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KELP BIOMASS PRODUCTION

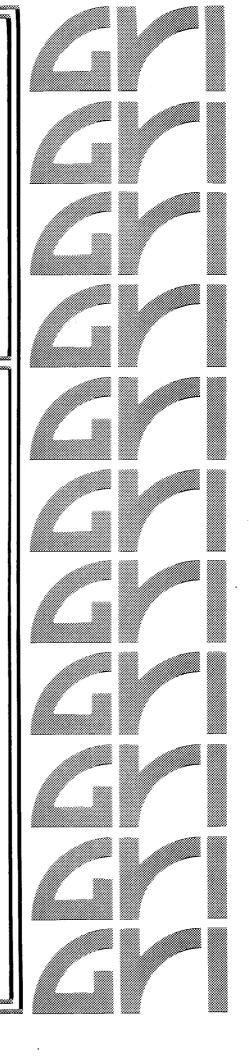
YIELD, GENETICS AND

PLANTING TECHNOLOGY

ANNUAL REPORT

January 1983 - August 1984

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



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# KELP BIOMASS PRODUCTION YIELD. GENETICS AND PLANTING TECHNOLOGY

ANNUAL TECHNICAL REPORT January 1983 - August 1984

## Prepared by:

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#### For:

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Advanced Biomass Technology

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#### 16. Abstract (Limit: 200 words)

Progress has been made toward the long-term goal of growing macroalgae in the sea as a future source of substitute natural gas. This annual report discusses progress made to: 1) measure macroalgal yield, 2) enhance yield by row planting and selective harvesting, 3) genetically breed high-producing plants, 4) devise methods for planting kelps and 5) maintain and extend collaborative research efforts and communication with scientists working on macroalgal biomass production in Japan, China and elsewhere. report discusses kelp biology and macroalgal mariculture in general terms, the theories that have been proposed and the existing data base in the scientific literature. Particular attention is given to new techniques used to make in-the-sea hydrodynamic and light-climate measurements and microspectrophotometric measurements of DNA levels in kelp sporophytes and gametophytes. New and effective tank, dish and in-the-sea planting and culture methods have been successfully employed in a co-funded program to establish a new kelp bed. Other co-funded work in genetics and marine farm engineering is mentioned in the report. A list of ten publications resulting from this work is included. The report suggests that in the distant future, the 1,000 acre natural kelp forest, recently leased to NMI by the State of California, could be used along with on-shore facilities provided by Southern California Gas Company, for pilot-scale demonstration project.

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#### RESEARCH SUMMARY

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KELP BIOMASS PRODUCTION

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GRI Contract Number:

5083-226-0802-4

Principal Investigators:

M. Neushul and B. W. W. Harger

Time Span:

January 1 to April 30, 1983 (GE Subcontract)

May 1, 1983 to August 31, 1984

Objectives: This annual report discusses progress made on the five tasks undertaken, these being to: 1) measure macroalgal yield, 2) enhance yield by row planting and selective harvesting, 3) genetically breed high-producing plants, 4) devise methods for planting kelps and 5) maintain and extend collaboration and communication with foreign scientists working on macroalgal biomass production.

Achievements: Growth rates of 7% per day increase in wet weight were

Major

measured for genetically-defined, pedigreed gametophytic strains of Macrocystis. The gene bank of uni-algal Bay. Mexico) North American Macrocystis

gametophytic strains was extended to include material from the most northerly (Sitka, Alaska) and southerly (Turtle populations. Environmental measurements were made to document the adverse effects of persisting El Niño conditions, and new methods were developed for computer-based measurements of light and hydrodynamic conditions on the test farm and in natural populations. A new kelp bed was established in Ventura County. Two major kelp beds (1.650 acres) were leased from the State of California for future work.

## LIST OF PRESENTATIONS, VISITS, REPORTS AND PUBLICATIONS

#### PRESENTATIONS

World Mariculture Society, Washington, D. C. - January 12, 1983 Effects of Waste Disposal on Kelp Communities, La Jolla, California -January 25, 1983

Energy from Biomass and Wastes, Lake Buena Vista, Florida - January 26, 1983 GRI Project Managers, Newport Beach, California - March 7, 1983 Philip Morris Symposium, Richmond, Virginia - April 11, 1984

GRI Project Advisors, Sheraton Harbor Island, San Diego, California - July 20, 1983

Third International Artificial Reef Symposium, Newport Beach, California - November 4, 1983

Marine Biomass Contractors Meeting, Key West, Florida. - April 26, 1984 Southern California Academy of Sciences, Los Angeles, California -May 13, 1984

GRI Project Advisors, Sheraton Harbor Island, San Diego, California - August 22, 1984

## PUBLICATIONS - 1983 and 1984

- Neushul, M. 1983. Morphology, structure, systematics and evolution of the giant kelp Macrocystis. In: C. K. Tsung (ed.), Proceedings of the Joint China-U. S. Phycology Symposium. Science Press: Beijing, China pp. 1-27.
- Neushul, M. 1983. New Crops from the sea. <u>In:</u> J. W. Rosenblum (ed.), <u>Agriculture in the Twenty-First Century.</u> John Wiley & Sons: New York. pp. 149-156.
- Neushul, M., B. W. W. Harger and G. A. Brosseau 1983. Studies of biomass yield from a near-shore macroalgal test farm. <u>In:</u> D. L. Klass (ed.), Energy from Biomass and Wastes. VII. pp. 169-183.
- Harger, B. W. W. and M. Neushul 1983. Test-farming of the giant kelp,

  Macrocystis, as a marine biomass producer. J. of the World Mariculture
  Society 14: 392-403.
- Harger, B. W. W. 1983. A historical overview of kelp in southern California.

  In: W. Bascom (ed.), Effects of Waste Disposal on Kelp Communities.

  Proceedings of a symposium presented by the Southern California Coastal Water Research Project and the Institute of Marine Resources at the University of California, San Diego. pp. 70-83.
- Neushul, M. 1983. An overview of basic research on kelp and the kelp forest ecosystem. In: W. Bascom (ed.), Effects of Waste Disposal on Kelp Communities. Proceedings of a symposium presented by the Southern California Coastal Water Research Project and the Institute of Marine Resources at the University of California, San Diego. pp. 282-300.

- Lewis, R. J. and B. W. W. Harger 1984. Production and mortality of the giant kelp <u>Macrocystis</u> in California during adverse climatological conditions. J. of Phycology 20 (supplement): 12. (Abstract).
- Fei, X. G. and M. Neushul 1984. The effects of light on the growth and development of giant kelp. Hydrobiologia 116/117: 456-462.
- Neushul, M., B. W. W. Harger, D. L. Carlsen, R. J. Lewis and G. A. Brosseau 1984 (in press). The siting, design and biomass production of an artificial reef planted with kelp. Proc. Third Intl. Artificial Reef Conference. (In press).
- Neushul, M. and B. W. W. Harger 1984 (in press). Studies of biomass yield from a near-shore macroalgal test farm. J. Solar Energy Engineering. (In press).

#### ACKNOWLEDGEMENTS

Many people offered advice and provided assistance as this program progressed. Dr. Kimon Bird, formerly the Marine Biomass Program Manager for GRI, provided wise guidance and encouragement. His appreciation of the problems resulting from the extreme weather conditions that destroyed Ellwood Pier and the need to establish a controlled recirculating seawater system, are particularly appreciated. Dr. W. J. North very kindly provided us with warm-water, Mexican strains of Macrocystis, while Mr. Brian Paust collected and sent kelp sporophylls from Sitka, Alaska. Visitors from China (T. C. Fang and N. N. Kiang from the Shandong College of Oceanography) provided cultures of high-yielding Chinese strains of Laminaria and discussed their work with our staff. X. G. Fei, of the Institute of Oceanology in Qingdao, completed his collaborative work on the effects of light on kelp growth and presented it at the XIth International Seaweed Symposium in China. Professors Jiaxing Chen and Ru-ying Suo from the Yellow Seas Fisheries Research Institute visited Goleta and brought gametophytic isolates of the "Chinese" strains of Macrocystis now being experimentally grown there, as well as providing the color photograph of their coastal test farm (see Figure 47). Drs. Y. Sanbonsuga and H. Yabu of the Hokkaido Fisheries Research Institute, and the University of Hokkaido, Japan, kindly provided chromosome counts and illustrations of Macrocystis strains isolated from collections made in Goleta. Drs. Richard Snow and Sabodh Jain of the University of California at Davis served as consultants in the development of the Macrocystis breeding plan (Appendix D) that we are following.

The historical review of kelp utilization in California by P. Neushul, (Appendices A, B and C) was supported by a grant from the California Energy Commission to Dr. C. Purcell, of the Department of History of the University of California at Santa Barbara. The work on this project was administered through the Social Process Research Institute at UCSB.

We greatly appreciate, and would like to acknowledge important contributions made by visitors to NMI and UCSB. Mr. S. Kennerly (Australia) visited and made several useful suggestions about the potential future of underwater microscopy in kelp studies. Mr. S. Fain and Dr. W. Wheeler visited from Canada and discussed in-progess work on the isolation of kelp plastid DNA, and on macroalgal seasonality and physiology. We particularly appreciate the encouragement provided by Mr. Ken Wilson and Mr. Emil Smith of the California State Department of Fish and Game. The former worked closely with us in the successful site-selection study, installation and planting of the new kelp bed in Ventura County, while the latter made it possible for us to present a successful proposal to the California Fish and Game Commission, leading to the granting of leases to beds 26 and 29, in Goleta Bay and near Ellwood, to NMI.

As in the past, we must acknowledge the contributions made by D. A. Coon, A. W. Ebeling, Mahmood Shivji, A. Gibor and M. Polne and others (whose contributions are cited elsewhere in this report) at the Marine Science Institute and in the Marine Botanical Research Program in the Department of Biological Sciences at the University of California at Santa Barbara. Finally, we have benefitted from provocative and constructive discussions with Drs. W. J. North, V. A. Gerard, S. Manley, J. Ryther, B. Brinkhuis, and other colleagues in the Marine Biomass Program.

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#### INTRODUCTION

This annual report summarizes the work completed under contract 5083-226-0802 for the Gas Research Institute (GRI) at Neushul Mariculture Incorporated (NMI) from January 1983 through August 1984. Parts of the report should also be considered as the first draft of a chapter on yield in the forthcoming GRI-sponsored book on marine biomass production. We have also appended to this report a summary of the history of kelp harvesting and utilization in California (Appendices A, B and C), for possible inclusion as a chapter in the GRI marine biomass production book.

Some of the work done by NMI under GRI sponsorship since 1980 has recently been published or is in press, and has attracted co-funding from such diverse agencies and companies as the National Science Foundation, the National Institutes of Health, the Ministry of Education of the Peoples Republic of China, the California Department of Fish and Game and Southern California Edison Company. This co-funded work and other collaborative studies are discussed in detail in this report.

There has been a noteable "thinning of the ranks" of those involved in the GRI marine biomass program over the period this report covers. The final report submitted by the General Electric Company (GE) (Tompkins and Bryce 1984) concluded a period of maximum effort, unfortunately marked by some disappointments. Nonetheless, an assessment of progress made to-date, when compared with work done in China, Japan, the Philippines, Sweden and elsewhere is encouraging. It has become obvious that the difficult problems that are encountered, when farming in the open sea or even in coastal waters, will not be easily solved or even very well defined without long-term, persistent effort. It has also become obvious that NMI, now working directly with GRI rather than as a subcontractor, must help define and overcome the major barriers to commercial production of gas from kelp.

Briefly, this report first considers farming the sea in a general way (in Part II). Next, the kelp biology data base is reviewed (Part III). We briefly review specific NMI contributions to the data base (Part IV). Then, we review our 1983 - 1984 tasks and progress (Part V). Next, theoretical aspects of kelp growth are discussed (Part VI). New research techniques that have been developed and applied to kelp are reviewed (Part VII). In these sections, we emphasize the many different data sets that have been accumulated. Many of these are illustrated by the figures and tables in the report. It is important to remember that in nearly all instances the figure or table presented in this report is the first and as yet the only analysis done to-date on the data being discussed. Obviously, much more can be done with the data in hand in every case. We pose a number of questions about ways that these data sets can be interrelated and effectively used to guide future work. It is clear that the data base is reaching a point where the whole is greater than the sum of its individual parts. We conclude this report with a brief, speculative look toward the future (Part VIII).

## II. FARMING THE SEA AND THE MARINE BIOMASS PROGRAM

The ecosystems that man now uses for food production on land were originally deciduous forests and grasslands. With cultivation and the application of fertilizer, their productivity has been maximized in the last half century. It is now widely recognized that the successful modification of natural ecosystems into agricultural ones changes the patterns of energy flow and succession, while at the same time it retains and exploits the natural mechanisms of fertility, productivity and regulation that support the original forests or grasslands.

The "forests and grasslands" of the sea are unmodified ecosystems in which the marine farmer seeks to grow undomesticated plants and animals. In order to effectively domesticate and cultivate these organisms, the marine farmer must be familiar with their natural biological requirements and features. Marine farming techniques must be developed for genetic strain selection, seedstock storage, seedstock propagation, planting, maintenance, feeding or fertilizing, optimizing growth and ultimately harvesting. We must also learn how to identify good natural sites for marine cultivation. The question of what the marine farms of the future might be like, and what crops they might produce was addressed in a special symposium entitled "Agriculture in the 21st Century" (Rosenblum 1983) where results from the GRI coastal test farm work undertaken by NMI were presented as an example of a marine farm of the future (Neushul 1984).

## Marine Farms of the Future

Marine farming on a large scale, as envisaged by GRI when the marine biomass program was started, will certainly be a reality in the future. There will be a world population of 6 billion by the turn of the century, which will require 50% to 60% higher food production than in 1980. Clearly, a global food production and distribution system must be established before the end of the present century when the shortage of land will be a critical restraint on land farming for about two thirds of the countries of the world. Drought conditions and increasingly expensive fertilizer will make farming the vast, untamed areas of the sea essential.

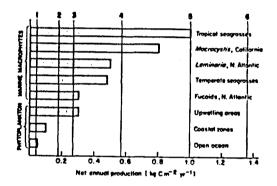
In 1981, the Food and Agriculture Organization of the United Nations published a book entitled "Agriculture: Toward 2000," which focuses on world population growth and the productivity of agriculture, forestry and fisheries, in attempting to predict what the future holds. The potential of large-scale marine farms is not considered, nor is information on aquaculture in China mentioned. Chinese aquaculture has been discussed by Ryther (1979), who points out that the Chinese catch only 3.1 million tons of marine fish yearly, while in contrast, they farm and harvest 17.5 million tons of freshwater fish. If this type of fresh water intensive fish cultivation could be extended into coastal waters it is likely that marine fish production would be similarly enhanced. Macroalgal cultivation in Chinese coastal waters has been very successful.

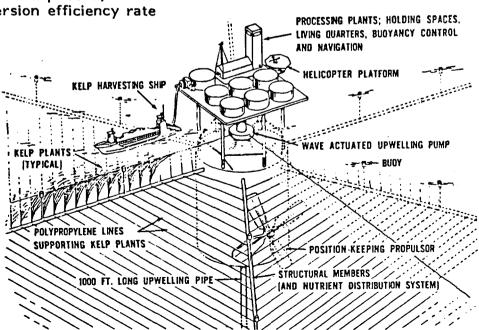
## Macroalgal Productivity

It is known that macroalgae are very productive. Natural populations of Laminaria off Nova Scotia can produce 90 dry metric tons per hectare per year (calculated from Mann 1973 - Figure 1). Gracilaria grown in tanks in Florida has produced 112 dry metric tons per hectare per year (Ryther, DeBoer and Lapointe Natural populations of the giant kelp, Macrocystis pyrifera, off California produce up to 55 dry metric tons per hectare per year (Neushul Mariculture 1980). The giant kelp has long been considered a good candidate for large scale cultivation (North and Neushul 1968). Wilcox (1980) calculated that an open ocean kelp farm could yield as much as 109 to 218 dry metric tons per hectare per year, but he treated the kelp plant as a black box, calculating only the energy that reached it's fronds and assuming a 2% rate of photosynthetic efficiency (Figure 2). Wilcox at that time had no physiological data to support this assumption and did not consider the environmental growth requirements nor the patterns of carbon assimilation and translocation that occur in the plant. Obviously, one must seek to understand how the giant kelp functions in the sea, how the morphology and anatomy determine carbon allocation in the plant, and how plants in nature and under cultivation interact with each other.

Figure 1. Net annual production of different kinds of marine vegetation compared (vertical lines) with terrestrial vegetation (1= desert scrub 2=lake and stream, 3=temperate grassland, 4=temperate forest, 5=tropical rain forest, 6= swamp and marsh vegetation)

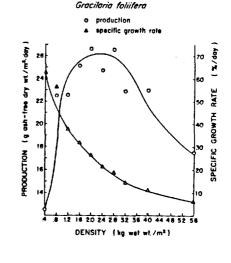
Figure 2. The quarter-acre module of the large-scale ocean food and energy farm visualized by H. A. Wilcox. Estimates of production were based on the known productivity of natural kelp beds, and a photosynthetic conversion efficiency rate of 2%.





A critical aspect of marine farm production estimates is the interaction between biomass density and production. Seaweeds grown in tanks show a reduced growth rate with increased density (Figure 3, from Ryther, DeBoer and Lapointe 1979) but natural populations of seaweeds growing in the sea have shown the opposite response to increased density (Figure 4, from Schiel and Choat 1980). Schiel and Choat showed that individual plant length, dry weight, stipe length, reproductive dry weight and total yield increased with increasing density. One-year-old Sargassum plants and three-year-old Ecklonia plants were studied growing in a shallow (2 to 6 m deep) open coast, tumbled-boulder area. Sargassum germlings were artificially settled on asbestos plates at four different densities, outplanted and studied. Brawley and Adey (1981) have disputed the claim that high density had a positive effect in this community and have put forth an alternate hypothesis that this effect was due to micrograzers. The question of how macroalgal density influences production is not yet fully resolved, but in order to farm macroalgae an optimum density must be determined.

Figure 3. The effect of plant density in tank culture of the red macroalga, Gracilaria was studied by Ryther, DeBoer and LaPointe (1979) show showed that as density in the tanks increased aove 2.0kg/ sq. meter, productivity decreased.



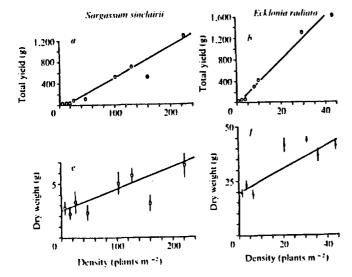


Figure 4. Studies of macroalgae in the sea by Schiel and Choat (1980) suggested that, in contrast to the findings of Ryther et al. (1979), total yield increased with increasing density for Sargassum and Ecklonia. Note that the Sargassum plant density of 240 plants/m2 is approximately equivalent to a biomass density of 13 kg wet wt./m2 and the Ecklonia plant density of 45 plants/m2 is approximately equivalent to a biomass density of 16 kg wet wt./m2.

The Chinese and Japanese marine farmers now plant, cultivate and harvest macroalgal crops valued at between 871 million and one billion US dollars annually (Doty 1982, Tseng 1981a, Tseng 1981b). Their ocean farms, used for growing both plants and animals, spread over many thousands of acres of sea surface are fabricated from ropes, nets, floats and other fittings anchored to the sea floor (Figure 5 and 6). Even larger farms are proposed by the Japanese who allocated some 43 million dollars in 1981 for open-ocean marine farm research, and who have spent even more for artificial reefs. There are no commercial macroalgal farms now operating in United States (US) waters, even though substantial quantities of Oriental seaweeds are imported every year.

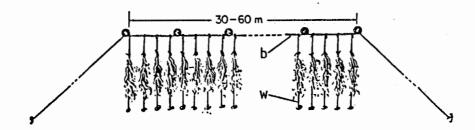


Figure 5. Macroalgae are grown on marine farms in the coastal waters of China, Japan and the Philippines. With the Chinese long-line system, non-floating kelps are grown on weighted curtain lines, suspended from buoyed surface lines (from Tseng 1983).

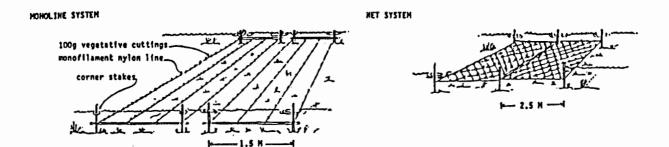


Figure 6. Philippine farms employ monolines (left) and nets (right) to farm the non-floating red alga Eucheuma on shallow tropical reef flats.

While the technology to farm the sea is being developed in other countries, efforts in the US are being curtailed. In 1980, Congress passed the National Aquaculture Development Act, which unfortunately has never received the necessary funding, and the Office of Management and Budget is unlikely to seriously consider funding the program now. Moreover, the Department of Interior, which previously has endorsed the development of aquaculture, has now decided to close some 33 hatcheries, and the National Marine Fisheries Service has proposed to terminate some 4 to 5 million dollars of aquaculture research. Furthermore, the National Sea Grant Program was proposed for phase-out and termination in the 1982 budget, and was similarly targeted in the 1983 budget. Fortunately there are far-sighted members of Congress who have insisted on continuing this program, but its fate is still uncertain. Hopefully, these difficulties will ultimately be seen only as a temporary pause in our progress toward learning how to farm the sea.

There are obvious advantages in developing a strong US marine farming program. The Sea Grant Aquaculture Plan for 1983-1987 proposes to support the legislative mandate of the Aquaculture Act of 1980 to ensure the development of a national aquaculture industry (Crowder 1982) which would produce valuable seafoods and industrial feedstocks to be consumed domestically. More than 60% of the fish products consumed in the US are now imported which creates a trade deficit of more than 2.5 billion dollars, or about 28 percent of the US trade deficit exclusive of petroleum products. It is likely that increasing demand and further world wide limitations of wild fishery stocks will result in even more expensive imports. Food production from aquaculture is now only three percent of the total US fisheries catch, and comprises only two percent of the total consumption of fishery products. The US is clearly the world's leading agricultural producer; it should also become a world leader in farming the sea. The GRI marine farming program, even in its present reduced form, is the largest single US effort to learn how to farm the sea.

## The GRI Marine Biomass Program

The goal of the marine biomass program is to grow marine macroalgae in large quantities for use as a renewable source of biomass for the production of substitute natural gas and other products. At the time that Howard Wilcox (1972, 1980) first proposed that large marine farms could be used for this purpose, the US was facing shortages of petroleum and natural gas. His theory that kelp was a suitable source of biomass was based on a simple estimate of the amount of solar energy per-unit-area that penetrates into the sea and how efficiently the kelp plant might trap this solar energy input. He was able to generate a great deal of enthusiasm and even optimism about the potential of macroalgal mariculture. This led to the initiation in 1972 of a large, cooperative effort to farm the open ocean called the Marine Biomass Program.

NMI first became involved in the Marine Biomass Program in 1979 as a reviewing agency, under contract to the Office of Technology Assessment of the US Congress. Our task was to evaluate the potential of large-scale open-ocean farms for the production of biomass energy. This was done in the context of assessing all potential biomass-energy sources (Office of Technology Assessment 1980a, 1980b) However, a separate NMI report dealt specifically with macroalgal biomass. This was used by the OTA and published separately as a Committee Print (Neushul Mariculture Incorporated 1980).

Ever since the Marine Biomass Program was first started, participants and observers would first be enthused by the optimistic proposals of those who used simplistic theories on one hand, and then alarmed by others who would raise major objections to the whole concept on the other. There were many theories, but there was little real data to use in testing them. After advice was sought from knowledgeable scientists (who did not encourage a restrained approach), expensive, large-scale and generally inconclusive experiments were undertaken. Some of these disappointments can be attributed to conceptual and/or engineering errors, but many were caused by the adverse weather conditions in California that resulted in a major, unprecedented decline in kelp bed production, that can be seen in the graph provided here of kelp harvests over this century (Figure 7). In fact, in retrospect it is remarkable that any kelp yield data at all was collected during a period when harvests from natural kelp beds in California fell from 100,000 to less than 5,000 tons per year. Assuming that the adverse conditions of the last few years are atypical, we anticipate working in an "improving" marine climate over the next few years, as progress is made toward learning how to farm the sea.

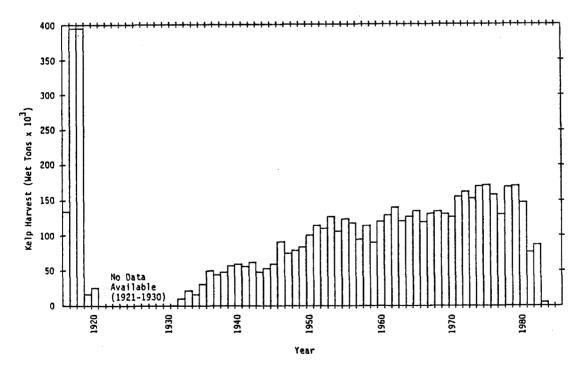


Figure 7. California Kelp harvests 1916-1983. Harvests from 1916-1918 were extremely high because harvesting availability, demand and effort were high. The demand for kelp decreased in 1919. The demand for kelp and effort increased gradually through the 1970's increasing harvests, although kelp availability declined somewhat from the 1920's to the 1950's. The kelp availability declined dramatically in the 1980's due to very low availability because of the El Niño climatic event, while demand remained high.

## III. KELP BIOLOGY DATA BASE

The theoretical maximum production potential of various macroalgae in the sea could best be determined from the production of macroalgal farms and of the individual plants grown on them. Until such farms become commonplace, the production of natural populations can be used as a baseline against which to compare estimates based on theory. The biennial and annual seaweeds cultivated in Japan and China for the past four decades (see Tseng 1981a and 1981b) are grown on lines and nets supported at the sea surface. The giant kelp, Macrocystis which has been grown on the NMI test farm is an ideal crop plant in that it is a perennial in nature and needs only to be planted once and has floats which obviate the need for vulnerable farm structures at the sea surface. This section of the report will first consider the natural ecosystem of the kelp bed and the individual kelp plants within this ecosystem.

## The Stability and Yield of Natural Kelp Beds as Model Ecosystems

Theoretical yield estimates can be compared with the productivity of natural kelp beds. Submarine forests of the giant kelp, Macrocystis, are found in the Northern Hemisphere only along the Pacific Coast of North America. This unique near-shore ecosystem was first surveyed and mapped from boats between 1911 and 1912 by the U. S. Department of Agriculture (Crandall 1912, Cameron 1915). The giant kelp plants produce masses of vegetation that floats to the sea surface forming a canopy (Figure 8). The California Department of Fish and Game was charged with the task of leasing and monitoring these resources belonging to the people of California. The California kelp beds were harvested early in the century as a source of potash, which was in short supply. During the First World War, the beds were harvested for energy-containing compounds, like acetone. The history of these large-scale harvesting efforts, when huge harvesters were operated 24 hours per day, is discussed in appendices A, B and C.

With the advent of aviation, aerial photographs were taken of the Pacific coast kelp beds and today the beds can be seen in satellite images. Most of the beds have been studied underwater by SCUBA-equipped divers, who began to explore them in the 1950's. These first mid-century studies were stimulated by concern that kelp harvesting for the production of valuable alginates was causing the once lush beds to disappear. Subsequently, there was and continues to be concern that sewage outfalls, offshore oil development and nuclear generating stations adversely affect this unique and vulnerable ecosystem.

Natural kelp beds such as the 1,000 acre kelp bed in Goleta Bay (Bed # 26 in the State leasing records) in Santa Barbara County have been relatively stable since they were first surveyed in 1911 and serves as a useful model for a large-scale near-shore marine farm. The Goleta Bay bed has ranged in size from 650 to 1,160 acres since 1955 although in the last two years it has disappeared completely due to the "El Niño" event. In 1980, the Goleta Bay kelp bed had a standing crop of about 44,000 metric tons and about 400 plants per acre (Coon 1981b). From 1972 through 1973 about 5,700 tons of material was dislodged each year during the winter storms and deposited on the beach (DeWitt unpublished data). The average drift plant including holdfast weighed about 100 kg. This would mean that about 57 plants were lost per acre or about 25% of the plants were detached by winter storms and deposited on the beach.

In the same period of time only 4,400 metric tons was harvested each year from this bed. Approximately one half of all plant material in natural beds is lost to grazing animals, to decay and sloughing and never does drift up on the beach. Kelp beds normally recover rapidly from such losses and where a plant is torn from a natural bed, fragments of the holdfast may remain and grow and the rapid re-seeding of juveniles from spores ensures the replacement of lost plants under normal conditions.

One of the factors in natural beds that was found to reduce the harvestable material substantially was sloughing. A detailed analysis of a single plant showed that as much as one third of the blade tissue on a frond having a surface area of 15 square meters could be lost due to partial or complete sloughing of the blade. If each frond in the harvest zone is approximately 19 m long, and has lost one third of its biomass at the time of harvesting, any reduction of this sloughing process could substantially increase yield. The prevention of whole plant loss could also substantially increase farm yield.

Prior to 1982, it was assumed that the kelp forests of Santa Barbara County were very stable, since records of beds such as bed #26 in Goleta Bay showed little change from 1911 to 1979 (Harger 1983). Recently, this assumption has been tempered by the effects of the El Niño event of 1983 and 1984 which has resulted in the virtual disappearance of most kelp beds in Santa Barbara County including bed #26. It remains to be seen whether these beds will re-appear. Clearly, the commercial farmer cannot rely exclusively on the self-regenerating capabilities of kelp plants to maintain a successful commercial farm.

In 1981 (Neushul, Harger and Woessner 1981), it was estimated that the potential yield from bed #26 at Goleta was 3.7 dry ash-free tons per acre per year. If these large plants could be cultivated at a density of 1 plant per 4 square meters they would yield 15.4 dry ash-free tons an acre if they grew at the same rate as plants measured in field studies of kelp bed #26. That is about four times the yield that could be achieved from a natural kelp bed.

In view of the published work of Schiel and Choat (1980), who claim that increased plant density produces increased plant growth and yield in natural algal populations in the sea, the "natural thinning" which occurs in kelp beds may not result in an optimally spaced kelp forest. Nonetheless, the beds appear to be healthy at their natural densities and this should be the first basis for a theoretical model. The theoretical systems analysis of marine farms is hampered by the lack of standardized methodology for the measurement and recording of data necessary to the design process. However, there is some terminolog which is commonly used: most phycologists agree that "density" refers to the number of individuals per square meter and that the "standing crop" refers to the amount of wet tissue per square meter. The term "stipe index" refers to the number of stipes (fronds) per square meter measured at 1 m from the bottom is less easy to measure and standardize but has been widely used (North 1957, McFarland and Prescott 1959, Coon 1981b).

Plant density studies made in the Goleta Bay kelp bed (Figure 9) by Coon (1981b) indicate that there are between 0.024 and 0.166 individuals growing per square meter. The stipe index of these plants was from 4.3 to 5.2 stipes per square meter while the standing crop was from 7.5 to 9.1 kg per square meter. The standing crop reported at White's Point was from 0.1 to 0.6 kg per square meter and at La Jolla was from 5 to 6 kg per square meter (North 1964). Studies at Paradise Cove have shown the kelp bed to have a stipe index of 3.5 to 5.0 and a standing crop from 4.4 to 5.8 kg per square meter (McFarland and Prescott 1959).

There is a long standing controversy as to what effect kelp harvesting has on kelp. Commercial harvesting of the California kelp beds is limited to the first three feet below the sea surface and has had no obvious adverse effects on their size, stability and density (Harger 1983). Aerial photographs and information received from the captains of kelp harvesters indicate that the beds are harvested about two or three times a year. Miller and Geibel (1973), who compared kelp plants in harvested and unharvested kelp beds, showed that hapteral growth rates were reduced among harvested plants. Coon and Roland (1980) found that frond growth rates were reduced among harvested plants while the frond initiation rate remained unchanged. Obviously, further work is needed to better understand the effects of harvesting on kelp.

