MARINE FARMING:
MACROALGAL PRODUCTION
AND GENETICS

FINAL REPORT
MAY 1980 - DECEMBER 1986

Gas Research Institute
8600 West Bryn Mawr Avenue
Chicago, Illinois 60631
MARINE FARMING: MACROALGAL PRODUCTION AND GENETICS

FINAL TECHNICAL REPORT
May 1980 - December 1986

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The work accomplished with funding from GRI from May, 1980, through December, 1986, is reviewed in this report. Two float-bearing macroalgae, Macrocystis and Sargassum, were domesticated and grown in the sea, in tanks, and axenically in dish culture. The giant kelp, Macrocystis, has been successfully farmed on a pilot scale in coastal waters under controlled conditions for the first time, and its productivity is as great as sugar cane. The gulf-weed, Sargassum natans, a major component of the Sargasso Sea, has been grown and maintained for three years, in dish and tank culture. This is the first time that long-term culture of this giant has been possible. A seedstock collection consisting of over 800 vegetatively-propagated macroalgal isolates has been established and maintained. New methods for applying mutagens and isolating mutant strains of the giant kelp have been successfully employed, and hybridization trials have shown that morphological variants are expressed in the sporophytic but not in the gametophytic life-history phase. New marine farm engineering principles have been developed and applied. A detailed study of the effects of environmental conditions on farmed macrophytes in the sea, has been completed. Finally, NMI has assembled a list of valuable new co-products and by-products, that would be generated if macroalgal farms were established in coastal waters.
Title: MARINE FARMING: MACROALGAL PRODUCTION AND GENETICS

Contractor: NEUSHL MARICULTURE INCORPORATED

GRI Contract Number: 5083-226-0802-5

Principal Investigators: M. Neushul and B. W. W. Harger

Period of Performance: May 1, 1980 to December 31, 1986

Objectives: This final report discusses the work completed at all phases of the GRI contract including measuring the growth of individual kelp plants in the sea, and the installation of an experimental coastal test farm. Subsequently, a seedstock collection was established, and methods for manipulating algae genetically were developed and mutants were produced and tested. Research programs were established and maintained with scientists and marine biologists in Japan, China, Korea and the Philippines.

Major Achievements: The domestication of two float-bearing macroalgae has been completed and a vegetatively-propagated seedstock collection of some 800 strains has been established. The giant kelp, *Macrocystis*, has been farmed on a pilot scale in coastal waters for the first time, and engineering principles have been developed and applied (with co-funding from NSF). A six year study of the effects of oceanographic conditions on the growth of giant kelp in natural kelp beds has been finished. The gulf-weed, *Sargassum*, has been cultivated for over three years in the laboratory. New methods for applying mutagens to *Macrocystis* have been developed and tested and mutants have been isolated and cloned. Cloned mutant-stocks have been crossed and morphologically distinctive sporophytes have been produced for the first time.
ACKNOWLEDGEMENTS

We would like to acknowledge the generous contributions to this program by those who were the first to cultivate macroalgae in the sea. It has been nearly thirty years since Dr. Wheeler J. North authored the first Institute of Marine Resources Annual Report for the Kelp Investigation Program, to which the author of this report also contributed (July 1, 1957, IMR Reference 57-4). For three decades Dr. North has generously given advice, assistance, and encouragement to us. His scientific "courage" to tread first where others have not, serves as an example for all. We also owe a debt of thanks to Dr. H. A. Wilcox whose vision of open ocean kelp farms was matched by an unusual ability to transfer his enthusiasm to others. Similarly, Dr. A. Flowers of the Gas Research Institute recognized that the development of an entirely new, renewable source of energy was a major undertaking that required substantial levels of funding. For the first time, kelp research was well funded. He also recognized the need, after touring the vast Chinese and Japanese marine farms, to establish a scientific exchange program, which will most certainly continue long after the termination of this project.

Dr. C. K. Tseng, of the Institute of Oceanology, Qingdao, China, is another pioneer who can justly claim to be among the very first to have farmed the sea. He has been very generous with his assistance to us, provided advice, encouragement and gave us an invaluable historical perspective on the problems involved with farming the sea. He also made it possible for his talented co-workers X. G. Fei, C. Y. Wu, the late T. C. Fang and N. N. Kiang to visit and work with us. We look forward to a visit in 1987 from the noted geneticist, Giajun Li, who has been studying kelp genetics for twenty years. The Japanese phycological community has also been helpful to our research projects. We would like to acknowledge the advice and assistance of Y. Sanbonsuga and H. Yabu, who taught us invaluable cultivation and cytological techniques. Scientists at Ishikawajima-Harima Heavy Industries were also very generous in keeping us up-to-date with their recent progress, which is discussed in this report.

Colleagues and co-workers at the University of California, Santa Barbara have been particularly contributory, and their advice and assistance is greatly appreciated. Finally, we would like to acknowledge the financial and scientific guidance provided by our GRI program managers, including Drs. P. Benson, K. Bird, and J. Frank. Most recently we have been fortunate to have Dr. H. R. Isaacson as our Program Manager. He has adeptly and tactfully dealt with the problems of closing down this program, and shares our hope that one day the task of large-scale farming of macrophytes in the sea will be taken up again in the U. S. to the extent that it is now done in the Orient and Philippines.
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I. INTRODUCTION

As this and our other G. R. I. reports show, we are convinced that the Marine Biomass Program has been a major success. Nonetheless, when some twenty million dollars of rate-payers' and tax-payers' money is spent over a twelve-year period, and then the entire program is dropped, it is obvious that someone must have raised questions about the value of the research expenditure. It is our hope that our reports and published papers will convince G. R. I. management, and the Gas Industry, that their substantial investment along with that of the National Science Foundation and the Department of Energy was worthwhile. It has set the stage for the development of a new U. S. marine farming industry that may ultimately provide a vast renewable source of energy and other products for human use.

Many people had grave doubts about this program from the very beginning, which perhaps explains why so many outside reviews and analyses were solicited and offered gratis by our critics. We view these critics as the major component of an inverted pyramid, with many people (most of whom had never seen a seaweed underwater) looking critically at the efforts of those few who actually got wet.

Of course, it is logical that there should be considerable skepticism about a totally new, renewable source of energy. Atmospheric studies have documented an increase in the carbon dioxide level of our atmosphere, which has been traced to our use of fossil fuels, and as a result it has been predicted that the worldwide climate will warm (the "Greenhouse Effect", Bernard 1980, Barth and Titus 1984, Kerr 1986). Future use of abiogenic methane, if it in fact exists, would add to the atmospheric carbon dioxide load as much as fossil fuels do today, while growing seaweed as a source of methane would incorporate as much carbon dioxide as it releases, as well as provide a renewable resource.

Critics' doubts about the energy-from-seaweed concept were reinforced by a series of discouraging setbacks. An unimaginative business assessment made by General Electric (Marler 1982) further compounded the problem by suggesting that marine farms would not be commercially successful. It is our opinion that we should still be working toward the goal of establishing large-scale marine biomass farms along our coasts and ultimately in the open sea. We feel that within a few years the products of these farms will make them self-supporting.

Was the whole idea a bad one, as many of our critics claimed? Were the research milestones unrealistic and the program direction by the program managers intrusive and erratic? Has the money spent been wasted? Why have marine farms been commercially successful in China, Japan and the Philippines, while at the same time unsuccessful here in the United States? Why are the Japanese, Chinese, and Phillipinos continuing to invest in large-scale marine farming, while we are not? We return to these questions in the concluding section of this report.

Early in the program, it was felt that nearshore farming was of no interest, because only offshore could one establish farms that were large enough to make a significant contribution (at least 5%) to the future U. S. energy needs. Consequently, vast sums were spent building and installing the first open-ocean farm module to prove that the offshore concept would work,
that is, that one could pump deep, nutrient-rich water to the surface and grow mature kelp plants in it. In the end, this quarter-acre module produced no yield data, and was widely criticized. Then, the program directors of General Electric organized a meeting to solicit the advice of a panel of academic "experts". This panel endorsed a concept proposed by North and Gerard to build a rubberized bowl, the "Hemidome", at Santa Catalina Island. In it, kelp plants would be grown under "controlled" nutrient-enriched conditions, using deep, nutrient-rich water, to determine what the "optimum" kelp yield is that could be achieved. N. M. I. staff participated in these meetings and voiced misgivings about this approach. We were worried about possible fouling and bacterial growth on the walls of the "Hemidome" and the low water motion that the plants would be subjected to inside the bowl, citing the work of Wheeler (1980) on water motion effects on nutrient uptake. Unfortunately, other views (Gerard 1982) prevailed.

We proposed that a less controlled approach be taken, wherein an experimental kelp planting, patterned after a natural kelp bed, be established in the sea and fertilized in part. D. O. E./S. E. R. I. Program Managers stipulated that their funds be used solely to support the "Hemidome" work. Fortunately, we were able to convince some of the G. R. I. Program Managers that our approach, although "flawed" scientifically in the eyes of some, would serve as a parallel path in case the main experiment, for some unlikely reason, did not produce yield data. G. E. Program Managers tried very hard to change our minds and to reduce the scope of our proposed project. They considered ours the "throw-away" project, while the "Hemidome" was the main project that would give the program the yield results that were so critically needed. In the end, the "Hemidome" failed to produce kelp yield data while this report summarizes the yield data which our nearshore test farm produced.

We feel that the failures of the "Quarter-Acre Module" and the "Hemidome" were due to the fact that the participants in the Marine Biomass Program were unaware of basic hydrodynamic "facts of life" that influence the ways that floating macroalgae grow and reproduce in the sea, and how they interact with other organisms and with the farm. The Japanese, whose recent work is reviewed here, still seem to be unaware of these problems. While nearshore farms in the future may resemble existing natural kelp forests (Neushul 1984), open-ocean macroalgal farms are more likely to resemble the Sargasso Sea, and the plants cultivated may well be genetically-engineered to meet the specific hydrodynamic demands of that environment (Neushul 1985). We are sure that regardless of whether nearshore or open ocean farms are being operated, a detailed record of environmental conditions will be essential. We have included in this report, such a record, made over a six-year period at our farm sites.

In order to answer critical questions like those posed above, we have taken a broad view of our specific contributions over the past six years as they relate to the efforts made by others during this century. Fortunately, much of the technical information produced by the G. R. I. marine biomass program will be covered in the soon-to-be-published, multi-authored book, "Seaweed Cultivation for Renewable Resources" edited by P. Benson and K. Bird. Here, we examine the Marine Biomass Program as a scientific process, while at the same time examining the products of that process. For this reason, some chapters of this report are introduced and referenced separately, as "stand-alone" sections. Some chapters have been published elsewhere. This report is offered with the hope that the challenges of domesticating marine crop plants and animals, and farming them in the open sea, will again be taken
up. Clearly the task is not one for the faint of heart, or those who are short of funds. On the other hand, the rewards for success will be enormous.

Milestones

As a concise summary, it is useful to list the milestones achieved by N. M. I. during the 1980-1986 period, starting with the study commissioned first by the Office of Technology Assessment, as a component of their larger study of bio-energy, which was issued as a committee report by the Government Printing Office (see Neushul Mariculture Incorporated 1980).

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<tr>
<td>1984</td>
<td>Yield verification studies undertaken, the effects of storms and nutrient drought measured. Tissue culture studies performed on <em>Macrocystis</em> and <em>Sargassum</em> producing calluses and some regeneration.</td>
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<tr>
<td>1985</td>
<td>Kelp genetics studied, seedstock collection developed.</td>
</tr>
<tr>
<td>1986</td>
<td>Mutagenized gametophytic lines established and crossed. DNA measured and modern genetic techniques reviewed and tested. A six year study of the effects of environmental conditions on natural beds, and cultivated <em>Macrocystis</em> was completed.</td>
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II. BACKGROUND AND HISTORY OF THE MARINE BIOMASS PROGRAM

The Marine Biomass Program, as a scientific undertaking, has produced a substantial data base. However, in some instances, projects were undertaken without knowledge of preceding work, due to the obscurity of the historical record. Since research in the sea is both expensive and hazardous, it pays to avoid repeating mistakes and "re-inventing the wheel." Hopefully, those who carry on this important work, will start with a careful study of what has been done previously, both in the U. S. and elsewhere in the world. This chapter and appendix B to this report, are offered as such a starting point. As the Marine Biomass Program developed and matured, it supported projects in California, New York and Florida. The book edited by Benson and Bird and cited earlier will summarize the findings of the Marine Biomass Program.

Kelp Studies in California and Elsewhere 1900-1986

The G. R. I. Marine Biomass Program kelp work did not begin, de novo, but was preceded by several major research and development programs that had commercial as well as scientific objectives. In fact, over fifty different projects dealing with kelp and kelp beds had been completed, before the G. R. I. Marine Farm Program began. This body of information is dispersed and difficult to assess, consisting of thesis work, unpublished reports, and material published abroad that is not commonly available in libraries. Of course, much of the work done on the development of products was, and still is, proprietary.

The large-scale Marine Biomass Program, funded by G. R. I., D. O. E., S. E. R. I. and others, was preceded early in this century by the similar large program of Hercules Chemical Company in San Diego, in which the basic problems of harvesting natural kelp beds and processing kelp were overcome. Hercules Co. harvested over 400,000 wet tons per year for the production of energy and other by-products from marine biomass, for a comparatively brief period (Figure 1). It is appropriate to note here that events abroad, specifically the First World War, produced a market for energy (explosives) produced from kelp.

The sudden shortage of potash, due to Germany's refusal to sell it to other countries, led to Congressional funding for a survey of the kelp beds in 1911. Fortunately, the kelp act of 1915 established the beds as a state resource, and provided for the recording of kelp harvests by the California Department of Fish and Game. Unfortunately, the U. S. D. A. experimental kelp harvesting plant and research station at Summerland, near Santa Barbara, was a short-lived venture, as it could have been the basis for the development of several new industries early in the century. After the war, when the less expensive European potash was again available, Hercules shut down the largest kelp biomass processing plant in the world. The global market for kelp products has always changed, and even today the Chinese entry into the world alginate market may have dire consequences for the aging U. S. alginate extraction industry.

From 1925 to mid-century little research was done, except for some basic studies of kelp biology undertaken in response to complaints from citizens about drift kelp on the beaches. Of course, during this period rapid advances were made in the development commercial products by the alginate industry by the Kelco Company of San Diego. With the advent of commercial aviation and aerial photography, used for surveying coastal land, aerial pictures of the
Figure 1. California kelp harvests, 1915-1986, show the remarkably large harvests at the beginning of the century, and the gap during the 1921-1930 period, before Kelco learned how to produce alginates. Then there was a gradual increase in harvested amounts until the 1970s when the yield of natural beds seemed to level off. For the four years (1980-1983) that NMI was carrying out yield studies, environmental conditions worsened, and a record low natural harvest of 5,000 tons was recorded for 1983. Since then there has been a rapid recovery of the natural kelp beds, particularly those on rocky bottoms.
Kelp beds were taken and archived. N. M. I. in 1980 found that these could be used effectively to map the beds retroactively, and was able to produce a clear historical record using them (Harger 1983). Some pioneering oceanographic work done by Allen at the Scripps Institution of Oceanography in La Jolla, provided some of the first environmental records of seasonal variability in growing conditions in nearshore waters. Later records made by the Southern California Coastal Water Research Program (SCCWRP), add additional environmental information, that when combined with the N. M. I. environmental measurements and records of kelp harvests, provide a useful view of the effect of environmental conditions on biomass production in cultivated and natural kelp beds.

Other kelp research efforts in California, were responses to more local needs. For example the Institute of Marine Resources of the University of California, was funded by the California Department of Fish and Game, starting in 1957, to study the effects of kelp harvesting on sportsfishing. This was extended with support from the Water Pollution Control Board, to study the effects of nearshore pollution on the kelp forests. The principal investigator for most of this work has been Dr. W. J. North, and the results of the work have been described in a series of Institute of Marine Resources reports, and elsewhere in the published scientific literature.

Support from the California Department of Fish and Game, and Kelco Company, made it possible to begin working on kelp growth and reproduction, and M. Neushul made one of the first attempts to grow kelp on floating platforms in deep water near the Scripps Institution of Oceanography. Subsequently, the National Science Foundation supported basic research on the exploration of benthic algal communities, and the effects of environmental conditions on plant growth and reproduction (Neushul 1981).

Recently, an extremely well-funded kelp research program has been pursued by the Marine Review Committee, based at U. C. Santa Barbara. Their program, called the Kelp Ecology Project, started in 1975, and focused on studies of the effects of cooling water effluent from the San Onofre Nuclear Generating Station on the giant kelp, Macrocystis. N. M. I. reviewed this program, as well as earlier work supported by the Southern California Edison Company, which dealt with the environmental impact of this large power plant. The total amount spent by the Marine Review Committee and its antecedents, on kelp research, approaches that spent by G. R. I., and is continuing. In contrast with the G. R. I.-sponsored work; however, relatively few published papers have been produced by this research group. Most of the results obtained have been described in annual reports to the Marine Review Committee.

The Marine Biomass Program was initiated by Dr. Howard Wilcox at the Naval Undersea Center and received funding from the Navy in 1972 and 1973. The program continued under the direction of Wilcox with funding from the Energy Research and Development Agency and American Gas Association from 1974 to 1975. In 1976 the American Gas Association and Department of Energy/Solar Energy Research Institute selected General Electric to direct the program through a competitive "Request for Proposal." The program support was transferred from the A. G. A. to the Gas Research Institute which provided the main support from 1976 to December 1986. There was intermittent support from D. O. E./S. E. R. I. in 1976 to 1978, 1981 to 1982 and 1986. General Electric directed the program from 1976 until April 1984. From April 1984 to December 1986 the program was directed directly by Gas Research Institute Program Managers.
Neushul Mariculture Incorporated received program support from April 1980 to December 1986.

Important research concerning kelp cultivation has been carried out in other countries as well. From mid-century to 1975, the Chinese kelp farms were established and developed by C. K. Tseng (1981a and 1981b), with research support from the Chinese Academy of Sciences. It is noteworthy that the Chinese support was not erratic like that in the U. S., but steady and continuous, with an increase in the level of funding of about 10% per year (Tseng, personal communication).

A program that has been virtually ignored by U. S. kelp research workers was started in 1975 in Argentina, which has luxuriant kelp forests (Neushul 1971a). Several conferences and ultimately a well-run and productive research program was sponsored by the Centro Nacional Patagonico. Much of the work was based in Puerto Madryn, in Chubut province. Kelp was, and still is, harvested in limited amounts from this coast. In 1975 Fernando Gutierrez, reported work on seasonal variability of alginic acid in Argentine kelps, and in 1975 Martin Hall published the first of several excellent studies dealing with the assessment of the kelp bed resources of this coast, basing the work on aerial photography using infrared film. Hall and Alicia L. Boraso de Zaixso described cyclical patterns of growth and reproduction and in 1980 four reports were issued dealing with kelp measurement, growth and reproduction, where large and regular samples were taken to produce a unique data-base. Given the cold and uncomfortable working conditions of the Patagonian coasts, this was certainly a major achievement. As far as we know, Dr. Hall's involvement with the program ended in 1981, and progress has been minimal since then. Nonetheless, some 120 million dollars U. S., was provided by the International Development Bank to Argentina to establish research centers and fund fellowships for study abroad. Major research laboratories are under construction in Ushuaia, Puerto Madryn and Bahia Blanca, along the Argentine coast, (as well as four new inland research centers). Thus it seems likely that more kelp research will be done in the future along this coast.

Work in Tasmania by C. Sanderson is more recent, and has focused on establishing the structure of kelp communities, following productivity and seasonality and making underwater transects and observations of the extensive kelp beds that occur there. Exploratory studies of kelps in Chile have also been done by Dr. P. Dayton of the Scripps Institution of Oceanography. He has also studied natural kelp beds in California and Alaska. Dr. J. Cain has worked on European kelps for many years, being one of the first to use SCUBA gear to study them first hand. She began experimentally farming Laminaria after the oil crisis of the mid-1970s. Her projects, involve experimental farms off the Isle of Man, and are continuing up to the present, with yields of as much as 50 tons per hectare being predicted from small scale plantings.

The above-mentioned studies can be seen to begin with resource surveys of various sorts, prompted by the desire to utilize the resource. Little management of the resource is provided until concerns are raised about the overexploitation of natural populations. Then when pollution or harvesting interferes with other uses of the resource, like sport-fishing or abalone harvests, more detailed ecological studies are undertaken. With so many different programs going on, it is difficult to remain aware of the progress made in each area, and the implications of this for other projects. Fortunately, G. R. I. program managers felt that it was important to remain
aware of research activity world-wide, and assigned this task to N. M. I.. It is particularly interesting that since there were no native kelp forests along the coast of China, work there focused immediately on cultivating the plants, a problem not faced by those working in U. S. waters until recently. However the problem of cultivation, and cultivation on a large scale, came up when H. Wilcox suggested that marine biomass could be a renewable source of energy.

At present, macroalgal mariculture seems to be entering a new phase, where new biotechnological tools offer the possibility of producing genetically engineered cultivars, adapted for either attached or free-floating farms. The question of whether the marine farms of the future will be structurally engineered in the traditional sense, or genetically engineered is still to be answered. Perhaps an answer to this question will be obtained before the end of the century. This century will most certainly be known as that in which the foundations for large-scale macroalgal mariculture were established.

The Marine Biomass Program

H. A. Wilcox, in a paper addressing the prospects for farming the ocean (1972), estimated that given a 2% efficiency for converting solar energy into plant material, 5% efficiency for production of human food, and 50% efficiency for the production of fuels and other products, one square mile of sea surface would produce enough food to feed 3,000 to 5,000 persons, and enough energy to support more than 300 persons at current U. S. per capita consumption levels. Since the oceans contain 80 to 100 million square miles of arable surface water, the marine farms could support a world population of more than twenty billion persons (the latter being a particularly unpleasant prospect). N. M. I., in it's first review of the potential of marine biomass farms for energy production (1980), undertaken for the Congressional Office of Technology Assessment, took a much more conservative view. S.V. Smith has compared marine macroalgal productivity with that of other plant life on earth (Figure 2).

The contrast between optimistic, and conservative views persisted and are pictorially "summarized" by the illustrations of existing and future farms that have been published. These, and the text accompanying them, are particularly revealing. They serve as "samples" of past views and draw our attention to specific aspects of the G. R. I. program. For example, a 1974 article in Newsweek, with an illustration, carries the heading, "Four-H Frogmen" and a quotation from Dr. Wilcox to the effect that marine farming is an easy, low technology task (Figure 3). This initial optimism was found to be misleading to say the least, since the program began in 1972 with the primary goal being to prove the concept that kelp could be farmed. N. M. I. produced the first (and only) yield data in 1982-3, having joined the project in 1980. Our assumption was that the plant was exceedingly complex, and that it would be difficult to cultivate. We have found that marine farming must be built on a sound program of hydrodynamic measurements, and that this is most certainly a high-technology task.

As the illustrations on the following pages demonstrate (Figures 4 & 5), there is a general lack of knowledge in the U. S. about marine farming. For example, the Washington Post cartoon, makes fun of the loss of 100 kelp plants on an early test farm (Figure 6). No one bothered to find out that mature kelp plants are ephemeral, even though they are large and tree-like. Nearly one-third of all the plants in local beds are lost to storms and grazing damage in a single year (N. M. I., unpublished data). The loss of all plants in an experimental planting is not unusual. Nonetheless, this illustration alone caused great damage to the program.
Figure 2. Carbon dioxide fixation by macroalgae is shown here, compared with the amounts fixed by plankton, and land vegetation. It is clear that natural macroalgal fixation is substantial, and that the use of macroalgal biomass for energy would not add to the alarming increase in carbon dioxide in the atmosphere, attributed to the burning of fossil fuels. (From S.V. Smith)

Fig. 2. Primary production, biomass, and turnover time for carbon in the biosphere, adapted from (9). Letters A to N (italic) indicate terrestrial ecosystems and remaining letters O to S (roman) indicate marine ecosystems: A is tropical rain forest; B is tropical seasonal forest; C is temperate evergreen forest; D is temperate deciduous forest; E is boreal forest; F is woodland and shrubland; G is savanna; H is temperate grassland; I is tundra and alpine meadow; J is desert scrub; K is rock, ice, and sand; L is cultivated land; M is swamp and marsh; N is lake and stream; O is open ocean; P is upwelling zones; Q is continental shelf; R is seafloor and reef, and S is estuaries. The cumulative fossil carbon input and the input rates (?) are shown as a dashed line. Since the source is not constant in size or input rate, the line represents a locus of points through time.
The 4-H Frogmen

Kelp is a form of seaweed native to colder ocean waters and has been cultivated for centuries by the Japanese as a basic item of their diet. It is also used on a small scale in the West, providing a chemical binder that appears in paints, toothpastes and such foodstuffs as ice cream and salad dressing. Now, a group of marine researchers in southern California is studying the feasibility of large-scale farming of a local giant kelp for an entirely different purpose—an economical new source of energy.

The kelp in question, a plant known technically as Macrocystis pyrifera, has a twin appeal for energy experts. First, it can absorb nutrients from the sea throughout its entire surface area, and thus it is one of the world's fastest-growing plants. Typically, the giant kelp can grow at a rate of 2 feet a day until it attains an average length of 60 to 100 feet. Just as important, the plant is a rich source of organic materials, and therefore can be readily converted to methane gas, by the action of bacteria, or to petroleumlike compounds, by a simple heating process.

The California kelp farmers are a team from the Naval Underseas Center in San Diego, headed by engineering physicist Howard Wilcox. They have begun their farm as a rectangular grid of 2-inch-thick ropes anchored 40 feet beneath the surface in the 300-foot waters off a small island 60 miles west of San Diego. Since they completed assembly of the framework in May, Navy frogmen have been attaching transplants of the giant kelp to it, at intervals of 10 feet along the ropes. The plants are anchored to the grid by tiny rootlike growths known as holdfasts, which normally serve to attach the plants to convenient rocks; the frogmen thread the holdfasts through the rope with large needles, and then tie them securely. "As long as you tie or anchor the kelp somewhere, it'll grow," notes Wheeler North, a marine biologist from Caltech who developed the transplantation technique.

West Coast kelp farm: A fast-growing new source of economical energy?

Eat: Now, about 100 plants are in place. And North plans to make regular dives to check on the kelp's growth. The kelp farmers' main worries are that disease might develop among the plants, that such fish as Catalina perch may eat the growing kelp, and that the waters around the framework may not contain enough nutrients to support the plants' high growth rate.

