PERFORM PHYSIOLOGICAL STUDIES ON GROSS PRODUCTIVITY VS NET PRODUCTIVITY.	MODEL DEVELOPMENT BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT LOSS MECHANISMS ENVIRONMENTAL IMPACT	NMI LAB CIT	NMI/CIT	3/1	B/B	NOT PERFORMED
8. DETERMINE LOSSES BY SLOUGHING AND MECHANICAL DAMAGE BEFORE HARVEST	PERFORM SERIAL IMAGE ANALYSES OF FRONDS TO MEASURE GROWTH AND SLOUGHING. EVALUATE INCREASE IN DAMAGE SENSITIVITY IN PRESENCE OF SLOUGHING.	MODEL DEVELOPMENT BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT LOSS MECHANISMS ENVIRONMENTAL IMPACT FARM CONCEPT DEVELOPMENT	GTF	NMI	1	B	NOT PERFORMED

Table 2.1-1. Research Matrix (cont'd.)

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
9. CURRENT AND WAVE ATTENUATION BY KELP POPULATION	MEASURE CURRENT ATTENUATION AT VARYING DISTANCES INTO INSHORE KELP FORESTS OF KNOWN FROND AND CANOPY DENSITIES.	FARM DESIGN DATA EDGE EFFECTS DEFIN. FARM CONCEPT DEVEL. ETF SIZE REQTS ENVIRONMENTAL IMPACT	NATURAL BEDS	TBD	1	A	COMPLETED 1982. REF. PROVIDED BELOW*
10. PLANT SUBMERGENCE BY CURRENT.	MEASURE PLANT ANGLE AND CANOPY SUBMERGENCE AT DIFFERENT CURRENT VELOCITIES. OFFSHORE PLATFORM AND INSHORE KELP FORESTS.	FARM DESIGN DATA EDGE EFFECTS DEFIN. FARM CONCEPT DEVEL. ETF SIZE REQTS	NATURAL BEDS OSTP	CIT/GE	1	C	COMPLETED 1981. REF. GRI-81-0118 (MYRTLE EXPERIMENT)
11. EFFECTS OF SUBMERGENCE ON PRODUCTIVITY	COMPARE INDIVIDUAL PLANT PRODUCTIVITY WITH CANOPY AT SURFACE AND SUBMERGED, ISOLATED AND IN GROUP; OFFSHORE PLATFORM AND INSHORE KELP FORESTS. PREDICT VALUES OF YIELD FOR EDGE AREAS VS CENTER AREA OF LARGE FARM.	FARM DESIGN DATA EDGE EFFECTS DEFIN. FARM CONCEPT DEVEL. BIOLOGICAL MODEL INPUT SYSTEM MODEL INPUT	NATURAL BEDS OSTP	CIT/GE	3	B	PARTIAL EVALUATION PERFORMED 1981, 1982. REF. GRI 81-0118 81-0182
12. EFFECTS OF SUBMERGENCE ON PNEUMATOCYST COLLAPSE	DETERMINE COLLAPSE OF PNEUMATOCYSTS HELD AT DIFFERENT DEPTHS FOR VARYING TIME PERIODS. OFFSHORE PLATFORM AND INSHORE KELP FORESTS.	FARM DES REQTS FARM CONCEPT DEVEL. SYSTEM MODEL INPUT	OSTP NATURAL BEDS	CIT/GE	3	C	NOT PERFORMED
13. DETERMINE DRAG AND BUOYANCY CHARACTERISTICS OF KELP PLANTS	MEASURE THE DRAG AND BUOYANCY CHARACTERISTICS OF VARIOUS SIZES OF KELP PLANTS OVER A RANGE OF WATER VELOCITIES, PLANT ANGLES, AND CANOPY SUBMERGENCE LEVELS.	FARM DESIGN DATA FARM DESIGN REQTS FARM CONCEPT DEVEL. SYSTEM STUDY INPUTS	OSTP NATURAL BEDS	GE/CIT	1	A	COMPLETED 1982; REF. GRI 81-0182, SECTION 2 THIS REPORT
B[1]. ENGINEERING							
14. DISPERSION OF DEEP UPWELLED WATER	MEASURE THE DISPERSION OF UPWELLED WATER INCLUDING SINKING AND/OR RISING, AND HORIZONTAL DISPERSION OF VARIOUS CONFIGURATIONS OF VERTICAL AND HORIZONTAL COLD WATER PLUMES AND JETS. DETERMINE CONCENTRATION PROFILES, ETC., AND DEVELOP, VALIDATE A MODEL.	SYSTEM MODEL INPUT MODEL DEVELOPMENT NUTRIENT REQTS. FARM CONCEPT DEVEL. WAVE PUMP REQTS. ENVIRONMENTAL IMPACT	OSTP/TBD	GE/TBD	1	A	COMPLETED 1981 REF. GRI 81-0118
15. WAVE PUMP FEASIBILITY AND TECHNOLOGY	EXAMINE WAVE PUMP CONCEPTS TO MEET NUTRIENT UPWELLING REQTS. PERFORM ANALYSES. CONDUCT MODEL TESTS AS REQ'D AND VERIFY DESIGN WITH LARGE SCALE PUMP MODEL IN OCEAN TEST.	SYSTEM MODEL INPUTS FARM CONCEPT DEVEL. HARDWARE DESIGN DATA VERIFY FEASIBILITY PERFORMANCE DATA	GE/TBD	GE/TBD	3	A	PRELIMINARY EVALUATION PERFORMED 1979, 1980 BY GENERAL ELECTRIC CO.
16. FARM DESIGN CONCEPT STUDIES	UPDATE EARLIER DESIGN CONCEPT STUDIES FOR LARGE SCALE COMMERCIAL FARM STRUCTURES ON BASIS OF NEW AVAILABLE DATA. TRADE OFF VARIOUS STRUCTURAL/MOORING (OR DYNAMICALLY POSITIONED). CONCEPTS TO IDENTIFY MOST PROMISING CANDIDATES FOR TEST EVALUATION. EVALUATE COMPATIBLE UPWELL PUMP/DISPERSION SYSTEM CONCEPTS.	MODEL DEVELOPMENT SYSTEM MODEL INPUT COMMERICAL FARM CONCEPTS ETF CONCEPTS TEST/DATA REQTS	GE/TBD	GE/TBD	1	B	COMPLETED 1983. REF. FINAL REPORT TO GRI BY RALPH M. PARSONS COMPANY & SECTION 1 THIS REPORT
17. DRAG OF LARGE FARM STRUCTURES WITH KELP	EVALUATE CONFIDENCE IN AVAILABLE MODEL AND PREDICTION TECHNIQUES. DETERMINE REQTS FOR ADDITIONAL MODEL DEVELOPMENT (ANALYSIS) AND MODEL TEST DATA. CONDUCT ANALYSES AND TESTING AS REQUIRED TO PROVIDE NECESSARY CONFIDENCE IN DRAG PREDICTIONS INCLUDING FOULING EFFECTS.	MODEL DEVELOPMENT SYSTEM MODEL INPUT FARM DESIGN DATA FARM DESIGN CONCEPTS ETF REQTS	GE/TBD	GE/TBD	2	A	NOT PERFORMED

*NOTE: Reference: Jackson, G.A. and Winant, C., Effects of a Kelp Forest on Coastal Current in Continental Shelf Research, Vol. II, pp. 75-80 1983

Table 2.1-1. Research Matrix (cont'd.)

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
18. FARM STRUCTURE CONCEPT TEST DATA	PERFORM OCEAN TESTS ON SELECTED COMPONENTS, SUBASSEMBLIES, MODULES MATERIALS, ETC OF VARIOUS FARM STRUCTURE CONCEPTS. ACQUIRE OCEAN ENGINEERING DATA ON LOADS, DYNAMICS, ETC. FOR VARIOUS DESIGN APPROACHES.	MODEL DEVELOPMENT SYSTEM MODEL INPUT FARM DES. DATA/REQTS CONCEPT EVALUATION ETF REQTS.	TBD	GE/TBD	2	A	COMPLETED 1981. REF. GRI-81-0118
19. NUTRIENT DISPERSION SYSTEM CONCEPT TEST DATA	PERFORM OCEAN MODEL TESTS USING DEEP UPWELLED WATER (OR ADEQUATE SIMULATION) TO PROVIDE COMPARATIVE PERFORMANCE DATA ON CANDIDATE DISTRIBUTION SYSTEMS. MEASUREMENTS WILL BE MADE TO DETERMINE EFFECTIVENESS OF NUTRIENT DISTRIBUTION PATTERNS FOR VARIOUS ENVIRONMENT CONDITIONS, FARM AND DISTRIBUTION SYSTEM GEOMETRIES.	MODEL DEVELOPMENT SYSTEM MODEL INPUT FARM DESIGN DATA/REQTS CONCEPT EVALUATION ETF REQTS	TBD	GE/TBD	3	B	COMPLETED 1981. REF. GRI-81-0118
20. UPDATE SYSTEM REQ'TS MODEL AND ANALYSIS	REEVALUATE THE SRA RESULTS/MODELS ON THE BASIS OF NEW DATA AND STUDY RESULTS. UPDATE GAS COST PREDICTIONS AND SENSITIVITIES. UPDATE CAPTIAL COST PREDICTIONS AND ENERGY BALANCE ANALYSES.	CONCEPT EVALUATIONS COST ANALYSES DECISION INFORMATION FOR COMMERICALIZATION DECISION	GE	ALL	3	A	COMPLETED 1983. REF. R.M. PARSONS REPORT & SECTION 1 THIS REPORT
21. ENGINEERING TEST FARM (ETF) DESIGN CONCEPT STUDY AND DEFINITION	PRELIMINARY DEFINITION OF ETF REQTS AND OBJECTIVES. DEFINITION OF ETF DESIGN CONCEPTS SHOWING RELEVANCE TO POTENTIAL COMMERICAL DESIGN CONCEPT AND FEASIBILITY FOR ETF IMPLEMENTATION EARLY IN NEXT PROGRAM PHASE. PRELIMINARY EVALUATION OF SITING REQUIREMENTS.	ETF OBJECTIVES/REQTS ETF DESIGN CONCEPTS ETF ENG'G FEASIBILITY DECISION INFORMATION FOR COMMERCIALIZATION DECISION	PHILA/TBD	ALL	3	B	NOT PERFORMED

Table 2.1-1. Research Matrix (cont'd.)

DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY LOCATION	EXPERIMENTER	TIMING	TECH VALUE	COMMENTS
C. CONVERSION BIOLOGY							
1. ULTIMATE DIGESTIBILITY	CONDUCT EXPERIMENTS TO IDENTIFY REASONS FOR LIMITED DIGESTIBILITY AND DEVELOP METHODS FOR IMPROVEMENT, E.G. - NUTRIENT LIMITATION - SPECIFIC CULTURE REQUIREMENT	(Δ-COST) VS. (Δ-METHANE) YIELD TRADEOFFS ANALYSIS SYSTEM MODEL INPUT	GE	GE	1	A	IN PROGRESS. REF. SEE NOTE BELOW*
2. KELP COMPOSITION/METHANE YIELD VARIABILITY STUDIES (CONTAINS TASK A10)	COMPILE AVAILABLE DATA AND CONDUCT EXPERIMENTS ON KELP GROWN UNDER CONTROLLED CONDITIONS TO DEVELOP RELATIONSHIPS BETWEEN COMPOSITION AND METHANE YIELD	DEVELOP RELIABLE METHANE YIELD DATA FOR SYSTEMS ANALYSIS SYSTEM MODEL INPUT	ALL	ALL	1	A	INITIATED 1982 REF. GRI-81-0182, DELETED 1983
3. KELP DIGESTION BIOCHEMISTRY	INVESTIGATE SINGLE KELP COMPONENT DEGRADATION USING PURE STRAINS. DEVELOP SINGLE COMPONENT KINETICS. DEVELOP COMPLETE FOOD-CHAIN KINETICS. CHARACTERIZATION OF NON-METHANOGEN MICROBIAL ISOLATES INCLUDING METABOLIC REGULATION.	IDENTIFICATION OF YIELD- AND RATE-LIMITING CONDITIONS. DEVELOP MODELS FOR PROCESS DESIGN. DEVELOP PROCESS METABOLIC REGULATION CONTROL STRATEGIES. SYSTEM MODEL INPUT.	GE	GE	1	A	IN PROGRESS 1982 REF. OP. CIT. DELETED 1983
4. METHONGENS ISOLATION AND CHARACTERIZATION	ISOLATE METHANOGENS FROM KELP DIGESTERS AND CHARACTERIZE THEM FOR METHANE PRODUCTION PROCESSES.	NEED PURE METHANOGENS IN ORDER TO COMPLETE - TASK 3.	UCLA-LAB	UCLA	1	A	IN PROGRESS 1982 REF. OP. CIT. DELETED 1983
5. EFFECT OF KELP PROCUREMENT METHODS ON CONVERSION PROGRAM	DEVELOP REQTS FOR EACH OF THE STEPS INVOLVED, E.G. PRE-HARVEST MONITORING AND KELP COMPOSITION ANALYSIS, HARVESTING TECHNIQUE, FREEZING AND MONITORING DURING THE USE PERIOD.	ACCEPTABILITY OF KELP SAMPLES. DATA VALIDATION	WRRC-LAB	WRRC	1	A	PRELIMINARY EVALUATION COMPLETED 1982. REF. OP. CIT.
6. DIGESTER SYSTEM DESIGN	DESIGN AND TEST ALTERNATE DIGESTER CONFIGURATIONS. EVALUATE MULTIPHASE DIGESTION SYSTEM. DEVELOP DIGESTION SYSTEM KINETICS. DEVELOP INPUTS FOR PROCESS CONTROL STRATEGIES.	DEVELOP IMPROVED ESTIMATES OF METHANE YIELD, METHANE PRODUCTION RATES, AND DIGESTER SYSTEM COSTS. SYSTEM MODEL INPUT.	IGT-LAB CORNELL	IGT CORNELL	1	A	IN PROGRESS 1982, 1983. REF. OP. CIT
7. EFFECT OF FEEDING FREQUENCY	COMPARE BASELINE DIGESTER PERFORMANCE FOR ONCE-A-DAY AND MORE FREQUENCY FED DIGESTERS.	ELIMINATE BIAS INTRODUCED BY EXPERIMENTAL MENTAL PROCEDURE. TEST DATA VALIDATION.	IGT-LAB	IGT	1	C	COMPLETED 1981 REF. GRI-81-0118
8. EFFECT OF TEMPERATURE	OBTAIN DATA ON BASELINE DIGESTION AT MESOPHILIC AND AMBIENT-TEMPERATURES.	PERFORM TRADEOFFS ON DIGESTER TEMP. VS. METHANE PRODUCTION RATE AND SYSTEM NET ENERGY PRODUCTION EFFIENCY. SYSTEM MODEL INPUT.	GE	GE	1	B	COMPLETED 1981 REF. GRI 81-0118
9. PRETREATMENT (CHEMICAL, BIOLOGICAL)	SCREEN AND TEST TECHNIQUES TO IMPROVE METHANE YIELD AND PRODUCTION RATES.	(Δ-COST) VS. (Δ-YIELD) (Δ-PRODUCTION RATE) TRADEOFFS. SYSTEM MODEL INPUT	WRRC-LAB IGT-LABS	WRRC KOHLER IGT	1	B	COMPLETED 1981 REF. OP. CIT.

Note: Reference: Marine Biomass Program; Anaerobic System Development and Stabilization Study; Final Report for the Period 2/1-12/31/82, Institute of Gas Technology, Chicago, Illinois, June 1983

Table 2.1-1. Research Matrix (cont'd.)

DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/ LOCATION	EXPERIMENTER	TIMING	TECH VALUE	COMMENTS
10. PROGRAM STANDARD DIGESTER SYSTEM EVALUATION	INTEGRATED EVALUATION OF DIGESTER PARAMETERS INCLUDING PERFORMANCE EVALUATION OF A CONTINUOUSLY-FED SYSTEM.	TOTAL DIGESTER SYSTEM PERFORMANCE EVALUATION SYSTEM MODEL INPUT	IGT-LAB	IGT	1	A	COMPLETED 1981; REF. GRI-0118
11. PARTICLE SIZE EFFECT	CONDUCT DIGESTION EXPERIMENTS ON DIFFERENT SIZE KELP PARTICLES AND CORRELATE METHANE PRODUCTION DATA WITH ENERGY REQUIRED FOR SIZE REDUCTION.	(Δ-ENERGY COST) VS. (Δ-YIELD) AND (Δ-PRODUCTION RATE) TRADEOFFS, SYSTEM MODEL INPUT.	WRRC-LAB	WRRC	1	B	COMPLETED 1981 REF. OP. CIT.
12. PUMPING ENERGY REQUIREMENTS	CONDUCT EXPERIMENTS ON PUMPING RAW KELP OF DIFFERENT PARTICLE SIZES	VALIDATE SYSTEMS ANALYSIS ASSUMPTIONS ON KELP TRANSPORT ENERGY REQUIREMENTS. SYSTEM MODEL INPUT.	WRRC-LAB	WRRC	2	C	COMPLETED 1981 REF. OP. CIT.
13. EFFECT OF STORAGE	CONDUCT EXPERIMENTS TO EVALUATE DEGRADATION OF RAW KELP IN AN ANAEROBIC ENVIRONMENT. CONDUCT BIOASSAY TYPE TESTS TO DETERMINE EFFECTS ON METHANE PRODUCTION.	VALIDATE SYSTEMS ANALYSIS ASSUMPTIONS ON STORAGE DESIGN. ANALYZE DATA FOR IMPACT ON DIGESTER SYSTEM DESIGN. SYSTEM MODEL INPUT.	WRRC-LAB	WRRC	1	B	COMPLETED 1981 REF. OP. CIT.
14. CONVERSION SYSTEM MODEL	INTEGRATE AND ANALYZE DATA ON A SYSTEM LEVEL INCLUDING SIZE REDUCTION, PRETREATMENT, TRANSPORTATION, GAS PRODUCTION, BYPRODUCTS RECOVERY, AND WASTE DISPOSAL.	UPDATE EXISTING ANALYTICAL TOOL. INVESTIGATE ALTERNATIVE CONVERSION SYSTEM CONCEPTS. SYSTEM MODEL INPUT.	GE-LAB	GE	2	B	COMPLETED 1983 REF. R.M. PARSONS REPORT TO GRI. IN PROGRESS 1984 AT INSTITUTE OF GAS TECHNOLOGY
15. BYPRODUCTS RECOVERY	DETERMINE POST TREATMENT REQUIREMENTS AND DEVELOP TECHNIQUES FOR BYPRODUCTS RECOVERY FROM DIGESTER EFFLUENTS. DEVELOP UTILIZATION SCHEMES.	SYSTEMS ANALYSIS FOR IMPACT ON ENERGY EFFICIENCY, GAS COST, CAPITAL REQUIREMENTS, SYSTEM MODEL INPUT	TBD	TBD	2	A	PRELIMINARY EVALUATION COMPLETED 1982. REF. GRI 81-0182
16. WASTES ASSESSMENT	IDENTIFY AND CHARACTERIZE POTENTIAL WASTE STREAMS. DEVELOP TREATMENT AND UTILIZATION SCHEMES.	SYSTEM MODEL INPUT.	TBD	TBD	2	A	PRELIM. EVAL. COMPLETED REF. OP. CIT. & R.M. PARSONS REPORT

Table 2.1-1. Research Matrix (cont'd.)

REQUIRED DATA/ANALYSIS	RESEARCH APPROACH	DATA UTILIZATION	FACILITY/LOCATION	EXPERIMENTERS	TIMING	TECH VALUE	COMMENTS
9. ENVIRONMENTAL IMPACT							
1. EFFECT OF KELP CULTIVATION ON OTHER SEA LIFE	PRELIMINARY DATA WILL BE ACQUIRED TO ASSESS THIS AREA OF ENVIRONMENTAL IMPACT BY MONITORING AND DOCUMENTING RESPONSES OF EPIPHYTES, WEEDS, PESTS, ANIMALS IN THE VARIOUS FARM EXPERIMENTS WHERE PLANTING AND FERTILIZATION ARE IMPLEMENTED.	ENVIRONMENTAL IMPACT CO-PRODUCTS POTENTIAL	CTF, GTF OSTF, NATURAL BED AREAS (?)	CIT/NMI	1	C	NOT PERFORMED
2. GENERATION AND DISPERSION OF LOST ORGANIC TISSUE	ESTIMATE AMOUNTS OF TISSUE LOSS FROM LARGE FARMS BASED ON BIOLOGICAL PRODUCTIVITY AND YIELD EXPERIMENTS. ESTIMATE INTERACTION OF MATERIAL WITH OCEAN CURRENTS OF VARIOUS SCALES TO PREDICT POTENTIAL TRANSPORT OF WASTE MATERIALS BOTH LOCALLY AND TO MORE DISTANT AREAS. DEPOSITION ON THE OCEAN BOTTOM TO BE CONSIDERED AS A POTENTIAL DEPOSITION SITE.	ENVIRONMENTAL IMPACT CHANGE OF FARM ENVIRONMENT RECOVERY OF LOST TISSUE	CTF, GTF, NATURAL BEDS	CIT/NMI/TBD	3	C	NOT PERFORMED
3. DISPERSION AND TRANSPORT OF UPWELLED WATER	ESTIMATE CHANGES IN LOCAL AND LARGER SCALE OCEAN TEMPERATURE/CIRCULATION PATTERNS DUE TO UPWELLING AND DISTRIBUTION OF LARGE AMOUNTS OF COLD NUTRIENT-RICH WATER. ALSO DISTORTION OF NORMAL NUTRIENT DISTRIBUTIONS.	ENVIRONMENTAL IMPACT CHANGE OF FARM ENVIRONMENT	TBD	TBD	3	C	NOT PERFORMED
4. GENERATION AND DISPERSION OF OTHER CHEMICALS, COMPOUNDS AND MATERIALS INTO THE ENVIRONMENT	ESTIMATE TYPE AND AMOUNT OF OTHER CHEMICALS, ETC., RELEASED FROM FARM, STRUCTURAL MATERIALS, PRODUCTS OF OPERATIONS, ETC., AND DISPERSION OF THESE MATERIALS WITHIN THE LOCAL AND LARGER SCALE OCEAN ENVIRONMENT.	ENVIRONMENTAL IMPACT	TBD	TBD	3	C	NOT PERFORMED
5. EFFECT OF FARM MATERIALS RELEASE ON PLANT AND ANIMAL LIFE	MAKE PRELIMINARY EVALUATION OF POTENTIAL EFFECTS OF RELEASED CHEMICALS AND MATERIALS ON THE PLANT AND ANIMAL LIFE IN THE OCEAN ENVIRONMENT. UTILIZE DISPERSION AND CONCENTRATION ESTIMATES GENERATED IN ABOVE ENVIRONMENTAL STUDIES.	ENVIRONMENTAL IMPACT COMMERICAL, SPORT FISHERIES POTENTIAL IMPACT POSSIBLE CO-PRODUCTS STUDIES			3	C	NOT PERFORMED
6. EFFECT OF FARM STRUCTURE AND OPERATIONS ON LOCAL AND LARGER SCALE OCEANOGRAPHY AND AIR/SEA INTERFACE	PERFORM ANALYSES TO ESTIMATE COMBINED EFFECTS OF FARM STRUCTURE, MOORINGS, KELP PLANTS, UPWELLED WATER AND WAVE PUMPING ON LOCAL CONDITIONS. PARAMETERS TO BE CONSIDERED INCLUDE: CURRENTS, STRATIFICATION AND THERMAL STRUCTURE BELOW THE SURFACE, WAVE CONDITIONS, THERMAL, WIND AND ATMOSPHERIC CONDITIONS ABOVE THE SURFACE, AND COASTAL CONDITIONS ON THE SHOREWARD SIDE OF THE FARM.	ENVIRONMENTAL IMPACT OCEANOGRAPHIC IMPACT ATMOSPHERIC IMPACT COASTAL AND NEARSHORE IMPACT	TBD	TBD	3	C	NOT PERFORMED
7. WASTE AND BYPRODUCT GENERATION AND DISPOSAL FOR LAND PROCESSES	EVALUATE DESIREABLE AND UNDESIRABLE PRODUCTS GENERATED IN CONVERSION PROCESSES AND INVESTIGATE POTENTIAL UTILIZATION/DISPOSAL SCHEMES. ALSO SEE LAST TASK OF CONVERSION EFFORTS.	ENVIRONMENTAL IMPACT CO-PRODUCTS/BYPRODUCTS ASSESSMENTS	GE	GE	3	C	NOT PERFORMED
8. EVALUATION OF LEGAL AND COMMUNITY ACCEPTABILITY ISSUES	PERFORM PRELIMINARY EVALUATION OF SOCIAL AND LEGAL ISSUES ASSOCIATED WITH ENVIRONMENTAL IMPACTS IDENTIFIED ABOVE. IDENTIFY POTENTIAL PROBLEM AREAS ASSOCIATED WITH ENVIRONMENTAL IMPACT AND DEVELOP PLAN FOR IMPLEMENTATION IN THE ETF PHASE OF THE PROGRAM (I.E., AFTER 1983).	IDENTIFICATION OF POTENTIAL ENVIRONMENTAL PROBLEM ISSUES PLANNING FOR NEXT PHASE	GE/TBD	GE/TBD	3	C	NOT PERFORMED

3. 1983 ACTIVITIES AND RESULTS

3. 1983 ACTIVITIES AND RESULTS

3.1 Yield Studies

1. Hemidome Experiment - (California Institute of Technology)
The Hemidome experiment results from 1982 were extensively reviewed by GRI, CIT, and GE. The conclusion reached was that in view of the unresolved factors negatively affecting long term kelp growth in the experiment enclosure, this experiment equipment would not be operated in 1983. Repeated (three) attempts to achieve stable operations within the Hemidome had failed due to an apparent acceleration of competing biological activity within the enclosure. This activity resulted in perforation of the kelp blades, degradation of the holdfasts and accelerated build up of various species of macrobiota. (A complete description of these phenomena can be found in the 1982 Marine Biomass Program Annual Report). It was felt, on review, that to continue study of these factors-to-resolution would most likely result in the loss of the yield and physiological data that would be most useful to the Program. As a result of these considerations, the CIT work was restructured to delete all Hemidome operations and to perform a series of tasks that acquired physiological data in a nearshore environment.

These experiments were structured in order to meet two specific needs. In the first case, the preparation of specifications for the Systems Analysis had emphasized the need for a comprehensive Kelp Growth Model. Preliminary work in concept and specification development showed that a tool that could describe the interrelations among the many physiological and oceanographic parameters affecting kelp growth and development was required. In the absence of such a tool, the Systems Analysis would be site specific, and in addition, would not be able to take full advantage of all of the data that had been collected to date. A second major objective of the restructured study was to provide inputs to development of the

model which would verify key physiological assumptions that had been used in the Systems Analysis. These included, assumptions on the variability of photosynthetic rate with depth; photosynthetic activity as a function of tissue type, age and location; carbon allocation strategies; and the effect of nutrients other than nitrogen on carbon fixation.

The Kelp Growth Model (KGM) development activity was performed by the University of California - San Diego, Scripps Institution of Oceanography (SIO). Model development is anticipated to extend through 1984. The KGM will be based on two models which had previously been developed by SIO. These are the Kelp Plant Model; which treats growth and development of individual Macrocystis plants and the Kelp Bed Model; which describes the interactions of a large kelp forest with waves, currents and nutrients. Progress on the Kelp Growth Model is described in detail in Section 4. Supporting laboratory and field experiments for the KGM were performed by the California Institute of Technology. The results of these studies are also reported in Section 4.

2. Nearshore (Goleta) Test Farm - (Neushul Mariculture Inc.)
The 1983 objective for the Nearshore Test Farm was to confirm the yield results of the 1982 experiment and to investigate methods for increasing average yields. The 1983 test plot consisted of 2 half acre plots; one on which the 1982 experiment was to be repeated with the exception that lost plants were not to be replaced. On the second plot, high density planting would be performed but with widely separated rows in order to increase the available subsurface light climate. An additional activity was to cultivate and measure the production of a number of high-yielding plants (3-4 times higher than population average) which had been isolated during the 1982 study.

Severe storms occurred early in 1983 which precluded initiation of the planned experiments until the second quarter. Damage from the storms included partial destruction of the pier from which the test

plots were to be serviced, sanding-in of the test areas and extensive damage to the main workboat. January through March were, therefore, dedicated to repair and replacement activities. The experimental work was initiated during the second quarter of 1983 under direct contract to GRI. Progress and results will be reported separately by GRI.

3. Systems Analysis Support - (General Electric Company)
During this period, GE provided support to GRI and The Ralph M. Parsons Company, (RMP) for preparation of the Systems Analysis. Key activities included integration between RMP and other program participants, acquisition and review of scientific and engineering data and assistance to GRI in reviewing interim reports. Other activities included provision of technical direction and monitoring of the CIT and NMI experiments.