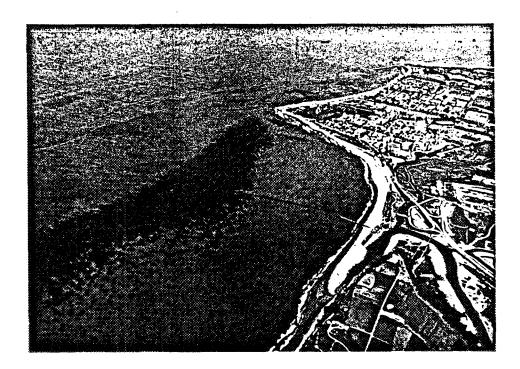


Figure 9. A 1,000 acre kelp bed (bed #26) in Goleta Bay, California, with Campus Point and the University of California at Santa Barbara, shown in the background, and Goleta Pier in the foreground. This bed, and bed #28 to the west has been leased to NMI by the Fish and Game Commission of the State of California.

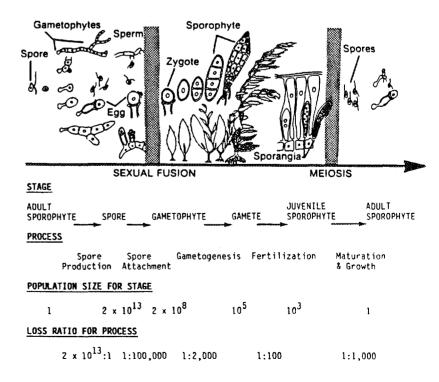
## The Life-History Strategy of the Kelp Plant

The continued survival of natural kelp beds, and of the floating masses of Sargassum, in benthic and open-sea habitats, depends on the natural fertility of the plants. The cost of planting a large-scale farm is substantial and the the Parsons study (Brehany 1983) indicated that it would take nearly two years of continuous work to plant a 6,000 acre biogas-producing farm. However, these cost estimates could be substantially reduced once it is possible to exploit the natural fecundity of the plant in the initial planting process. The replacement of lost plants was part of the experimental work conducted at the NMI test farm but it is also obvious that plants are replaced by natural recruitment.

The successful cultivation of the giant kelp depends on a complete understanding of the life history stages of the plant (Figure 10). In the sporophytes, meiosis occurs in the zoosporangia of the sporophylls producing zoospores. The zoospores are released into the water column, swim, settle into the boundary layer at the water/rock interface on the sea floor and attach. These then germinate to form microscopic male and female gametophytes, which grow, mature and undergo gametogenesis. The male gametophytes produce sperm which are released and swim to and fertilize the female-gametophyte-produced eggs. The zygotes germinate and grow into the macroscopic sporophytes.

In quantitative terms, the giant kelp is very fertile and has a huge reproductive potential (Figure 10) because it produces many zoospores. Yet, in spite of this production of a huge number of progeny, adult plants only replace themselves and the number of adult plants is relatively stable. Kelp populations sustain considerable losses at each stage in their life history. The management of kelp farms could reduce these losses in order to take advantage of the reproductive potential of the plant and increase the number of plants on the farm. Each adult sporophyte produces approximately 2 x 10 exp 13 zoospores in its lifetime, assuming a 6 year

The life-history of Figure 10. Macrocystis in qualitative and quantitative terms, showing the sporophytic and gametophytic the life-history stages (above), and potential, and estimated actual numbers of individuals at each stage, and their reproductive potential (below). The losses at each transition point is estimated high indeed. to be very Cultivation and improved planting technology is likely to reduce these losses and consequently to greatly increase yield over that seen in natural populations.



lifespan, (calculated from Neushul 1963). The settlement rate has been estimated from laboratory experiments where a given number of zoospores were introduced into flowing water in a device called a water broom (Charters and Neushul 1979). The zoospores that attached to a flat surface were counted after the population of zoospores passed over it. For example, 2.8 x 10 exp 8 zoospores in 400 ml of water were passed over a glass slide with an area of 19 sq cm at a rate of 7 cm/sec. Counts showed that of this total, 3,890 zoospores had become attached to the surface. Therefore, approximately one zoospore in 100,000 settled. If natural settlement occurs somewhat like what was observed in this settlement experiment, in its lifetime an adult sporophyte could produce 2 x 10 exp 8 zoospores that would successfully settle.

The zoospore germinates to form either a male or female gametophyte, but environmental conditions are such that only a few survive to produce eggs and sperm. Although multicellular and multi-gametangial gametophytes can be grown in the laboratory, none have been found yet in the sea. For this discussion, we will assume that spores germinate quickly to form one to two-celled gametophytes. Of these, each female produces one egg and the males form one or more sperms which are released and swim to and fertilize an egg. Probably only one female in one thousand ever survives to produce an egg, and only one in one hundred eggs is ever fertilized. With 2 x 10 exp 8 zoospores settling, and half of them producing female gametophytes, this would mean that the adult sporophyte could produce 1,000 fertilized zygotes. Therefore, only one in one thousand sporophytes needs to survive to maturity for direct replacement of the parent.

## The Theoretical Kelp Lifespan

Plant life expectancy is a critical factor in farming a perennial macroalgal crop. A "half-life" defined as the time it takes to lose one half of a group of individuals, assuming a uniform logarithmic mortality rate (North 1964) can be used to compare mortality rates of plants in experiments of various durations.

Estimates of the half-lives of plants growing in the natural kelp beds of Macrocystis angustifolia in the Santa Barbara area varied widely but were significantly greater than plants growing in the Los Angeles and San Diego kelp beds which had a half life of 4.0 to 15.9 months (North 1964). Plants growing near Gaviota had half-lives of 69.1 to 85.8 months (North 1964). Shorter half-lives have been reported more recently for plants in the Goleta Bay bed, between 4.1 and 10.6 months (calculated from Coon 1981). Life expectancy probably increases with increasing age. When one cohort of recruits was followed in the Del Mar bed in the San Diego area, the half-lives increased from 0.6 months for two-month-old plants to 83.9 months for nine-month-old plants (calculated from Rosenthal, Clarke and Dayton 1974). Several adults of a group of Del Mar plants with a half-life of 10.5 months grew to be five to seven years old (calculated from Rosenthal, Clarke and Dayton 1974). Some of the Santa Barbara plants with half-lives of 70 to 85 months could live 20 years or longer (using the projected time for 10% survival rather than 50%).

The studies of natural populations in Argentina, by Hall (Hall 1980, Hall and de Zaixso 1979), and in Chile by Santileces (Santelices and Ojeda 1984) are important to the study of kelp lifespans. Hall studied kelp beds in the Bahia de Camarones (44deg 48min 30sec south), over a period from 1975 through 1979. He measured 100 plants each month, taken from the central region of the huge offshore kelp bed at this location. He recorded growth rates of 44.4 g/per day per plant, and observed three cycles of regeneration after severe storm damage. Hall's measurements suggest that kelp plants can persist for many years despite having to replace themselves almost every year. These results were confirmed in data collected from bed 26, in Goleta Bay where plants torn out in a storm were replaced by sexual reproduction in as little as 12 months.

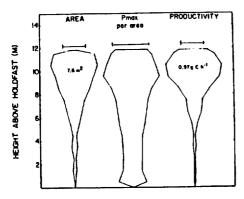
## Physiological Ecology of Kelp

The coastal marine environment varies seasonally, just as the terrestrial environment does. Indeed, in southern California, the marine seasons are much more pronounced than those on land. Underwater irradiance in the winter is 22% what it is in summer, while surface irradiance is 43% in the winter what it is in the summer. Water motion drag effects are much more severe than are atmospheric winds (Charters, Neushul and Barilotti 1969). An important effect of water motion is its effect on the uptake of nutrients. The ocean in southern California is a marine desert, in the sense that for most of the year, the plants are limited in their productivity by an adverse chemical environment. Nutrients in the form of nitrogen, phosphorus, and carbon are simply not present in quantities great enough to support optimum growth. On land, desert plants compensate by storing water, while in the sea, marine plants compensate by storing nitrogen and carbon. The analogy can be carried further. Many annuals appear in the desert only after spring rain storms whereas in the ocean, a "storm" often results in upwelling which provides nitrogen and, with clearer upwelled water, more light. Both of these give rise to high plant productivity. The effects of light, temperature, nutrients and water motion are considered here.

#### A. LIGHT

During the winter, when water clarity is generally low, a combination of wind and tide can cause the phenomenon known as upwelling. Cold, clear, upwelled water allows more light to penetrate into the subtidal. Since plants often integrate light, such upwelling can provide a good portion of the light needed for plant maturation (Lüning and Neushul 1978) or can result in the storage of carbon. Lüning and Neushul (1978) have determined that between 40 and 60 uE/sq m - sec are required for gametogenesis. One of the requirements for gametogenesis is blue irradiance, and this necessary blue light is available throughout much of the year off Santa Barbara.

Fain (1979) indicates that photosynthesis of gametophytes is saturated at quantum irradiances between 35 and 70 uE / sq m - sec. A quantum irradiance level in excess of 140 uE / sq m - sec inhibited photosynthesis. Compensating irradiances (the level at which photosynthesis just matches respiration) were on the order of 1.4 uE / sq m - sec (Fain 1979). Embryonic sporophytes have been studied by Fain and Manley (1979). Fain (1979) determined that irradiance compensation, saturation and inhibition occurred at 2.8, 35 to 70 and 210 uE / sq m - sec respectively. Manley (1979) determined that the irradiance saturation level for embryonic sporophytes was between 54 and 68 uE / sq m - sec. Wheeler (unpubl.) determined that the saturation levels for adult sporophytes was between 125 and 300 uE / sq m - sec. Both Manley (1979) and Wheeler indicate that the saturation level appears to be related to the nutritional status and age of the blade tested. These factors also affect the maximum photosynthetic rate (Pmax) and the Ks value (the irradiance necessary to produce a photosynthetic rate of half the maximum value). Meristematic tissue and senescent tissue have lower photosynthetic rates than mature tissue. The Pmax distribution along a kelp frond is bell shaped (Wheeler 1980, Figure 11). Because most of the tissue on a kelp frond is within the top one-third of the water column (Wheeler 1978), most photosynthesis takes place within the canopy near the surface where irradiance is the highest. However, the embryonic sporophyte and gametophytes must utilize only dim irradiances on the sea floor. Because these plants can store energy in the form of photosynthates, growth is not inhibited by lack of continuous irradiance throughout the growing period.



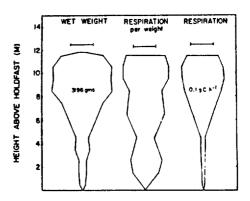


Figure 11. Diagrammatic representation of a kelp plant (left), as though it were a single, Laminaria like blade, showing the distribution of area, photosynthetic capacity and (by calculation using these two measurements) productive capacity with depth. The diagrams shown on the right also represent the plant as a single blade, showing the distribution of wet weight, respiration rate per unit wet weight, and calculated rate of respiratory carbon loss rate with depth. A comparison of the rate of carbon gain (left) with the respiratory rate (right) illustrates the great capacity of the plant to use light to fix and retain carbon (from Wheeler 1978).

Lüning and Neushul (1979) have shown that gametophytes of Macrocystis survive at quantum irradiances as low as 20 uE / sq m - sec. There are no published records for the minimal survival irradiance levels for young or mature sporophytes of Macrocystis. However, in Laminaria and other laminariaceous sporophytes, the irradiance range has been determined to be approximately 0.7% of the surface irradiance, or 15 MJ / sq m - year or 70 E / sq m - year (Lüning and Dring 1979).

#### **B. TEMPERATURE**

There are relatively few studies of the temperature tolerance of brown algae generally and of Macrocystis in particular. Fain (1979) has shown that the photosynthetic rates of Macrocystis gametophytes are a function of temperature, and that temperatures in excess of 25 C were deleterious. Embryonic sporophytes also showed decreased rates of photosynthesis at these high temperatures. But these were short-term experiments (24 hrs incubation) and so it is likely that tolerance levels would be even lower if the plants were exposed for long periods of time. Growth is a better indicator of long-term temperature effects. Lüning and Neushul measured growth of Macrocystis gametophytes growing at different temperatures and found maximum growth at 17C, with growth rates decreasing rapidly at higher Reproduction is even more sensitive to temperature levels as temperatures. gametogenesis occurs at a maximum level of 12C, and the rate of gamete production decreases rapidly at higher temperatures. Lüning has shown that Laminaria sporophytes cannot tolerate temperatures over 20C for longer than a week. southern California water temperatures reach these levels every summer, but while local species of Macrocystis tolerate these levels they do not grow well. It is possible that a warm-water tolerant strain of Macrocystis could be developed and introduced to circumvent this problem, as has been done in China with Laminaria.

#### C. NUTRIENTS

Nitrogen plays an important role in regulating the metabolism of kelps and high nitrogen levels may well influence the turnover time of protein synthesis, making it possible for nutrient-enriched plants to better withstand high temperatures. The rate of scenescence and sloughing accelerates at high temperatues and low nitrogen levels. For example, Laminaria uptake rates for nitrogen saturate at around 10 micromoles nitrate per liter and growth is maximized, but nitrate levels above 10 micromoles do not increase growth rates. Both Laminaria and Alaria accumulate nitrate in their tissues to levels 3,000 times greater than those found in the surrounding seawater (Chapman and Craigie 1977, Buggeln 1978). Laminaria has been shown to store excess nitrate for as long as two months, while Alaria stores it for only a few days. Preliminary data suggests that Macrocystis, like Alaria, stores nitrogen for only a few days. Efforts to fertilize various kelps in the sea have been successful on both an experimental level and in China for commercial kelp production.

## D. WATER MOTION

Preliminary laboratory experiments to measure nutrient uptake by <u>Macrocystis</u> blades under controlled hydrodynamic conditions, indicated that when water flow over the plant blade was laminar rather than turbulent, uptake of nutrient was sub-optimal. It is clear that kelp plants are "hydrodynamically-adapted" to a wide range of water motion conditions.

## Seasonality and Ocean Variability in Southern California

Empirical studies over the last ten years have provided a great deal of information about the conditions in the Pacific Ocean and the overlaying atmosphere and it is now clear that the sea and air act as a system. Nothing demonstrates this more clearly than the recent (1982 to 1983) "El Niño" event (Cane 1983).

There is a continuing effort to define cause and effect relationships between natural oceanographic conditions and biological responses (Barber and Chavez 1983). Fish catches, for example, fluctuate widely in almost all of the world's fisheries with time. These fluctuations are attributable to fishing intensity, as well as to variations in the oceanographic conditions in a given fishing ground. Lasker (1978) has illustrated the seasonal cycles of physical and biological parameters, particularly with regard to fish and plankton in southern California.

The upwelling index used by Lasker (1978) may be important in kelp ecosystem studies, since upwelling provides nutrients for kelp growth. Tont (1976) showed the relationship between climatic variations and diatom production in southern California. This study, based on measurements made from 1928 through 1939, indicates that 85% of each year's diatom biomass results from three major blooms lasting about 5.5 weeks each, and that these blooms coincide with upwelling. Mearns (1978) has also documented the variation in the marine climate of southern California.

## The Response of Kelp to the Environmental Complex

The metabolic processes that occur in the giant kelp plant can be summarized graphically (Figure 12, from Black 1948). This figure illustrates the different pathways that lead to the production of mannitol, alginic acid, laminarin, cellulose, pigments and other plant components which are synthesized from combined light-energy, carbon and nitrogen sources. These materials come from the environment and are obviously not present in constant amounts throughout the year and the rates at which they are taken up are a function of temperature which also changes with season.

The pioneering work of Black (1948) showed that Laminaria composition changes with season and this observation has been confirmed with respect to the chemical composition of Macrocystis by Lindner, Dooley and Wade (1977) (Figures 13 and 14). A study by Shokes and Callahan (1978) of kelp canopy development, also illustrates that the amount of biomass varies greatly by season. North (1967) discussed how Macrocystis adapts to changes in environmental conditions although he also pointed out that kelp growth can be stimulated or depressed in localized situations for inexplicable reasons. In a study by Harger (1979), the variation in kelp harvesting rate follows the solar irradiance and nutrient availability (Figure 15).

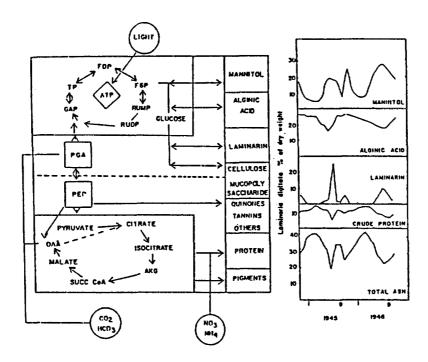


Figure 12. A graphic representation of kelp metabolism, showing how light, carbon dioxide, bicarbonate, nitrate and ammonia ions (circles) are taken up and metabolized to produce pigments, proteins, carbohydrates and other materials. The pioneering work of Black in 1945-6 on Laminaria (illustrated to the right) showed for the first time that the chemical composition of the kelp plant varies with season, presumably because of differences in the availability of light and nutrients (from Black 1948).

Dayton and Tegner (1984) recorded the adverse effects that the "El Niño" winter storms of 1982-3 had on the kelp forests near San Diego, which were severely damaged. Subsequently, large scale recruitment occured, but these juveniles, along with the few storm survivors were subsequently damaged by high temperatures and low nutrient levels present during the summer of 1983. They point out that this combination of storm, and temperature/nutrient damage may have long-lasting consequences for the kelp communities of California, since in a natural kelp forest undergrowth can become established that can resist subsequent recruitment by Macrocystis.

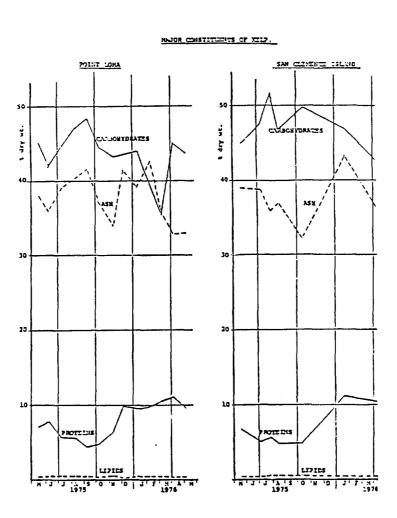
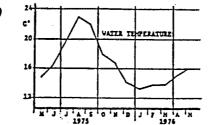
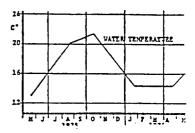


Figure 13. The major chemical components of <u>Macrocystis</u> (carbohydrates, ash, proteins and lipids) have been found to vary with season (from Lindner, Dooley and Wade 1977).

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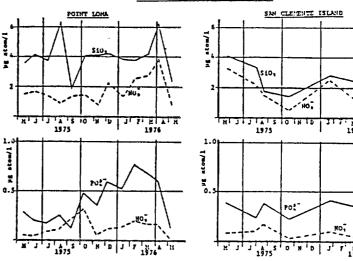
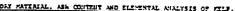
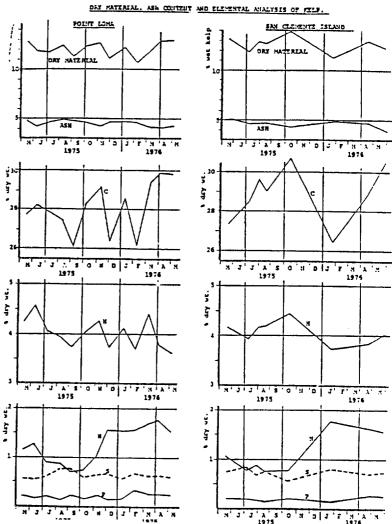
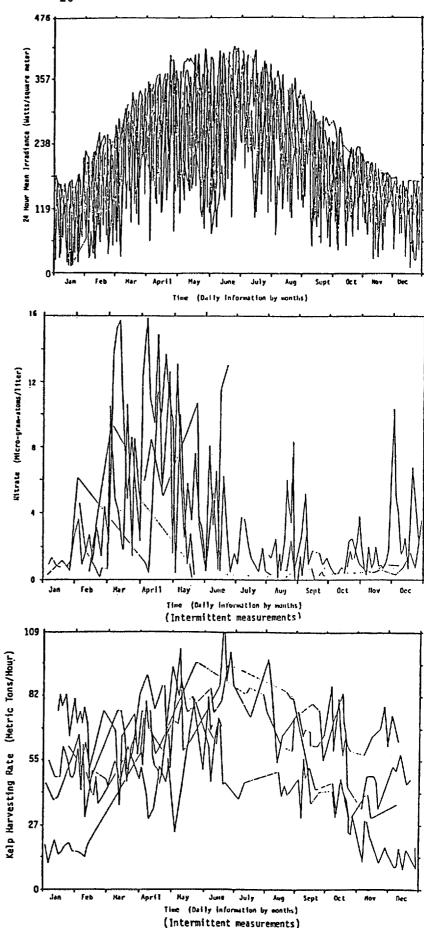


Figure 14. Measurements of silicon, nitrate and phosphate available to kelp plants in the sea, were made by Lindner, Dooley and Wade (1977) in nearshore (Point Loma) and an offshore (San Clemente Island) kelp beds, where samples were taken for analysis of dry weight, elemental ash content and composition. These results show how seasonal variation in the availability of these substances (along with temperature) influence the chemical composition of kelp plants, substantiating the concepts of kelp metabolism illustrated in Figure 12.





The availability of Figure 15. light (top) and nitrate (middle) affects the amount of kelp that could be harvested from one thousand acres of kelp in Goleta This Bay (shown in figure 9). that the time illustrates greatest production (May-June) corresponds to the time of peak light intensity, and is preceeded by a period of upwelling nutrient enrichment. Seasonal variation in growing conditions influences yield in a natural kelp The Stauffer Chemical forest. Company harvesting rates, given in metric tons per hour, show that rates vary seasonally as the canopy formation rate changes, and that as much as 100 metric tons per hour can be harvested under the ideal growing conditions (from Harger 1979).



#### IV. 1980 - 1982 PRIOR STUDIES

NMI joined the GRI-sponsored Marine Biomass program in May 1980, as a subcontractor to the General Electric Company. NMI was first assigned long-term tasks dealing with kelp characterization, cultivation and genetics, topics that are still part of the work that is now funded directly by GRI. During this research period it was determined that as kelp juveniles grew larger their growth rate declined (Figure 16). Preliminary experiments in nutrient uptake were also designed using a "water tunnel" to determine whether plants would sustain the same uptake rate with lower nutrient concentrations but higher water motion (Figure 17).

## The Yield Question

In 1981 it was decided that the key activity for the marine biomass program for the 1981 - 1983 period would the determination of the sustainable yield of adult Macrocystis plants. Subsequently, NMI stopped working on individual, genetically-defined plants and began work on the "yield question" in April 1981. Experiments were designed and initiated to answer several long-standing questions about the potential yield of the giant kelp under cultivated conditions. For example, would kelp plants stand up to repeated, severe harvesting? What amount of biomass would they produce? Would all the plants respond in the same way to growing conditions? What would the plant-to-plant variability be? What would the season-to-season variability be? How could the kelp plants be best "cultivated" in the sea to enhance their health and yield? How would "wild" plants respond to high-density planting and fertilizer application?

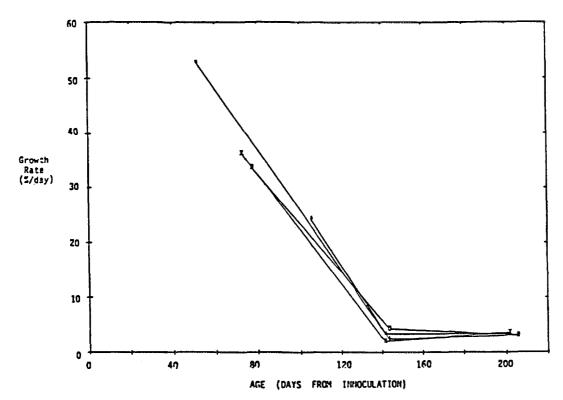


Figure 16. It is known that juvenile kelp plants can grow at exceptionally fast rates, (ca 50% per day increase in wet weight). NMI studies of juvenile kelps show that, as would be expected, these rates drop as the plants mature, presumably because of the formation on non-photosynthetic tissue and self-shading.

These and other questions became the focus of presentations that were made to the GRI Advisors, at seminars held with other members in the Marine Biomass Program and at meetings with GE specialists and consultants. The questions could not be answered in a definitive way as the necessary data base was inadequate and the theoretical framework necessary to effectively cultivate Macrocystis in the sea had yet to be developed. Consequently, the first task was to provide at least some answers and workable hypotheses from data gathered from prior studies of natural kelp forests (as reviewed in section III). Finally, the process of selecting a site on which to install a coastal test farm, which could be planted with kelp plants and experimentally cultivated and harvested was also begun.

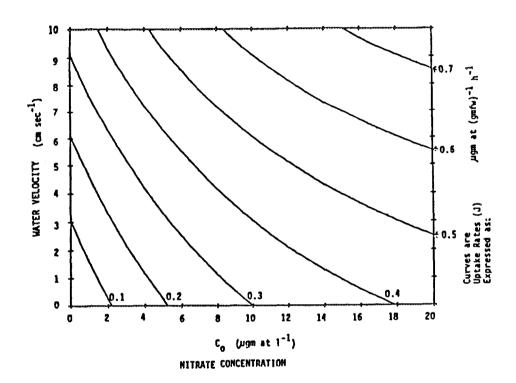


Figure 17. Measurements made of nutrient uptake rates of kelp blades under defined hydrodynamic conditions in the laboratory, show that uptake rates are influenced both by nitrate concentration and water velocity, showing that a given assimilation rate can be achieved either by rapid water motion or by high nutrient concentration. The curves are constant rates of nutrient uptake expressed as microgram atoms taken up per gram of fresh weight per hour. These "rate curves" intercept the ordinate and abscissa where water velocities and nitrate concentrations are listed. It can be seen that there is a tradeoff between flow rate an nutrient concentration. For example at a flow of 4cm/sec a nutrient concentration of 1 microgram-atom per liter will give an uptake rate of 0.2 ugm at per gram fresh weight per hour, while a lower flow rate 1cm/sec will require a concentration of 4 ugm at/l to produce the same uptake rate. These preliminary results illustrate the importance of making accurate and detailed hydrodynamic measurements in selecting a farm site and planting in the sea.

## The Coastal Test Farm

The coastal test farm was installed to test the predictions of stability and yield that had been made based on studies of natural kelp-forests. The farm was planted with 722 mature Macrocystis angustifolia plants transplanted from the in-shore area of bed 28 near Ellwood, California. Each plant was cut loose from the sea floor with a pruning saw and its fronds were cut off at aproximately 1 meter below mean tide level. This pre-planting pruning established a uniform initial starting point for all the plants. The plants were placed in nylon-mesh bags and taken to the surface where both the plant and holdfast were weighed and fitted with plastic tags. They were then transported by boat to the farm.

At the farm site, the holdfasts were enclosed in gravel-filled mesh bags which were dropped to the sea floor and tied to one-half inch chain and rope lines already laid out in a planting grid with rope-chain intercept points serving as sites for each plant. The farm consisted of two one-half acre plots each divided into five sections. There were two low-density sections each with 0.0625 plants per square meter on a four-meter grid, two medium-density sections each with 0.250 plants per square meter on a two-meter grid and one high-density section with 1 plant per square meter on a one-meter grid. Each plot was established at a depth of about 23 feet (7 m) and was oriented perpendicularly to the prevailing swell. Of the total 722 plants, 72 were chosen as "sample plants" to be studied extensively. The fronds of each of these sample plants were marked with a plastic tag on a quarterly basis and the growth of individual fronds and the initiation of new fronds was recorded. Of the remaining 650 plants, 6 were used as handling controls for the sample plants and were measured underwater to avoid damage due to handling. The location and position of the farm is shown in Figure 18 and Figure 19.

## Stability of, and Yield from a Coastal Test Farm

The original farm was planted with 12 tons of wet biomass and in a fifteen month period five harvests were made at the site which yielded a total harvest of 61 tons. This farm yield experiment indicated that the giant kelp is a very productive and resilient plant. Since the yield of a kelp farm is the collective product of many individual plants, the study of these individual plants was important to the study of farm yield.

The study of plant growth on the farm site involved growing a group of individuals of the same age and then harvesting them at a specific time interval to determine their whole plant increase or decrease in weight over time. The process of measuring growth and frond initiation in a whole giant kelp plant is difficult given the ocean environment and the mass of heavy vegetation which Macrocystis plants produce. As discussed in section III, before this study most scientists have measured only a part of the plant, usually the number of stipes near the holdfast or the length, weight and area of the fronds and their various parts, and then extrapolated these measurements to give the total estimated plant yield. The wet weight measurement undertaken in this study involved harvesting the canopy from individual plants on the farm, bagging this material and taking it to the surface where it was weighed.

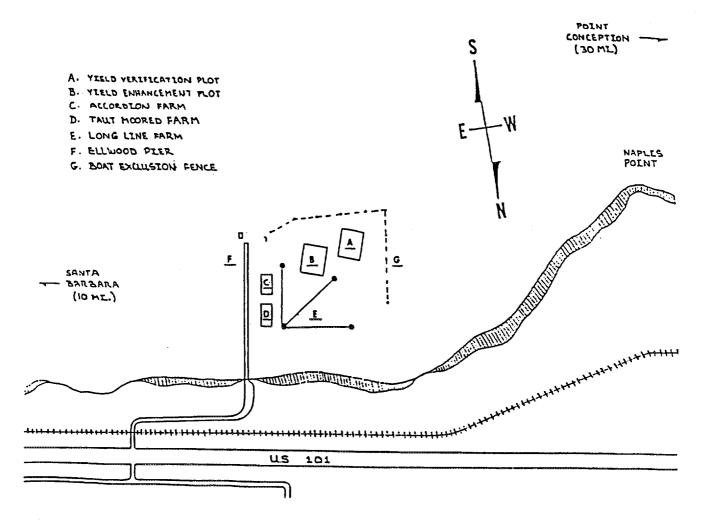


Figure 18. The Goleta test farm is located 10miles from Santa Barbara, near Ellwood Pier, NMI kelp bed 26 in Goleta Bay is to the east, and NMI bed 29 is west of this experimental site. Yield-verification and enhancement plots are shown along with other experimental in-the-sea plantings at this location.

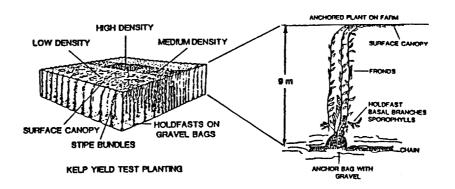


Figure 19. A diagrammatic representation of a  $40 \times 60m$  experimental plot, showing plants at low, medium and high density plantings, and an individual plant attached to an anchor bag, tied to a chain and rope-line grid layed out on the sea floor at a depth of 9 meters. Each plot was planted with 722 plants, that were harvested every three months.

In the density-yield experiment, all the plants in each plot were harvested at one meter below the mean low tide level on a quarterly basis. The number of fronds on each plant were counted at one meter above the holdfast and at the harvest point. The canopy was then cut off and placed in a mesh bag by a diver and taken to a boat where the excess water was drained from the plants. The bags were then weighed to the nearest 0.5 kilograms. The loss or death of individual plants was recorded each quarter and these were replaced at the end of the harvest. Plants that were living, but had no fronds that reached the harvest point, were recorded as plants with no canopy but not replaced.

A fertilizer application experiment which involved applying fertilizer to the yield farm on a regular basis was carried out in 1982. One of the two half-acre plots was sprayed with fertilizer four to five days a week depending on the weather, while the half-acre control plot was not sprayed. The fertilizer used was 91 kg of solid ammonium sulfate disolved in sea water and applied at a concentration of 3.02 M/I (199.6 g/I) at an average of 37.8 grams per square meter. The fertilizer was sprayed from a boat adapted to pump the fertilizer from a spray-gun directly onto the kelp canopy (Figure 20).

Density affected plant frond production (Figure 21), plant wet weight (Figure 22) and projected yield (Figure 23). Plants grown at low levels of density had the highest number of fronds and highest wet weight and the lowest projected yield. Plants grown at high density had the lowest number of fronds, the lowest wet weight and the highest projected yield. The average wet weight production per day of individual plants was found to be 0.63%, 0.69%, 1.3% and 0.73% for the fall, winter, spring and summer harvest respectively. The average whole-plant growth per day for the entire year was calculated to be 0.71%. Although fertilizer was applied all through the year, the only significant increase in yield that resulted from this application took place in the late summer of 1981 (Figure 24). There was considerable plant-to-plant variability. Some plants grew consistently more rapidly than the average, and in some cases they produced as much as one kilogram of wet biomass per day (or 91 kg harvestable yield for an average 91 day growth period, Figure 25).