But if the present 7-acre experimental farm is as successful as it presently appears, the next step will be the planting of two 1,000-acre farms in the deep waters of the Atlantic and Pacific oceans. By 1985, Wilcox thinks, a 100,000-acre kelp farm might be providing enough energy to power an American city of 50,000 residents. Perhaps the biggest advantage of this new approach to the energy problem is its basic simplicity. "It's not high technology," says Wilcox. "We're just talking about plain old plants growing."
Figure 4. Prior to the establishment of the Gas Research Institute, contributions to the Marine Biomass Program were made by the American Gas Association, whose "artists impression" of a kelp farm illustrates several "hydrodynamic" misconceptions (note the diver swimming with a plant showing no buoyancy). Unfortunately this lack of appreciation for moving water, and it's effects on a large buoyant plant, like Macrocystis, was not limited to the artists associated with the program.
That's not an oil rig or a sunken ship you're looking at. It's a man-made underwater farm located five miles off the coast of California. Here General Electric is working with the Department of Energy and the Gas Research Institute to grow energy.

The actual crop is the giant kelp, a kind of seaweed. Kelp, believe it or not, can be processed into a gas like natural gas. Kelp grows naturally at a fantastic rate, as much as two feet some days. On this experimental farm, GE is trying to make it grow that much every day. If that can be done, ten acres of kelp could be harvested and turned into as much as six million cubic feet of gas a year. And someday, with more and bigger farms, kelp might make a dent in America's energy problems.

This energy farm is one example of how General Electric is applying many different technologies to help solve problems that face us all: in medicine, transportation, pollution control, as well as in energy.

Because that's how progress for people happens.

Progress for People

Figure 5. The General Electric Farm Illustration and accompanying text suggests that G.E. was "growing energy in the sea" and shows an actual photograph of the farm, with plants attached (and already tangled with, and presumably abrading against the farm structure). No production data was obtained, which unfortunately is not how "progress for people" happens.
The $1.2 Million Seaweed Saga, and Other Ideas

By Jerry Knight

When General Electric Co. asked the Department of Energy for $1.2 million to grow seaweed in the Pacific Ocean and try to extract energy from it, DOE scientists were dubious.

It may be possible to get energy from seaweed, they said, but it would be better to try growing the weed "under well-controlled conditions...on land-based aquatic test sites."

But Energy Department higher-ups approved the project anyway, and General Electric in December 1978 carefully transplanted 100 kelp plants onto a quarter acre of Pacific Ocean floor.

Within two months, all the kelp was gone.

The kelp case was just one of 10 exotic energy research projects criticized in an internal DOE report accusing the agency of wasting millions of dollars on studies with little promise of solving the nation's energy problems.

The department ignored the recommendations of its own evaluators and gave grants to projects the experts said were a waste of money, passed out funds without evaluating other applications, and paid for "research" on equipment that could be bought right off the shelf, the report says.

The department's inspector general looked at the performance of only one small office—the Biomass Energy Systems Program—and concluded the whole DOE research funding systems needs to be tightened up.

Energy officials blame the biomass boondoggles on growing pains in a research budget that has jumped from $400,000 to $56 million in four years. The system had already been tightened up—and several staff members transferred—by the time the inspector general's report was prepared, they insist.

But by then millions of dollars had been spent.

The seaweed scientists not only had trouble keeping track of their kelp, they also apparently got their money for the project from the government when private funds were readily available.

The idea was to turn the seaweed into a gas that could replace natural gas. The Federal Energy Regulatory Commission allows gas companies to spend money collected from customers on such projects.

But DOE grant-givers decided to spend the taxpayers' money, without determining that their colleagues at FERC had already told the gas companies to pay for the project themselves.

This Washington Post Cartoon had an indelible, and unfortunately adverse, effect on marine biomass funding, labeling the project as "the kelp calamity" and describing differences of opinion within DOE at the time. It is interesting to recall that the Washington press ridiculed Langly's efforts to fly his amphibious "aerodrome" off the Potomac, and a Congressional investigation was threatened, since he was also accused of wasting public money trying to fly. That press release was in 1903, nine days before the Wright Brothers' first flight at Kitty Hawk.

Figure 6.
After several farm losses, General Electric decided to call in the "experts" and invited phycologists from Canada and elsewhere in the U. S. to a seminar, held in Goleta. As noted earlier, the consensus at the seminar was hemidome and Druehl-cylinder experiments should be conducted. Thus, even though the scientific "community" was asked for advice, in retrospect this advice turned out to be bad.

As a result of the G. E./C. I. T. farm failures there was much negative publicity and pressure to shut the program down. D. O. E./S. E. R. I. withdrew support in 1981. It was only through the valiant efforts of several Program Managers at G. R. I. that the program was kept alive past 1981, though it cost some of them their jobs. In 1982 advisors from several major gas companies insisted that the program be stopped. Funding was phased out through 1986.

This is just when N. M. I. was gathering the data and facilities which could have proven that the kelp biomass farming concept could work. Yield data was collected that showed that the nearshore kelp farm strategy was viable. A 1,000-acre kelp forest was leased (by N. M. I.) from the State of California that could form the basis for a pilot farm. Kelp harvesting and research boats were in the water and ready for use. A talented and trained staff was ready to move forward with the program. The interest of the Southern California Gas Company had been engaged. This is when an alternative route was pursued, that of focusing attention on longer term kelp genetics goals. The final blow to the U. S. Marine Biomass Program was dealt by the Solar Energy Research Program, in response to criticisms made by the Department of Energy. S. E. R. I. had proposed to continue the program, but in late 1986 all D. O. E. funding via S. E. R. I. was focused on microalgal, not macroalgal, energy production.

In spite of the fact that the program has now been stopped, it is possible to say that the Wilcox projection of 2% efficiency is not entirely out of line (our calculations from actual farm tests, show values around 1%). It is possible to say that giant kelps have an exceedingly high rate of biomass production (ca 15 dry ash-free tons per acre per year), and that the plants can withstand quarterly harvests where nearly half of their biomass is removed, so that substantial yields are possible without changing the standing crop. The experimental farming also provided valuable information about the chemical changes in cultivated plants under light stress (where they were planted close together) and for the first time gave us information on frond initiation rates, as these relate to whole plant growth.

One of the final studies done by General Electric was a review of co-products and by-products of kelp. As with the prior G. E. economic evaluation work, nothing innovative was produced. Indeed, the list of products that they considered was shorter than that produced by Hercules Chemical Company in 1919, when they were considering the options of continuing their harvesting activity. Valuable co- and by-products beyond those mentioned by G. E. justify further work on the farming of macrophytes in the sea.

The G. R. I. Marine Biomass program was constrained in various ways. At first, nearshore work was not felt to be worthwhile, since there was not enough area to grow a huge amount of biomass and not within the Wilcox concept. Later, the work was expanded to include nearshore concepts. Work on alcohol and by-product development was not emphasized since biogas was the focus of the project. Many small projects were funded, where no field work was done. The resulting reports, such as the Batelle marine farm engineering study, were often very interesting, but these "dry" studies were not translated into in-the-sea work, where their practical value could be tested. Nonetheless, this broad approach was useful in that the need to explain our research to outside reviewers gave us many opportunities to produce "review" documents. The following section of this report is such a review document and deals in general terms with macroalgal farming and products.
III. MACROALGAL CULTIVATION AND GENETICS

A. MARINE FARMING

The farms on land that now produce our food were originally deciduous forests and grasslands. The transformation of these natural ecosystems into productive farms involved clearing the land and cultivating it and introducing new crop plants. The natural fertility of the original ecosystems was enhanced by crop rotation or the application of fertilizer, and eventually the crop plants themselves were modified to increase the size and quality of the harvest. The forests and grasslands of the sea are still unmodified ecosystems in most parts of the world. One of the challenges facing our generation is to devise methods for cultivating and harvesting the open ocean (Neushul, 1959, 1984). The transformation of nearshore marine ecosystems into productive farms has already begun.

Nearshore Kelp Cultivation

Nearshore kelp cultivation, over the last four decades, has progressed through the experimental stage and is now a commercially viable industry both in China (Tseng and Wu, 1962) and in Japan (Kawashima, 1984). The red alga, Porphyra, is extensively cultivated as a food in Japan and Korea, while a tropical red alga, Eucheuma, is grown commercially in the Philippines. The annual value of all these brown and red macroalgal crops grown in nearshore waters at present falls between $871 million and one billion U.S. dollars, (Doty, 1982; Tseng 1981a,b). In contrast, offshore farming of macroalgae has only recently been attempted, and as yet the open sea remains uncultivated, even though large masses of floating brown algae often grow there naturally, as in the Sargasso Sea.

Nearshore commercial macroalgal farms presently cover thousands of acres of sea surface in Asia. They are generally made of rope, nets, floats and other fittings anchored to the sea floor. The Japanese government, working with their private industries, plans to provide funds to develop farms specifically to produce energy. They have also funded the large-scale installation of artificial reefs that enhance both plant and animal life in nearshore waters (Mottet 1982). Ishikawa-Harima Heavy Industries Corporation of Japan has recently embarked on an ambitious fifteen-year project to grow kelp for methane production near Uchiura Bay on the Island of Hokkaido (The Economist, October 19, 1985; Honja, personal communication: Figures 7, 8 & 9).

There are no commercial-scale macroalgal farms in U.S. waters, even though substantial quantities of macroalgae are harvested from natural kelp beds every year and are used for the production of alginates. With the exception of the 1980-1983 El Nino period, when harvests of kelp from natural beds plummeted (from 150,000 to 4,000 tons), annual harvests of around 150,000 tons of wet Macrocystis had been common since mid-century. Nearly 400,000 wet tons per year were harvested from the southern Californian kelp beds between 1917 and 1918 (see P. Neushul, Appendix B). The Chinese now harvest around one million wet tons of cultivated Laminaria yearly from their nearshore Laminaria farms.
Figure 7. Japanese farms, like the General Electric farm, are planned as anchored, brute-strength units, with nutrient-upwelling pumps to provide fertilizer.
Figure 8. Japanese farms, are planned to operate on a strict seasonal schedule where seedstock is produced, outplanted and harvested, mechanically, as illustrated here.
Figure 9. Japanese marine farm products and by-products are listed, and are remarkably broad, with no "programmatic restrictions" to the production of gas alone. Clearly commercial success is a central theme of this on-going project.
As might be expected, the size of the Californian kelp beds has fluctuated (Harger 1983), due to climatological factors and coastal pollution (see Chapter IV-B). Accordingly, there have been several successful efforts to thicken and extend the kelp beds, and to replace them in locations where they have been lost (Wilson and North 1983, North 1972, 1976, 1981).

The Giant Kelp, Macrocystis

The term "kelp" was first used in Scotland between about 1720 and 1830 as a name for seaweed ash. The word was later used to describe large brown algae generally, and today is given specifically to brown algae that are classified in the order Laminariales, since these plants produce large blades, or lamina. All kelps consist of three basic parts: a root-like holdfast, a stem-like stipe, and either a single leaf-like blade, or multiple blades. The smaller, simpler kelps consist only of these three basic parts, while the more complex kelps, like the giant kelp, form complex branched holdfasts, basal branching systems and many stipes and blades. Some of the largest kelps, like Macrocystis produce buoyant gas-filled floats. The largest giant kelp found to-date on the North American Pacific Coast was 43 meters (140ft) long (Frye, Rigg and Crandall, 1915). The leaf-like blades are 30-35cm (12-14 inches) long and 8-10cm (3-4 inches) wide, each having a pyriform float at its base. Stipe, blades and floats collectively form a fern-like frond, at the tip of which is either a falcate meristic mation point if the frond is still growing, or a terminal vesicle and blade if the frond is mature. The kelp frond is a determinate structure, in that like a fruit, such as an apple, it cannot grow indefinitely. The average life of a giant kelp frond is only 6 months, after which time it decomposes and is shed. The basal branches that form new fronds also give rise to dichotomously-branched, cylindrical, root-like haptera that grow downward. These form adherent tips, from which rhizoids grow to attach a given hapteron to a rock or other solid substrate. The haptera will grow down into sand or sediment for a short distance in places where there is no rock. Collectively, these haptera form a large clump, into which sediment and sand drifts and accumulates. The weight of this material serves to produce a heavy anchor which holds the plant in place on a sandy sea floor. Nearly 60% of the kelp beds in southern California grow on sandy, not rocky, sea floor, as was originally thought. The distribution of biomass in a kelp plant harvested from Goleta, California is shown in Figure 1.

Since the giant kelp, Macrocystis, grows its own holdfast-anchor, anchoring ropes (the stipes), and even produces its own buoys (the pneumatocysts), it duplicates in vegetable form some of the features found in man-made oriental kelp farms. Since the giant kelp is also perennial and very productive, it is widely recognized as a good candidate for both nearshore and offshore cultivation. Domestication and experimental cultivation of giant kelp has been attempted on the U.S. west coast where it grows naturally (Neushul and Harger, 1985) and in China, where it has recently been introduced.

Early (incorrect) theories about kelp reproduction held that the reproductive bodies of kelps were formed within the thick stipes. Thus it was rather surprising when Sauvageau (1915) discovered that kelps produced spores in sporangia that formed thickened fern-like sori on the surfaces of their blades, and that these spores grew not into another kelp plant but into microscopic,
filamentous plants. Even more surprising was the discovery in subsequent studies by Sauvageau and others that the gametophytes of all the kelps were remarkably similar, even though the sporophytes were vastly different in size and morphology. It was later found that the microscopic gametophytes had a haploid chromosome number, while the macroscopic sporophytes of kelps were diploid.

Haploid zoospores are produced following meiosis in the sporangia on the diploid sporophyte and are released into the water. Relatively little is known about the zoospores of *Macrocystis*, except that they are small, about 5 μM long, and short-lived (Amsler, personal communication 1985). They are not phototropic (Haxo, personal communication) but are photosynthetic and can be sensitive to too much light; they can be killed by exposure to five minutes in full sunlight (Luning and Neushul, 1978). They contain two plastids as well as reserve materials and "adhesion" vesicles (Chi and Neushul, 1972) and readily attach and recruit near parental sporophytes (Anderson and North, 1966). They are easily washed away by water motion, and sediment greatly reduces their attachment and survival rates (Devinny and Volse, 1978).

Spores swim, and settle into the boundary layer at the water-rock interface on the sea floor where they attach, growing to form either ephemeral or perhaps perennial male and female gametophytic plants, which in due course form eggs and sperm. Spores can attach to, and produce gametophytes that grow epiphytically on other plants. On a sandy sea floor they will grow on the tube worm, *Diopatra*, which provides a firm starting point for the young kelp plant that subsequently anchors itself in the sand. The gametophytic phase in the life history can take as little as two weeks, and a new spore-producing kelp plant can be formed in little over one year.

It has generally been assumed that the microscopic gametophytic phase is ephemeral and short-lived. In fact, we have found that while a few of the diploid giant kelp plants can survive for many years most are uprooted and cast ashore in one or two years. While it is still not possible to describe the field-grown gametophyte as they have seldom been found in the sea, we can assume, based on observation of outplanted gametophytes that most are indeed ephemeral (Deysher and Dean, 1984). However, experiments on gametophytes cultivated in the laboratory, suggests that perennial gametophytes may also occur in the sea. Studies of the natural recruitment patterns of the sea-palm *Postelsia* (Paine, 1979) suggest that perennial gametophytes are formed by this kelp species.

*Macrocystis* gametophytes require only low levels of light to survive (Fain and Murray, 1982; Deysher and Dean, 1984). About 260 μE/cm² blue light is required for the gametophytes of *Macrocystis* to produce eggs and spermatozooids in two weeks (Luning and Neushul, 1978). As with *Laminaria*, temperature and nutrients affect the amount of blue light required for gametogenesis. More light is required at higher temperatures (Luning, 1980) while higher nitrate concentration decreases the light required for gametogenesis (Hsiao and Druehl, 1971). High concentrations of iron stimulate gametogenesis while high concentrations of boron inhibit gamete formation (Motomura and Sakai, 1984). Muller and Maier (Msc.) have found that the female gametophytes of *Macrocystis*
produce a sexual hormone called lamoxirene, which influences antherozoid release and chemotaxis in some 26 other kelp species as well (Luning and Muller 1978; Muller, Gassmann and Luning, 1979).

An average adult sporophyte has 0.29 square meters of sporophyll area (Neushul, 1963) and the sporophylls produce about 20.7 million zoospores per minute per square meter of sporophyll area (Anderson and North, 1966). This means that an average adult produces 6 million zoospores per minute, about 9 billion zoospores per day and 16 trillion zoospores per average five year lifetime. Of course, most of these are lost. Half the surviving zoospores produce male and half female gametophytes, consequently only one zoospore in 6 trillion needs to successfully produce an adult sporophyte for the natural population to replace itself. This is a very high reproductive rate and a very low success rate.

Points in the life history of Macrocystis where the greatest losses occur, have been called "environmental turnstiles" by North, since environmental conditions determine how many kelps survive to pass through the turnstile between one life-history phase and the next (North, 1972). While it seems likely that the highest losses probably occur in the microscopic stages, a natural kelp bed can experience severe losses during storms or periods of nutrient drought. For example, a thousand acre kelp bed in Goleta Bay (California Fish and Game Bed #26) was completely destroyed during the El Nino storms of 1983-4. In order to compare mortality rates in populations of adult kelp sporophytes, a "half-life" has been defined as the time it takes to lose one half of a group of individuals of the same age, assuming a uniform logarithmic mortality rate (North, 1964). Half lives of plants vary with age, locality and season. Beds in more exposed locations have plants with half lives that range from 4.0 to 15.9 months (North, 1964; Rosenthal, Clarke and Dayton 1974), while beds in more protected areas have plants with half lives that range from 4.1 to 85.8 months (North, 1964; Coon, 1981a).

It has been possible to complete the kelp life history entirely in the laboratory by growing sporophytes to maturity in laboratory tanks, although this requires specialized marine culture facilities (Sanbonsuga and Neushul, 1975). Growing and measuring kelp sporophytes in the sea can be difficult, as an individual kelp plant can weight up to a ton, and since the many fronds tangle into a confusing, spaghetti-like stipe bundle which is almost impossible to disentangle. Consequently, experiments with kelp sporophytes usually focus on one or at best a few plants. In the few cases where larger numbers of plants have been studied, very significant plant-to-plant variation has been encountered. This variability makes it difficult to generalize from the results of experiments where only one, or a few plants have been measured. Nonetheless studies based on small samples provide the basis for most of our present knowledge of the physiology and environmental requirements of the plant (Wheeler and Neushul, 1981; Lobban and Wynne, 1981; Lobban, Harrison and Duncan, 1985).
B. PLANTING, CULTIVATION AND YIELD

KELP CULTIVATION

The task of culturing kelps involves growing both the microscopic gametophyte and the macroscopic sporophyte. Therefore, both laboratory and field cultivation methods must be employed.

Laboratory Cultivation and Seedstock Production

It is comparatively simple to isolate single spores and to grow filamentous gametophytes from them. The resulting plants can be vegetatively propagated for many years. Our oldest cultures have been maintained in the laboratory for ten years.

Gametophytes can be isolated in the laboratory using a simple technique developed by Sanbonsuga and Neushul (1978). A fertile sporophyll is collected, cleaned, folded in a moist paper towel and left in a refrigerator overnight. The next day, the undamaged, uncut parts of the blade are suspended in sterile seawater in a shallow dish, whilst the cut edges are kept out of the water, to prevent the release of mucilage from the damaged edges. The spores are allowed to release for 30 minutes and then the parental sporophyll is removed. The upper water layer is then decanted from the dish into a graduated cylinder which is placed in a refrigerator for five hours. During this time non-swimming spores and contaminating diatoms settle toward the bottom of the cylinder. After five hours, drops are pipetted from near the top of the graduated cylinder onto 50x50mm square pieces of glass cut from a microscope slide. The spores are given five minutes to attach to the slide and then a stream of water from a wash-bottle containing sterile seawater is sprayed over each glass square to wash away any unattached spores.

The glass squares with attached spores are then placed in a petri dish containing PESI medium (Provasoli's enriched seawater medium plus iodine, described in Tatewake, 1966). Five days later, after the spores have germinated to form dumbbell-shaped germlings, a drawn out pipette is used under a high-powered dissecting microscope, or the low-power objective of a compound binocular microscope, to dislodge and suck up individual germinated gametophytes from the glass pieces. Individual gametophytes are then transferred with a pipette to test tubes containing PESI where they grow vegetatively into a filamentous ball. If the clumps that grow in the test tubes are not perfectly round they are discarded as this indicates the presence of more than one gametophyte. The gametophytes can be maintained in a vegetative state and will not undergo gametogenesis if they are kept under red lights, limiting the amount of blue light that they receive (Luning and Neushul, 1978).

Spores can also be isolated by using a sterilized glass chromatography sprayer filled with a solution of spores. The spores are sprayed onto agar or into liquid culture medium and allowed to germinate and grow. Clumps of gametophytic branches arising from single spores are usually symmetrical, while those from two or more spores are assymetrical and are discarded.
As noted earlier, vegetatively growing gametophytes can be held for many years. The cultures are subcultured as necessary, or every two to four months, by breaking a few filaments from a ball and placing them in a culture tube containing PESI medium. The tubes are held in the following conditions: 15°C, 12:12 LD photoperiod, and a filtered red light intensity of 10 μE/m²·sec. Our laboratory now has a seedstock collection of over 800 distinct kelp gametophyte isolates from locations as far away as Japan, China, Mexico, Argentina, and Alaska.

If there were only four zoospores in each sporangium, instead of 32, each spore, because of the independent assortment and segregation of chromosomes, would be genotypically different from the others. In such a case, the analysis of genetic differences between spores from a single sporangium, would be similar to tetrad-analysis in the fungus Neurospora or the green alga Chlamydomonas. Although the process of separating the 32 spores from a single sporangium should not, theoretically, be particularly difficult, it has not yet been achieved. If mutagen-induced variants are to be identified as true mutants, then tetrad analysis is essential.

The mitotically produced eggs from a single female gametophyte and spermatozoids from a single male gametophyte should be (barring mutations) genetically identical. Herein lies the usefulness of isolating individual spores and growing cloned gametophytes from them. When gametogenesis is induced in one specific male clone and one specific female clone and the resulting eggs and sperm are mixed, many genetically identical progeny are produced and this crossing can be repeated over and over again. To produce a specific cross of one female with one male, clumps of each are fragmented together and placed in blue light (Neushul, 1983) or in a culture solution with a low salinity (Sanbonsuga and Neushul, 1978).

A culture collection of kelp gametophytes has some advantages over land plant tissue culture collections. It is a laborious process to produce haploio tissue cultures from anthers or pollen grains of land plants and such cultures tend to have a limited life-span. Also, if land plants are repeatedly subcultured, they are susceptible to somaclonal variation, genetic drift and mutations (see discussions in Gressel, 1984; Maliga, 1984). The gametophytic cultures of Macrocystis appear to have none of these disadvantages and the collection has been used to provide material for chromosomal studies in Japan (Yabu and Sanbonsuga, 1985), for studies of sex hormones (Muller & Maier, msc.), for studies of the effects of pollutants on kelp fertilization (Steele, pers. comm.), and for studies of the photosynthetic apparatus of kelps (Smith, pers. comm.). In addition, the cultures have been used to produce intergeneric and interspecific hybrids.

Another technique for propagating kelp sporophytes is by tissue culture. Callus tissue has been produced from meristematic tissue of Laminaria blades (Fries, 1980) and stipes (Saga and Sakai, 1983). But only Fang, Yan and Wang (1983) have reported the complete regeneration of a sporophyte from a callus. Others have reported the production of aposporous gametophytes, i.e. gametophytic branches growing out from sporophytic tissue without spores being produced (Fries, 1980; Lee, 1985).
Successful tissue culture of sporophyte callus or aposporous gametophytic filaments, depends upon the tissue being completely free of bacteria. A variety of methods have been used to clean macroalgal tissues including: antibiotics (Druehl and Hsiao, 1969); ethanol (Fries, 1980); untrasound (Polne, Gibor and Neushul, 1981); Betadine (Gibor et al., 1981) and sodium hypochlorite (Lee, 1985). Bacteria-free cultures of Macrocystis gametophytes have been successfully grown, although the sporophytes produced from them were irregular in morphology and short-lived (Zarmouh, 1985), which suggests that surface bacteria produce growth hormones that in turn influence the development of the young sporophyte.

The easiest way to produce kelp sporophytes in quantity for outplanting is sexually, using either known gametophytic lines or by obtaining spores. After grinding up and mixing a specific male and female gametophyte, gametogenesis and fertilization takes place and young sporophytes are formed. When these plantlets are visible to the eye, they are transferred to "bubble flasks" where filtered air is pumped through a glass pipette with its tip resting on the bottom of the flask. Air bubbles up through, and mixes with, the culture medium. Macrocystis sporophytes grow with a crisp, rigid blade at 12°C and below, but form a lax, flexible blade at 15°C and above. The plants float free in the bubble flasks until they are 2 to 3 cm long (Sanbonsuga and Neushul, 1978).

The plants are then transferred to greenhouse tanks with flowing seawater (Sanbonsuga and Neushul, 1978) until they reach 10 cm or more in size (Charters and Neushul, 1979; Devinny and Leventhal, 1979). They are then outplanted into the sea, by inserting the holdfast into the lay of a rope (Harger and Neushul, 1982). The time in the greenhouse strengthens the plants and increases their survival rates when they are eventually transferred to the sea. The plants can be held in the greenhouse for long periods if the available light is greatly reduced.

The commercial method for producing Laminaria sporophytes for outplanting in the sea works well with Macrocystis. Sporophytes are grown from a population of gametophytes, each arising from a single spore. As noted earlier, the giant kelp produces prodigious numbers of spores (North and Neushul, 1968; Jackson and North, 1973). Zoospores can be released either by the gentle overnight treatment of sporophylls in paper towels, or by grinding whole sporophylls, and then spraying this mixture of tissue and sporangia onto a suitable substrate. The Japanese have developed a smooth woven "Kuremona" string which is especially useful as a substrate for spore attachment. The Chinese use twisted palm fiber that has been singed to remove excess small fibers for the same purpose. The strings are held for a month in incubator tanks during which time the spores germinate, the gametophytes mature, and gametogenesis and fertilization take place. The strings are then transferred to a seawater supplied greenhouse where water temperatures are controlled, and the plants are "hardened." Juvenile sporophytes are then ready for outplanting.
In-the-Sea Planting and Cultivation

The selection of an optimum site for kelp cultivation in the sea is of primary importance. Proper site selection depends on a combination of laboratory information, field measurements and, if possible, information produced by experimental test farming (Harger and Neushul, 1982). The most important parameters to be considered by the mariculturist are: ocean light levels, temperature levels, nutrient concentrations, water motion, sedimentation, fish predation, and the interaction and variation of these factors with season.