4. WORK TASKS FOR CURRENT YEAR

4. WORK TASKS FOR THE CURRENT YEAR

The key 1983 output of the Marine Biomass Project was the publication by GRI of an economic and systems assessment which was prepared by The Ralph M. Parsons Company (Economic and Systems Assessment of the Concept of Nearshore Kelp Farming for Methane Production, Final Report June 1982-May 1983 - Gas Research Institute). This study was based on previously developed project data and was supported by all project participants. The report represented a comprehensive examination of the technical and economic feasibility of commercial production of methane on nearshore farms. Various scenarios were developed for feedstock production, harvesting, and gas production. These scenarios were subjected to detailed engineering, scientific and financial tradeoffs and evaluated for resultant gas costs. In addition to product gas cost estimation, a significant output of the study was identification or verification of the major technical uncertainties affecting product cost. This output defined the critical elements of R&D which would be necessary in order to reduce the identified uncertainties or to fill gaps in the extant data base. These issues provided the basis for structuring the balance of the 1983 program and for the development of future data.

In summary, the report developed product gas costs on a baseline system and an advanced sytem. The baseline system parameters were founded on observed scientific and engineering data and produced gas at an estimated cost of $13.50/million BTU. The advanced system was based on reasonably optimistic extrapolations of the data base and produced a gas cost of $6.00/million BTU (both values represent levelized costs). The reader is referred to the complete report for a full treatment of the financial and technical analyses.

The balance of the 1983 project, which was significantly reduced in scope relative to previous Marine Biomass activity, consisted of a number of investigations which had been identified by the Systems Analysis as being critical to increasing the strength of, or increasing the range of utilization of the published study. These included development of a Kelp Growth Model which would allow projection of the study results to other than the specific

sites considered in the systems analysis and would also provide analysis of the harvest yield data accumulated during previous research. Additional 1983 work included those financial and contractual administrative tasks that were required to bring the integrated program to an orderly completion.

As an adjunct to the GRI sponsored Systems Analysis, The General Electic Company performed an analysis of the potential for commercial production of by-products and co-products from Marine Biomass. The GE analysis examined several scenarios in which bulk chemicals were produced in parallel with methane produced from the conceptual commercial system that was specified in the GRI Systems Analysis. In one case, where all of the kelp feedstock went into gas production, it was estimated that the methane cost could be reduced from $12.50/MMBTU to $11.30 through by-product retrieval. In a second case, where 15% of the incoming raw kelp feedstock was used for chemical recovery, methane cost was reduced from the baseline $13.50/MMBTU to $6.00/MMBTU. The complete General Electric study is described in A Technical and Economic Evaluation of Production of Chemicals as By-Products/Co-Products of Methane Production from Kelp - GE-BIO-1868, General Electric Company - AEPD, King of Prussia, Pa., April 1983.

EXPERIMENTAL RESEARCH

4.1 1983 Specific Tasks

Specific tasks for 1983 included the following items which are reported in this section.

A. Catalina Test Facility - (Hemidome)

B. Nearshore Test Facility (Goleta)

C. Engineering Support

D. Kelp Growth Model Development

1. Kelp Modeling
2. Field Studies - Kelp Photosynthesis
3. Field Studies - Measurement & Prediction of Marine Farm Light Environments
4. Laboratory Studies - Phosphorous and Growth of Juvenile *Macrocystis pyrifera* (Phaeophyta) Sporophytes
5. Laboratory Studies - Carbon Allocation in *Macrocystis pyrifera* (L.) C. AG.
   I. Intrinsic Variability in Photosynthesis and Respiration

A. Catalina Test Facility (Hemidome)

Operation of the Hemidome was terminated in January 1983 due to combined biological and technical problems that surfaced during the 1982 operations. A review of this task determined that it would be more cost effective to allocate resources from this task to an alternate activity (Kelp Modeling Development and supporting laboratory and field studies).

The Hemidome and all supporting equipment was subsequently transferred by GRI to the University of Southern California Catalina Marine Sciences Center. Formal transfer to USC occurred in July 1983. USC assumed all responsibility for use or disposition of the equipment at that time. The General Electric Company effected details of the transfer including formal notification of shutdown of operations to cognizant regulatory authorities.

B. Nearshore Test Facility - Goleta

The Goleta facility was non-operational from January through March 1983. Severe winter storms prohibited experimental operations. The storms also resulted in the need for refurbishment of the test plots and operating equipment.

As of May 1, 1983, management of the Goleta facility was undertaken directly by GRI. Repairs and refurbishment of the test facility as well as experimental work after this time period were performed under GRI's direction and will not be reported herein.

C. Engineering Support

The engineering support provided by GE during 1983 consisted of the following:

- Assistance to The Ralph M. Parsons Company.

  Detailed specifications to be used in the systems analysis of a conceptual commercial farm were provided. These specifications included the definition of chemical, physical, and biological parameters which were used as a basis for the RMP engineering/economic analysis.

- Arrangements for transfer of the Hemidome facility.

  In this task, GE provided for orderly shutdown of operations and assured that the equipment was properly secured until transfer was effected. GE made initial contacts with USC regarding the transfer and assisted GRI in subsequent transfer negotiations. In addition, all spares were accumulated and transported to USC, and outstanding engineering action items were closed.

- Program support for California Institute of Technology

  GE with GRI, defined the alternate Kelp Growth Model Development program and reallocated resources for its implementation. A new work statement and program plan were developed and implemented to support this task.

- Offshore Test Platform Insurance Claim

  All financial and engineering data relative to the OSTP claim were transferred to GRI. GRI assumed future responsibility for pursuit of this claim.

D. Kelp Growth Model Development

The kelp modeling task was initiated during 1983 following a program decision to discontinue studies at the Catalina Test Facility (Hemidome). The modeling task represented a new direction for the program and consisted of three phases:

- o Mathematical Modeling
- o Field Studies Supporting Modeling
- o Laboratory Studies Supporting Modeling

Activity in the mathematical modeling phase was delayed by negotiations involved in acquiring a lead investigator. The program was extremely fortunate in securing collaboration by Dr. George A. Jackson of Scripps Institution of Oceanography. Dr. Jackson had had previous experience in kelp modeling, as well as a good background in physical and chemical oceanographic studies of kelp beds. Kelp modeling activities were initiated in September and are oriented toward improving and expanding existing models. The objective is to develop a general model which can address practical questions concerning siting design and operation of kelp farms. Current activities by Dr. Jackson's group included refining information concerning the submarine light field within kelp beds, incorporating recent findings regarding photosynthesis (PS) and respiration (R) by kelp tissues into the existing Jackson model, and incorporating additional details relating to kelp morphology and tissue distribution throughout the water column.

Principal efforts during 1983 involved developing a general mathematical expression relating blade area to position on the frond. Previous attempts by others had succeeded in defining these relationships by separating fronds into several size classes with a separate equation for each size class. Dr. Jackson was able to improve on this approach significantly by developing a single mathematical expression to accomplish the task. Variability within a

natural frond population can be assessed by comparing morphometric measurements on actual fronds with predictions by Dr. Jackson's mathematical representation. Actual frond measurements were derived from a large pre-exising data base.

Past kelp models have been seriously hampered by deficiencies and inaccuracies in the large and diverse body of physical, chemical, and biological information that underpin such models. The 1983 activities included considerable field and laboratory research directed toward providing the most urgently needed data for our modeling activity. This work included review, organization, and preparation for publication of earlier Marine Biomass Program laboratory studies with relevance to the informational base needed for modeling.

Dr. Valrie Gerard was the lead investigator supervising the field studies which centered around a small artificial kelp bed constructed off Laguna Beach. Intensive measurements of submarine light and in-situ PS and R were conducted, as well as frequent determinations of nutritional status, growth rates, morphometrics, and production for the experimental plants. The objective was to document all critical parameters affecting kelp growth and productivity over a significant time period in order to provide information for guiding model design and for testing model predictions. Also examined were the effects of simulated canopy harvesting on the kelp bed light climate and on PS in the remaining tissues. An exponential relationship was found between canopy density and underlying light intensity within an artificially fixed canopy. A complex system for measuring submarine illumination, consisting of an array of sensors linked to a shipboard computer was developed for this task. The system recorded short term fluctuations in irradiance, storing the data for future analysis. Using this system, the effects on PS and photoinhibition of tissue type and of acclimation by kelp blades to their environment were assessed. The work developed PS vs I

relationships for various blade types from *in situ* studies. Advantage was also taken of a major El Nino condition to document nutritional effects on PS.

Drs. Steven L. Manley and Keith E. Arnold established ranges for PS, R, and dark carbon fixation in their laboratory studies involving various tissue types and ages. Considerable variability was encountered, but the highest values determined during this study, at the upper ends of the ranges, represent rates higher than any others previously reported. Arnold and Manley found gradients in PS and R along the lengths of immature, mature, and senescent blades. These findings suggested that caution must be used when interpreting findings of earlier workers who generally assumed that a single excised disc could be used as representative of an entire blade. Direct relationships were shown between PS and chlorophyll content.

Earlier laboratory studies investigating kelp growth and phosphorus availability were reviewed and prepared for publication. These studies relate to a macronutrient that might occasionally limit kelp growth (nitrogen is probably growth-limiting more frequently than phosphorus). Luxury uptake, storage, and consumption of phosphorus were demonstrated. A P-content of 0.2 percent dry weight appeared to be the critical level for juvenile *Macrocystis*, representing freedom from P-limited growth.

1. Kelp Modeling - (by Dr. George A. Jackson)

The goal of the modeling project has been to develop a quantitative description of seaweed photosynthesis and growth which can be used to predict yields for differing management strategies. The seaweed that is being worked with is *Macrocystis pyrifera*. The model being developed will incorporate a description of the light field around a plant, morphological relationships that describe the distribution of biomass and photosynthetic area along a frond, and physiological relationships among light irradiance, tissue type, photosynthesis and respiration. A simpler model to describe daily photosynthesis, the kelp photosynthesis model (KPM), already exists. The KPM forms the start of the present work.

The KPM treats a kelp plant as a collection of unconnected fronds of different lengths. Photosynthetic properties of the fronds are derived from morphometric characteristics (e.g. blade area and biomass as a function of blade position and frond length) and from physiological properties measured in the field and laboratory (e.g. photosynthetic and respiratory rates). The light field has been described as a simple function of density of vegetation at the sea surface, plant spacing, sun angle, and background light absorbance by the water. A FORTRAN-coded computer realization of the KPM provides the vertical distribution of net and gross photosynthesis as functions of frond-length distributions in a plant, and of plant density.

This work is being done in cooperation with the California Institute of Technology.

One function of the Kelp Growth Model is to represent growth at high plant densities where light becomes limiting. Researchers at Neushul Mariculture Incorporated (NMI) have been conducting

field experiments at plant densities where light irradiance rather than nutrient concentration or plant standing stock determine havested yield (NMI 1983). They found that stands of plants at the highest density studied, 1 plant/m^2, had smaller harvested yields than plants at lower densities and that the plants decreased in size over the course of the experiment by decreasing the number of fronds (Figure 4.1-1). Plants at lower densities increased their average number of fronds. The result was that while the frond densities varied by a factor of 15 at the start of the experiment, they were within a factor of 4 at the end. Plants that were planted at 0.25 plants/m^2 and at 1.0 plants/m^2 differed in frond density by a factor of 4 at the start of the experiment but were within 50 percent by the end of the experiment. The implication is that plants adjust the frond density to a value which is optimal when light limitation exists.

If a kelp plant is receiving enough light so that photosynthesis is greater than respiration, then it will add fronds and thereby increase its self-shading; if there is too much plant tissue, respiration will exceed photosynthesis, it will shed fronds until the self-shading is low enough so that it can grow. In a world where only light is limiting, plants over a range of densities should increase or decrease in size until the frond density is such that there is neither a surplus nor a debit of photosynthetic material. The results of the NMI experiments suggest that at densities of 8 fronds/m^2, the plants are still growing or have equilibrated; at densities of 11.5 fronds/m^2, they are either reducing or have stabilized. Thus, the frond density when respiration and photosynthesis seem to balance is between 8 and 11 fronds/m^2.

Such a balance between photosynthesis and respiration in kelp should be amenable to prediction by modelling. A test of the KPM suggests that models will be able to do so. When the KPM was run for depth and plant density conditions similar to those of the

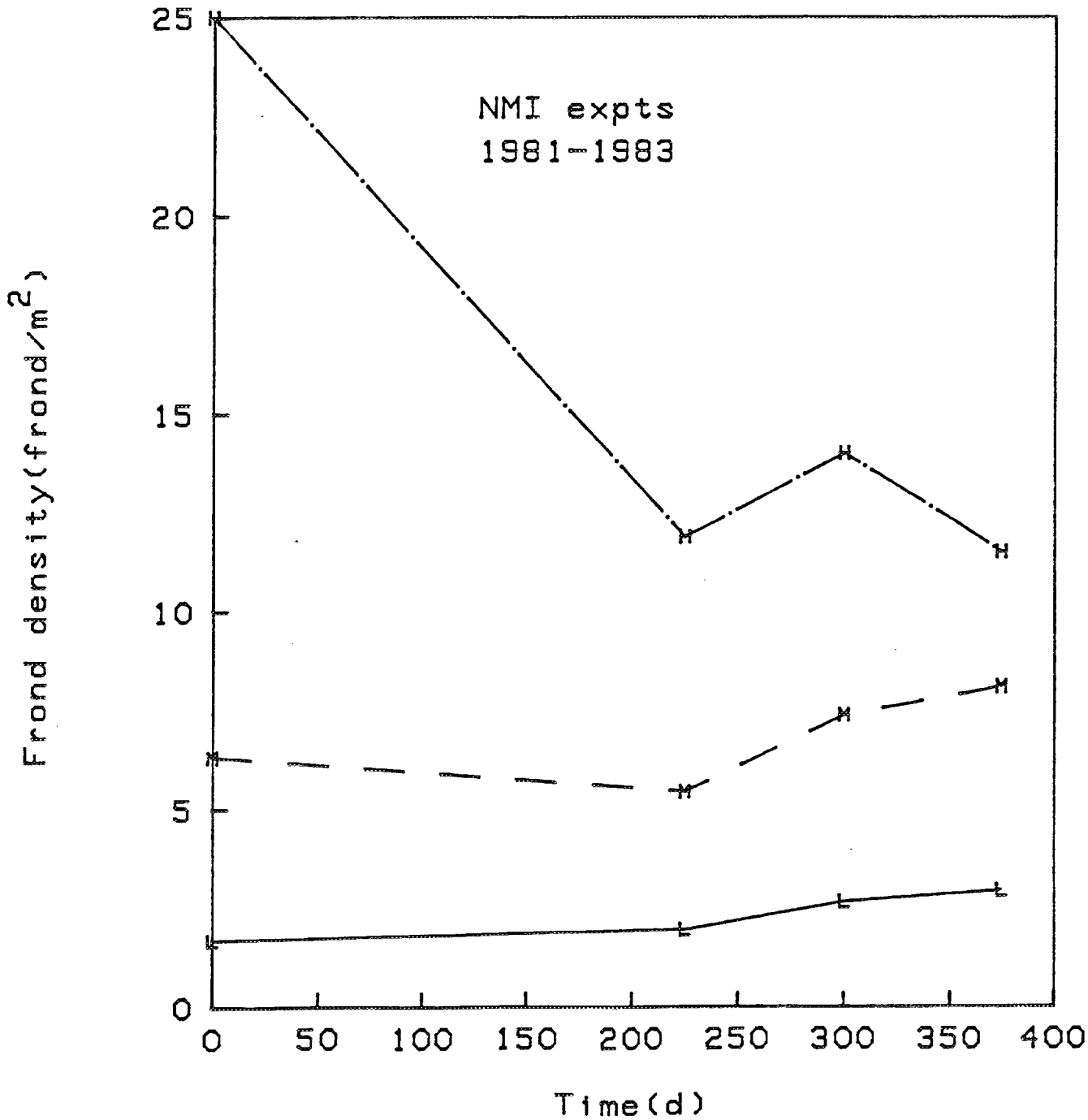


Figure 4.1-1 Frond densities as a function of time in experiments made by NMI (1983). Plotting symbols indicate the plant densities: H-1.0 plants/m^2; M-0.25 plants/m^2; L-0.06 plants/m^2.

NMI experiment, results showed that the net production (photosynthesis - plant respiration) per unit area should be at a maximum when the frond density is about 3 fronds/m^2 and 0 at a frond density of about 8 fronds/m^2 (Figure 4.1-2). The frond density at which net production is 0 is the frond density at which plant growth should stabilize. The model prediction of 8 fronds/m^2 is remarkably close to the value of 8-11 fronds/m^2 that the field experiments suggest.

It is important to remember that the original kelp photosynthetic model is a very simple description of a complex biological system. It does not have the means to include the effects of harvesting on the plants. Even so, the model prediction is within the range of results found in field experiments. It is expected that more sophisticated models will be very useful in helping to explain the results of field work and to suggest new directions for experimental study.

The KPM is a useful start for the modeling work but has weaknesses that must be improved before it is incorporated into a growth model. At present, efforts are being made to improve the light description. The work of NMI emphasizes the importance of light in determining seaweed production.

Field experiments by Dr. V. Gerard show that the light model needs more work. Dr. Gerard measured the light scalar irradiance below kelp tissue forming a canopy at the surface. Her purpose was to measure the effect of canopy density on sub-surface light levels. By changing the canopy density, she was able to determine the effect of different surface canopy densities. Her results showed that the light one meter below the canopy decreased exponentially with the lamina area index (a measure of the number of lamina layers covering the surface), LAI. The simple light model of the KPM predicts such an exponential decrease with increasing LAI, but it underestimates the light flux. One reason for this flaw in the KPM light model is that it does not

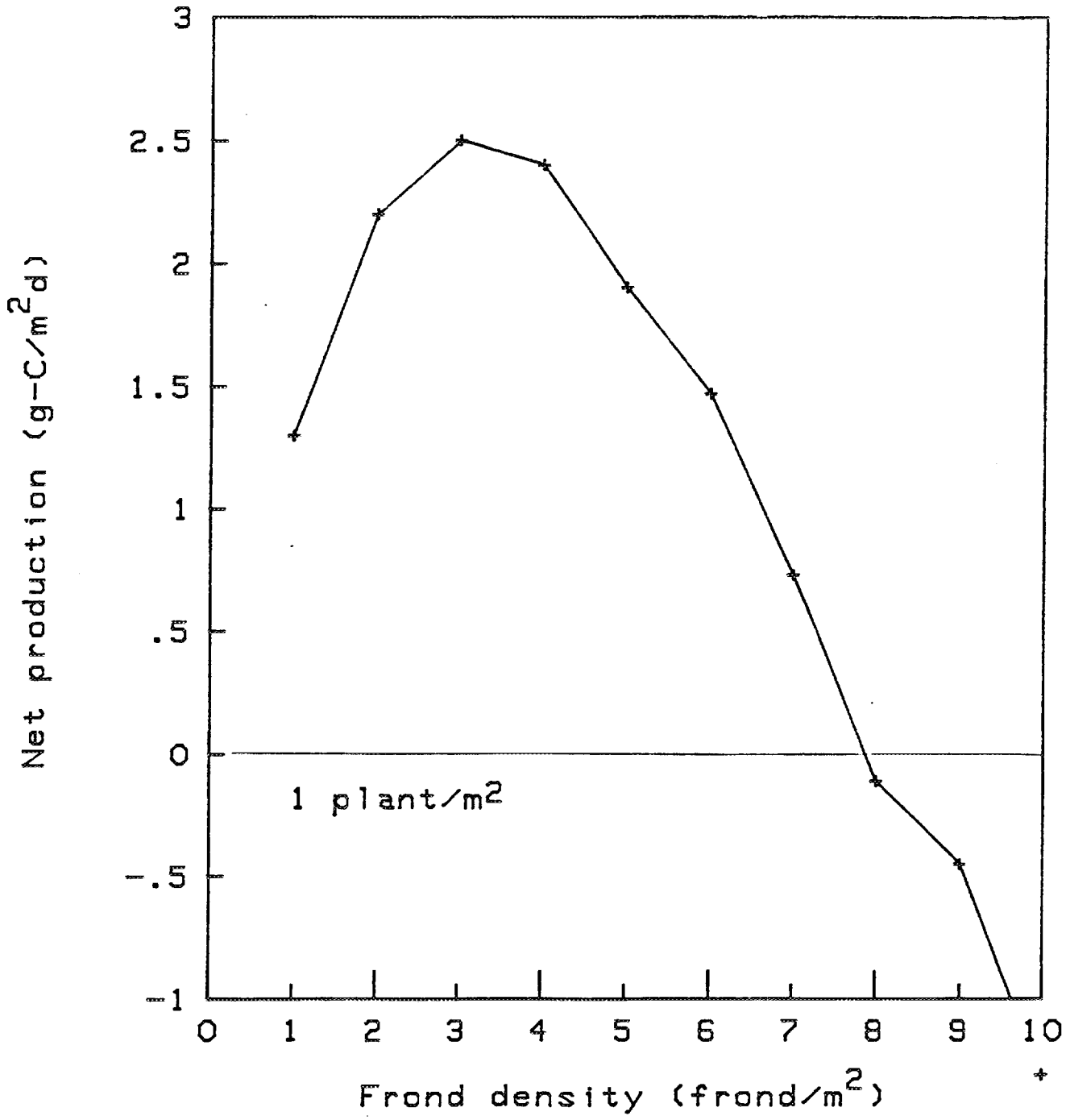


Figure 4.1-2 Net production predicted by the KPM as a function of frond density. The calculations were made for a plant density of 1.0 plants/m^2. Calculations made for the other plant densities shown in Figure 4.1-1 show little difference. One gram of carbon is about equal to 2.5 grams of ash-free dry weight or 33 grams of wet weight kelp.

incorporate the fact that a seaweed is different from a unicellular alga, that two blades lying on top of each other allow more light to pass than two non-overlapping blades, even though the LAI is the same in the two different cases. A a new light model for the canopy is being formulated to account for this discrete nature of seaweeds. The discrepancy between model predictions and field measurements highlights the importance relating the two.

An important part of the Kelp Growth Model is a good description of a kelp plant. With the right description, it is possible to develop rules to determine the distribution of photosynthetic tissue, to predict the shading patterns, and to predict how the plant grows. Improvements have been made in the morphometric description of giant kelp to use in the new kelp models. Our data consist of measurements made by Dr. North on individual fronds taken from natural kelp stands off Southern California. Ways have been found of describing fronds that are valid over large ranges of frond size. As an example, consider the distribution of blade area along the length of a kelp frond. A comparison of the blade area as a function of position along a frond shows that there is great variability among fronds (Figure 4.1-3). This variability has several sources, including systematic changes of blade area with position along a frond, systematic differences in blade sizes for different sized fronds, and natural variability along one frond. A good morphological description should account for the systematic changes and average out the natural variability. One technique for averaging out natural variability along a frond is to examine a running sum of total area along a frond. This has the effect of smoothing out the effect of aberrant blades. The cumulative blade area plot (Figure 4.1-4) shows less variability than does that of individual blade areas (Figure 4.1-3). There is still variability caused by differences in frond size that is not accounted for. This variability is reduced by normalizing the cumulative blade area using the total blade area of a frond and

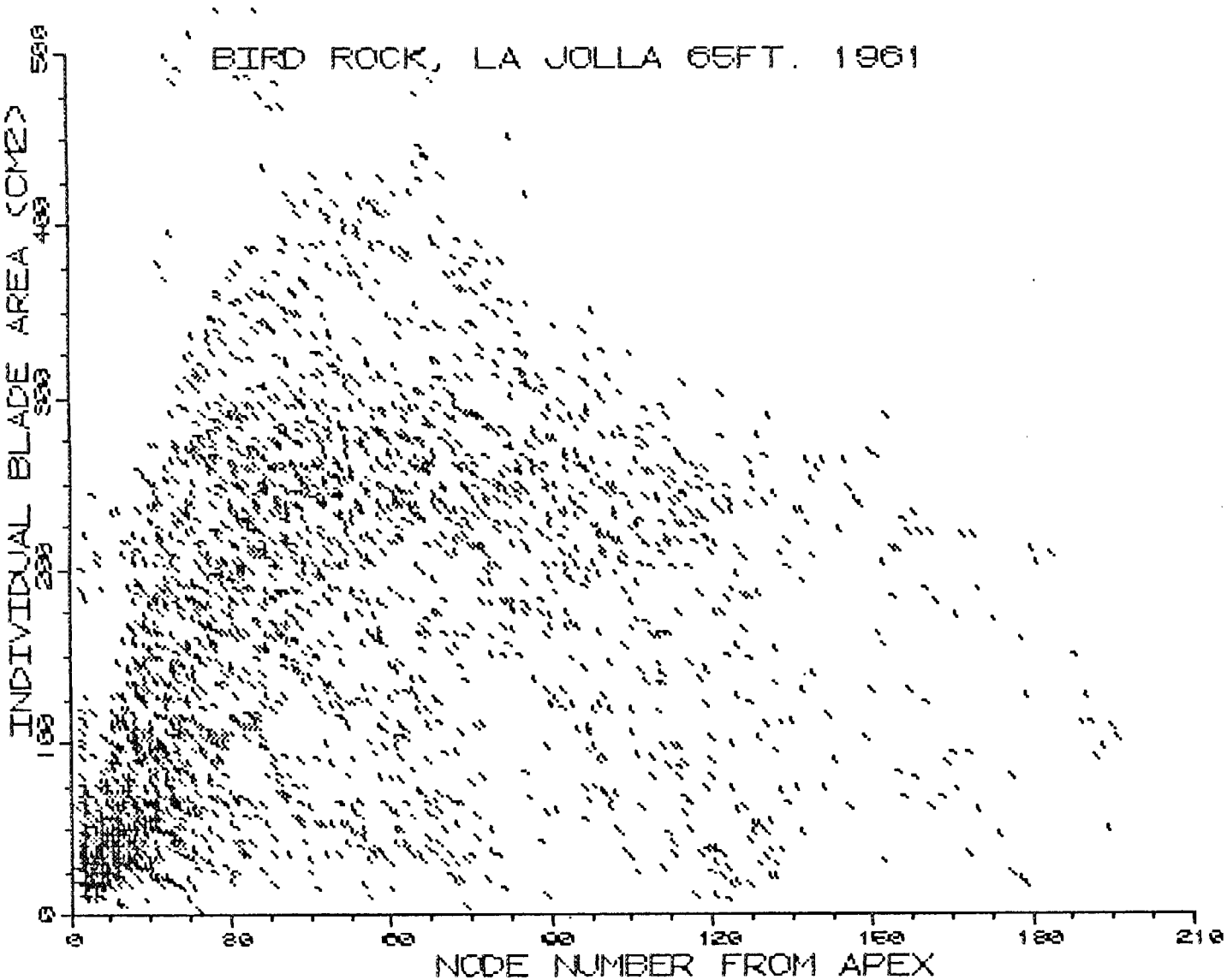


Figure 4.1-3. Relationship between blade area and its position on a frond, given as node number from the apex. Blades get larger away from the apex, smaller near the base. Data are for 37 fronds, ranging in length

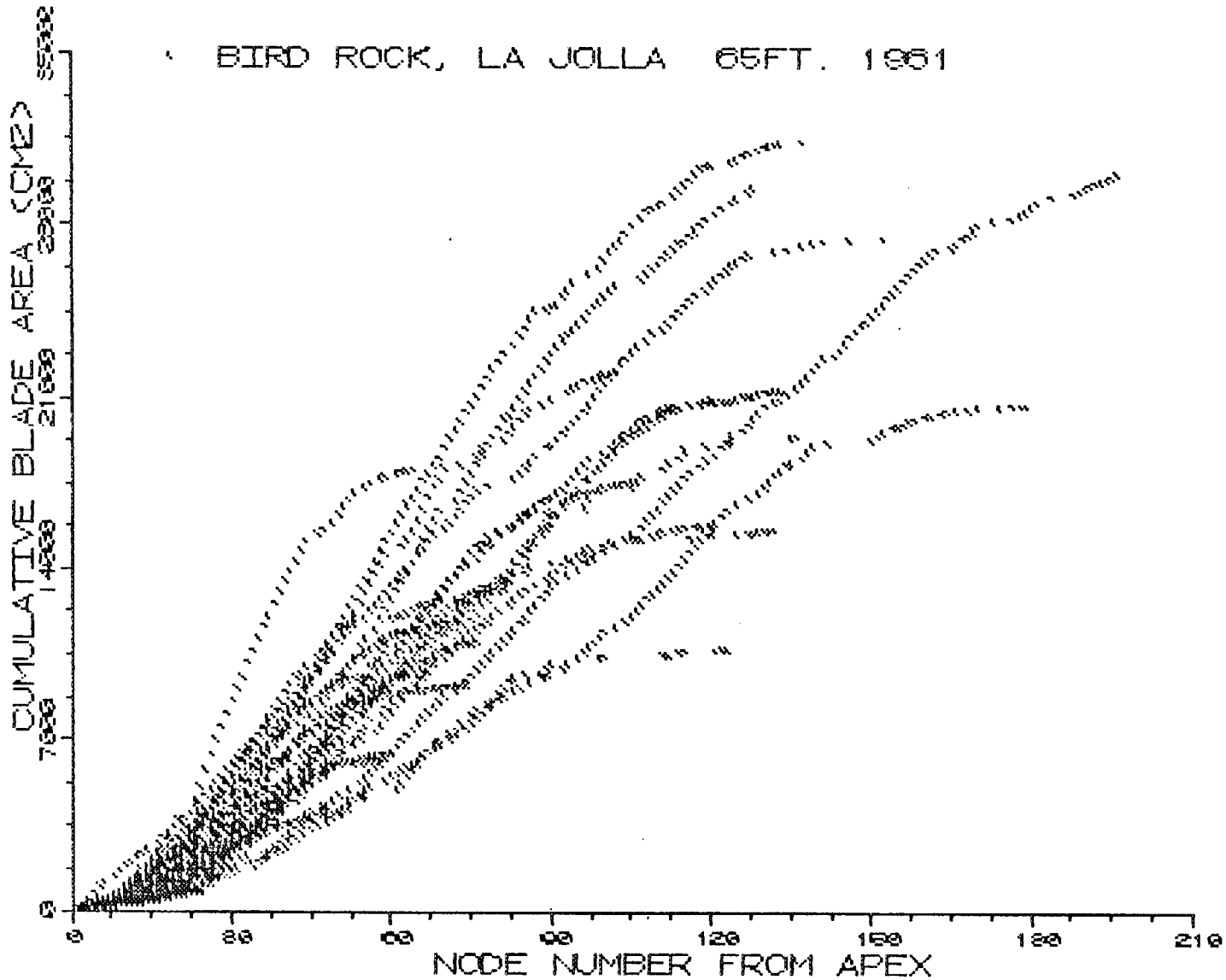


Figure 4.1-4. Cumulative blade area as a function of node number. The integration of blade area greatly reduces the scatter associated with missing blades. Data shown are for the 37 fronds plotted in Figure 4.1-3.

normalizing the blade number using the total number of blades on a frond (Figure 4.1-5). A power series fit to data in this normalized form gives results that are the same for different frond lengths. There is also a relationship between total number of nodes on a frond and the total blade area (Figure 4.1-6). Thus, it is possible to derive power series curve fits that express much of the variability in blade area along different sized fronds.