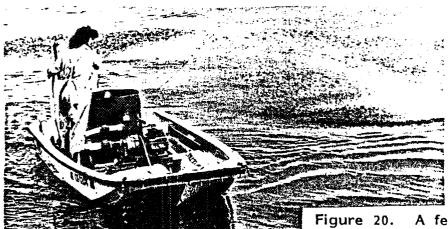


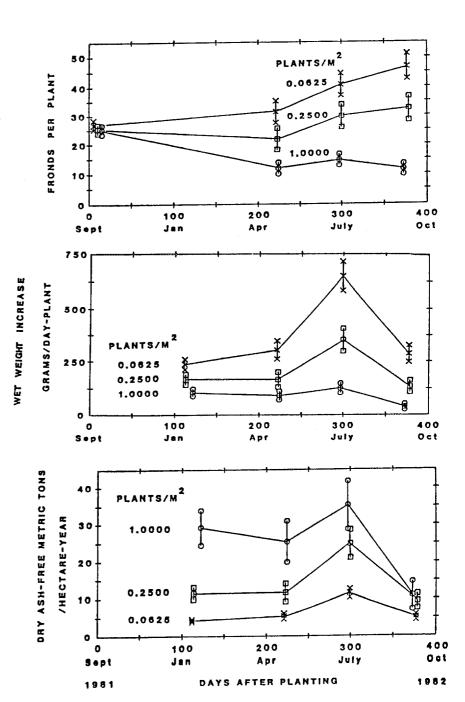
Figure 20. A fertilizer sprayer, copied from a Chinese design, was used to suck water from the sea, mix it with fertilizer in a platic tank and then spray the nutrient-enriched mixture out onto the kelp fronds at the sea surface. During low-nutrient periods of the year, this "cultivation" technique quadrupled kelp yield.

It was of considerable interest to compare the stability of the test farm with that of a natural kelp bed. Losses from different density plantings on the test farm ranged from 0.4% loss in the spring of 1982 in the low density plot to a 38% loss in the fall of 1982. These results were very similar to those gathered by Rosenthal, Clarke and Dayton (1974), and Hall and de Zaixos (1979) in that the adult plants were lost in higher numbers during the winter storm periods. These plants were also lost in higher numbers as they grew larger. Just after the plants were planted on the farm the loss rate for the average plant (25 fronds average) was less than 5% whereas the loss rate in the same planting density after one year was 35% (40 fronds average). The loss from the Goleta Bay kelp forest was estimated to be approximately 5,760 tons per year or 25% of the total population of the bed. The losses recorded at the farm site in 1982 averaged approximately 22% which indicates that plant loss is a natural part of the life cycle of the bed and comparable to that of a natural bed at the same depth.

Figure 21. Measurements of the growth of kelp plants in the sea on the coastal test farm, show that planting density influences the number of fronds each plant produces. At the beginning of the experiment all the plants had the number of fronds, but 400 later the low density plantings had increased, medium density plants stayed about the same and high-density plants had produced fewer fronds to replace those harvested.

Figure 22. The test-farm yield experiment showed that planting density influences wet weight production, expressed here in grams produced per day per plant. Again low-density plants were the most productive. Seasonal variation in production by individual plants is also seen in this graph, superimposed on the differences attributable to planting density.

Figure 23. The projected yield, in dry ash-free metric tons per hectare per year, shows that high-density plants cannot sustain a high yield, while low and medium-density plants steadily produce biomass at high rates even when harvested quarterly.



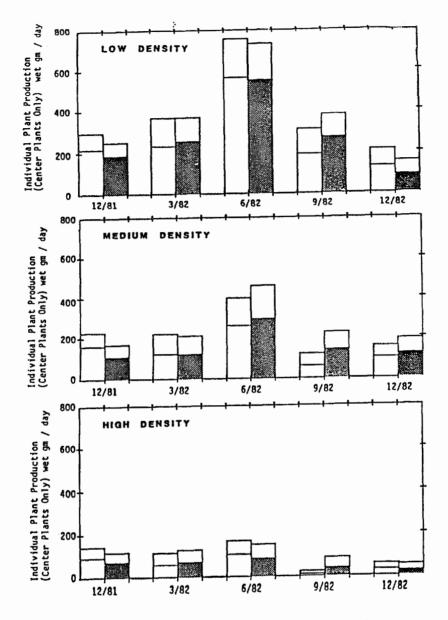


Figure 24. As noted earlier, fertilization (see figure 20) of low, medium and high density plantings, produces substantial increases in yield of the fertilized individual plants (shaded bar) compared with unfertilized bars (unshaded bars), expressed as wet weight increase in grams per day, when nutrient levels are low. A significant difference is seen in these graphs when medium and high density plants for the September 1982 harvest are compared. This period was preceded by a summer "nutrient drought" period. The high density plants quadrupled their yield, and the medium density plants doubled in yield, in the 9/82 harvest. At all other times of year, and at all planting densities, no significant differences in yield between fertilized and unfertilized plots were seen, as the comparisons of shaded and unshaded bars show. The 95% confidence interval is shown at the top of each bar.

The results of the 1981-82 yield experiments can be described both in terms of individual whole-plant production, and in terms of fronds produced. The final yield of a kelp farm is influenced by the number of kelp fronds produced by the plant and the rate which they grow up into the harvest zone. The yield experiment results indicated that the number of fronds per plant showed a net increase for plants grown at low density while the number of net fronds per plant decreased for plants grown at high density. This decrease indicates that the rate of yield achieved at high density was not sustainable over the long term.

If the assumption is made that no fronds were lost, and all fronds that were produced were eventually harvested, it is possible to calculate an estimated frond production rate for plants grown on a farm. The plants grown under low density conditions yielded an average of 6.5 fronds harvested per month with a net increase of 1.7 fronds per month. Their total gross production was 8.2 fronds initiated per month. The plants grown at medium-density yielded an average harvest of 4.8 fronds per month with a net increase of 0.6 fronds per month. Their total gross production was 5.4 fronds initiated per month. The high-density plants yielded an average harvest of 2.0 fronds per month with a net decrease of 1.1 fronds per month. Their total gross production was 0.9 fronds initiated per month. These rates all fall within the range reported by Gerard (1976) for natural kelp beds in central California.

The results of the 1981-1982 yield experiments show that the giant kelp Macrocystis angustifolia responds to cultivation and withstands repeated harvesting. Planting density affects the behavior of individual plants significantly. Plants grown in conditions of high density have a reduced frond initiation rate and yield. Plants grown at medium density maintain their frond initiation rate and yield and plants grown at low density increase their frond initiation rate and yield. The test farm proved as stable as a natural kelp bed and showed similar patterns of plant growth and loss. The plants on the test farm grown at different densities responded to fertilizer application during the low-nutrient, high-irradiance months of late summer, with the medium-density yield being doubled and the high-density yield being quadrupled with the application of fertilizer.

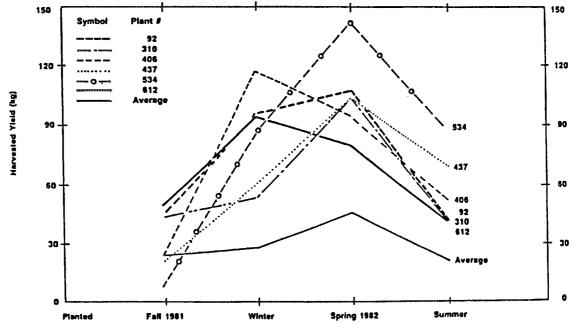


Figure 25. Since all the plants grown were individually numbered and harvested, on the test farm, it was relatively simple to identify the "super kelps" in test plots. Some of these plants, shown here by number, produced over a kilogram of wet biomass per day, which was significantly greater than the average rate of biomass production. This data set suggests that high-yield plants can be obtained through genetic selection and breeding programs.

## V. 1983 - 1984 TASKS AND PROGRESS

The "yield" studies started in 1981 were continued in 1983 - 1984, including yield verification and yield enhancement work. The coastal test farm was maintained and environmental conditions were monitored at the farm site. Preliminary genetics and plant characterization work and the international cooperative program continued. Table 1 lists the 1983 - 1984 tasks, and the progress that has been made on each of them. Work continues in the 1984 - 1985 period on the tasks coded in Table 1 with an "IP" for in-progress work.

The results of the work completed in 1983 - 1984 will be discussed under four categories: 1) yield studies which occupied 71% of the time, 2) genetics of Macrocystis at 16% time, 3) planting technology for Macrocystis at 7% time and 4) assessment of foreign research efforts and collaboratove studies at 6% time.

TABLE I. TASKS UNDERTAKEN 1983 - 1984

TASK	CUD DC= data co IP= in prog	llected
1.	YIELD VERIFICATION STUDIES	
	Urganize and Set-Up Plot	υC
	Harvest and Replace Dead Plants in First Half of Plot	ÐC
2.	Harvest and Do Not Replace Dead Plants in Second Half of Plot YIELD ENHANCEMENT STUDIES	DC
	Organize, Plant and Set-Up Plot	ÜC
	Harvest and Replace Dead Plants	DC
3.	FARM MAINTENANCE	IΡ
4.	GENETICS OF MACROCYSTIS	
	Develop and Review a Kelp Genetic Improvement Breeeding Plan	DC
	Perform Mass Selection from Yield Farm Plants	ΙP
	Organize and Develop a Set of Pedigreed Lines	ΙP
	Evaluate Progeny of "High-Yield" Kelps	IP
5.	Evaluate Modern Techniques - Cell Fusion / Genetic Engineering . PLANTING TECHNOLOGY FOR MACROCYSTIS	IP
	Broadcast Trials	DC
	Planting Line Trials	DC
	Subadult and Adult Plantings	DC
	Ventura Artificial Reef	DC
٥.	ENVIRONMENTAL MONITORING	ΙP
7.	DATA BASE MANAGEMENT	ΙP
გ.	INTERNATIONAL COOPERATIVE PROGRAM	ĪΡ
9.	MEETINGS, CONSULTING, MANAGEMENT AND REPORTING	ĬΡ

## Yield studies

The main purpose of the yield studies conducted in 1983 -1984 was to illustrate that the results obtained from the 1981 - 1982 yield farm were verifiable and repeatable. The original unfertilized plot became the yield verification plot. In the first experiment, dead plants were replaced with new live ones at the end of each harvest. If these had not been replaced, it is possible that the planting density would "run down" to a level at which it would maintain itself. To test this idea, we divided the plot into two halves. In one half, dead plants were replaced with new live ones (as was done in the first experiment), while in the other half, dead plants were not replaced and the population was allowed to "run down."

In the first experiment, yield was significantly increased by adding fertilizer to the plants in one plot. In this second experiment, certain modifications were made to the original grid farm planting and harvesting plan to make better use of the available light. Light levels in the original grid farm were severely reduced by the thick surface canopy in the higher density plantings directly prior to harvest. The new "yield enhancement" farm was designed in rows rather than on a grid, and each alternate row was harvested every six weeks, rather than all the plants being harvested every 12 weeks. This scheme was planned to more efficiently utilize the light because a thick canopy would not develop and cover the whole plot, and after a harvest not all plants were newly cut with no canopy at all. The old "fertilized" plot became the new "yield enhancement" plot.

As in previous reports we have compared the results of the yield experiments with what is known about natural kelp beds like the Goleta Bay bed, since it is likely that future, large-scale kelp farms will be similar in some ways to these natural forests. The 1983-4 studies have also provided valuable insights into how individual kelp plants respond to cultivation and repeated harvesting. Individual plant responses are discussed in view of what is known about kelp and plant growth generally.

The very severe winter storms of 1983 dislodged and killed 98% of our farm plants while destroying the outer 100 feet of Ellwood Pier. After this, the experimental plots had to be completely replanted. By the end of June, 1983, 519 plants were under cultivation, 219 in the "dead plant replacement" half of the yield verification plot and 300 in the yield enhancement plot. During the course of these efforts, natural nutrient levels remained low for longer periods than recorded in years. As a result of these unusual conditions, the natural kelp beds off the coast all but disappeared. The harvest fell from approximately 150,000 tons per year to approximately 5,000 tons per year (see Figure 7) and Merck Chemical Company of San Diego was forced to import kelp from South America and China to operate their alginate factory. Only now in January 1985 are the beds beginning to recover. The following table (Table 2) illustrates harvested amounts of biomass, and rates of plant survival on the coastal test farm and these data parallel that obtained from the commercial kelp harvesters.

The environmental data taken daily since the program began in 1980 illustrates the dramatic change in farming conditions that occurred within a one year period. One can see from the following series of figures, how periods of maximum temperature have greatly increased (Figure 26) and how nutrient rich periods have been very few and infrequent, as compared with a "good" year in 1980-1 (Figure 27). These monthly mean figures are based on the daily and weekly measurements that have been made by NMI in the growing area, since 1979. This interesting data set is now large enough to show year-to-year trends in growing conditions, and will be an important baseline for retrospective analyses done with the yield, and frond-production data sets. The severe storms in the Winter of 1983 are shown in the monthly means for wave height (Figure 28) and current speed (Figure 29). This water motion decreased visibility (Figure 30) and increased sedimentation rate (Figure 31). A plot of the production (Figure 32) and survival (Figure 33) measured for our quarterly harvests illustrates the impact of first the winter storms and later the high temperature and low nutrient conditions.

TABLE 2. SUMMARY OF YIELD FARM HARVEST DATA

Month /Year	Plot Half	Dead (%)	No Canopy (%)	With Canopy (え)	Total Harvest (kg)	Average Harvest (kg/plt)	Largest Plant Harvest (kg)
12/81		6.3	4.7	89.0	13,140.4	18.2	102.0
3/82 6/62		16.5 5.4	4.8 4.7	78.7 89.9	13,501.4 20,938.U	18.7 29.0	189.Ú 177.U
9/82		9.3	14.4	76.3	9.097.2	12.6	111.0
12/82		27.3	4.4	68.3	5.920.4	8.2	58.0
3/83		98.5	1.1	0.4	11.6	0.0	4.9
198ء	84 YIELD	VERIFIC	ATION RESU	ILTS			Largest
			No	With	Total	Average	Plant
donth	Plot	Dead	Canopy	Canopy	Harvest	Harvest	Harvest
Year	Hal f	(2)	(%)	(%)	(kg)	(kg/plt)	(kg)
7/83		8.2	34.7	57.1	1.061.0	4.80	37.0
0/83		21.5	72.6	5.9	20.0	0.09	3.0
12/83		47.0	32.4	20.6	25.6	0.57	6.0
84/د		61.6	27.4	11.0	62.3	0.28	8.7
7/84		27.8	23.3	48.9	973.0	4.40	77.0
1983-19	84 YIELD	ENHANCE	MENT RESUL				Largest
			No	With	Total	Average	Plant
Month	Plot	Dead	Canopy	Canopy	Harvest	Harvest	Harvest
/Year	Half	(%)	(%)	(%)	(kg)	(kg/plt)	(kg)
8/83	1	16.0	36.7	47.3	504.0	3.4	26.0
9/83	2	6.0	71.0	23.0	78.3	0.5	8.0
1/83	1	40.7	59.3	0.0	0.0	0.0	U.0
1/84	Ż	72.0	13.3	14.7	64.0	0.4	4.0
2/84	1 2	61.3	16.0	22.7 52.7	115.0	0.8	15.0
5/84 6/84	1	30.6 28.6	16.7 12.0	52.7 59.4	612.8 1.195.5	4.1	41.0
	1	20.0	12.0	27.4	1.190.5	8.0	49.0

When production and survival numbers were directly compared (Figure 34) it appears that plants under stress have a mechanism for survival. In some of the harvests, there was very high survival among plants combined with very poor production. This would indicate that the plant may have a growth-production control mechanism which allows it to become dormant under adverse conditions.

When production was compared with quarterly mean temperature (Figure 35) in the first harvest period to the second, even though the temperature dropped, the production remained about the same. Moving from the second to third harvests, the temperature rose but so did the production. Moving to the fourth harvest the temperature continued to rise but production was greatly reduced. There seems to be a lag in the affect on production of temperature changes, high nutrient periods and high irradiance periods. Production seems dependent on the "antecedent" environmental conditions.

The 1983-4 yield-verification and yield-enhancement studies were not as instructive as the preceeding 1981-82 work, because of the severe environmental damage suffered by both the natural kelp beds, and the test farm. However, some plants that survived the storms and high-yielding plants from the populations were maintained under cultivation and have isolated spores and cultivated gametophytes from them. The apparent stability of natural kelp beds in the Santa Barbara area is deceptive. The areas of kelp beds here, as seen when aerial photographs are re-assembled into measureable photo-mosaics (Harger 1983), have been constant for a half-century. However, the damage that has occurred in the last two years illustrates that the plants are not immune to severe environmental stress.

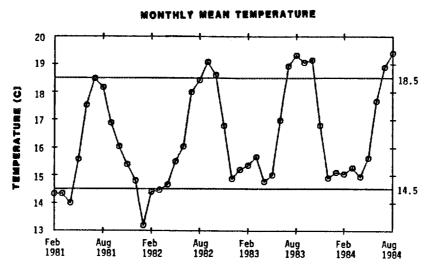


Figure 26. In-the-sea temperature records from February 1981 to August 1984, for Goleta and Elwood, showing the gradual lengthening of the duration of time that the seawater reached temperatures above 18.5 C tempeature level, when plants were damaged. It can also be seen that there has been a decrease in the total time that water temperatures fell below the 14.5 C level when upwelling and nutrient enrichment often occurs. This record illustrates how growing conditions have deteriorated over the past three years when harvests have fallen dramatically (see Figure 7).

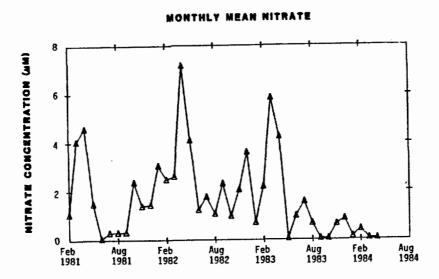


Figure 27. In-the-sea measurements of surface nitrate, experessed in micromoles per ml, from February 1981 to August 1984, showing good nutrient, and hence growing conditions, in 1982, and increasingly low nutrient levels and correspondingly poor growing conditions in 1983-4.

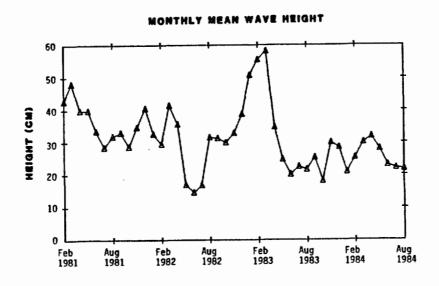


Figure 28. Measurements of wave heights from 1981 to August 1984 (in centimeters) showing the very high waves in the winter of 1982-3, when a series of record storms destroyed most of the southern California kelp beds, severely damaged the coastal test farm, and tore 100 feet off the end of Elwood Pier.

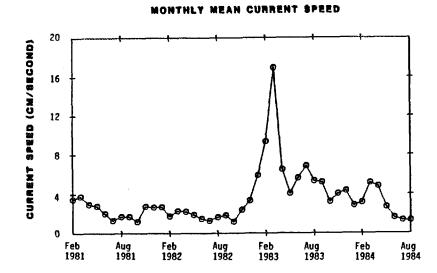


Figure 29. In-the-sea current records, from the NMI environmental monitoring data base, show the strong currents measured in the winter of 1982-3. The higher current speeds encountered after the December-February storms, are thought to be due to the destruction of the offshore kelp beds, which previously served as a barrier to high, near-shore currents.

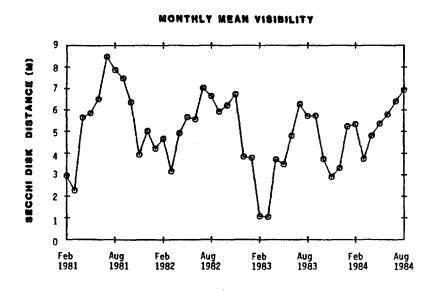


Figure 30. In-the-sea transmissivity records from secchi-disk measurements, show a period of two months, during the 1982-3 winter storms, when sea-floor plants were in complete darkness, due to the high sediment load picked up and suspended by the turbulent sea. This was much more extreme than the normal winter decrease in water transparency due to storm activity.

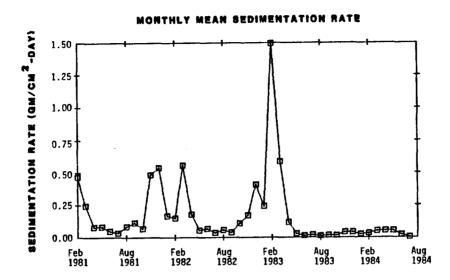


Figure 31. Sedimentation rates, measured weekly with sediment-collecting tubes, and expressed as grams collected per square centimeter per day show that the winter of 1982-3 was much more severe than the following mild winter of 1983-4.

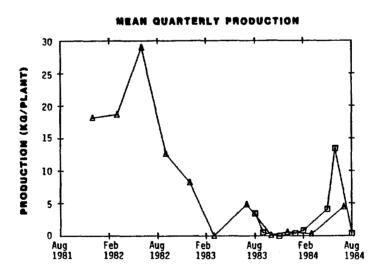


Figure 32. The mean quarterly production records from the Coastal Test Farm, showing the high Spring production levels of 1982, compared with the disasterous levels of 1983, and the beginnings of recovery in 1984. The diamonds on the graph shown represent the original yield experiment, continued as the yield-verification experiment in 1983-4. The squares show the results of yield enhancement efforts, where production is still relatively low, but the survival rates of individual plants has been increased. These results suggest that the plants respond to adverse conditions by reducing their frond-production rates.

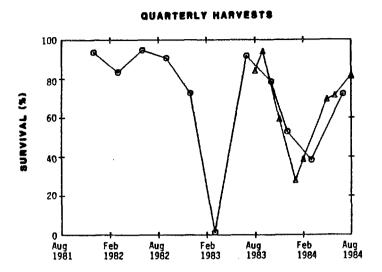


Figure 33. A history of plant survival rates from August 1981 to August 1984, showing the major losses seen after the 1982-3 storms, and during the low-nutrient summer of 1983, with subsequent survival rates approaching those seen in the "normal" 1981-2 period. Squares are used to plot the results of the original yield-and continuing verification experiments, while the diamonds representing the survival of plants in the yield-enhancement plot.

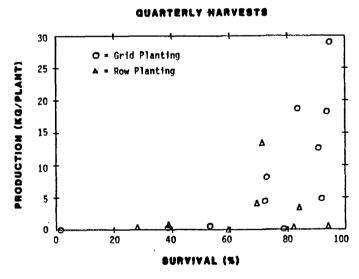


Figure 34. A scatter diagram showing corresponding measurements of production and survival, showing that high survival rates are not always associated with high production. This suggests that under low-light and/or low-nutrient conditions plants can survive by becoming "semi-dormant" for periods of time during which no new fronds are produced.

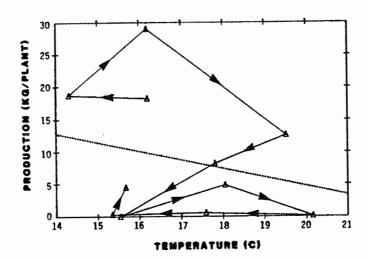


Figure 35. A sequential-vector graph, where production (in kilograms per plant per unit time) is plotted against temperature. The dotted line shows the gradual decrease in productivity, while the vectors show two peaks of production in a downward trend with time.

## Genetics of Macrocystis

## A. DEVELOP AND REVIEW KELP BREEDING PLAN

The total variability among organisms is a function of both environment and heredity, and only heritable characters are subject to selection. A characteristic like flower color may be inherited simply, being determined by a single gene, but a quantitative character like yield is likely to be determined by several genes. The kelps can be viewed as a storehouse of genetic diversity which has been accumulated This diversity has allowed individual kelp as these plants evolved and spread. plants to grow and reproduce under a broad range of environmental conditions. Indeed, the plant-to-plant variability that illustrated in our yield study work (see Figure 25) suggests that kelp plants are a genetically diverse population from which a variety may be selected and bred which consistently produces high yield. Over the course of geological time, gene and chromosomal changes have produced very The genetic diversity in Macrocystis is seen in the three different plant types. distinctive species found in California: <u>Macrocystis pyrifera</u>, <u>Macrocystis angustifolia</u> and <u>Macrocystis integrifolia</u> (Figure 36). Pedigreed lines can be produced from all of these species and they are all interfertile.

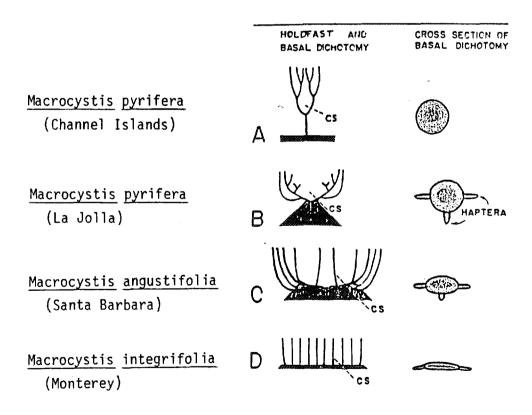


Figure 36. Three species of Macrocystis occur along the pacific coast of the U.S. These differ in the degree to which haptera form on the basal branching system, ranging from a rhizomatous northern species to an upright southern species as shown. Gametophytes of all three species are presently under cultivation at NMI.

In developing a selection and breeding program, it is assumed that natural selection has operated to select the kelp strains that survive and grow well along the coast of California. No attempt has yet been made to produce giant kelp plants with heritable characters such as high yield. However, the Chinese have bred Laminaria plants taken from Japan which have a higher growth rate, a higher iodine content and greater tolerance to high water temperature than is usual.

A kelp breeding plan was developed in consultation with Dr. Richard Snow and Dr. Subodh Jain of the University of California at Davis to: 1) determine those traits that might be indicators of high production, 2) develop a mass selection breeding scheme, 3) develop pedigreed plants and 4) develop techniques for inducing autoploidy. The breeding plan is attached to this report as Appendix D.

#### B. MASS SELECTION FROM YIELD PLANTS

Mass selection involves culling inferior plants and collecting seed from the rest. Often the breeder of land plants will select the superior plants in an open-pollinated place and allow only these to reproduce. Selection from a population of cross-fertilized plants gives the breeder no control over the sources of egg and sperm nuclei. Lack of strict genetic control also makes it difficult to distinguish subtle differences between plant traits which are attributable to environmental factors versus those due to heredity. Nonetheless, the simple mass-selection approach to breeding has been very effective.

The focus of this work was to identify and select of high yielding plants from the coastal test farm and generate open-pollinated inbred progeny from these plants. These progeny would then be grown to reproductive maturity. The highest producing plants from this generation would be selected to produce another generation of open-pollinated inbred progeny. This cycle would continue, resulting in inbred lines of consistently high-producing plants.

Six plants were selected from the coastal test farm that showed superior survival and production. In addition, two survivors of the 1983 storms were selected, one a coastal test farm plant that was a high producer and the other a fast-growing recruit. The greenhouse culture of excised sporophylls from these plants was used to assure a supply of spores over an extended period of time. Sporophylls that had no sporangial sori when collected from the sporophyte would produce sori for 2 to 3 months in culture. The best results were obtained by growing them in flowing nutrient rich seawater at 14 deg C under low levels of light. This technique was especially useful in the summer when the environmental conditions were adverse to kelp growth and few ripe sporophylls were available.

An attempt was made to produce sporophytic progeny from the 1983 storm survivors and grow them on strings to be outplanted to the sea but the adverse climatological conditions resulting from "El Niño" prevented them from growing to maturity. The other six plants that were selected from the coastal test farm were also used to initiate sporophyte progeny which will be used in the next phase of the project.

#### C. ORGANIZE AND DEVELOP PEDIGREED LINES

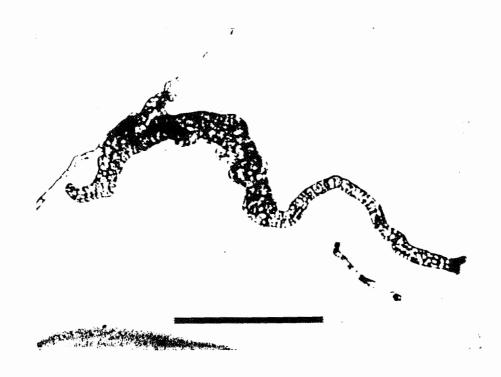
Pedigree selection involves the isolation of pure lines which are then tested. This can be very time-consuming since it may take three or more years to evaluate a trait such as yield and then select promising lines that can be tested on a larger-scale. The process of producing a new variety of wheat or barley can take from eight to fifteen years, but the dividends of such a careful program can be substantial.

It is relatively easy to create a set of pedigreed lines of kelp because of the nature of its life history. The haploid gametophytic phase can be isolated, cloned and grown in the laboratory or stored until needed to propogate the genes of superior strains of kelp. If sporophytes produced from the cross of a specific male isolate with a specific female isolate are found to have special characteristics, sporophytes of this same genetic makeup can be repeatedly generated using the same gametophyte parents.

The culture collection of gametophyte isolates was maintained and expanded during this research period. The basic collection consisted of 406 kelp gametophyte isolates, 308 from Macrocystis spp. and 98 from other kelps. At the end of this project, the collection had expanded to 499 isolates. The additional 93 isolates were all of Macrocystis including isolates from a recruit plant of Macrocystis angustifolia from the coastal test farm which survived the winter storms of 1983 and proved to be a fast-growing plant. Gametophytes were collected from a Macrocystis integrifolia plant collected from Baranof Island in Alaska. Gametophytes from a Macrocystis pyrifera plant from Punta Eugenia, Baja California, Mexico, were added to the collection. These additions extend the geographic range of Macrocystis isolates in the culture collection to the northern and southern extremes of its distribution along the West Coast of North America.

A useful technique was also developed for producing large quantities of a genetically defined gametophytic isolate for experimental purposes. The isolate was ground up and the fragments were grown in large amounts of enriched seawater medium under white light at 40-50 uE/sq m - sec. This method produced ample material for experimentation in just two months. The fragmented material had an exponential growth rate of 11% a day while unfragmented material had an exponential growth rate of only 5 % per a day.

The careful selection and repeated inbreeding of preferred strains of kelp will finally result in homozygous true-breeding lines. There are two benefits from producing these lines. First, progeny of each line will have more uniform superior, true-breeding characteristics. Second, by crossing two homozygous parents with different sets of genes, the offspring may have heterosis or hybrid vigor. In China, genetic experimentation with Laminaria japonica, has resulted in the development of homozygous strains by the production of parthenosporophytes from female Parthenosporophytes have been observed in a few of our bulk gametophytes. cultures of Macrocystis gametophyte isolates. These have been abnormally shaped, as shown in Figure 37 and 38. None of the parthenosporophytes obtained thus far have developed into large plants, but have died while young and small. If such plants could be grown to maturity, homozygous strains could be obtained in one generation and a useful tool for studying the heredity of traits in Macrocystis would be available. The pedigree selection approach, while more time consuming than mass selection, can also produce valuable results more quickly.



Figures 37 and 38. Sporophytes that have arisen from unfertilized eggs, (parthenosporophytes) of a female strain of <u>Macrocystis pyrifera</u> (Mp-A:F-1). Both examples show an abnormal pattern of cell division producing elongated, or mishapen plants. A 100um scale is shown.



# D. EVALUATE PROGENY OF "HIGH-YIELD" KELPS

Because of the adverse environmental conditions in the sea during 1983 – 1984, the sporophytic progeny generated in this program did not grow well and eventually died when they were outplanted into the sea. Therefore, it was not possible to determine which subadult and adult sporophyte plants grew best. However, several gametophytic progeny were evaluated for growth rate in the laboratory under an array of temperature and light level combinations on a crossed gradient table. In addition, previous data on genetically defined field grown kelp progeny were analyzed to determine if their genetic background affected the growth rate of these plants.

Three gametophyte isolates were used for growth experiments on the light and temperature crossed gradient table. Cultures were grown at 20 light and temperature combinations, with temperatures of 6, 10, 14, 18 and 22 deg C and light levels of 0.5, 2, 6 and 15 nE/cm2/s. The results of these experiments are shown in Figures 39 to 41.