One of the first attempts to compare different coastal farm sites in Japan was made by Matsumoto (1959). This study is exemplary in that the levels of light, temperature and water motion necessary for the optimum growth of Porphyra were first measured in the laboratory, and these measurements were then used to evaluate different coastal sites and to select an optimum site for commercial cultivation.

The evaluation of sites for kelp culture in California involved a three stage test-outplanting process (Neushul, 1981). First, kelp sporelings were produced in the laboratory and hardened in a marine greenhouse. Then they were outplanted to three sites in the sea. Finally, the growth and survival of these outplants in different environments were compared. The outplanting sites were either rocky or sandy, and exposed to or protected from waves. The best survival was in well-lighted, rocky areas. Since plants growing in California in near-shore farm sites almost always suffer from some degree of nutrient "drought", attempts have been made to provide fertilizers, which when successful have greatly augmented plant growth (Neushul, 1982).

Juvenile kelps that are grown in a greenhouse and then outplanted to ocean sites are particularly susceptible to storm damage and loss until they develop a holdfast and become firmly attached to a substrate (Harger and Neushul, 1983). If the outplanting site is near a pier, platform or similar man-made structure, the fish populations that thrive there often eat the juveniles before they get established. Several species of fish have been observed feeding on kelp juveniles, including: surfperch (Phanerodon furcatus and Hyperprosopon argenteum), kelp bass (Paralabrax clathratus), halfmoon (Medialuna californiensis) and opaleye (Girella nigricans). In natural stands, juvenile kelps growing among the turf-like algae, Ectocarpus and Giffordia, are protected from grazing fishes (Ebeling, personal communication).

The growth requirements of juvenile kelps have been studied extensively. Given an adequate supply of nitrogen (Wheeler and North, 1980), light (Shivji, 1985), and optimum temperature conditions (Dean and Jacobsen, 1984), and given an absence of predators, kelp juveniles grow in excess of 10% day (wet weight). Juvenile kelp plants have been grown very effectively on rope structures near the sea surface at higher than recommended light levels (North and Neushul, 1968; Wheeler, Neushul and Harger, 1981). When the juvenile kelp reached a four- to eight-frond size, the holdfasts increased rapidly in size and the plants were transferred to a site near, and eventually on the sea floor.
Several techniques have been developed for planting adult giant kelp plants on rocky ocean bottom. North used a long knitting needle attached to twine to penetrate the holdfast and attach it to a buoy or line, in effect sewing the plant to a support structure. Medium-sized adult kelp plants have been successfully planted on the sea floor by attaching the holdfast to a rock (North and Neushul, 1968; Wilson and North, 1983). Wilson and McPeak (1983) cut off Pterygophora or Eisenia plants and then attached juvenile kelps to the stumps with rubber bands.

Attaching kelp to a sandy bottom requires different techniques. Neushul and Harger (1985) have used nylon mesh bags filled with gravel as anchors for 25-frond kelp plants. These anchors with the kelp plants attached were rolled over the side of a boat and sank to the ocean floor, thereby "planting" the kelp. The bags soon accumulate drift sand and silt and become firm anchors. If the transplanted kelp is dislodged or dies, juvenile kelps will develop on the gravel-filled bag to replace it.

Another propagation method first employed by W. J. North is called "broadcast seeding." Here zoospores, gametophytes or young sporophytes are cultured in mass and poured into a pipe extending from the sea surface to the sea floor. The masses of gametophytes with attached sporophytes are spread by a diver moving the end of the pipe over the sea floor (North, 1972). Results from the broadcast, mass-cultured plants have varied, ranging from complete failure to the appearance of excellent stands of juvenile plants in the months following dispersal (North 1972, 1976, 1981; Wilson and North, 1983). However, it has been very difficult to prove that the plants that do grow successfully are the same ones that were originally broadcast.

Kelp Yield

The potential for high growth rates in benthic marine macroalgae was suggested many years ago by Ryther (1959), but few actual production measurements have been made to verify this. Intensive confined culture in tanks on land has produced 31 dry gm/m²-d for Gracilaria (Ryther et al., 1979) and 16 dry gm/m²-d for Chondrus (Craigie, 1985). Natural populations of Laminaria off Nova Scotia produced 25 dry gm/m²-d (Mann, 1973). Natural populations of the Californian giant kelp, Macrocystis pyrifera, are known to produce up to 15 dry gm/m²-d (Neushul Mariculture Incorporated, 1980).

Theoretical Yield Estimates

Wilcox (1972, 1979) calculated that an open-ocean kelp farm could yield as much as 30 to 60 dry gm/m²-d. However, these optimistic production estimates did not go unquestioned. Wilcox had no physiological or production data to support his estimates and he did not consider the environmental growth requirements nor the patterns of carbon assimilation and translocation that occur in the plant. Nonetheless, great interest in marine biomass production for energy was generated by the high rates of production estimated by Ryther and Wilcox, and kelp yield studies were supported, beginning in the 1970's, by the U.S. Navy, the Energy Research and Development Administration (later re-named the Department of Energy), the National Science Foundation, American Gas Association, the Gas Research Institute and the Solar Energy Research
Institute. The early work was supervised by Howard Wilcox at the Naval Undersea Center. Scientists were employed at the California Institute of Technology by General Electric Company and Global Marine Development Incorporated to work in a "Marine Biomass Program." However, since no yield measurements were made to verify Wilcox's rather optimistic growth yield rates the program was reviewed critically. Ashare et al. (1978), working on a report for the Department of Energy, said that "in every respect open-ocean farming of aquatic biomass does not merit further development." Knight (1980) quoted an internal Department of Energy report that said "the agency was wasting millions of dollars on studies with little promise of solving the nation's energy problems." Goldman (quoted in Budiansky, 1980) saw the "tremendous mismanagement of a program and a tremendous waste of money... There is not one piece of data on anything done in the ocean. I don't think we've learned anything." Smil (1983) went as far as to say that the program was based on a "sci-fi tale of kelp-run civilization."

A very important aspect of any marine farm production estimate is the relationship between the original planted biomass density and the ultimate yield. Macroalgae grow at reduced rates with increased density in tanks (Ryther et al., 1979). This response is probably due to self-shading and competition for light and other resources in a limited space. However, natural populations of macroalgae growing in the sea have shown the opposite response, with higher growth rates seen at higher densities (Schiel and Choat, 1980). The causes for this response are not yet clear. The planting densities in both cases were similar, but the depth of water was obviously greater for the natural populations than those grown in tanks.

Extrapolated Yield Estimates

Several workers have based yield estimates on extrapolations from physiological or growth measurements made on parts of kelp plants, or on measurements of one or two plants. These include: the use of cut disks for photosynthetic measurements (Clendenning, 1964; Wheeler, 1980); using whole blades for photosynthetic measurements (Arnold and Manley, 1985); measuring radioactive carbon assimilation (Towle and Pearse, 1973; Lobban, 1978) and measuring frond elongation (North, 1971; Coon, 1981b; Gerard, 1982). Results from these approaches have been conflicting. Disk-cutting and even the removal of whole blades produce wounding effects (Arnold and Manley, 1985). These authors suggest that the kelp plant has "intrinsic variability in PS (Photosynthesis) and R (Respiration)" among its parts, consequently it is very difficult to take an adequate sample for an accurate estimate of whole-plant kelp productivity. The general pattern of decreasing frond elongation rate, on a percent basis, with increasing size has been used to establish and compare relative growth rates and estimate total plant growth rate (North, 1971). However, in fitting the curve, the "slow growing" fronds are not considered (North, 1972), making the measurements a biased indicator of the optimum and not the mean of the actual population that can be used for statistics, estimates and projections. There is also a major unresolved question as to whether all fronds grow at the same rate.
Some studies show that all fronds grow at the same rate during each fast- or slow-growth period (e.g., Gerard, 1982), while in another study measurements of individual tagged fronds pulled out of the stipe bundle underwater showed that there was no overall synchrony in frond growth (Coon, 1981b). Instead, it has been found that some fronds grew faster at a given time while others grew more slowly. Later, the same slow-growing fronds were found to be transformed into fast growing ones and vice-versa. The only pattern that seemed to emerge was that there was a symmetry of growth, where fronds on one side of a basal branching system seemed matched in growth rate responses to matching fronds on the other side of the plant.

Yield From A Coastal Test Farm

In 1981, a coastal test farm was planted in Goleta (Neushul and Harger, 1985) to supply the yield information that was lacking. Instead of estimates of yield based on extrapolation, or on the measurements of a few plants, the aim was to take measurements from a reasonable number of plants grown in the sea. A total of 722 plants having 25 fronds each were transplanted from nearby kelp beds into two plots, using the gravel-filled bag planting method mentioned earlier (Figure 10). One experimental plot was fertilized, while the other was not. Each plot had subsections planted at three different densities: 1 plant/square meter, 1 plant/4 square meters and 1 plant/16 square meters. As might be expected, plants at the highest density produced the highest yield per unit area, an annual average of 7 gm/m²-day (Neushul and Harger, 1985). However, these plants decreased in size as the experiment progressed. The rate at which new fronds were produced was much lower than that measured for plants in less-dense, and hence less-shaded, plantings. Plants at the lowest density produced the most biomass per plant, about 350 wet gm/day. Some individual plants produced much more than this average (see Figure 11). If all the plants were high-yielding, then project yields would be much greater than those we measured. Also, fertilizing the farm increased production during the low-nutrient slow-growth period in late summer. Production was highest in spring, following the natural upwelling of nutrients and increasing day length. This test showed how much kelp could be harvested on a quarterly basis from a coastal test farm, and that the estimates of Ryther and Wilcox were not unrealistic. The test plots planted in 1981 are still supporting substantial amounts of biomass today some five years later, although the plots have not been cultivated, thinned or harvested since 1984.

Fertilizers

One of the most important innovations introduced by the Chinese, in the development of their extensive nearshore kelp farms, was the application of fertilizer. The Chinese first applied fertilizers by putting them in porous clay pots attached to the wickerwork holders on which plants were planted. In California perforated plastic pipes were used to apply fertilizer (Harger and Neushul, 1982) and on a small scale plants were periodically taken from planting lines and dipped in nutrient-enriched water.
Figure 10. A perspective view of the planted farm. This shows the chain and rope attachments for the gravel-bag plant anchors with attached plants that formed a surface canopy.

Figure 11. 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.
On a larger scale, liquid fertilizer has been applied by spraying it from a helicopter (North, Gerard and McPeak, 1981) or small boats (Tseng 1981a; Neushul and Harger, 1985). The practice of applying fertilizer in pellet form was not as effective, since the pellets tend to get trapped in the horizontally-positioned blades of the kelp for long enough to burn holes in them. In China fertilizer application has been very effective when the area of kelp cultivation is large, and the water mass is calm (Tseng and Wu, 1962), since the fertilized water remained in the farm long enough for the plants to effectively absorb the nutrients. The residence time of water in natural kelp beds has been found to be a matter of days (Jackson and Winant, 1983), suggesting that large-scale fertilizer application in kelp beds would be effective.

Diseases, Epiphytes and Grazing

Kelp diseases, which have been attributed to physiological factors or pathogenic organisms by Tseng (1981a) or to bacterial or fungal infection by Goff and Glasgow (1980) can affect kelps grown in marginal environments under crowded conditions. Kelp diseases have been categorized as black, white and green rot. Black rot in Macrocystis occurs at high temperatures (Scotten, 1971). White dot disease which spreads rapidly from plant to plant often occurs when nutrient levels are low and temperatures are high in greenhouse tanks. Tseng has found that increased light and nutrient levels often cure green and white rot in Laminaria (1981a). To eradicate white dot disease completely in a recirculating greenhouse culture system, it is usually necessary to shut down the system, fill it with freshwater and chlorine and then refill it with seawater only after the tank has been thoroughly cleaned.

Epiphytes and competitors can affect both young and old kelps. Very young kelps must compete with many other algae in order to grow. Cultivation conditions are usually managed to favor the growth of kelp over that of the competing weeds. In old kelps, epiphytization by bryozoans can be a problem and can eventually sink fronds. This is especially true during late summer when growth conditions are sub-optimal.

Grazers can be a problem for large and small kelps. Areas where there are high concentrations of fish and sea urchins should be avoided, as these can decimate a kelp plant or an entire bed (Bernstein and Jung, 1981). Wilson and North (1983) describe methods used to control grazing sea urchins, including crushing with hammers, suction dredges, quicklime and commercial fishing. North has observed that the presence of sea lions, which feed on kelp-grazing fish, seems to help.

Harvesting

There is considerable controversy as to whether harvesting natural kelp beds has a detrimental effect. North (1968) and Coon (1981a) were unable to discern any detrimental impact of harvesting on natural kelp beds that they studied. Coon and Roland (1980) showed a reduced growth rate at one time and not at another. Barilotti, McPeak and Dayton (1985) could find no difference in hapteral elongation and branching, while McCleneghan and Houk (1985) found
harvested plants had reduced hapteral division and growth rates. It is our opinion that cutting the canopy off of a kelp plant has a negative impact on the future growth potential of that plant, simply because it reduces the translocation of photosynthate down to the basal branches. Nonetheless, the removal of a dense surface canopy would allow more light to penetrate, thereby (possibly) stimulating photosynthesis and growth of sub-surface fronds. However, in our studies of harvested yield (Neushul and Harger, 1985), plants at the low density grew in one year from having an average of 25 to an average of 40 fronds and having a much larger holdfast. These plants were harvested every three months, with each harvest removing as much as one-third of the total biomass present. Thus it seems clear that kelp plants in a spaced-planting configuration, where adequate light penetrates into the sea, have an enormous production capability even when they are heavily harvested.
The late T. C. Fang and his co-workers were the first to ask whether the strains of Laminaria cultivated near Qingdao might be replaced with fast-growing, heat-tolerant, high-iodine-yielding strains (Fang, et al., 1962, 1963). He collected plants with these preferred characteristics to use as spore donors in the large refrigerated greenhouses that are used in China for seedstock-production. This first attempt to genetically improve a macroalgal crop plant was successful although several problems were encountered. For example, the Chinese were unable to cross isolated gametophytic strains because of male sterility in Laminaria and as a result had to select from diploid plants that came from mixed populations of genetically diverse spores. Nonetheless, this mass-selection approach, made possible by the large labor force available to work on the project and substantial government funding over a period of many years, resulted in the production of new high-yielding, high-iodine containing strains that can tolerate high water temperatures. Californian kelps are not male-sterile, and so a different approach was taken in the present study.

THE SPECIES OF MACROCYSTIS IN CALIFORNIA

In California, three species of Macrocystis are recognized (Neushul 1971a): Macrocystis pyrifera (L.) C. Agardh; Macrocystis angustifolia Bory and Macrocystis integrifolia Bory. These are distinguished by different holdfast characteristics. The holdfast of Macrocystis pyrifera has haptera which grow from the base of the primary stipe below the basal dichotomy. The holdfast of Macrocystis angustifolia has terete or slightly flattened rhizomatous branches (the basal dichotomy) with haptera arising from all sides. The holdfast of Macrocystis integrifolia has rhizomatous branches that are flattened with haptera at the edges. Macrocystis pyrifera and Macrocystis angustifolia in California are considered to be conspecific by Nicholson (1976) while Brostoff (1977) recommended that Macrocystis angustifolia be recognized as a form of Macrocystis pyrifera. The question of whether or not these species are interfertile was not addressed by these authors.

Hybridization

Kelps are particularly responsive to growing conditions in the sea. Norton, Mathieson and Neushul (1981) drew attention to the extreme morphological plasticity of these and other seaweeds and the fact that different genera exhibit similar responses to light, water motion, temperature, salinity, chemical factors and damage. Mathieson, Norton and Neushul (1981) cited and discussed 48 instances of attempts to deliberately hybridize algae or to explain unusual morphological types found in the sea as interspecific or intergeneric hybrids. Twenty of these examples involved kelps.

The suggestion, that interspecific or even intergeneric kelp hybrids could occur naturally in the sea, was initially met with considerable skepticism, even though an unusual plant, intermediate in morphology between Macrocystis and Pelagophycus, was found in 1957 by the senior author and James Stewart while diving at 24m depth in the south fork of the La Jolla submarine canyon.
Subsequently, two other similar "intermediate" plants were found in mixed populations of the two possible parents (Neushul, 1971b). Putative interspecific kelp hybrids were described by Silva (1957) and Chapman (1961), but no attempt was made then to verify such hybridization experimentally.

Subsequently, Sanbonsuga and Neushul (1976) were able to produce intergeneric hybrids between Macrocystis and Pelagophycus in 22 out of 23 attempts, starting with vegetatively propagated gametophytes grown from single isolated spores from the two genera (Figure 12). The plants produced were unlike either parent, having two unusual, coiled pneumatocysts, and reaching only a few meters in length. When these hybrids were grown in greenhouse seawater system tanks, they matured and one plant ultimately produced sporangia that were sterile (Neushul, 1981). The laboratory-produced hybrids were very similar, if not identical to those found twenty years earlier in the sea. The presence of naturally-occurring kelp hybrids in substantial numbers has since been reported by Coyer and Zaugg-Haglund (1982), which further suggests that morphologically distinctive kelps may well be very closely related to one another. Coyer (personal communication) has recently found a fertile intergeneric hybrid that has produced viable spores, from which gametophytes have been isolated. He kindly provided sporangia from this plant, and single spores were hand-isolated. Clonal stocks of gametophytes from this "fertile intergeneric hybrid" are now ready for experimental hybridization tests.

The unusual intergeneric hybrids both found in the sea and produced in the laboratory may be just a first suggestion of what might be learned about gene expression when kelp genomes are manipulated and variously combined in the future. Hybrid sterility might be of considerable economic value if it is possible to introduce a giant float-bearing kelp to a new locality without fear that it could spread out of control.

Neushul (1981) showed that Dictyoneuropsis, a member of the Lessoniaceae that does not produce floats, could be hybridized with Pelagophycus, a float-bearing member. Reciprocal intergeneric crosses were strikingly different. The float-producing characteristic was seen only when the Pelagophycus egg was fertilized by the Dictyoneuropsis sperm. In addition, Dictyoneuropsis (female) x Pelagophycus (male) sporophytes were found to be rounded and "penny-shaped" while the Pelagophycus (female) x Dictyoneuropsis (male) sporophytes were not (Neushul, 1983). These findings suggest that maternal, cytoplasmic inheritance may well be a significant factor in kelp genetics. The implications of this strong maternal inheritance are not yet fully appreciated.

GENE EXPRESSION

Gene expression in kelps is not well understood, but the phenotypic plasticity of the sporophytes at least raises questions of interest to scientists and mariculturalists concerning the effects that environmental cues might have. The complex tissues of the kelps, and the presence of perennial and ephemeral organs, enables the plants to respond to environmental stress by becoming dormant, by accumulating pigments (Shivji, 1985) and by storing nutrients. In land plants, responses of this nature have been traced to the
Figure 12. The genetic manipulation of kelps starts with isolated, and vegetatively propagated gametophytes (panel 1), which can be propagated and grown on agar (2) or in liquid (3). An intergeneric hybrid between Macrocystis and Pelagophycus is shown in panel 4, illustrating the genetic compatibility of the genomes of these two very different kelps.
production of plant growth regulators: auxins, kinetins, gibberellins, and ethylene. Recent studies of land plant morphogenesis (Van et al., 1985) and gene expression (Razin and Riggs 1980, Dynan and Tjian 1985) provide insights that might be applicable to kelps.

Some marine algae have been examined for the presence and activity of plant growth regulators. Cytokinins have been found in the brown algae Sargassum (Mooney and Van Staden 1984) and Ecklonia (Featonby-Smith and Van Staden 1984). In both algae, the cytokinin concentration varied with season. Such seasonality could be a response to a number of seasonal cues. Among these, photoperiod seems one of the most likely because it is the most stable from year to year. Experiments on another species of Sargassum support the hypothesis that photoperiod influences the seasonal cycle of growth and senescence in this plant (Gellenbeck 1984).

Mr. R. Lewis, working with GRI-developed seedstock at U. C. S. B., has used a new enzyme immunoassay, based on a specific monoclonal antibody, to measure zeatin riboside (a cytokinin) in Sargassum muticum. The plant assayed contained 79 nm of this zeatin riboside per kilogram of fresh weight. Commercial seaweed extracts (claimed by the manufacturers to have growth stimulatory effects on plants) were also assayed and contained levels ranging from 7 to 21 nm/l. This is the first time that monoclonal antibodies have been used to measure and detect cytokinins in seaweeds and seaweed extracts. This suggests that commercially valuable agrichemicals might be profitable by-products of a macroalgal biomass farm.

**GENETIC MANIPULATION OF KELPS**

Several new methods for increasing the efficiency of genetically manipulating macroalgae, including: mutagenesis, spray seeding, cluster analysis, image analysis for characterization, spore packet isolation, and tetrad analysis, have been evaluated, and are discussed here.

**Mutagenesis**

Gametophytes obtained from spores, that were exposed to the mutagen ethyl methanesulfonate (EMS), were observed. All gametophytes appeared to grow normally, forming brown fuzzy balls in culture. Isolates, that were previously reported as being yellowish in color, attained a brown color after the medium was renewed. These isolates may not be able to grow well at low nutrient concentrations, which may explain the yellowish color observed earlier.

Gametophytes isolated from spores treated with EMS were crossed with untreated gametophytes to determine if any of these gametophytes have a mutation that prevented them from reproducing. Female gametophytes from untreated (control) spores and from those treated with EMS for 10, 20 and 60 minutes were combined with isolate Mi-S: Male 4 to determine if normal sporophytes would be produced. Male gametophytes were tested with isolate Mp-A: Female 3. Refer to Table 1 for a summary of the results.
Sporophytes were first visible 8 days after combining female and male gametophytes. However, the final results of the experiment were recorded after 30 days in order to ensure that reproduction had occurred, as crosses involving Mp-A: Female 3, which only produced very small sporophytes. The control cross between Mp-A: Female 3 and Mi-S: Male 4 had small sporophytes at 30 days, so small sporophytes were counted as normal in crosses with Mp-A: Female 3. All controls with the single isolates that were not crossed with other isolates had no sporophytes. The results of these test crosses are shown in Table 1.

Table 1. Results of crosses between EMS-treated gametophytes and normal gametophytes. The number of isolates of EMS-treated gametophytes that produced normal, small or no gametophytes is listed.

<table>
<thead>
<tr>
<th>EMS-Treatment</th>
<th>Females X Mi-S: Male 4</th>
<th>Males X Mp-A: Female 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 minutes</td>
<td>2 normal sporophytes</td>
<td>11 normal sporophytes</td>
</tr>
<tr>
<td>10 minutes</td>
<td>2 normal sporophytes</td>
<td>19 normal sporophytes</td>
</tr>
<tr>
<td></td>
<td>1 small sporophyte</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 no sporophytes</td>
<td></td>
</tr>
<tr>
<td>20 minutes</td>
<td>5 normal sporophytes</td>
<td>18 normal sporophytes</td>
</tr>
<tr>
<td>60 minutes</td>
<td>7 normal sporophytes</td>
<td>7 normal sporophytes</td>
</tr>
<tr>
<td></td>
<td>1 no sporophytes</td>
<td></td>
</tr>
</tbody>
</table>

Normal sporophytes were obtained in most of the crosses attempted in this experiment. Of particular interest are the isolates that formed small sporophytes or none at all. The female isolate that produced small sporophytes will be investigated further in order to quantify the size of the sporophytes and determine if they are actually smaller than normal. The presence of one or more mutations may prevent reproduction in those female and male isolates that do not produce any sporophytes.

Spray seeding

Spores were spray-seeded on agar plates. In spite of initial bacterial contamination on the plates, numerous gametophytic growths could be distinguished as single thick or thin filaments (the males are thin and females thick). The differentiation of the gametophytes into single thick or thin filaments, rather than into clusters of filaments of different sizes, suggests that each had arisen from a single spore and are clonal isolates. Dr. M. Stekoll, working at U. C. S. B. as a Visiting Professor, was able to isolate gametophytic strains from these sprayed spores and now has them growing in liquid medium.

Cluster analysis

A crop of wild-type, spore-initiated Macrocystis sporophytes was grown by Mr. D. Reed of the Marine Botany Group at U. C. S. B., on April 8, 1986. A total of 90 rock plates were seeded in the laboratory using sporophylls of
Macrocystis and Pterygophora collected from Naples Reef. Fifteen treatments were tested, using spore settling densities ranging from as low as 2.5 to as high as 601 spores per square millimeter. These were outplanted into the sea on April 9th.

Observations of these seeding probes were made in April, May and June during diving trips to Naples Reef. The first kelps were visible to the eye on June 9th. The test plates were returned to the laboratory on June 24th, and counts made on the 25th. Plants under 2cm in length were not identifiable as being either of the seeded genera, however the larger plants could be distinguished. From June 25th to July 21st the plants were grown in running seawater in a seawater-supplied greenhouse, so that they could develop further.

Twenty clusters of sporophytes were collected from the plates and photographed. There was a total of 111 plants in this sample. Video images were also made on July 21st. This collection serves as a wild-type, genetically diverse sample against which genetically-uniform crosses can be evaluated. The basic question is whether or not the great differences seen in this sample are due to ontogenetic drift in response to environmental conditions or to genetic diversity in the spores from which the parental gametophytic stages arose.

**Image Analysis**

Considerable progress was made during July in using the newly-purchased TARGA 14 computer image analysis system. The A.T. & T. Targa-16 (TARGA= Truevision Advanced Raster Graphics Adapter) image analysis system now operating at NMI has been attached to an HP Laser Jet printer (purchased by NMI) and has been used to "grab" an image from a video tape. Work on both the hardware and software for this new system is progressing well. It is now possible to zoom in on an image and visualize the individual color pixels. This will make it possible to analyze specific plants for color variation, since the TARGA-16 can recognize 32,716 colors. A total of 400K of memory is required to store a single image.

Clusters of kelp sporophytes were photographed and the same images were video-taped. From the tape, the computer was able to "grab" a specific image and reduce this to a digital format, of approximately 200 x 200 picture elements, or pixels. The areas of the kelp sporophytes were then measured by counting the pixels that they covered.

The next stage of the process is to carry out what is called a blob-analysis in order to break down and characterize each blob. Also, we want to measure the color of each pixel. With the assistance of Dr. Susan Hackwood and Mr. Laurent Bouchereau of the U. C. S. B. Center for Robotic Systems in Microelectronics, it has been possible to do both a blob-analysis and a color analysis, using their proprietary software. We now face the task of developing similar software ourselves, or obtaining it from U. C. S. B. At present the latter course seems to be beyond our present budget.