These relationships can be used to calculate the distribution of area along a representative frond. Given the total number of nodes on a frond, one first calculates the total blade area for a plant. Then, one calculates the normalized node number for each node along the frond. For each normalized number, one then calculates the normalized cumulative area and, using the value of the total area, the cumulative area at each node number. The actual blade at a node is the difference between the cumulative area at that node and the previous one. Thus, one can calculate representative blade areas along fronds of different sizes with two simple curve fits.

These morphological relationships are important to determining the distribution of tissue at different locations along a kelp plant for the kelp model. They make it possible to describe the vertical and horizontal distribution of photosynthetic tissue, self-shading of kelp plant, kelp respiration, and rules for kelp growth. A set of these are being built for the kelp model.

The kelp modeling project is well underway. Efforts are being made to describe kelp morphology in a compact way to use in the kelp models. Experience with different field measurements has shown areas where more work needs to be done, such as the description of the light field around a kelp plant, as well as to show that the model will be a useful tool to study kelp growth. During the next year, modifications of the KPM will be completed and will include the ability to simulate kelp growth over periods longer than a day.

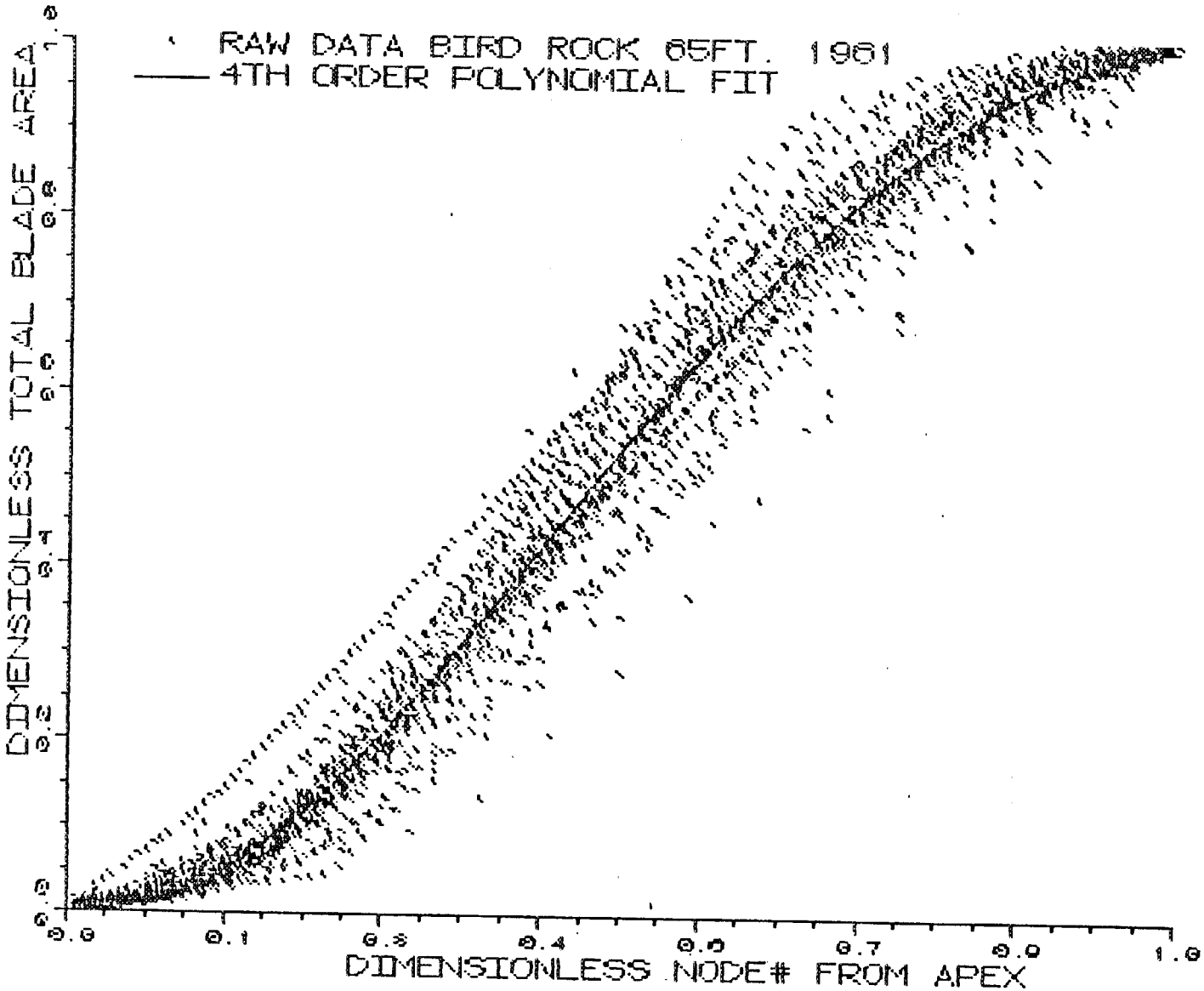


Figure 4.1-5. Normalized blade area as a function normalized node number. This treatment of the data reduces most of the scatter seen in Figures 4.1-3 and 4.1-4. The line drawn through the points is for a fourth order polynomial fit to the data.

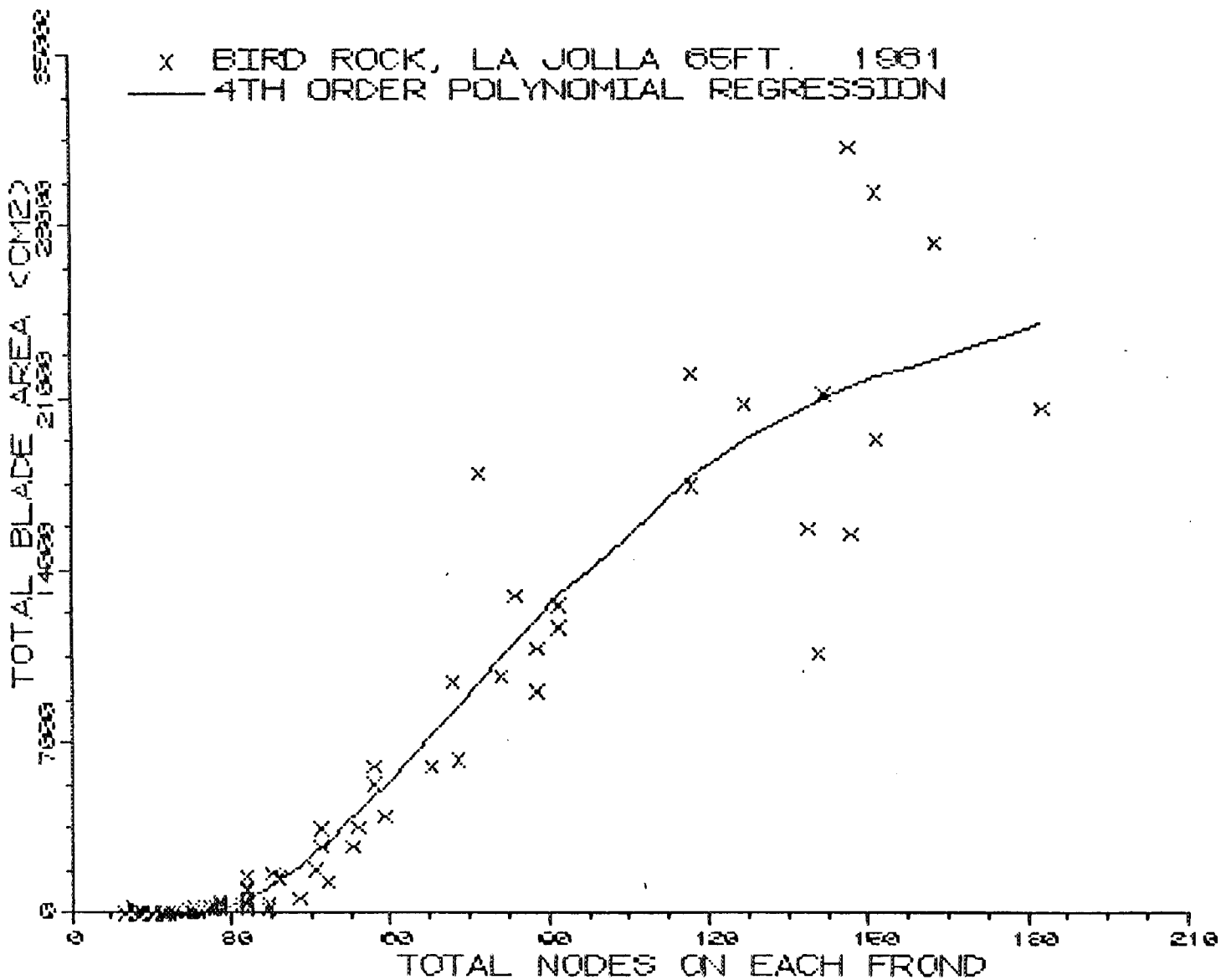


Figure 4.1-6. Total blade area as a function of total number of nodes on a frond. The line drawn through the points is for a fourth order polynomial

REFERENCES

1. Neushul Mariculture Incorporated, 1983 - Kelp Biomass Production Coastal Test Farm Program, Annual Report for 1982.

2. Field Studies - Kelp Photosynthesis - (by Dr. V. Gerard)

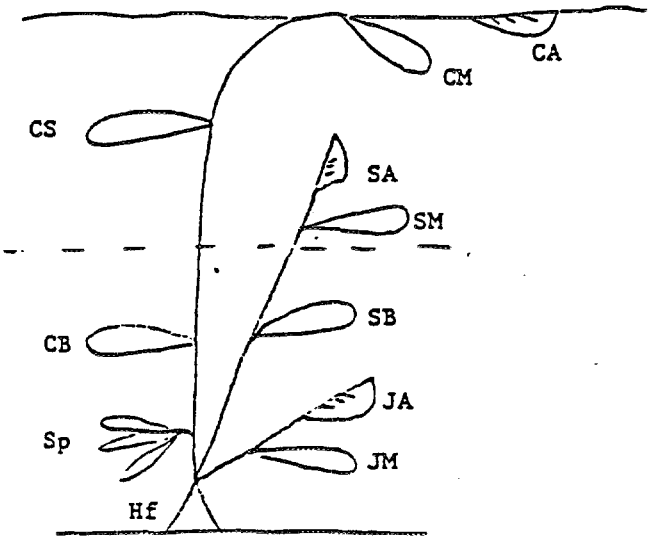
The relationship between light and photosynthesis has been determined for the giant kelp, *Macrocystis pyrifera* by a number of investigators. Physiological differences between portions of an individual blade were amply demonstrated. Only one study (Zimmerman, R., 1983. Ph.D. Thesis, University of Southern California) utilized *in situ* photosynthesis measurements and natural light conditions, but that study concentrated on nutrient effects and did not attempt to define the relationship between photosynthesis and light (P vs. I). Other studies used photosynthetic determinations in the laboratory under artificial light conditions, which are easily controlled but may have artifactual effects on photosynthetic rates. Comparison of photosynthesis under stant and rapidly fluctuating light, for example, showed significant effects on a number of terrestrial plant and phytoplankton species. The kelp forest light environment is characterized by great variability, and photosynthesis *in situ* may differ significantly from laboratory rates. Photosynthetic rates are probably the most important parameters of the kelp production and yield model. Accurate prediction of biomass production must depend on accurate estimation of photosynthetic rates and on integration of individual blade rates to provide frond, whole-plant, or population rates. The goals of this research, therefore, were to accurately determine P vs. I relationships for various blade types of *M. pyrifera* under natural light conditions. The results, briefly described herein, will be used in the kelp production model, in conjunction with results of laboratory esperiments designed to determine effects of physical factors which are difficult to control in the field (e.g. temperature).

Photosynthetic and respiratory rates were determined *in situ* using individual kelp blades enclosed in clear polyethylene bags. Blades from various parts of an adult sporophyte were divided into different categories based on age and position in

the water column (Figure 4.1-7). Incubation procedures were similar to those described by Gerard (1982, J. Exp. Mar. Biol. Ecol. 62:211-224), and rates were measured as change in dissolved O_2 determined by Winkler analysis. Most of the incubations used intact blades, but blades were detached from the stipe and moved to different depths or into open water for incubations at non-ambient irradiances. Mean irradiance (PPFFR) was measured during each incubation using the LDAS described by Gerard in 4.1 D-3 of this report entitled "Measurement and Prediction of the Marine Farm Light Environments". Respiratory rates were measured in black bags, and incubations were carried out between dusk and dawn to avoid artifactual effects of recent light history.

P vs. I relationships were similar for different blade types located at similar depths in the water column, indicating that acclimation was more important than tissue age in determining photosynthetic rates. Tissues from canopy, subcanopy, and basal depths showed significant differences in photosynthetic rates under similar light conditions. Canopy blades had the highest photosynthetic capacity (Pmax) and showed no photoinhibition at high irradiances (open water, 1 m depth). Deeper tissues had lower photosynthetic capacity and reduced photosynthetic rates at high irradiances. Nocturnal respiratory rates were similar for all blade types, except apical blades which have a high proportion of meristematic tissue and showed higher respiration on a dry weight basis. These results can be used directly in the kelp production model.

Two experiments tested the effects of different acclimation conditions on photosynthetic capacity. Mature blades on juvenile fronds, detached and held for 2, 7 and 11 days at subcanopy depths, showed significantly higher chlorophyll content and photosynthetic rates at high irradiance than similar blades held at basal depths. The photoinhibition response, therefore, was



Frond type

canopy frond = apex at surface

subcanopy frond = apex in upper half of the water column

juvenile frond = apex in lower half of the water column

DESIGNATION	FROND TYPE	TISSUE TYPE	SPECIFICATIONS
CA	canopy	apex	apical 50 cm
CM	canopy	mature	2-3 m from apex
CS	canopy	subcanopy	2-5 m below the surface
CB	canopy	basal	2 m above base
SA	subcanopy	apex	(see above)
SM	subcanopy	mature	
SB	subcanopy	basal	
JA	juvenile	apex	
JM	juvenile	mature	

Figure 4.1-7. Tissue Sample Nomenclature
Revised 1983 Version

reduced by acclimation at increased light levels. The second acclimation experiment examined the effect of harvesting on photosynthesis by subcanopy blades. The canopy in one 10 meter X 10 meter plot of the Palos Verdes kelp forest was harvested at 1 meter depth, and the canopy remained intact in another plot. After 7 days, photosynthetic capacity was compared for mature blades on subcanopy fronds collected from the two plots. Blades from the harvested plot showed significantly higher Pmax. These results suggest that the kelp production model can assume similar photosynthetic characteristics for all mature blade types with similar acclimation histories. Changes in the P vs. I relationship may then be predicted on the basis of the light environment during the previous days or weeks.

The influence of diurnal light history on photosynthetic capacity was examined using mature blades from canopy fronds. Detached blades were held at 1 meter depth in open water or beneath the kelp canopy at Laguna Beach for 24 hours. Photosynthetic rates under high irradiance were compared for the two treatments during the morning, before exposure of the open water treatment to high light, and during the late afternoon. Photosynthetic capacities of the two treatments were similar in the morning, but differed significantly during the afternoon incubation. Similar patterns have been domonstrated for other algal species (Ramus J., and G. Rosenberg, 1980, Mar. Biol. 56:21-28). In the case of M. pyrifera, the demonstrated effect is probably important only to canopy blades which are not shaded during the morning period of high light. The effect may be of limited significance, however, since a tagging experiment showed that very few individual canopy blades remain in the uppermost layer of the canopy for more than an hour at a time.

The 1982-1983 El Nino provided a natural demonstration of the effects of nutrient starvation on photosynthetic pigments and rates. During July 1983, nitrogen content of mature, canopy blades was below the critical level (1% of dry weight, indicating

depletion of internal reserves. Chlorophyll content of those blades decreased significantly between June and July, and photosynthetic capacity decreased by almost 50% during that period (Gerard, manuscript submitted). These results emphasize the importance of nutrient supply, and fertilizing under low nutrient conditions, to maintenance of optimal biomass production rates. These results will be used to couple the photosynthesis and nutritional portions of the kelp production model.

3. Field Studies - Measurement and Prediction of Marine Farm Light Environments

Optimum production of biomass on a marine farm will occur when light is the primary factor limiting kelp growth. Biomass production, therefore, depends to a large extent on the light environment, and accurate prediction of the light environment in a kelp forest or farm population is critical to a predictive model of biomass yield. Site-specific surface irradiance can be predicted for different seasons using weather data, and subsurface light in open water can be estimated for various sea states and turbidity conditions using relationships defined by physical oceanographers. Little information exists, however, on the shading effects of the kelp itself. This is an important factor, since the entire kelp plant is photosynthetic, and all but the uppermost canopy layer is subject to self-shading. Furthermore, the shading effect depends at least partly on biomass density and population structure, so knowledge of these relationships is necessary for selection of the optimal planting density for a specific site. Early attempts to predict optimal density using a mathematical model clearly demonstrated the inadequacy of available data describing the kelp forest light environment. The goals of this research, therefore, were to examine the light environment in natural and experimental kelp populations and to experimentally determine relationships between kelp density, population structure, and shading effects. The results, briefly summarized herein, are now undergoing final analysis and will utlimately be used to help formulate and to test the light portion of the kelp production model.

Light was measured *in situ* using the Light Data Acquisition System (LDAS) which consisted of two quantrum meters, two spherical underwater sensors, and a spherical surface sensor. Quantum irradiance in the 400-700 nm range, or photosynthetic photon flux fluence rate (PPFFR), was recorded simultaneously for

the three sensors on an IMS computer with special interface hardware. All hardware and appropriate software were provided by Biospherical Instruments, Inc. Response time of the sensors was approximately 10 microseconds, and a 1 second interval was used for all recordings. The underwater sensors were deployed at various depths and configurations. Most often, one sensor was placed within a stand of kelp, and the other was placed at a similar depth in open water adjacent to the kelp forest. Recording periods ranged from 10 to 200 min.

PPFFR was recorded at 1, 4, 7-8, and 11 m depths in two kelp populations. A relatively homogeneous group of 60 adult Macrocystis pyrifera sporophytes, averaging 35-40 fronds per plant, was established off Laguna Beach, California. Plants were collected from several sparsely populated sites and transplanted to a central location where they were attached to concrete anchors with uniform 2 meter spacing. Monthly determinations of plant and frond size distributions and morphometric data defining lamina area per meter of frond length were used to estimate canopy density (blade area per bottom area) for this population at various times during April-August 1983. The Laguna Beach population was designed to approximate a marine farm in spatial homogeneity of biomass. The second kelp population used for PPFFR determinations was a natural kelp forest at Palos Verdes, California. The canopy of this forest remained relatively dense through August 1983, when the canopies of most other southern California populations had already disappeared due to anomalously high surface temperatures and low nutrient concentrations associated with the 1982-1983 El Nino. The Palos Verdes population was more heterogeneous than the Laguna Beach population in plant size and spacing. Mean PPFFR ranged from 25 to 1295 E m^{-2} S for various depths and locations within the two forests. In order to distinguish variation due to biotic factors, such as canopy density, from the influence of physical

factors, such as irradiance, turbidity, and sea state, PPFFR measured within the forests was expressed as percent penetration, or percent of irradiance at the same depth in open water.

An artificial kelp canopy was used to empirically define the relationship between canopy density and light penetration. Apical 5 meter sections of kelp fronds were attached to a 5 meter X 5 meter PVC frame which floated at the surface. The number of fronds on the frame ranged from 26 to 104, and canopy density (blade area per bottom area) ranged from 0.9 to 6.2. PPFFR was recorded at 1 meter depth below the artificial canopy and simultaneously at 1 meter depth in open water adjacent to the frame. The relationship between kelp density and percent penetration was exponential. Light penetration through the artificial canopy was higher than predicted from light transmittance values for individual kelp blades, however, due to the uneven spatial distribution of tissue. Percent penetration through the natural kelp canopies at Laguna Beach and Palos Verdes was higher than predicted from results of the artificial canopy experiments, again due to greater spatial heterogeneity of canopy tissue. These results show that accurate prediction of the kelp canopy shading effect cannot be based on canopy density and blade transmittance values alone but require a relatively complex model of tissue distribution. The PPFFR measurements made at Laguna Beach and Palos Verdes can be used to test the accuracy of such a model.

The nonuniform distribution of kelp plants and canopy tissue in natural forests resulted in high spatial variability in light penetration. A 20X range was measured at 1 meter depth within a 100 m^2 area of the Palos Verdes forest. Predictably, the shading effect was greatest immediately adjacent to a kelp plant and diminished with increasing distance from the nearest plant. The shading effect deeper in the water column also varied with

proximity to individual kelp plants. At 4 and 7 meter depths, the canopy accounted for most of the shading effect if the nearest plant was more than 1 meter away. Shading by deeper tissues was significant, however, adjacent to a plant. These results indicate the importance of selfshading to overall production by a kelp plant and the importance of planting density in optimizing biomass production on a marine farm.

Short term temporal variation in light has been shown to influence the photosynthetic efficiency of plants. Variability in PPFFR recorded at 1 second intervals was high in open water due to rapid changes in surface reflection and refraction. Short term variability was greatly enhanced, however, below a kelp canopy. Continuous motion of kelp fronds and blades superimposed a fluctuating shading effect on the already variable light environment. Short term peaks of beam radiation, equivalent to the sunflecks which are important to photosynthesis in terrestrial forest plants, occur frequently in the kelp canopy but diminish with increasing depth. The relative importance of diffuse radiation increases at greater depths, and short term variability at 7 meters was similar for open water and kelp forest measurements. Temporal variation in PPFFR averaged over longer intervals (>60 s) did not correspond to changes in surface or open water irradiance but was due to changes in the spatial configuration of canopy fronds. These results not only provide a test of the light portion of the kelp production model, but also may provide the basis for variability functions if long term average PPFFR data prove insufficient for predicting kelp productivity.

4. Laboratory Studies - Phosphorus and the Growth of Juvenile *Macrocystis pyrifera* (Phaeophyta) Sporophytes (by Steven L. Manley and Wheeler J. North, California Institute of Technology)

(See pages 4-31 through 4-49)

LABORATORY STUDIES - PHOSPHORUS AND THE GROWTH OF JUVENILE *MACROCYSTIS PYRIFERA* (PHAEOPHYTA) SPOROPHYTES

By

Stanley L. Manley and Wheeler J. North

California Institute of Technology

ABSTRACT

The effect of phosphate (P_i) supply on growth rate and tissue phosphorus content of juvenile Macrocystis pyrifera (L.) C. Ag. sporophytes was examined. Sporophytes were batch cultured in aquaria with flowing recirculated seawater enriched by 30 µM nitrate, shown to be above growth saturation. Each aquarium was supplemented with a different seawater P_i concentration, 0, 0.3, 1, 2, 3, and 6 µM. Sporophyte mean specific growth declined with time in all cultures presumably due to the normal developmental decrease in the proportion of meristematic tissue of each plant. Growth declines were more pronounced in cultures that were nutrient limited. Sporophyte growth was P-limited after 2-week exposure to P_i less than 1 µM, corresponding to a tissue P concentration of less than 0.20% dry wt. Plants cultured at 6 µM P_i contained tissue P levels of 0.53% dry wt after 3 weeks. Luxury consumption and storage of P occurred.

Key index words: growth rate, Macrocystis, nutrients, phosphorus, sporophyte.

INTRODUCTION

The growth of Macrocystis pyrifera in southern California may, at times, experience N-limited (6,7,13,14,18,19) or Mn-limited (11) growth. Nitrate, usually the most abundant form of nitrogen available to Macrocystis, and orthophosphate (P_i) enter kelp beds via upwelling and runoff and thus display similar temporal and spatial concentration patterns (7,14). The large temporal variation in P_i concentration of seawater from Macrocystis beds, 0.2 to 1.3 μM (7), and for P-levels in Macrocystis tissue, 0.15-0.33% dry wt (8), raises the possibility that P-limited growth might also occur. Examining the possibility, we have defined P-limited growth of juvenile M. pyrifera sporophytes based on tissue analysis.

MATERIALS AND METHODS

Each of our experiments utilized sporophytes of Macrocystis pyrifera (L.) C. Ag. that were rope cultured from spores (12) obtained from sporophylls collected from a single plant. Juvenile sporophytes were at a similar developmental stage prior to preconditioning having a single lamina, ranging in length from 2 to 5 cm, and ranging in fresh weight from 50-200 mg. Seven plants were held in each 40-l Plexiglas aquarium and preconditioned in continuously recirculated (10-13 cm s^{-1}) offshore surface seawater, supplemented daily with $NaNO_3$ and K_2HPO_4 to produce concentrations of 15 and 1 μM, respectively. Water was replaced every other day. Plants were grown under continuous illumination

(cool white fluorescent lamps, 138 $\mu E \cdot m^{-2} \cdot s^{-1}$) which was above growth-saturating levels (1.1×10^2 $\mu E \cdot m^{-2} \cdot s^{-1}$, Manley, unpublished). Temperature ranged between 10-14°C. Experiments were begun 10 days after the preconditioning period. Mean specific growth rates were computed from weekly measurements of wet weight according to the equation,

$$\text{specific growth rate} = \frac{100}{t}(\ln\frac{W_t}{W_o})$$

where W_o and W_t represent wet weights before and after elapsed time t in days. After each weighing, 1/3 to 1/2 of the apical laminar tissue was trimmed for tissue analysis. New sets of juvenile sporophytes were used for each experiment.

In a first experiment, P_i concentration was increased to 2 μM in all aquaria after the preconditioning period. Nitrate concentration was adjusted in each aquarium to either 3, 6, 15, or 30 μM and growth was followed for 38 days.

The second experiment involved increasing nitrate concentration to 30 μM (a concentration above growth saturation found from the previous experiment) after the 10-day preconditioning. Phosphate concentration was adjusted to either 0.3, 1, 2, 3, or 6 μM. Growth was followed for 4 weeks.

For the third experiment, nitrate concentration was increased to 30 μM and P_i concentration adjusted to either 0, 0.3, 1, or 2 μM. The initial P_i concentration of surface seawater was 0.1 μM; P_i became undetectable after a 1-day exposure to plants. Growth was followed for 3 weeks.

Nitrate and P_i seawater concentrations were analyzed by the methods of Strickland and Parsons (17). Tissue analysis was performed by Galbraith Laboratories (Knoxville, TN) on the excised tissue dried at 80°C for 2 days and pulverized through a 40-mesh screen (18). Total tissue N (% dry wt) was determined using a nitrogen analyzer (Carlo Erba 1400). Analysis is based on the complete combustion of the sample followed by thermal conductivity detection. Total tissue P (% dry wt) was determined by a modification of standard method used on sediments and sludge (1). The sample was rigorously digested and P_i determined colorimetrically. Triphenylphosphine (2-6 mg) was used as a control standard. Tissue (5-40 mg, corresponding to approximately 400 µg P) was placed in a 50-ml vol flask (with boiling chips), and 20 ml of 1% w/v Na_2MoO_4 solution containing 12.5% v/v HNO_3, 67.5% v/v H_2SO_4, and 7.5% v/v $HClO_4$ was added. The mixture was boiled until only H_2SO_4 remained, at which time the bubbling ceased. After cooling, the total volume was adjusted to 25 ml with H_2O, 5 ml of 0.25% w/v NH_4VO_3 solution in 2% v/v HNO_3 was added, and the solution mixed. A 5% w/v $(NH_4)_2MoO_4$ solution was then added, the total volume adjusted to 50 ml with H_2O, and the contents mixed. Absorbance was read at 460 nm after 30 min.