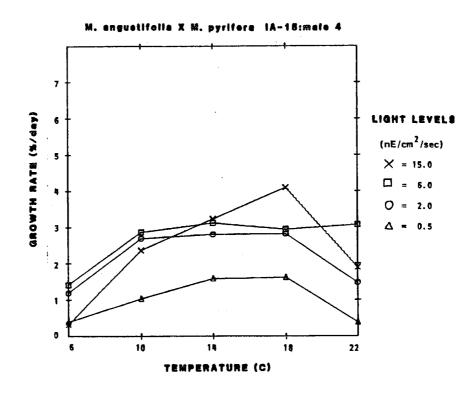


Figure 39. Laboratory experiments where gametophyte growth is measured for a specific gametophytic strain (in this case a male strain from a M. angustifolia M. pyrifera hybrid) show that optimum growth rates of 4% per day increase in wet weight can be achieved at 15.0nE per sq. centimeter per second at a temperature of 18 deg. C. This type of experiment can be used to select high-yield, pedigreed gametophytic strains (super-gametophytes) just as in-the-sea selection can identify high-yield sporophytic (super-sporophyte) strains (see figure 25).

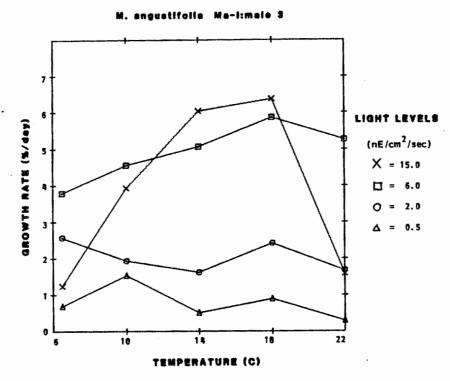


Figure 40. A high yield male gametophytic strain of M. angustifolia, showed growth rates approaching 6% per day wet weight increase over a 14 to 18 deg. C. temperature range. This type of experiment could be used to select high-yielding, high-temperature-tolerant pedigreed gametophytic lines.

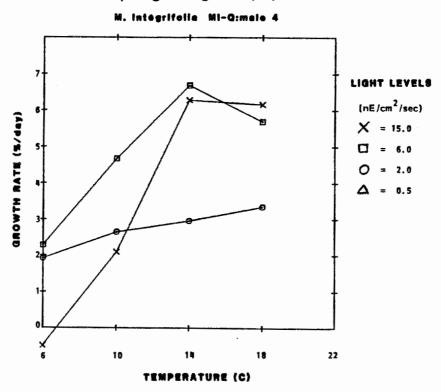


Figure 41. A high-yielding (6% per day wet weight increase) gametophytic strain of M. integrifolia, shows optimum growth at 14 deg. C, which would be expected in this northerly species.

Growth rate information that was taken from genetically defined plants in 1980 and 1981 was further analyzed to determine if the genetic makeup has a significant effect on growth rate. The object of this analysis was to determine if genetic background has a significant effect on a production trait such as growth rate and to determine what genetic background was most favorable for such a trait. All possible crosses were made between female and male gametophytes of Macrocystis pyrifera (Mp) from Santa Catalina Island, Macrocystis angustifolia (Ma) from Santa Barbara and Macrocystis integrifolia (Mi) from Santa Cruz. The genomic formulas are listed as crosses between female X male. Significance levels reported below are at the 0.05 level of significance.

These sporophytes varied widely in respect to initial plant size. A regression of growth rate on initial plant size showed that growth rate significantly decreased with larger initial plant weight. In order to determine whether differences in growth rate could be attributable to genetic makeup independent of initial plant size, an analysis of covariance (ANCOVA) was performed on these data. Tests using statistics generated in the ANCOVA were performed to determine homogeneity of slopes and homogeneity of elevation. The data on six of the crosses were found to be suitable for this analysis. The test for homogeneity of slope of regression lines showed that all slopes are homogeneous. This indicates that the effect of initial plant size on the growth rate is the same for all groups tested. This allows the testing of homogeneity of elevations of the regression lines, which showed that the elevations are not homogeneous. A multiple range test, using the Student-Newman-Keuls procedure, was performed to determine which groups were significantly different from the others. The results of this test are presented in Table 3.

Table 3. Pairwise comparison of mean exponential growth rates (percent per day) for juvenile sporophytes of 6 different genetic makeups.

• = Significantly different (p < 0.05)

NS = Not significantly different (p > 0.05)

	Cro Mea Num	n (%/day)	Ma X Mp 4.055 n = 42	Ma X Ma 3.774 n = 11	Mi X Mp 3.157 n = 47	Mp X Ma 2.886 n = 21	Ma X M 2.834 n = 43
Cross	Mean (%/day)	Number					
ria X Ma	3.774	n = 11	NS				
Mi X Mp	3.157	n = 47	*	*			
Пр х На	2.886	n = 21	*	*	NS		
Na X Mi	2.834	n = 45	*	*	*	NS	
ili X Ma	2.688	n = 47	*	*	•	иЅ	NS

The data on the three crosses not included above were unsuitable to include in this analysis because of small sample size or heterogeneous variance. These growth rates were: Mp x Mp, 3.717 (n=9); Mp x Mi, 3.129 (n=2); Mi x Mi, 2.120 (n=2). These results are represented diagrammatically in Figure 42.

# Growth of Genetically Defined Macrocystis

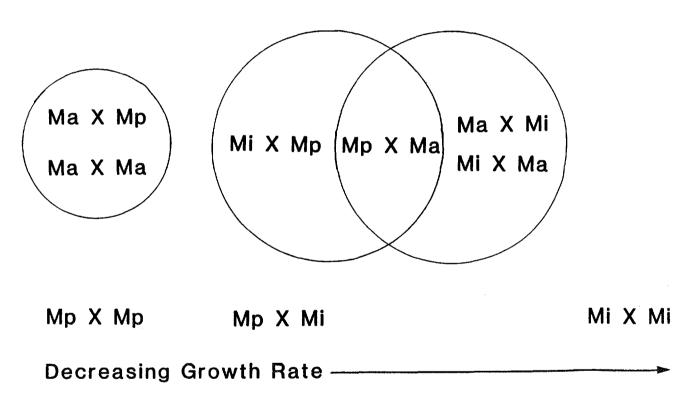


Figure 42. A graphic comparison of the growth of sporophytic plants of known genetic background, (based on data in table III), showing that M. pyrifera has the highest, and M. integrifolia have the lowest growth rates. Hybrids have intermediate growth rates. Those crosses falling within a circle are not significally different from each other, while those not encircled are significantly different.

Plants from the Ma X Mp and Ma X Ma crosses had much higher growth rates than those of other genetic backgrounds. The M. angustifolia gametophyte isolates used in these investigations was isolated from an adult sporophyte collected at Campus Point. Therefore, sporophytes derived from these gametophytes may be better suited for growth in the Santa Barbara vicinity than sporophytes derived from other gametophytes. Mi X Mi sporophytes grew the least well of all crosses, with plants showing weight loss during the summer growth period. This may be due to the difference between the environment from which the parental sporophytes were collected and the environment in which they were grown. Water temperature may have been the most important environmental factor here. The depth of outplanting may also have significantly affected the growth of these sporophytes, since Macrocystis integrifolia grows naturally in shallower areas than Macrocystis angustifolia and Macrocytis pyrifera.

Therefore, genetic background does significantly affect growth rate. Also, sporophytes that were initiated from gametophytes that were collected from southern California Macrocystis generally had higher growth rates than those from northern California isolates. This shows that the development of improved strains of Macrocystis, and indeed all crop plants, is dependent on the locality at which the plants are grown.

Morphometric and weight data that were taken from mature genetically defined sporophytes in September 1982 were analyzed for differences in these characters with respect to their genetic background. These data were taken from one plant of Macrocytis pyrifera X Macrocystis pyrifera, two plants of Macrocystis pyrifera X Macrocystis and one wild plant at Ellwood Pier (presumably Macrocystis angustifolia X Macrocystis angustifolia). Morphometric characters of mature lamina lengths and widths and mature pneumatocyst lengths and widths were examined. Weight characters of mature lamina weight, mature pneumatocyst weight and internodal stipe weight were also examined. These characters were analyzed for statistical differences by analysis of variance (ANOVA). Where the ANOVA showed a statistically significant difference at the p = 0.05 level, pairwise comparisons were made using the Student-Newman-Keuls procedure.

The results of these analyses are presented in Figures 43 and 44. The data on laminae dimensions and weights is of particular interest. The greater weight of the Mp X Mp laminae can be attributed to their width, which is greater than that of all others, and their length, which is greater than that of the Ma X Ma plant. The laminae from the two plants of Mp X Ma are not significantly different from each other, as would be assumed since they are genetically identical. The weight of these laminae can be attributed to a greater length and width than Ma X Ma laminae. The pneumatocysts show a few significant differences, in particular in width and weight. Internodal stipe weights, taken from the internodes near the mature blades, show significant differences, with the two Mp X Ma plants having different weights. This character is greatly influenced by environmental and age factors which may make it difficult to use to determine differences between plants of different genetic makeups.

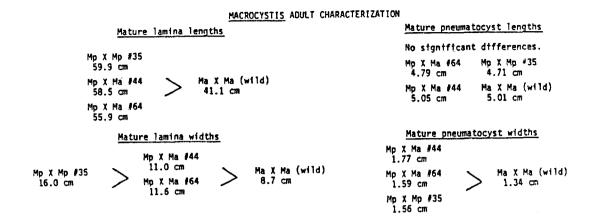


Figure 43. The analysis of pedigreed kelp sporophytes grown to reproductive maturity together on the test farm, shows that blade and pneumatocyst lengths and widths differ, and suggests that these differences are due to genetic rather than environmental factors.

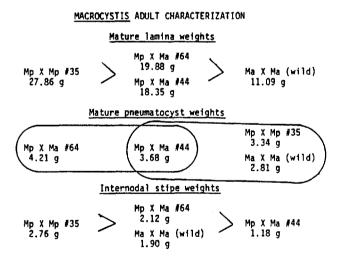


Figure 44. The weights of lamina, pneumatocytis and internodal stipe segments of mature, pedigreed kelp sporophytes, grown in the sea at the same site, show significant differences, suggesting that genetic rather than environmental conditions are responsible for the differences seen.

The weight data taken from these plants reveal some of the potential of using particular genetic strains for greater biomass production. The mean weight of a mature lamina with its pneumatocyst and the length of internodal stipe attached to it is 33.96 g for Mp X Mp, 24.71 g for Mp X Ma and 15.80 g for Ma X Ma. Therefore, given the right genetic strain, more than twice the biomass per node could be produced. These measurements were taken in the late summer, when many laminae show a great deal of sloughing. The greater size and weight of laminae on plants with Macrocystis pyrifera genes may be a result of these plants being able to perform better in summer conditions than the local Macrocystis angustifolia.

#### E. EVALUATE MODERN TECHNIQUES

No way of maintaining, propagating and certifying kelp breeding stock has yet been devised as it has in land agriculture where it is possible to define breeder, foundation, registered and certified seed. However, the kelp gametophyte stocks that are maintained at NMI can be treated in the same way as a seed bank. The recent development of bacteria-free gametophytic strains by Mr. Mufta Zarmouh (now working at the University of California, Santa Barbara (UCSB)) may well make it possible to use these haploid strains to produce cytoplasts and in turn to use these to produce cell, and nuclear fusions, and hence new genetic combinations.

The development of kelp plants (from homozygous gametophtic strains) which exhibit hybrid vigor certainly merits further experimentation. If they can be produced then they will be tested for their ability to combine with other inbred lines to produce vigorous hybrid plants. The preliminary crosses made in the laboratory at NMI suggests that the combinational ability of gametophytic strains will be very high.

There is a possiblity that kelp may be induced to produce multiple sets of genes, that is, to be made into a polyploid plant. Sometimes the addition of an entire new genome results in a plant with more luxuriant growth. Chromosome numbers can be experimentally doubled to produce very large flowers in ornamental plants such as the Easter lily. Macrocystis integrifolia has a diploid chromosome number of 32 (Walker 1952 and Cole 1967). However the diploid number according to Yabu and Sanbonsuga (unpublished manuscript) is 64, not 32. Therefore, the strains are being worked with at NMI may already be polyploid. Our crosses of Macrocystis angustifolia (with 64 chromosomes) with Macrocystis integrifolia with chromosomes, should have produced a triploid plant of low fertility. Although this cross was made only once, it was found that the hybrids that were produced were The matter of chromosome number, and that of intra-specific normal and fertile. compatability are areas which require further detailed study. The development of new microspectrophotometric methods for measuring DNA in kelp nuclei (discussed in section VII of this report) will undoubtedly advance the study of kelp genetics considerably, and will be basic to any efforts to produce polyploid lines.

There are a number of challenges which face any potential kelp breeder. The plants are highly variable and represent an unexplored gene pool which makes the selection of favored genotypes difficult. Once superior varieties are selected and bred, then "seed" certification of the gametophytic strains that produce these varieties will be possible. Both traditional plant breeding methods and modern genetic engineering approaches are possible. Any approach that is used will start with evaluation and selection work, like that now under way at NMI.

## Planting Technology for Macrocystis

The Parsons company (see Brehany 1983) economic feasibility study of the cost of farming Macrocystis for energy contains some suggested methods for reducing the cost of planting a kelp farm. A planting scheme was proposed in that report which envisioned workers on a barge attaching juvenile plants to rocks and attaching the rocks to long lines that formed beds. As part of this task in 1983 -1984 NMI has compared several ways to economically plant kelp and has tested them at the artificial reef installed by the California Department of Fish and Game in Ventura.

#### A. BROADCAST TRIALS

An inexpensive to establish a kelp bed is to "broadcast" many spores or small plants over the area to be seeded. Although the mortality rate of plants or spores seeded in this way is likely to be high, some of the many spores will survive and the cost of planting kelp in this way is minimal. NMI used this approach in seeding rocks at the Ventura artificial reef. A suspension of spores was made to spray on the rocks by drying sporophylls slightly to induce them to release spores or by grinding sporophylls up to force spore release. The spore suspensions obtained were sprayed onto rocks using a weed sprayer. In initial trials, rocks were placed in the ocean or in enriched seawater in an aquarium. Both methods of obtaining spore suspensions were successful and many sporophytes developed on the rocks that were placed in an aquarium and later transferred to greenhouse tanks. However, they did not develop on rocks placed directly in the sea. The rocks at Ventura Reef which were marked and sprayed with spores had no more kelp sporophytes than those that were not sprayed.

#### B. PLANTING LINE TRIALS

A useful method to start kelp plants in the laboratory and outplant them in the sea is to initiate cultures of kelp spores onto strings which can be attached to lines for outplanting. This involves allowing the spores to settle onto strings in culture dishes or aquaria, allowing the spores to grow into gametophytes which will then produce sporophytes on the string, transferring the strings to a flowing seawater system where the sporophytes grow larger and become "hardened," and finally, outplanting the young sporophytes in the sea. The research conducted in this program was designed to refine this procedure in order to make it more efficient.

In the course of this research, it was found that the strings with attached sporophytes can be transferred to the greenhouse while the sporophytes are still very small (less than 5mm) and these will subsequently grow larger than sporophytes kept in dishes or aquaria. A reason for this faster growth may be that in dishes there is no water motion, while in the greenhouse there is. The seawater system must be supplied with adequate nutrients for sporophyte growth. In the greenhouse seawater system, plants should reach a size of approximately 100 mm before being outplanted in the sea. Plants of this size are growing rapidly and are sufficiently "hardened." Results from outplanting experiments show that optimal growth can be obtained by outplanting at 5 m water depth (see Table 4). Plants in shallower depths receive too much light while plants in deeper depths receive too little.

Table 4. Initial and end weights of young sporophytes outplanted at Ellwood at various depths from 5/10/83 to 5/25/83, exponential growth rate (EGR) and approximate irradiance level.

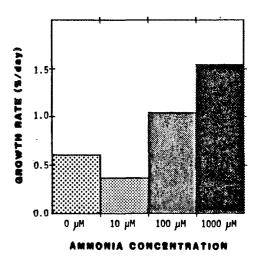
	UEPTH (m)	INITIAL WT. (g)	END WT. (g)	EGR (%/day)	IRRADIANCE (uE/m2/s)	
-	0.5	1.7	1.2	-2.32	2000	
	1.0	1.5	6.7	9.98	1510	
	3.0	1.2	18.7	18.31	667	
	5.U	1.5	25.2	18.81	283	
	7.0	1.5	11.7	13.69	116	

## C. SUBADULT AND ADULT PLANTINGS

Young sporophytes were attached to small rocks from 0.5 to 10 kg in weight with rubber bands (see Wilson and North 1983 for details of this method). These were placed at 9 m depth at the Ellwood farm site, at 7, 9 and 11 m at the Ventura Artificial Reef site before reef installation, and on the reef at 9 m after installation. At Ellwood, all sporophytes were eventually lost, either due to fish grazing or detachment of plants from the rubber bands. At Ventura before reef installation, all sporophytes and some rocks were lost, possibly due to sand scour and burial. Plants that were placed on the reef grew well for a couple of months. These plants died with the onset of high water temperatures in the summer of 1984.

During the El Niño period of poor climatic conditions in 1983, nutrients were supplied to young sporophytes by dipping them in a nutrient solution once a week for four weeks. Plants were dipped in solutions containing 10, 100 and 1000 uM ammonium in seawater. The resulting growth rates for these plants are shown in Figure 45. A clear trend of increasing growth rate with increasing ammonium concentration is apparent, although the differences in growth rate are not significantly different because of the high variability and small sample number. It is likely that a significant difference would have been obtained with a larger sample size. Plants that were dipped in the higher nutrient concentrations continued to grow larger than the other plants, even when the nutrient treatments were discontinued, indicating that this nutrient treatment earlier in their growth favorably affected growth later on as well.

Figure 45. In an attempt to enhance juvenile kelp survival during low-nutrient summer months, young plants were taken from the sea once per week and dipped into a nutrient solution. Fertilized juveniles showed an increase in growth rate.



Young sporophytes were also transplanted to the Ventura Artificial Reef site at 0, 1, 2 and 4 m from the bottom in 8.7 m water depth. The growth rates of these plants is shown in Table 5. The best growth was obtained at the shallowest depth. Plants growing on the bottom had little light and lost weight during this growth period.

Adult plants were placed at the Ventura Artificial Reef site before and after installation. Before installation, 15 adult plants were transplanted using gravel bags, with 5 each at depths of 7, 9 and 11 m. These plants were observed periodically. The number of fronds present decreased over time (Table 6). They all eventually died, which was attributed to sand burial of the holdfasts.

Table 5. Mean exponential growth rate (EGR) of juvenile sporophytes grown at various depths at the Ventura Artificial Reef site, 1/10/84 to 2/15/84, and irradiance levels.

HEIGHT (m)	n	MEAN EGR (%/day)	MEAN IRRADIANCE (uE/m2/s)
4	30	3.96*	125.4*
2	30	1.44	46.6*
1	27	1.11*	24.0*
ð	29	-0.80	13.9

<sup>\*</sup> Significantly different (p = 0.01) from the next deeper depth.

Table 6. Number of fronds on adult plants at 7, 9 and 11 m depth at the Ventura Artificial Reef site.

DEPTH	MEAN FRUND NUMBER				
( m)	11/15/83	12/15/83	01/18/84		
7	27.2	14.2	5.4		
9	21.4	10.2	3.2		
11	27.5	6.2	lost		

Fifty adult plants were transplanted in gravel bags on the Ventura Artificial Reef soon after it was installed. The number of fronds on these plants was measured monthly, as shown in Figure 46. The number of fronds decreased after transplantation, after which the number of fronds appeared to stabilize. However, another measurement made in 10/84 showed that the frond numbers decreased and mortality increased dramatically. This can be attributed to extraordinarily high water temperatures during the month of September, and possibly fish grazing.

#### D. VENTURA ARTIFICIAL REEF

The decision to place an artificial reef constructed of quarry rock in the nearshore of the Ventura County coast was made by the California Department of Fish and Game (CDFG) in order to provide a suitable substrate for the establishment of a kelp bed and to enhance fish populations. CDFG administers the kelp beds in California and has a long history of kelp bed research, management and enhancement. The Ventura County coast has very few kelp beds because there is very little rock bottom and the coast is relatively exposed so the kelp cannot grow on the sand bottom in this area. Southern California Edison Company (SCE) funded an NMI study to assist CDFG in the selection of the artificial reef site.

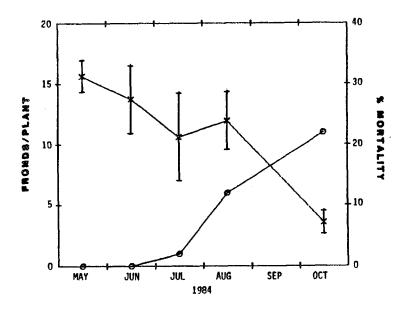


Figure 46. Mortality (o) and frond production (x) per plant with time for 50 plants on the Ventura artificial reef, showing the effects of adverse summer growing conditions in 1984. A survey of this reef showed many single-bladed juveniles had recruited, and the reef is recovering well from the summer damage.

The Ventura Artificial Reef is situated 0.9 km offshore of the Dulah Creek Overpass and 2.8 km southeast of Pitas Point at 9.2 m depth. It was constructed by dumping 4 barges containing 6.5 X 10 exp 6 kg of quarry rock. These rocks were placed in four piles or modules. Two bargeloads were installed on 4/15/84 and two on 5/2/84. These were installed by dumping a base of "filter rocks" about 30 cm in diameter after which they were topped with "anchor rocks" approximately 120 cm in diameter.

The biological study of the reef is divided into a kelp study, a benthic study and a fish study. Three methods to establish kelp on the reef were tested in the kelp study: 1) planting adults, 2) planting juveniles, and 3) spraying spores on the rocks before installation. These were performed on three of the modules, with the fourth module serving as a control. The results of these methods were reported in prior sections. Planting adults was marginally successful, with plants surviving for some time, producing many spores and with juveniles recruiting from spores released from these plants. This recruitment is a significant success, showing that young kelp plants can be established on an artificial reef by providing mature spore-bearing plants. Planting juveniles was initially successful, but the plants died in the adverse environmental conditions of summer. Spraying spores was not successful in this attempt. Natural recruitment of kelp, including Macrocystis and Egregia, occurred on all modules of the reef. These spores for these recruits came from nearby kelp beds. Natural recruits and juvenile and adult transplants were all visibly grazed by fish.

Benthic studies were conducted at the reef site before installation and in August and October after installation. During surveys made, community development was noted. The species of algae and invertebrates observed in these surveys are shown in Table 7. All of the algae and most of the invertebrates are new recruits on this virgin rock. Several large lobsters (Panulirus interruptus), sheep crabs (Loxorhynchus grandis) and sea stars (Pisaster brevispinus) were observed on the reef and are immigrants.

Fish surveys were conducted before and after reef installation. The water at the reef site is normally turbid, making fish observation difficult. However, a large number of fish of many species were observed at the reef that were not at the sandy bottom site before reef installation. The fish that were observed at the reef are listed in Table 8. Most of these fish are immigrants to the reef. However, young-of-year and half-grown individuals of many species were observed, indicating that the reef may enhance fish populations by increasing recruitment. More fish were observed at the module with adult kelp plants present than at a module without adult kelp. This can be attributed to the presence of the plants in midwater, providing a structure which attracts the fish, and which may provide food for some of the fish.

 $\ensuremath{\mathsf{Table}}$  7. Species of algae and invertebrates observed on the Ventura Artificial Reef.

#### Algae:

Brown algae:
Macrocystis angustifolia
Egregia laevigata
Giffordia granulosa
Pachydictyon coriaceum
Green algae:
Ulva sp.
Enteromorpha sp.
Chaetomorpha sp.

Red algae:
Gigartina sp.
Pterosiphonia dendroidea
Sarcodiotheca gaudichaudii
Polysiphonia sp.
Polyneura latissima
Coeloseira parva

#### invertebrates:

Panulirus interruptus
Loxorhynchus grandis
Balanus sintinnabulum
Balanus sp.
Pisaster brevispinus
Unidentified articulate bryozoans
Unidentified encrusting bryozoans
Unidentified tube worms
Unidentified tube worms
Unidentified scallop

Table 8. Species of fish observed at the Ventura Artificial Reef after installation.

## Scientific name:

Paralabrax clathratus
P. nebulifer
Sebastes auriculatus
S. serranoides
Semicossyprius pulcher
Embiotica jacksoni
Knacochilus toxotes
Damalichtnys vacca
Hyperprosopon argentum
Phanerodon furcatus
Girella nigricans
Hedialuna californiensis
Scorpaenichthys marmoratus
Uxyjulius californica
Chromis punctipinnis
Trachurus symmetricus

#### Common name:

Kelp Bass
Sand Bass
Brown Rockfish
Olive Rockfish
Sheephead
Black Surfperch
Rubberlip Surfperch
Pile Surfperch
Walleye Surfperch
White Surfperch
Upaleye
Halfmoon
Cabezon
Senorita
Blacksmith
Jack Mackerel

## Assesments of Foreign Research Efforts, and Collaborative Studies

The foreign technology task undertaken in 1983 and 1984 has been very successful, involving exchanges of information at scientific meetings, and visits to NMI by Chinese, Japanese, and Canadian scientists that have been particularly productive. In addition NMI staff have visited China, Japan, France, Senegal and England during this reporting period, as well as attending research meetings in Colorado and a useful contractors meeting in Florida.

Although the idea of using marine biomass as a source of energy is relatively new, it has not only been espoused in the United States, but scientists in Sweden, and Japan have started programs focused on growing macroalgal biomass as a source of bio-gas energy. When Howard Wilcox first introduced the concept of marine biomass as an energy source, it was assumed that the United States was in the vanguard of research in this area! In fact, at that time government-financed research dealing with in-the-sea farming of seaweed for food and energy was far more advanced in Japan and China than in the United States. It was obvious to many, that the U.S. program should not develop in isolation.

An important aspect of opening, and maintaining lines of communication and even collaborative studies with foreign groups studying marine biomass production has been to eliminate the costly and unnecessary business of starting research from scratch, as it is metaphorically called, "re-inventing the wheel." As will be seen in reviewing the historical information presented in appendix A, the same might be said for carefully studying prior uses of kelp energy from Californian kelp harvests. It is not necessary to re-invent the wheel in matters of farm design as the Japanese and Chinese have worked out planting and harvesting techniques which are easily adaptable to use in the coastal waters of the United States. Given a basis for farm design and seedstock-production, operating for kelps in China and Japan, the task of farming a perrenial kelp plant does not seem so daunting.

In cooperation with the GRI international program, headed by Dr. A. Flowers, NMI has established and maintained close ties with Japan and China where some most significant research is ongoing. For example Yoshiashi Sanbonsuga who heads the Hokkaido Regional Fisheries Laboratory at Yoichi is continuing his important studies on kelp genetics and propogation. He conducted research in Dr. laboratory at the University of California at Santa Barbara for a month in October, 1983 and is a continuing source of information and assistance concerning Japanese The Japanese are very interested in growing research into seaweed cultivation. Macrocystis, the largest known marine plant in Japanes waters without the risk of it spreading uncontrollably. To this end, they plan to produce sterile hybrids like those produced in a collaborative study in California (Sanbonsuga and Neushul 1979) which were fund to be sterile. NMI exchanged plant material with Dr. Sanbonsuga and continues to communicate with him about this research. He also gave NMI a detailed report on Japanese plans for research on the cultivation of marine biomass for energy. The isolates obtained in California have recently been studied by the noted Japanese cytologist, Dr. H. Yabu, who has determined the chromosome number for the first time. As will be seen later, this datum is extremely interesting and useful, and does not agree with previous work.

Mr. Tetsuo Fuseya and his associates Mr. Sawada and Mr. Nishitani also visited Neushul Mariculture in September 1983 to communicate their interest in using powdered Macrocystis and Nereocystis as a food additive. Discussions with these industrialists revealed the extent to which the Japanese government is willing to fund job-producing programs, particularly in areas of low employment such as in Hokkaido or coastal regions of Chiba provence, where they offered to establish algal processing plants. Similarly there is great concern in Japan about a slacking in fishing and other maritime activity and the surplus shipping that is available. Whether or not these Japanese programs will mature or not is in question.

The GRI research program also sponsered the two-year visit of Mr. X. G. Fei of the Institute of Oceanography in Qingdao, China to the United States. He returned to China in June, 1983 and gave a paper entitled "The Effects of Light on the Growth and Development of Giant Kelp" which was co-authored with Dr. Neushul at the International Seaweed Symposium held in Qingdao, China in June, 1983. The Chinese are particularly interested in large-scale cultivation of the giant kelp, Macrocystis in China. They introduced plants from Mexico in 1978. They are now attempting to cultivate it on long line farm structures in the sea (see Figure 47).

Dr. Fang, the Provost of Qindao University visited UCSB under the auspices of the National Science Foundation in August, 1984 and was able to give us the latest information concerning the current research into seaweed farming in China. GRI has not only benefited from the expertise of the most senior experts in seaweed cultivation in China but has also sponsered in part the education of some junior scientists such as Shu-li Cao who visited UCSB between April and September 1983 before moving to Florida and Lin Chai who is currently working towards a Masters Degree at UCSB and working under the direction of Dr. Neushul.

NMI contacts with foreign scientists is not limited to the Orient. Scientists from South America have visited NMI in the past eighteen months with questions about the farming of Macrocystis. Dr. Ribiero Dos Santos of Brazil is interested in the production of agar and agarose from Gelidium and made several visits to Santa Barbara to visit the NMI farm and facilities. He will be involved in planning and running the 1986 International Seaweed Symposium to be held in Brazil.

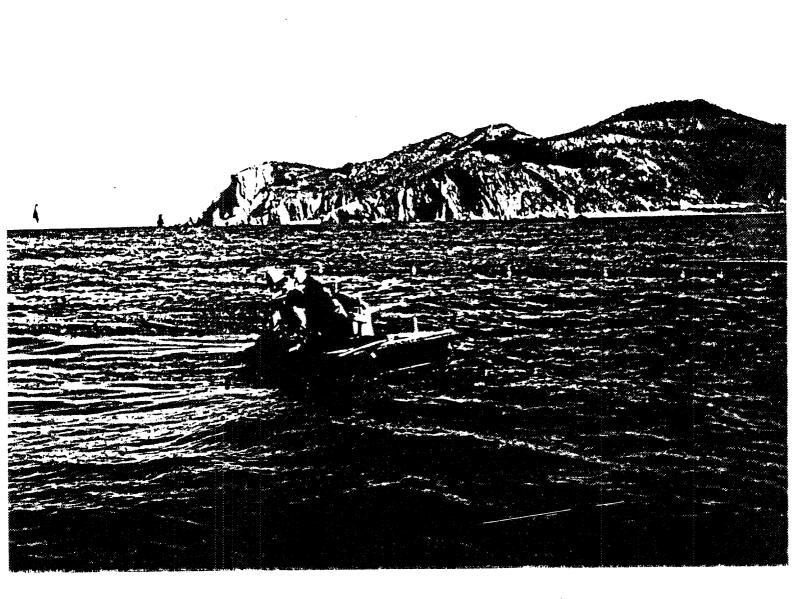


figure 47. Experimental biomass-production farming of the giant kelp, Macrocystis in the Peoples Republic if China, in the waters off Huangcheng Island, north of the Shandong Peninsula, 1984. Giant kelp was first introduced into Chinese waters from Mexico in 1978 by Tianjing Liu. A farm boat and the kelp canopy near it can be seen, and on the shore in the background a seedstock production facility can be seen. (photograph provided by Jiaxing Chen of the Yellow Seas Fisheries Research Institute, Qingdao, PRC)

## VI. TOWARD A THEORY OF KELP GROWTH AND PRODUCTION

The kelp biology data base collected at NMI provides a basis for the first step in the difficult process of producing a general, experimentally-testable theory of kelp growth and production. The heuristic value of attempting to model kelp growth, even when the data base is minimal, has long been recognized. The first kelp growth models were produced by Anderson (1974) and subsequent models have been proposed by Nyman and Neushul (unpublished msc.) and more recently by George Jackson (unpublished msc., personal communication). An adequate data base will eventually allow us to resolve some major disagreements. For example, does a frond grow at a constant rate as suggested by Gerard (1982), or show spurts of growth interspersed with periods of no growth, as suggested by Coon (1981a)? important role of sloughing as it influences plant productivity in general, has been considered only peripherally. Unexpected "semi-dormancy" has been seen in both adult plants (see Section V) and in juveniles grown under laboratory conditions (see Section Vii), and the significance of these growth- and survival strategies is not yet fully appreciated. It is likely that plant growth regulators (auxins, cytokinins, gibberellins, or their phycological analogues) will be found to play an important role in growth and resource allocation within the kelp plant. These and other questions can only be answered in a satisfactory manner by the gradual accumulation of experimental data over time.