**Tetrad Analysis**

Sporangia of Macrocystis are produced on specialized blades called sporophylls. Each sporangium arises from a diploid cell that divides meiotically to form 4 haploid cells, each of which undergoes 3 mitoses to form
a total of 32 spores. Sporophylls were dissected in order to determine how these spores might be separated in order to follow the development of each of them.

In order to show that a specific characteristic of a gametophytic line such as low-iron requirement, weak-walled bulging cells, or a specific branch thickness or frequency, is a genotypic rather than simply a phenotypic character, it is important to show that this character is subjected to independent assortment and segregation during meiosis. This is usually done by showing that a given character appears in a specific ratio in the products of meiosis, using a process called tetrad analysis. While in some red and brown algae tetrads (with 4 spores) are formed, in the kelps the sporangium contains a multiple of 4 spores (32), each of which is 5-7um in size. The size and number complicates attempts to analyze genetic segregation, which as far as we know has never been accomplished via tetrad analysis with kelps.

Considerable effort was made in 1986 to develop simple but effective methods to isolate single sporangia for tetrad analysis. Several methods were tested. Viable sporangia were obtained by grinding sporophylls which were filtered through nylon mesh to remove any large fragments. After washing, the sporangium solution was sprayed onto hydrophobic plastic culture dishes (provided gratis by Falcon Plastics) using a sterilized chromatography sprayer.

The EMS trials indicated that spores are released in packets, and these packets could be seen when fresh sporophylls were sliced and examined under the microscope. An attempt was made to collect these spore packets by releasing them on prepared concentrations of soft calcium alginate gel and soft agar. The spores released onto the soft agar which was then cut in strips and sliced into sub-fractions. These were spread on a microscope slide and examined under the microscope which indicated that packets of spores had been released and were germinating. The initial spores became contaminated, but subsequently, when agar containing antibiotics was used rather than untreated agar, the spores did not become contaminated. The germinating spore clusters have not yet been successfully separated into a single spore cluster.

We have been able to grind up fertile sporangia, and by subsequent screening and filtering to produce a suspension of isolated single sporangia. These were sprayed with a chromatography sprayer onto hydrophobic plastic culture dishes. We found that single sporangia could be isolated in this way, and held in hanging drops (produced simply by inverting the dishes) for several weeks.

While working with ripe sporangia, we have found that hand-made sections through ripe sporangia can be mounted (without water) on a slide, so that one can look with a microscope at a large number of ripe sporangia. When water is added, spore release occurs. Careful microscopy has revealed that the spores are initially released in packets, much like those shown in Figure 1, page 4, of our February 1986 monthly technical report. The spores soon begin to swim and disperse. If we can interfere with spore movement, as for example by vinblastin treatment, it is possible that the spore packets will remain together and perhaps, like the sporangia themselves, can be isolated and spread on agar or plastic surfaces, for tetrad analysis.
One of the major objectives of the GRI-sponsored work was to see if mutants could be obtained using chemical mutagens, and if these could be raised and evaluated. We are very pleased to be able to report that gametophytic lines obtained after the treatment of sporangia with ethylmethane sulfonate (EMS), have been raised, grown into bulk cultures and then crossed. We have been able to produce sporophytes from these EMS-treated strains, which in gametophytic form do not differ greatly from normal un-modified gametophytes. However, the sporophytes produced are not normal, illustrating for the first time that unexpressed "sporophyte" genes carried by the gametophytic strains that we have isolated and have in culture, are modified, and when put in a sporophytic context, are expressed.

**Mutagenesis and Polyploidy**

Various mutagenic agents have been used experimentally on macroalgae (Fjeld and Lovlie 1976). Striking pigmentation variants were produced in red algae by Van der Meer (1977) using ethylmethylsulfonate (EMS). These variants were isolated and crossed, and genetic segregation demonstrated, showing that the variants were true mutants.

The nuclei and chromosomes of the kelps have seldom been studied. Kelp nuclei are ultrastructurally similar to those of other plants, but do show distinctive conformational changes depending on whether they are dormant or active (Neushul and Dahl 1972, Chi and Neushul 1972). The chromosomes of kelps are very small and difficult to count, which has led to some confusion as to the number of chromosomes in these plants. For example, Yabu and Sanbonsuga (1985) working with freshly collected and cultured material from Santa Barbara, found 30 chromosomes in the gametophytes of *Macrocystis angustifolia*. Sporophyte counts ranged from 30 to 60. They concluded that the haploid chromosome number was 32 and the diploid 64 in this species. The chromosome number for *Macrocystis integrifolia* had been given by Walker (1952) as n=16 and 2n=32, and by Cole (1968) as n=4 to 16 and 2n=28 to 32. Yabu and Sanbonsuga have suggested that *Macrocystis angustifolia* is a natural polyploid of *Macrocystis integrifolia*. However, this seems unlikely since Lewis, Neushul and Harger (1985) have been able to isolate ten female and ten male gametophytic lines from interspecific hybrids between *M. integrifolia* (2n=32 or 64) grown to maturity in the sea.

Fang and his co-workers (1983) used x-rays and ultraviolet light as mutagenic agents. C. Lin (personal communication) has recently exposed ripe kelp sporangia to ultraviolet light and has obtained striking reductions in spore germination numbers, presumably because of the mutagenic effect of such treatments. He also found photo-reversion after ultraviolet exposure. The late T.C. Fang (personal communication) found that the gametophytic strains of Laminaria, grown from UV-treated spores, are much more prone to develop sporophytes parthenogenically, than untreated control lines. These studies are not yet definitive, but do suggest that several mutagenic agents can be effectively used with kelps.

In 1986, it was finally possible to evaluate the gametophytes that had been grown from sporophylls exposed to the mutagen, ethyl-methane sulfonate (EMS). After exposure, spores were released and germinated, but germination rates were low, and most of the gametophytes produced died, particularly when long exposure times were used. Nonetheless a few did survive, were cloned and ultimately crossed. To our surprise, gametogenesis and fertilization took place...
and sporophytes were produced. Initially these were thought to be normal, but subsequent developmental abnormalities were seen, particularly after the plants were put into bubble culture. Sporophytes from the same cross were grossly different, ranging from single blades to multi-bladed sporophytic clusters. It is not unusual to see larger sporophytic damage in bubble culture, and since some of the EMS sporophytes showed signs of deterioration when they were approaching 3 cm. in length, they were transferred (on day forty-one) to a seawater-supplied culture table, to allow further growth and development. Twenty-nine sporophytes were placed in the water table, but abnormal growth eventually occurred and these were ultimately lost.

Table 2. Crosses made with EMS-treated gametophytes. Assessment of crosses on day 7. + = sporophytes present; – = no sporophytes; C = contaminated; D = dead.

<table>
<thead>
<tr>
<th></th>
<th>O male1</th>
<th>10 female2</th>
<th>20 male1</th>
<th>20 male7</th>
<th>60 male9</th>
<th>60 male11</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 female2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 female2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 female3</td>
<td>–, C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>20 female1</td>
<td>–, C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–, C</td>
<td>–</td>
</tr>
<tr>
<td>20 female2</td>
<td>–, C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–, C</td>
<td>–</td>
</tr>
<tr>
<td>60 female4</td>
<td>–, C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–, C</td>
<td>–</td>
</tr>
<tr>
<td>60 female10</td>
<td>–, C</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–, C</td>
<td>–</td>
</tr>
<tr>
<td>Control</td>
<td>–, C</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–, C</td>
<td>–</td>
</tr>
</tbody>
</table>

As a new task, in response to a SERI request that NMI take some new initiatives, a review of techniques for DNA measurement in vivo and in vitro has been completed, and systems for field-inversion-electrophoresis, and pulsed-field-gel electrophoresis have been reviewed. Also microspectrophotometric techniques have been tested, and new filters obtained for detailed measurements of cellular DNA using DAPI staining techniques. A Hoeffer submarine gel electrophoresis system with a DNA* controller and software package is to be used, with a Biorad power supply to separate and measure enzymatically treated DNA (restriction enzymes) from macro- and micro-algae. A new image-analysis approach to gel analysis is being marketed by Amersham, and literature on this was obtained and reviewed.
Equipment for field-inversion-electrophoresis, and pulsed-field-gel electrophoresis have been purchased, and set up with a small (64K) computer that will control the electrical input with a DNA* program which is on order. Microspectrophotometric techniques have been tested, and standards (nucleated red blood cells of chicken) have been obtained. We are now able to quantitatively measure nuclear DNA using DAPI staining methods, as reported in an earlier GRI report. Thus it seems that modern genetic techniques can be combined with more traditional mass- and pedigree-selection methods.

**STRAIN SELECTION**

Strain selection has been accomplished for several red algae. For example fast growing strains of Gigartina exasperata have been selected (Waaland 1979). Chondrus crispus strains were compared based on frond width (Chen and Taylor 1980) and Gracilaria tikvahiae strains were selected based on thallus morphology (Patwary and Van Der Meer, 1983).

In a plant like Macrocystis, which produces morphologically distinctive haploid and diploid life-history stages, strain selection can be undertaken starting with either. Some gametophytic strains are able to grow rapidly (7 to 9%/day increase in wet weight) and bulk cultures of these specific lines can be produced for experimental purposes.

Strain selection among sporophytes growing in the sea is laborious but possible. For example the records of harvested amounts from each of the 700 plants grown by Neushul and Harger (1985) made it possible to identify those plants with consistent high yield. Some of these produced as much as a kilogram of wet biomass every day. Obviously, it would be useful to cultivate these high-yield genotypes as well as those that survive best during the warm nutrient-poor summer months.

The problem of selecting desirable characteristics in kelps is complicated because little is known about which kelp characteristics are inherited and which are not. The extent of mucilage canal development in Laminaria has been shown to have a significant genetic component, as well as a strong environmental component (Chapman, 1975). Laminaria stipe length has been shown to have high heritability (Chapman, 1974). In contrast, the heritability of algin content in Laminaria has been shown to have a low genetic component (Chapman and Doyle, 1979). Lewis, Neushul and Harger (1985) showed that in Macrocystis, like Laminaria, certain morphological characteristics were inherited, as well as the multiple gene trait of growth rate.

**Bulk cultures, iron effects**

The gametophyte clumps from these cultures were fragmented in a blender at high speed for 45 seconds. The fragmented gametophytes were grown in PESI medium with no Fe-EDTA added. Gametophytes require iron in order to reproduce and withholding iron in the medium will keep the gametophytes growing vegetatively, so that they will grow faster. The fragments were grown in erlenmeyer flasks and bubbling was supplied through cotton-plugged 1-ml disposable pipettes with an aquarium pump. The medium was changed weekly.
The suspension of fragments is poured through a 95-um Nitex screen and the fragments that pass through the screen are used for crossing. The density of fragments is measured by counting the density with a Sedgwick-Rafter counting chamber, so that a known number of fragments can be inoculated into a culture chamber. They are inoculated into 500-ml erlenmeyer flasks with 400 ml of PESI medium with iron. After two weeks, the cultures are provided with bubbling for better sporophyte growth.

Spore cultures are started from spores released from sporophylls collected in the sea. The sporophylls are treated with 10% Betadine in seawater for 10 minutes, rinsed with sterile seawater, and allowed to desiccate overnight wrapped in moist paper towels at 15°C. The sporophylls are then placed in sterile seawater until spore release is obtained. The density of spores is measured by counting the spore suspension on a hemacytometer. Spores are inoculated into culture chambers to allow germination, gametophyte growth and reproduction, and sporophyte growth. After 2-3 weeks, these cultures are provided with bubbling for better sporophyte growth.

The above methods were used to initiate bulk cultures of gametophyte isolates, spore cultures and gametophyte isolate crosses. Gametophyte isolates that were used are Ma-377: Female 1 and Male 1; Mp-C: Female 6 and Male 10; and Mi-S: Female 1 and Male 4. When bulk cultures that had been growing for two weeks in PESI medium, to which iron was not added, were used to initiate crosses, it was observed that isolate Ma-377: Female 1 produced eggs while the other two female isolates did not. Therefore, this isolate requires little or no iron to undergo oogenesis. Sporophytes were obtained in all cultures within 6 days, with the sporophytes in the Ma-377: Female 1 cultures were larger than those in the other two cultures.

One female isolate, Ma-377: Female 2, was observed to produce oogonia in iron-free medium. Further culture of this isolate yielded many sporophytes, presumably by parthenogenesis. These sporophytes are abnormally shaped and have grown to a maximum size of ca. 2 mm. This behavior is similar to that of Ma-377: Female 1, reported above. These isolates were all obtained from yield farm plant #377 and the ability of female gametophytes isolated from this parental sporophyte to produce parthenosporophytes indicates that this is genetically determined.

Clusters of genetically diverse sporophytes were grown in culture starting with spores released from a wild sporophyte collected near Ellwood Pier. Contamination was prevented by treating the sporophylls with 10% Betadine for 10 minutes prior to allowing the sporophyll to desiccate. After overnight desiccation, many spores were released within 5 minutes of placing the sporophylls in seawater. Spores were released on August 26, 1986 and inoculated in a range of concentrations into deep culture dishes with PESI. It has been found that bacterial contamination of the cultures can be reduced by using a modified culture medium. The PESI has been modified to reduce bacterial infection by replacing sodium glycerophosphate with an equivalent amount of inorganic phosphate (NaH₂PO₄) and the Tris buffer has been removed. In 12 days, the first sporophytes were observed. At 17 days, these sporophytes were transferred to 1-1 flasks and bubbled. At 28 days, the sporophytes appeared healthy and were growing well. Many were preserved by pressing them on herbarium paper. At 35 days, additional sporophytes were pressed for later analysis.
The spore concentrations used in initiating cultures ranged from $1.0 \times 10^6$ to $5.0 \times 10^6$ spores/300 ml PESI. The fewest sporophytes were obtained in the highest concentration of spores. Intermediate concentrations of $1.0$, $2.0$ and $5.0 \times 10^5$ spores/300 ml PESI yielded the greatest numbers of sporophytes. Fewer sporophytes were noted in the lowest concentration than in the intermediate concentrations. The lower number of sporophytes obtained in the highest concentration of spores indicates that competition between gametophytes in culture inhibits sporophyte production.

The gametophyte culture collection has been assessed, and a completely up-dated summary has been made of all stocks in hand. This is attached to this report as appendix B. The list of taxa in culture is as follows:

<table>
<thead>
<tr>
<th>Genus and Species</th>
<th>code letters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macrocystis pyrifera</td>
<td>Mp</td>
</tr>
<tr>
<td>Macrocystis angustifolia</td>
<td>Ma</td>
</tr>
<tr>
<td>Macrocystis integrifolia</td>
<td>Mi</td>
</tr>
<tr>
<td>Pelagophycus porra</td>
<td>Pp</td>
</tr>
<tr>
<td>Nereocystis laetkeana</td>
<td>NL</td>
</tr>
<tr>
<td>Dicthyoneurum californica</td>
<td>Dc</td>
</tr>
<tr>
<td>Pterogophora californica</td>
<td>Pc</td>
</tr>
<tr>
<td>Alaria marginata</td>
<td>Am</td>
</tr>
<tr>
<td>Dicthyoneupsis reticulata</td>
<td>Dr</td>
</tr>
<tr>
<td>Laminaria setchellii</td>
<td>Ls</td>
</tr>
<tr>
<td>Laminaria saccharina</td>
<td>Lsa</td>
</tr>
<tr>
<td>Laminaria japonica</td>
<td>Lj</td>
</tr>
<tr>
<td>(Pelagophycus x Macrocystis)</td>
<td>PM</td>
</tr>
</tbody>
</table>

Collection data for all the parental lines has been reviewed and verified, as have the health of all of the monoclonally-isolated lines. Out of a total of 841 genetically distinct lines now in culture, 625 are from wild stock. Cultivated plants and hybrids made from crosses of wild types were used as the source for 132 lines, and 84 lines were produced from mutagenized sporophylls of wild-type Macrocystis plants. There are eight lines isolated from the PM intergeneric hybrid, not yet added to the strain list.

**DISCUSSION**

The establishment of nearshore and perhaps even offshore farms of macroalgae is possible. In a sense farmers growing kelp in the ocean will be faced with the same problems encountered by farmers on land: seasonal nutrient deficiencies; adverse light conditions; adverse temperature conditions and destructive storms. However the solutions to these problems must be based on a better understanding of the physiological requirements of the plants being grown. Obviously more basic information about the genetics of these plants will also be very important. The implications of studies of kelp DNA are presently only dimly perceived, but are likely to add valuable new insights (Fain, 1985). Similarly, it may one day be possible to isolate and mutagenize isolated kelp genes, and to move these genes from one taxon in another, as has been done with land plants by using the Ti plasmid to transfer the phaseolin gene from the bean to the sunflower where it is expressed (Murai et al., 1983). Even the transfer of genes, that function in land plants, to the algae has been attempted. The report that Agrobacterium genes can be transferred into and expressed in the
green alga Chlamydomonas are particularly provocative, although as yet unconfirmed (Rochaix et al. 1984). It seems that it will be only a matter of time until kelp genes are isolated and transferred.

The ease with which traditional hybridization and breeding can presently be undertaken with the kelps, has opened exciting new vistas. This suggests that Smil's (1983) critical view that open ocean cultivation of kelps for energy is "science fiction" is in a sense correct. We seem to be witnessing the transformation of hard-to-imagine concepts of ocean farming into reality. In view of the progress made by the Chinese in particular, it seems safe to predict that large-scale cultivation of marine macroalgae will have begun to provide new sources of energy and chemical feedstocks in many places in the world by the end of this century. Domesticated, float-bearing brown algae like *Macrocystis* and *Sargassum* will no-doubt become important marine crop plants of the future.
Collaborative research with colleagues abroad began soon after NMI joined the Marine Biomass Program. Program managers from General Electric and GRI were invited to attend the IXth International Seaweed Symposium at Santa Barbara, where a special evening discussion session on open-ocean mariculture was held. Unfortunately, even though there was a large Japanese delegation, political events in China prevented C. K. Tseng from attending (although he was invited). This situation was soon corrected, and NMI arranged for a GE/GRI/Global Marine group to make an extensive trip to both Japan and China, where the marine farms in these countries were studied firsthand and in considerable detail, thanks to the hospitality of our hosts. This trip set the stage for a series of important, and lasting scientific exchanges. As an examination of the list of publications provided with this report will clearly illustrate, the scientific expertise of Chinese and Japanese scientists was focused on the problems of large-scale macroalgal farming, with considerable success.

In August of 1986, we were very pleased to have two distinguished phycologists from the Peoples Republic of China visit Santa Barbara. Drs. C. Y. Wu and C. K. Tseng both gave a seminar on seaweed cultivation in China that was attended by all NMI staff members. This was particularly informative since Drs. Tseng and Wu graciously allowed us to interview them extensively about the history of the macroalgal cultivation program in China. Significant funding was first provided for their project in the early 1950s and continues to the present day. In marked contrast to the way research funding is provided in the U.S., this research support was routinely provided, and even increased gradually over the years. There were no drastic changes in funding levels, even when farms were wrecked by storms and other setbacks were encountered. Since the basic research phases of the work were essentially complete by the time of the cultural revolution, even this social upheaval did not interfere with work on large-scale production farming in the sea. It is clear that collaborative research with the Chinese will extend beyond the termination of the GRI Marine Biomass Program, as X. G. Fei has recently obtained Chinese funds for continued collaborative work on macroalgal farming. While in Washington in 1986, Dr. Neushul visited Dr. Alice Hogans of the NSF U.S.-China Program to report progress made, since NSF funded some of the foreign exchange studies. The possibility of extending this program with a new Chinese co-principal investigator, to replace the late Dr. Fang, was discussed, and prospects seem bright for continued work.

Several other visitors came to UCSB and NMI in 1986, including Dr. Yasuyuki Yamada, Director of the Center for Cell and Tissue Culture of Kyoto University, Japan. Dr. Yamada gave a seminar at UCSB, attended by NMI Staff. He discussed recent developments in plant biotechnology in Japan. Dr. F. Gavino C. Trono of the Marine Science Institute, University of the Philippines, visited on June 20th. He was interested in using NMI facilities for the cultivation of Sargassum and Eucheuma seedstock. A major problem in the Philippine Eucheuma industry is the lack of seedstock. Dr. Trono would like to develop a seedstock collection and production facility, similar to ours, in the Philippines. Dr. Trono observed the macroalgal culture systems in operation, reviewed the
computer monitoring systems and facilities for media preparation, tissue culture, mutagenesis and image analysis. He suggested that students from the Philippines would like very much to study macroalgal genetics and cultivation in Santa Barbara.

Mr. J. C. Sanderson, from the University of Tasmania visited NMI and UCSB. Working with B. Light, he has recently completed a survey of the *Macrocystis pyrifera* stocks along the east coast of Tasmania. This important work extends the initial studies by A. B. Cribb of Tasmanian records (Sanderson, personal communication) and more recent work on the oceanographic conditions around Tasmania by D. J. Rochford of the CSIRO Division of Fisheries and Oceanography. It is clear that only a minimal harvest can be expected from these waters unless effective planting and cultivation methods are employed. For this reason, Mr. Sanderson was particularly interested in the GRI/SERI funded work on in-the-sea cultivation and genetics. He kindly offered to supply us with genetic seedstock from Tasmania in the future.

Dr. F. Catarino, University of Lisbon, Portugal, visited, and examined the seedstock collection and greenhouses both at UCSB and NMI. We are presently doing collaborative work on DNA levels and germling development of macroalgae with the Portugese (Professor R. Melo is working with us).

In summary, it seems fair to say that GRI support both for foreign travel and for foreign visitors to work here in the United States had a major impact on the program. The relationships that have been established have been profitable for all concerned, and are likely to continue to be so in the future.
IV. ENVIRONMENTAL FACTORS AND MACROALGAL MARICULTURE

This chapter contains three separate sections. The first introduces the aquatic environment in general terms, and is taken from a paper by W. Wheeler and M. Neushul. The second section is a chapter written by B. W. W. Harger on the history of kelp forests of southern California. The final section deals specifically with physical and chemical measurements of the marine environment made by NMI since 1980, and the effects of the measured conditions on kelp growth and production. It is surprising that this type of multi-year data-base has not been more frequently assembled, but at least in southern California, there are only a few comparable sets of data. Measurements of the chemical composition of plants sampled during the 1981-83 GRI yield studies can be related to climatological and oceanographic conditions at that time. Also, given the long data collection period and the fact that it bridges the severe storms and nutrient drought of the El Nino years, this data base becomes particularly interesting. As with any other large data-base of this sort, there are many possible relationships to examine. A simple visual inspection of the 32 pages of multi-year plots, presented here for the first time, clearly shows a regular pattern of seasonal variability in physical and chemical factors. Superimposed on this regularity are infrequent events like large storms, long periods of calm water, or times when nutrient levels were abnormally low. Of course, the regular seasonal changes are reflected by the kelp-harvest records kept by the California Department of Fish and Game and the kelp harvesters themselves. The ability to predict seasonal changes in environmental factors that influence biomass production would obviously be important if a gas-production plant were being run on this biomass.
The Aquatic Environment
by
W. N. Wheeler and M. Neushul

The plant scientist who seeks to evaluate quantitatively the various environmental factors that influence plant life in aquatic habitats faces a formidable task. For those interested in a more extensive discussion, the marine aquatic environment is introduced in Kinne's Marine Ecology (1975, 1976) vols 1, 2, while Hutchinson's Treatise on Limnology (1957, 1967) vols 1, 2 covers the freshwater habitat. We will concentrate here on a description of macronutrients, water motion and irradiance in both fresh and salt water. For a treatment of such factors as salinity, dissolved gases and micronutrients, the reader is directed to such works as Wetzel (1975), Riley and Skirrow (1975) and Hill (1963).

The energy driving the aquatic environment is external. The ultimate source is the sun which heats the water while generating winds which move the water. Irradiance influences the plant through photosynthesis and photomorphogenesis. Water motion affects the transport of mass, heat and momentum on both a very small (boundary layer) scale and on a very large (planetary) scale.

It is important to describe fluctuations in the energy sources with time since these fluctuations will influence the behavior of plants. It is also important to consider the size spectrum of the plants themselves. In the aquatic environment, plants range in size from nanoplankton and algal spores around $1 \times 10^{-5}$ m to the giant kelp at $5 \times 10^{-1}$ m, which is a span of almost 7 orders of magnitude.

Water motion: Turbulence

Movement in natural bodies of water is predominantly turbulent. Turbulence is a property of fluid motion that consists of irregular fluctuating movements which are superimposed on the general pattern of flow. These variations in the current velocity are three-dimensional and may be thought of as packets of randomly moving fluid called eddies. These randomly fluctuating vortices can also be thought of as groups of waves covering a large spectrum of wavelengths corresponding to eddy diameters.

Since turbulent fluctuations are the result of interactions with outside energy sources, the eddy diameter depends on the "wavelength" of the energy source. Another property of turbulence is the dissipation of its energy through viscous shear stresses which increase the internal energy of the fluid at the expense of its kinetic energy. The result is a transformation of the energy into ever decreasing wavelengths. These two properties together define the two ends of a spectrum of diameters with frequencies on the order of days$^{-1}$ (tidal influence) to millisecond$^{-1}$ and smaller. Of most interest to oceanographers and limnologists are the medium and long length frequencies. The medium-length frequencies (min$^{-1}$ to h$^{-1}$) have been correlated with phytoplankton patchiness (Therriault et al. 1979) and implicated in modelling studies (Kemp and Mitch 1979) designed to establish turbulent levels which allow the coexistence of a number of phytoplankton species in the same "niche" or patch as first hypothesized by Hutchinson (1961). Long-period fluctuations are due to climactic variability caused primarily by local variation in solar radiation which produces sea surface
temperature anomalies (Cushing and Dickson 1976, Lasker 1978) and variability in current movements due to fluctuations in winds and climate (Lasker 1978).

Turbulent fluctuations with much smaller frequencies are found in the region of the benthos (bottom-dwelling organisms) where interactions between the water, the bottom, the animals, and the plants have dissipated many of the larger frequencies (Gust and Schramm 1974). These smaller frequencies are important in terms of mass transport and drag which are considered later (3.2.4, 3.2.5).

The spatial scale on which turbulence takes place can be directly measured through the diffusive properties of turbulent flow. Here, rate of transport of a substance is defined by how far it moves by eddy diffusion in a given direction through time. The rate is proportional to the eddy diffusion coefficient. The coefficient is determined through the physical transport of labelled molecules, radionuclides of naturally occurring elements such as Rd, Sr and Rn or through macronutrients such as phosphorus.