RESULTS

At the end of preconditioning, mean specific growth rates from each aquarium were never significantly different within an experiment (Figures 4.1-8A, 4.1-9A). After 31 days of growth (10 days

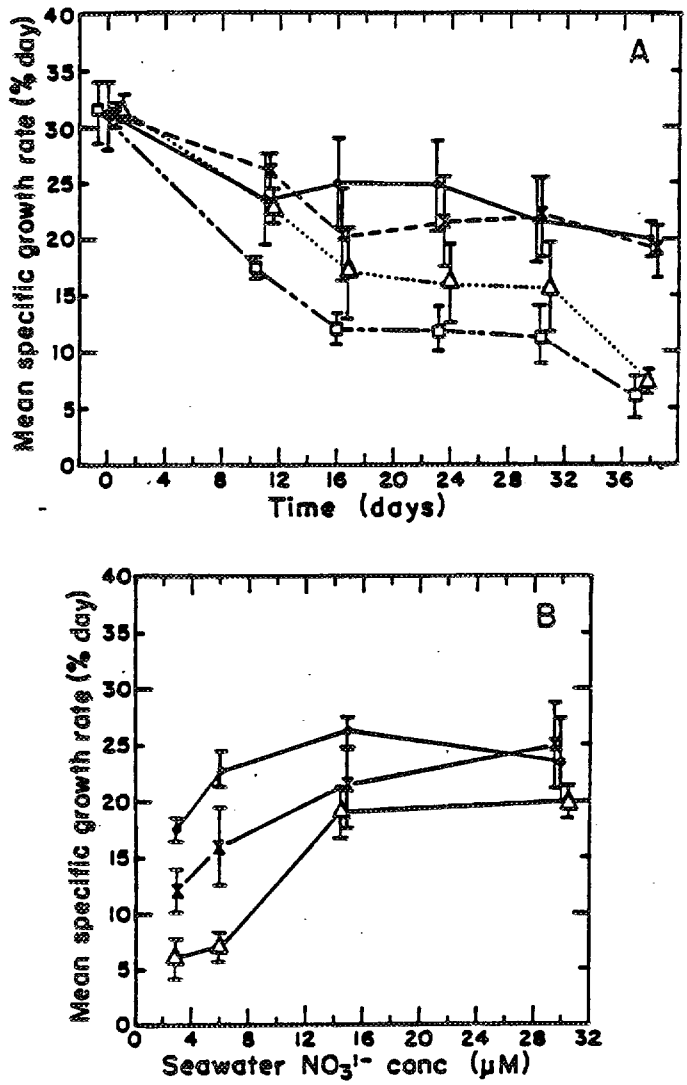


Figure 4.1-8. Growth of juvenile sporophytes cultured in different nitrate concentrations. Bars represent 95% confidence intervals (n = 6). A) Specific growth rate vs culture time, t = 0 after 2 weeks preconditioning in 15 μM nitrate, 1 μM P_i. Experimental $[P_i]$ = 2 μM. Experimental [nitrate]: •—• = 30 μM, x---x = 15 μM, Δ...Δ = 6 μM, and □-·-·-□ = 3 μM. Note: initial points at t = 0 offset for clarity. B) Specific growth rate vs seawater nitrate concentration. Days after preconditioning: • = 11, x = 24, Δ = 38.

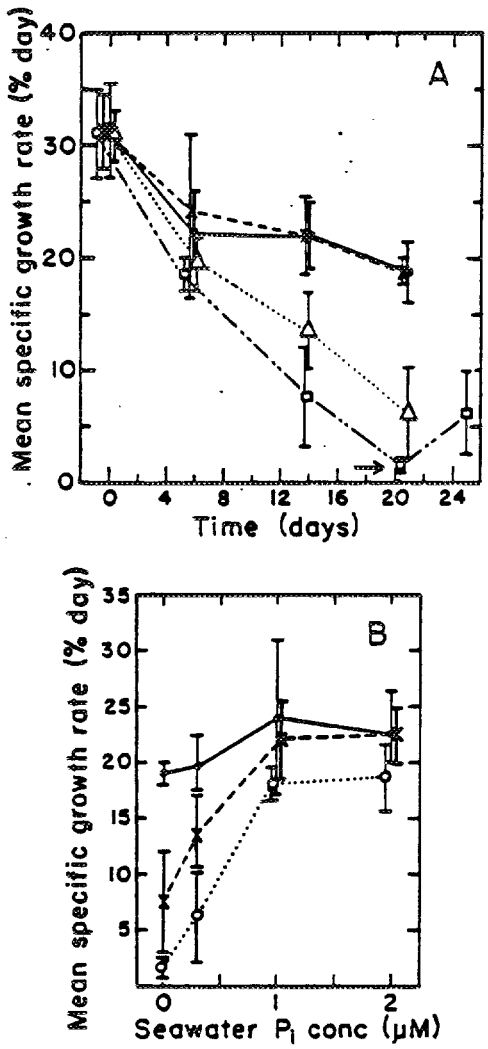


Figure 4.1-9. Growth of juvenile sporophytes cultured in different P_i concentrations. Bars represent 95% confidence intervals (n = 6). A) Specific growth rate vs culture time, t = 0 after 2 weeks preconditioning in 15 µM nitrate, 1 µM P_i. Experimental [nitrate] = 30 µM. Experimental [P_i]: •—• = 2 µM, x---x = 1 µM, Δ...Δ = 0.3 µM, □-·-·-□ = 0 µM. Arrow indicates addition of P_i to 2 µM. Note: initial points at t = 0 offset for clarity. B) Specific growth rate vs external P_i concentration. Days after preconditioning (15 µM nitrate, 1 µM P_i): •—• = 7 days, x---x = 14 days, o---o = 21 days.

preconditioning and 3 weeks experimental), all of the single-bladed juveniles had undergone their first primary division with enlargement of stipe and holdfast.

Growth vs external nitrogen concentration. Mean specific growth rates always decreased with time in all aquaria (Fig. 4.1-8A). The rate of decrease was more pronounced as the external nitrate concentration decreased. Effects of external nitrate concentration on growth became more pronounced as exposure time increased (Fig. 4.1-8B). Growth was saturated between 6-15 µM nitrate. Plants exposed to 3 and 6 µM nitrate became noticeably pale after 3 weeks compared to plants at higher nitrate concentrations.

Growth vs external phosphate concentration. Both experiments involving plants cultured in different P_i concentrations yielded similar results. We here present data relating growth to various parameters from the second P_i experiment (Fig. 4.1-9 & 4.1-10).

Mean specific growth rates decreased with time in all aquaria (Fig. 4.1-9A). Phosphorus-limited growth occurred after 2-week exposure to less than 1 µM P_i (Fig. 4.1-9B) corresponding to a tissue P level of less than 0.20% dry wt (Fig. 4.1-10B). Tissue P_i was high (0.32% dry wt) immediately after preconditioning and remained high in those plants exposed to 2 µM P_i (Fig. 4.1-10A, B). Tissue P declined in the others. Tissue P was hyperbolically related to external P_i concentration, ranging from 0.12% to 0.53% dry wt (Fig. 4.1-11). Analyses from the first P_i experiments indicated there was no significant difference ($p = 0.05$) in tissue N

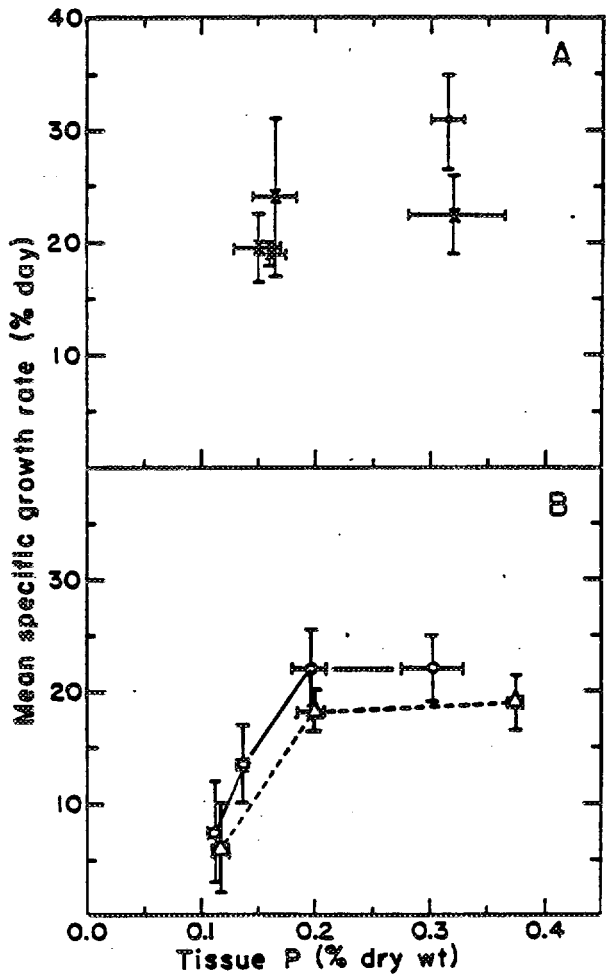


Figure 4.1-10. Specific growth rate (n = 6) vs tissue P (n = 4). Bars represent 95% confidence intervals. A) ● = at end of preconditioning, x = 7 days after preconditioning. B) o = 14 days after preconditioning. Δ = 21 days after preconditioning.

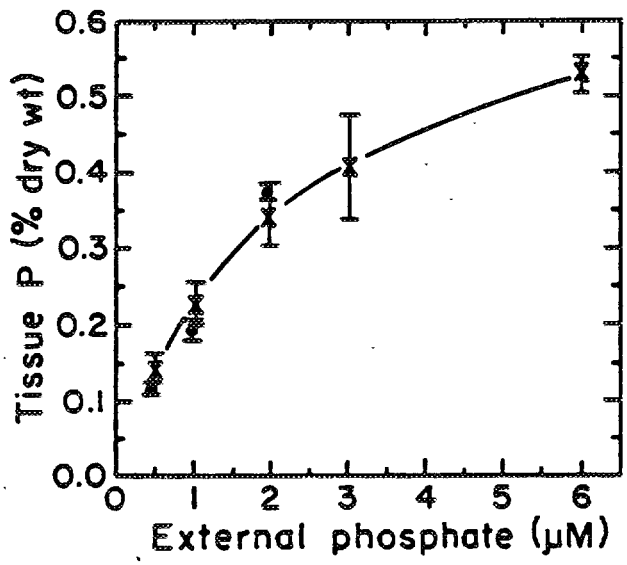


Figure 4.1-11. Tissue P (n = 4) vs seawater P_i concentration for tissue after 3 weeks culturing from preconditioning period. Bars represent 95% confidence interval. x = data from first P_i experiment. ⊕ = data from second P_i experiment.

between plants in the 5 different aquaria after 3 weeks. Percent dry wt tissue N for 20 plants analyzed was 3.1±0.1 (mean ±95% CI).

Plants deprived of P_i for 3 weeks were noticeably pale with abnormally thin blades. Some plants sloughed tissue. Growth rate increased after external P_i concentration was raised to 2 μM for a week (Fig. 4.1-9A) indicating that plant growth was previously P-limited. The distal P-starved blade tissue eventually disintegrated leaving a zone with a granular surface. The blade's basal zone retained a normal appearance.

Plants cultured for 2 weeks in 6 μM P_i were much darker than the remainder. Some, however, suffered necrosis of the basal cleft. After 3 weeks, the entire basal cleft was destroyed on these plants and others contracted the disease. Necrosis plagued all plants in this 6 μM P_i aquarium after 4 weeks. Substantial reductions and wide variations in growth resulted. These plants did not survive the fifth week. High external P_i concentration or high external P_i and nitrate was deleterious to the sporophytes. We did not determine whether necrosis and resulting growth declines were direct toxic responses or bacterially mediated.

DISCUSSION

A standardized culturing methodology must be used to reduce variability in growth among plants in the same aquarium and to assure uniform culturing conditions when determining effects of nutrient concentrations on growth of juvenile Macrocystis sporophytes. Essential features of such a methodology are as follows:

an unchanging source of seawater should be used to ensure that growth effects can be attributed to the nutrient under investigation; actual testing should be preceded by one to two weeks of preconditioning in a constant environment to encourage uniformity of growth rates and tissue chemistry among the plants; replicate plants should be as genetically uniform as possible and at similar developmental stages (same age); light intensity should be above growth-saturating levels to minimize effects from changes in light intensity due to light source variability and "self-shading," occurring when laminae overlap.

Our method for computing growth lumped slow or nongrowing tissues with rapidly growing (meristematic) portions of the plant. Proportions of rapidly growing tissues in juvenile Macrocystis decrease with time during normal development. This slow change may, in part, explain the declining growth rates we observed, although weekly trimming of laminal apices removed a large fraction of the slowly growing tissues. Apical portions of laminae do not seem critical for growth and development. They are often lost in nature during storms or may be sloughed during periods of warm water or low nutrients. Removal of these distal parts did reduce total photosynthetic areas of the plants. This presumably minor effect would be similar for all individuals in an experiment, permitting within-experimental comparisons.

The P distribution along the length of a single- or double-bladed juvenile Macrocystis sporophyte was not determined. The

tissue P concentration showed an insignificant to slight decrease from the lamina base to tip of Laminaria hyperborea (4), L. digitata (5), and Macrocystis (mature blade, Manley, unpublished). Such tissue P gradients have been attributed to intralamina translocation (5) and probably do not reflect differences in P growth kinetics between basal and apical tissue.

Growth was apparently saturated between 6 and 15 µM external nitrate concentration. A similar range was obtained for Laminaria saccharina (2, 20). Wheeler and North (18) found that growth did not saturate up to 30 µM nitrate; however, the culturing methodology mentioned previously was not followed. Data were combined from batch, continuous, and field grown juvenile sporophytes, and culturing conditions were not uniform. Preconditioning parameters were different for the two cultures, and plants obtained from the field at different seasons were probably of different genetic stock. Also, the data were combined from sporophytes batch cultured for different periods of time (1-29 days). Thus a part of the decrease in growth rate attributed to a decrease in tissue N may actually have been a developmental effect. It is, therefore, not surprising that they did not find significant N-saturated growth and corresponding surplus nitrogen accumulation. The relationship between tissue N and juvenile sporophyte growth should be reinvestigated. Adult sporophytes do accumulate surplus nitrogen (6,14,19) if growth is saturated above the tissue N level of 1.5% dry wt, as suggested by Gerard (6).

Fronds among adult sporophytes arise from basal meristems (frond initials) and elongate by the unilateral division of the apical scimitar. Apical and basal meristems are morphologically very similar to single- and double-bladed juveniles (9). They are probably physiologically comparable being primarily composed of young meristematic tissue. Growth by basal and apical meristems (and thus frond initiation and growth) is probably saturated above a tissue P level of 0.20% dry wt similar to juvenile sporophytes. Juvenile sporophytes can only obtain nutrients from the surrounding water. Meristems on an adult plant can also obtain P from other tissues of the parent plant via translocation. Translocation to meristems of N, as amino acids (16), and P, as organic P and P_i (10), maintains high growth rates during short periods of low external nitrate and P_i concentrations. Adult Macrocystis can maintain growth for 2 weeks on internal N reserves (6). Phosphate storage probably also allows for similar growth maintenance.

The concentration of P_i in seawater of the Pt. Loma, CA, kelp bed was below 0.6 µM in the upper 4.5 m (where most of the canopy resides) for an entire year (1975) while the concentration between 4.5 and 9 m was greater than 0.6 µM for 1 month of the year (7). Nitrate (NO_3 + NO_2) concentration, however, was never above 10 µM (7). Another study of the same area from 1975-76 showed similar results; concentrations of P_i and nitrate in the top 1.4 m of the water column were never above 0.8 and 4 µM, respectively (8). It seems possible that adult sporophytes may become nutrient limited by N and/or P. North et al. (14) have indicated that N

probably becomes growth limiting to Macrocystis before P because inshore water can contain 0.35 µM (mean) P_i when nitrate concentration is undetectable (<0.05 µM).

The preferred method of determining the nutrient status of an alga is by tissue analysis (3). If the relationship between the tissue concentration of a given nutrient and growth is known, tissue analysis of field material can determine whether algal growth is nutrient limited. It is difficult to determine relationships between growth and internal nutrient concentration for adult Macrocystis sporophytes because of their large size and the existence of multiple meristems and tissue types. A study relating growth of a single adult sporophyte to tissue N has been done (6).

The only published tissue P data for adult sporophytes is from monthly elemental analyses of homogeneously mixed samples of dried fronds (8). Tissue P levels were below the level of 0.20% dry wt for 5 months of the year, reaching minimum values of 0.15% dry wt in August and December and suggesting P-limited growth. (It is possible, however, that meristematic tissue may have been above critical levels relying on translocation from other tissues.) For 3 of these 5 months, tissue N was below 1.5% dry wt. Assuming that this represents the critical tissue N level (6) and that there is no simultaneous N and P growth limitation, then P-limited growth was indicated for at least 2 months--December (P = 0.15% dry wt, N = 1.56% dry wt) and January (P = 0.17% dry wt, N = 1.52% dry wt).

Phytoplankton growth is regulated by the nutrient in shortest supply; there is no simultaneous N and P growth limitation (15).

There are no comparable experiments performed on macroalgae. There is no compelling physiological reason, however, to presume that at the cellular level Macrocystis should display multiple nutrient limitation.

The entire adult sporophyte with meristems at different depths, exposed at times to different nutrient concentrations and ratios, could conceivably experience multiple nutrient limitation because the various meristems might be limited by a different nutrient. This would be a transient phenomenon lasting until translocation could equalize nutrient ratios among the growing tissue. In such a scenario, whole plant growth would be considered limited by multiple nutrients, but a given meristem would be limited by a single nutrient.

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REFERENCES

1. Am. Public Health Assoc., Am. Water Works Assoc., and Water Pollut. Control Fed. 1975. Standard Methods for the Examination of Water and Wastewater (M.C. Rand, A.E. Greenberg, and M.J. Taras, Eds.). 14th ed. APHA, AWWA, and WPCF, Washington, D.C. 1193 pp.

2. Chapman, A.R.O., Markham, J.W. & Lüning, K. 1978. Effects of nitrate concentration on the growth and physiology of Laminaria saccharina (Phaeophyta) in culture. J. Phycol. 14:195-8.

3. DeBoer, J.A. 1981. Nutrients. In Lobban, C.S., and Wynne, M.J. [Eds.] The Biology of Seaweeds. Univ. of Calif. Press, Berkeley, pp. 356-92.

4. Floc'h, J.Y. & Penot, M. 1980. Variation de la composition minérale et transport á longue distance au cours de la croissance de la lame chez Laminaria hyperborea (GUNN) FOSL. Z. Pflanzenphysiol. 96:377-85.

5. Floc'h, J.Y. 1982. Uptake of inorganic ions and their long distance transport in Fucales and Laminariales. In Srivastava, L.M. [Ed.] Synthetic and Degradative Processes in Marine Macrophytes. Walter de Gruyter & Co., Berlin, pp. 138-65.

6. Gerard, V.A. 1982. Growth and utilization of internal nitrogen reserves by the giant kelp Macrocystis pyrifera in a low-nitrogen environment. Mar. Biol. 66:27-35.

7. Jackson, G.A. 1977. Nutrients and production of giant kelp, Macrocystis pyrifera, off southern California. Limnol. Oceanogr. 22:979-95.

8. Lindner, E., Dooley, C.A. & Wade, R.H. 1977. Chemical Variation of Chemical Constituents in Macrocystis pyrifera. Ocean Food and Energy Farm Project Final Report. Naval Undersea Center, San Diego, CA, 25 pp.

9. Lobban, C.S. 1978. The growth and death of the Macrocystis sporophyte (Phaeophyceae, Laminariales). Phycologia 17:196-212.

10. Manley, S.L. 1983. Composition of sieve tube sap from Macrocystis pyrifera (Phaeophyta) with emphasis on the inorganic constituents. J. Phycol. 19:118-21.

11. Manley, S.L. 1984. Micronutrient uptake and translocation by Macrocystis pyrifera (Phaeophyta). J. Phycol. In press.

12. North, W.J. 1977. Growth factors in the production of giant kelp. Proc. Symp. Clean Fuels from Biomass and Wastes. Institute of Gas Technology, Chicago, IL, pp. 128-40.

13. North, W.J. 1980. Trace metals in giant kelp, Macrocystis. Am. J. Bot. 67:1097-101.

14. North, W.J., Gerard, V.A. & Kuwabara, J.S. 1982. Farming Macrocystis at coastal and oceanic sites. In Srivastava, L.M. [Ed.] Synthetic and Degradative Processes in Marine Macrophytes. Walter de Gruyter & Co., Berlin, pp. 247-62.

15. Rhee, G.-Y. 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. Limnol. Oceanogr. 23:10-25.

16. Schmitz, K. & Srivastava, L.M. 1979. Long distance transport in Macrocystis integrifolia. I. Translocation of ^{14}C-labeled assimilates. Plant Physiol. 63:995-1002.

17. Strickland, J.D.H. & Parsons, T.R. 1972. A practical handbook of seawater analysis. Bull. Fish. Res. Bd. Can. 167:1-310.

18. Wheeler, P.A. & North, W.J. 1980. Effect of nitrogen supply on nitrogen content and growth rate of juvenile Macrocystis pyrifera (Phaeophyta) sporophytes. J. Phycol. 16:577-82.

19. Wheeler, P.A. & North, W.J. 1981. Nitrogen supply, tissue composition and frond growth rates for Macrocystis pyrifera off the coast of southern California. Mar. Biol. 64:59-69.

20. Wheeler, W.N. & Weidner, M. 1983. Effects of external inorganic nitrogen concentration on metabolism, growth and activities of key carbon and nitrogen assimilatory enzymes of Laminaria saccharina (Phaeophyceae) in culture. J. Phycol. 19:92-6.

5. Laboratory Studies - Carbon Allocation in *Macrocystis pyrifera* (L.) C. AG. I. Intrinsic Variability in Photosynthesis and Respiration (by Keith E. Arnold, California State Polytechnic University and Steven L. Manley, California Institute of Technology)

(See pages 4-51 through 4-110)

CARBON ALLOCATION IN *MACROCYSTIS PYRIFERA* (L.) C. AG.

I. INTRINSIC VARIABILITY IN PHOTOSYNTHESIS AND RESPIRATION

By

Keith E. Arnold
California State Polytechnic University

and

Steven L. Manley
California Institute of Technology

ABSTRACT

Measurements of net photosynthesis (O_2 evolution), dark respiration (O_2 consumption), and light and dark carbon fixation (^{14}C) were conducted on whole blades, isolated blade discs, sporophylls, apical scimitars, and representative portions of stipe and holdfast of the giant kelp Macrocystis pyrifera L. C. Ag. Macrocystis tissue discs can be used to assess photosynthesis (PS) and respiration (R) if the parental blade is collected and handled correctly and if the discs are used within one to two hours after excision.

On a dry weight basis, highest photosynthetic rates were observed in apical scimitar segments and whole blades (3.81 and 3.07 mgC·g dry $wt^{-1}\cdot h^{-1}$, respectively), followed by sporophylls (1.42 mgC·g dry $wt^{-1}\cdot h^{-1}$) and stipe segments (0.15 mgC·g dry $wt^{-1}\cdot h^{-1}$). No photosynthetic capacity was observed in holdfast material. Dark respiration rates showed similar ranking ranging from 1.22 mgC·g dry $wt^{-1}\cdot h^{-1}$ for apical scimitar to 0.18-0.22 mgC·g dry $wt^{-1}\cdot h^{-1}$ for holdfast material. Tremendous within blade variability in both PS and R was also found. Steepest PS and R gradients on both an areal and weight basis were found within immature blades followed by senescent and mature blade material. Highest PS rates were associated with the blade tips ranging from 6.46-10.09 mgC·g dry $wt^{-1}\cdot h^{-1}$ for immature and mature blades. Highest rates of R generally occurred towards the basal portions of blades and ranged from 1.03-1.80 mgC·g dry $wt^{-1}\cdot h^{-1}$ for immature

blades. The variability within and between blades was high, with coefficients of variation approaching 50%. The observed patterns can be related to the decreasing proportionment of photosynthetic to structural tissue from the blade tip to the blade base. Rates of light carbon fixation revealed similar longitudinal profiles for the different blade types, with the absolute rates being slightly lower, while patterns of dark carbon fixation (DCF) were less easily interpreted. Highest rate of DCF (0.04-0.06 mgC·g dry $wt^{-1} \cdot h^{-1}$) occurred at the basal portions of immature and senescent blades. Longitudinal profiles of total chlorophyll (a + c) on both an areal and weight basis were very similar to the profiles of PS. Normalized to chlorophyll a, PS displayed an unusual longitudinal profile in immature tissue; however, such profiles for mature and senescent tissues were similar to those for PS on an areal basis.

In this study, it can be clearly demonstrated that it is difficult, if not impossible, to select single tissue plugs that are representative of whole blades, as has been done in many recent studies. Furthermore, the observed metabolic longitudinal profiles reveal a characteristic developmental pattern; the previous working definitions of immature, mature, and senescent blades, based on morphology and frond position, have now been shown to have a physiological basis.

Keywords: carbon allocation, dark carbon fixation, kelp, *Macrocystis*, metabolic variability, photosynthesis, respiration.

INTRODUCTION

The giant kelp, Macrocystis pyrifera (L.) C. Ag. is one of the more important primary producers in shallow, hard bottom coastal waters off southern California. Little is known about relationships among growth, development, and productivity of the mature sporophyte. Attempts to relate basic aspects of photosynthetic and respiratory carbon metabolism to growth have been incomplete and very speculative (Sargent and Lantrip 1952, Clendenning 1971, Wheeler 1978). Estimates of Macrocystis photosynthesis (Sargent and Lantrip 1952, Clendenning 1971, Towle and Pearse 1973, Arnold 1980, Willenbrink et al. 1979, Wheeler 1980a) are extremely variable, ranging on a weight basis from 0.11-3.51 $mgC \cdot g$ dry $wt^{-1} \cdot h^{-1}$ and on an areal basis (one side of blade) from 0.004-0.482 $gC \cdot m^{-2} \cdot h^{-1}$. This appears to be a result of both the different sampling and incubation methods employed as well as a reflection of natural intrinsic variability both within fronds and between fronds of different plants.

Measured rates of photosynthesis (PS) and respiration (R) of Macrocystis are dependent upon tissue type (apical scimitar, blades, sporophylls, stipe, holdfast), tissue age, physiological state (nutritional and light history), and physical injury (abrasion, sloughing, epiphytes, etc.). Rates of PS and R have been determined on different tissue types: Sargent and Lantrip (1952), blade and stipe; Clendenning (1971), stipe, pneumatocyst, sporophyll, and blades of various ages; Willenbrink et al. (1979),

young and old blades; and Wheeler (1980a), blades of various ages. In all cases, only "representative" tissue segments (discs) were incubated. To date, only Towle and Pearse (1973) and Arnold (1980) have incubated whole blade samples.

Two factors must be assessed when using tissue discs: the effects of tissue excision (wounding) and variability within a tissue type. None of the previously mentioned authors assessed the possible effects of wounding on photosynthetic and respiratory performance. A possible wounding effect is increased oxygen consumption due to release and oxidation of phenolic compounds (Ragan and Jensen 1977, Sieburth 1968, Dromgoole 1978, Arnold 1980). Hatcher (1977) observed that in freshly cut blade discs of the kelp *Laminaria longicruris*, even after 12 h of acclimation, respiratory rates were 1.8 times that of whole plants; a second effect of cutting was to increase the variability among replicates for both respiration and photosynthesis. Both Hopkins and Kain (1978) and Hawthorne *et al*. (1981) have found similar respiratory increases following wounding of the kelp *Laminaria hyperborea* and the green siphonous alga *Caulerpa simpliciuscula*, respectively.

Although the blades of kelps are the primary site of PS, their large surface area to volume ratio and their high photosynthetic rate make it difficult to incubate the whole blade (or frond) under optimal (Littler 1979) conditions. Hence, many investigators have chosen to conduct physiological measurements on

isolated tissue discs punched from blades. Both Hatcher (1977) and Johnston et al. (1981) have expressed the difficulty in selecting "truly representative" tissue segments from Laminaria sp. blades because of the wide variation of PS and R rates within a thallus. Similar large within-blade variability of photosynthetic performance has been found by Küppers and Kremer (1978) for three additional species of Laminaria. It can therefore be anticipated that sampling problems would be extreme in Macrocystis since large mature plants can bear up to 60 or more fronds, each of which can bear more than 100 blades of various ages (Clendenning 1971).

The blades of Macrocystis, like those of Laminaria species, can be considered "moving belts of tissue" (Mann 1972) because younger tissue is produced at the basal meristem of the blade pushing older tissue distally where it commonly erodes. Light and electron microscopical observations (Parker 1971) have revealed large structural differences (on the basis of cross sections) between the young and old portions of Macrocystis blades. The entire blade is covered with three to eight layers (Parker 1971) of meristodermal cells, which in other kelp species have been shown (Kremer 1980) to contain most of the photosynthetic pigments and carboxylating enzymes. The tissue is thicker near the blade base (Parker 1971) as compared to the apex because of the relative increase in the nonphotosynthetic structural and transportive tissues of the cortex and medulla. Rates of PS and R per unit weight should, therefore, vary down the central axes of a blade,

and these profiles should also be affected by blade age. Clendenning (1964, 1971) was the first to investigate this intrablade variability using what he considered a mature blade. He found a marked increase in PS and R from blade base to blade tip on a weight basis (fresh weight), while on an areal basis, R decreased from base to tip and PS remained relatively constant. Unfortunately, because his methods are not stated in great detail and because in many cases the data are presented on a relative basis, it is difficult to accurately interpret his results.