## Frond Life-Spans and Sloughing Losses

The leaf-like photosynthetic organs of macroalgae like <u>Macrocystis</u> and <u>Sargassum</u> appear to be formed and lost in response to the availability of nutrients and light and may respond to seasonal differences in photoperiod. The loss of macroalgal "leaves" is not a well-defined process as is abscission and leaf fall in deciduous land plants. The loss of blades of <u>Macrocystis</u> is called "sloughing" which is an ill-defined process of decay, fragmentation and dissolution of blade material over time. However, this sloughing process is responsible for a major loss of plant tissue. Kelp yields would be increased very significantly if sloughing losses could be modified. In land plants, roots and microbes interact in what is called the "rhizosphere", similarly in macroalgae similar but less-well-understood processes occur on the surfaces of macroalgae in what has been called the "phycosphere." The processes of sloughing are those that occur in the "phycosphere."

Many broadly-applicable hypotheses have been advanced to explain the different patterns of leaf life-span in land plants (Chabot and Hicks 1982) and some of these land-plant hypotheses are usefully applied to the study of biomass-producing marine macroalgae. Leaf loss occurs in northern temperate climates to reduce snow and ice loading which can damage the plant. In the sea, the loss of epiphyte-loaded or senescent fronds which occurs prior to the turbulent winter period may also reduce hydrodynamic stresses on the plants, and reduce the levels of whole-plant loss during storms. Sloughing of blades also appears to occur when epiphytic loading interferes with the efficient productivity of the blade to the detriment of the rest of the plant.

Long-lived, evergreen leaves of land plants appear to act as carbohydrate and mineral storage organs. In the same way, the longer-lived and perennial parts of marine macroalgae, like holdfasts and stipes, may act as storage organs. In environments where low nutrient levels limit growth, land plants that retain their leaves conserve carbon and do not invest limited photosynthetic resources in the production of new leaves. In land plants, the life span of a leaf is thought to be related to the cost of producing a leaf versus the benefits derived from it. Once the cost of maintaining a leaf through unfavorable growth periods exceeds the profit it provides, it is discarded. These "costs" are measured in terms of carbon and nutrient "investment" in a leaf.

The loss of tissue in Macrocystis may also be an adaptive strategy related to plant survival in adverse conditions. When a macroalga is under stress the nutrients from the blades of the plant may be translocated to the longer-lived stipe or the perennial basal holdfast for storage (see Manley 1983) and the blades may eventually be lost. However, in macroalgae the "leaf" is a functional combination of leaf and root since the leaf photosynthesizes and the root takes up nutrients. Therefore, both the net carbon gain and the net nutrient uptake by a blade (or a frond, if this is the morphological unit selected) has to be considered in the cost-benefit ratio. In order to calculate the net total carbon gain by a blade or frond, and the net total nitrogen taken up by the same frond, a good deal data must be collected, including:

- 1. The daily carbon exchange rate per leaf of age I, expressed in days of leaf life span or length of season favorable for positive photosynthesis in days.
- 2. The net carbon export or loss in units of grams of carbon lost per blade of age I per day.
- 3. The length of the favorable and unfavorable seasons in days.
- 4. The intial cost of producing a blade in grams of equivalent photosynthate and amount of respiration needed to produce a blade of a given chemical composition.
- 5. The loss of leaf tissue and productivity due to stress during times of low photosynthesis, summed over the leaf life span. This is a double cost since the actual biomass lost has to be added to the reduced future photosynthetic capacity of the blades which remain.
- 6. Loss of tissue to herbivors, summed over the leaf life span and expressed in grams of equivalent photosynthate.
- 7. Photosynthate stored and not transported.

The leaf "profit" of mg of carbon dioxide fixed can be estimated by measuring the maximum net photosynthesis, in mg carbon dioxide per square decimeter per hour, the specific leaf weight in mg per square centimeter, and the leaf area in square centimeters, and taking into account the age of the leaf and its life span in days. Simply by moving a plant from a shaded habitat in a kelp farm into open water, the maximum photosynthesis rate can be doubled, and the duration of the time of maximum net photosynthesis is increased.

Using higher plant data, where leaf payback time is a function of the environment, calculations show that a leaf of Fragaria virginiana pays for itself in about 12 days, in an unshaded habitat. In a shaded habitat, as in a forest, the payback time would be more than 30 days. If the growth increase in wet weight of an apical blade of Macrocystis is 10% per day, and if, as Fei and Neushul (1984) have shown, the mature blades do not grow (but presumably continue to photosynthesize and export their product) it would take approximately ten days of such export to "pay back" the initial cost of blade production in Macrocystis.

Differences in foliar nutrient content are known to be associated with differences in the internal use of, and translocation of nutrients within the plant body. As leaves are lost in leaf-fall, so are nutrients. It is not known if macroalgae export nutrients from blades prior to sloughing, as do land plants. Selection for efficient nutrient utilization would favor those plants with perennial parts (like Macrocystis) over plants that have a comparatively brief annual or biennial life-span (as with Laminaria, or even the closely-related macroalga Pelagophycus).

#### Plant Growth Regulators and Kelp Production

A more detailed theoretical kelp yield projection is now possible given the physiological data-base accumulated by Manley, Gerard, Wheeler and NMI. Hydrodynamic blade-water-interactions have been studied in a preliminary way (Anderson and Charters 1982 and Section IV). Growth and production measurements have been made and the preliminary harvesting of farmed kelp plants has resulted in solid data that can be used to measure kelp production and yield. Doty (1971) has considered the impact of antecedent events on macroalgal growth in general, Chapman and Craigie (1977) have studied the impact of antecedent events on kelp growth, while Harger (1979) conducted a study of Macrocystis in particular. Clearly, the multicellularity of the giant kelp, and its complex tissue system, is an adaptation that enables the plant to respond to changes in environmental conditions with periods of dormancy, programmed frond initiation, nutrient storage and pigment accumulation. In higher plants, this kind of response has been traced to plant growth regulators (auxins, kinetins, gibberellins, and ethylene). A careful review of kelp growth studies by algolgists indicates that little is known about how plant growth regulators influence kelp growth and production, but it is an area where further work could be very rewarding indeed.

Plant growth and development is controlled by simple organic chemicals; Tatewaki, Provasoli and Pintner (1983) have shown that some marine bacteria and species of red and brown algae release active extracellular compounds that play an important role in the growth and morphogenesis of other macroalgae in the sea. Using bacteria-free unialgal and bialgal cultures, they studied 15 macroalgal genera and showed that morphogenetic substances, as yet unidentified, are actively synthesized and secreted in bulk into the sea. The amount present in coastal waters was shown to be sufficient to stimulate normal algal development under culture conditions. While it has long been known that marine algae secrete pigments into the sea, the so-called "yellow substance, or gelbstoff", the suggestion that they also secrete large amounts of developmentally-active compounds is particularly interesting.

#### VII. NEW RESEARCH TECHNIQUES

The tools and techniques available to the marine phycologist often determine data accuracy and the rate of data acquisition. In some instances, for example, in the measurement of all the fronds and blades on a single kelp plant, the task is so laborious if the measurements are made by hand with a planimeter that it is not often done, and if done, seldom repeated. The hand harvesting of over 60 tons of wet kelp from the experimental kelp farm by NMI was also laborious and time consuming. The measurement of light and hydronamic conditions directly in the sea has traditionally been done by divers using hand held instruments in conjunction with an underwater tape recorder. More sophisticated instrumentation is available but it is often very large, costly and not suitable for use in kelp beds.

Less-laborious and more accurate techniques for studying biomass producing macroalgae, as they grow in the sea, are needed to adequately document how they respond to conditions in the underwater environment. Considerable progress has been made at NMI by using microcomputers rather than divers to take measurement in the field. Image analysis, which results from interfacing a computer with a television camera, has been used in a preliminary way to study plant morphology and growth. A computer is now used to constantly monitor and record temperature and light conditions in the greenhouse culture facility at NMI. In the laboratory, improved methods have been introduced for producing and growing seedstock under controlled and semi-controlled conditions.

### Data-Base Acquisition, Management and Archiving

A common problem in research programs which accumulate large amounts of different types of data is keeping it easily available for analysis. NMI has an effective data-acquisition and storage system which is used to acquire and process data on physiological responses, growth rates, responses to light and nutrients, climatic and oceanographic factors and reproductive processes on a regular basis.

The cornerstone of the NMI data-processing system is the Vector System B computer system which is versatile enough to interface with more powerful computers at the University of California at Santa Barbara via a modem. The system is also portable enough to be used at sea. The in-the-sea data-acquisition system that has been developed for use on the Research Vessel "Triton" is built around both the Vector System B, and a California Computer system unit with a separate disk drive. The unit collects light, wave and hydrodynamic data via sensors, which are recorded by the computer and stored on disks. These are returned to the laboratory where complex analyses are possible with both in-house computers, and by using a modem telephone connection that can transmit the field-collected data to the larger Digital Equipment Corporation VAX computer at UCSB.

The Vector System B system is also used for more conventional data-processing and word-processing tasks. The large collection of unpublished reports (some 413 items so far) in the NMI collection has been organized and cataloged on the computer so that this "gray" literature is now accessible for use.

Environmental measurements have been collected at Goleta Pier since 1980 and these include the daily measurement of wave height, water temperature, ambient air temperature, salinity, water clarity, wind speed, currents and the general sea state measured on the Beaufort scale. Detailed weekly measurements are made by divers at Ellwood Pier and this information is archived and analyzed, to produce seasonal records that make year-to-year comparisons of ocean conditions possible. This data has recently been supplemented by in-the-sea measurements fed directly to an on-board computer on the Research Vessel "Triton." These real-time measurements of light, wave height and period, and water motion will make it possible to relate plant performance to environmental conditions. Currently, the theoretical analysis based on "cylinders" provided by Battelle (Wang and Ditmars 1982, see Figure 59) is now being tested using this on-board hydrodynamic test equipment.

#### Morphometry and Image-Analysis

An image analysis system has been operated in the past to measure blade areas and sloughing. This image analysis system operates from video-images stored on tape which is subsequently analyzed using the Vector System B microcomputer system. The system has also been used to measure areas of surface kelp canopy visible in aerial photographs and photo-mosaics of kelp beds. With this unique data-acquisition and analysis system for computer-acquired and stored images, the area and sloughing of large, mature kelp plants can be measured.

#### Cytological, Ultrastructual and Anatomical Techniques

A microspectrophotometric method has recently been developed for measuring DNA levels in kelp gametophytes and sporophytes (Figure 48). This employs a nano-spec-20 microspectrophotometer attached to an epi-flouresence microscope which can differentiate diploid and haploid nuclei by their relative flouresence. This new instrument will be used to compare DNA levels in various kelp strains, which will be more accurate and less time consuming than counting the tiny chromosomes of kelps to determine ploidy levels.

Ultrastructural methods including traditional fixation, embedding and sectioning have been employed in the past at UCSB by E. Y. Chi and M. Neushul to study kelp spore formation. These methods were also used to study sporangial dysfunction in hybrid kelps, and are now being used by Mr. C. Amsler at UCSB, to study kelp spore release, distribution and attachment. Work done by H. Rahimian on critical-point-dried kelp blades, using the scanning electron microscope will be extended to illustrate the extent to which kelp surfaces are colonized by bacteria and fungi, which can cause problems in seedstock production and outplanting. A novel method of gluing kelp fronds to aluminum foil, and then pulling them apart, has provided an alternative to more traditional sectioning methods, and provided some insight into how kelp conductive tissues are arranged.

#### Physiological Techniques

The use of calorimetry, pigment-extraction methods, and methods for measuring photosynthesis and respiration are critical to any physiological study. The study by M. Shivji of juvenile Macrocystis plants grown under defined light and nutrient levels was made possible by the use of the flow-injection-analysis system developed by NMI, which measures nitrate levels at the rate of one sample per minute.

The literature on plant growth regulators in kelps has recently been reviewed, and co-funding has been sought to purchase a new cryo-sectioning device at the University, for use in developing immunohistochemical techniques. These techniques will be used to study the functioning of the basal branching system of Macrocystis and to test theories about marginal epigenesis and tissue senescence (Neushul 1983).

Professor X. G. Fei concluded his two year stay sponsored by the GRI international collaborative research program in 1984. Professor Fei designed and built a unique raceway culture system with controlled light levels to grow whole kelp plants and parts of kelp plants under defined light conditions, in order to measure growth and sloughing rates. He concluded (Fei and Neushul 1984) that immature surface, and subsurface kelp blades have different growth and sloughing rates (Figure 49). This data will be useful in determining the leaf payback time of Macrocystis and in analysing the internal allocation of resources in the kelp plant.

Professor Fei produced an additional data set comparing the response of juvenile kelp plants to various light levels in raceway culture (Figure 50). The growth and sloughing of very small (0.3mm long) sporelings, juvenile plants (10cm long) and parts of the mature kelp plant were compared at five different defined light levels (Figures 49 and 50). The optimum light levels for mature kelp growth are much higher than those for young sporelings. A summary of the definition of optimum light levels for kelps is shown in Table 9.

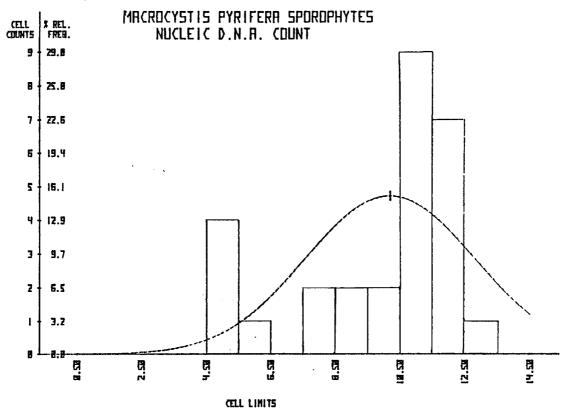
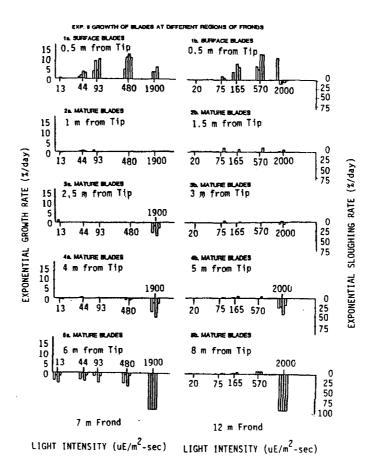


Figure 48. Measurements of nuclear DNA in sporophytic nuclei of Macrocystis pyrifera made with an epiflourescent microscope equipped with a microspectrophotometer, showing that this new technique is capable of measuring nuclei-acid changes during the normal cell cycle. The plants studied were cultured from warm-water Mexican plants, kindly provided by W. J. North. Gametophytic isolates of these plants and sporophytes derived from them are presently under cultivation in Goleta at NMI.

Work on the physiology of kelps was not a part of the NMI task list (see Table 1), but NMI did assist in the work of M. Shivji who worked in M. Neushul's laboratory at UCSB. A flow-injection nutrient analyzer for taking field measurements was developed at NMI and used by M. Shivji for his thesis work. His findings provide some new insights into the interaction of light and nutrients in controlling kelp growth. Indeed, the cessation of kelp growth in the GE hemidome experiments under low light, high-nutrient conditions may be a more general phenomenon in the sea than was apparent before (Figures 51 to 55). For these figures, high, medium and low light correspond to 305, 132 and 25 uE / sq m - sec. High, medium and low nitrate levels correspond to 20, 7 and 1.5 uM nitrate.



graphic 49. **Figure** representations of growth (in % gain per day) and sloughing-loss (in % loss per day) of parts of a Macrocystis plant held under defined light conditions in a raceway, comparing how the plant parts respond at five different light levels. The results of four to five experiments (multiple bars) are shown, illustrating that deep-water mature blades light consistently slough at high intensities, while mid-water and surface blades do not. (from Fei and Neushul in press)

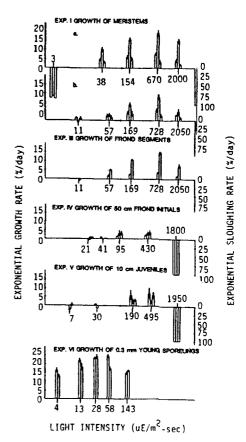
# Culture Methods, Seedstock Production and Planting

The culture techniques that have been employed at NMI combine the advantages of uni-algal, and more recently bacteria-free laboratory culture, with tank- and in-the-sea methods of cultivation. The growing collection of seedstock is maintained by NMI in Percival plant growth incubators and in two walk-in cold rooms. Gradient tables, similar to those designed by Yarish, Lee and Edwards (1979), have been used to grow microscopic gametophytes and juvenile sporophytic germlings under crossed gradients of light and temperature.

The tank culture system at NMI (Figure 56) is used to harden the the tube and dish-cultured plants grown under controlled incubator and cold-room conditions before they are transplanted into the sea. The NMI system diagrammed in Figure 57 consists of two insulated 400 gallon sumps from which cooled and filtered water is pumped up into two 100 gallon glass water tables. Gravity flow returns the water to the sumps. In addition, fresh seawater held in two 1000 gallon holding tanks flows into the sumps by gravity. All water lines and fittings used in the system are made non-corroding, non-metallic polyvinyl chloride material. This system is an improved, self contained version of the hydrodynamically defined culture system designed for benthic seaweeds by Charters and Neushul (1979). The system can be operated as two completely separate systems, and nutrients can be added independantly to the two 1,000 gallon sumps and then released in pulses or gradually to the recirculating systems.

Control of temperature is achieved with two Frigid Unit immersion coolers that are capable of maintaining water temperatures of 15 deg C. during the warmest summer months. Unfortunately, these units corrode after about a year and are expensive to replace so that NMI has developed a new system that has larger compressor and plastic cooling coils. Preliminary trials with this unit have been successful.

Figure 50. A graphic representation of growth and sloughing losses seen in five experiments with meristems, frond segments, frond initials, juveniles and very small sporelings grown under defined light conditions (from Fei and Neushul 1984).



Water motion in the culture system is essential, and this has been achieved so far with an oil-free, high-volume low-pressure blower. Air is introduced into the tanks from plastic pipes running the length of the tank. Filtration is achieved with Purex High Rate sand filters, and Sta-Rite vertical-grid diatomaceous earth filters. This double-filtration and chilling delivers very clean cold water to the high-tempered glass tables. These are easily cleaned, and without screens provide high light levels. The addition of screens and covers allows the cultivator to control light levels and to exclude dust from the system. Light levels in full sunlight can reach 2100 uE / sq m - sec, a level that is reduced to 900 under the plastic greenhouse roof. Several perennial macroalgae have been held successfully for nearly one year in the system.

Table 9. Optimum Light Levels for the Growth of Giant Kelp

Plant or Plant parts	Optimum range (uE/sec M <sup>2</sup> )	Response to 100% Daylight	Response to 1% Daylight
Adult Meristems			
Length	86-1600 (843)	Inhibiting	Sloughing
Wet Weight	190-1800 (995)	•	
Splitting	36-940 (488)	•	M
Adult Frond Segments (se			
Length	120-1500 (810)	*	R
Wet Weight	160-1500 (830)	*	×
Adult Immature Blade (si			
Length	140-900 (520)	*	No Change
Width	140-1100 (570)	*	Ħ
Area	180-1250 (715)	•	
Wet Weight	130-1400 (765)	Ħ	19
Adult Mature Blade	222 0 (,,	No Change	No Change
Adult Mature Blade (bots	tom)	Dead	N
50cm Frond Initials	•		
Length	80-930 (505)	Dead	Sloughing
Wet Weight	70-740 (450)	#	9
Splitting	80-720 (400)	*	н
10cm two branch juvenile	•		
Length	140-1000 (570)	•	Ħ
Wet weight	110-700 (405)	*	и.
Splitting	140-900 (520)	H	11
New Holdfast	500		
0.3mm young sparelings			
Length	4-149 (45)	• ,	Growing we
Width	9-90 (30)		· · · · · · · · · · · · · ·

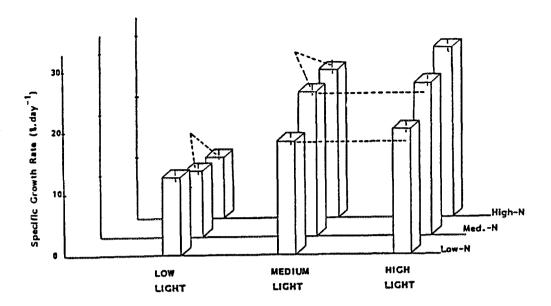
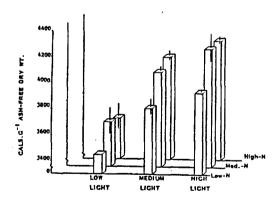
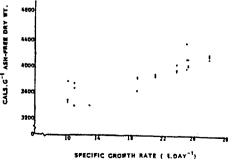


Figure 51. The results of laboratory experiments on juvenile Macrocystis plants grown under three light, and three nutrient levels, the nutrient levels being maintained by controlled flow and measured with the NMI flow-injection-nutrient-analyzer. This data-set shows that, contrary to what might be expected, growth at low light levels is not increased by the addition of nutrients, but in fact decreases, at low light levels, as nutrients are added. Rates joined by dashed lines are not significantly different. (from Shivji, J. Expt. Mar. Biol. in press)

Figure 52. The caloric value of juvenile Macrocystis plants grown under three combinations of light and nitrate, showing that caloric value decreases with increased nutrients at low light levels, but increases at medium and high light levels. (from Shivji, in press)

Figure 53. A scatter diagram showing the relationship of specific growth rate and caloric value of juvenile Macrocystis plants grown under controlled conditions, showing that rapidly growing plants have a high energy content. (from Shivji, in press)





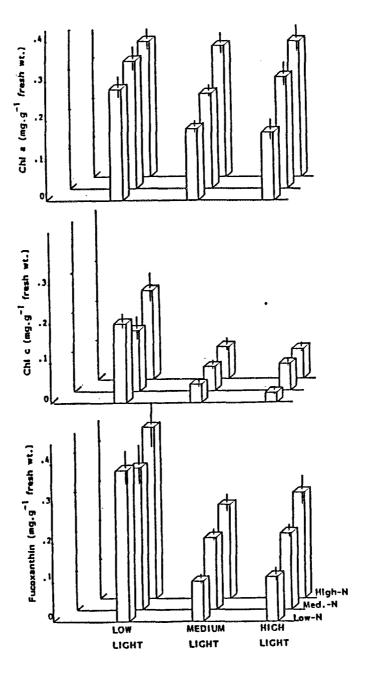


Figure 54. It has long been recognized that nutrient enrichment produces a color change in kelps. Measurements of chlorophyll a, chlorophyll С fucoxanthin in juvenile Macrocystis plants grown under different conditions of light and nutrients, show that those grown under low light, high nutrient conditions become both visibily, and measureably browner in color. This is illustrated by the amounts of chlorophyll a, c and fucoxanthin produced per unit fresh weight. (from Shivji, in press)

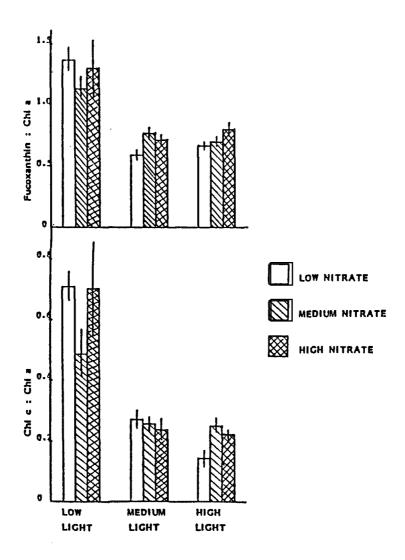
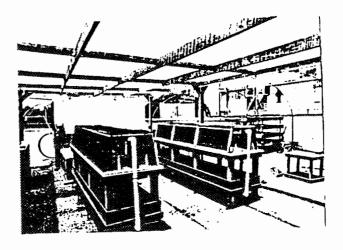


Figure 55. A plot of the molar ratios of accessory-to-primary-pigments in juvenile Macrocystis grown at low, medium and high nitrate levels in the laboratory, shows how the photosynthetic apparatus changes at low light levels. (from Shivji, in press)

Figure 56. The NMI tank-culture system, showing the corrugated fiberglass overhead that protects the tanks, which are on tables as shown, each table having additional light-modifying screens. Water enters the growing tanks through 2 "diam. pipes and returns to a sump at the far end. Large resevoirs are shown in the background.



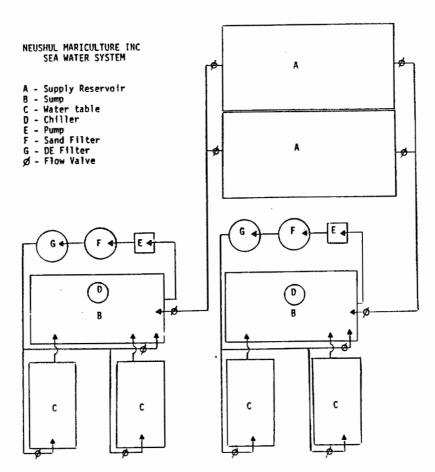


Figure 57. A diagram showing two sets of two culture tables (C) each having an independent sump (B), with two large resevoirs (A) serving as the seawater supply. Nutrients are mixed in the resevoirs and fed into the sumps to be recirculated onto the plants on the tables. The entire system is computer-monitored.

The one acre GRI test farm used large <u>Macrocystis</u> plants which were anchored to the sea floor using gravel bags. Preliminary work has also been attempted in 1984 to spray rocks with algal spores which are then placed in culture tanks before being transferred to the sea. Spray-seeded rocks produced sporophytes under laboratory and tank-culture conditions but these did not survive outplanting into the sea. However, this method with further refinement could be useful for future in-the-sea planting on a large scale.

Providing fertilizer to plants in the sea has been found to be effective at times of the year when the natural supply of nutrients is low. Yield in a farm plot to which fertilizer was applied yielded four times the amount of biomass than a control plot which received no fertilizer. Laboratory experiments by M. Shivji (see Figures 51 to 55) have shown that both growth rates and specific energy content (caloric value) are increased when fertilizer is applied to juvenile kelp plants. Pigment extractions showed that molar ratios of primary and accessory pigments changed as the plants grew in nutrient-enriched as opposed to nutrient-depleted water. The number of experiments carried out in the laboratory have, however, been limited and more work of this sort is needed.

#### Hydrodynamic Measurements in the Sea

Water motion is very important to the success of experimental culure of plants in controlled tank culture systems and in the sea. Measuring water motion in the sea has always been difficult and labor intensive before the development at NMI of a computerized system for taking hydrodynamic measurements from a boat. The system uses a Marsh-McBurney electromagnetic current meter (Figure 58) which provides a two-channel output measuring velocity in cm/sec in a flat plane. Two units are interfaced with a recorder and a California Computer Systems computer on board the Research Vessel "Triton" from which it has been possible to measure hydrodynamic conditions on the test farm at Ellwood and in natural populations nearby. Another study sponsored by GRI (Wang and Ditmars 1982) included some interesting diagrams of hypothetical wave action over a kelp farm (Figure 59). This new system makes it possible to test this kind of model by making direct in-the-sea measurements of natural sites (Figure 60) and actual farms (Figure 61).

The water motion conditions in several natural sites and on the NMI farm structures used for seedstock growout, is shown in Table 10 which compares the wave height, current and plant motion. This new information provides valuable insights into how farm sites might be selected, how arrays of plants might best be planted relative to prevailing waves and currents, and how dense plantings might influence wave and current patterns in a cultivated area.

#### Harvesting

The mechanisms used for harvesting kelp have changed very little since 1900 (Figure 62). The NMI kelp cutter (Figure 62) uses this cutter-bar, conveyor-belt system to cut and load fronds from the surface canopy. Because of the labor-intensive nature of hand harvesting, a mechanical harvester is essential for studying the effects of repeated harvesting on genetically-defined kelp strains. The very small 20ft NMI experimental harvester developed in 1982 was not stable enough to be useful in the initial farm yield studies. A new, larger harvester engineered for stability is being developed independently by NMI for future experimental harvesting work.

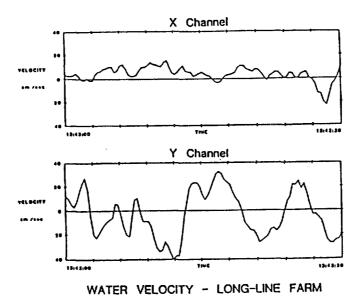
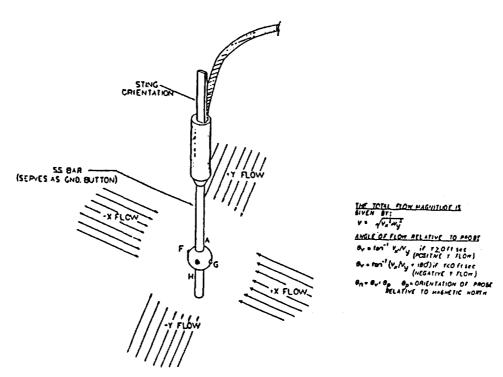
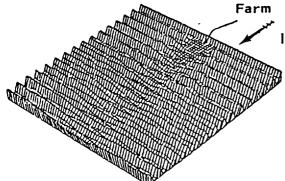


Figure 58. A Marsh-McBirney electromagnetic current meter shown diagrammatically (below) and water velocities measured on a Chinese-type, long-line outplanting farm in December 1982, illustrating the sensitivity of this instrument and it's usefulness in defining hydrodynamic conditions in the sea.



ECM sensor orientation.



Incident wave direction

Figure 59. A hypothetical modification of moving train through experimental kelp farm, showing waves moving diagonally from the right. Wave modification is based on the concept of the farm consisting of a series of cylinders.

Table 4. Relative responses of various test sites to ambient or modified flow.

LEYEL - FLOW ------ SITE OR STRUCTURE ----- PLANT

Wave-track 2 Std. De (cm)		Current Met Avg. Water Mo (cm/sec)		Movie Camera Max. Excursion Area (cm²)						
Naples Reef	37.38	Naples Reef	20.8	Naples Reef	1397.74					
LLF(par)8/12	28.35	LLF(par)8/25	15.4	LLF(par)8/12	748.87					
TMF(tuned)	28.19	AF	11.0	Ellwood Pier	740.35					
LLF(par)8/25	26.75	Ellwood Pier	10.8	LLF(par)8/25	722.66					
LLF(per)	24.23	TMF (tuned)	9.8	ΛF	722.31					
TMF(n.t.)	24.12	TMF(n.t.)	9.4	LLF(obl)	703.40					
LLF(obl)	23.24	LLF(obl)	8.2	LLF(per)	678.08					
Ellwood Pier	22.14	LLF(par)8/12	8.0	TMF (tuned)	233.14					
AF	21.41	LLF(per)	7.0	TMF(n.t.)	222.75					

LLF(par)8/12 = Long-line farm parallel to the prevailing swell tested 8/12/83. LLF(par)8/25 = Long-line farm parallel to the prevailing swell tested 8/25/83. LLF(obl) = Long-line farm oblique to the prevailing swell.

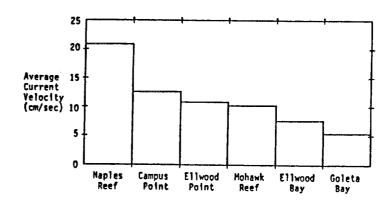
LLF(per) TMF(tuned) Long-line farm perpendicular to the prevailing swell.

Taut-moored farm tuned.

= Taut-moored farm not tuned. TMF(n.t.)

- Accordion farm.

Figure 60. The definition of optimal hydrodynamic conditions for farming can be based in part on the ranking of macroalgal habitats in Santa Barbara County, where measurements of average current velocity (in cm/sec.) have been made using а Marsh-McBirney electromagnetic current meter, and a wave tracker. Underwater photographs were made of plant movement under surge conditions as well. Naples Reef is a highly productive macroalgal habitat (see data on water movement in natural habitats and on farm structures in the preceeding Table).