Although three-dimensional, the scale and intensity of the vertical component of the turbulent eddy diffusion often differ greatly from the horizontal component (Bowden 1964), being generally 1-4 orders of magnitude smaller than the corresponding vertical component (Bowden 1964; Imboden and Emerson 1979). Values of the coefficient from the Greifensee in Switzerland vary from 0.05 to 0.2 cm$^2$ sec$^{-1}$ in the vertical direction to 100-1000 cm$^2$ sec$^{-1}$ in the horizontal direction. For comparison, still water in a bottle has a coefficient near 0.25 cm$^2$ sec$^{-1}$.

Mixing

The stability of a water column is a function of two interacting components: 1) the work against gravity produced by the density gradient in the water and 2) the energy supplied to the eddies from the shearing flow (Mortimer 1974). The Richardson number (Ri) is a non-dimensional number reflecting the ratio between these components. Thus,

$$Ri = g(d\rho/dz)/(dU/dz)^2$$

where $d\rho/dz$ is the density gradient where $\rho$ is the density and $(dU/dz)^2$ is the energy component in terms of velocity ($U$). When the ratio falls below 0.25, the flow becomes unstable and mixing occurs. With a given density gradient, an increase in external energy applied to the system will decrease the ratio with a tendency toward mixing.

Solar radiation warms the surface water creating a temperature gradient and concomitantly a density gradient. Wind increases the kinetic energy of the system through shear stresses and the production of breaking waves. In temperate regions in the winter, solar irradiance is weak, and its effects in producing a density gradient are easily overcome by winter winds which mix the water (in the ocean to a depth of between 50 and 300 m) and cool the mixed water through convection. In the summer, the reverse occurs, a much higher solar irradiance creates a steep density gradient which the weaker summer winds cannot overcome. This sets up a seasonal discontinuity layer or thermocline.

There is, then, a seasonal variation in the formation and breakdown of
the thermocline typical of high and middle latitudes in the ocean and freshwater lakes (Monin et al 1977; Hutchinson 1957). The depth of this layer increases in winter and decreases in the summer. Variability in this pattern with respect to freshwater lakes led Hutchinson and Löffler (1956) to a now widely held lake classification scheme based on the degree of mixing.

Within the mixed layer, phytoplankton populations are for the most part homogeneously distributed (Bougis 1976). When the depth of this layer rapidly becomes thinner relative to the phytoplankton compensation depth (photosynthesis-respiration), phytoplankton blooms often occur (Sverdrup 1953). Thus, as the mixed layer becomes shallower in spring, and solar irradiance increases, phytoplankton blooms should and do occur (Bougis 1976). The sedimentation rate of phytoplankton is also greatly influenced by the depth of the mixed layer.

The mixing processes in the ocean are generally related to large-scale winds which move large bodies of water. Upwelling is one such form of mixing. Upwelling takes place when seasonal winds blow towards the equator along western coasts (California, Peru and Benguela currents). The stress of the wind on the water combined with Coriolis forces move the water away from the shore. The water is replaced by water which "upwells" from deeper water (50-300 m; Picard 1963). Away from the coasts, in oceanic habitats, the interactions of currents moving in opposite directions may lead to large-scale upwelling in the open ocean, for example along the equator in the eastern tropical Pacific.

Boundary layers

The exchange of mass (dissolved gases, nutrients, etc) as well as heat and momentum between the water and plant surfaces occurs on a very short length scale. The exchange takes place through a thin layer of fluid adjacent to the surface which is produced as frictional forces act to slow down fluid motion in that region (see also chapter 11, Vol A).

The thickness of this layer is dependent upon characteristics of the fluid, the flow, and the surface. Because the boundary layer is a region where viscous forces predominate over inertial forces, it is reasonable to assume that the characteristics of the boundary layer are determined by the ratio of these forces. This ratio is known as the Reynolds number (Re),

\[ Re = \frac{n l}{\rho U} \]

where \( n \) is the viscosity, \( \rho \) is the density, \( U \) is the free-stream velocity of the fluid and \( l \) is the characteristic dimension (e.g. diameter or length) of the surface. Although experimentally determined for only such simple shapes as flat plates, rods and spheres, it is possible in a general way to guess at the flow characteristics over a surface by calculating the Reynolds number. Experiments (Schlichting 1968) have shown that Reynolds numbers less than \( 10^4 \) for a flat plate (with a zero pressure gradient) indicate laminar flow and boundary layers. Reynolds numbers greater than \( 10^4 \) indicate turbulent flows and boundary layers. The transition between laminar and turbulent is, however, critically dependent on the surface roughness (Schlichting 1968). It is not surprising, then, to find that the rugose nature of the blades of the giant kelp *Macrocystis pyrifera* produce
turbulent flow with a Reynolds number less than $10^3$ (Wheeler 1980).

The surface mentioned above can be either a plant or a part of the substrate. When the latter, it is called the benthic boundary layer. Because of their size, some plants, juveniles, and plant propagules inhabit this region. Neushul (1972) has called this community the boundary layer community. Propagules must sink (sediment) through this region, settle and attach (Sewada et al 1972; Charters et al 1973). Nutrients which are regenerated in the sediments must diffuse through the benthic boundary layer to the waters above.

**Drag**

At very low Reynolds numbers ($< 1$), a laminar boundary layer persists over the entire surface of a plant (substrate) without separation. The frictional force of the moving fluid against the surface (drag) is, in this case, entirely due to skin-friction (viscous drag).

Stokes law can be modified to calculate the intrinsic terminal sinking rate of particles the size and shape of phytoplankton or plant propagules due to viscous drag. Here,

$$\mathbf{U_t} = \frac{2/9}{g} \mathbf{r}^2 (\rho' - \rho)/n\theta$$

where $g$ is the acceleration due to gravity, $r$ is the radius of the particle, $(\rho' - \rho)$ is the fluid viscosity and $\theta$ is a shape factor (Hutchinson 1957). Physiological state (fat accumulation and the ionic composition within the cell vacuole) and the change in physiological state with age play large roles in the modification of $(\rho' - \rho)$ and thus the intrinsic sinking rate (Smayda 1970). For particulates, surface coatings, electrolytic forces and dissolved organic substances may reduce drag—giving sinking rates an order of magnitude greater than Stokesian predictions (Chase 1979). Aggregations of particulates, propagules, or plankton may also alter the intrinsic sinking rate (Smayda 1970; Reynolds 1979; Booker and Walsby 1979). The intrinsic sinking rate may or may not be related to the effective (actual) sinking rate. Reynolds (1979) found good agreement between Stokesian predictions and effective sinking rates of Lycopodium spores only through a highly stratified lake enclosure. Turbulence within the mixed layer slowed the effective sinking rate. According to Reynolds (1979) the greater the mixed depth, the slower the effective sinking rate or,

$$\frac{dP}{dt} = -\mathbf{U_t}/z_m$$

where $dP/dt$ is the loss in population (spores or phytoplankton) with time, and $z_m$ is the depth of the mixed layer.

As Reynolds numbers increase beyond a few hundred, flow separation occurs and the drag becomes proportional to the square of the velocity and the cross-sectional area (pressure drag). Thus, the drag force ($F_d$) can be expressed by

$$F_d = (C_d/2) U^2 A.$$ 

Here, $(A)$ is the cross-sectional area of the plant normal to the flow and
$C_d$, is the drag coefficient. Using measurements of the drag of the kelps, *Eisenia* (Charters et al. 1969) and *Nereocystis* (Koehl and Wainwright 1977) it is possible to estimate the stipe breakage velocities. This gives an indication of the forces that can be found in the near shore and intertidal regions. Where these plants occur, *Eisenia* would have a breakage velocity of 9 m sec$^{-1}$ and *Nereocystis* 3 m sec$^{-1}$. These plants are not adapted to very rough exposed intertidal areas where waves can generate calculated velocities of 14 m sec$^{-1}$ (Jones and Demetropoulos 1968). Mechanical adaptations such as high moduli of elasticity (Delf 1932; Table 2) and a tendency to clump or otherwise become modified in form enable these plants to survive in subtidal areas where although 14 m sec$^{-1}$ velocities are rare, breakage point velocities do occur with relatively high frequency.

Mass transport

On the other end of the water velocity scale, stagnation and small water velocities can create diffusion stresses. Under these conditions, Fick's first law governs the rate of diffusion of metabolites to and from plant surfaces. It can be described as

$$J_j = \frac{D_j (\Delta C_j)}{\Delta X}$$

where $J_j$ is the flux of the diffusing molecule $j$, $D_j$ is the diffusion coefficient of species $j$, $(\Delta C_j)$ is the concentration gradient and $\Delta X$ is the diffusion boundary layer thickness, which is somewhat smaller than the physical boundary layer thickness.

Fick's law can be simplistically modified to work under more realistic conditions in nature. The diffusion coefficient $D_j$ is replaced by another $D_j$ which is the eddy diffusion coefficient (or turbulent diffusion coefficient).

When the flux, $J_j$, is smaller than the plant's ability to take up species $j$, the plant is said to be under a diffusion or mass transport stress. Phytoplankton, although small in size, do experience mass transport stresses. Munk and Riley (1952) first called attention to the fact that various shaped phytoplankters would sink with variable speeds, influencing their mass transport abilities. Since then, the problem of mass transport (carbon, nitrogen, phosphorus and other metabolic molecules) to and from phytoplankton cells has been well documented (Gavis 1976).

Larger aquatic plants can also face severe mass transport stresses under low water motion conditions. Wheeler (1980) has demonstrated that the giant kelp, *Macrocystis*, encounters such stresses when currents over the fronds are less than 6 cm sec$^{-1}$. Westlake (1967) has shown such stresses for river plants in flows less than 1 cm sec$^{-1}$, while Lock and John (1979) found 5 cm sec$^{-1}$ a critical speed for river periphyton phosphate uptake.

Nitrogen

Inorganic macronutrients are distributed throughout the aquatic environment by the movement of water. However, the transformation of these molecules within the aquatic environment is the result of biological cycling. The period and amplitude of these cycles varies depending on the scale being considered. For instance, on a global scale, Stevenson (1972)
has estimated that $1.7 \times 10^{20}$ g of nitrogen (N) are present on earth. Of this, 97.6% belongs to the lithosphere, slightly less than 2.3% to the atmosphere and the remainder to the hydrosphere and biosphere. The approximately $3.9 \times 10^{18}$ g N in the atmosphere are in the elemental form, which is not directly available to plants. Only a few prokaryotes can fix N$_2$. In spite of this fact, organisms fix $2.2 - 2.3 \times 10^{14}$ g of N yr$^{-1}$ (Soderlund and Svensson 1976) of which somewhere between 0.09 and 37% is fixed in the aquatic environment.

N fixation by blue-green algae and bacteria occurs in anaerobic environments, either within the plants themselves (heterocysts) or in anaerobic areas surrounding the plants (Carpenter and Price 1976). Values range from $65.7 \text{ g N m}^{-2} \text{ yr}^{-1}$ (Wiebe et al. 1975) for blue-green algal mats on Enewetak Atoll to $7 \times 10^{-4} \text{ g N m}^{-2} \text{ yr}^{-1}$ in the Sargasso Sea (Carpenter and McCarthy 1975).

Although large quantities of N are fixed, this accounts for only 3-10% of the N utilized by plants. Recycling accounts for the other 90-97% made available for plant production (Soderlund and Svensson 1976). Thus, N turnover rates can be important indicators of plant production. On a global scale, N has been estimated to turnover in between 1 to 20 days.

Within freshwater and coastal communities most of the regeneration takes place in the sediments. Regeneration rates from sediments vary, being turned over in some small ponds at the rate of 67% d$^{-1}$ (Sugiyama and Kawai 1978) to 0.7% d$^{-1}$ in the Baltic (Hallberg et al. 1976).

Nitrogen contributions from zooplankton vary, being dependent on the amounts already present in the water. In an estuary near Beaufort, North Carolina, U.S.A., ammonium excretion by zooplankton accounted for 16% of the utilizable N (Smith 1978). In oligotrophic (nutrient poor) environments, excretion can account for as much as 90% (Jawed 1973).

Within the ocean most of the regeneration takes place in the top 200 m of the water column. Fecal pellets or marine snow produced in the upper layers are micro-habitats for bacterial regeneration of N within the upper layers. These micro-habitats can produce micro-patches of available N where traditional methodology would show no nutrients. Such micro-scale patches are immediately exploited by the phytoplankton, contributing to phytoplankton patchiness (McCarthy and Goldman 1979).

Inorganic nitrogen is made available through fixation, mineralization and excretion. It is removed from the environment through denitrification, assimilation and sedimentation. Denitrification is a microbial process. Rates range from 4.6 mg N m$^{-2}$ yr$^{-1}$ in a Danish fjord (Oren and Blackburn 1979) to 8-16 mg N m$^{-2}$ d$^{-1}$ in a polluted river (Nakajima 1979).

Plants assimilate inorganic N primarily as nitrate (NO$_3$). Soderlund and Svensson (1976) and Bougis (1976) estimate that 12 and 30 g N m$^{-2}$ yr$^{-1}$ respectively are assimilated from the ocean alone. Ammonium may also be sorbed by the sediments and not released to the interstitial water under anoxic conditions (Hallberg et al. 1976; Rosenfeld 1979).

Laboratory studies and field correlations indicate that N is the most limiting nutrient (Dugdale 1967; Ryther and Dunstan 1971; Eppley et al. 1979) in the coastal marine environment. This is, perhaps, the reason that some marine macrophytes have been shown to store N (Chapman and Craigie 1977) and others have been found in symbiotic relationships with N fixers (Hanson 1977).
Phosphorus

In freshwater communities, phosphorus (P) rather than N is generally limiting. In contrast to N which is primarily in the atmosphere, inorganic P is found primarily in the lithosphere. In natural waters, P occurs in solution in both inorganic (soluble reactive P: SRP) and organic forms (soluble unreactive P: SUP), as well as adsorbed to organic, colloidal and inorganic particles. Although SRP is for the most part orthophosphate, SRP levels can be as high as 6 x the orthophosphate levels (Lean and Charlton 1976).

SUP can be transformed to SRP either by the plants themselves through hydrolysis via alkaline phosphatase or via photodegradation with UV radiation (Francko and Heath 1979; Morse and Cook 1978). Phosphorus in sediments is present mainly as apatite or in association with free cations (usually iron and calcium). Under anoxic conditions phosphates are released from sediments (Mortimer 1942; Martens et al 1978).

Under aerobic conditions, the sediment may act as a sink for P. Phytoplankton and macrophytes are also a temporary sink for P. They assimilate to a large degree only orthophosphate, although in special circumstances other forms are also assimilated (Kuhl 1974). Phosphorus assimilation can be in quantities far greater than amounts immediately required by plants. The excess P is either stored or excreted as unreactive polyphosphates.

The turn-around time between assimilation and excretion and reassimilation can be on the order of seconds for some phytoplankton (Pomeroy 1960). Because of its fast turnover rate and general scarcity in freshwaters (Hutchinson 1957,1967), it is generally held that P is the most limiting nutrient in freshwaters although inorganic carbon has also been implicated (Schindler et al. 1972). Adding (loading) phosphates to freshwaters usually results in increased biomass production (Schindler 1971). Phosphate loading of natural waters occurs through introduction of man-made detergents, fertilizers and sewage. This eutrophication process results in tremendous blooms of phytoplankton and macrophytes (see Schindler 1972). Thus, changes in physical factors can bring about large-scale responses from the associated flora.

Plant response

Because the interactions between water and plant determine survival and influence plant function, it is not surprising to find relationships between the type of water motion in a given habitat and plant morphology. Thus, phytoplankton shape (Smayda 1970) and blue-green algal colony shape (Booker and Walsby 1979) have been correlated with intrinsic sinking velocity. The blades of the giant kelp, Macrocystis, produce turbulent boundary layers in non-turbulent water flow, which, by decreasing the effective boundary layer thickness, enhances mass transport (Wheeler 1980). High and low water motion morphologies are common in macrophyte habitats (Norton 1969; Gerard and Mann 1979), as well as different morphologies for submerged and emerged leaves (see Sculthorpe 1967). Depending on the size of the plant, different morphologies, even in the same location, are a response to different hydrodynamic habitats. Neushul (1972) has classified marine macrophytes according to their hydrodynamic environment, and Aleyev
(1976) has classified the plankton according to size and hydrodynamic environment.

Irradiance

Solar radiation is the source of energy utilized by plants in the manufacture of complex organic substances. The degree of availability of this energy directly controls the amount of organic matter synthesized. The energy that is not utilized by photosynthesis contributes to the potential and kinetic energy of the aquatic environment.

This radiant energy is carried by electromagnetic waves, and may be described in terms of its spectral composition. Visible light is the radiant energy within the wavelength range from 350 to 750 nm. The range utilized by plants for photosynthesis, photosynthetically active radiation (PAR), is restricted to the range from 350 to 700 nm.

Definitions and units

The quantity of energy transferred by radiation (in Joules, J) is represented by the symbol \(Q\). Radiant flux (F), \(\text{d}Q/\text{d}t\), is defined as the time rate of flow of radiant energy (measured in watts, W). The irradiance, \(E(z)\) at a depth \(z\) is the total radiant flux per unit area incident on an element of surface (the flux from the hemisphere covering the element's surface). \(E_a(z)\) is the downwelling (downward) irradiance (flux incident per unit area measured on a horizontally oriented surface facing upward). The upwelling (upward) irradiance \(E_u(z)\) is the flux per unit area measured on the downward facing side of the surface.

While the SCOR working group (Tyler 1974) has recommended the use of the above definitions for oceanographers, photobiologists have defined the energy incident on a given surface area as energy fluence (\(F; \text{J} \cdot \text{m}^{-2}\)). The time rate of flow of the energy fluence is the energy fluence rate and has the units of \(\text{W} \cdot \text{m}^{-2}\). These terms can also be defined in terms of quanta, e.g. photon fluence or photon fluence rate (Rupert 1974).

Rough conversions can be made between the radiometric and quantum aspects of irradiance. Morel and Smith (1974) found the ratio, \(Q/E\) (total quanta/total energy), remained constant above water at \(2.5 \pm 0.25 \times 10^{18}\) quanta sec\(^{-1}\) watt\(^{-1}\), regardless of sun elevation (above 25°) and meteorological conditions. Below the surface the \(Q/E\) ratio exhibited greater variability (±10%) but was predictably dependent on the optical properties of the water. The average \(Q/E\) for natural waters is \(2.5 \pm 0.25 \times 10^{18}\) quanta sec\(^{-1}\) watt\(^{-1}\). For lake Kinneret, Dubinsky and Berman (1979) further divided this into 3 water types characterized by the plant pigment in them (abundant chlorophyll, low chlorophyll and abundant peridinin, a red pigment of dinoflagellates) with respective \(Q/E\)'s of 2.7-2.8, 2.5-2.6 and 2.96 x 10\(^{18}\) quanta sec\(^{-1}\) watt\(^{-1}\). For waters with high gilvins, yellow substance, content, Spence et al (1971) found 3.07 x 10\(^{18}\) quanta sec\(^{-1}\) watt\(^{-1}\).

Absorption

Water, itself, absorbs radiant energy throughout the visible spectrum, absorption being the strongest in the red wavelengths. Seals have
little influence (Morel 1974) and so the absorption of light in pure seawater, as well as in distilled water, is minimal in the blue and maximal in the red region. Light is absorbed in aquatic habitats by pigmented phytoplankton, particulate matter (Jerlov 1976) and dissolved substances (Kirk 1976b). In many inland and coastal waters a yellow substance called gilvin or gelbstoff can be present in large quantities, influencing the spectral irradiance immensely (Kirk 1976b). Gilvin is thought to be composed of humic substances that are found in waters runoff from land (Kalle 1966) or phenolic compounds released from algae (Sieburth and Jensen 1970).

Scattering

Scattering does not in itself attenuate light, but increases the optical pathlength followed by the light, thereby increasing absorption. The scattering of wavelengths by water and by small particulates is generally dependent upon the reciprocal of the fourth power of the wavelength. However, phytoplankton and larger particulates scatter light almost independently of wavelength (see discussion in Jerlov 1976).

In the clearest natural waters scattering plays a minor role, but there are minor differences between fresh and saltwater. The scattering coefficient, which is 30% higher in seawater (35-39o/oo) appears to be related to the presence of dissolved ions in seawater (Morel 1974). Particulates in clear natural waters are present only in small quantities (0.02-0.17 mg m\(^{-3}\); Jerlov 1976). Phytoplankton are therefore the major contributor to scattering and absorption. Light absorbed by phytoplankton and gilvin virtually eliminates blue light from deeper natural waters. The red end of the spectrum is absorbed by the water itself, creating a situation where in deeper waters, the irradiance spectrum is nearly monochromatic (Jerlov 1976).

Attenuation coefficient, K

The attenuation of radiant energy in natural water has been found to obey the Bouguer-Beer law:

\[ \text{dE} = -K \ E \ \text{dz} \]

where K is the attenuation coefficient. If K is constant with depth (assuming a mixed water column) then, the irradiance at depth z (E(z)) can be defined as

\[ E(z) = E(o) \ e^{-Kz} \text{ or } K_d(z) = \ln (\ln E(z) - \ln E(o))/z \]

where E(o) is the energy incident on the surface of the water and K_d is the diffuse attenuation coefficient measured in the vertical direction. Another way to measure attenuation is through beam transmission. Here the energy of a beam of light measured before and after being transmitted through a fixed distance of water,

\[ T = e^{-Cz} \]
C is a total of the absorption of the water, particulates and dissolved substances but only a portion of the scattered energy. That energy which is scattered away from the beam's path is not measured. The beam transmittance coefficient, C, and the $K_d$ can be related by including the forward scattering.

Spectral variation in $K_d$

The range of water conditions produced by variable amounts of particulates and gilvin has led Jerlov to propose a system to classify a body of water according to its transmission or attenuation spectrum (Jerlov 1976, 1977 Fig 2). In this system, there are 5 types of oceanic water characterized by transmission windows in the blue with corresponding high transmission coefficients approaching those of pure water (Fig 2). These are types I, Ia, Ib, II, III. For areas with increasing gilvin and particulates, he defined 9 gradations of coastal water, from the most transparent at 1 to the least at 9 (Fig 2). There appears to be little difference between fresh and salt water. The clearest water measured to date is that of Crater lake ($K_d = 0.037$ m$^{-1}$; Smith et al. 1973) and the least clear is that from an East African lake ($K_{540} = 100$ m$^{-1}$; Melack and Kilham 1974).

Temporal variation in $K_d$

The temporal variation in the $K_d$ can be quite large. Both the $K_d$ and the spectral distribution of underwater irradiance are dependent on changes in the pathlength of the light as the sun's angle changes, and on phytoplankton patchiness and wind related processes (Clarke 1938; Kain 1971; Kirk 1977; Harger 1979; Lüning and Dring 1979). Runoff during storms in winter as well as wind related mixing can drastically change the near-shore underwater light climate. Harger (1979) has demonstrated that the submarine irradiance in a kelp forest (15 m deep) can change by orders of magnitude within 1 day. These variations can and in many cases do exceed the range observed seasonally. In the winter months the irradiance was correlated more with hydrographic conditions than to atmospheric ones as was the case in summer. Lüning (1971) related loss of water clarity to winds over Beaufort scale 6 ($10.8-13.8$ m sec$^{-1}$). Changes in tidal height with the above factors caused variations as much as 250 x in the daily irradiance near the Isle of Man (Kain 1971). Bindloss (1976) and Dubinsky and Berman (1979) in seasonal studies on freshwater lakes found that phytoplankton blooms were the most important factors determining underwater irradiance. In more open waters where hydrographic conditions are much more uniform, seasonal changes in submarine irradiance seems less noticable (Poole and Atkins 1929), although little data exists for this region.

Spatial variation in irradiance

Irradiance levels can change drastically with the microhabitat of an organism. Sandy bottoms reflect more irradiance (Brake 1979). Large boulders, crevasses or reef structure in general and vegetation can greatly modify the light impinging on a given surface area (Ernst 1957; Forstner and Rutzler 1970).
Vegetation can change not only the irradiance, but the spectrum as well. Phytoplankton in concentrations greater than 10 mg m$^{-3}$ can significantly alter the underwater irradiance and spectra (Talling 1960). Size and shape of the phytoplankter also play a significant role in their light absorbing characteristics (Kirk 1976a). Canopies of the giant kelp, *Macrocystis* can cut the irradiance by 2 orders of magnitude depending on the thickness of the surface canopy (Neushul 1971), much in the same way irradiance in forests is attenuated (Tasker and Smith 1977). However, in the marine environment, pigments other than chlorophyll a can be dominant. Fucoxanthin, a brown pigment absorbs primarily in the blue-green, while phycerythrin and phycocyanin absorb mainly in the green and blue-green region respectively. Little work is available dealing with this effect on undergrowth algae.

Plant response

The distribution of red and blue light in the aquatic environment may be more important than previously considered. Research on phytochrome and blue light responses may apply to aquatic plants. Lüning and Dring (1975) have noticed blue light photoperiodic responses in some brown algae, while Müller and Clauss (1976) have demonstrated other photomorphogenetic responses in brown algae. So far, these effects have been found to be limited to the phaeophyta, but investigations with other divisions are continuing. The levels of irradiance necessary to induce photomorphogenetic effects, or to sustain growth have been generally considered to lie in the former case below the 1% level of surface irradiance and the latter case above the 1% level. However, recent research (Lüning and Dring 1979) has shown that many species of algae have photosynthetic compensation points between 0.01-0.05%. Red algal crusts have been found in the sea at depths representing 0.01% surface irradiance calculated over a year's time. Kelps such as Laminaria hyperborea have been shown exist to depths representing 0.05% in many places around the world. Because their pigmentation is complementary to most green coastal waters, red algae have been postulated to have evolved as a deep water division (Engelmann 1883). In contrast, however, red algae do equally as well in the high intertidal zone. Although the pigmentation of an alga confers an advantage in the absorption and conversion of incident energy of specific wavelengths, recent research has shown that factors such as morphology (Ramus 1978) and internal organisation of the photosynthetic unit (Ramus et al. 1977; Prézelin 1976) play much larger roles.

Concluding remarks

We have attempted in this review to provide a view of the aquatic environment which is a dynamic one. To emphasise this, we have considered the manifold effects of water motion on the aquatic plant community. The dynamic aspect of the environment can be thought of as being periodic- in the sense that the energy sources of the aquatic environment manifest themselves in cycles. The cycles or period of the energy input vary on a temporal scale from milliseconds to centuries, and spatially from microns to planetary in scope. An understanding of the flora and its productivity
necessitates, then, an understanding of the periodic behavior of the energy source. In this review we have tried to define a number of the more important cycles influencing the ecosystem and associated flora.