High rates of light independent carbon fixation or dark carbon fixation (DCF) have been associated with young meristematic tissue of kelps, and as a result, distinctive thallus profiles of DCF and phosphoenolpyruvate carboxykinase (PEP-CK; EC 4.1.1.32) activity, the enzyme responsible for DCF, have been observed for various species of Laminaria (Weidner and Küppers 1973, Küppers and Kremer 1978, Kremer and Markham 1979, Kremer 1979). Blade profiles of Macrocystis DCF have not been determined. Rates of DCF by Macrocystis pyrifera and Macrocystis integrifolia have been determined on isolated discs from mature blades and apical scimitars (Willenbrink et al. 1979).

We feel that much of the previous research on photosynthesis and respiration in the giant kelp Macrocystis has been carried out without adequate consideration of methodological problems associated with (1) incubation techniques, (2) environmental differences, and (3) intrinsic aspects of variability. Thus attempts to model growth and productivity in Macrocystis are severely limited by the lack of

accurate quantitative descriptions of the important aspects of carbon allocation (PS, R, and DCF) for the plant as a whole. We have, therefore, reexamined in greater detail variability in PS, R, and DCF due to tissue type and age, and the intrablade variability (longitudinal profiles) of these metabolic processes. Special attention was given to the methodological problems associated with photosynthetic and respiratory measurements of intact blades and isolated blade discs.

MATERIALS AND METHODS

Experimental material. Fronds of *Macrocystis pyrifera*, representing various stages of maturity, were collected from shallow subtidal habitats (~-10 m relative to mean lower low water) off Corona del Mar, California. Experimental material (whole small fronds, whole blades, and large representative sections of stipe and holdfast) was collected in the morning (9:00-11:00 a.m.) during the summer, fall, and early winter. All material was selected to be relatively free of obvious epiphytes. As suggested by Johnston *et al*. (1981), great care was taken not to expose the thallus material to drastic changes in salinity and temperature, and to protect the tissue from "light injury." All material was transported to the laboratory (within 30 min of collection) in large, light tight, insulated polyethylene containers. Prior to experimentation, material (whole blades and large representative sections of stipe and holdfast) was stored at ambient temperature (17-20°C) and salinity (34.5 o/oo) in either the light (100 $\mu E \cdot m^{-2} \cdot h^{-1}$) for photosynthesis experiments or in the dark for dark carbon fixation and respiration measurement. Preincubation routinely lasted 1 h to reduce the effects of any possible endogenous gas exchange transients (Dromgoole 1979).

Blade types were characterized by appearance and position on nonterminated canopy fronds: juvenile blades - below apical scimitar to 2 m; mature blades - 2 to 5 m from apical scimitar and more richly pigmented as compared to juveniles; senescent blades - greater than 5 m from apex, less pigmented and showing signs of tissue decay; sporophylls - basal blades growing above the

frond initials usually without a pneumatocyst. These terms are meant to be merely operational. More thorough definitions can be found in the excellent review by Lobban (1978).

Incubation procedures. All incubations were performed in large walk-in environmental chambers adjusted to temperatures corresponding to those of collection (17-20°C) which were found to be optimal (Fig.4.1-12A)for mature blade photosynthesis. For photosynthesis experiments, Cool-White fluorescent lights were located perpendicular to the sides and tops of incubation chambers and offered a photon flux density of 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, which was previously determined (Fig 4.1-12B)to be above light saturation for mature blades.

Incubation vessel volume varied (0.08 to 8.5 l) depending on the thallus size, expected incubation time, and metabolic activity according to predetermined linearity runs. For the most metabolically active tissues (mature blades), tissue weight:incubation volume ratios were less than 0.3 g dry wt per liter for 1 h incubations (see Fig. 4.1-13). Incubation times for photosynthesis experiments ranged from 1-2 h while those for dark respiration and dark carbon fixation ranged from 2-4 h. Chambers were stirred constantly throughout the incubation period by magnetic stir bars powered by electric stirrers. Discs were suspended in vessels on brass swivels which allowed free rotation and exposed both sides of the disc to identical light fields while preventing any damaging interaction with the stir bar (see Fig.4.1-14 for details). To minimize heat transfer, cork insulation was provided between the chambers and

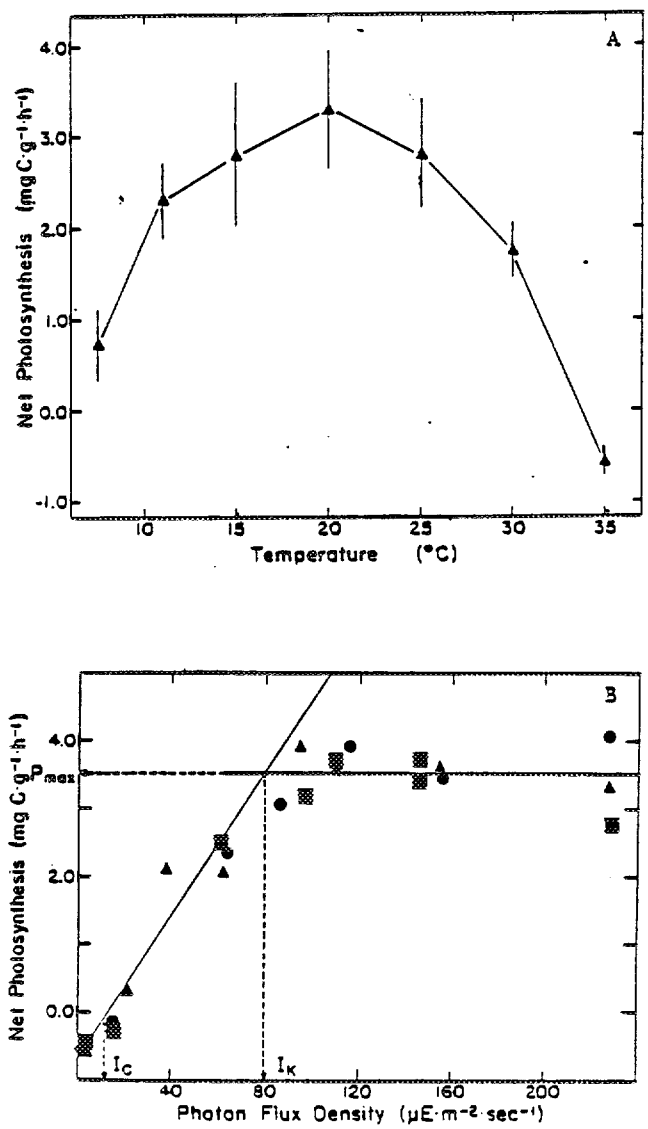

Figure 4.1-12. A. Photosynthesis-temperature relationships for mature blade discs taken 19 cm from the blade base. Means ± standard deviation for three replicates. B. Net photosynthesis-irradiance relationships for mature blade discs (taken as above). Horizontal line drawn through the average rate of net photosynthesis for determinations above 80 $\mu E \cdot m^{-2} \cdot sec^{-1}$. Initial rates determined least-squares linear regression (see Arnold and Murray 1980). Each different symbol represents different blade disc samples. P_{max} = 3.5 mgC·g dry $wt^{-1} \cdot h^{-1}$, compensation intensity (I_c) = 12 $\mu E \cdot m^{-2} \cdot sec^{-1}$, saturation intensity ($I_k$) = 80 $\mu E \cdot m^{-2} \cdot sec^{-1}$.

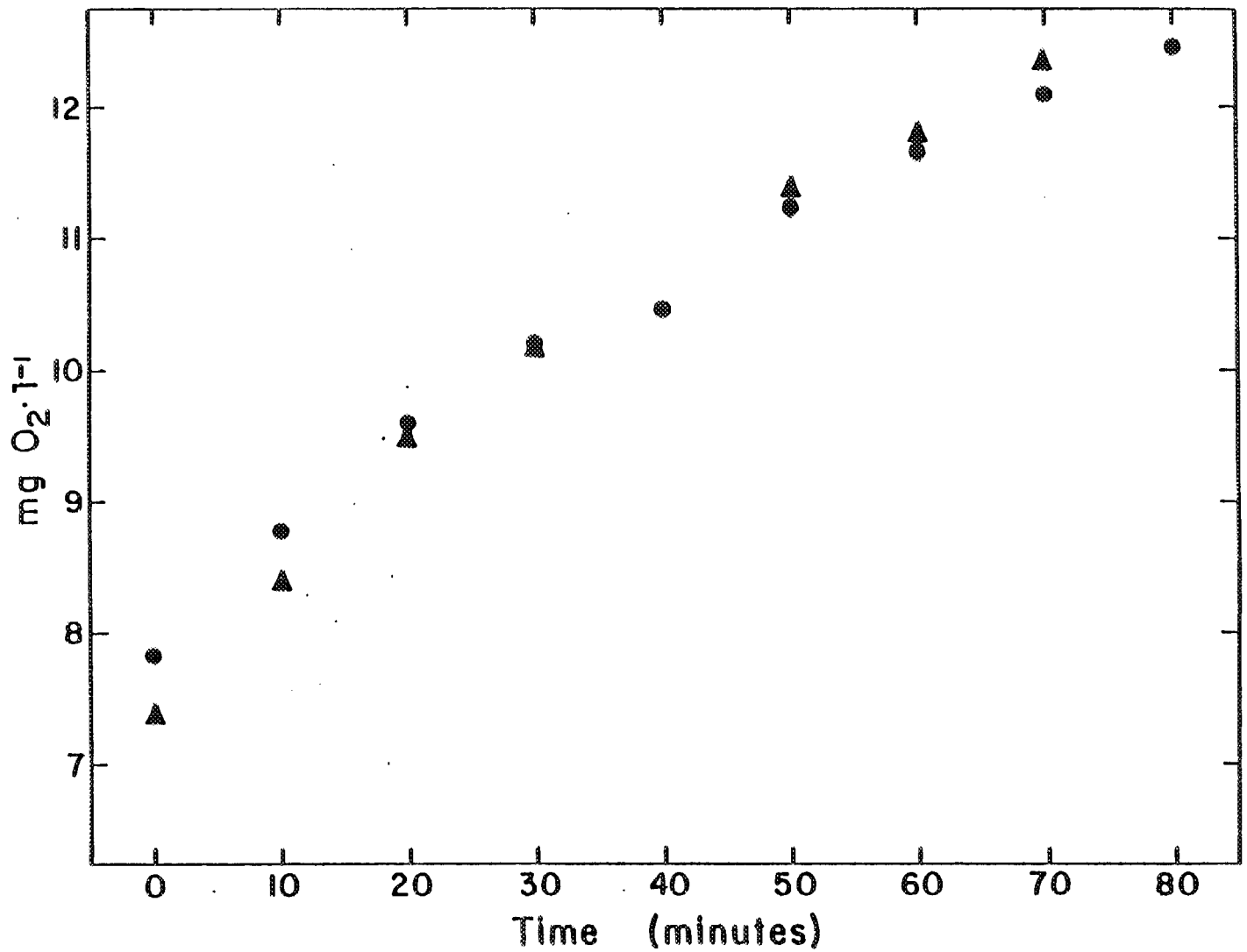

Figure 4.1-13. Net photosynthesis-time course runs for mature blade discs sampled 19 cm above the blade base. Different symbols represent different sample plugs. Disc size was 79.7 cm^2.

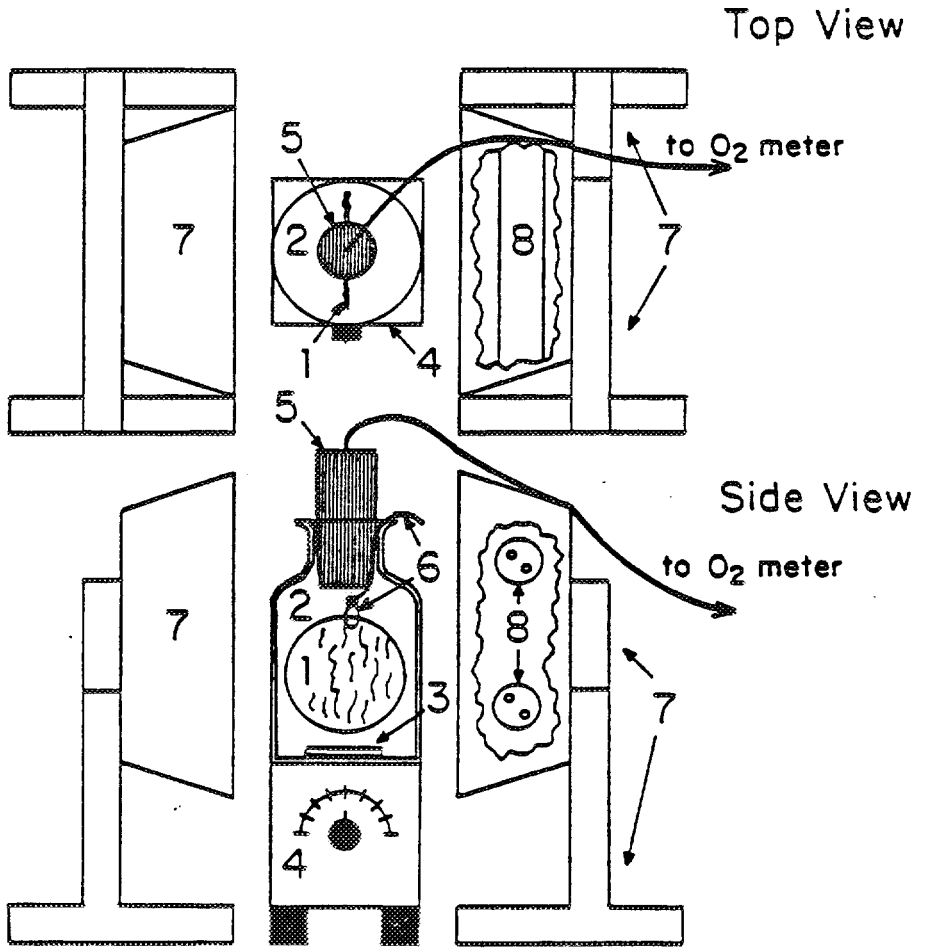

Figure 4.1-14. **Experimental setup for measuring photosynthesis of isolated blade discs.**

stirring motors. All incubations were performed in filtered seawater (Millipore type HA, 0.45 μm) which was collected offshore from the kelp beds and stored in the dark until used. Filtration under vacuum resulted in water being O_2 undersaturated by 10-20%. Therefore, air was bubbled through water 4 h prior to use to insure saturation.

For isolated incubation of blade discs in O_2 experiments, the effects of wounding and preincubation on photosynthesis and respiration were assessed in four different experiments. In the first two experiments, six isolated (10.5 cm^2) discs were each taken down the central axes of two mature blades. One set of six discs was immediately incubated in the light and one set in the dark to measure initial rates of photosynthesis and respiration during a 1 h incubation. Upon termination of incubation, the discs were removed and held under constant conditions of light or darkness for different periods of time and then reincubated for further metabolic measurements. The results from these experiments (Fig 4.1-15) show that both photosynthesis (PS) and respiration (R) decrease with time after initial plug excision with an average decrease of 16% in PS and 53% in R after 50 h. In the next two experiments, 12 discs were each taken from the central portion of 12 different mature blades. Six discs (10.5 cm^2) each were incubated for 1 h to measure PS and R immediately after excision from the parental blade. The parental blades in this and the previously mentioned experiment were preincubated for 1 h to the experimental conditions before plug excision. After the initial PS and R

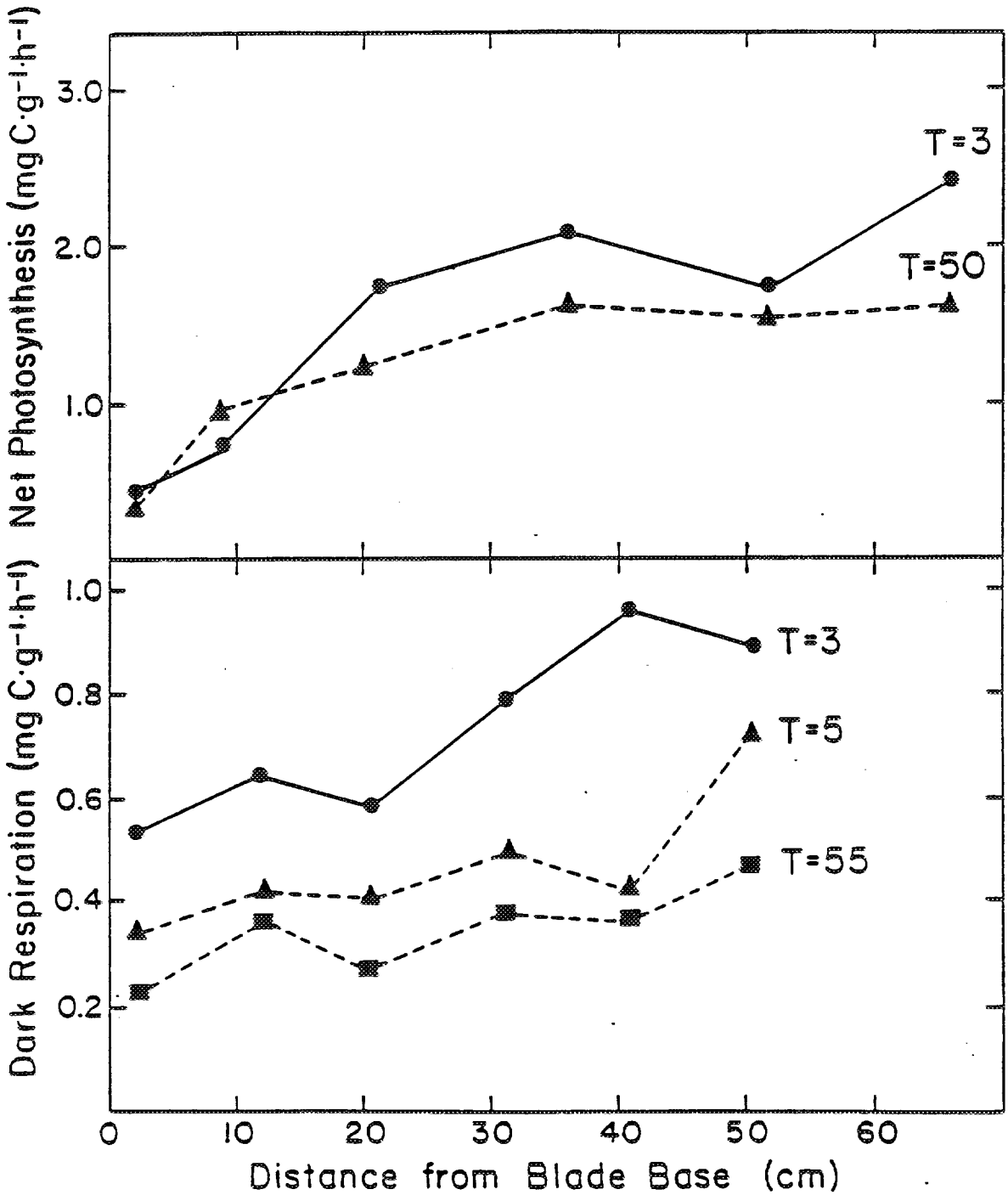

Figure 4.1-15. The effects of wounding on net photosynthesis and dark respiration of mature blade discs taken down the longitudinal axis of a single mature blade. Times are given in hours after the discs were excised from the parental blade. Different blades were used for the photosynthesis and respiration experiments.

readings were recorded, additional wounding was created by slicing the discs six times perpendicularly to the center of the disc. The discs were then reincubated for various periods of time to examine the effects of excessive wounding on PS and R. Between incubations, discs were held in either constant darkness (R) or constant light of 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (PS). Table 4.1-1 shows that excessive wounding of discs had relatively little immediate effect on either PS or R, and only after two days did discs exhibit a large (82%) reduction in photosynthetic capacity. Our overall conclusion, as will be discussed in more detail later, is that tissue discs can be used with confidence during short-term metabolic experiments if they are not preincubated for long periods of time.

Oxygen measurements. Net photosynthesis and dark respiration were measured by assessing changes in dissolved oxygen using the light/dark bottle technique of Gaarder and Gran (1927). Oxygen exchange was monitored using YSI Model 57 oxygen electrodes (Clark-type) and amplifiers using the techniques of Arnold (1980). Electrodes were calibrated in air-saturated distilled water and gave a linear response (Fig 4.1-16) over the range of oxygen tensions encountered. Upon termination of incubation, thalli material was gently removed; dry weights (80°C) and area were calculated from disc diameter or determined with a Li-Cor area meter (Model LI-3100, Lambda Instruments, Lincoln, NE). Calculations of PS and R were done according to the procedures as outlined in Strickland (1960) assuming a PQ (photosynthetic quotient; moles O_2 evolved/moles CO_2 fixed) of 1.0 and a RQ (respiratory quotient; moles CO_2 evolved/moles of O_2 taken up)

Table 4.1-1. The effects of excessive wounding and preincubation on photosynthesis and dark respiration of mature blade plugs in *Macrocystis pyrifera*. Mean values of 6 replicates ±95% confidence limits.

	$mgC \cdot g^{-1} \cdot h^{-1}$	% Difference	$gC \cdot m^{-2} \cdot h^{-1}$	% Difference
Photosynthesis				
Before excessive wounding	2.66±0.31	0	0.192±0.021	0
Immediately after excessive wounding	2.87±0.72	+8	0.200±0.044	+4
2 days later	0.49±0.10	-82	0.036±0.008	-81
Respiration				
Before excessive wounding	0.719±0.062	0	0.034±0.002	0
Immediately after excessive wounding	0.612±0.037	+17	0.029±0.001	+17

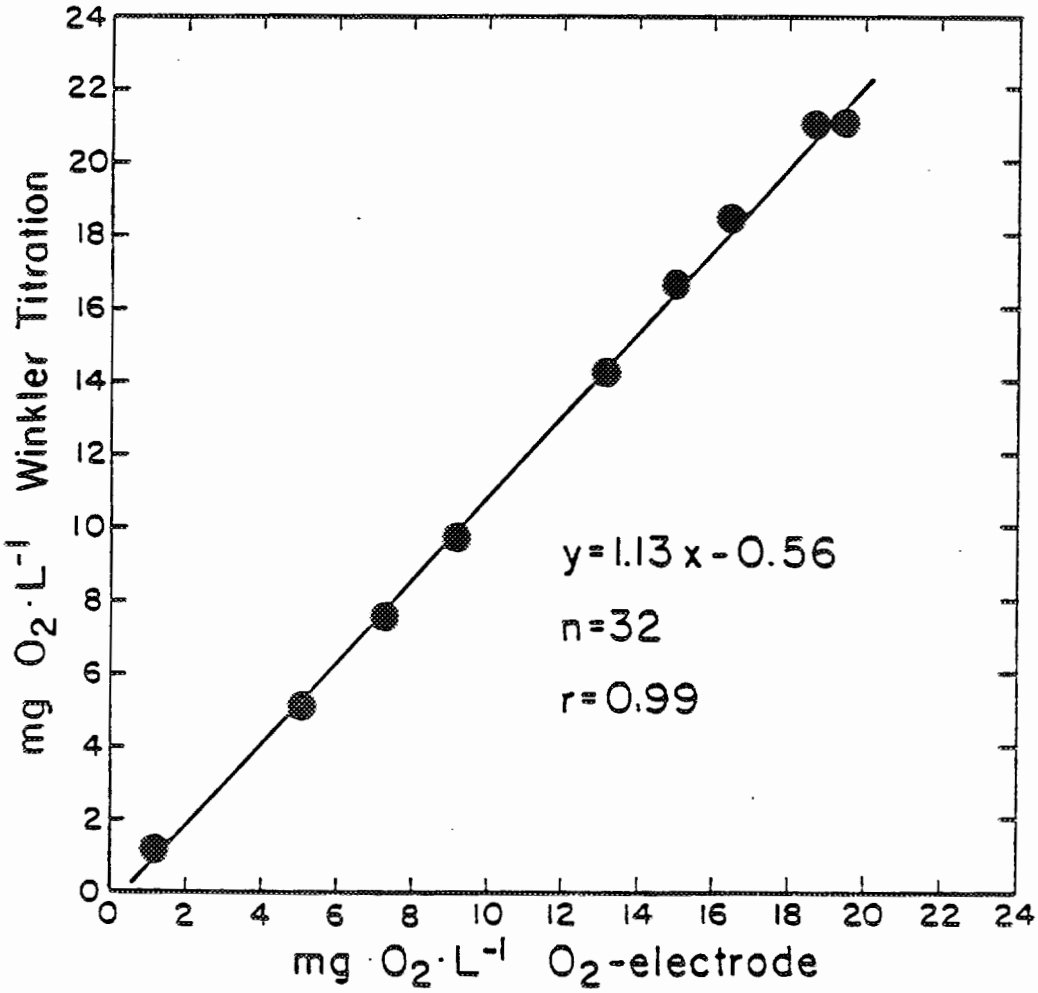

Figure 4.1-16. A standard calibration curve showing the linearity of O_2 measurements using our YSI Model 57 oxygen analyzer calibrated according to the manufacturer's recommended method using air-saturated distilled water. High O_2 tensions were produced by bubbling seawater with pure oxygen for short time intervals. Lower O_2 tensions were produced by degassing under vacuum or bubbling with nitrogen gas. The linear regression equation ($P = 1.13x-0.56$) for $n = 32$ had an r value of 0.99.

of 1.0 to facilitate interconvertibility in comparisons with other studies. Hourly metabolic rates were normalized to dry weight, m^2 of thallus area (one side), and in some experiments to mg chl*a*.

^{14}C-Uptake measurements. Rates of carbon fixation were measured in the light and dark on whole incubated blades (with pneumatocysts removed) of various ages. Generally, techniques were similar to those of Wassman and Ramus (1972) and Brinkhuis and Jones (1974) with specific modifications as discussed below.

Each incubation vessel was innoculated with 10-50 µCi of $^{14}C\text{-}NaHCO_3$ (New England Nuclear; specific activity, 6.2 mCi/m mol). After introduction of the ^{14}C, the medium was stirred for at least two min, and three initial 0.5 ml activity samples were pipetted into 22 ml low potassium, borosilicate glass vials containing 3 ml of the CO_2-trapping agent NCS (Amersham). Contents were gently mixed, followed by addition of 10 ml of Aquasol II (New England Nuclear) and further mixing. Preliminary experiments found this trapping agent/cocktail mixture superior to that proposed by Iverson *et al*. (1976) since we have observed excessive precipitation at larger sample/cocktail ratios with this latter technique, while trapping efficiency was similar. Blanks of stock $^{14}C\text{-}NaHCO_3$ solutions were checked for organic ^{14}C contamination by acidifying subsamples to pH 2.0 with HCl, followed by aeration. In all cases, no significant acid-stable ^{14}C-label (Williams *et al*. 1972) was detected. All vials were dark adapted at 40°C for 24 h prior to counting to avoid problems (Peng 1981) of chemical and photoluminescence.

Counting efficiency as determined from quench curves using the external standard channels-ratio method (Peng 1981) routinely ranged between 82-87%.

Total available "CO_2" was determined using the methods of Strickland and Parsons (1972), following the recommendations of Smith and Kinsey (1978) for pH measurement in seawater.

After incubation, thalli were washed for several minutes in filtered unlabeled seawater. Tissue samples (1.4-79.7 cm^2) were taken down and across major blade axes with metal cork borers or baking cutters. Contiguous samples were also taken for dry weight determinations. Incubated stipe and holdfast tissues were sectioned longitudinally with one half being used for dry weight/fresh weight determination and the other for extractions of activity.

Subsampled tissues were exhaustively extracted with 80% ethanol acidified with 2N HCl to remove any residual cell wall bound $H^{14}CO_3^-$ (Willenbrink et al. 1979). Ethanol insoluble material was solubilized using the procedure of Gagne et al. (1979) modified by Manley (1981). Preliminary experiments comparing the techniques of Lobban (1974) and Gagne et al. (1979) are given in Table 4.1-2. Even though the method of Lobban offers a 2% higher recovery efficiency, we preferred the method of Gagne et al. This latter method visually offers much more complete digestion, lower chemiluminescence, and higher counting efficiency. Both ethanol soluble and insoluble concentrates were corrected for quench (ESCR) and background and added to obtain the

Table 4.1-2. A comparison of solubilization techniques for determining total carbon fixation in kelp tissues.

Method	DPM (X 10^5)		Counting efficiency	Percent recovery[c]
	Fractionated digest[a]	Total digest[b]		
Lobban	8.7±0.3	8.1±0.4	60.0±2.0	93
Present	8.2±0.4	7.5±0.3	89.0±0.3	91

[a]Fractionated into ETOH soluble and insoluble.

[b]Tissue not fractionated but subjected to digestion.

[c]Percent recovery = total digest/fractionated digest.

total net particulate ^{14}C-fixed. Calculations of net particulate carbon fixation (Peterson 1980) and dark carbon fixation followed those as outlined in Strickland and Parsons (1972) and were normalized to g^{-1} dry wt and m^{-2} of thallus area (one side).