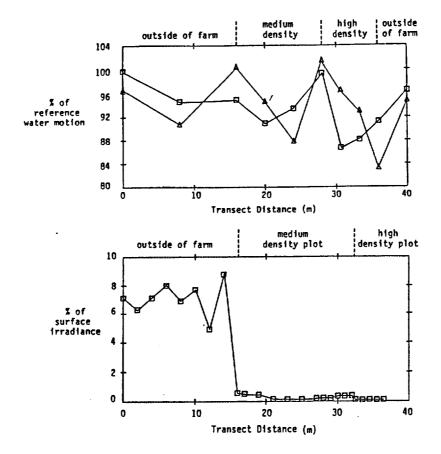
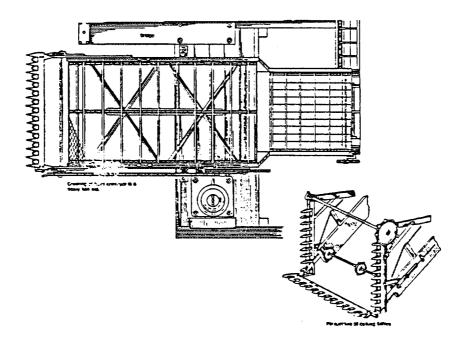


figure 61. A diver-carried current meter, attached by cable to an on-board computer, has been used to define hydrodynamic conditions in an experimental kelp farm, showing that as predicted (see figure 59) water motion is decreased as one swims from the outer edge into more densely-planted regions. A similar modification of the environment within a planted area can be seen when light measurements are made with with a diver-carried irradiance meter.



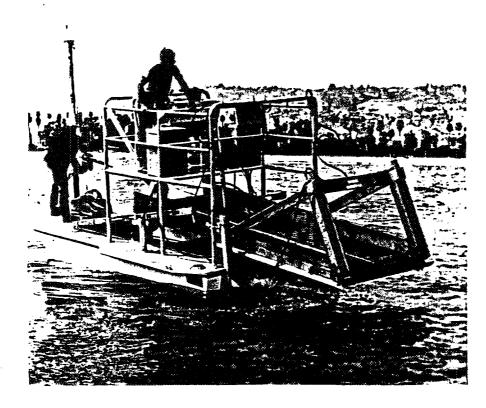


Figure 62. Harvesting techniques have not changed a great deal since the first kelp cutters were built around 1900. The early design is shown above (from Cameron 1915), the small NMI experimental cutter is shown below. A barge and tug system visualized by Breheney (in the Parsons systems study) was tried and found to be ineffective in 1915 (see appendix A).

#### VIII. FUTURE PERSPECTIVE

Looking back at the progress made since 1980, when NMI joined the marine biomass program, it is useful to examine both the successes of the program and its shortcomings and to reflect on the lessons learned. Certainly, there has been no shortage of criticism, indeed at times it seemed that there were more people employed to review the marine biomass program such as Dynatech, Battelle, Aquaculture Associates, Ralph M. Parsons and the Electric Power Research Institute, than were working in it

One of the most significant successes of the marine biomass program is that it has kept the United States at least current with the remarkable advances made by the Japanese and Chinese in marine farming of seaweeds and shellfish. The success of the Chinese system for farming Laminaria was made abundantly clear in 1983 when Merck Chemical Company imported kelp from China since the natural kelp beds off the coast of California were devastated by the El Niño climatic conditions. In 1983, the Chinese cultivated and harvested over one million wet tons of kelp biomass for internal consumption due in part to the decades of research by C. K. Tseng and his colleagues at the Institute of Oceanology in Qingdao. The introduction of Chinese alginates to the European market is now in progress and could eventually significantly undercut the present price of alginates in the world market. Harvesting the natural Californian kelp beds for alginate production in its present form will soon be unprofitable, since the Chinese can already produce and sell alginate at half the current world market price.

It is interesting to see whether Smil (1983) will prove correct in his opinion that the marine biomass program envisioned by Wilcox (1980), Show et al. (1979) and Flowers (1980) is in the realm of science fiction. Given the significant progress in the large-scale farming of the sea made by Japan, China and Korea in the last decade, this is unlikely. The progress made in learning how to farm the sea in the United States made possible by the GRI marine biomass program is significant and will prove invaluable in the next decade. Alternate sources of food and fuel will ultimately be farmed in the sea and the GRI marine biomass program has at least made it possible for the United States to maintain parity with the Japanese and the Chinese in learning how to best use these resources.

#### Prospects for the Future

China and Japan, unlike the United States, both have a long history of hunting and gathering food from the sea. In the last two decades, scientific research has established the life cycles of the major marine crop plants and made seaweed farms for food and by-products a profitable venture in both Japan and China. Farming the sea for biomass and by-products could also be profitable in the United States once the basic research on marine crop plants and products has been completed.

The GRI program has also encouraged the free exchange of information between the United States and other nations in the vanguard of scientific research in marine macroalgal farming, especially with China, and Japan which have commercially-successful macroalgal farm industries. NMI has also maintained close communication with Van Wachenfeld who heads the marine biomass program in Sweden which is also rapidly moving toward commercialization (Van Wachenfeld, personal communication).

The feature common to all these successful ventures into marine farming in Japan, China and Sweden is the initial resistance and scepticism of the funding agencies who provided the long-term support necessary to establish the industry. C. K. Tseng, who envisioned the planting and harvesting of giant kelp beds along the 4,000 mile long coastline of China was lucky to survive the cultural revolution to see his dream come true. The Chinese continue to expand their "science fiction" (Smil 1983) marine farms to include the cultivation in a coastal test farm of the giant kelp, Macrocystis, recently introduced from Mexico in addition to their farms of Laminaria which was introduced from Japan.

It is very encouraging indeed, to reflect on the fact that the successes of the GRI program have attracted a considerable amount of co-funding. Specific aspects of the overall GRI project, like marine farm engineering, genetics, and kelp planting in the sea have been of sufficient interest to agencies like the National Science Foundation, the National Institute of Health, the California Department of Fish and Game, and others, to attract co-funding.

Another encouraging aspect of the GRI program is the technological advances that have been made possible by this program. The only way that the United States can effectively compete with China and Japan in farming the sea is by using scientific techniques to design and operate marine farms rather than a large labor force. The research tools developed at NMI with GRI funding now make it possible to make computer recorded measurements of hydrodynamic conditions in natural macroalgal communities and the NMI test farm from a boat. Prior to this, it has only been possible to collect this kind of information by using divers with various rudimentary equipment such as the "fishpole tensiometer" and tape recorder used by Charters, Neushul and Barilotti (1969), or the "clod card" developed by Doty (1971). In addition, significant advances have been made in developing new culture methods for producing and outplanting macroalgal seedstock using gradient-tables, hydrodynamically-defined tank culture (see Charters and Neushul 1979) and tissue culture systems.

An area of research that has provoked a good deal of interest at NMI currently is the presence of plant growth regulators in algae reported by Mooney and Van Staden (1984) who studied the changes in cytokinin levels in Sargassum with season and Featonby-Smith and Van Staden (1984) who studied Ecklonia. This work is significant to the marine biomass program because it could give some insight into how algae control dormancy, frond-initiation, and the production of sporogenous tissue. The basal branching system in algae apparently functions to allocate, and perhaps even to store resources but it is also possible that plant growth regulators are involved in defining sources, sinks and patterns of translocation. The difficulties encountered in culturing Sargassum both in tank and tissue culture may be overcome by effectively isolating and applying plant growth regulators to the cultures. Some further work on macroalgal hormones has been done in Scandinavia by Lizbeth Fries (1970), and in Washington by Susan Waaland (1975, Waaland and Watson 1980).

## A 1,000 Acre Alternative Natural-Gas-Production Facility in Goleta Bay

The report by the R. M. Parsons Company (Brehany 1983) concluded after detailed and careful analysis, that methane production from a nearshore Macrocystis farm could be economically feasible. This study did not include the historical data-base that has been re-discovered by P. Neushul (see Appendices A, B and C). The conclusions of the Parsons Report were based in large part on kelp yield results obtained by NMI, from a small hand-harvested, one-acre coastal test farm operated at Ellwood for two years. Clearly, much more yield data are needed before a large-scale pilot coastal test farm is attempted. The Parsons model for a coastal macroalgal gas-production system was based on planting in and thickening the existing kelp beds in Santa Barbara County which are leased to commercial kelp harvesting companies.

NMI has recently concluded negotiations with the California Department of Fish and Game to lease beds 26 and 29 which have just become available for lease. These beds have yielded harvests of as much 100 tons of biomass per hour in the past and have collectively occupied some 1,600 acres of sea surface at times of maximum development. Presently, the beds have been denuded of kelp by the El Niño conditions and represent a unique scientific laboratory to use the marine farming techniques developed at NMI in the last four years to plant a coastal kelp farm.

Southern California Gas Company operates a large gas-storage facility immediately on shore next to the Goleta Bay kelp bed Lease #26. This facility is managed by Mr. Carl Anderson. Mr. Anderson was approached in September, 1984, about the possibility of providing on-shore space for NMI to use for a seawater system and greenhouse. The ultimate goal of the project could be to assemble a pilot-scale facility to produce pipeline-quality biogas for commercial use. Although the development of a pilot program at present may not be feasible, NMI has available the two major components needed for such a marine biomass gas production project, namely the leases for two large natural kelp beds and access to land for an on-shore gas production facility.

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# APPENDIX A

Potash From Kelp: The California Kelp Industry 1914-1922

Peter Neushul

Large scale harvesting of California kelp for conversion into potash began in 1914, when Germany placed an embargo on the export of potash to the United States. In 1913, the United States was the largest importer of German potash in the world purchasing 1,092,588 short tons of potash at a cost of over \$13,000,000. During World War I, potash from the kelp harvested in California was used both as fertilizer, and in the manufacture of black gunpowder. The potash production process could also be modified to yield acetone. This important by-product, was used in the production of cordite, a smokeless gunpowder used extensively by the British during the war. Most of the California kelp companys used an old processing technique first developed in the 17th century by seaweed burners in Scotland, but on a larger scale. In contrast, the Hercules Powder Company developed a completely new process for extracting potash, acetone, and other chemicals from the kelp. In 1917, the United States Department of Agriculture built an experimental plant at Summerland, California, in order to aid private industry in the development of efficient processing techniques and new products from kelp. Despite this effort, the California kelp industry, which had flourished from 1914 through 1919, collapsed in the face of renewed importation of potash from Germany and French Alsace.

During the 17th-18th centurys, the dwindling supply of wood in many European countries forced them to import large quantities of potash, prompting a search far cheaper domestic sources. The kelp industry began in France during the 17th century, where seaweeds were processed for their soda content, which was used in the manufacture of glass and the glazing of pottery. By the turn of the century, the industry had spread to Ireland, Scotland, the Orkneys, and Norway, where seaweed was harvested for both its soda and potash content. Potash extracted from kelp was a vital ingredient used in the production of soap, alum, saltpetre, and as a bleach (1). For centuries, farmers had also made extensive use of seaweeds as a fertilizer and mulch. In Scotland, as the supply of ash derived from wood became scarcer, production of kelp was increasingly important to the economy, until eventually "kelping" became the predominant occupation in the Highlands and Islands. The Scottish kelp industry experienced two boom periods the first during the War of American Independence (1775-83), and the second during the Peninsular War (1808-14). major source of wood ash was from her American colonies, a supply which was cutoff after the outbreak of hostilities, creating an increased demand for potash from the Scottish industry. The use of kelp as a source of potash continued up until 1814, when foreign imports and Le Blanc's development of a method for extracting soda from salt, resulted in the decline of the In 1841, the European industry revived itself briefly, when seaweeds were processed for their iodine content. However, the discovery of extensive saltpetre mines in Chile which offered a far more abundant source of iodine (from the mother-liquors of gunpowder manufacture), dealt a final blow to the European kelp industry (2). It was not until the 20th century, that a kelp industry re-emerged in the United States, due to the potash shortage created by World War 1.

Prior to World War I the majority of the world's supply of potash for fertilizer came from the Stassfurt mines, in Northern Germany, where in 1852, immense subterranean deposits of potash salts were discovered. In 1861, the eminent soil scientist Justus von Liebig proclaimed the efficacy of "bitter salts" as a fertilizer. Liebig and other chemists during the 19th century discovered that the presence in the soil of potash, nitrogen, and phosphates provided elements which were vital to plant growth, and that intensive farming rapidly depleted this supply. Beginning in 1861, potash salts were widely used as fertilizer in the sandy soils of Northern Germany. Realizing the commercial value of this enormous natural resource, German producers increased the number of mines, and began to export potash fertilizers. The mining and sale of potash was regulated by the German Government while the marketing of the product was handled by the Kali Syndikat, which represented all the mine owners under the supervision of Government officials (2).

Nitrogen, Phosphate, and Potassium are the three most important nutrients required for plant growth. Although Potassium is ranked third in value, no other element can more than partially replace it in a plant, because it is essential to the formation and translocation of starch and in the synthesis of other carbohydrates. During the war, it was feared that the potash shortage in the United States was becoming acute in localities which normally required large quantities of the fertilizer. In 1918, investigations by representatives of the United States Department of Agriculture and the Maine Agricultural Experiment Station showed that soil in Aroostook County known as "Washburn loam" was suffering from a distinct shortage of potash. Potatoe plants were discolored or bronzed, and in some cases blackened and dying as a result of the fertilizer deficiency (4). Fortunately, not all Maine soils were as demanding as the Washburn loam, however, an officials at the Maine Agricultural Experiment Station was still inclined to state that Maine farmers would need all the potash they can get, and that "there is no question that on potatoes and root crops in general, potash pays agriculturally in this State" (5).

In the United States, potash was widely used to improve the light sandy soils found in the Gulf and South Atlantic States and in New England (Table 1).

Table 1. Numbers represent the average number of pounds of pure potash (K20) employed per hundred acres of cultivated land:

										Pound s
Florida										2,131
South Carolina										1,317
New Jersey										1,307
Massachusetts .										966
Maine										900
North Carolina										837
Rhode Island .										810
Georgia										764
Connecticutt .										757
Delaware										747
Maryland										690
Alabama				•						517
Virginia										426
New York										307
New Hampshire .			•						•	288

<sup>\*</sup> Taken from Thomas H. Norton, "The Potash Famine: Its Magnitude and Effects, and Remedies Promised for the Future," <u>Scientific American</u> 114 (Feb. 5, 1916): p. 145.

Potash was particularly useful in the intensive cultivation of cotton, corn, potatoes, beets, and tobacco, crops which require heavy applications of fertilizer (6). Although the majority of potash imports were mixed in fertilizers, a large quantity was also used by the manufacturers of glass, gunpowder, soap, matches, and various dyes. Sales of potash for use as a fertilizer in the United States were so large, that State Department officials began to fear that a potash embargo by Germany, or a failure in production, could seriously effect the nation's agricultural productivity. America was particularly dependent upon the imported German potash, as it had no natural supply, and purchased over half the total amount of potash exported from German mines. In 1910, American importers tried to increase potash imports to the U.S., a move which was opposed by the German Government, leading to a series of diplomatic exchanges between the two countries. Because of the considerable attention which the press gave to the United States dependence on German potash, Congress resolved in 1910, to make a special investigation of domestic sources. Government interest also stimulated private companies to investigate the feasibility of producing potash in the United States (7).

In 1902, long before the production of potash from kelp became an issue in the U.S., David M. Balch, a chemist from San Diego, California, had "noticed that masses of giant kelp, lying above the surf line on Coronado strand, were covered with a heavy efflorescence of air-dried salts . . . Analyses in my private laboratory led to the unexpected, in fact amazing result that the said salts were potassium chloride, almost chemically pure" (8). Balch pointed out the vast amounts of kelp available, and suggested several methods for commercially exploiting the new source of potash. He carried out numerous analyses of the inorganic constituents of the giant kelps by drying and charring samples, in order to determine the relative advantages and disadvantages of extracting potash from kelp. In December 1909, Balch wrote an article for the Journal of Industrial and Engineering Chemistry, in which he gave a chemical analysis of California kelps (9).

Balch considered the development of efficient methods of kelp harvesting to be vital if it was to become an economically viable source of potash. In the past, kelp had been collected by hand along beaches or cut from reefs and transported to land in boats. Once on land, the material was spread out to dry in the sun, and then burned, leaving a residue of impure potash. Balch described these methods as "costly in the highest degree", and suggested that steam power be substituted for hand labor. Balch envisioned a mechanized harvester which could move quickly from place to place, selecting the best locations, cutting the plants, drawing them on board and then carrying the harvest to shore. Kelp plants are 87.5 percent water, and consequently require a extremely efficient method of drying before being converted into potash. Balch suggested that an artificial source of heat be used, rather than relying only on the relatively inefficient and often unreliable sun and wind. Although Balch received no financial rewards for his research, his suggestions for the commercial exploitation of the vast kelp resources of California were the impetus for future harvesting of kelp for potash (8).

During the spring and summer of 1911, the Federal Government, through its agencies, the Bureau of Soils and the Geologic Survey, began reconnaissance surveys to determine the potash resources of the United States. Early in the summer of 1911 three field parties were organized to evaluate the California kelp beds as one possible source of potash. Professor George B. Rigg observed and mapped the kelp beds or groves in Puget Sound; Professor Frank M. McFarland surveyed the groves from San Francisco Bay to Point Sur; and Captain W. C. Crandall, of the La Jolla Station of the Marine Biological Association of San Diego (now the Scripps Institution of Oceanography), surveyed the groves of the main shore and outlying islands from Point Loma to Point Conception. The results of this government survey were published in 1911, in a Senate Document entitled "Fertilizer Resources of the United States" (9).

One conclusion of the Government survey, was that the Pacific coast kelp groves might yield 8 million tons of potash annually, worth about \$300,000,000. In addition, kelp also could provide valuable byproducts such as iodine, ammonia, and algin. In 1915, the Department of Agriculture published a second report under the authorship of F. K. Cameron, W. C. Crandall, G. B. Rigg, and T. C. Frye, which embodied the results of surveys of all the main areas of kelp in American waters from the coast of Mexico to the northwest peninsula of Alaska. Although potash from kelp was not expected to entirely replace the material imported from Germany (811,000 short tons in 1913), a domestic source of fertilizer protected agricultural interests in the U.S. from being completely at the mercy of imports from Germany (10).

The 1915 U.S.D.A. report showed that there were four pricipal varieties of kelp growing along the coast of California, these being Macrocystis, Nereocystis, Alaria, and Pelagophycus. These were found growing at depths of over 100 feet, but most often grew at depths of 15 to 60 feet. Beds of "Giant Kelp" or Macrocystis were most frequently harvested, as the plants grow so quickly and in such great quantity, that it was possible to harvest the beds several times a year (11).

From 1911-1919 a nine-year kelp boom raged along the California coast, reaching its height after the U.S. entry into World War I. The first commercial organization to attempt to utilize the Pacific kelps as a source of potash was the Coronado Chemical Co. of San Diego, California, founded by engineer, Henry S. Firman. In 1911, a plant was built at Cardiff-by-the-Sea, a town 20 miles north of San Diego, and a "secret process" devised by Dr. Firman was used in an attempt to produce a material containing soluble potassium salts and phosphates, together with other substances. In 1913, Harry Wilson of San Francisco, another pioneer in the kelp industry, founded the Ocean Products Co., and built a plant at Half Moon Bay near San Francisco. Neither of these businesses were successful until 1913 when they combined to form the American Potash Company with a factory in Long Beach where kelp was harvested for conversion into postash and other products. The American Potash Company was joined in the Los Angeles area by the California Fertilizer Co. at Terminal Island, which produced a fertilizer made up of 75 percent kelp, 15 percent sardine meal, and 10 percent bone phosphate. Also in 1913, two other companies were formed near Point Fermin, in San Pedro; the Pacific Products Co. and the Pacific Kelp Mulch Co. Other concerns were the Pacific Kelp Co. with an experimental plant at Pillar Point, Half Moon Bay, and the Kelp Products Co., with a factory in San Diego. These pre-war kelp companies were small and often shortlived enterprises, a great many of which were stock schemes that never materialized into an actual businesses (12).

With the outbreak of World War I in August 1914, the price of German potash more than doubled and U. S. investors began to put their capital into the California kelp industry which could provide an alternate source of fertilizer. In 1913, the U.S. imported 1,000,000 short tons of potash at a cost of \$13,200,413. In contrast, in 1916, the U.S. was importing 1,726 short tons of German potash salts per year, valued at \$453,091 (13). By 1916, a total of 11 kelp processing plants were operating in Southern California at San Diego, Wilmington, and Long Beach. The largest of these were the Hercules Powder Co. Chula Vista; Swift and Co. Kelp Works, San Diego; Diamond Match Co., Wilmington; and American Products Co., Long Beach (formerly American Potash).

In late 1915, S. G. Smith and J. E. Sellers, representatives of the Swift & Company, leased the small processing plant of the Kelp Products Company at Roseville, San Diego, and began experimenting with kelp processing on a small scale. Their investigations showed that "the amount of potash secured from the dried kelp ran about 12 percent per ton" (14). In July 1916, C. S. Churchill, general superintendent for Swift & Co. supervised the construction of a large scale kelp processing plant and a 250 foot wharf at a cost of \$250,000. Extraction of potash fertilizer from kelp was a temporary project for Swift, which planned to discontinue operations at the end of World War I. Swift used the basic "dry" method for processing kelp, in which the raw material was harvested and then dried in "nine large revolving drums by direct heat . . . ground like feed, sacked and shipped" (15). Upon its arrival, the ground kelp was mixed with other ingredients for the manufacture of fertilizers. The Diamond Match Company also used the "dry" method for potash extraction, however they carried the process further, crystallizing the potassium chloride for use in the manufacture of matches (16).

By 1912, the "dry" method for extracting potash from kelp, which was used by the majority of potash producers in California, was divided into six stages: harvesting, air-drying, oven-drying, destructive distillation (burning), crystallization of the potassium chloride, and marketing. It was estimated that in order to produce 1,000,000 tons of potash, 300,000,000 tons of kelp had to be harvested. Mechanized harvesters were developed, which vastly increased the industry's production capabilities. Kelp drying required the construction of drying sheds equipped with shelves upon which the raw material could be spread. In order to produce a ton of potash, about 27 tons of water had to be evaporated from the kelp. Air-drying in open sheds did not completely remove the necessary amount of water and consequently, the partly dried kelp was further dried in ovens. About 40-50 percent of the potash effloresced through the outermost cells of the kelp, and was collected by shaking the dried material. The remaining potash was extracted by breaking up the organic matter of the kelp through destructive distillation (17). Although most companies used the "dry" method for potash production, the Hercules Powder Company, whose major interest was in the production of acetone from kelp, developed a far more productive "wet" kelp processing technique.

Hercules Incorporated was created in 1913, following a U.S. Government antitrust suit against the Du Pont de Nemours Powder Company. At the conclusion of the lawsuit, Hercules was awarded eight black powder mills, three dynamite plants, and several patents for the manufacturing of smokeless sporting powder, (the explosives used in rifle and shotgun shells). The court order also required that Hercules compete vigourously with its parent company in the area of explosive manufacturing and marketing. During World War I, the two year old company produced 46 million pounds of cordite for the British Government, demonstrating its ability to develop new production methods to fill vital wartime requirements, a trend which continued after World War I, until today Hercules Incorporated is one of the largest diversified chemical producers in the world (18).

As noted earlier, the Hercules Powder Company's kelp processing plant was by far the most innovative of those designed during the 1911-1919 kelp boom. Hercules did not use the "dry" method for extracting potash, but developed a unique fermentation process to extract potash, acetone, and other products from the kelp (19). This process was similar in some ways to the lixiviation process developed by Scottish chemist E.C. Stanford in 1883, for the extraction of alginic acid from seaweed (20). Stanford's process required that the raw kelp be heated and stirred in order to promote maceration, and then filtered, all methods which were later adopted by Hercules. However, although alginic acid was a by-product of the Hercules process, it was was extracted using Sodium Carbonate, rather than with through the addition of either sulfuric or hydrochloric acid as in Stanford's process.

Kelp was an ideal source of various constituents used in arms production during W.W. I. Potash was an ingredient of crude, "black powder", while acetone, another derivative of kelp, was a key component used in the manufacture of cordite, a smokeless explosive powder used extensively by the British army and navy. On February 6, 1915, the British Government, desperate to expand the firepower of its war machine, gave Hercules a contract to produce two million pounds of cordite. Hercules successfully filled this order and two others as well, producing a total of 10,000,000 pounds of cordite by May 25, 1915. In order to meet these early contracts, Hercules relied upon the U.S. supply of acetone produced by the wood chemical industry. The British were also purchasing acetone from the U.S. to manufacture cordite, and consequently were in direct competition with Hercules. 1915, they turned down an offer by the powder company to produce 24,000,000 pounds of cordite, as it would have been foolish to buy powder abroad at the cost of decreasing their own output. Hercules realized that in order to sell large volumes of cordite to Britain, they would have to create an entirely new source of acetone. After negotiations, the British agreed to contract Hercules for the 24,000,000 pounds of cordite, provided that they obtain their acetone from a new source, and that each pound of cordite must be accompanied by 1 pound of acetone. The contract was rejected by many larger firms as impossible to fill, mainly because at that time the total production of acetone in the U.S. did not exceed 7,000,000 pounds, a far cry from the 60,000,000 pounds required by the British contract (20).

Hercules' initial solution to the problem was to make acetone out of vinegar. The largest vinegar plant in the world was built near Baltimore, Maryland, and plans were made to extract vinegar from a mixture of alcohol and wood shavings. However, despite the huge size of this plant, there were only about 100,000 gallons a day coming out of the great vinegar casks, whereas 150,000 or 200,000 gallons a day were needed to manufacture the amount of cordite needed. While searching for a new source of acetone, Hercules discovered that "it had been produced on a small scale from kelp, a giant seaweed that grows along the rocky shores of our Pacific Coast" (21). This discovery led to the construction of a \$7,000,000 kelp processing plant on a 30-acre tract of land at Chula Vista, near San Diego, California. Herbert Talley was sent to Chula Vista from Wilmington, Delaware, Hercules' headquarters, as manager of the new kelp plant. The plant was completed in the spring of 1916, by Charles C. Morse and Company of San Francisco, under the direction of Hercules' engineers. At this time, a method for the efficient extraction of acetone from kelp had not been developed, and as a result, Hercules engineers had to experiment, spending "one and a half million dollars on this plant, recklessly throwing out machinery and tanks and stills for old processes when something better was stumbled on" (22).

The fermentation or "wet" process developed by Hercules' engineers in 1916, relied upon a bank of 156 giant 15' x 18' redwood "digestive" tanks, open at the top, and with a total capacity of 7,800,000 gallons. Each tank was equipped with an air agitation pipe and heating coils, used to speed the fermentation of the kelp and water mixture pumped into them. After 30 days, the remaining kelp was screened aside, and the liquor was pumped into other tanks where lime was added to sterilize the mixture, stopping the fermentation process. Next, the liquor was heated and run through a series of filters and separators which removed a large percentage of the mud and water. The remaining liquor was subjected to an evaporation crystallization process, in which the acetone bearing portion (calcium acetate) was removed by heating the liquor, and the potash after cooling. Distillation of these two products produced both the acetone necessary to fulfill Hercules' contract with the British, and a huge quantity of potash, which was sold to the chemical industry rather than as fertilizer due to its high level of purity (90-95 percent). The Chula Vista plant processed 1200 tons of wet kelp daily, obtaining 13 tons of 95 percent potassium chloride and over 1500 liters of acetone. During their two and a half year period of operation, Hercules produced 18,618,533 pounds of potash, and enough acetone to manufacture 46,000,000 pounds of cordite (23).

The task of developing effective mechanical harvesters was one of the more difficult problems encountered by the California kelp companies. The Coronado Kelp Company cut kelp with a barge, allowing it to wash up on the beach, before gathering it for processing. This method was too wasteful for industrial purposes, because "so much material was lost by sinking and by coming ashore in inaccessible places" (24). An expensive alternative was to pull entire plants into a boat using a cable and hoist, or to drag a chain or length of piano wire through the bed, collecting the severed kelp plants from the sea surface. These methods were inefficient, as they destroyed the holdfasts of many plants, and much of the harvested material drifted to the ocean bottom. The most commonly employed harvesting method was "to drag fronds into a skiff or small barge by means of a boat hook, and cut them off with a long knife" (25). This method required a large amount of hand work, and consequently was not practible on a large scale. In 1909, David Balch had recognized that harvesting kelp could only be profitable if it was done on an extremely large scale, using mechanical harvesting machines.

The first successful mechanical harvester was designed by the Pacific Kelp Mulch Company of San Pedro between 1913-15 for use in the kelp beds near Point Fermin. The Mulch company's machine was patterned after an ordinary horse-drawn hay-mowing machine to be found on every farm. A flat decked barge was fitted with a 16 ft. wide endless belt device which extended over one end of the barge down to a depth of four feet beneath the surface. At the submerged end of the belt was a horizontal cutting bar, with a vertical bar at each end. Each blade was fitted with a gear device to give it a cutting stroke of about four inches. Onboard the barge, the end of the endless belt extended over a hopper with revolving blades powered by a separate gasoline engine. An undecked towing barge moving along side the cutting barge, was used to carry the kelp once it passed through the chopper. Neither barge was self propelled, and a launch had to be used to push them both through the kelp groves at a speed of about four miles per hour (26).

In order to supply their plants both Hercules and Swift had to harvest kelp in unpredecented amounts. At first Hercules attempted to build a machine similar to that of the Pacific Kelp Mulch Company's, but found that it was unable to harvest enough kelp to supply the Chula Vista plant. Instead Hercules' engineering department produced a new design for a kelp cutter which was self propelled, and had a far greater carrying capacity than the combined cutting and carrying barges of the Mulch Company. However, Hercules' new design still utilized barges for the final transportation of the chopped kelp from the cutters to the plant. Three of these giant machines, the Joplin, Kenvil, and Bacchus (named for Hercules' U.S. powder plants in New Jersey, Missouri, and Utah) were built at a cost of \$75,000 each. Each machine could harvest 500 tons of kelp every 24 hours (27). Hercules' harvesters operated continuously day and night, returning to port only in very heavy weather. According to Captain Robert Morris, supervisor of the Hercules harvesting operations, "each bed was be cut from the edge to center . . . in this way, loose pieces cut and not taken into the boat are usually picked up on the next time around" (28).

The largest harvester operated by any kelp company was the <u>Aliceil</u>, owned by the Swift Company. Swift eliminated the need for barges by building huge compartments aboard the cutter, in which the harvested material was stored until the harvester returned to port. The enormous cutting bar on the 150 ft. Aliceil cut a 40 ft. swath through the kelp. On a calm day, the 150 ft. Aliceil could harvest up to 500 tons of kelp. The excellence of the Hercules and Swift harvester designs is evident in modern kelp harvesters used today by Kelco Company of San Diego, which bear a striking resemblance to those developed during the early 20th century (29).

In order to encourage the blossoming California kelp industry, the Federal Government designated funds for research and the development of new technology. In late 1915, legislation was introduced which instructed the Bureau of Soils to develop methods for the commercial production of potash from an American source. A fund of \$175,000 was appropriated for the fiscal year 1917-18, to finance potash research. In 1918, a further appropriation of \$127,600 was made. In the Fall of 1916, Bureau of Soils potash expert J. W. Turrentine began to investigate the U.S. potash industry "at that time under development due to a critical shortage of potash and the consequent extremely high prices" (30). In order to avoid competing with private interests in San Diego and San Pedro, the Government plant was built at the small town of Summerland, California, on the coast near the city of Santa Barbara. Summerland was centrally located with large kelp beds to the east, west and directly in front of the village. Offshore, the Santa Barbara Channel Islands were also fringed by kelp beds. Summerland's coastal location was adjacent to the Southern Pacific Railway, which offered a means for transporting construction materials, and for delivery of goods produced at the plant.