As botanists, we can see certain periodic phenomena (variability) within the plant ecosystem (variability in population dynamics, growth rates, productivity, etc.) for which we cannot identify any single physical factor. This is the result of plant behavior. Aquatic plants and plants in general have been shown to be integrators of environmental variables (Evans 1972) and further, to respond to changes in environmental factors with a certain lag phase (Doty 1971; Cushing and Dickson 1976). Thus, storage of essential nutrients by many of the aquatic plants as well as the effect of physical factors in influencing the distribution of plant propagules determine the behavior of an individual or a plant population at a later date. Although it is important to identify the driving factors, it is almost impossible to significantly correlate plant behavior with fluctuating environmental variables (Evans 1972). An understanding of the variability within the system, however, can only come about from an elucidation of the driving function(s).

The aquatic botanist should be aware then, of the problem of scale. Spatial, temporal and energy scales interact to define a given set of circumstances to which the plant must respond. The nature of this response is the subject of intensive research.
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Table 3
Intrinsic sinking rates of a number of marine plants and spores

<table>
<thead>
<tr>
<th>Species</th>
<th>Velocity</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agardhiella tenera</td>
<td>116</td>
<td>Coon et al 1972</td>
</tr>
<tr>
<td>Callophyllis flabellulata</td>
<td>15</td>
<td>&quot;</td>
</tr>
<tr>
<td>Cryptopleura violacea</td>
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<td>&quot;</td>
</tr>
<tr>
<td>Gelidium robustum</td>
<td>43</td>
<td>&quot;</td>
</tr>
<tr>
<td>Myriogramme spectabilis</td>
<td>59</td>
<td>&quot;</td>
</tr>
<tr>
<td>Anabaena flos-aquae (straight form)</td>
<td>3.9-4.5</td>
<td>Booker and Walsby 1979</td>
</tr>
<tr>
<td>Anabaena flos-aquae (helical form)</td>
<td>4.5-8.1</td>
<td>&quot;</td>
</tr>
<tr>
<td>Asterionella japonica</td>
<td>5</td>
<td>cited in Smayda 1970</td>
</tr>
<tr>
<td>Chaetoceros curvisetus</td>
<td>12-29</td>
<td>&quot;</td>
</tr>
<tr>
<td>Coccolithus huxleyi</td>
<td>15</td>
<td>&quot;</td>
</tr>
<tr>
<td>Coscinodiscus concinnus</td>
<td>708</td>
<td>&quot;</td>
</tr>
<tr>
<td>Dunaliella tertiolecta</td>
<td>2</td>
<td>&quot;</td>
</tr>
<tr>
<td>Leptocylindrus danicus</td>
<td>0.9-5</td>
<td>&quot;</td>
</tr>
<tr>
<td>Monochrysis lutheri</td>
<td>5</td>
<td>&quot;</td>
</tr>
<tr>
<td>Noctiluca miliaris</td>
<td>300</td>
<td>&quot;</td>
</tr>
<tr>
<td>Phaeodactylum tricornutum</td>
<td>0.02-0.05</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

$1 m s^{-1} \times 10^{-6}$
A Historical Overview of Kelp in Southern California

by Bruce H. Harger

The giant kelp, *Macrocystis*, forms beds off the coast of southern California. In this century these beds have decreased in size. The decreases have been attributed to kelp harvesting, sewage pollution, other human activities, sea-urchin-population outbreaks, natural warm-water periods and natural low-nutrient periods. All of these probably impact kelp populations at different times and in different localities. The present study is based on heretofore unused aerial imagery and kelp-harvesting-rate information. The focus of the work was to determine what the variation in kelp bed area and cover is and if this variation can be assigned to specific causes.

Existing aerial imagery was used to determine the kelp-bed areas and kelp-canopy areas for the kelp beds of southern California over the past sixty years. There were consistent differences through time and there are consistent differences between beds in different geographic locations. Parts of beds have been destroyed by boat traffic. Kelp beds reached a low point between 1959 and 1963. This could have been due to high temperatures, low nutrients, high numbers of sea urchins or large volumes of toxic sewage pollution. It is not possible to determine the cause. Santa Barbara beds are larger, have higher cover, and are more stable. Los Angeles and San Diego beds are smaller, have lower cover and are less stable. These differences are probably due to different exposures of these localities to winds and storms.

A statistical model was formed that can predict 46% of the variation in kelp-harvesting rate given the prior month’s average surface irradiance, swell height and sea temperature. Probably over half the variation in kelp-canopy growth is determined by light, temperature (nutrients) and water motion (swell height).

INTRODUCTION

The giant kelp, *Macrocystis*, forms beds off the coast of southern California in water from 6 to 20 m deep. The history of these beds is poorly known. Only during this century have they been carefully studied. During the last thirty years vertical aerial photographs have been used to map the canopy area of these kelp beds and document the gradual decrease in kelp area. The present study presents some of the results of an extensive study of aerial
photographs of the kelp beds of southern California taken over the last sixty years. Details of this study are available from the Southern California Edison Company as Research Report Series Number 91-RD-9A. Since little ground truth or physical environmental information is available for the beds and their environment over this time period, interpretation of the cause for specific changes in kelp-bed area is difficult. In an attempt to link kelp canopy changes with environmental conditions, the present study compares kelp harvesting rate information for the Goleta Bay kelp bed with ground-truth environmental information for that period. A more detailed presentation of this study can be found in Harter (1979). The results of this study should be of interest to those who manage and harvest the natural kelp-bed resources of southern California.

Nautical charts from the last century indicate that the southern California kelp beds were extensive at that time. European sailors used to look for detached drifting kelp plants as an indication that they were near land (Dana 1841).

It is difficult to tell what the kelp beds were like before man settled along our coast. Sea otters used to live here and may have benefited kelp by consuming many kelp-grazing sea urchins. Stellar sea cows may have also inhabited our coastline and could have consumed large quantities of kelp (see Dayton 1975). When the local Chumash Indians settled here, they consumed sea otters (see Tompkins 1967 and Doran 1980) and may have kept their populations at low levels, allowing sea urchins to settle and graze.

The first comprehensive mapping project that dealt specifically with the kelp beds was initiated by the United States Department of Agriculture prior to World War I (Cameron 1915 and Crandall 1912) when potash supplies from Germany were restricted (Scofield 1959). This survey extended all along the continental west coast of North America from Mexico to the Aleutian Islands. Maps were drawn from bearings made from boats following the kelp bed margins. A sextant, compass, and "three point apparatus" were used to measure the areas of the kelp beds. The large sizes that were reported, compared to the present sizes of the beds (Hodder and Mel 1974), have led others to question the validity of these early studies. However, Cameron claimed that at the time of his survey the kelp beds were in relatively poor condition, compared with what had been seen in previous years. Thus, it is likely that the kelp beds of the Southern California Bight were larger in the last century than they are now.

Kelp beds have more recently been mapped and the areas projected from vertical aerial photographs. When these are properly exposed they can clearly show kelp-bed canopy. Vertical aerial photography was first used to map kelp beds by Dr. Wheeler J. North in the 1950's. Since the 1950's, commercial seaweed harvesters, particularly the Kelco Company of San Diego, have used the areal extent of kelp canopy to estimate the health and potential yield of the kelp beds. The California Department of Fish and Game regulates kelp harvesting activities and has numbered the beds for the purpose of leasing them. North has regularly mapped the Palos Verdes (1975, beds #13 and #14), La Jolla (bed #4) and Point Loma kelp beds (1974, bed #3). He has also photographed and mapped most of the beds between Palos Verdes and La Jolla (beds #5 to #10). His work is still continuing. The other beds in the Southern California Bight have not been studied in such detail.
Kenneth Wilson of the California State Department of Fish and Game has used techniques similar to North's to monitor kelp beds in the Palos Verdes region. The Department of Fish and Game is involved in an effort to restore the formerly rich kelp beds in Palos Verdes by transplanting kelp plants into this depleted area. Wilson's group is also destroying dense sea urchin populations in the transplant areas and near the borders of other kelp beds. Wilson's maps drawn from vertical aerial photographs show expanding kelp beds and confirm the efficiency of their transplanting efforts (Wilson, Haaker, and Hanan 1977).

Esca-Tech (Hodder and McEl 1978) has done the most comprehensive job of photographing and mapping the kelp beds of the Southern California Bight to date. The Bureau of Land Management, through Science Applications Incorporated, contracted with Esca-Tech to make flights on a quarterly basis for two years from 1975 to 1977, a total of eight flights. These new images were analyzed and related to the data obtained by Crandall (1912) and others in 1967 and 1972.

**MATERIALS AND METHODS**

Jensen, Estes, and Tinney (1980) have recently reviewed the methods used for remote sensing of kelp beds. In the present study, we collected images from several sources, converted them to a standard size, mapped the kelp beds, measured their sizes, measured their canopy cover, and studied the variation of the size and configuration of all of the kelp beds of the Southern California Bight. We found that maps we made using an optical-transfer microscope were less accurate than those we made using the photographically enlarged mosaic method. We also found that photo-enlarged imagery was very close to the quality of the original photographs. This method was also more convenient because we were able to take all the imagery to our facility to work with it and were able to convert images in several different formats to the same size.

We located several sources of existing images. These collections were evaluated and several sets were selected for processing. We found so many sets of images available that we focused on sample years spaced every four years since 1955 rather than covering all years. When photographs of specific beds were not available for the "sample" years, photographs from the prior or subsequent year were used instead. We also included photographs taken in 1977 because there were so many good photographs available.

The photographic copying was done with a copy stand and a Hasselblad F1/M camera with a Carl Zeiss S-Planar 120mm f5.6 lens and a 4.5 x 6 cm format film back. H&W VTE 120 and Ilford Pan F 120 films were used. The collected images were printed with a Beseler 23CIT enlarger with a 100 mm lens. All prints were scaled to a reduction of 1:24,000 by superimposing the image on a standard U.S. Geological Survey (7.5') topographic map under the enlarger. Coastline and other features in the negative image were matched with those on the map. Kodak and Agfa resin coated (RC) papers were used for printing. Prints taken as part of the same flight were assembled into mosaics. Transparent, acetate versions of the U.S. Geological Survey topographic maps were used to assure that the individual photographs in the mosaics were correctly positioned with respect to one another and land features. Maps were made for each of the numbered kelp beds, using the California State Department of Fish and Game kelp-bed numbering
system. Clear acetate was first taped over the mosaics. The configuration of the kelp beds were then drawn on these acetate sheets. Canopy cover within the configuration boundary was estimated as well as measured with an Digital Graphics CAT-100 image analysis system in a Vector Graphics System B Microcomputer.

Because individual kelp plants are irregularly spaced within a kelp bed, it is sometimes difficult to decide what to include within and what to exclude from the drawn kelp-bed boundary. Hodder and Mel (1978) used subjective criteria to define the margins of the beds. In order to be more objective we made the decisions needed to define the boundary of a kelp bed by following the guidelines listed below. The mapper would draw boundaries around any group of two plants or more and exclude single plants if they were separated by more than 2 mm (measured with a 2 mm dot) from the main body of a bed or patch (2 mm at that scale of reduction represents 40 meters at the water surface). Nearshore _Egregia_ beds were excluded.

Area measurements were made with a Lasico L50D digital planimeter directly from the acetate maps. The measuring technique was straight forward. Care was taken by the data takers to periodically recalibrate the instrument by measuring a standard area. We found that the same observer repeatedly measuring the same area was consistent within ±1%. Different observers were consistent to within ±2.5%.

Statistics were calculated on untransformed data for kelp-bed areas and kelp cover, for each of the 33 beds over the sample time periods studied between the 1930's and 1979. The statistics that were calculated were the mean, standard deviation and coefficient of variation (100 x standard deviation / mean). Beds in the San Diego, Los Angeles, Santa Barbara, and Santa Catalina Island geographic areas were compared using means, standard errors, and t-tests. The bed length, width, area, variation in area, cover, and variation in cover were compared.

The kelp harvesting rate (catch-per-unit-effort) in the Goleta Bay kelp bed was used as an indicator of the amount of kelp canopy that was available for harvest at the surface. All harvests were done under near-calm conditions. The rates were compared with the means for the prior month's measurements of surface irradiance, wind speed, swell height, sea temperature at Platform Holly, sea temperature at Santa Barbara Harbor, surface nitrate concentration, and bottom nitrate concentration (at 12 m deep). A stepwise multiple-linear-regression model was determined for the 70 observations (of 256) for which we had information for all eight variables. Three variables were selected for the model. A second model was determined to verify the first one (as suggested by Cooley and Lohnes 1971) using the 195 observations for which we had information for the three variables that appeared in the first model. The contribution that each variable made to the regression was calculated as the product of the regression coefficient and the standard deviation of the variable (not standard deviation of the regression coefficient).
### Table 4. Mean Kelp-Red Areas and Kelp-Canopy Cover of 33 Southern California Right Kelp Reds from the 1930's to 1979, Including Standard Deviations and Coefficients of Variation

<table>
<thead>
<tr>
<th>Kelp-Red Areas (hectares)</th>
<th>Kelp-Canopy Cover (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Periods</strong></td>
<td><strong>Mean</strong></td>
</tr>
<tr>
<td>1</td>
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</tr>
<tr>
<td>2</td>
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<td>32</td>
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</tr>
<tr>
<td>75</td>
<td>5</td>
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</table>

**Total**: 5,791.502

**Note**: Numbers in this table are shown to three places, not because they are that accurate, but because we wished to not introduce "rounding error" when statistical tests are made. These numbers are accurate to one place beyond the decimal point.
RESULTS

In the course of this study, we examined over 15,000 aerial photographs and copied and printed more than 6,000 of these, from which we assembled 269 photomosaics. We made approximately eight photomosaics for each of the thirty-three California Department of Fish and Game designated kelp beds that we studied. We measured the areas of the kelp beds and estimated the kelp canopy cover within each bed. Means of the kelp-bed areas and kelp-canopy cover measurements were calculated for each bed (Table 1.1). Standard deviations and coefficients of variation were also calculated for the data for each kelp bed.

The areas of the kelp beds were compared between sample periods by using the measurements of each bed for the two periods as a paired sample. There was a significant decline in area from 1959 to 1963. There was a significant decline in area from 1971 to 1975 followed by a very significant expansion from 1975 to 1977. The 1955 kelp-bed areas were the highest since 1911.

The cover of kelp canopy in the kelp beds was compared between sample periods by using the measurements of each bed for the two periods as a paired sample. There was a significant increase in cover from the intermediate period to 1955 and a very significant decrease in cover from 1955 to 1959. There was a significant decrease in cover from 1967 to 1971 and a significant increase from 1971 to 1975. There was a significant decrease in cover from 1977 to 1979. The 1955 kelp cover values were the highest in the present study.

We separated the beds that we studied into four geographic areas: San Diego, Los Angeles, Santa Barbara, and Santa Catalina Island (Figure 1 and Table

![Figure 13](image-url)

*Figure 13.* Changes in area of the kelp beds of three regions of the Southern California Bight. Note that the Santa Barbara area beds have covered more area and been more stable than the Los Angeles and San Diego area beds. There has been a decline and then a recovery in the Los Angeles and San Diego beds in this century.
We compared the following information for kelp beds in these geographic areas: kelp bed length, kelp bed width, kelp bed area, coefficient of variation of kelp bed area, kelp canopy cover, and coefficient of variation of kelp cover canopy (Table 3). We measured the kelp bed length as the linear distance from one California Department of Fish and Game bed boundary to the

<table>
<thead>
<tr>
<th>CDFG Red Number</th>
<th>Length (km)</th>
<th>Width (m)</th>
<th>Area (Hectares)</th>
<th>Variation -Area (%)</th>
<th>Cover (%)</th>
<th>Variation -Cover (%)</th>
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<tbody>
<tr>
<td>San Diego to San Onofre</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>75</td>
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<td>11.272</td>
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### TABLE 6. COMPARISON OF KELP REDS IN FOUR GEOGRAPHIC AREAS OF THE SOUTHERN CALIFORNIA BIGHT

<table>
<thead>
<tr>
<th>Information</th>
<th>San Diego</th>
<th>Los Angeles</th>
<th>Santa Barbara</th>
<th>Santa Catalina Island</th>
<th>All</th>
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<td>9-19</td>
<td>20-32</td>
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<td>Linear Distance (km) along Coast (Total for Reds)</td>
<td>126.18</td>
<td>212.45</td>
<td>93.49</td>
<td>78.72</td>
<td>510.84</td>
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<tr>
<td>(% of Total)</td>
<td>24.70</td>
<td>41.59</td>
<td>18.30</td>
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<tr>
<td>Kelp Red Area (Hectares) (Total for Reds)</td>
<td>1,217.03</td>
<td>752.49</td>
<td>3,753.39</td>
<td>69.60</td>
<td>6,791.51</td>
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<tr>
<td>(% of Total)</td>
<td>21.01</td>
<td>12.99</td>
<td>64.81</td>
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<tr>
<td>Kelp Red Width (m) (Average for Linear Distance of Coast)</td>
<td>96.45</td>
<td>35.42</td>
<td>401.48</td>
<td>8.71</td>
<td>113.37</td>
</tr>
</tbody>
</table>

| Linear Distance (km) along Coast (Average for Reds) | 15.77     | 19.31       | 7.19          | 78.72                 | 15.48       |
| Standard Error (n)                                | 2.49(8)   | 3.02(11)    | 1.22(13)      | (1)                   | 2.51(33)    |
| Kelp Red Width (m) (Average for Beds)             | 111.90    | 45.19       | 354.89        | 8.71                  | 182.26      |
| Standard Error (n)                                | 48.38(8)  | 15.20(11)   | 48.45(13)     | (1)                   | 33.52(33)   |
| Kelp Red Area (Hectares) (Average for Reds)       | 152.13    | 68.41       | 288.72        | 69.60                 | 175.50      |
| Standard Error (n)                                | 52.49(8)  | 25.36(11)   | 76.28(13)     | (1)                   | 36.95(33)   |
| Kelp Bed Area Variation (Coefficient of Variation) | 79.09     | 69.62       | 22.30         | 11.27                 | 51.26       |
| (Average for Reds)                                | 19.19(8)  | 17.82(11)   | 4.22(13)      | (1)                   | 8.72(33)    |
| Kelp Red Cover (%) (Average for Beds)             | 47.73     | 39.85       | 69.86         | 55.33                 | 53.66       |
| Standard Error (n)                                | 7.57(8)   | 6.68(11)    | 2.06(13)      | (1)                   | 3.66(33)    |
| Kelp Red Cover Variation (Coefficient of Variation) | 23.07     | 37.09       | 13.90         | 20.31                 | 24.65       |
| (Average for Beds)                                | 4.74(8)   | 10.46(11)   | 1.21(13)      | (1)                   | 3.98(33)    |
next. This distance was measured as straight as possible, at a distance from shore where the kelp beds grow, rather than following all of the undulations of the shoreline. We calculated the bed width as the area divided by the length. This gives an average width across the length of the bed. If a bed only occurs along part of its California Department of Fish and Game designated length, this measure will show a narrow width compared to those sections of the coast where kelp actually occurs.

The following relationships were noted for kelp-bed lengths and kelp-bed widths. The San Diego beds were significant longer than the Santa Barbara beds ($t = 3.46$, d. f. = 19, $p = 0.033$). The Los Angeles beds were significantly longer than the Santa Barbara beds ($t = 3.95$, d. f. = 22, $p < 0.001$). The San Diego beds were significantly narrower than the Santa Barbara beds ($t = 3.34$, d. f. = 19, $p = 0.004$). The Los Angeles beds were significantly narrower than the Santa Barbara beds ($t = 5.67$, d. f. = 22, $p < 0.001$).

Kelp-bed areas and cover also differed for beds in the four geographic areas. Statistical tests of the kelp-bed area means showed that the Los Angeles beds were significantly smaller than the Santa Barbara beds ($t = 2.55$, d. f. = 22, $p = 0.020$). The San Diego bed areas varied significantly more than the Santa Barbara beds ($t = 3.55$, d. f. = 19, $p = 0.003$). The Los Angeles bed areas varied significantly more than the Santa Barbara beds ($t = 2.79$, d. f. = 22, $p = 0.011$). The San Diego beds had significantly lower cover than the Santa Barbara beds ($t = 3.29$, d. f. = 19, $p = 0.043$). The Los Angeles beds had significantly lower cover than the Santa Barbara beds ($t = 4.45$, d. f. = 22, $p < 0.001$). The kelp cover of the San Diego beds varied significantly more than the kelp cover of the Santa Barbara beds ($t = 3.46$, d. f. = 19, $p = 0.033$). The kelp cover of the Los Angeles beds varied significantly more than the kelp cover of the Santa Barbara beds ($t = 3.95$, d. f. = 22, $p < 0.001$).

The kelp harvesting rate varied seasonally as well as from year to year (Figure 2). The lowest monthly average kelp harvesting rate was 12.9 metric tons per hour in December while the highest was 74.6 metric tons per hour in May. The lowest annual average kelp harvesting rate was 40.2 metric tons per hour in 1976 while the highest was 66.8 metric tons per hour in 1973.

The stepwise multiple-linear regression of kelp harvesting rate dependence on environmental variables was highly significant ($p = 0.001$, Table 4.). Three of the independent variables significantly increased the coefficient of determination and so were included in the final regression. Together they accounted for 59.5% of the variation of kelp harvesting rate. High surface irradiance for the prior month increased the kelp canopy available for harvest. Higher swell heights and temperatures for the prior month decreased the kelp canopy. The verification multiple-linear regression resulted in a significant model ($p = 0.001$) based on 76% of the data. The second model accounted for 46% of the variation in kelp harvesting rate, with similar contributions by the environmental variables.

The positive affect that high surface irradiance has on kelp growth is through increasing the photosynthetic rate. The negative affects that high swell heights have on kelp growth are to increase water motion around the plant and increase tissue and plant loss. High temperatures can directly damage kelp, however, since there is a negative correlation between nutrient levels and temperature, some of the negative contributions high temperatures may have on canopy growth can be attributed to low nutrient concentrations.
Figure 14. The rate at which kelp was harvested in the Goleta Bay kelp bed (number 26) from 1973 to 1977. The rate was higher in Spring and early Summer while it was lower in Fall and Winter (from Harner 1979).
TABLE 7. MULTIPLE LINEAR REGRESSIONS OF THE DEPENDENCE OF KELP HARVESTING RATE ON THE AVERAGE OF THE PRIOR MONTH'S PHYSICAL ENVIRONMENTAL DATA

Seven Variable Comparison  
# of Observations = 70

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<th>Correlation Matrix</th>
<th>Surface Irradiance</th>
<th>Wind Speed</th>
<th>Swell Height</th>
<th>Sea Temp. at Holly</th>
<th>Harbor</th>
<th>Bottom Nitrate</th>
<th>Nitrate</th>
<th>Holly Temperature</th>
<th>Sea Temperature at Platform Holly</th>
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Analysis of Variance Table

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<th>MS</th>
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Variables Selected  
Regression Coefficient  
Standard Error  
Contribution

- Surface Irradiance: 0.313  
  0.089  
  19.75 (27.9%)  
- Swell Height: -0.395  
  0.658  
  6.30 (9.4%)  
- Sea Temp. at Holly: -6.240  
  0.676  
  15.00 (22.3%)

Three Variable Comparison  
# of Observations = 195

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Analysis of Variance Table

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Variables Selected  
Regression Coefficient  
Standard Error  
Contribution

- Surface Irradiance: 0.311  
  0.089  
  17.16 (19.9%)  
- Swell Height: -39.355  
  8.658  
  7.18 (8.3%)  
- Sea Temp. at Holly: -6.240  
  0.676  
  15.33 (17.8%)
The results of the present study, based mostly on heretofore unused imagery and kelp-harvesting-rate information, provide a detailed historical record that shows kelp variability within the Southern California Bight as a whole. There are significant differences between kelp beds in different geographic areas, presumably because of differences in exposure to currents and storms. In short term studies of the Goleta Bay kelp bed, one half the variation in the amount of kelp canopy at the surface can be predicted knowing the average solar irradiance, swell height, and water temperature over the last month.

The characteristic shapes of the southern California kelp-beds that one can see today are recognizable even in the nautical charts drawn in the 19th century. Since the depth contours are generally in the same location in these old nautical charts as they are today, so the kelp canopy configurations are probably correct. In re-measuring Crandall's (1912) maps we have found that in light of these old charts they appear in general to be accurate in spite of the contention in the literature that his maps have grossly overestimated the extent of canopy area. The smaller areas obtained by measurements from more recent maps of southern California kelp beds probably reflect both a slight increase in mapping precision over Crandall's methods, and an actual decrease in kelp-bed areas.

We have found significant differences between our measurements of Crandall's (1912) maps and those found in Hodder and Mel (1978). We also found that there are noteworthy differences in the 25-year-mean area that we calculated for each of the beds as compared to the current area values provided by the California Department of Fish and Game. The total mean area we have measured is 5,791.5 hectares while the California Department of Fish and Game cites an area of 8,547 hectares (48% higher).

We have arbitrarily divided the kelp beds of the Southern California Bight into four geographic areas: 1) San Diego, 2) Los Angeles, 3) Santa Barbara, and 4) Santa Catalina Island. The kelp beds in the San Diego area are long, small in area, and have a low cover. The variation in their area and cover is relatively high. They are made up of Macrocystis pyrifera. Most of these beds occur on cobble bottoms and are found far offshore. They are exposed to winter storms from the Northwest and summer storms from the Southwest. During the early 1960's their areas reached a minimum and their areas have increased since then.

The kelp beds in the Los Angeles area are long, very small in area and have a low cover. The variation in their area and cover is relatively high. They are made up of Macrocystis pyrifera that has been transplanted from the offshore islands. These beds were originally made up of Macrocystis angustifolia, as found in the Santa Barbara area, but this all died off in the late 1950's and early 1960's (M. Neushul, personal communication). The Macrocystis pyrifera transplantation work from the offshore islands was performed in an attempt to restore the kelp beds. These beds are mostly on high-relief rock bottoms but are rather rare along the coast. The beds are partially sheltered from the summer storms from the Southwest by the offshore islands.
The kelp beds in the Santa Barbara area are short, large in area, and have a high cover. The variation in their area and cover is low. They are made up, for the most part, of *Macrocystis angustifolia* (for this paper the separation of the two species presented in Neushul 1971 is used rather than calling all the populations *Macrocystis pyrifera* as was suggested in Abbott and Hollenberg 1976). Some of these beds are on sand and some on high-relief rock bottoms, but most are on sand. They are the most stable, being in the lee of the Santa Barbara Channel Islands. Often the beds are sheltered from the storms from the West by being to the East of prominent points of land.