Estimates of excreted ^{14}C-DOM were obtained for whole blades and isolated blade discs using the technique of Smith (1975). After incubation, 100 ml of the remaining seawater was filtered and acidified to pH 2.5 to 3.0 with concentrated HCl and bubbled with N_2 for at least 10 min. Preliminary experiments showed this was sufficient time to remove all inorganic ^{14}C as $^{14}CO_2$ gas. Replicate 3.0 ml subsamples of this acid-stable label were added to 15 ml of Aquasol, dark adapted, and counted for at least 20 min and corrected for quench (ESCR). Rates of excretion were expressed as a percent of the corresponding light carbon fixation rates.

Pigment analysis. Chlorophylls a and c were determined by the method of Duncan and Harrison (1982). Tissue discs were placed in dimethylsulfoxide (spectrophotometric grade) at 5-7 $ml \cdot g$ wet wt^{-1} or 0.29 $ml \cdot cm^{-2}$ and stirred by placing on a rotary shaker for 5 min after which they were added to absolute MeOH (14 $ml \cdot g$ wet^{-1} or 0.83 $ml \cdot cm^{-2}$) and stirred for 20 min. All extractions were performed in a darkened room.

RESULTS

Net photosynthesis and respiration of isolated frond parts. Estimates of net photosynthesis (PS) and dark respiration (R) for various thallus parts are given in Figure 4.1-17. On a dry weight basis, highest rates of PS were observed in apical scimitar segments ($\bar{x}$ = 3.81 mgC·g dry wt^{-1}·h^{-1}) and whole blades (3.07 mgC·g dry wt^{-1}·h^{-1}), which make up the bulk (Lobban 1978) of the photosynthetic portions of fronds. Surprisingly, sporophylls, which are most often located at the base of fronds, had appreciable photosynthetic capacity (1.42 mgC·g dry wt^{-1}·h^{-1}). Stipe segments, sampled throughout a mature frond, exhibited little net photosynthesis (0.146 mgC·g dry wt^{-1}·h^{-1}), while young lightly pigmented portions of holdfast material showed no photosynthetic capacity. The same general patterns of PS are observed when the data are calculated on the basis of surface area.

Dark respiration rates showed similar but less pronounced differences among the various thallus portions. Highest R was found in apical scimitars (1.22 mgC·g dry wt^{-1}·h^{-1}), while blade and sporophyll tissues exhibited lower values (0.70 and 0.64 mgC·g dry wt^{-1}·h^{-1}, respectively). Although stipe and holdfast tissue showed only 1/4 to 1/5 of the respiration of blades, the respiratory capacity of these thallus portions integrated over an entire frond can be considerable.

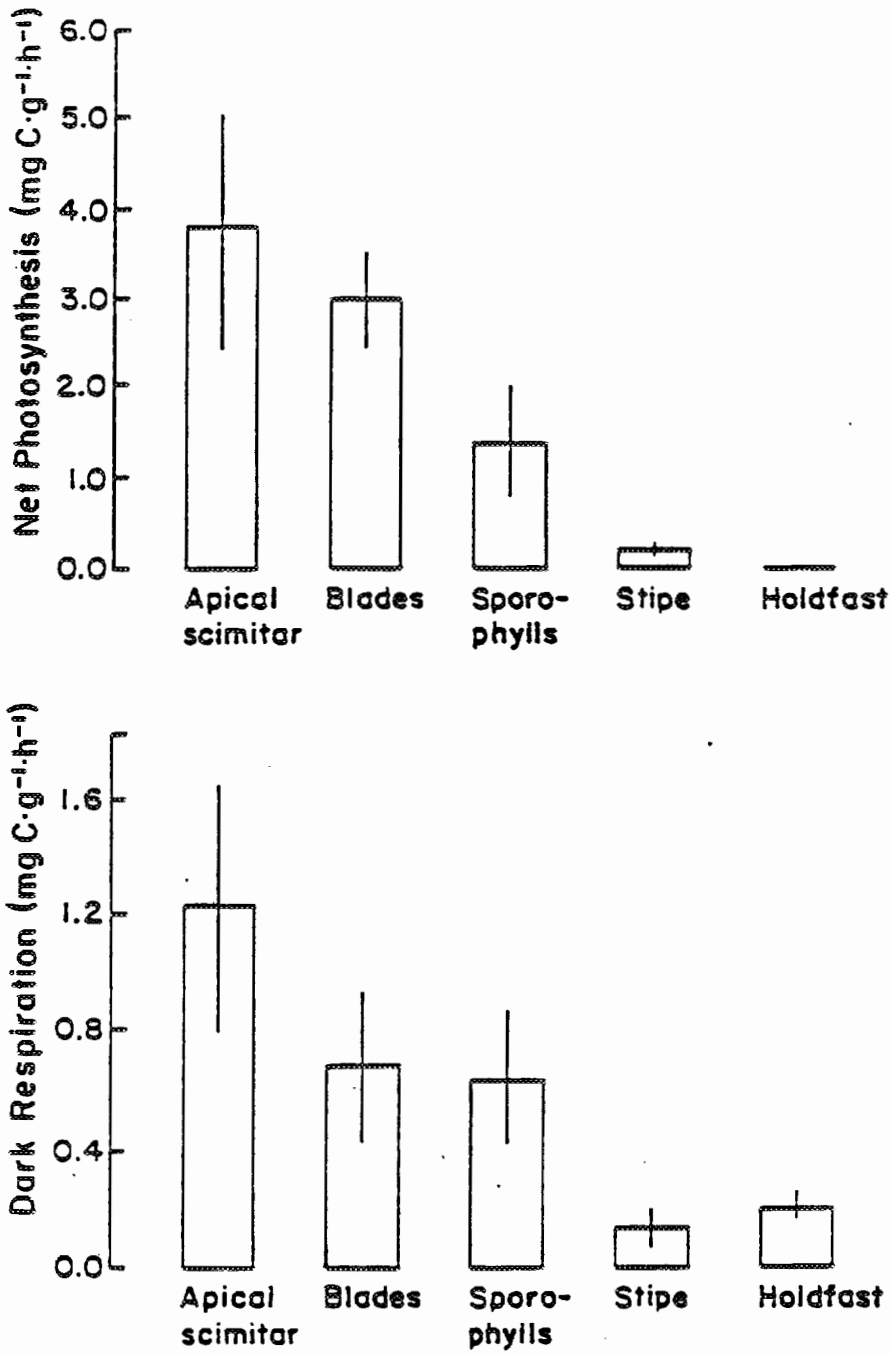

Figure 4.1-17. Net photosynthesis and dark respiration of various thallus parts. Descriptions follow those as given in Lobban (1978). Whole blades were incubated without pneumatocysts. Stipe and haptera were sampled from two separate fronds and holdfasts, respectively. Values represent means of at least six replicates ± their standard deviations.

Within blade variability of PS and R. Tremendous within blade variability in both PS and R was found (Figs 4.1-18 & 4.1-19) in different aged blades. Steepest photosynthetic gradients on both an areal and dry weight basis (Fig 4.1-18) were seen in immature blades followed by mature and senescent blades. Highest PS rates within immature (4.27-10.09 mgC·g dry $wt^{-1} \cdot h^{-1}$) and mature (2.54-6.46 mgC·g dry $wt^{-1} \cdot h^{-1}$) blades were associated with the terminal portions of blades. In the case of senescent blades, the tips were almost always highly abraded. These senescing tissues generally represented dying portions of the thallus and, consequently, exhibited much lower photosynthetic activity (0.16-1.74 mgC·g dry $wt^{-1} \cdot h^{-1}$). Overall within blade differences in PS on an areal basis were much less pronounced because, as Parker (1971) has shown, there is less difference in the thickness of the meristoderm (the outer photosynthetic tissues) from the tip to the blade base than in the thickness of the cortex and medulla (the relatively nonphotosynthetic inner supportive and conducting tissues). The cortex and medulla represent the bulk of the internal blade biomass in the basal portions of the blade and result in higher thallus densities (g dry wt/cm^2) than those found at the terminal ends.

The average coefficient of variation (C.V.) in the PS rates of a single plug taken from any position along the main blade axis was found to be highest for immature and senescent blades (49 and 44%, respectively) and slightly lower for mature blades (39%). Clearly, it is difficult, if not impossible, to select a single tissue plug that is "representative" of the blade as a

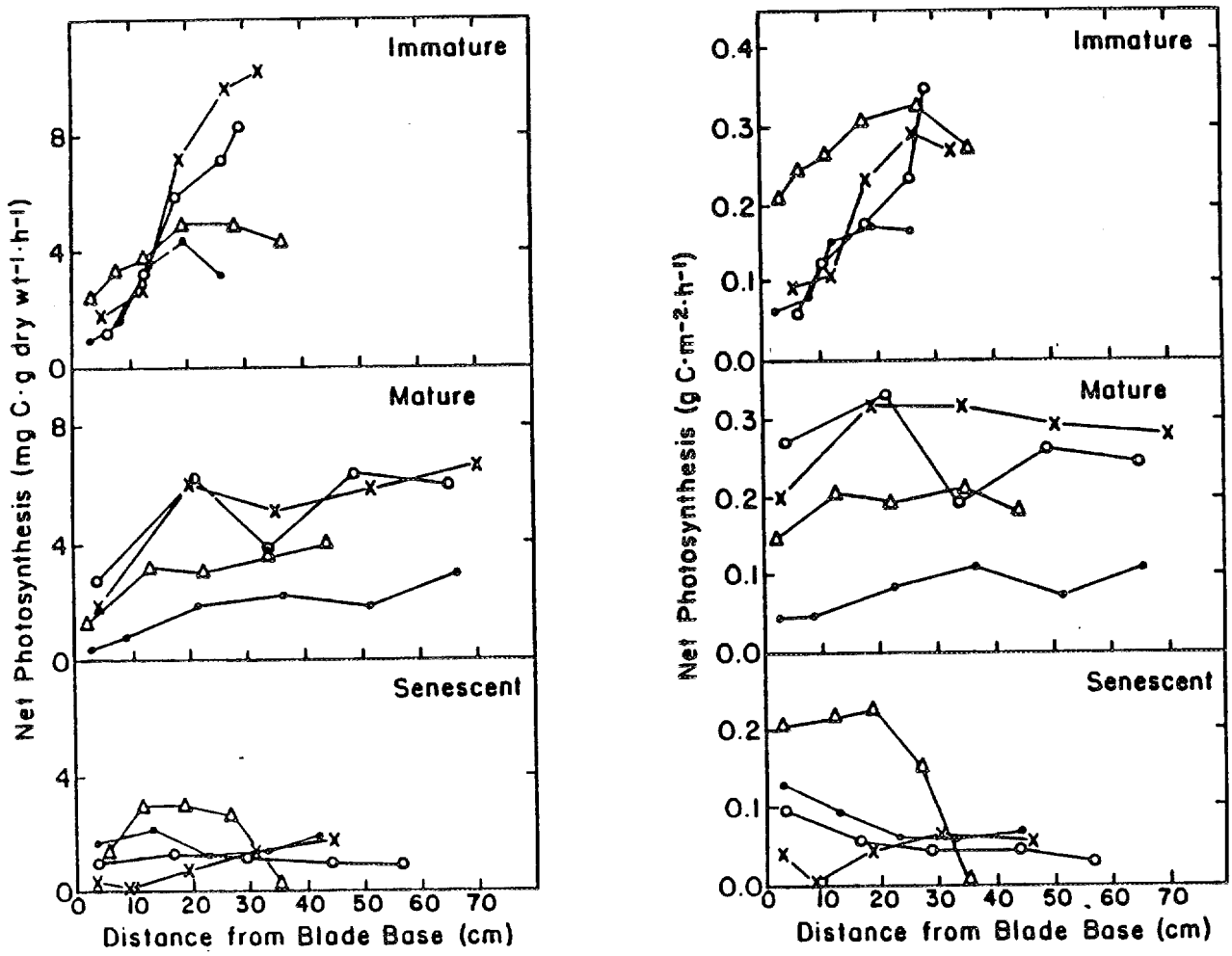

Figure 4.1-18. Longitudinal profiles of dry weight and area-based net photosynthesis determined from O_2-evolution experiments. Blades were sampled in late fall and early winter. Different symbols represent discs taken from different blades. Blades were grouped into immature, mature, and senescent based on the definitions given in the materials and methods section.

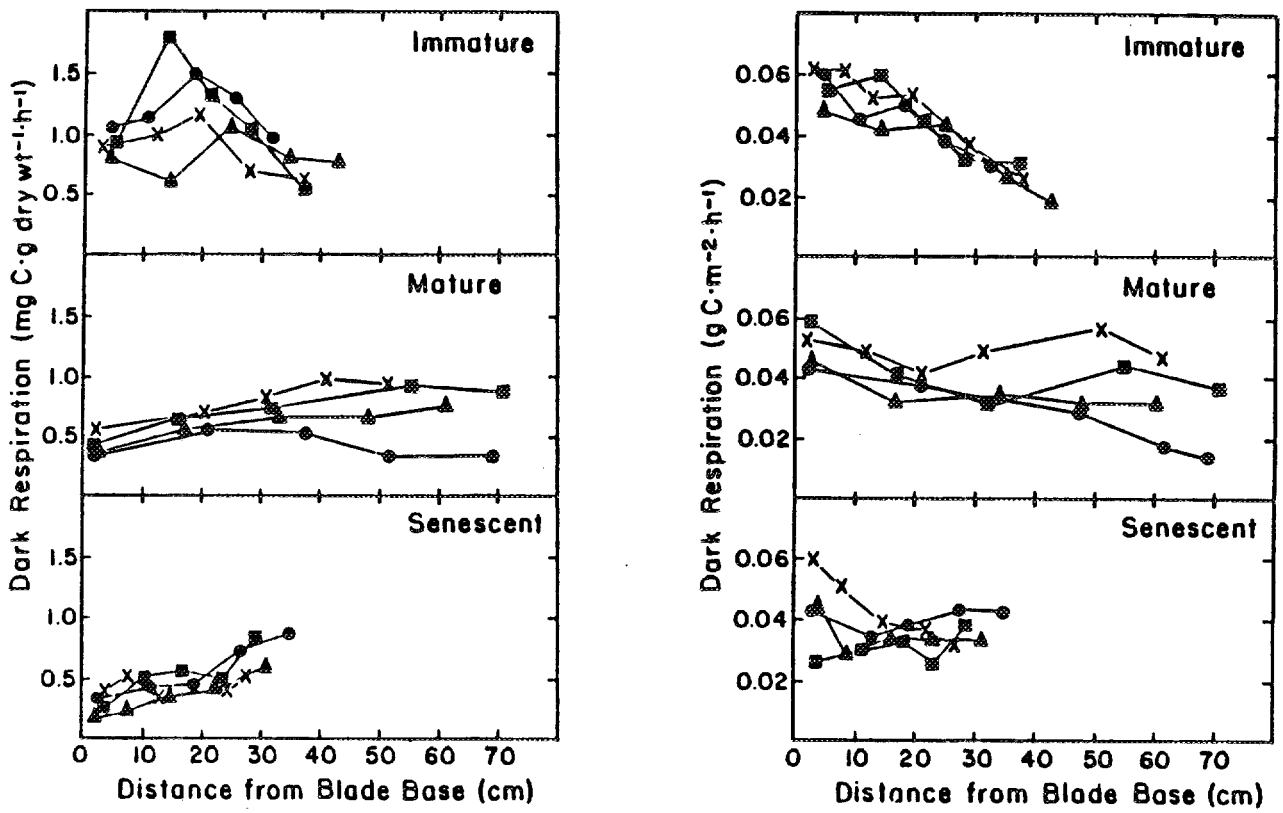

Figure 4.1-19. Longitudinal profiles of dry weight and area-based dark respiration determined from O_2-uptake experiments. Details the same as in Figure 4.1-18

whole. To illustrate this point further, six single discs were taken (19 cm from the blade base) from six different mature blades (of the same length) from different fronds and incubated in one-hour PS experiments. The PS rate ranged from 1.56 to 2.98 mgC·g dry $wt^{-1} \cdot h^{-1}$ with an average of 2.52 mgC·g dry $wt^{-1} \cdot h^{-1}$ and a C.V. of 20.4%. Thus even when identical areas or nearly identical types of blades are incubated, variability is still high.

Profiles of R (Fig 4.1-19) displayed a more variable pattern. Highest respiratory activity generally occurred towards the basal portions of all blades on an areal basis, and these patterns were found to be much more consistent for immature blades. Less clear-cut profiles were observed when R rates were expressed on a dry weight basis. For immature blades, highest rates (1.03-1.80 mgC·g dry $wt^{-1} \cdot h^{-1}$) occurred between the mid and basal portions of the blades while the pattern was somewhat reversed in mature and senescent blades. Overall blade respiration was highest in immature blades followed by mature and senescent blades. Within blade variability of R was less than that observed for PS, with the C.V. for single plugs ranging from 26% for juvenile and mature blades to 31% for senescent blades.

Light and dark fixation of carbon. Light carbon fixation (LCF) experiments (Fig 4.1-20A) revealed longitudinal profiles for the different blade types (collected from different fronds) that were almost identical to those observed for O_2-evolution (Fig. 4.1-18) experiments. The absolute rates along blade axes were somewhat

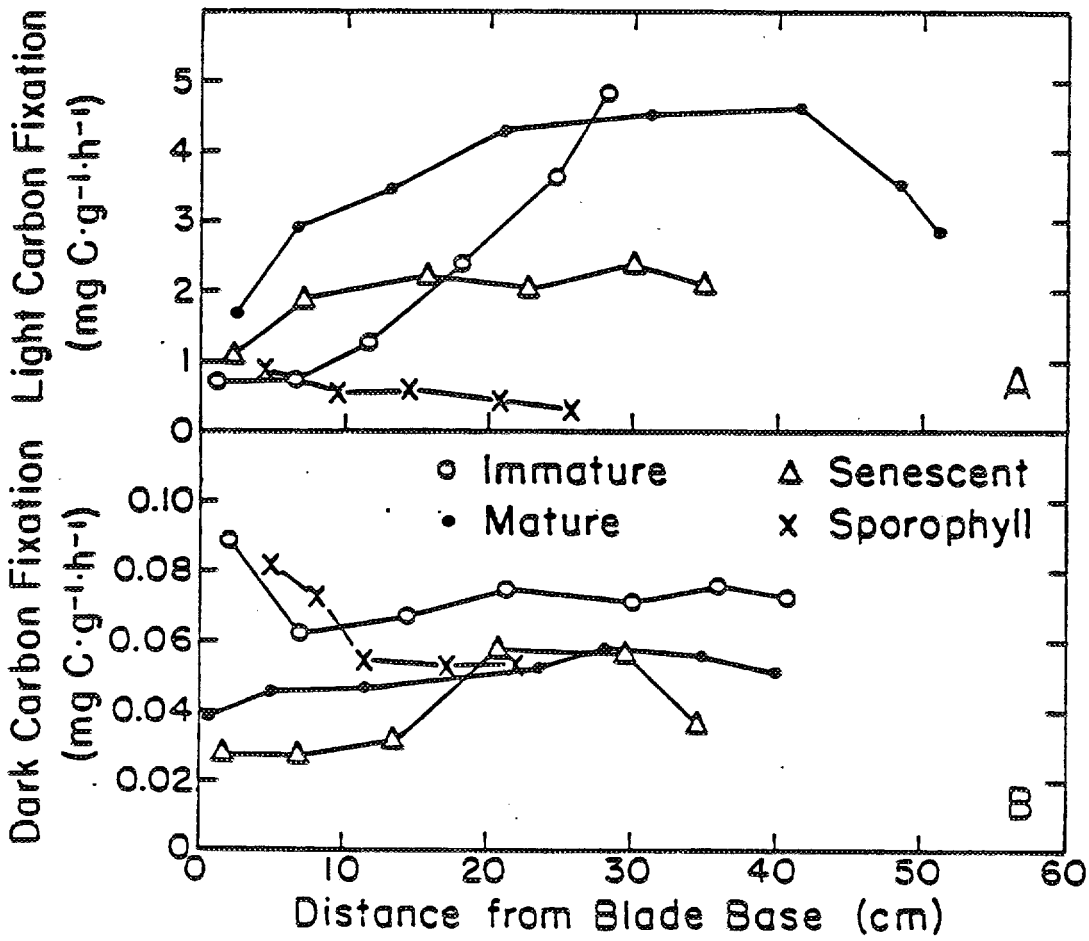

Figure 4.1-20. Longitudinal profiles of light (LCF) and dark (DCF) carbon fixation. Different symbols reflect different types of blades. Blades sampled in late summer.

lower but well within the range of those found in O_2 experiments where a photosynthetic quotient of 1.0 was assumed in calculations. Simultaneous experiments of O_2 evolution and ^{14}C uptake in the light were conducted on mature and immature blade discs (44 cm^2), and the resultant PQs were 1.5±0.3 and 1.1±0.1, respectively ($p = 0.05$, $n = 5$). Using the PQ value of 1.5, the O_2-evolution rates (Fig 4.1-18) would be reduced by 30% and much closer to those values observed in Figure 4.1-20A for LCF rates.

Irregular patterns of dark carbon fixation (DCF) were revealed for the four types of blades as seen in Figure 4.1-20B Overall, highest rates of DCF (0.08-0.09 mgC·g dry $wt^{-1} \cdot h^{-1}$) were seen in the basal portions of immature and senescent blades, while lowest within blade rates were found in these regions of mature and senescent blades. Only in the basal portions of immature and senescent blades do rates of DCF approach 10-15% of the corresponding LCF rates. In other blade types and positions, DCF values are normally much less than 4% of the LCF values.

Transverse patterns of LCF (Fig. 4.1-21) within different aged blades reveal a much more uniform photosynthetic response across the blade as opposed to down the major blade axes. Some slight variability is apparent, and this can be a reflection of minor differences in specific blade weight across this axis as well as abrasion at sides of blade tips (see mature blade data, Fig. 4.1-21).

Obtaining blades of the same precise age is a difficult sampling problem. In the previous experiments, individual

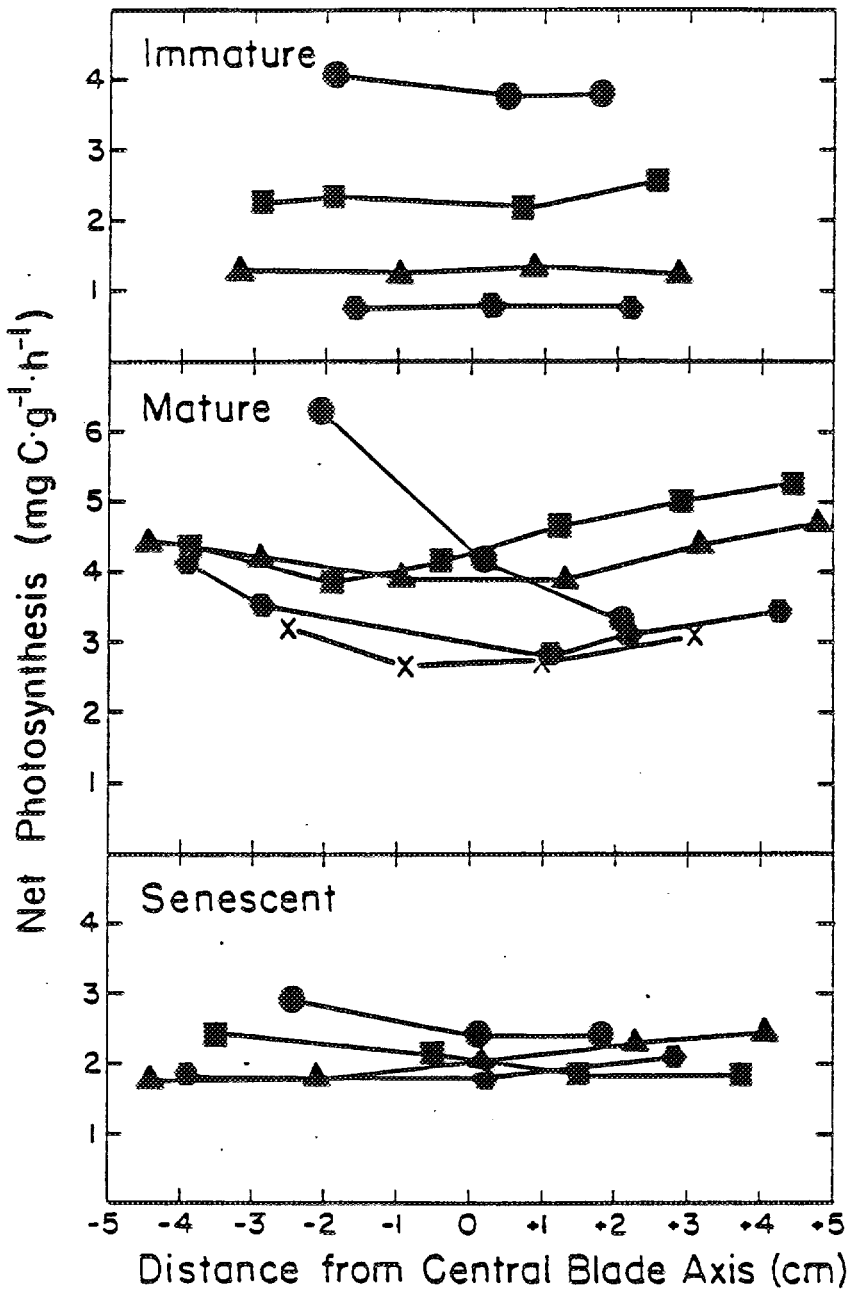

Figure 4.1-21. Transverse profiles of LCF across different aged blades. Different symbols represent discs taken at different positions across the blade with respect to the blade base. Order is as shown for immature plugs with circles representing discs close to the tip and hexagons representing discs close to the base of the blade. Disc size was 1.4 cm^2.

blades were taken from randomly sampled canopy fronds because our primary aim was to examine the variable nature of metabolism both within blades and between blades on different fronds. In our final LCF experiments, we chose to examine whether similar profiles and variability exist within and between blades sampled from the same frond. We selected a subcanopy frond (with a total of 14 blades) from a shallow-growing plant and incubated blades of various ages obtained from the entire frond length. The longitudinal profiles (Fig 4.1-22) were quite comparable to those shown in Fig. 4.1-20 for randomly sampled blades. The absolute rates, however, were about 30% greater than those observed previously and were probably a result of the overall immature nature of the frond and most of its blades.

Rates of LCF on isolated holdfast and stipe tissue (Fig. 4.1-23) were considerably higher than the corresponding PS values obtained from O_2-evolution experiments (Fig 4.1-17). LCF values of the combined ages of stipe were 0.65 mgC·g dry $wt^{-1} \cdot h^{-1}$, which was over four times higher than the corresponding PS measurements. Holdfast material exhibited no O_2 evolution (Fig 4.1-17) and had relatively insignificant rates (0.05 mgC·g dry $wt^{-1} \cdot h^{-1}$) of LCF. Immature stipe had significantly higher rates of LCF than did immature or mature samples, which were not significantly different from each other. Dark carbon fixation of stipe and holdfast tissue (Fig. 4.1-23) was in the same general range (0.02-0.07 mgC·g dry $wt^{-1} \cdot h^{-1}$) as that found along the longitudinal axes of different aged blades (Fig. 4.1-19). Immature stipe material had significantly higher rates

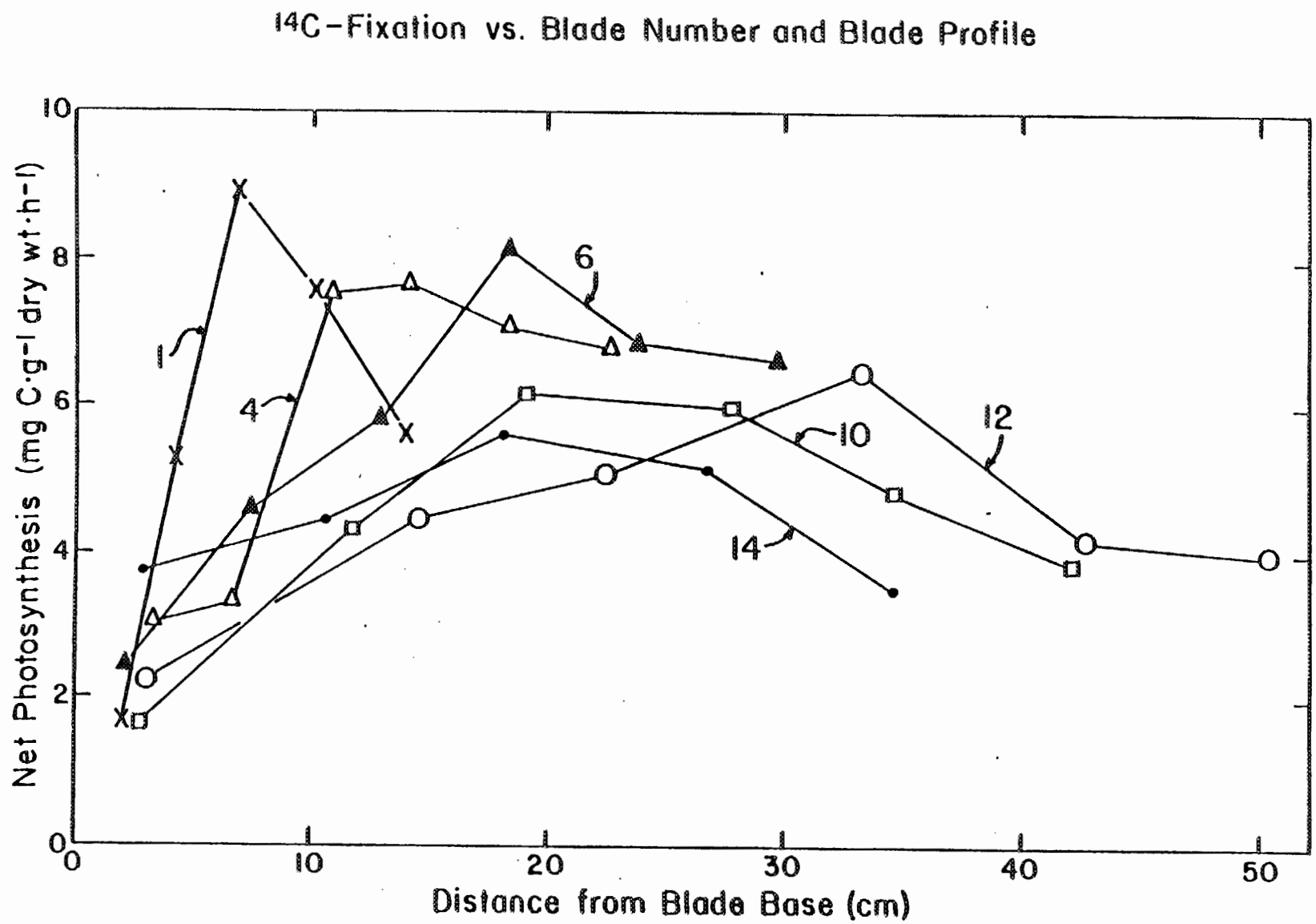

Figure 4.1-22. Longitudinal profiles of LCF for different aged blades taken from the same juvenile frond. Numerals indicate blade number on frond from apex.