The Government plant, constructed in 1917, was "designed to treat about 100 tons of raw material per 24 hours" (31). Two harvesters, the Joseph Priestly, of 100 tons capacity, and the Mayflower, of 150 tons capacity were built to harvest kelp along the coastlines of Santa Barbara and the Channel Islands. Both cutters were built by the Simmons Hardware Co. of St. Louis, Missouri, owned by J. W. Turrentine's friend and frequent correspondent, Mr. L. H. Thompson. These twin-screwed vessels were equipped with internal combustion engines which were set in the stern of the cutter. At the bow of each was "a cutting mechanism made up of a ten-foot horizontal and two five-foot vertical reciprocating knives, with a vertical knife at either end of the horizontal one" (32). Once on board, the harvested kelp was distributed within the large hold space between the cutter on the bow and the pilot house on the stern. The government vessels could harvest at a rate of 25-50 tons per hour, depending on the thickness of the kelp, the weather, the tides, and the current.

The kelp was unloaded from the harvesters using a crane, which lifted the kelp with a clam-shell bucket, dropping each load into a chopper which cut it into 6-in. lengths. From the chopper, a conveyor carried the kelp to a storage bin which was divided into two compartments, each capable of holding 300 tons of raw kelp. Once filled, the contents of the bins was discharged through wells at either end. While sitting in the holding bin, the kelp began to ferment, and as a result produced a large quantity of juice. When chemically analyzed, this juice was found to have the same potash and nitrogen content as the kelp itself. Consequently, the fresh harvest was allowed to sit in the bins which were charged and discharged in rotation, in order to allow fermentation of the raw kelp before delivery to the dryers. The kelp was dried in three steel cylinders, 50 ft. long and 5 ft. in diameter, mounted in a sloping position on trunions, and encircled with a gear ring so that they could be rotated. The inside of each dryer was lined with longitudinal ledges which lifted and dropped the drying kelp as the cylinder rotated. Oil burners were used to generate hot air which was blown into the ends of the dryers at a temperature of 800 deg C (33).

Once it was fully dried, the kelp was processed in four different stages, each designed to extract different products from the kelp. A portion of the kelp was conveyed to a set of four clay retorts, where it underwent destructive distillation. Each retort was made of fire-clay tubes of 12 in. internal diameter, joined together to form a vertical tube of 18 ft. long. All four retorts were enclosed in a furnace which heated them to a temperature of 980 deg C. Dried kelp was fed slowly into the retorts from bins located above the apparatus. Products obtained from this stage of the process were heavy and light tar, creosote, pitch, an aqueous distillate containing ammonia, and a combustible gas. Excess kelp material not burned in the retorts, was incinerated for the production of kelp ash, a crude fertilizer with a 35 percent potash content. The kelp charcoal remaining in the retorts underwent "lixiviation" a process which used mechanical filter presses to remove all soluble material from the kelp char. The remaining charcoal was used as a decolorizing agent. Brine left over from the lixiviation process underwent evaporation and crystallization using a vacuum evaporator joined in circuit with a vacuum crystallizer. Materials produced by this process were sodium chloride from the evaporator, and potassium chloride from the crystallizer (34).

Ironically, despite the success of the government plant, private industry which might have benefited from the government research was no longer able to compete in the production of potash once the embargo on German potash was lifted in 1919. A bitter J. W. Turrentine, described the signing of contracts by practically all of the big American purchasers of potash with the German Kali Syndicate for 75 percent of their consumption and with the French for their other 25 percent as "not only directly aimed at the American industry but effectually designed to kill it" (35). Turrentine felt that potash production in California was short-lived due to the inefficiency of processing techniques developed by the majority of the private kelp companys, which severely limited the ability of the industry to compete once the embargo on potash from Germany was lifted. However, it is unlikely, even with the added value of by-products and the new processing methods developed at the Government plant, that California kelp companies could have remained price competitive after World War I.

The California kelp industry emerged between 1910-1919, as a result of the extraordinary circumstances created by the embargo of German potash at the beginning of World War I. Private industry seized the opportunity of make a healthy profit from producing potash which was no longer available from any other source. The successful extraction of potash and acetone from kelp by the California Kelp Industry, exemplifies the wave of chemical engineering skill with which U.S. industry met the challenge of World War I. Without the products of the kelp industry, the U.S. and her allies would have been deprived of vital ingredients used both for fertilizing crops, and in the manufacture of gunpowder. Although the extraction techniques developed by the California kelp industry were effective, when the embargo on German potash was lifted, the price of potash produced from kelp was no longer competitive and the industry died out. Despite the successful efforts of the Government researchers at Summerland, California, to develop more efficient technology for the extraction of potash and numerous other more valuable by-products from kelp, no further Government support for the California kelp industry could be justified. However, the California kelp industry did revive itself in the late 1920's on a much smaller scale to produce alginates, a multi-million dollar industry which is still operating today in California.

### Notes

- (1) The following was taken from V. J. Chapman, "The Kelp Trade," Nature 3944 (1945): p. 673.
- (2) Archibald Glow and Nan L. Glow, <u>The Chemical Revolution</u>. A Contribution to Social Technology (London: Batchworth Press., 1952), pp. 65-91.
- (3) Verkaufsyndikat der Kaliwerke, A profile of the German potash mines published by the Kali Syndikat in 1910. In the fertilizer file of the Romaine Catalogue Collection at the University of California at Santa Barbara libarary.
- (4) Thomas H. Norton, "The Potash Famine: Its Magnitude and Effects, and Remedies Promised for the Future," <u>Scientific American</u>, 114 (Feb., 5, 1916): p. 145. H. R. Tosdal, "The Kartell Movement in the German Potash Industry," <u>The Quarterly Journal of Economics</u>, 28 (Nov., 1913): pp. 140-190. H. R. Tosdal, "The Potash Law of 1910," <u>The Quarterly Journal of Economics</u>, 28 (May, 1914): pp. 579-580.
- (5) Frank K. Cameron, "Potash from the Pacific Kelps," The Journal of Industrial and Engineering Chemistry, 4 (Feb., 1913): pp. 76-77. Frederic P. Dewey, "The Business Aspect of the Kelp Proposition," The Journal of Industrial and Engineering Chemistry, 4 (April, 1912): p. 311.
- (6) David M. Balch, "Father of Kelp Tells History of Valued Discovery," San Diego Union, (Jan., 1, 1917): n.p.
- (7) David M. Balch, "On the Chemistry of Certain Algae of the Pacific Coast," The Journal of Industrial and Engineering Chemistry 1 (December, 1909): p. 783.
- (8) Ibid., 785.
- (9) "Fertilizer Resources of the United States," Senate Document No. 190, 62nd Congress, 2nd Session (April 1911): Appendix K.
- (10) "Potash From Kelp", U.S. Department of Agriculture, Report, No. 100, (April, 1915): p. 10.
- (11) E. Yale Dawson, <u>Seashore Plants of California</u> (Berkely: University of California Press, 1982): pp. 1-30. V. J. Chapman and D. J. Chapman, <u>Seaweeds and their Uses</u>, (London: Chapman and Hall, 1980): pp. 1-30.
- (12) W. L. Scofield, "History of Kelp Harvesting in California," California Department of Fish and Game, (July, 1959): p. 139.
- (13) J. W. Turrentine, <u>Potash</u>, A <u>Review</u>, <u>Estimate</u>, and <u>Forecast</u> (New York: John Wiley and Sons, Inc., 1926): pp. 1-7.

- (14) Allen H. Wright, "Kelp Industry of the Pacific Coast," The American Fertilizer 45, (Nov. 25, 1916): pp. 31-33.
- (15) Mary L. Gulliver, "Fortunes in the Kelp Beds of Southern Seas," <u>San Diego</u> Union, (Jan., 1, 1917): Annual Midwinter Number p. 131.
- (16) "Starting From Scratch, About Matches and How Diamond Makes Them," A history of Diamond Match Co. published by Diamond National Corporation in 1962."
- (17) W. C. Phalen, "Potash Salts-1915," <u>The American Fertilizer</u> 45 (Mr. 3, 1916): pp. 27-28.
- (18) Werner C. Brown and Alexander F. Giacco, Hercules Incorporated, A Study in Creative Chemistry, read at the Newcomen Society Meeting at the New York World's Fair on August 5, 1939. (Princeton University Press, 1977): pp. 5-11. Alfred D. Chandler, Jr., and Stephen Salsbury, Pierre S. Du Pont and the Making of the Modern Corporation, (New York, Harper & Row, Publishers, 1971): pp. 296-299.
- (19) "Chemicals From Kelp," A pamphlet published in 1918 by Hercules Powder Co. of Wilmington, Delaware which describes their kelp products plant, manufacturing processes, and products. In the Turrentine Papers: National Archives, Agricultural Section, Wash. D.C. Record Group 54.
- (20) "A War Bride Whose Story Reads Like A Romance Of Aladdin," <u>Current Opinion</u> 62 (July, 1917): p. 439. <u>Ibid</u>.
- (21) This quotation is from an unpublished description of the Hercules Powder Co.'s plant at Chula Vista, San Diego, no mention is made as to who discovered the acetone content of the kelp. In the papers of J. W. Turrentine, at the National Archives, Agricultural Section, Wash. D.C.
- (22) Edward C. Crossman, "Sea-Weed for War," <u>Scientific American</u> 114 (September, 1918): p. 237.
- (23) "Hercules' Achievements During the Period of the War," The Mixer (Hercules Powder Co. Publication): (April, 1918): p. 24.
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- (25) "California's Kelp Industry", <u>Standard Oil Bulletin</u>: (December, 1918): pp. 3-7.
- (26) "Potash From Kelp", U.S. Department of Agriculture Report, No. 100, (April, 1915): p. 12.
- (27) Mary L. Gulliver, "Fortunes in the Kelp Beds of Southern Seas."

- (28) Interview conducted by Terminal Island Fish and Game Official, C. H. Groat, with Captain Robert Morris, 18 Aug. 1931. From Calif. Fish and Game interdepartmental correspondence files, Sacramento, Ca.
- (29) "Getting Potash Kelp with a Submarine Lawn Mower is a War Industry," American Machinist, 47 (Oct. 4, 1917): pp. 599-600.
- (30) J. W. Turrentine, "Potash From Kelp: The Experimental Plant of the United States Department of Agriculture," <u>Journal of Industrial and Engineering Chemistry</u>, 2 (September, 1919): p. 867.
- (31) Ibid., p. 870.
- (32) J. W. Turrentine, H. G. Tanner, and P. S. Shoaff, "Potash From Kelp VII-The Manufacture of Potash Salts," <u>Journal of Industrial and Engineering Chemistry</u>, 15 (Feb., 1923): p. 159.

(33)

- (34) For a detailed review of the history of the U.S.D.A.'s kelp plant at Summerland, CA, see G. Anderson, "Kelp Entangles Santa Barbara County," Unpublished Senior Thesis, University of California at Santa Barbara, 1968. pp. 1-34.
- (35) Letter from J. W. Turrentine to L. H. Thompson. (Feb., 13, 1922): In the Turrentine Papers, Agricultural Section, National Archives, Wash. D.C.

### APPENDIX B

Alginates and Animal Feed From Kelp: The Calfornia Kelp Industry 1927-1984

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The early California kelp business, was a shortlived but highly profitable industry, which emerged as a result of the shortage of potash for fertilizer in the United States during World War I. California kelp was one of the largest sources of domestic potash, second only to natural brines, and consequently attracted the interest of big business. Thousands of dollars were spent on processing plants and harvesting machinery needed to handle large quantities of kelp. In 1918, four hundred thousand wet tons of kelp were harvested by Calfornia kelp companies, with an average potash content of 1.5 percent per ton. In addition to potash, considerable quantities of iodine, nitrogen, and other byproducts such as acetone Two processing techniques were developed for the extraction of were also produced. chemical constituents from kelp. The majority of companies such as Swift Co. and Lorned Co., used a "dry" processing method in which the kelp was dried first in shacks, and then in ovens, and then burned in large clay retorts, before being sold as fertilizer. In contrast, The Hercules Powder Co. produced acetone in addition to potash by a wet processing method in which the kelp was first fermented in large holding tanks followed by filtration to yield a clear liquor which was concentrated by evaporation before the crystallization of its various constituents. While the World War I industry was concerned with the production of potash and acetone, after the war it turned to the processing of kelp for use in animal food additives and the development of an improved method for the extraction of algin.

Four principal varieties of kelp grow along the coast of California, these being Macrocystis, Nereocystis, Alaria and Pelagophycus, which grow at depths of over 100 feet, but most often are found at depths of 15 to 60 feet. The "Giant Kelp" Macrocystis pyrifera formed the basis for the alginates and animal feeds industry during the 1920's as it was possible to harvest the plants several times a year (1). There were approximately 75 square miles of kelp beds of the Southern California coast.

In 1881, chemist E. C. Stanford, discovered algin while experimenting with new methods for extracting potash and iodine from kelp in Scotland. Stanford found that after undergoing destructive distillation, many brown seaweeds contained a viscous substance which when treated first with sodium carbonate and then with mineral acid, produced a new compound which he called alginic acid. Stanford began his process for algin production by soaking the seaweed in a 10 percent solution of sodium carbonate. After 24 hours the plants completely disintegrated, leaving behind an extremely viscous, semi-gelatinous mass. This material was heated and passed through filter bags which retained the particles of sand and plant matter remaining in the solution. Next, the mixture was combined with either sulfuric or hydrochloric acid, which caused the alginic acid to precipitate from the solution in "light gray albuminous flocks" (2). The precipitated algin was placed in a filter press and then washed, forming a compact cake resembling new cheese. Stanford recognized the commercial possibilities of his discovery, and described his attempts to isolate the new material in scientific literature (3). Although he eventually started a business for the manufacture of algin in Scotland, Stanford was unable to overcome the problems involved in isolating a pure and uniform product. Stanford experienced difficulties in filtering the raw alginate mixture, and with handling the raw gelatin procipitate produced when he attempted to concentrate the algin. Several factories were built, but unfortunately the laboratory processes developed by Stanford were not efficient enough to be applied on a commercial scale (4).

The first successful commercial development of alginates was started in 1927, when the Thornley and Walsh Company, (later renamed the Kelp Products Corporation) was established in San Diego, California. Irish-born chemist Michael J. Walsh began his work with algin at a laboratory in London, England, where he developed new commercial uses for the seaweed derivative. Walsh established a his factory in San Diego because of its close proximity to the large beds of giant kelp extending from Point Conception to the Mexican border. Thornley and Walsh was heavily subsidized by the American Can Company which employed algin as a viscocity regulator in the latex gasket compound used for sealing cans. By using the algin as a sealer, American Can was able to increase the speed of their canning operation from 60 to 250 cans per minute" (5). Unfortunately, this was the only successful application of algin at this time, and consequently the survival of Thornley & Walsh was dependent on the financial support of the American Can Co. The new company was unable to develop additional products from the algin because of the difficulty of refining the material to the point where an odorless, consistent product could be produced. This situation continued until 1929, when the company was purchased by Arnold Fitger, and renamed "Kelco Company" (6).

Fitger was the owner of the Celite Corporation of America which had a major interest in the diatamaceous earth mines at Lompoc California, the largest of their kind in the world. During the early 1920's, the Celite Corporation merged with Johns-Mansville Corporation, and the Fitger sold his interest in the company (7). Shortly after the sale of Celite Co., Fitger contracted tuberculosis, and on the advice of his doctor, he moved to the Mount Helix area behind San Diego. Fitger loved boating and fishing, along San Diego coastline, and came in frequent contact with the large kelp beds in the area. As a result, he began to speculate on the uses of this bountiful natural resource. In August 1929, Fitger bought the Kelp Products Co. (Thornley and Walsh), and arranged to repay its debts to the American Can Co. Although Kelco continued to develop an effective process for algin extraction, they also began producing animal feed additives from kelp.

By 1931, the new company was producing ground kelp (Kelco Meal) at a small plant in National City, San Diego. The kelp was harvested by hand off Point Loma, with men using long hooks to cut and haul the kelp up into a small boat. At first the wet kelp was trucked to nearby El Cajon, where it was spread on open fields to dry before being returned to National City for milling and packaging. Later, an oil fueled rotary dryer was installed at the National City plant, which was capable of turning out as much as 5 tons of dried kelp per day.

After purchasing Kelco, Arnold Fitger immediately hired an assortment of skilled directors who had worked for Celite Corporation, including Harlan Green, as vice-president, general manager, and head of finance, Don Clark, as engineer, and head of research, J. R. Foster as plant controller, and Russ Kiely in charge of sales. Between 1929–36, Clark headed a research team that developed processing techniques designed to refine algin to a point where it could be used in foods without adversley effecting their flavor or color. Fitger, with Kiely as his assistant, financed and structured a national sales organization to sell alginates with representatives at key spots across the United States (9).

From 1929-36, Don Clark researched and developed a new "batch process" for extracting Clarks's method retained the same basic techniques originally algin from kelp (10). developed by E. C. Stanford, but added improved filtering, precipitation, and drying processes, which enabled Kelco to introduce algin into the food stabilizer market. In the first step of Clark's process, the raw kelp was mixed with a weak hydrochloric acid solution to lower the calcium content. After removal from the acid, 40-50 pounds of soda ash was added to the kelp, reducing the material to a gelatinous mass. Next, the crude paste was pulverized in a hammer mill, mixed with water, and pumped into a series agitator tanks where diatemaceous earth was added as a filter aid. The remaining raw alginate liquor was filtered through a plate frame filter press. Clark was able to overcome Stanford's problem of concentrating the alginic acid, by treating the filtrate with a 10 percent calcium chloride solution which caused the alginate to precipitate in a hard fiberous form, rather than as a gel. The fibers were bleached and acid washed, leaving enough calcium in the mixture so that it still retained some of its original form. The algin was then dewatered by being squeezed between rollers until it was reduced to about 20 percent solid, making it as dry as paper. The resulting product was insoluble alginic acid which had no chemical value until it was converted back into soluble form. In this final step, the alginic acid was mixed in huge dough mixers along with soda ash, forming a heavy sodium alginate paste which was dried, ground, and packed for shipment (11).

Arnold Fitger realized the potential for algin as an icecream stabilizer, and understood the need for a strong marketing network in order to sell the new product. Under his direction, Russ Kiely organized a nationwide sales department which was backed by Don Clarks Technical Service Department. The technical service department was responsible for the study and opening up of new fields in which algin could by successfully used. Kelco's sales and technical service combination enabled them to modify algin to fit the demand of a large variety of markets, and has much to do with their future success. In 1939-40, Dr. Arnold Steiner and his assistant Dr. Ken Gibson was hired to head up a research department, which began conducting animal feeding tests using kelp meal, in addition to the development of algin products.

Kelco continued to produce kelp meal up until 1943, when World War II resulted in the expansion of the navy yard, which took over the National City site. The Kelco office building was floated on a barge across the bay to its present site in San Diego, the dryer, however, could'nt be salvaged, was bulldozed into the bay. After the move from National City, Kelco concentrated its efforts on the production of algin (8). Algin production was increased when in 1943, mechanical engineer and dryer specialist Spencer Coleman joined the company. Coleman came up with the first alginates production line. Using a machine like a meat chopper. A new alginates extraction plant was built, using the same basic process developed by Clark, but with several improvements in efficiency.(tape) Between 1944-45, Kelco expanded their research efforts, hiring Dr.'s W. H. McNeely, Aaron Miller, and A. W. Saddington. Together with A. B. Steiner, McNeely masterminded the development of a new propylene glycol alginate, which up until today accounts for 50 percent of Kelco's business, and up to 75 percent of its profit.

One of the more difficult stages in kelp processing was harvesting the raw material. Although kelp contains a large quantity of algin while growing in th ocean, once it is cut, bacteria immediately begin to consume the algin content. Consequently, it was very important that the Kelco harvesters deliver kelp as fresh cut as possible. The Hercules Powder Company, Swift Fertilizer Co., and Lorned Manufacturing Co. all designed efficient harvesting machines for the potash and acetone industry. These designs were used again by Kelco to build harvesters for the alginates industry. Kelco's early harvesters, the Elwood and El Capitan were very similar to the Swift Companys' harvester Aliceil, which was the largest used by the early industry (12). These vessels had a 10' horizontal cutting bar, with 5' bars at either end, mounted on a mechanism in the bow of the cutter, which could be lowered three feet into the water. The cutting mechanisms were composed of one 10 foot horizontal cutting bar, with five-foot vertical bars at either end. As the kelp was cut, it was automatically hoisted aboard the cutter by an inclined chain conveyor. The earlier Kelco vessel retained the square, barge-like shape which characterized the cutters of the potash extraction industry (13). During the 1950's, Kelco moved the cutting mechanism to the stern of their harvesters the Kelmar and Kelstar, and replaced the square bow with a conventional wedge shaped bow to increase the seaworthiness of the harvesters, and enable them to harvest up to 300 tons of kelp (stern first) and return with a full load of kelp in a much shorter period of time.

In 1936, Kelco began to manufacture an alginate stabilizer for ice cream. James R. Moss, a recent graduate in Dairy Science, and an employee of the Foremost Dairy Company, was among the first to suggest the use of this new product to prevent the formation of ice crystals and to maintain a finely textured ice cream even though the icecream was repeatedly frozen after partial thawing during retail distribution. Although the alginate stabilizer was "reasonably successful at first, it suffered from problems with solubility in milk" (13). In 1937, Kelco began to produce another food stabilizer called "cocolloid", which was used to suspend the chocolate in chocolate milk. Although this product had some initial problems with solubility, it was superior to any stabilizer that had been offered up to that time. Kelco's algin products were instantly successful when developed for use in ice cream and chocolate milk. Between 1935-39, the U.S. yearly consumption of algin rose to approximately 1 million pounds per year, the majority of which was used in dairy products, and as a latex additive (14).

With the outbreak of World War II in 1939, gelatin which had been the conventional food stabilizer and the major competitor with alginates, was in very short supply as it was used in the manufacture of film needed by the army. This caused an even greater demand for alginates as a replacement for gelatin in dairy products. Throughout the war years Kelco grew very rapidly due to the momentum established during the prewar organizational period. Kelco further consolidated its position as a major producer of food stabilizers by increasing its technical staff. In 1946, Kelco began a concerted effort to expand and diversify the uses of algin in other industries, including textiles, printing, and paper sizing where compatible chemicals such as modified starch and various gums were used extensively (15).

Between 1950-55, two technical consultants, Vance Vallandingham, a specialist in paper manufacturing and sizing, and Vern Morell, a specialist in textile printing, were hired to develop the use of alginates in these industries. By 1948, algin was being used in the screen printing of textiles, where a thick alginate solution mixed with the coloring dyes, controlled the coloring as it passed through the silk screen, holding the design being printed in the exact position desired. The alginate not only carried the coloring through the screen at a precise rate, but also prevented the color from flushing and blurring the design once it reached the cloth. Beginning in 1950-51, Kelco began to receive a substantial number of orders from roller printers, including the Rockhill Printing and Finishing Co. in South Carolina, which was the largest textile printer in the world, the Pacific Mills Co., Magog Co., and S. A. Fairforth Finishing Co. Kelco sold approximately two million pounds of powdered alginates annually to printing industries, with the algin ground to precise particle size specifications (ranging between .80 and .200 mm.) in order to meet the needs of various printing businesses (16).

In 1950 Vallandingham and Aaron Miller were able to interest some small businesses in the use of algin for paper sizing. Companies making wax coated paper boards found that if they applied three to five pounds of algin to the surface of one ton of paper board prior to wax application, they could control the penetration of the wax into the paper board. The depth of penetration of waxes used on the boards was very important, especially when these were subsequently made into milk cartons or frozen vegetable boxes. If the wax penetrated too deeply, the board would become brittle and often cracked under stress, particularly after being refrigirated. With algin, the degree of penetration of the wax into the board could be precisely controlled just as dye penetration was controlled in the textile industry. molecular size of the algin was very important in this process, since larger algin molecules allowed less wax to penetrate into the paper. During the early 1950's, algin was used extensively in the manufacture of wax paper board, however, in 1958, this use was curtailed when it was discovered that some of the cellulosic gums, such as epoxymetholcellulose (EMC) could be used instead of algin. Later, plastic sizing compounds were found to be even more efficient. However, large amounts of algin continued to be used to regulate the viscosity and adhesive efficiency of the starch adhesives used in the production of corrugated boards. During the 1950's alginates were also used to pre-coat for papers designed for glossy printing; algin in the paper keeps the ink near the surface, producing a better color yield, and a more glossy finish. A substantial volume of alginate was also used in dry wall joint cements, and similar plaster wall coatings (17).

Kelco continued to produce alginates for use as a latex stabilizer and as a latex consecrating agent. In 1939, use of alginate as a latex stabilizer and as a latex concentrating agent, was well established, and substantial shipments were being made to the plantations in Indonesia and other rubber producing areas. Low levels of algin stabilized the latex emulsion, while a large quantity splits the emulsion, concentrating the latex in the water phase. Alginates were also used in combination with various other rubber compositions (18).

In about 1950, the technical group at Kelco presented the sales corps with a modified alginate known as Kelcosol designed for use as a desert gel, and in the fine foods industry. Kelcoloid, another derivative of alginic acid, was made by reacting the acid with propalene oxide under pressure. After several years of trial and error, it developed into the largest dollar volume product of the company. It was widely used as a stabilizer in beer, in salad dressings of the french dressing type, and in a multitude of other food products. Today Kelcoloid still accounts for 40-50% of Kelco's business today (19).

During the late 1950's, Kelco lost its hold over the ice cream and chocolate milk stabilizer market as new additives were discovered. A combination of Carageenan (A red seaweed derivative), and guar gum or epoxymetholcellulose (EMC), or a combination of all three made a stabilizer for ice cream which was superior to algin. This new blend is now used in all but Briar and Haagen-Daz ice creams which profess to use no stabilizer at all. Consequently the "no additive" producers must charge a much higher price in order to manufacture, deliver, and sell their ice cream within a very short period of time, otherwise it will turn icy and be rejected by the consumer. The usual complaint is that "it tastes like water," as it has no fat, and is not rich. The use of the stabilizer prevents the development of ice crystals and enables the manufacturer to retail his ice cream without spoilage (20).

Throughout their history Kelco has used the alginate molecule in a variety of different ways in order to produce products with different chemical and physical properties. Although colloids, gums and other chemical stabilizers were developed which competed with alginates, Kelco was able to develop new uses for algin so that as it was replaced in one area, another new application was being developed. Kelco prospered through the manufacture of alginates, and up until the 1980's, the company was the largest producer in the world (21). In 1972, the now multi-million dollar company was purchased by Merck Corporation. In recent years, higher energy costs, and the fluctuation of local kelp availability has severely affected Kelco's production of colloids, and consequently an all-out effort to find substitutes was initiated. Kelco reduced its production of alginates products and began to manufacture xanthangums, a stabilizer produce by bacteria which is less costly to manufacture than algin (22).

Unfortunately, although Kelco was technically proficient in the development of new uses for alginates, they were far less concerned with their source of raw material, the California kelp beds, which at first seemed inexhaustible but which have decreased in size since the turn of the century. This decline has been attributed to decreases in water clarity in urban coastal areas, increased boat traffic, and perhaps in part to the large amount of indiscriminate and often destructive harvesting which took place between 1916-1919, at the height of World War I when kelp was used to produce potash and acetone (23). Although Kelco has expressed concern at the decline of the beds, and provided limited funding for research on damage perpetrated by sea urchins which feed on the kelp, no attempt has been made to insure the renewability of this vast natural resource (24). In the Peoples Republic of China, the development of thousands of acres of seaweed farms now provides large amounts of kelp for alginates extraction. The Chinese now harvest up to 1,000,000 tons of seaweed a year, far more than the 150,000 tons harvested from the California kelp beds. The key to the Chinese success has been their development of seedstock production methods and new genetic strains of seaweed that are able to grow at high temperatures or produce high concentrations of desired elements such as iodine. Chinese alginates, which are being produced in great quantities for both domestic and foreign use, will undoubtably be far less expensive than the xanthan gums produced by Kelco, which have replaced algin as the major stabilizing agents in U.S. food products. Throughout Kelco's history, the company has been known for its abilility to adapt to changes in the market for alginates, ironically, in their endeavors they have overlooked the source of these diverse products, and consequently are now faced with the prospect of competition from Chinese alginate producers (25).

In 1928, the Philip R. Park Company of San Pedro, California built a \$100,000 plant for the manufacture of kelp additives for animal feed (26). While living in the east, Philip R. Park made a fortune by introducing new feed materials such as dry-mash for poultry, and fish meal as a cattle feed additive. After his retirement in the late 1920's, Park visited Scotland where he "observed cattle pass up lush grasses along the coast and go to the beaches to eat seaweed" (27). Analysis of the seaweed revealed a rich mixture of iodine, potassium, phosphorous, and vitamins A, B, C, and D. Realizing the potential value of seaweeds as a livestock food additive, Park began investigating the possibility of using kelp for food production. At San Pedro, Park could harvest raw material from the coastal California kelp beds, which at this time were the largest source of seaweed in the world. In 1928, Park hired chief chemist, Gertrude Beckwith with whom he developed the new feed additive "ManAmar" which was widely used in dairy, beef, swine and poultry feeds. P. R. Park's kelp company prospered, and in April 1928, the first shipment of kelp stock feed was made to Mid-western stock farms (28).

P. R. Parks' harvester, the C. L. Arques was much smaller than the Kelco vessels, and harvested a maximum of only 125 tons per day. Upon its arrival at the plant, the kelp cutter was unloaded with a crane using a double clawed shovel. The processing technique used by P. R. Park was similar to the "dry" process developed by the Swift Fertilizer Company during the earlier potash extraction industry (29). In Park's process, the kelp was placed in a wooden hopper from which it slid into a chopping machine, was ground, and then pumped as a slurry into a large storage tank. From the tank, the kelp was pumped into large rotary gas-flame dryers, where most of the 90 percent water content was evaporated. Once it was dry, the kelp was combined with fish flour, fish solubles, and other feed additives from the sea. By 1953, Park was processing 18,000 tons of kelp per year. Although many livestock and poultry farmers purchased the ManaMar, the food value of the product was never fully established. P. R. Park's sales staff distributed reprints of publications supporting the nutritional value of his feed additive, often including the testimony of dairy and poultry producers. In 1961, Dr. Robert Appleman stated that the economic value of kelp had never been supported by sound research, and that there were "cheaper sources of vitamins, potassium, and iodine" (30). In his reply, Park stated that in addition to known chemical constituents, "the ocean provides trace minerals and unidentified growth factors which cannot be measured" (31).

During the 1940's, P. R. Park began to manufacture a line of kelp products for human consumption. The most famous of these was "Parkelp", which was sold in both tablet and granular form, and was advertised as a "dietary supplement of nutritionally important iodine" (32). When processed for human consumption, the kelp was put through a series of hammer mills before being fed into the rotary driers. The final product was composed of tiny green flakes which were granulated for use as a food supplement, or compressed into tablets. Other products manufactured for food use were "Sea Zun", a mixture of kelp and other additives which was desinged for use as seasoning on salads, meats, vegetables or fish, and "Sea Mar", bulk laxative tablets made from a mixture of kelp and other extracts.

Although P. R. Park eventually grew into a multi-million dollar business, by 1960 the supply of kelp adjacent to the San Pedro processing plant dwindled due to urban pollution which increased the sediment content of coastal waters, cutting off the light supply necessary for survival of the kelp beds. During the late 1960's the business was forced to move to Port Hueneme, where it was purchased by the Ocean Nutrients Company, of Chaska, Minnesota which continued to manufature Parkelp for food flavoring, although production of ManaMar was discontinued. In 1974, Ocean Nutrients was purchased by the Stauffer Chemical Company, which began to compete with Kelco in the production of alginates, while continuing to produce Parkelp tablets. Although sales of Parkelp continued to produce profits, Stauffer was unable to compete with Kelco in the alginates business, and was reputedly losing \$1,000,000 a year before going out of business in 1980 (33).

Although the first California kelp industry collapsed with the removal of the German potash embargo in 1919, it was revived once more in 1927, when kelp was harvested as a source of animal feeds and of algin, a valuable stabilizer widely used by the food, textile, and printing industries. The largest, and most successful companies engaged in kelp processing during the late 1920's were the Kelco Company of San Diego, and the Philip R. Park Company of San Pedro. Although Kelco developed numerous applications for their alginates, they did not insure their continued natural supply of kelp from the California kelp beds by developing farming techniques as have the Chinese. In fact, during the Summer of 1983 the Kelco plant was run for a time on kelp imported from China. Consequently, although Kelco has produced substitutes for algin such as xanthan gums, they are now faced with the prospect of competing with a major Chinese alginates industry, which is based on a farmed supply of The P. R. Park company was immediately successful in its production and sale of animal feed additives from kelp and eventually the company expanded to produce kelp products for human consumption. However, the decrease in size and number of the California kelp beds due to adverse environmental conditions resulted in the eventual decline of Park's feed additive production. By processing kelp, the California industry could indirectly tap the vast number of chemical and mineral constituents which are contained in Unfortunately, kelp is a limited natural resource, that has not yet been cultivated extensively in California. It is interesting to note that the Chinese have imported living kelp plants from Mexico and are now growing them experimentally in China, promising even more future competition for Kelco.