The kelp bed off Santa Catalina Island is very long, small in area, and has medium cover. The variation in its area and cover is low. It is made up of a special variety of *Macrocystis pyrifera* that looks slightly different from the mainland variety. Some of the largest kelp beds in the Southern California Bight occur off the offshore islands, but none of these is off Santa Catalina Island. The beds face all compass directions. The south facing beds are exposed to severe winter and summer storms, while north facing beds are relatively sheltered the year round. The oceanographic conditions are different from those found near the coast of the mainland. These beds occur, for the most part, on high-relief rock bottoms.

Our maps have documented damage to kelp beds due to boat traffic near piers and harbors. However, many of the disputes about damage done to the kelp beds by kelp harvesters, by nearshore sewage pollution, and by other human activity (dredging, thermal effluents, etc.) have been prompted by the gradual disappearance of kelp beds earlier in this century. This disappearance has been attributed to the direct effects of sewage (or DDT), sewage enhanced populations of sea urchins, natural warm-water years, or natural low-nutrient years (Jackson 1977). In the present studies, it was difficult to determine which of man's activities was damaging to kelp beds and how damaging they were. Each activity is probably damaging to kelp in different places and at different times. Our short-term monitoring of the Goleta kelp bed has shown that natural kelp growth is seasonal, being affected by seasonal climatic variables, and varies from year to year. Unless one monitors and determines what the natural variation is and what causes it, one cannot determine which changes are man-induced and which ones are natural. This study has provided some new insights into the natural variation of kelp beds in southern California and what causes that variation.
I would like to acknowledge Dr. M. Neushul, Dr. J. W. Woessner, and Mr. G. A. Brosseau, who did much of the kelp-bed mapping work. This work was supported by Southern California Edison Company (Contract C2000903 to Neushul Mariculture Incorporated) under the direction of Mr. Jay N. Stock and Dr. John Palmer. Many persons and agencies generously made their aerial imagery available to us for this work, without which we could not have done it.

Mr. B. Szylenyi of Stauffer Chemical Company was helpful in providing their kelp harvest information for the Goleta Bay kelp bed. The Atlantic Richfield Company kindly provided the sea temperature data from Platform Holly. Mr. D. A. Coon, Dr. J. W. Woessner, and Dr. W. N. Wheeler assisted in phases my dissertation work that, in part, analyzed the kelp harvest information (see Harger 1979). This work was directed by Dr. M. Neushul of the Marine Science Institute at the University of California at Santa Barbara. It was supported, in part, by grants to Dr. Neushul from the National Science Foundation (NSF GH 43, GH 95 and GA 27484) and the U. S. Department of Commerce, Office of Sea Grant (R-FA-10).


Dana, R. H. 1941. Two Years before the Mast. 235 pp.


C. THE MARINE ENVIRONMENT AND FARmed KELP

It has become very clear that the kelp plant is a complex one, physically spanning the full range of conditions found from the sea floor to the sea surface, and surviving through seasonal changes for several years as a perennial plant. In order to measure the effect(s) of the environment on farmed macroalgae, we made daily environmental measurements at Goleta and Ellwood piers, starting in 1980. In addition to the daily measurements, detailed weekly measurements were made at three depths. These measurements included the weight of sediments in collecting tubes and weekly maximum and minimum temperatures. The divers also recorded horizontal visibility, using secchi disks, and collected water samples for nutrient analysis. When these daily and weekly data are combined with information collected by Harger (1979), they comprise regularly-collected and analyzed environmental data spanning nearly two decades. The total environmental data-base for the GRI/SERI project from 1980 to the present consists of 52 files. The computerized data has all been checked against the original hand-recorded data for errors in transcription. Computer-based processing makes it possible to analyze and plot all of this information.

These data indicate that wave activity is generally greatest in Winter, decreases in Fall and Spring and is lowest in Summer. However, 1984 was an exceptionally calm year, and in contrast, very large winter waves occurred in 1983, resulting in extensive damage to Ellwood Pier, the location of our experimental marine farm. In February 1986, severe storm waves were again recorded. It is interesting that large summer storm waves were also measured in 1981 and 1985. Wave periods appear to be stable year-round, except during storm periods, when they become longer during both winter and summer storms.

As these measurements taken at the surface, in mid-water and at the sea floor show, conditions can change greatly as one progresses from the surface to the sea floor. Light measurements show this clearly, as do measurements of turbidity and sedimentation. Chemical measurements (made by flow injection analysis, CHN analysis, Mannitol content analysis etc.) reveal less-obvious differences, since one cannot see nutrients in the water, or get more than a vague idea of the chemical composition of a plant from its level of pigmentation.

The physiological ecology of Macrocystis

The physiological ecology of the kelp plant, and theoretical aspects of kelp growth and production are discussed at length in our 1983-4 annual report, where prior work by Tont, Black, Harger, Lindner et al. and others is reviewed. A more recent paper, by K. E. Arnold and S. L. Manley on carbon allocation in Macrocystis reports work done with GRI support, that shows that different tissues taken from a single blade, and tissues from different blades had a coefficient of variability, in photosynthetic capacity, approaching 50%. They also show that the relative amounts of photosynthetic versus structural (respiratory, but not photosynthetic) tissues, produce this variability. Wheeler, Smith, Lobban, Kremer, Willenbrink and others cited by these authors have used "representative" samples in their measurements, and these have
sometimes been used to extrapolate production rates. Clearly, this can only be done if more than a few such samples are used.

Our studies of kelp yield focused first on the rates (tons per hour) at which kelp was harvested from the Goleta Bay kelp bed. This could be as much as 109 tons per hour. Peak harvests occur in May, June and July, which are the months with the highest light levels. The highest nutrients are found earlier, in March and April when upwelling occurs. The time lag that when nutrients are highest, (March-April) to the time when record harvests are made (June-July) is obviously one of rapid growth and development, when the nutrients taken up earlier must be translocated, perhaps stored, and then re-distributed and used to produce new tissue. These processes are no-doubt triggered by environmental factors, and regulated by internally produced and distributed plant growth regulators (like cytokinin).

If we could find out what stimulates frond initiation and growth in these plants, it might be possible to farm them more effectively. For example when grid and row-plantings were compared in 1984, we found that often under adverse conditions that prevailed, there could be close to 100% survival of cultivated plants, but no production. The plants just seemed to "sit" on the sea floor, "refusing" to initiate new fronds. This "dormancy" phenomenon was also seen by M. Shivji, working with the Marine Botany group at U.C.S.B., who found that whole sporophytes exposed to high nutrients, but low light levels, increased in pigment and caloric content, but did not grow as rapidly as plants at higher light levels. Shivji did not measure mannitol or CHN ratios in his plants.

As noted earlier, as a continuation of work started with GRI support, Mr. R. Lewis, a member of the Marine Botany Group at UCSB, employed a new enzyme immunoassay to make measurements of the plant hormone, zeatin riboside (a cytokinin) in Sargassum muticum. The plant assayed contained 79 nm of this zeatin riboside per kilogram of fresh weight. Commercial seaweed extracts were also assayed and contained levels ranging from 7 to 21 nm/l. This is the first time that monoclonal antibodies have been used to measure and detect cytokinins in seaweeds and seaweed extracts. This approach should make it possible to approach the question of how hormones regulate growth and development in Sargassum and perhaps Macrocystis as well.

A major objective of the hemidome experiments undertaken by G.E., and Caltech, was to simulate the center of a large, open-ocean farm where low light levels and high nutrient levels might be found. Unfortunately the plants in the hemidome were infected by damaging bacteria and epiphytized by unusual bryozoans and other organisms, and did not grow. Fortunately the nearshore test-farm experiment can also be used to simulate a larger-scale farm, without the deleterious "container effects." Because the system was an open one, it was not always possible to control the nutrient levels around the plant to any great extent. However when a surface canopy had developed, and when near-surface vegetation was present, the experimentally-applied fertilizer was retained, as though there were a "perforated vegetable-equivalent" of the hemidome operating. For this reason, experimental fertilization at some times produced a significant increase in yield, and as the results shown below illustrate, also may have influenced the chemical composition of the plants.
It is particularly interesting to compare the production and the wet-to-dry weight ratios of tissues sampled in plants grown at the three different densities used. The chemical composition (CHN ratio and Mannitol content) of low, medium and high-density plantings can also be compared with season, in fertilized and unfertilized planting plots, to give some clues as to how plants under light stress (in the dense plantings) and at low nutrient levels respond. The tedious work involved in launching a boat, mixing fertilizer and spraying the kelp every working day is justified by the data obtained, which allows one to see the differences in plant composition between the fertilized and unfertilized plots, at different seasons and at different planting densities (Tables 8, 9 and 10).

The many figures (Fig. 16-47) illustrating the daily and/or weekly measurements of environmental conditions might be considered to be "padding" by some. However they provide the background against which field-experiments, like the above-mentioned in-the-sea fertilization tests, must be viewed. The graphs are arranged so that one can simply look down the page, to compare similar seasons for a six year period. It is also possible to plot them together, as for example when comparing kelp harvests year-by-year (compare figure 14 and 15c). Of course, these visual comparisons can also be made statistically, using multiple regression analyses. Harger (see Chapter IV, part B) has used environmental data of this sort to examine the effects of antecedent events on the productivity of natural kelp beds. The same can be done with the large NMI data base.

The environmental data graphs first show air temperatures, to give an idea of the basic seasonal pattern of change, then water temperatures are presented. Here direct measurements were made of water-samples taken from the surface, mid-water and bottom water, to show stratification. These were taken daily, and also on a weekly dive, where maximum-minimum thermometers and sediment traps were recovered and replaced. This detailed temperature analysis provides an opportunity for internal verifications, and illustrates the advantages of "fine scale" measurements.

Daily wind-speed measurements, and records of wave heights and periods, give a good picture of seasonal variability in water turbulence. Of course the major storms of the El Nino period are clearly shown, one of which (1983) tore out 1,000 acres of kelp in Goleta Bay, and 100 ft off the end of Ellwood Pier. The staff member who recorded values just before the pier was destroyed was recognized for his bravery. Sediment values, and water visibility measurements illustrate turbulence and stratification. Vertical visibility can be transformed into light attenuation estimates, and horizontal visibility measurements at surface, mid-water and bottom depths, clearly show stratification and may also be relatable to nutrient levels recorded at different depths. These chemical measurements for nitrate, ammonia and phosphate in water samples taken weekly, give a very specific history of nutrient levels around the plants, and are particularly useful in evaluating the percent dry weight, mannitol, and nitrogen values for kelp samples (blades, vesicles and stipes) taken during field experiments. It is also possible to relate seawater and plant chemistry to frond production rates and yield, using this new data base. By knowing how the kelp plant responds to its environment, new and more effective farming strategies can be developed in the future.
Figure 15. The availability of light (top) and nitrate (middle) affects the amount of kelp that could be harvested from one thousand acres of kelp in Goleta Bay (shown in figure 9). This illustrates that the time of greatest production (May-June) corresponds to the time of peak light intensity, and is preceded by a period of upwelling and nutrient enrichment. Seasonal variation in growing conditions influences yield in a natural kelp forest. The Stauffer Chemical 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).
Figure 16. NMI 1980-86 air temperature measurements illustrate summer warming, and the sub-tropical climate of the region.
Figure 17. NMI 1980-86 surface water temperatures, taken daily, show that as with air temperatures, water warms in the summer. However, pulses of cold water were recorded for comparatively brief periods, coinciding with winds, upwelling and nutrient enrichment (for example, see March and May 1981).
Figure 18. NMI 1980–86 middle water temperature measurements, made daily with a Van-Dorn water sampler, do not show temperature stratification in the water column at these shallow sites.
Figure 19. NMI 1980-86 bottom water temperature measurements made daily with a Van-Dorn water sampler, do not show temperature stratification in the water column, while sedimentation and visibility did vary with depth, as other measurements show.
Figure 20. NMI 1980-86 records of surface water temperatures, taken weekly, show summer warming and winter cooling, as expected.
Figure 21. NMI 1980-86 weekly middle minimum temperature, like the daily measurements, show no stratification.
Figure 22. NMI 1980-86 weekly records of bottom water temperatures, showing less seasonality than seen in surface waters.
Figure 23. NMI 1980-86 weekly records (made with a max-min thermometer) of surface maximum temperature, gives a record comparable with the daily record, but without the fine detail that catches short-term upwelling events.
Figure 24. NMI 1980-86 weekly surface minimum temperature records, made with a max-min thermometer, can show that cold water did occur but gives less time resolution than the daily measurements.
Figure 25. NMI 1980-86 weekly records of middle maximum temperature, is comparable with daily records.
Figure 26. NMI 1980-86 records of mid-water temperatures, made with a maximum-minimum thermometer, showing the expected seasonality.
Figure 27. NMI 1980-86 weekly records of bottom maximum temperature, is comparable with daily records, and also shows no striking stratification.
Figure 28. NMI 1980-86 weekly bottom minimum temperature, like daily measurements, show no stratification, but make it possible to produce a "temperature envelope" for the waters of the region.
Figure 29. NMI 1980-86 wind speed measurements can be visually compared and show storm winds as high as 40 mph, and that windy periods are more common in the first quarter of the year, while summer winds are usually below 10 mph. Wind speeds influence waves and the upwelling of nutrients.
Figure 30. NMI 1980-86 daily wave height measurements made with a wave staff, clearly illustrate the major storms, that usually occur in the first quarter of the year, and show stormy winter and calmer summer conditions.
Figure 31. NMI 1980-86 daily wave period measurements made with a wave staff and a stop watch, show no striking seasonal pattern as with wave heights. Long period waves are seen when both temperate and tropical storm waves impact the coast.
Figure 32. NMI 1980-86 sedimentation rates were determined from weekly collections of sediment from collection tubes held at three depths. The weekly surface collections, show high- and low-sediment seasons.
Figure 33. NMI 1980-86 sedimentation rates from mid-water collection tubes show more sediment than collected closer to the surface.
Figure 34. NMI 1980-86 sedimentation rates from tubes placed near the sea floor, show the highest levels of sediment, illustrating the entrainment and re-suspension of sediment by wave- and current-induced water motion.
Figure 35. NMI 1980-86 made vertical visibility measurements daily using a secchi-disk, showing that warmer summer water was generally clearer, but that short-term pulses of clear oceanic water were encountered even during the generally more turbid winter months. These measurements can be correlated with upwelling, and nutrient and plant chemistry.
Figure 36. NMI 1980-86 made horizontal visibility measurements weekly by measuring the maximum distance at which a secchi-disk could be seen. Comparisons between surface, middle and bottom visibility clearly show the combined effects of decreased light and increased turbidity as one approaches the sea floor. The turbidity measurements can be related to sediment trap collections.
Figure 37. NMI 1980-86 made horizontal visibility (Secchi-disk) measurements in mid-water, which were generally less than at the surface.
Figure 38. NMI 1980-86 made horizontal visibility (secchi-disk) measurements near the sea floor, illustrating the generally-turbid waters there.

BOTTOM HORIZONTAL VISIBILITY (m)

MONTH
Figure 39. NMI 1980-86 measurements of nitrate concentrations from surface water samples, show major pulses of enrichment in March and April, except during 1983, the nutrient-drought year, and 1986, which has been very calm.
Figure 40. NMI 1980-86 measurements of nitrate in mid-water samples, show that when surface waters were low, mid-water was enriched, and more clearly show the March-April enrichment occurs every year.
Figure 41. NMI 1980-86 measurements of nitrate in bottom-water samples, show that pulses of nutrient-rich water usually occur in March and April, and with less regularity in October and November corresponding to the classical pattern of Spring and Fall enrichment of surface waters associated with the loss of water column stability.
Figure 42. NMI 1980-86 measurements of ammonia made from water samples collected weekly by divers, shows patterns of variation that are not clearly related to upwelling, but may provide a measurement of regenerated nutrients in the nearshore region.
Figure 4.3. NMI 1980-86 measurements of ammonia in mid-water are higher than those near the surface, and occur at the same times, suggesting regeneration of nitrogen from the sea floor.
Figure 44. NMI 1980–86 measurements of ammonia from samples taken weekly, show the highest ammonia levels again suggesting that regeneration may be occurring at or near the sea floor, where particulates, and presumably bacterial populations on them and on the sea floor, are involved in the breakdown of organic matter and the release of ammonia.
Figure 45. NMI 1980-86 measurements of phosphate show a less distinct seasonal pattern than is seen with nitrates. The occasional peaks are difficult to explain, nonetheless surface and mid-water levels are lower than bottom water levels, as the following figures show.
Figure 46. NMI 1980-86 measurements of mid-water phosphate concentrations are somewhat higher than those at the surface, and show the same general pattern.
Figure 47. NMI 1980-86 measurements of phosphate concentrations near the sea floor show the highest levels, suggesting that some regenerated phosphate is being produced and re-cycled.
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% Dry Weight Data for Vesicles

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Table 10. C : H : N % Nitrogen Data for Blades

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C : H : N % Nitrogen Data for Vesicles

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C : H : N % Nitrogen Data for Stipes

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V. GENETICS AND ALGAL PRODUCTION

A. BIOMASS PRODUCTION BY MARINE CROPS:
GENETIC MANIPULATION OF KELPS

M. Neushul, Ph.D.
Professor of Marine Botany
Department of Biological Sciences
University of California, Santa Barbara, 93106

ABSTRACT

The purpose of this paper is to call attention to the fact that the large scale use of kelps for energy and chemical feedstock production began in California in 1914, and then to consider efforts made since to cultivate these unique plants in the sea, particularly where these have involved genetic selection and hybridization. It has been found that cultures of the microscopic gametophytic phase of the kelp life history can be maintained in liquid culture for many years. As reported here for the first time, cultures isolated from single spores (i.e. monoclonal cultures) ten years ago, have been tested and were found to still be capable of producing gametes that after fertilization give rise to normal sporophytic plants, suggesting that genetic drift is minimal. New methods for collecting kelp spores and for applying mutagens and screening for variants have been developed and are described. Interspecific and intergeneric hybrids have been made, showing that Macrocystis species from Alaska and Mexico are interfertile, and that all three species of Macrocystis from California can be intergenerically hybridized with Dictyoneurum. These results show that the genetic manipulation of these plants via mass- and pedigree selection, mutagenesis and screening of variants is very likely to produce unique, and patentable gametophytic strains that can be crossed to produce high-yielding sporophytes with valuable characteristics.

INTRODUCTION

The implications of genetically manipulating giant kelps are beginning to be appreciated, and reliable methods for handling these
unique plants are being developed. A great deal has been learned in the past few years, as a result of work done both in the U.S. and abroad. The Gas Research Institute has encouraged and funded collaborative research on kelp for several years (15), that has involved visits by U.S. Scientists to Japan and China, as well as work by visitors from China, Japan, and elsewhere (1,2,13,33,39,41) in my laboratory at the University of California Santa Barbara on various aspects of kelp biology. I am also privileged to be working at present with a number of outstanding graduate students and Post-Doctoral visitors who have made and are presently making significant contributions to our knowledge of kelp biology (19,30).

Support from the National Science Foundation and the Chinese Ministry of Education made it possible to initiate collaborative genetic studies at Santa Barbara in September 1984 with the pioneering Chinese geneticist, T. C. Fang, whose death in July 1985, in China, was a great personal and scientific loss. Professor Fang, who worked collaboratively with N. N. Kiang (Mrs. Fang) and others was the first person to produce genetically-distinct kelps, and to show that the mass-selection and breeding of these superior strains has a very significant impact on the yield, the temperature tolerance and the iodine content of plants farmed in the sea (8,9,10,11,12). This paper is dedicated to the memory of Dr. Fang (Figure 1).

BACKGROUND

The first large-scale use of float-bearing macroalgae for energy and chemical feedstocks began in California in 1914, when the Hercules Chemical Company signed a contract with the British government to produce acetone for the manufacture of cordite, and potash for black gunpowder to be used in the First World War (28). They harvested nearly 400,000 wet tons of kelp from the offshore kelp forests of California and fermented this biomass in a large on-shore plant on aptly-named Gunpowder Point near Chula Vista, California. At this time the method by which kelp reproduced was still unknown, however one year after the Hercules effort began, Savageau (34) discovered that the kelps had a heteromorphic life-history, involving a microscopic sexual stage and a macroscopic spore-producing, sporophytic stage. Gametophyte development, gametogenesis and fertilization has been studied by a number of workers who have considered both the chromosome numbers involved (3,4,5,38,39) and the potential use of tissue culture (40, 32,18) as well as the effects of hormones on gamete release and fertilization (20). Clearly much has been learned since the turn of the century.

The experimental manipulation of the kelp life-history, and the experimental cultivation of the giant kelp, Macrocystis in the sea began in 1956 in the laboratory of F. T. Haxo at the Scripps Institution of Oceanography in La Jolla, California (22). Speculation about possible intergeneric kelp hybrids that were found in the sea by
Figure 48 The late T. C. Fang collecting marine algae at Piedras Blancas Point on the California coast.

Figure 49 K.N. Kiang (Mrs. Fang) operating a microspectrophotometer used with fluorochromes to quantitatively measure DNA levels in kelp gametophytes and sporophytes (G).
the author and James Stewart while diving near the La Jolla submarine canyon at this time, (24) were confirmed after collaborative work with Y. Sanbonsuga when intergeneric hybrids were made in the laboratory from cloned gametophytes (33). It is now known that intergeneric hybrids between the multi-vesiculate genus Macrocystis and the single-vesicle-forming genus Pelagophycus are commonly found in mixed populations of these two very different plants (7). Subsequent hybridization experiments with these and other kelps (25, 19) have shown that it is not difficult to make intergeneric and interspecific kelp hybrids, using cloned gametophytes. It may also be possible to produce, select and hybridize mutant clones.

It is known that ultraviolet light induces mutants in the eucaryotic marine alga, Ulva (14) and that the giant kelp, Macrocystis can be damaged by ultraviolet light (29). Recent work in my laboratory by Chai (1) and unpublished work by the late T.C. Fang, has suggested that kelp spores can be mutagenized by exposure to ultraviolet light. Post-exposure treatments showed that photoreactivation occurs. Chemical mutagenesis of kelps also seems possible. The production of mutants in the red alga Gracilaria has been achieved by van der Meer (36) using ethylmethanesulphonate (EMS). His treatment times ranged from 0 to 100 minutes, with spores being killed after 90 minutes exposure to a 0.2m solution of EMS. Many green and pink color variants were produced and shown, through tetrad analysis, to be true mutants.

The historical record (28) as well as recent advances in macroalgal mariculture in the United States, China and Japan, clearly indicate that marine macroalgae can be used for large-scale production of both chemical feedstocks and energy (21,26,35). Those familiar with the recent spectacular advances made in morphogenetically manipulating land plants (37) will find morphogenetically-abnormal kelps (17) and kelp hybrids (33,25) (Figures 3-6) to be provocative. It seems likely that the application of modern genetic techniques to marine macroalgae (14) may allow us to genetically control morphogenesis in these plants, which would have a significant impact on the economics of both near-shore and off-shore farming (26).

As the findings discussed here will show, we have learned how to select and culture specific kelp germ lines, and hold these in laboratory culture for ten years, after which they are still capable of producing seedstock for outplanting in the sea. We have also found that these germ lines can be used to produce interspecific and intergeneric hybrids of plants from as far away as Alaska and Baja California. This suggests that we will not be limited to working with the morphological and physiological characteristics of a single kelp species. Preliminary results discussed below suggest that mutant lines can be produced using physical and chemical methods. These findings lead us toward the conclusion that those who develop, maintain and ultimately license the use of specific macroalgal germ lines, are likely to be rewarded for their efforts.
MATERIALS AND METHODS

In the present study we have employed methods for the cultivation of gametophytes in the laboratory and their hybridization developed by Sanbonsuga and Neushul (33). Methods employed for the planting, cultivation and harvesting of the large kelp sporophytes in the sea, are those reported by Harger and Neushul (16) and Neushul and Harger (27). New methods have been developed for the collection of kelp spores, and for the application of mutagenic agents to sporangia and spores and determining their effects. These are discussed here.

Kelp Spore Collection

Since spores of all the kelps are produced in sporangia that form extensive sori, special attention has been given to developing methods for exposing these sporangia to mutagens and to collecting spores from them. Small plastic capsules (called Beem-capsules) used for embedding materials for electron microscopy, were held in a plastic framework consisting of centimeter squares, one centimeter thick, so that the open end of each capsule was pressed against the sorus. For Macrocystis this apparatus was roughly the same size as a sporophyll, so that a single sporogenous blade could be held against a row of open capsules. Before being pressed against the sporophyll each capsule was filled with filtered seawater. The capsules, capsule holder, and sporophyll were then clamped between two solid plastic sheets, held together with screws. By compressing the sporophyll-capsule-framework sandwich, spores from a given position along the long axis of the sporophyll were released into a specific capsule. After a period of spore release, the collection apparatus was removed from the water, allowed to drain and then carefully disassembled so as not to disturb the contents of the capsules, which were removed and capped. Subsequently the contents of the capsule was stirred with a glass pipette to suspend any settled spores and a hemocytometer was used to count the numbers of spores present in 1 ml samples taken from each capsule.

Ultraviolet-Radiation Mutagenesis

Spores were collected from mature sporophylls that had been abscised from plants in the sea and held in seawater-supplied aquaria. These were wiped clean with paper towels and then placed in an American Scientific Products, ultrasonic cleaner (ME 4.6) and given three five-minute periods of sonication in filtered seawater, to remove surface epiphytes. The sporophylls were then held in darkness at 10 deg. C, for two hours. Pieces of cleaned sporophyll were placed in pyrex culture dishes in Provasolli's enriched seawater (1,33) and held for 10-20 minutes in the dark, during which time spores were released. The spore-containing medium was then poured into shallow plastic petri dishes containing 18mm square microscope cover glasses, onto which the
spores settled and attached. Both radiation- exposed and control spore populations were fixed, stained and made into permanent slides, by fixing first in 5% formalin in seawater, washing and then staining in 1:10 alizarin viridine in distilled water. The cover glasses were mounted on slides in an aqueous mounting medium (Permount). Exposure to ultraviolet light was accomplished at room temperature in a specially-fabricated box where a General Electric G8T5 254 germicidal ultraviolet lamplight source was suspended above the culture dishes, containing the settled spores, at distances of 20 and 26cm. The tube was masked so that only a 4cm length was exposed. Times of exposure varied from 1 to 10 minutes. After irradiation cover slips were moved into new petri dishes containing fresh medium, and held at 17 deg C, under 52 uE/MF sq. per sec. light, after a 24 hour period in the dark to prevent photoreactivation. After a growth period of two weeks, germinating spores were counted under a Zeiss dissecting microscope. The number of germinating spores growing into gametophytes was counted in a spore-germination-assay, to measure the effect of ultraviolet radiation and the study the process of photoreactivation.