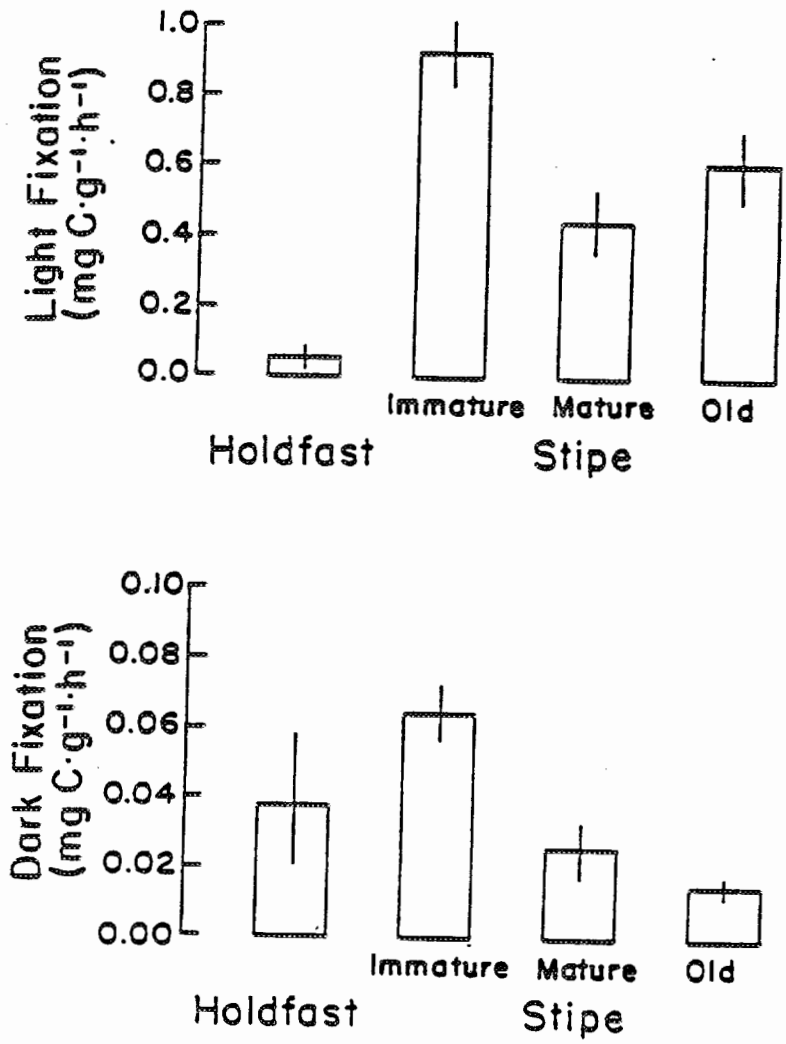

Figure 4.1-23. Rates of light and dark carbon fixation in holdfast and stipe samples of different age. Values represent means ± standard deviations of three replicates.

of DCF than did either mature or old stipe material. The young growing tips of holdfast haptera had moderately high values (0.04 mgC·g dry $wt^{-1} \cdot h^{-1}$) of DCF and were 65% of the corresponding LCF rates.

Loss of ^{14}C-DOM (acid-stable label) during the incubation period was minimal in all experiments conducted. Incubation of isolated mature blade plugs (40.5 cm^2) during short-term labeling experiments revealed less than 5±0.2% of the total net particulate carbon fixed was either excreted or leaked into solution. Incubation of whole isolated blades showed an average excretion rate of only 3.4% (range 1.8-6.5, n = 5) of the corresponding LCF rates.

Chlorophyll profiles and photosynthesis normalized to chlorophyll a. Longitudinal profiles of total chlorophyll (chl*a* + *c*) on a dry wt and areal basis were distinctive for each blade type (Fig. 4.1-24). The more informative profiles are those on an areal basis because the bulk of the chloroplasts are contained in the meristoderm. The most immature tissue displayed a characteristic decline in total chlorophyll from 0 to 10 cm from the base followed by a rapid increase towards the tip. Mature tissue showed a relatively constant chlorophyll concentration base to tip. Senescent tissue displayed no consistent profile because of the randomness of decay. In general, total chlorophyll concentrations ($\mu g \cdot cm^{-2}$) were highest in senescent blades and lowest in immature blades. Chlorophyll *a*:*c* ratios were constant throughout the length of both mature and senescent blades (Fig. 4.1-25). Immature blade profiles of chlorophyll *a*:*c* ratio consistently formed a peak approximately two-thirds from base

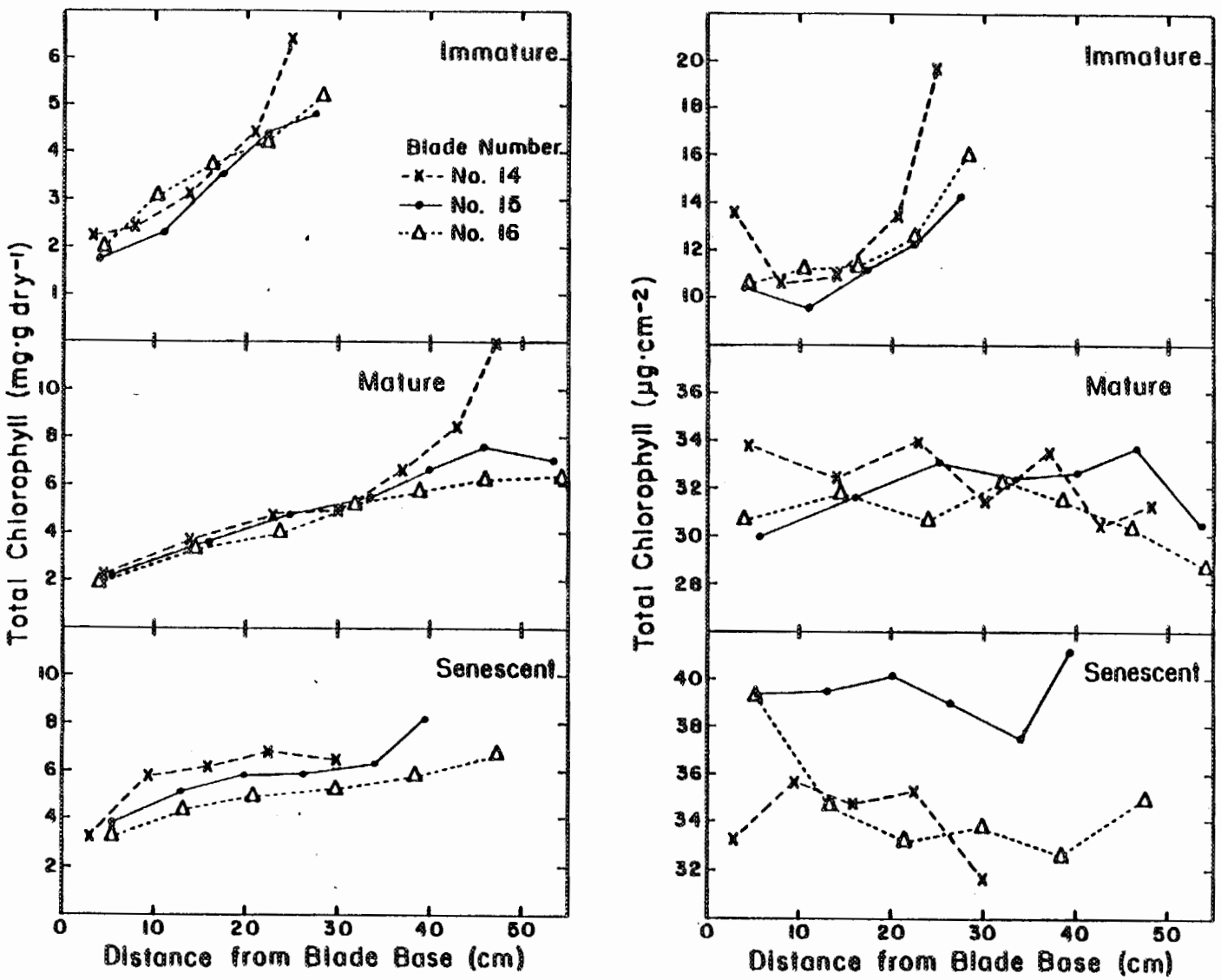

Figure 4.1-24. Longitudinal profiles of total chlorophyll (chl*a* and *c*) for different aged blades on the bases of dry weight and area. Each different symbol represents plugs sampled from different

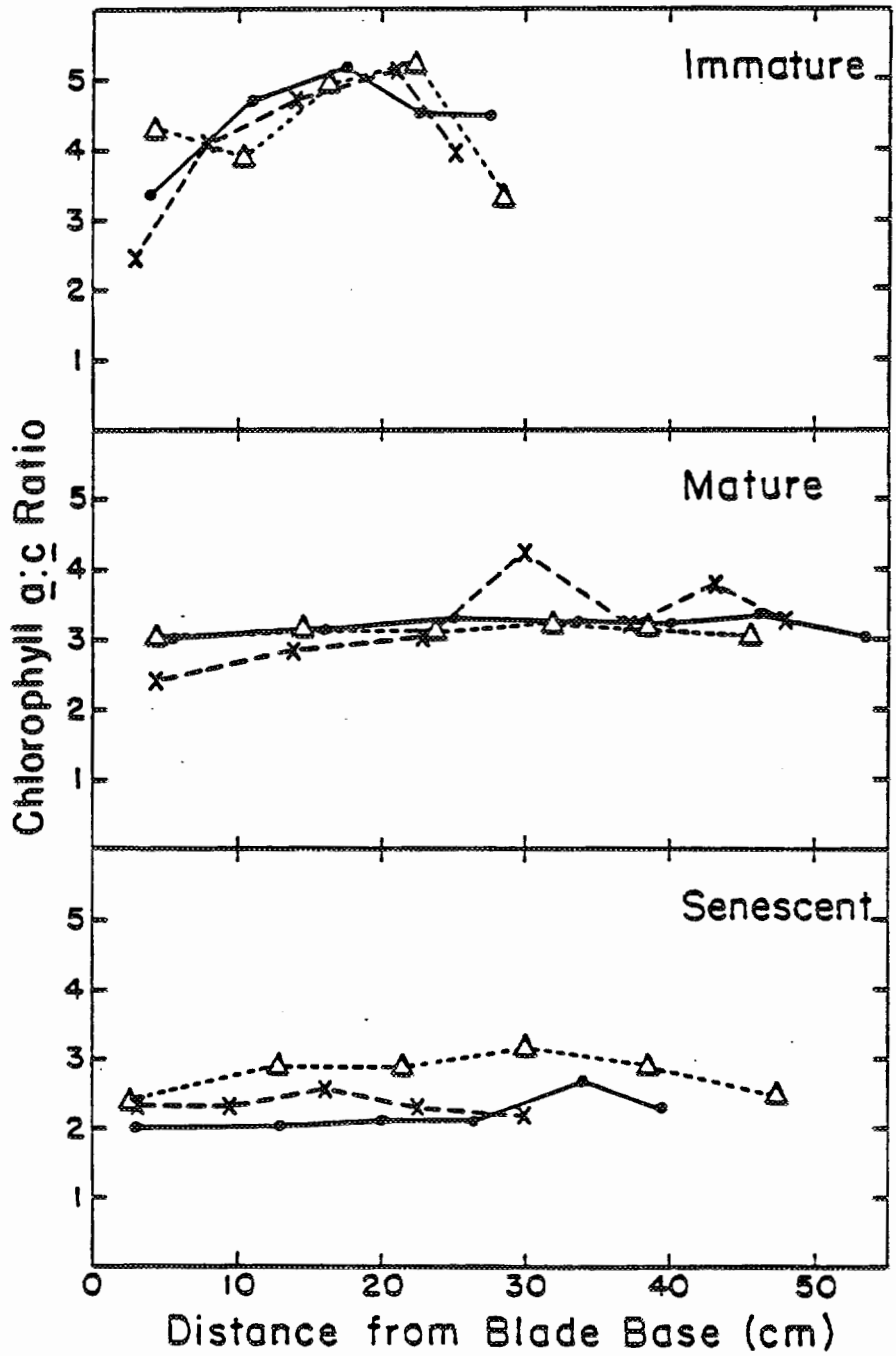

Figure 4.1-25. Longitudinal profiles of chl*a*:chl*c* ratios as a function of blade age. Data are for those blade profiles presented in Figure 4.1-24

to tip (Fig. 4.1-25). This increase in the ratio was due to the greater increase in chlorophyll a as compared to chlorophyll c. Immature blades had the highest chlorophyll a:c ratio; senescent blades, the lowest.

Simultaneous measurements of PS and chla concentrations were conducted on representatives from the three blade age classes (Fig. 4.1-26). For clarity, only single sets of data are shown (with replicates, identical patterns were observed). Within immature blades, the close correlation of PS with both area and weight as PS decreases down the blade axes was consistently encountered as has been shown in other (Fig 4.1-18) experiments. Photosynthesis normalized to chlorophyll a was fairly consistent at the basal and terminal ends of the blades but exhibited a precipitous bulge in the center of the blade. This bulge was characteristic of all our replicates. Overall chla-based PS rates in immature blades were higher (1.44-2.05 $mgC \cdot mg\ chla^{-1} \cdot h^{-1}$) than mature (1.05-1.44 $mgC \cdot mg\ chla^{-1} \cdot h^{-1}$) or senescent (0.47-0.95 $mgC \cdot mg\ chla^{-1} \cdot h^{-1}$) tissues. In both mature and senescent blade profiles, however, chlorophyll a-based PS was found to co-vary with those rates based on area, increasing from the blade tip to the blade base. while weight-based PS rates exhibited the opposite trends.

Again, it is apparent that "representative" plugs are difficult to sample because of the within and between blade variability. Immature and senescent blades display the greatest variability in chlorophyll content, chlorophyll a:c ratio, and PS even when expressed on an areal basis. This is also true when PS is normalized to chlorophyll a.

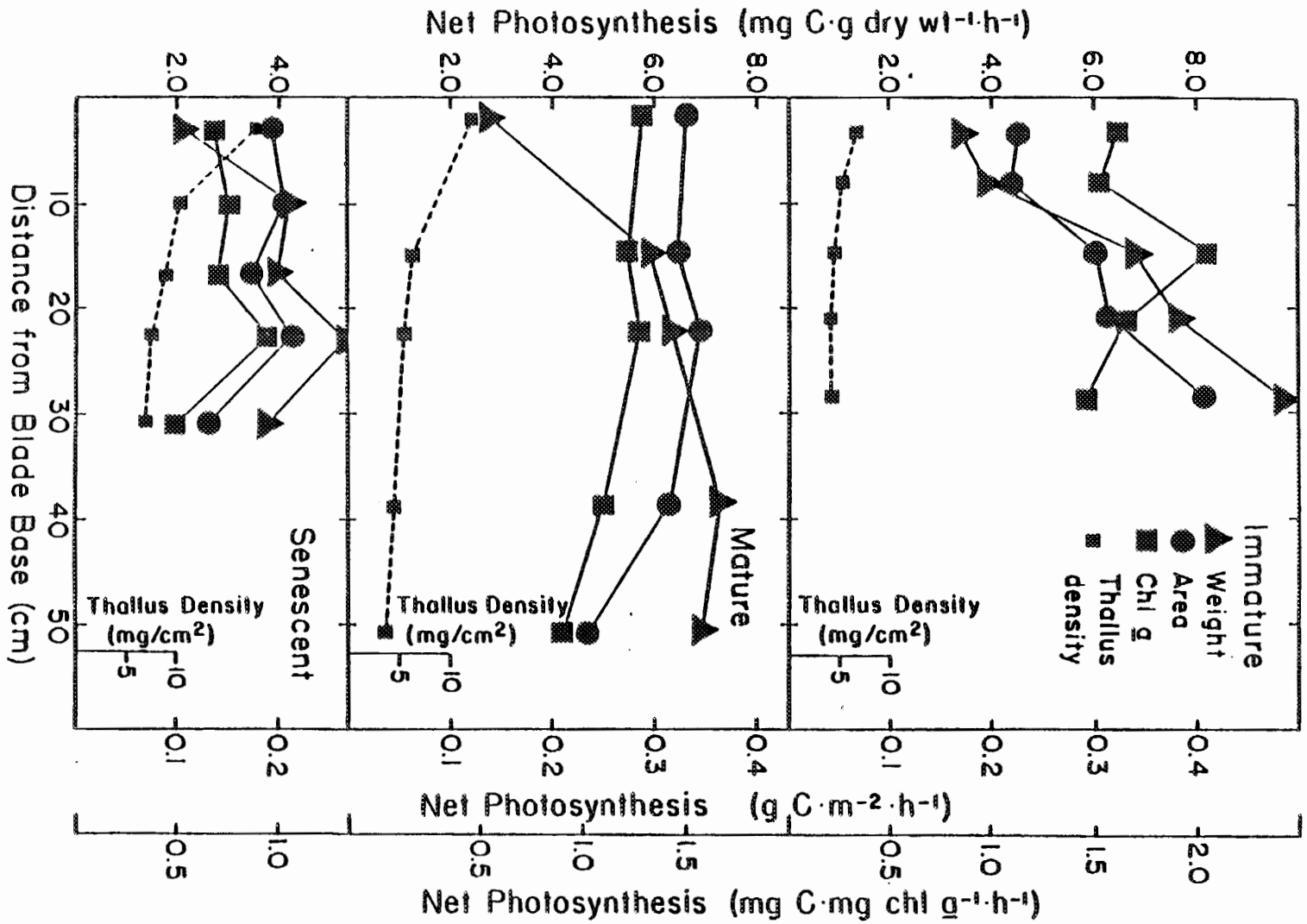

Figure 4.1-26. Longitudinal profiles of net photosynthesis normalized to dry weight (triangles), area (circles), and chl$\underline{a}$ (large squares) for different aged blades. The thallus density (mg dry wt·cm^{-2}) is presented (small squares) for each disc position.

DISCUSSION

Accurate estimates of kelp productivity are difficult to obtain, primarily because of their large size and extreme morphological and physiological complexity. Even though initial appreciation for this variability came from the original studies of Sargent and Lantrip (1952) and Clendenning (1964, 1971), many subsequent researchers (Johnston et al. 1977, Gordon and De Wreede 1978, Lüning 1979, Willenbrink et al. 1979 Wheeler 1980a, Drew et al. 1982, Matsuyama 1983) have chosen to estimate kelp productivity from short-term photosynthesis experiments based on representative discs or tissue subsamples. Even Clendenning (1971) failed to show the full extent of the large potential within blade variability of PS and R as a function of blade type. Furthermore, most of his tissue samples were taken from a "sampling zone" (Clendenning 1971, Fig. 59) located between 15 to 45 cm from the blade base on mature blades. Generally, within this mature blade sampling zone, areal-based PS rates (present study, Figs. 4.1-18 and 4.1-20) exhibit little variation, which is in agreement with Clendenning (1971). It can be clearly seen, however, that such a consistent "sampling zone" cannot be found in most immature and some senescent blades (Figs. 4.1-18 and 4.1-20).

Accurate estimates of whole blade PS and R cannot be derived from single disc samples taken from a single blade. Where this has been done (Clendenning 1971, Fig. 61; Wheeler 1980a, Fig. 3) to determine the vertical distribution of PS and R along a frond,

serious errors were probably made, especially for estimates of immature and senescent blade PS and R. Similarly, accurate estimates of whole blade pigment concentrations, even on an areal basis, cannot be obtained from single discs punched from a blade; this is particularly true for immature and senescent blades (Figure 4.1-24). Vertical distributions of pigment concentrations based on such an unrepresentative sampling scheme (Wheeler 1980a) are, therefore, questionable.

We recognized, as did Clendenning (1971), that plug areas based on the diameter of the sampling borer may slightly underestimate the true photosynthetic area, due to the corrugated nature of the blade. Immature blades show less deep corrugations than do mature or senescent blades, and in all blades, the corrugations become much deeper towards the blade base.

A further difficulty in obtaining accurate estimates of PS and R on kelp tissues when employing isolated plugs is the effect of wounding and excision of the tissue from the parental blade. The response to wounding appears to be variable among algae and even within different kelp species. For Caulerpa simpliciuscula, a tropical siphonous chlorophyte, Hawthorne et al. (1981) found that R increased (more than 50%) to a peak 2 h post wounding and then returned to the rate of the control after 6 h. Photosynthesis, on the other hand, decreased about 58% after 2 h and returned to normal after 6 h. These workers also discovered alterations in the distribution of ^{14}C-fixed during photosynthesis into various end

products throughout a 48-h post-wounding period. The flow of carbon (post wounding) increased the labeling of sulfated polysaccharides, which were found to have a direct involvement in the formation of structures formed during the wound healing process. Both Hatcher (1977) and Hopkin and Kain (1978) have observed similar respiratory increases following wounding of kelp tissues during short-term incubation experiments. Hatcher's (1977) observations, however, compared excised tissue disc respiration to that of the whole plant (*Laminaria longicruris*). This is ambiguous because the whole plant, stipe, and holdfast tissues probably have much lower R rates, and thus a direct comparison of weight-based rates would obviously show lower rates of R in the whole intact plant. Thus the exact magnitude of wounding on R in their experiments is difficult to assess. Drew *et al*. (1982) conducted time course studies (similar to ours) on R of tissue segments of the kelp *Laminaria ochroleuca*. Their studies showed a rapid decrease (~40%) in respiration after 5 h from excision from the parental blade, and their findings generally agreed well with those found in the current study. Immediate wounding effects on PS appear to be less dramatic; Buggeln and Bal (1977) found that in *Alaria esculenta* rates of LCF in excised tissue segments from the young meristematic regions located close to the base of the blade were not significantly different in short-term incubations from similar sections observed within the intact blade (incubated whole and subsampled after incubation termination). However, 24 h after excision, Buggeln and Bal (1977) found that these young blade

tissue segments showed a dramatic increase (50%) in the rate of LCF. From this, they hypothesized that the photosynthetic rate of meristematic tissue may be regulated by assimilate level, with the importation of photosynthate into meristematic tissues from source areas (mature blade areas) exerting an end product inhibition of PS, similar to that which has been suggested (Guinn and Mauney 1980) in higher plants. Thus when this photosynthate supply was removed after 24 h of excision from the parental blade, rates of LCF increased because negative feedback was relieved. This might also explain the decrease in R upon wounding, as was observed in our experiments and those of Drew *et al*. (1982) because the import of photosynthate used as the substrate of respiration (primarily mannitol, Kremer 1981a) would be stopped and respiration would drop as substrate becomes limiting with the tissue plugs. The effects of wounding would thus depend on the ontogenetic characteristics of the plugs within a blade as well as the position of the blade on the frond. It also appears that other kelp species behave differently, since in contrast to the current study, Arnold (unpublished) found no significant effects of wounding on short-term (2 h) incubations of whole blades and blade segments in different aged blades of the southern sea palm *Eisenia arborea*. Generally, we believe for kelp studies which require incubating discs that long preincubations (Gordon and De Wreede 1978, Lüning 1979, Wheeler 1980a) be avoided and the effects of wounding on PS and R be assessed.

Undoubtedly, because kelps have a high degree of morphological and histological complexity, this has contributed significantly to the difficulty in obtaining accurate rates of PS and R. In complex thalli, such as Macrocystis, ontogenetic gradients can be found both within a blade and between different aged blades on the same frond. Internally, blades are composed of three types of tissues, the meristoderm, cortex, and medulla (Parker 1971) whose composite structural complexity is allied with their functional specialization for carbon allocation, support, and transport, respectively. The relative proportionment of these tissues within blades, stipe, holdfast is dependent upon a strong developmental factor. For instance, Parker (1971) has found that the relative ratio of photosynthetic (meristoderm) to structural (cortex) tissue is highest at the oldest portions of blades (tips) and decreases in the younger growing basal areas. More recently, Kremer (1980) has shown for three species of Laminaria that 53-60% of the chl*a* and 63-73% (calculated from his data) of the activity of the primary carbon-fixing enzyme, RuBPCase, is found in the meristoderm, which only comprises about 2.7% of the total dry weight of a blade. The metabolic gradients of O_2 evolution and LCF fixation observed in Macrocystis (present study), Eisenia arborea (Arnold 1980), several species of Laminaria (King and Schramm 1976, Johnston *et al*. 1977, Küppers and Kremer 1978, Drew *et al*. 1982) and Undaria pinnatifida (Matsuyama 1983) are clearly ontogenetically related. In all cases, weight-based photosynthetic rates are highest towards the distal portions of blades (lower thallus density, mg dry wt/cm^2 and

a higher ratio of photosynthetic to structural tissue), dropping to lower rates at the basal, rapidly growing portions of blades (higher thallus density and lower ratios of photosynthetic to structural tissues). There also appears to be a general tendency for these gradients to be steeper (Arnold 1980, this study) in younger immature blades. Profiles of total chlorophyll (present study, Fig. 4.1-24 on a dry weight basis also show this same pattern.

Our photosynthetic rates (determined by either O_2 evolution or LCF) for both whole blades and isolated blade discs are comparable Table 4.1-3 to those found by other researchers. Such comparisons are, however, of limited value since different researchers have employed different techniques of sampling tissues and estimating photosynthesis. It is instructive to note that for isolated blade discs our values reflect a much broader range of variability (on the basis of weight) as compared to others. The full range of variability on the basis of whole blades (Table 4.1-3) ranges more than one order of magnitude (0.1 to 3.9 mgC·g dry $wt^{-1} \cdot h^{-1}$) with even higher variablity seen on the basis of isolated plugs (0.0 to 10.3 mgC·g dry $wt^{-1} \cdot h^{-1}$).

Canopy blades display a characteristic pattern (on an areal basis) of chlorophyll production Fig. 4.1-24. Synthesis rapidly occurs in immature blades, as exemplified in the steep longitudinal gradient base to tip and reaches approximately 30 $\mu g\ cm^{-2}$ in mature blades. This pattern probably represents a developmental process, and the differences in chlorophyll content within and between blades was not due to differences in the light environment

Table 4.1-3. A comparison of photosynthetic rates of whole blades and isolated blade discs in *Macrocystis pyrifera*.

Thallus portion incubated	Net PS		Method[a]	Author
	$mgC \cdot g\ dry\ wt^{-1} \cdot h^{-1}$	$gC \cdot m^{-2} \cdot h^{-1}$ [b]		
Whole blades (immature)	0.5-3.3[c]	.04-0.13[d]	^{14}C (in situ A,P)[e]	Towle & Pearse 1973
Blade parts	2.9	0.14	O_2 (outdoors B,M)	Littler 1980
Whole blades (various ages)	0.1-2.6	0.003-0.094	O_2 (outdoors B,M)	Arnold 1980
Whole blades (various ages)	2.3-3.9	--	O_2 (lab)	Present study
Immature & mature blade discs	0.56-3.4	0.05-0.35	O_2 (in situ B)	Sargent & Lantrip 1952
Apical & mature blade discs[f]	0.9-1.8	0.061-0.132	^{14}C (lab)	Willenbrink *et al*. 1979
Discs from all blade types	--	0.067-0.43	O_2 (lab)	Wheeler 1980a
Discs from all blade types	0.0[g]-10.3	0.0[g]-0.35	O_2 & ^{14}C (lab)	Present study

[a]A = blades attached, B = bottles, P = plastic bags, M = mechanical stirring.
[b]Area of one side of disc.
[c]Assuming 13.2% solids (Clendenning 1971).
[d]Assuming 4 mg dry $wt \cdot cm^{-2}$ (blade tip) and 7 mg dry $wt \cdot cm^{-2}$ (blade base).
[e]Discs punched after incubation.
[f]*Macrocystis integrifolia*.
[g]Senescent blade tips.

because all immature and mature blades resided at all times at the water surface. The increase in chlorophyll found in senescent blades may be partially light induced because during high tides they were submerged.