### Notes

- (1) For a detailed description of these methods see A. W. Allen, "Potash From Seaweed in California," <u>Journal of Chemical and Metallurgical Engineering</u>, 17, (July, 1923): p. 49. and J. W. Turrentine, "Potash From Kelp: The Experimental Plant of the United States Department of Agriculture, <u>Journal of Industrial and Engineering Chemistry</u>, 2 (September, 1919): p. 867.
- (2) Stanford's discovery of alginic acid was first described in British Patent 142 of 1881. Detailed descriptions of the process are found in E. C. Stanford, "On Algin: A New Substance Obtained From Some of the Commoner Species of Algae," Chemical News, 47, (Jan. 1883): pp. 254-257, 267-269.
- (3) D. K. Tressler, Marine Products of Commerce: Their Aquisition, Handling, Biological Aspects and the Science and Technology of Their Preparation and Preservation. (New York: Rheinhold Publishing Co., 1951): pp. 97-96.
- (4) Stanford's attempt to start an alginates industry is described in W. L. Stephensen, Seaweed in Agriculture and Horticulture. (London: Faber Press, 1968): pp. 48-49.
- (5) Interview with James R. Moss, President of Agro-Mar Inc. Moss was a sales manager at Kelco up until 1958 when he founded the Marine Colloids Company, now a Division of the Food and Minerals Corporation of America, one of the largest producers of agar and agarose (other seaweed extracts) in the world. (Feb. 11, 1984).
- (6) Ibid.
- (7) Ibid.
- (8) E. Yale Dawson, <u>Seashore Plants of California</u>. (Berkely: University of California Press, 1982): pp. 1-30. V. J. Chapman and D. J. Chapman, <u>Seaweeds and their Uses</u>. (London: Chapman and Hall, 1980): pp. 1-13.
- (9) Mary L. Gulliver, "Fortunes in the Kelp Beds of Southern Seas," <u>San Diego Union</u>, (Jan., 1, 1917): Annual Midwinter Number p. 131. "California's Kelp Industry," <u>Standard</u> Oil Bulletin: (December, 1923): p. 38.
- (10) W. L. Scofield, "History of Kelp Harvesting in California," California Department of Fish and Game, (July, 1959): p. 146.
- (11) V. J. Chapman, <u>Seaweeds and their Uses</u>. (New York: Pitman Publishing Co., 1952): p. 196-197.
- (12) Interview with D. A. Sattington, engineer at the Kelco plant from D. K. Tressler, Marine Products of Commerce, pp. 98-99.
- (13) Interview with James R. Moss.

- (14) Ibid.
- (15) <u>Ibid.</u>
- (16) C. K. Tseng, "Seaweed Colloids in the Textile Industries", reprinted from <u>Textile Age</u>, (June and July, 1946): n.p.
- (17) C. K. Tseng, "Seaweed Products and Their Uses in America," reprinted from the Journal of the New York Botanical Garden, 47 Numbers 533 and 554, (January and February, 1946): pp. 1-9, 32-39.
- (18) C. K. Tseng, "Utilization of Seaweeds", reprinted from The Scientific Monthly, 119 (July, 1944): pp. 37-46.
- (19) James R. Moss Interview.
- (20) Kelco Algin, a description of Kelco Company techniques and products, published by Kelco in 1961.
- (21) R. W. Krauss, The Marine Plant Biomass of the Pacific Northwest Coast, (Oregon: Oregon State University Press, 1977): pp. 301-312.
- (22) James R. Moss Interview.
- (23) R. P. Brandt, "Potash From Kelp: Early Development and Growth of the United States Department of Agriculture," <u>Journal of Industrial and Engineering Chemistry</u>, 2 (September, 1919): p. 867.
- (24) R. W. Krauss, The Marine Plant Biomass of the Pacific Northwest Coast, pp. 163-167.
- (25) James R. Moss Interview.
- (26) "Kelp Industry Revival Looms," Los Angeles Times, (January, 25, 1938): n.p.
- (27) G. E. Brown, "The Sea-Going Haywagon," and unpublished paper describing the early P. R. Park Co. At the San Pedro Bay Historical Society. Approx 1,100 words. Cat. # 82.908.
- (28) "First Shipment made of Kelp Stock Feed," Long Beach Press Telegram, (April 29, 1928): n.p.
- (29) Mary L. Gulliver, "Fortunes in the Kelp Beds of Southern Seas."
- (30) J. Smith, "Sea Farming," Farm Quarterly 16 (August, 1962): pp. 72-73.
- (31) Ibid.
- (32) "Parkelp Sea Kelp," Pamphlet distributed by the P. R. Park company in 1961.

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## APPENDIX C

Gas and Biomass From Kelp: The Calfornia Kelp Industry 1972-Future

### Peter Neushul

University of California at Santa Barbara

In 1972, the idea of using California kelp as a source of biomass for methane gas production was first proposed by Physicist Dr. Howard A. Wilcox of the Naval Undersea Center in San Diego (1). Wilcox envisioned large offshore Macrocystis pyrifera farms which would be a souce of chemicals, aquaculture, fisheries, and energy production. He was able to attract a large amount of financial support for his idea, both from the government (Department of Energy, U.S. Navy) and from industrial groups (American Gas Association, Gas Research Institute). Kelp Biomass offers an alternative to the finite supplys of fossil fuels upon which man presently relys for energy. Unlike biomass produced on land, kelp growth is not inhibited by shortages of suitable land, fresh water, and fertilizers. By cultivating and harvesting kelp biomass, man would profit from the vast supply of photosynthetic energy generated by the sun.

Wilcox cited problems of depth and plant nutrient supply as having curtailed the introduction of open ocean farms up until today. As a solution, he suggested the use of a "mesh some 50-100 feet down from the ocean surface, enabling the growth of attached seaweeds irrespective of the depth of the actual seabed" (2). Supplies of nitrate and phosphate compounds needed for plant growth are plentiful at depths of 300 to 1,000 feet, and could be made available for plant use if they were pumped upwards with wave or wind powered pumps, creating an artificial upwelling of the cool, nutrient-rich deep water. Wilcox assumed that the major questions confronting his idea were ecomomical, believing that the technical feasibility of many of the processes required by his ocean farm concept had already been established for use in other industries.

In 1975, Wilcox began a four-year marine farm research program at the Naval Undersea Center, with funding of approximately \$2,000,000 from the American Gas Association, Navy and the Energy Research and Development Association. In 1974, three open ocean farms were installed, as a test of Wilcox's "artificial bottom" theory. The first farm was moored 40 feet below the surface, off the northwest tip of San Clemente Island, California. A 300,000 square foot (about 7 acres) raft of polypropolene ropes was constructed, with ropes spaced 10 feet apart, and cross-linked every 50 feet. This rope grid, was planted with 100-150 Giant kelp plants (Macrocystis pyrifera) from nearby natural kelp beds. The plant holdfasts were attached to the grid by "sewing their clawlike tendrils onto the mesh", using a technique developed by Dr. Wheeler J. North of the California Institute of Technology (3). Later, two smaller farms were installed, one located near Corona Del Mar, and the other off of Catalina Island. Wilcox planned to observe the San Clemente farm for two years, however, in January 1975, a corner anchor broke loose from the bottom, and the submerged rope grid floated to the surface and was destroyed either by a passing ship, or by wave action (4). The other two farms experienced similar fates, as the Corona del Mar structure disappeared without trace, and the Catalina farm grid was destroyed by wave action. Although none of these early ocean farm experiments was successful, some data was collected from this first A more realistic interpretation of farm structure design, construction, and particularly anchoring requirements was gained. Preliminary results also indicated that "fish grazing pressures were low on the farm compared to those in natural beds nearby" (4). Wilcox also experimented with an upwelling device, designed to overcome the nutrient problems which he considered vital to the success of open ocean marine farming. Water was pumped through a pipe from a depth of 350 meters into a cone shaped structure in which a single Macrocystis pyrifera plant was located. Unfortunately, the cone collided with a Navy barge while being installed, and consequently leaks later appeared in the pipe. Wilcox was unable to make accurate measurements of possible plant growth, due to windstorms which caused the plant to chafe against the inside of the cone, tearing off any new growth. Following the unsuccessful attempts by Wilcox to install experimental marine farms structures and an upwelling device in the open ocean, the American Gas Association ended their management contract with the Naval Undersea Center, and concluded a new agreement with the General Electric Corporation.

General Electric subcontracted to Global Marine Development Inc., who continued work on open ocean marine farms, building a farm structure envisaged by Wilcox, but never actually This design resembled an open umbrella, suspended point first beneath the surface (see figure 2). In September 1977, a scale model was successfully tested at the Offshore Technology Corporation in Escondido, California. During the first six months of 1978, the final design of the test farm was completed, along with plans for its construction and deployment. The test farm was composed of four major sections. The mooring system, which was designed to hold the system in place, 5 miles off Laguna Beach, California, was composed of "three identical 16 foot diameter buoys each connected to a 15,000 pound anchor" (8). The three anchors were placed at the vertices of an equilateral triangel which was 550 feet on each The main part of the structure was composed of the machinery bouy and substrate The bouy contained the diesel engines used for artificial upwelling, and also provided support for the substrate system which was designed to hold up the 100 adult kelp The substrate structure consisted of six 16 meter stainless steel tapered poles, attached to the base of the machinery bouy. The substrate arms were connected to each other with polypropylene line, to which kelp plants were to be attached. Both the mooring, machinery bouy, and substrate sections of the test farm were constructed by the Bethlehem Steel Company at their San Pedro shipyard. The machinery bouy and attached substrate system had a dry weight of 110,000 pounds. The third main section was the upwelling pipe, "designed to draw 8,900 gallons per minute of nutrient rich water from a depth of 1,500 feet" (9). The 1,465 foot polyethelyene pipe was manufactured in 60 ft. by 2 ft. sections by the Dupont Company, under the trade name "Schlairpipe." The final major assembly component of the test farm was a current retardent curtain which was designed to act as a barrier around the machinery buoy, reducing surface currents, and enabling nutrients brought to the surface by the upwelling pumping system to be retained longer in the vicinity of the kelp plants. Test farm construction began at Long Beach, California on June 2nd, 1978, and was completed on September 13th.

Oceanographic Services Inc. (OSI), of Santa Barbara, California, a subsidiary of Global Marine, was contracted to search for a suitable site for the QAM. Initially, OSI was to examine an area 20 mile off the coast of Corona Del Mar, however, it was found that the region was frequently traversed by ship transit lanes, and consequently, the study area was expanded to 40 miles from Corona Del Mar, and included Santa Catalina and San Clemente Islands. The Corona Del Mar site was given top priority because it was close to support facilities, while weather and sea conditions were slightly milder than at the other two sites. OSI collected data on winds, waves, nutrients, the sea floor, and shipping lanes. Data on water clarity, and movement were also examined. At the end of their study, OSI recommended that the test site for the QAM be located four miles southeast in an area previously used by Dr. Wheeler North, of the California Institute of Technology whose research on the growth of Macrocystis pyrifera had been funded by the Naval Undersea Center. Their major reasons for selecting this site were that there was more oceanographic data available for this area than any other, water temperature and clarity were suitable, the site was away from commercial and pleasure vessel traffic, and it was a convenient distance from support facilities.

By August 1978, the QAM mooring system was installed, and in September, the machinery bouy/substrate assembley was anchored in 1,800 feet of water at the site reccommended by Oceanographic Services Inc. Although at first the installation of the upwelling pipe was delayed due to bad weather, it was successfully installed on by September 24th. Dr. North was contracted to assemble a team to plant and make growth observations of 100 adult Macrocystis pyrifera plants which were attached to the QAM during the first week of December. North's team began collecting data which included weekly measurements of growth of juvenile fronds, analysis of dissolved nutrients in water samples and blade tissue analysis. The protective curtain was also installed at this time, however, storms during December began tearing it at the points of attachment, until by the end of the month, it was completely gone. The effect of the storm on the unprotected farm was devastating, as strong waves and currents caused abrasion of plants against the farm structure, until by February, there were no transplants left on the farm. The first test of the QAM farm structure demonstrated a need for modification in order to minimize plant abrasion, and for a more rigid current inhibition system. There was also a need for improved plant selection, transplanting, and attachment techniques. Most importantly, it was realized that further data was needed in order to understand the dynamics of the kelp plants, which were crucial to its successful cultivation.

In 1977, General Electric began researching methods for extracting methane gas from kelp using anaerobic digestors. Dr. John Forro of G.E.'s Re-entry and Environmental Systems Division headed a research group which began to develop an anerobic inoculum (bacterial) for use in the digestors. Also in 1977, a research team headed by Dr. M. R. Hart from the Western Regional Research Laboratory of the United States Department of Agriculture, was contracted by General Electric to develop and design a mechanical and chemical process system for increasing the bacterial digestibility of Macrocystis pyrifera. The Institute of Gas Technology in Chicago, Illinois, was contracted to define and optimize the anaerobic digestion process for conversion of Macrocystis pyrifera into methane. During 1978, the Institute of Gas Technology began definition and optimization of the total anaerobic digestion process for conversion of kelp to methane. Research showed that kelp was degradable in saline culture and that addition of suplementary nutrients to the reactor was not necessary. The kelp digestion reaction rate was insensitive to drastic reductions in particle size, and current methane yields were at 71 percent per pound of volatile solid, greater than any other known biomass source at that time.

The QAM test farm was originally scheduled to be a two year project, however, the winter storms and the resulting loss of all adult transplants, limited the project to a two month period. Between May and August 1979, juvenile plants appeared on the QAM, presumably offspring arising from spores liberated by the adult transplants of five months previously (15). Taking advantage of this unexpected source of data, the research team headed by Dr. North of Cal Tech, collected data on growth rates, plant mortality, and nitrogen content of the juvenile plants. Researchers were also able to determine which of the farms components provided the best surface for plant attachment. The highest plant mortality rate occurred on the smooth plastic-coated cables, where plants were dislodged by water movement. Plants attached to the upwelling hoses were also shortlived, due to the large quantity of barnacle encrustations which abraded the juveniles. Although a higher survival rate was recorded for plants on the substrate arms and planting buoys, the best was among those attached to the moderately rough surface of the polyester support ropes.

APPENDIX D

Included in the June 1983 GRI Monthly Technical Scientific Report

### BREEDING PLAN FOR MACROCYSTIS

### I. INTRODUCTION

The purpose of the research on the genetics of the giant kelp, Macrocystis, to be carried out by Neushul Mariculture Incorporated (NMI), is to produce strains that consistently produce higher amounts of biomass when grown in nearshore farms. The goal is to produce methane gas from the kelp biomass by anaerobic digestion.

### A. Macroalgal Genetics

Enclosed are a number of papers on macroalgal genetics and the biology of Macrocystis. The paper by van der Meer (1983) provides a good overview of the progress and possibilities of genetic research as applied to marine macroalgae (seaweeds). The papers on Laminaria japonica (Anonymous, 1976; Fang, 1983) provide a summary of breeding research and results by the Chinese on this kelp species. This program has been one of only a few attempts to produce commercially useful domesticated seaweed varieties. The papers by Chapman (1974) and Chapman and Doyle (1979) on quantitative traits in populations of Laminaria spp. exhibit the type of characters that have been examined for heritability in these kelps. The paper by Waaland (1979) shows the potential for selecting for high growth rate in a red alga, Gigartina.

## B. Biology of Macrocystis

Macrocystis is a marine plant that grows in extensive nearshore beds. In southern California, these beds persist on rocky bottoms (and sometimes on sandy bottoms) in water depths of 20 to 60 feet. The biology and life history of this plant, as it relates to its domestication as a marine plant biomass producer, is described in Neushul (1978). Figure 3 in this paper and the table entitled "Selection points in seedstock rearing process" summarize the life history of this plant, which involves the alternation of independent gametophytic and sporophytic generations. The gametophytes are microscopic and dioecious. They can be easily grown in laboratory culture conditions, and can be vegetatively propagated. Therefore, it is possible to isolate single gametophytes and grow many individuals from this initial isolation. The gametophytes will grow vegetatively in red (blue lacking) light. Blue light will induce gametogenesis. Gametophytes can be maintained in laboratory culture for years. By crossing specific male and female gametophytes, it is possible to repetitively produce large numbers of sporophytes of defined, identical, genetic background.

Three species of Macrocystis are recognized to occur in California: M. pyrifera, M. angustifolia and M. integrifolia. M. integrifolia occurs exclusively north of Pt. Conception, while M. pyrifera occurs from Monterey to Baja California and M. angustifolia is only known in the Santa Barbara area. These species are difficult to distinguish using most morphological characteristics, because many of these characteristics show much variability in

response to environmental influences. Holdfast morphology has been considered to be a relatively conservative characteristic, and this is summarized in Figure 10 in Neushul (1978). Holdfast types B and C in this figure should be considered to be M. angustifolia, which is morphologically closer to M. pyrifera. In a previous kelp genetics and breeding program, all three species were interfertile, producing viable sporophytes. Therefore, these species (if they can still be called distinct) are genetically close. Intergeneric hybrids have also been formed between M. angustifolia and both Pelagophycus porra and Nereocystis luetkeana. These hybrids were viable, but did not reproduce. They did possess morphologies intermediate between the genera that were crossed (see Figures 12 and 13 in Neushul, 1978).

The sporophyte of Macrocystis is perennial, and the farming and harvesting strategy for this plant involves artificially anchoring individuals and periodically harvesting the biomass by cutting off the tops of the plants. Such harvests have been done commercially in southern California for alginate extraction since the beginning of this century. New fronds will grow from the holdfast, and these reach the surface in a relatively short time after harvest. The paper by Neushul et al. (1983, in press) summarizes the research on planting, fertilizing and harvesting carried out by NMI.

### II. BREEDING PLAN

Plants placed on the yield test plots provide the material for this section of the breeding plan. Previous studies have revealed that several individuals exhibited consistently higher biomass yields during quarterly harvests (see Figure 6 in Neushul et al., 1983). Most of these plants were lost in recent storms, but future work should result in the selection of more high-producing plants.

### A. Determination of Favorable Traits

First, an ideotype of <u>Macrocystis</u> must be determined. Increased biomass yield is the goal, and traits that contribute to this goal must be identified. Data from the species characterization measurements of the previous genetics program and from the yield farm plants will be reviewed and re-analyzed. Data from the yield farm plants on the number of fronds and biomass yield at each harvest should be useful to determine if any relationship exists between frond initiation rate, or some other frond characteristic, and biomass yield.

In addition, measurements of other traits that may be favorable will be undertaken. One trait that has been identified as being potentially favorable is a low rate of sloughing of material from the blades on a frond. A method to determine sloughing using a video system to measure surface area of blades will be developed in order to do this. Attempts will be made to identify other useful traits.

An approach that may be useful in defining the traits of a high-biomass-producing ideotype would be to model the growth of an adult sporophyte. Macrocystis responds to a complex of environmental factors in many ways. Responses to light, nutrients, and temperature have been examined. Translocation of reduced carbon resources, storage of inorganic and organic substances, and factors that lead to frond senescence are potential areas that

have been examined at least briefly, and that must be integrated in a form that will predict an ideotype. Growth and biomass production is an integrative response to such factors. One such model treats the fronds on a plant as a population, with each frond going through five developmental stages. The rate that the fronds are initiated, and the rate at which they move from one stage to the next is modeled in this approach. Mature fronds have a positive influence on the initiation of new fronds. The data from the yield farm plants could be used to model the growth of the fronds of plants.

### B. Mass Selection Breeding Program

Individuals that show superior yields on test plots will be selected in this breeding program. Two high-yielding plants from the previous test plots survived the winter storms, and will be used as standards in this program. Cultures of sporophytes will be started by inducing spore release from a sporophyll cut from high or low producing plants. The spores will be settled on string, and sporophytes (via gametophytes) raised from them. sporophytes on each string will be genetically diverse, because of recombination, but they will all be from one parental sporophyte. The fastest growing individuals from each high producing plant and the slowest growing from each low producer will be outplanted in the sea. Growth rates will be measured periodically on a fresh weight basis and compared to determine if growth rate (rate of biomass gain) is different between sets of sporophytes from different parental sporophytes and if this is related to the biomass yield of the parents. In addition, tests will be performed to determine if the variability of growth rates of these plants is different from variability in genetically uniform sporophytes that will be produced in the pedigree selection program (discussed below). If other traits are identified as useful from the kelp growth modeling described above, then these will also be measured and the above comparisons made. Once these sporophytes have reached maturity, they will be used to begin the cycle again, with each cycle being approximately 1 year long. If growth rate is significantly different between progeny of low- and high-producers after the first or second cycles, then growth rate is heritable, and can be selected in a breeding program.

After 3 or 4 cycles, it is possible that this selection program will reach a plateau, with little or no improvement in growth rate from one generation to the next. Germplasm from these strains will be isolated in the form of gametophytes and used to produce new genetic recombinations with other strains to attempt to produce significantly higher producing strains. Since the three species of Macrocystis that are recognized to occur in California are all interfertile, new genetic recombinations among them may be useful to produce superior strains. It would be interesting to determine if individuals that are more widely isolated geographically (from British Columbia or Alaska, New Zealand or Australia, South America, southern Africa) are also interfertile. If this were true, then a wide range of cultivars would be available for selection of specific traits that may be determined to be useful.

The production of biomass is a trait that may best be measured in adult plants that are greater than 1 year old. These plants will be maintained on in-the-sea structures on the ocean bottom, and they will be harvested every three months, as are the other plants that have been used on the test plots, to determine biomass yield.

### C. Pedigree Selection

This part of the breeding plan will involve producing sporophytes from clonal cultures of gametophytes. This will include gametophytes that have been used in a similar program in the past and gametophytes that will be isolated from high-producing individuals identified during the on-going mass selection program discussed above. Each batch of sporophytes produced from these crosses will be genetically identical.

There are currently 147 clonal cultures of female gametophytes and 161 of males being maintained in laboratory culture conditions. These are summarized in an enclosed table. Of these 308 cultures, 175 are from wild plants from a total of 15 parents, and 133 are from cultivated F1 generation sporophytes from a total of 9 parents. Many crosses could potentially be made (147 X 161 = 23,667), but this would not be practical. Selecting certain gametophytes at random would potentially exclude useful genetic potential, since haploid gametophytes do not contain the full genetic complement that sporophytes do. This section of the breeding plan, then, should be used to determine the heritability of certain traits through the use of genetically defined individuals, and not as a general strain improvement scheme.

In the previous kelp breeding program, certain crosses appeared to yield faster growing plants, and we will attempt to make sporophytes from these gametophytic lines again. The data from these crosses will be re-analyzed to determine if the growth rate differences were significantly different between crosses, which would indicate that these differences are due to genetic differences. F2 generation gametophytes were obtained from most of these pedigree crosses, and measurements of sporophytes produced from F1 and F2 generation gametophytes should enable us to estimate the heritability of growth characteristics.

After several generations of mass selection, the sporophytes may be more homozygous, which would make it more likely that individual gametophytes would contain more of the full genetic complement that contributes to high production traits. Therefore, clonal isolates of gametophytes will be established from the high producing plants from the mass selection program. These may be used in future pedigree selection programs.

It would be useful to be able to select gametophytes in the laboratory that produce sporophytes that would be fast-growing in the sea. If the genes that result in high production are expressed as fast growth in both nuclear phases of this plant, then gametophytes could be selected by their laboratory growth rates. This would be useful for a pedigree selection program.

### D. Autoploidy

Many crop plants are autoploids, and this portion of the breeding plan appears to have much potential, since autoploids tend to have larger cells, which may result in larger plant sizes. Colchicine will be applied to embryonic sporophytes in an attempt to obtain autopolyploid sporophytes. Colchicine will be applied in factorial concentrations of concentrations and exposure times to determine a suitable treatment. Initially, colchicine will be applied at 0.1 to 0.4% for 0.5 to 3 hours. Once a suitable treatment is determined, embryonic sporophytes from pedigreed crosses and female gametophytes will be treated. Female gametophytes have been found to produce sporophytes parthenogenically, but these have developed abnormally and do not

survive very long. Colchicine may induce a diploidization that may result in normal development of parthenosporophytes. Such parthenosporophytes would be homozygous and would be powerful tools in a breeding program.

Ploidy levels of plants treated with colchicine would be determined either using an acetocarmine squash to count chromosomes, or a quantitative staining procedure (DAPI) to determine relative levels of DNA. In previous cytological studies, M. integrifolia has been found to have n=16 and 2n=32 chromosomes. Therefore, it is possible that Macrocystis is already possesses a polyploid number of chromosomes. Chromosomes counts need to be obtained for the other two species as well. Chimeric plants may develop, with different parts having different ploidy levels, but this would probably be easily detected.

In summary, the proposed breeding program would involve mass selection, pedigree selection, and attempts to induce polyploidy. Both macroscopic sporophytes and microscopic gametophytes will be studied, and a gene-bank of long-lived gametophytes and sporophytes will be established and maintained.

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# Summary of gametophyte stocks of $\underline{\mathsf{Macrocystis}}$ spp. maintained by NMI.

# 8 June 1983

Species		Female	Ma1e
Мр		Mp-A:1-3 Mp-C:1-10	Mp-A:1,3,4 Mp-C:1-10
	-	Mp-D:2-3 Mp-E:1-6 Mp-F:1-4 Mp-G:1,3-17	Mp-E:1-5 Mp-F:1-5 Mp-G:1-19
	TOTAL	41	42
Ма		Ma-I:1-2	Ma-I:1-5 Ma-J:1-9
		Ma-K:1-10,12 Ma-L:2, 7-9 Ma-M:1-3 Ma-N:1-9	Ma-M:1-3 Ma-N:1-10
	TOTAL	29	27
Mi		Mi-Q:1-3 Mi-R:1-4 Mi-S:1-4,6-7	Mi-Q:1-11 Mi-R:1-5 Mi-S:1-7
	TOTAL	13	23
Mp X Mp			Mp-B:4,8 AA-10:1-2
	TOTAL	0	4
Ma X Ma		11-10:1-8	II-10:1-12
	TOTAL	8	12
Mp X Ma		AI-4:1-10 AI-27:1-10	AI-4:1-3 AI-27:1-10
	TOTAL	20	13
Ма Х Мр	•	IA-15:1-10	IA-15:1-10
	TOTAL	10	10
Ma X Mi		IQ-5:1-10	10-5:1-10
	TOTAL	10	10

Mi X Ma QI-24:1-7 QI-24:1-12 TOTAL 7 12 Mi X Mp QA-49:1-9 QA-49:1-8 TOTAL . 9 8

No Mp X Mi and Mi X Mi.

TOTALS:

FEMALES 147 MALES 161

OVERALL 308

Mp = Macrocystis pyrifera

Ma = Macrocystis angustifolia

Mi = Macrocystis integrifolia

Description of sporophytes of <u>Macrocystis</u> spp. from which gametophytes have been isolated.

### Wild Sources

## M. pyrifera

- Mp-A Sporophylls collected a wild sporophyte from Catalina Is. on 5/6/79. Spores released 5/7/79. Gametophytes isolated 5/9/79.
- Mp-C Sporophylls collected from a wild sporophyte off Santa Cruz Point in Santa Cruz Bay on 10/7/80 by S. Clabeusch and S. Fain. Spores released 10/10/80.
- Mp-D Sporophylls from a wild sporophyte (a.k.a. W#1) collected subtidally (12 m) from Anacapa Island by J. Woessner on 7/22/80. The sporophyte was characterized morphometrically. Spores released 7/24/80.
- Mp-E Sporophylls from a wild sporophyte (a.k.a. W#2) collected subtidally from Anacapa Island by J. Woessner on 7/22/80. The sporophyte was characterized morphometrically. Spores released 7/24/80.
- Mp-F Sporophylls from a wild sporophyte (a.k.a. W#3) collected subtidally from Anacapa Island by J. Woessner on 7/22/80. This sporophyte was transplanted to the NMI Campus Pt. farm after spore collection. Transferred to Ellwood Pier farm on 8/21/80 and lost on 2/2/81. Gametophytes isolated 1/2/81.
- Mp-G Sporophylls from a wild sporophyte (a.k.a. SD#10) collected subtidally from bed located off Pt. Loma by M. Neushul and J. Woessner on 7/3/80. Sporophyte transplanted to NMI Campus Pt. farm after spore collection. Transferred to Ellwood Pier farm on 8/21/80 and lost on 2/2/81. Gametophytes isolated 8/1/80.

# M. angustifolia

- Ma-I Sporophylls collected from a wild sporophyte from Campus Pt. on 5/1/79. Spores released 5/4/79. Gametophytes isolated 5/9/79.
- Ma-J Sporophylls collected from a wild sporophyte from Campus Pt. on 4/6/79. Spores releasesd 4/10/79. Gametophytes isolated 4/16/79.
- Ma-K Sporophylls collected from a wild sporophyte from Campus Pt. by Y. Sanbonsuga prior to 1977.
- Ma-L Sporophylls collected from a wild sporophyte from Campus Pt. on 11/3/79. Spores released 11/14/79. Gametophytes isolated 11/20/79.
- Ma-M Sporophylls collected from a wild sporophyte from Campus Pt. on 6/23/80. The sporophyte was collected at 9.4 m depth and was dissected into 1-meter lengths for modelling of growth of this plant. Gametophytes isolated 7/5/80.

Ma-N Sporophylls collected from NSF plant #F29, collected as a wild juvenile and outplanted on 10/15/79 at Goleta Bay. Gametophytes isolated 9/28/80. Sporophyte lost in storms 3/81.

## M. integrifolia

- Mi-Q Sporophylls collected from a wild sporophyte at Cannery Row, Monterey Bay, on 9/23/79. Spores released 9/27/79. Gametophytes isolated 10/3/79.
- Mi-R Sporophylls collected from a wild sporophyte collected subtidally off Soquel Pt. in Santa Cruz Bay on 10/9/80 by S. Clabeusch and S. Fain. Spores released 10/10/80.
- Mi-S Sporophylls collected from a wild sporophyte in the intertidal zone at Stillwater Cove, Monterey Peninsula by S. Clabuesch and S. Fain on 10/9/80. The sporophyte was discarded. Spores released 10/10/80.

### **Cultivated Sources**

## M. pyrifera X M. pyrifera

- Mp-B Sporophylls collected from Mp 5 X Mp 5 sporophyte cultivated at Campus Pt. Sea Grant site. Parent plant was outplanted 4/6/79 and was app. 10 m long at the time of collection. Sporophylls collected 7/27/79. Spores released 8/3/79. Gametophytes isolated 8/10/79.
- AA-10 Sporophylls collected from a Mp-A3 X Mp-A1 sporophyte cultivated at Ellwood Pier farm. Gametophytes isolated 1/2/81.

# M. pyrifera X M. angustifolia

AI-4, AI-27 Sporophylls colected from Mp-A3 X Ma-I3 sporophytes cultivated at Ellwood Pier farm. Gametophytes isolated 12/21/80.

# M. angustifolia X M. angustifolia

II-10 Sporophylls collected from a Ma-I1 X Ma-I3 sporophyte cultivated at Ellwood Pier farm. Gametophytes isolated 3/4/81.

# M. angustifolia X M. pyrifera

IA-15 Sporophylls collected from a Ma-I1 X Mp-A1 sporophyte cultivated at Ellwood Pier farm. Gametophytes isolated 12/31/80.

## M. angustifolia X M. integrifolia

IQ-5 Sporophylls collected from a Ma-I1 X Mi-Q9 sporophyte cultivated at Ellwood Pier farm. Gametophytes isolated 12/23/80.

# M. integrifolia X M. integrifolia

QA-49 Sporophylls collected from a Mi-Q2 X Mp-A1 sporophyte cultivated at Ellwood Pier farm. Gametophytes isolated 3/5/81.

## M. integrifolia X M. angustifolia

QI-24 Sporophylls collected from a Mi-Q2 X Ma-I3 sporophyte cultivated at Ellwood Pier farm. Gametophytes isolated 3/5/81.