An interesting developmental pattern within immature blades was seen for PS normalized to chl*a*. Net PS per chl*a* reached a maximum in the center of the immature blade. Although partially due to an unexplained decrease in chl*a* per unit area towards the center, this PS bulge may be due to the unsynchronized synthesis of components of dark and light PS reactions during early development. More data are needed (i.e., patterns of RuBP-C, EC 4.1.1.39, activity), however, to support this assertion.

Photosynthetic rates normalized to chl*a* (Fig. 4.1-26) for various positions along the three blade types ranged from 0.47 to 2.05 $mgC \cdot mg\ chla^{-1} \cdot h^{-1}$, while those observed for *Macrocystis integrifolia* (Willenbrink *et al*. 1979) and *M. pyrifera* (Wheeler 1980a) were less variable (0.35 to 1.02 and 0.69 to 1.77 $mgC \cdot mg\ chla^{-1} \cdot h^{-1}$, respectively).

Profiles of DCF, to a certain degree, can also be interpreted as showing ontogenetic patterns. High rates of DCF are characteristic of the Phaeophyta, particularly the kelps which can range (Kremer 1981a,b) up to 30-50% of the total carbon fixation potential. Highest rates are associated with the basal meristematic portions of blades, and recent estimates of DCF (summarized in Kremer 1981b) for the young blade tissues of *Macrocystis pyrifera* and *M. integrifolia*

represent 56.8 and 40.8% of the LCF rates, respectively. Our highest DCF rates appear, in comparison, considerably lower (10-15% of the LCF values); however, on an absolute basis, our data (0.08-0.09 mgC·g dry $wt^{-1}\cdot h^{-1}$) are slightly higher than those cited above (0.060 and 0.034 mgC·g dry $wt^{-1}\cdot h^{-1}$, respectively). Discrepancies can arise when reporting DCF rates as a % of LCF values, since for the previously cited values, the LCF rates were extremely low (0.106 and 0.087 mgC·g dry $wt^{-1}\cdot h^{-1}$), even for young tissues. In the present study, distinct longitudinal profiles were found in only immature blades and sporophylls. Both blade types have basal regions with intense meristematic activity. Mature blades displayed little variation in DCF down the blade. The major portion of DCF (>70%) occurs in the cortical cells (Kremer 1980) of blades and is believed to compensate for respiratory loss of CO_2 in these tissues.

Most measurements of $^{14}CO_2$-uptake in the dark probably seriously underestimate true rates of DCF, since simultaneously occurring respiratory processes give off CO_2. Some of this CO_2 may be refixed by PEP-CK while the rest leaves the thallus surface and enters solution. The real applicability of these measurements, however, lies in the ability to fingerprint the actively growing sink (Schmitz and Lobban 1976) regions within blades and fronds.

Respiratory profiles along blades are also correlated with active areas of growth as seen in the immature blades (present study), where highest rates are located towards the blade base.

Similarly, the apical scimitar, which initially produces new individual blades and is thought (Schmitz and Lobban 1976) to be a very strong carbon sink as are the developing sporophylls; both of these thallus parts exhibited very high rates of R. Observed profiles of intrablade R were similar to the patterns found by Clendenning (1971, Fig. 60) for what appears to be a mature blade, with the highest rates on an areal basis and lowest rates on a fresh weight basis occurring at the base of the blade. Overall our respiratory rates (all blades) for isolated plugs ranged from about 0.20 to 1.75 $mgC \cdot g\ dry\ wt^{-1} \cdot h^{-1}$ (0.014 to 0.062 $gC \cdot m^{-2} \cdot h^{-1}$) and are generally higher than those recorded (see review by Kremer 1981b) for isolated plugs of Laminaria sp. (0.036 to 0.396 $mgC \cdot g\ dry\ wt^{-1} \cdot h^{-1}$) and those found by Wheeler (1980a) in his studies of Macrocystis ($\approx$0.024 $gC \cdot m^{-2} \cdot h^{-1}$; recalculated on the basis of the area of one side of the disc). Oddly enough, Wheeler (1980a) states that his dark respiration rates varied little over the length of a frond; while in contrast, we found appreciable variation of R activity just within a single blade. Again, it seems that his unrepresentative sampling scheme has masked the truly variable respiratory responses that exist for whole blades down the developmental gradient of a frond.

The respiratory needs of stipe and holdfast are about 25% of those required by blade tissues; however, on a whole plant basis, the fresh weight biomass allocated to these parts is high and can represent over 30% (Clendenning 1971) of the total plant biomass.

So in this respect, the total biomass of stipe and holdfast represents an appreciable carbon sink, and this should be considered, especially when constructing annual carbon budgets. This was not considered in the annual carbon budget constructed by Johnston *et al*. (1977) for *Laminaria saccharina*.

In order to construct a carbon budget for a frond or whole plant, a statistically significant number of measurements must be made on the various tissue types. This, of course, includes measurements of the various blade types from juvenile, subcanopy, growing canopy (with apical scimitars), canopy (with terminal bifurcations), and senescent fronds. Rates of whole blade PS and R cannot be determined from incubations of single discs punched from single blades but can be constructed from disc incubations providing that longitudinal profiles are developed for each blade (as described herein) and the corresponding data regarding blade length and area relationships are gathered. Isolated whole blades may be incubated in the lab provided that the chamber satisfies all of the methodological criteria (Littler 1979) and the tissue is properly transported.

In situ measurements of PS and R by enclosing blades in plastic bags may severely underestimate rates unless the bag is found to be impermeable to inorganic carbon and H^+ (^{14}C or pH, alkalinity measurements) or O_2 (O_2 measurements) (Towle and Pearse 1973). Also, translocation of labeled products must be accounted for when measuring whole blade LCF or DCF *in situ* (Towle and Pearse 1973).

In situ-derived data are of marginal value unless vigorous quantitative description of water velocity, temperature, and light intensity are simultaneously determined. Natural fluctuations of these environmental variables are neither predictable, controllable, and are seldom repeatable; therefore, they must be accurately monitored during the *in situ* incubation period. Without such monitoring, conclusions will be based on the observer's estimation of conditions and lack strict scientific documentation. Temperature is the easiest parameter to monitor. The light field and water motion are most difficult. The light environment in and below kelp canopies can vary tremendously over short time intervals (seconds) due to wave focusing and blade movement and orientation. Wheeler (1980b) concluded that diffusion resistance can decrease *Macrocystis* productivity in large beds because current velocities were frequently less than 4 $cm \cdot sec^{-1}$ which is below the velocity which saturates PS. Gerard (1982a), however, concluded that current, wave surge, and blade movement (flagging) provided enough water movement to saturate nutrient uptake all of the time. This conclusion was based on 24-h dissolution rates of plaster buttons attached to blades *in situ* under varying sea states. These measurements, however, do not provide information as to short-term (1 h) fluctuations in water motion experienced by a blade bagged for *in situ* productivity determinations. No significant enhancement of nitrate uptake occurred over 1 h between bagged blades undisturbed or continually shaken (Gerard 1982b). The ocean conditions during these measurements, however, were not recorded, and it is possible that during calm

periods in dense kelp beds that there would be a significant difference. Although orbital motion velocities are transmitted through plastic bags (Gust 1977), it has not been demonstrated that a bagged Macrocystis blade experiences water motion due to surge or flagging; the blade enclosed with water is probably prevented from moving longitudinally in the bag during surge. Bag shaking experiments should, therefore, be performed during each day of incubations as a control. Laboratory investigations of kelp PS, R, and DCF are advantageous because the effect of a single variable can be easily ascertained in a controlled incubation. After the effects of these variables have been described, properly constructed in situ incubations may indicate that other unknown factors are important.

REFERENCES

Arnold, K.E. 1980. Aspects of the Production Ecology of Marine Macrophytes. Ph.D. Thesis, University of California, Irvine, 149 pp.

Arnold, K.E. & Murray, S.N. 1980. Relationships between irradiance and photosynthesis for marine benthic green algae (Chlorophyta) of differing morphologies. J. Exp. Mar. Biol. Ecol. 43:183-92.

Buggeln, R.G. & Bal, A.K. 1977. Effects of auxins and chemically related non-auxins on photosynthesis and chloroplast ultrastructure in Alaria esculenta (Laminariales). Can. J. Bot. 55:2098-105.

Brinkhuis, B.H. & Jones, R.F. 1974. Photosynthesis in whole plants of Chondrus crispus. Mar. Biol. 27:137-41.

Clendenning, K.A. 1964. Photosynthesis and growth in Macrocystis pyrifera. In Davey de Virville, A.D. & Feldman, J. [Eds.] Proc. IV Int. Seaweed Symp., Pergamon Press, New York, pp. 55-65.

Clendenning, K.A. 1971. Photosynthesis and general development in Macrocystis. In North, W.J. [Ed.] The Biology of Giant Kelp Beds (Macrocystis) in California, J. Cramer, Lehre, pp. 169-90.

Drew, E.A., Ireland, J.F., Muir, C., Robertson, W.A.A. & Robinson, J.D. 1982. Photosynthesis, respiration and other factors influencing the growth of Laminaria ochroleuca Pyl. below 50 metres in the Straits of Messina. P.S.Z.N.I.:Mar. Ecol. 3:335-55.

Dromgoole, F.I. 1978. The effects of oxygen on dark respiration and apparent photosynthesis of marine macro-algae. Aquat. Bot. 4:281-97.

Dromgoole, F.I. 1979. Photosynthetic and respiratory transients of oxygen exchange in Carpophyllum species (Fucales, Phaeophyceae). Aquat. Bot. 6:133-47.

Duncan, M.J. & Harrison, P.J. 1982. Comparison of solvents for extracting chlorophylls from marine macrophytes. Bot. Mar. 15:445-7.

Gaarder, T. & Gran, H.H. 1927. Investigations of the production of plankton in the Oslofjord Rapp. Proc. Verb. Cons. Int. Expl. Mer. 42:3-48.

Gagne, J.A., Larochelle, J. & Cardinal, A. 1979. A solubilization technique to prepare algal tissue for liquid scintillation counting, with reference to Fucus vesiculosus L. Phycologia 18:168-70.

Gerard, V.A. 1982a. In situ water motion and nutrient uptake by the giant kelp Macrocystis pyrifera. Mar. Biol. 69:51-4.

Gerard, V.A. 1982b. In situ rates of nitrate uptake by giant kelp, Macrocystis pyrifera (L.) C. Agardh: tissue differences, environmental effects, and predictions of nitrogen-limited growth. J. Exp. Mar. Biol. Ecol. 62:211-24.

Gordon, D.K. & De Wreede, R.E. 1978. Factors influencing the distribution of Egregia menziesii (Phaeophyta, Laminariales) in British Columbia, Canada. Can. J. Bot. 56:1198-205.

Guinn, G. & Mauney, J.R. 1980. Analysis of CO_2 exchange assumptions: Feedback control. In Hesketh, J.D. & Jones, J.W. [Eds.] Predicting Photosynthesis for Ecosystem Models, Vol. II, CRC Press, Florida, pp. 1-16.

Gust, G. 1977. Turbulence and waves inside flexible-wall systems designed for biological studies. Mar. Biol. 42:47-53.

Hatcher, B.G. 1977. An apparatus for measuring photosynthesis and respiration of intact large marine algae and comparison of results with those from experiments with tissue segments. Mar. Biol. 43:381-5.

Hawthorne, D.B., Dreher, T.W., Grant, B.R. 1981. The wound response in the siphonous alga Caulerpa simpliciuscula C. Ag.: II. The effect of wounding on carbon flow. Protoplasma 105:195-206.

Hopkin, R. & Kain, J.M. 1978. The effects of some pollutants on the survival, growth and respiration of Laminaria hyperborea. Estu. Coast. Mar. Sci. 7:531-3.

Iverson, R.L., Bittaker, H.F. & Myers, V.B. 1976. Loss of radiocarbon in direct use of Aquasol for liquid scintillation counting of solutions containing ^{14}C-$NaHCO_3$. Limnol. Oceanogr. 21:756-8.

Johnston, C.S., Ferrier, N.C. & Jones, R.G. 1981. A critical assessment of estimates of macroalgal primary production based on short-term measurements of photosynthesis. In Fogg, G.E. & Jones, W.E. [Eds.] Proc. VIII Int. Seaweed Symp., Marine Science Laboratories, Menai Bridge, pp. 372-6.

Johnston, C.S., Jones, R.G. & Hunt, R.D. 1977. A seasonal carbon budget for a laminarian population in a Scottish sea-loch. Helgol. Wiss. Meeresunters. 30:527-45.

King, R.J. & Schramm, W. 1976. Determination of photosynthetic rates for the marine algae Fucus vesiculosus and Laminaria digitata. Mar. Biol. 37:209-13.

Kremer, B.P. 1979. Light independent carbon fixation by marine macroalgae. J. Phycol. 15:244-7.

Kremer, B.P. 1980. Transversal profiles of carbon assimilation in the fronds of three Laminaria species. Mar. Biol. 59:95-103.

Kremer, B.P. 1981a. Metabolic implications of non-photosynthetic carbon fixation in brown algae. Phycologia 20:242-50.

Kremer, B.P. 1981b. Aspects of carbon metabolism in marine macroalgae. Oceanogr. Mar. Biol. Ann. Rev. 19:41-94.

Kremer, B.P. & Markham, J.W. 1979. Carbon assimilation by different developmental stages of Laminaria saccharina. Planta 144:497-501.

Küppers, U. & Kremer, B.P. 1978. Longitudinal profiles of carbon dioxide fixation capacities in marine macroalgae. Plant. Physiol. 62:49-53.

Littler, M.M. 1979. The effects of bottle volume, thallus weight, oxygen saturation levels, and water movement on apparent photosynthetic rates in marine algae. Aquat. Bot. 7:21-34.

Littler, M.M. 1980. Morphological form and photosynthetic performances of marine macroalgae: Tests of a functional/form hypothesis. Bot. Mar. 22:161-5.

Lobban, C.S. 1974. A simple, rapid method of solubilizing algal tissue for scintillation counting. Limnol. Oceanogr. 19:356-9.

Lobban, C.S. 1978. The growth and death of the Macrocystis sporophyte (Phaeophyceae, Laminariales). Phycologia 17:196-212.

Lüning, K. 1979. Growth strategies of three Laminaria species (Phaeophyceae) inhabiting different depth zones in the sublittoral region of Helgoland (North Sea). Mar. Ecol. Prog. Ser. 1:195-207.

Manley, S.L. 1981. Iron uptake and translocation by Macrocystis pyrifera. Plant. Physiol. 68:914-8.

Mann, K.H. 1972. Ecological energetics of the seaweed zone in a marine bay on the Atlantic coast of Canada. II. Productivity of the seaweeds. Mar. Biol. 14:199-209.

Matsuyama, K. 1983. Photosynthesis of Undaria pinnatifida Suringar f. distans Miyabe et Okamura (Phaeophyceae) from Oshoro Bay. II. Photosynthetic in several portions of the thallus. Sci. Rep. Hokkaido Fish. Exp. Stn. 25:187-93.

Parker, B.C. 1971. The internal structure of Macrocystis. In North, W.J. [Ed.] The Biology of Giant Kelp Beds (Macrocystis) in California , J. Cramer, Lehre, pp. 99-121.

Peng, C.T. 1981. Sample Preparation in Liquid Scintillation Counting, Amersham Corporation, Illinois, 112 pp.

Peterson, B.J. 1980. Aquatic primary productivity and the ^{14}C-CO_2 method: A history of the productivity problem. Ann. Rev. Ecol. Syst. 11:359-85.

Ragan, M.A. & Jensen, A. 1977. Quantitative studies on brown algal phenols. I. Estimations of absolute polyphenol content of Ascophyllum nodosum and Fucus vesiculosus. J. Exp. Mar. Biol. Ecol. 30:209-21.

Sargent, M.C. & Lantrip, L.W. 1952. Photosynthesis, growth and translocation in the giant kelp. Am. J. Bot. 39:515-8.

Schmitz, K. & Lobban, C.S. 1976. A survey of translocation in Laminariales (Phaeophyceae). Mar. Biol. 36:207-16.

Sieburth, J.M. 1968. The influence of algal antibiosis on the ecology of marine microorganisms. In Droop, M.R. & Ferguson Wood, E.J. [Eds.] Advances in Microbiology of the Sea, Academic Press, New York, pp. 63-94.

Smith, S.V. & Kinsey, D.W. 1978. Calcification and organic carbon metabolism as indicated by carbon dioxide. In Stoddart, D.R. & Johannes, R.E. [Eds.] Coral Reefs: Research Methods, UNESCO Paris.

Smith, W.O. 1975. The optimal procedures for the measurement of phytoplankton excretion. Mar. Sci. Comm. 1:395-405.

Strickland, J.D.H. 1960. Measuring the Production of Marine Phytoplankton. Bull. Fish Res. Board Canada 122, 172 pp.

Strickland, J.D.H. & Parsons, T.R. 1972. A Practical Handbook of Seawater Analysis. Bull. Fish. Res. Board Canada 167, 310 pp.

Towle, D.W. & Pearse, J.S. 1973. Production of the giant kelp Macrocystis, estimated by in situ incorporation of ^{14}C in polyethylene bags. Limnol. Oceanogr. 18:155-9.

Wassman, E.R. & Ramus, J. 1973. Primary-production measurements for the green seaweed Codium fragile in Long Island Sound. Mar. Biol. 21:289-97.

Weidner, M. & Küppers, U. 1973. Phosphoenolpyruvat-Carboxykinase und Ribulose-1,5-Diphosphat-Carboxylase von Laminaria hyperborea: Das Verteilungsmuster der Enzymaktivitäten im Thallus. Planta 114:365-72.

Wheeler, W.N. 1980a. Pigment content and photosynthetic rate of the fronds of Macrocystis pyrifera. Mar. Biol. 59:97-102.

Wheeler, W.N. 1980b. Effect of boundary layer transport on the fixation of carbon by the giant kelp Macrocystis pyrifera. Mar. Biol. 56:103-10.

Willenbrink, J., Kremer, B.P., Schmitz, K. & Srivastava, L.M. 1979. Photosynthetic and light carbon fixation in Macrocystis, Nereocystis, and some selected Pacific Laminariales. Can. J. Bot. 57:890-7.

Williams, P.J. LeB., Berman, T. & Holm-Hansen, O. 1972. Potential sources of error in the measurement of low rates of planktonic photosynthesis and excretion. Nature 236:91-2.

4.2 Major Achievements of Kelp Growth Model Development

Computations utilizing the most sophisticated existing kelp model were performed in order to support the evaluation of the effects of planting density on kelp production. The calculations yielded an optimal density of 8 fronds/m^2, which corresponded well with findings from the NMI test farm at Goleta on 8-11 fronds/m^2.

Modeling activities during 1983 included exploring methods for improving the current description of the submarine light field and expanding the morphological models of kelp fronds by incorporating additional details from existing data.

Manipulation of available morphological data yielded a single mathematical representation describing cumulative distributions of blade area along kelp fronds for all frond lengths. Previous studies had used several equations (Kirkwood & North), dividing fronds into five size-length classes, for their mathematical representations. Development of a single representation is a large improvement in methodology.

A relationship was also found between total blade area and total node number for fronds of any length.

The distribution of "representative blade areas" along "representative fronds" of any length can now be computed by combining the two relationships. It opens the way for mathematically constructing "representative kelp plants" and "representative kelp beds" for any given frond size distribution.

Field studies demonstrated that acclimation is more important than blade age in determining blade PS characteristics as well as manifestations of photoinhibition. Canopy blades yielded highest PSmax values and showed no photoinhibition. Changes in PS vs I relationships can now be predicted, knowing the recent history of a blade's environment.

Subcanopy blades residing in a harvested area within a kelp bed showed higher PSmax values than similar blades located nearby in an unharvested part of the bed.

Nocturnal R values were similar for all except apical meristematic blades.

Canopy blades constantly shift positions so that few remain fully exposed to full sunlight for longer than one hour.

Effects from El Nino caused nutrient depletion, chlorophyll reduction, and decreased PS in the experimental plants.

Percent penetration of light through an artificially fixed kelp canopy varied exponentially with canopy density.

Spatial heterogeneity in distribution of canopy tissue resulted in higher percent penetration light values beneath natural canopies than predicted from findings with the artificially fixed canopy.

Self shading was greatest immediately adjacent to a given plant, due to the combined contributions of canopy plus vertical frond bundle. Farther away, effects from the bundle decreased.

Short term (ca 1 sec) fluctuations in light intensity occurred both in open water and beneath kelp canopies but were considerably greater for the latter case. Radiation peaks or flashes decreased with increasing depth while the relative importance of diffuse radiation increased.

Long term (ca 1 min) fluctuations beneath canopies resulted from shifts in distribution of canopy tissues.

Apical meristematic blades and whole blades yielded the highest PS rates in the laboratory (3.81 and 3.07 mgC/gm dry. hr respectively). Sporophylls were 1.42 and stipe segments 0.15 mgC/gm dry. hr. Haptera showed no PS capability.

On both an areal and weight basis, the steepest PS and R gradients occurred in immature blades, followed by senescent and mature tissues.

Within a blade, the tip showed the highest PS rates (6.46 to 10.09 mgC/gm dry. hr.) for immature and mature blades.

Rates of R were generally highest towards the blade base (1.03 to 1.80 mgC/gm dry. hr.; immature blades).

Highest dark carbon fixation rates also occurred basally (0.04 to 0.06 mgC/gm dry. hr.; immature and senescent blades).

PS profiles were mirrored in profiles of chlorophyll a and c.

Studies indicated that it is difficult, if not impossible, to select single tissue plugs that are representative of entire blades.

Laboratory cultures of juvenile Macrocystis plants showed that sporophyte growth was phosphorus-limited following a two week exposure to P_i supplementations of 0.3 M or less. The critical level of tissue phosphorus appeared to be near 0.20 percent of the dry weight.

Luxury consumption and storage of phosphorus in juvenile Macrocystis was demonstrated.

4.3 Major Technical Problems of Kelp Growth Model Development

Severe weather during the first three months of 1983 including one storm that was rated as a 40-to-100 year event, seriously interfered with field activities during this period. The work that was accomplished was rendered useless by storm damage on several occasions.

A major El Nino event began in latter 1982 and extended its influence throughout 1983. A fairly well-developed upwelling season was experienced during April and May; however, June, July and August witnessed widespread deterioration of the California and Baja California seaweed resources as a result of the El Nino. The population of experimental plants at Laguna Beach was severely affected so that by August, further work at this site was not feasible, and experimental activities were transferred to Abalone Cove which lies centrally in the strong upwelling area off Palos Verdes. Reasonably healthy kelp canopies occurred in Abalone Cove. The site, however, lies 27 miles westerly from Cal-Tech's headquarters office, requiring two to three hours of travel time per visit.

In retrospect, the El Nino event probably impacted the later phases of the studies at the Catalina Test Facility in 1982.

4.4 Conclusions of Kelp Growth Model Development

The kelp modeling activity is well underway. A mathematical expression relating blade area to position on a frond has been formulated in simplified form. This represents a significant advance in characterizing this extremely important fundamental relationship which lies at the heart of kelp modeling. Deviations from this theoretical representation of a frond have been assessed by comparison to a large pre-existing data base of measurements made on natural fronds. Variability between real and predicted values was modest indicating that the new modeling procedure is valid.

Extensive records were obtained of subsurface irradiance levels vs time, both in open water and beneath kelp canopies of various densities, under a variety of conditions. These data are undergoing analysis and will assist in the development for modeling of a realistic mathematical description of light fields within kelp beds.

In situ determinations of PS and R were conducted under a variety of environmental conditions for different tissue types in an experimental *Macrocystis* population. PS vs I relationships, acclimation effects, and photoinhibition were studied. The investigations also included concurrent measurements of temperature, kelp nutrition, morphometry, and growth. Therefore, an extensive body of interrelated data extending over several months to assist in designing and testing the kelp model is available. Effects of kelp harvesting on PS rates among remaining blades was also investigated. A major El Nino occurred during these studies producing marked indications of nutrient starvation among the experimental plants. Relationships between PS and nutritional status of kelp blades were obtained, taking advantage of this rather infrequent phenomenon.

Detailed laboratory studies examined PS, R, and dark carbon fixation rates and their ranges. Longitudinal profiles of these parameters along lengths of fronds were obtained as well as profiles along lengths of individual blades. PS rates from hapteral and stipe tissues were measured.

PS values lying at the upper ends of the ranges observed were higher than any rates previously reported in the literature. Relationships between PS rates and contents of the photosynthetic pigments a and c were investigated.

Growth experiments utilizing juvenile Macrocystis plants indicated that the critical P-content (i.e. tissues were neither storing phosphorus nor were they starved) was approximately 0.2 percent on a dry weight basis. Like nitrogen, luxury uptake of phosphorus occurred with excess amounts being stored for utilization later when external sources might become growth-limiting.

5. MAJOR ACHIEVEMENTS

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The overall program objective was fulfilled by the provision of critical data which allowed subsequent preparation of a comprehensive systems and economic analysis of a conceptual Marine Biomass Farm.

- The first *Macrocystis* yield data on a large number of plants was accumulated, and subsequent yield verification studies were defined and implemented.

- Kelp Growth Model Development was initiated based on requirements identified by the systems and economic analysis.

6. MAJOR TECHNICAL PROBLEMS

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A major technical problem involved operation of the Catalina Test Facility (Hemidome). The problem was the intensification of deleterious biological effects which apparently resulted from enclosure of the plants in the test apparatus. Due to resource limitations, the problems could not be resolved, which resulted in termination of the Hemidome experiment project.

7. CONCLUSIONS AND RECOMMENDATIONS

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Conclusions

- Through analysis of detailed scientific and engineering data, using conservative projections, the results of the Marine Biomass Program indicate that production of methane from *Macrocystis pyrifera* on commercial sized farms is technically feasible.

- Projections of product cost using state-of-the-art production technology ($13.50/MMBTU) as well as using reasonably optimistic extrapolations of current technology ($6.00/MMBTU) indicate that the cost of gas produced on the commercial farm conceptualized would be higher than that produced from current conventional production sources, but competitive with other sources of substitute natural gas and unconventional natural gas.

- System and product cost can be reduced by utilization of improved methods of feedstock, production (genetic selection) and methane conversion (digestion system design).

- Methane from kelp systems may also become cost efficient and attractive in a business and financing sense if by-product and co-product recovery are incorporated into the system concept.

- A major unknown in all projections is caused by the fact that all studies have been performed with wild plants. This factor forces, probably, excessive conservatism on the entire concept. For example, improvement of crop yield as functions of planting and harvesting strategy, various nutrient management techniques, and the positive effects of hybridization and genetic selection could not be reasonably assessed. Any one of the above parameters could have major upward impact on yield with consequent reduction in cost. The combined result of optimization of all of these parameters may well yield a cost competitive system.

Recommendations

- Work on the Kelp Growth Model, now under development should continue in order to allow credible extrapolations to be made from the baseline systems analysis. This model should also facilitate the study and analysis of other types of biomass to methane systems.

- Kelp genetic studies should continue. Genetic composition influences yield, crop stability, digestibility, and many other factors influencing feedstock and product cost. As genetic research usually involves a relatively long-term effort, this work should be pursued at some level if successful commercial production is ever to be realized.

- Methane production from marine biomass should be considered as part of an integrated business. Subsequent financial analyses should consider by-products and co-products as integral parts of the system. Several potential ancillary products having values equal to or greater than the methane product can be generated by the system. Active development of these products would significantly increase the cost efficiency of the system by offering faster payback and the opportunity for using alternate financing strategies and sources.

- Engineering studies of kelp plant morphology - environmental interactions should be continued as an aid to development of commercialized systems. The data on buoyancy, drag and holdfast resistance in this study led to significant reductions in potential system cost. Hardware costs were reduced and lower cost fertilization strategies could be projected than would otherwise have been reasonably assumed. Future work should develop a model of the mechanical interactions occurring between kelp plants and their environment as well as those occurring among plants at various planting intervals. A basic question exists as to whether the plants should be morphologically modified in order to facilitate the design of least cost planting, cultivation and harvesting systems. (What is the ideal "design" for an individual kelp plant?) It has been shown,

for example, that photosynthetic rate in the canopy varies inversely as canopy depth, which is itself a function of drag and current. A model which explores these relationships and their potential effects on the design requirements of nearshore and offshore structures would be an extremely valuable tool for planning, design and cost projections.

- Hardware (plant and equipment) engineering studies should be pursued at some level. All of the previous work has shown that the system hardware is interactive with the plants; their physiology, structure and chemistry. Provisions should be made to assure that biological and hardware systems remain compatible. It should be assumed, for example, that successful development of a high yielding strain, which is now being investigated, would have a one-to-one impact on cultivation, harvesting, and transportation facilities. Higher yield may impact drag, harvesting and processing in non-linear ways.