Chemical mutagenesis

The chemical mutagen ethylmethansulfonate (EMS), was dissolved in Provasol is enriched seawater plus iodine (PESI) to make a 0.2M solution and placed in the wells of a 24 well culture dish. Disks, 5.5mm in diameter, were cut from the mature portion of a Macrocystis sporophyll with a cork borer and placed in the EMS solution for 10, 20 and 60 minutes and then removed and washed in PESI containing 0.1% sodium thiosulfate to neutralize the EMS. The treated disks, and untreated controls, were then transferred to a new 24 well dish with fresh PESI medium and a cover slip in each well and held for 1.5 hours during which time spores were released. Germinating spores on the cover slips were counted after two days of growth, using a spore-germination assay. The disks were then transferred to 0.3% or 1% agar plates for four days to allow further spore release. A simple spore-dispersal assay was used wherein measurements were made of the distances that spores swam out into the semi-solid agar from both the mutagen-exposed, and control disks of sporogenous tissue.

RESULTS AND CONCLUSIONS

The maturation of sporangia along a sporophyll offers a series of developmental phases where mutagens can be applied. Sporangium maturation has been studied ultrastructurally (2) and the meiotically dividing sporangium and the haploid spores produced both seemed a likely places to apply physical and chemical mutagens for maximum effect. Moreover it was felt that a simple, direct spore-killing curve could be established and that lethal damage to the haploid genome of the spore would be immediately expressed in the gametophyte, thereby screening out recessive lethal mutants that might not be evident in a diploid life-history phase.
As might be expected, maximum spore release occurred about midway between the base of the sporophyll where sporangia are forming to the tip, where spent sporangia are found. Between 250,000 and 400,000 spores were released per ml from the most mature sporangial regions. A total of 32 are released from each mature sporangium (Figures 3 and 4). The application of spore-germination and survival assays show that ultraviolet light was most damaging to spores from the basal and terminal regions of the sporophyll. A one-minute dose from a masked tube, with 4 cm exposed, 26 cm above the shallow culture dish containing the attached spores, inactivated 50% of the spores, while 92-98% of the unexposed control spores germinated. Increasing exposures of 3, 5, 7 and 10 minutes resulted in decreased rates of germination, and although the spores would survive they would not form normal germ tubes. Chai (1) found that spores treated, and presumably damaged by ultraviolet light could be photoreactivated by holding them in the light after UV exposure.

Spore-germination and survival assays, and a spore-dispersal assay were used to measure the effects of the chemical mutagen, ethylmethanesulfonate (EMS). Unfortunately pigmentation mutants, as were produce by EMS treatment of red algae (36) were not found. Swimming spores were observed around sporogenous disks exposed to 10 and 20 minutes in EMS, while no swimming spores were found after 60 minutes in EMS. In the spore-dispersal assay spores were observed nearly 9 mm away from the disk, while 10 and 20 minute exposure gave 2-3 mm dispersal zones. The disks exposed to 60 minutes of EMS had spores less than 1 mm around them, presumably due to physical shaking of the disk in the soft agar. As with ultraviolet exposure increasing mutagenic dose produced decreasing amounts of germination. Ten minutes of EMS resulted in 65% germination, while 20 minutes allowed only 4% germination. EMS treated germlings were stunted and mishapen. Nonetheless by careful searching 16 female and 49 male clones of EMS-exposed, but nonetheless growing, gametophytes were isolated and are now in clonal culture.

Tables 1 and 2 (on the following pages) show the results of intraspecific and intergeneric hybridization of kelps using clonal gametophytic stocks. The intraspecific results (Table 1) show that Macrocystis from Alaska and from Mexico are interfertile, extending the observation that all three of the species found in California will cross (19). It is notable that even some very old cultures are still capable of being crossed, illustrating the long-term stability of these clones. It is also interesting that while the massive kelp sporophytes can be grown in quantity only in the sea, and are considered to be perennial, they are very susceptible to storm and nutrient-drought damage, while the microscopic gametophytic strains, once thought to be ephemeral are in fact under laboratory culture conditions long-lived and perennial. These clones, not plants in the sea, now serve as a gene bank, much like a seed collection does for land plants.
### TABLE 11. HYBRIDIZATION OF CLONED MACROCYSTIS GAMETOPHYTES FROM VARIOUS LOCATIONS

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</tr>
<tr>
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1. Sporophytes very small, possibly abnormal.
2. A few small sporophytes observed after 28 days.

Note: Male and female gametophytes were isolated from Macrocystis angustifolia from Goleta, in October 1975, and clonally maintained for ten years. The male gametophyte strain (Ma-K) did not produce spermatozoids, but the female (Ma-K: Female 6) was still capable of gametogenesis and produced viable sporophytes.

<table>
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<td>M. integrifolia</td>
<td>Mi-Q: Female 1, Male 9</td>
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<tr>
<td>M. integrifolia</td>
<td>Mi-S: Female 2, Male 4</td>
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</tr>
<tr>
<td>M. pyrifera</td>
<td>Mp-C: Female 1, Male 5</td>
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</tr>
<tr>
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<td>Ma-I: Female 1, Male 1</td>
<td>Santa Barbara, California</td>
</tr>
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<td>M. angustifolia</td>
<td>Ma-K: Female 6, Male 1</td>
<td>Santa Barbara, California</td>
</tr>
<tr>
<td>M. pyrifera</td>
<td>Mp-A: Female 1, Male 1</td>
<td>Santa Catalina Island, Calif.</td>
</tr>
<tr>
<td>M. pyrifera</td>
<td>Mp-H: Female 10, Male 4</td>
<td>Baja California, Mexico</td>
</tr>
<tr>
<td>M. pyrifera</td>
<td>Mp-Ch: Female 1, Male 1</td>
<td>Baja California, Mexico</td>
</tr>
<tr>
<td>FEMALE</td>
<td>Mi-I</td>
<td>Ma-I</td>
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<tr>
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<tr>
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</table>

**Macrocystis gametophytes**

- *M. integrifolia:*
  - Mi-I: Female 3, Male 4

- *M. angustifolia:*
  - Ma-I: Female 2, Male 1

- *M. pyrifera:*
  - Mp-A: Female 1, Male 1
  - Mp-C: Female 1, Male 5
  - Mp-Ch: Female 1, Male 1

**Other kelp gametophytes**

- *Dictyoneuropsis reticulata:*
  - Dr-A: Female 9, Male 5

- *Nereocystis leutkeana:*
  - Ni-A: Male 1

- *Dictyoneurum californicum:*
  - Dc-A: Female 2

- *Pelagophycus porra:*
  - Pp-B: Female 3, Male 2
  - Pp-C: Female 3, Male 2
The intergeneric crosses made in this study (Table 2) show that a female strain of the strap-like, intertidal kelp Dictyoneurum californicum will cross readily with all five species of Macrocystis male plants tried, thus adding a new genus to the other two (Pelagophycus and Nereocystis) that have been crossed with Macrocystis (7,33).

It is particularly interesting to examine the process of intergeneric hybridization in some detail. For example, crosses between Dictyoneuropsis and Pelagophycus (Figures 5-8) are distinguishable from selfed Dictyoneuropsis at a very young age. It is also noteworthy that the sporophytes that are produced in these and other kelp breeding work remain attached to the female gametophyte from which the fertilized egg was produced, making a back-selection process possible, where a gametophytic strain giving rise to an abnormal sporophyte could be identified, isolated and cloned, without having to go through the time-consuming task of raising the sporophyte to maturity.

The potential of being able to clone gametophytes from high-yielding strains of Macrocystis have been considered (27) and appear to be considerable, since high-yielding strains have been found to produce two- to three-fold more biomass than average. Of course the realization of this potential will depend on successful isolation and cloning of gametophytes, and on improved field cultivation practices.

Several basic questions about the chromosome numbers and ploidy levels in the nuclei of both gametophytes and sporophytes remain unsolved, however the use of fluorochromes coupled with microspectrophotometry (Figure 2) appears promising. It is particularly interesting to consider the implications of gene-expression and repression in plants where the same genome produces a complex multicellular plant on one hand, and a simple filamentous one on the other.

In conclusion it seems clear that selection, mutagenesis, and the isolation and storage of gametophytic clones that can be hybridized, will make it possible to recombine specific genomes to produce new plants with high-yield, high-temperature-resistance, or perhaps even plants capable of producing specific chemicals, like the oligosaccharines (37) that will be of value as plant growth regulators. The development of large-scale nearshore, and perhaps even open ocean farms, where float-bearing kelps are cultivated, would seem to be well within the realm of possibility at the present time, raising the possibility that once again we will have extensive coastal facilities like those developed early in this century by Hercules Chemical Company, for producing energy and chemical feedstocks from kelps.

ACKNOWLEDGMENTS

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and the Chinese Ministry of Education. Invaluable technical support was provided by D. C. Coon, B. W. W. Harger and R. L. Lewis.

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Figure 50. *Macrocystis* sporangia contain 32 spores, which are released in a packet, as the thick-walled end of the sporangium dissolves.

Figure 51. After spores are released, they tend to remain either in a single clump or in chains, as shown here.
Figure 52. Male (thin) and female (thick, lower right) gametophytes can be vegetatively cloned, and grown separately. When mixed together sexual reproduction occurs and sporophytes with regularly-arranged small cells are formed. Normal, oblong sporophytes of Dictyoneuropsis are shown here.

Figure 53. When a female Dictyoneuropsis gametophyte is crossed with a male Pelagophycus line, as shown here, the sporophytes are not normal in shape.

Figure 54. A normal Dictyoneuropsis sporophyte, about 7mm long is shown. Traces of the parental gametophyte can be seen adjacent to the root-like holdfast.

Figure 55. An intergeneric Dictyoneuropsis x Pelagophycus hybrid, about 4mm long shows the characteristic rounded, or penny-like phenotype.
B. MARINE FARM ENGINEERING: GENETICS AND ALGAL PRODUCTION

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ABSTRACT

Marine farms in the Orient presently produce crops valued at nearly one billion dollars per year. The farms now in use in China, Japan and the Philippines have been developed mainly by trial-and-error, and the crops grown have been developed from wild stocks by genetic selection. It is now possible to use sophisticated hydrodynamic measuring tools to select optimum sites for farming and for measuring farm performance. It is also possible to produce and hold genetically-defined algal strains and derive hybrid plants from them. Marine plants grown on commercial and experimental farms are very productive. This suggests that the marine farms of the future and the crops grown on them, if properly engineered, will be a new source of fuels, feedstocks, food and perhaps even valuable pharmaceuticals and agrichemicals.
I. Introduction

According to Webster's New Collegiate Dictionary, an engineer is: "A person who carries through an enterprise by skillful or artful contrivance." The process of engineering is "The application of science and mathematics by which the properties of matter and the sources of energy in nature are made useful to man in structures, machines, products, systems and processes." The marine farms that exist today, in China, Japan and the Philippines, are "intuitive" and have been developed by trial-and-error methods, rather than through the application of science and mathematics. They are not "engineered."

The purpose of this paper is to briefly consider the past history and present status of marine farming, to provide an introduction to the present market for marine-farm products, and to discuss some experimental marine farms that have recently been designed. Some of the technical barriers facing the marine farm engineer and marine crop "genetic-engineer" are introduced.

II. The History and Present Status of Marine Farming

While agriculture began at least 10,000 years ago, the cultivation of aquatic plants and animals is much more recent. There are records of Chinese carp culture as early as the 5th century B.C., and the Japanese farmed oysters as early as the 3rd century B.C. Other organisms have been domesticated and farmed even more recently. The Japanese cultivation of nori (Porphyra), the most valuable marine crop at present began in a primitive way in Tokyo Bay in 1736. Laminaria, the largest-yielding crop, was introduced to China from Japan in 1927. Chinese marine farmers now grow one million tons of this crop every year.

The large marine macroalgal farms covering thousands of acres of sea surface, have been established in the last thirty years. The success of these farms in Japan, China and the Philippines is attributable to: 1.) Knowledge gained about the plant life-histories, so that the plants can be manipulated to produce seedstock at will, and 2.) Knowledge of the nutrients required to grow a sizeable crop, and the development of local and world markets for the products of these vast farms provided the economic incentive to move rapidly from experimental, to commercial scale farms.
III. The Market for Marine-Farm Products

The market for the products of the Chinese, Japanese, and Philippine macroalgal farms, is still small when compared to that for a major agricultural commodity like soybeans. For example, in 1981 there were some 70 million acres of soybeans planted in the U.S. which produced a crop worth 13 billion dollars. In contrast, the combined value of the Oriental and Philippine farms was between 871 million and 1 billion dollars (Doty, 1982, Tseng, 1981). The products of these farming efforts are macroalgae (seaweeds) eaten directly as a food, and as used as a source of the phycocolloids, carrageenan and algin. The most valuable of the phycocolloids, agar and agarose, have not yet been produced from a farmed crop, although efforts are being made to do this in China and elsewhere.

IV. The Principles of Marine-Farm and Crop Engineering

The marine farms that are in operation today have been developed largely by trial-and-error (Fei, 1983, Flowers et al, 1981) and are almost always labor-intensive and are operated in countries where wages have traditionally been low. If marine farming is to be successful in U.S. waters, basic design principles must be developed so that the farming process can be automated.

As practised today marine farming might be defined as the "controlled fouling" of man-made structures placed in the sea. Squires and McKay (1982) and Nath and Grace (1977) discuss marine farm construction and mooring. Matsumoto (1959) and Harger and Neushul (1982) discuss macroalgal growth requirements and the selection of optimum site for marine farms to grow this crop plant. Opinions among scientists differ on such basic matters as the design of marine farms and how crops should be planted and grown on them. Some theorists have suggested that "motion-averaging" and spring-like damping structures are needed to "protect" crops on a farm, while others hold that marine farms should enhance water motion, except of course during storms. Marine farms certainly can be "tuned" to extract wave energy from the sea to increase water motion over the crops, and thereby increase their growth. The different strategies suggested by these marine scientists can only be tested in the field and the careful observation and measurement of water motion both around marine farms where crops are grown and in the habitats where the plants grow naturally should resolve some of these basic questions.
An experienced seaman can look at the sea surface and can describe the sea state, using the Beaufort Scale. However, predicting the water-motion below the sea surface cannot be accomplished so simply. By simultaneously using both an inertial wave-sensor to measure motion at the sea surface and electromagnetic current meters installed on the sea floor, and by coupling these instruments with a small ship-board computer, it is possible to measure both wave and water-motion. The results of this exercise indicate that the Beaufort scale is not an accurate guide to conditions beneath the sea surface (Figure 1). The same instruments can also be used to compare loose and tensioned marine farms to illustrate that "tuning" a farm leads to increased water motion over the plants growing on it. In the future it may be possible to design plants that grow well on marine farm structures.

The genetic engineering of new marine crop plants involves the isolation and cultivation of known strains and their judicious crossing to produce varieties that grow rapidly or have other desirable characteristics. This is not a simple process and can have unexpected results. For example, it has been possible to make intergeneric hybrids of the two largest Californian kelps, (Macrocystis and Pelagophycus) and of float-bearing (Pelagophycus) and blade-like (Dictyoneuropsis) forms, Sanbonsuga and Neushul (1978) and Neushul (1983). Contrary to expectations, these first attempts to hybridize the giant kelps of California, while successful, did not yield large hybrid plants with many float-like vesicles and multiple growing points. The hybrids were uniformly small with only two growing points (Figure 2). Although the plants were very healthy and grew rapidly, they did not float effectively and hence did not survive well in the sea, and were ultimately found to be infertile as might have been expected from an intergeneric hybrid (Neushul, 1981). In spite of the counter-intuitive results of these first attempts to hybridize kelps, it does seem clear that the processes of isolating microscopic reproductive stages and making defined crosses will certainly make it possible to genetically engineer highly-productive plants with effective hydrodynamic characteristics. The genetic malleability of these plants also suggests that they might be screened for the production of useful pharmaceuticals and agrichemicals in the future.

Technical Barriers

The major technical barriers to marine farming in the U.S.
that exist today are conceptual. As yet, the basic principles of marine farm design have not been well defined. Similarly, the hybridization of potential crop plants shows that there is considerable genetic scope available, but the selection of strains with still to be defined "optimum physiological and hydrodynamic characteristics" will be difficult. The development of successful marine farm structures and marine farm-crop engineering will require the combination of elements of ecology, oceanography, genetics and physiology. Once the basic principles of marine farms have been established even in interim-form, it should be possible to select a suitable site for farming, to produce and plant genetically-defined seedstock, and ultimately to harvest bountiful crops from the sea.

V. The Future of Marine Farming

Some recent results of experimental farming in U.S. waters (Neushul and Harger, 1985) suggest that marine macroalgal productivity approaches that of sugar cane. These results support suggestions that the biomass fuels (methane, methanol, and alcohol) might be economically produced from marine farms within the next ten years. The domestication of floating macroalgae for cultivation in near-shore and open-ocean farms could well make it possible to develop a large, renewable energy source, although this goal will not be attained easily (Neushul Mariculture, 1980).

It is generally assumed that marine macroalgae only grow when attached to the sea floor or to a floating farm structure. However, marine farms of the future may well be planted in the vast areas of the open sea. For example, the Sargasso Sea occupies some 4 million square miles of sea surface in the mid-Atlantic where scattered, sparse patches of the floating brown alga Sargassum natans grow. This plant has now been cultivated in flasks under laboratory conditions from vegetatively-propagated fragments of the plant collected from Key West, Florida, in April 1984. The plant consistently shows an increase in wet-weight of 6.5% per day, under low nutrient conditions. This preliminary success with laboratory-scale culture suggests that it may ultimately be possible to produce seedstock that could be outplanted in the Sargasso sea, or perhaps more realistically in the more limited warm- or cold-core rings produced by the Gulf Stream, Wiebe(1982). This plant could form the basis of a true open ocean farm the structure of which would be formed from the floats, blades and stipes of the plant. In this case the problem of the marine farm structure and genetic-engineering are
are combined (Colwell et al, 1984).

The modification of both near-shore and open-ocean ecosystems by cultivation should eventually make it possible to use the highly-productive float-bearing macroalgae as renewable sources of chemical feedstocks and energy, (Neushul, 1983). In addition to the production of biomass, pharmaceuticals and agrichemicals, the products of these farms could also serve as a major food-source for cultivated marine animals.

Webster's dictionary rather-tersely defines "mariculture" as: "The cultivation of marine organisms by exploiting their environment." In contrast, "agriculture" is described as: "The science or art of cultivating the soil, producing crops, and raising livestock and in varying degrees the preparation of these products for man's use and their disposal (as by marketing)." It is encouraging to note that we are now moving toward a maricultural science, where maricultural engineers use science and mathematics to cultivate the sea, from which we will harvest genetically-engineered crops and livestock for man's use.
Figure 56. Water velocity and direction was measured using electromagnetic current meters and an on-board computer, at two sites under calm, moderate-swell and choppy conditions. The "bow-tie" shows alternating directions and degree of wave-induced flow, while dashed lines show net current flow in a given direction, a situation where "new" water flows into the sample zone. This type of hydrodynamic data is essential for farm operation.
Figure 57. An intergeneric (Pelagophycus x Macrocystis) hybrid, comparable to that shown in Figure 12 (page 47). In both these hybrids the fronds are "terminated" and do not continue to increase in length, whereas in the Macrocystis parent (shown on the right) the meristem continues to grow and a terminal vesicle does not form until the frond has reached the sea surface, producing a strikingly different, and much larger plant.
Bibliography


VI. CONCLUSIONS

As stated in the introduction to this report, the Marine Biomass Program has been a major success, but the lessons of history were at first ignored and the initial attempts to farm macrophytes in the sea were made without an assessment of farming techniques used elsewhere in the world. In the case of the Chinese, this would have been particularly difficult for political reasons, but the Japanese, Korean and Philippine farms were certainly available. The systems analysis made by the R. M. Parsons Company, was done without knowledge of the Hercules Chemical Company's successful, large, commercial-scale processing of kelp biomass for energy and other products. This inauspicious beginning of the Marine Biomass Program was further compromised by assumptions that marine farming would not be technically difficult, as Dr. Wilcox said, "It's not high technology, we're just talking about plain old plants growing" (see page 10). Ten years after Dr. Wilcox first suggested that open ocean food and energy farms were possible, and after farms had been installed and lost at San Clemente Island, Crystal Cove, and Ship Rock, and "container" experiments had been attempted off Corona del Mar ("ice-cream-cone") and at Catalina Island ("hemidome"), there was still no yield data in hand. Clearly, it was not going to be easy to grow these "plain old plants."

New program managers from General Electric assumed that the problem of open ocean farming could be solved by skillful engineers. They enlisted the help of naval engineers at Global Marine and proceeded to carry out plans that Dr. Wilcox had made, to anchor a large spar-buoy in open water. This amazing structure contained pumps that brought nutrient-rich water up from a depth of 1,500 feet, and survived for several years. But still there was no yield data, since for one reason or another the plants tangled with the farm, were eaten by fish, became infected, or were dislodged and destroyed by storms. Building a "false bottom" for the open ocean was not going to be easy. Also attempts to "protect" the plants with a fabric current shield (ripped away) or a rubberized bowl (torn and damaged by storms) were not successful.

Could it be that the "brute-force" approach, where man-made fabrics and structures were to resist the forces of the sea, was an unwise one in the first place? The Japanese and Chinese have successfully used this approach for nearshore farms for at least twenty years. At present, the Japanese are planning large open-ocean farms. Fortunately, the eastern coast of Japan is comparatively calm, so that perhaps their large-scale anchored farms will be successful.

One way to avoid the problems of farming in the sea itself, is to grow marine plants and animals in raceways and in ponds on land. This has worked well in Florida and Taiwan. The ability to control plant and animal reproduction and growth in tanks will be essential if large-scale seedstock production is to be achieved. The Chinese and Japanese seedstock production facilities are an important key to the success of their in-the-sea farms.

In-the-sea farming efforts at N. M. I. have not been without failure, but our approach has been much different from those taken by Wilcox, General Electric and North, in that we have taken a conservative approach by making our farm very similar to a natural bed, where we know kelp grows well. We did not assume that we could make large sweeping changes in the growth environment of the plants and still have them grow well. We did not assume that we could create an "optimal" growth environment in the sea. We only tried to optimize
growth by making slight modifications to the growing conditions. Our step-by-step approach, starting from a known base was the key to our ability to provide yield information, where others had failed.

At N. M. I., we have carefully measured the interactions between the crop and conditions in the sea. We have taken the position that the giant kelp and tropical *Sargassum* plants are themselves "farm structures" with floats, lines and even anchors produced vegetatively. The genetic work that we have done shows that the basic structure of the kelp plant can be drastically changed. The fact that float formation in at least one intergeneric hybrid (*Dictyoneuropsis* x *Pelagophycus*) is maternally inherited, suggests that it might be possible to genetically control float formation. Perhaps it would be easier to genetically "fabricate" or engineer open ocean farms than to grow the existing plants on man-made structures.

We should examine some of the questions raised in the introduction to this report. First, why have marine farms been commercially successful in China, Japan and the Philippines while unsuccessful in the U. S. The answer to this question lies in the different economic climates in these countries. Labor costs are markedly lower in China and the Philippines which allow them to farm seaweeds for a profit, while the same farm in the U. S. would probably be unprofitable. In Japan, where labor costs are more similar to in the U. S., the products derived from farmed seaweeds are high-priced foods. Since the Japanese strictly control their seaweed imports, we cannot compete in their market of high-priced food products. The U. S. market for high-priced seaweed food products remains small. In addition, the Japanese seaweed industry is Government subsidized.

The final reason why the U. S. seaweed industries have had limited success is because of the strategies used by many U. S. companies. The Diamond Match and Hercules Chemical Companies made their first big profits from their production of explosives from kelp during World War One. Kelco has profitably extracted alginate from kelp for decades. However, none of these companies has used the profit derived from kelp products to support the research and development effort necessary to continually develop new profitable product lines from kelp. Kelco has used its R&D funds to develop new more expensive gums that can be extracted from bacteria grown in digesters because the engineers there prefer the predictability of the raw materials from digesters to the unpredictable nature of having to rely on harvested kelp as a raw material. Commercial seaweed farms will be successful in the U. S. only when: 1) the worldwide wild natural supplies of the seaweed in question are limited and collection is more expensive than cultivation, 2) a profitable extraction regime and product mix is devised that will support the seaweed farming costs and 3) there is some form of protection (technological or species patents, secrets, etc.) from international competition, because labor and other costs are less in many other countries.

Finally, we must face the central question posed in the introduction to this report. Were the Marine Biomass Program goals overambitious and the money wasted? No matter how skillfully one presents the Marine Biomass Program results, the smell of failure is pervasive. Several of the engineering and biological technical research goals were oversimplistic and overambitious. Certainly, some of the money spent was wasted. In the final analysis, one major shortcoming was that there was no effort to analyze and determine what went wrong. As with the Challenger Space Shuttle tragedy, which is fresh in
our minds, the first step, after a farm was lost or an experiment failed, should have been to try to find out why, instead of ignoring the problem, glossing over the failure and trying a slightly different approach. We must agree with our critics, that most of the in-the-sea research was at best low on the learning curve. In addition, the failures have been poorly documented. Much of the money was probably wasted if we cannot learn from what was done in the Marine Biomass Program. In the future, scientists may repeat the mistakes made in this program because those who have been involved with the planning and execution of the work were unwilling to admit to their mistakes and openly discuss their problems.

N. M. I. has used N. S. F. support to define the basic principles of nearshore marine-farm design, with some success. But it is not likely that farms designed to work nearshore will also work in the open sea, where an entirely different kind of farm may be needed. Perhaps the best approach to designing an open-ocean farm would be to first study the hydrodynamics of free-floating Macrocystis and Sargassum, and then design a farming strategy based on the findings. We feel that "plain old plants" may not be what is really needed for large-scale biomass farming, and that very special (and patentable) plants may be the key to the development of the marine food and energy farms of the future.

Regardless of what kind of plant is used, basic physiological and oceanographic data are needed and should be collected over a multi-year period. Hopefully, the N. M. I. farming study will serve as an example of how this type of work is done. Regardless of whether the crop plant is large and complex, like Macrocystis or smaller and more easily manipulated like Sargassum, the growth strategy must be known, since these multicellular organisms are able to compete in the sea with the ubiquitous phytoplankton by effectively taking up and storing carbon and nutrients, and re-allocating these resources in an effective way. In fact, it is this ability to accumulate and store chemical energy, in a mechanically-harvestable "package", that makes these plants useful. Little or no thought was given, in this first phase of the Marine Biomass Program, to how the plant stores and allocates its reserves, or how it develops the vegetable anchors, lines and floats that are obvious adaptations for its survival. Any future attempts to farm macrophytes in the sea, will have to take these matters into account.
VII. REFERENCES


APPENDIX A

LIST OF MACROALGAL SEEDSTOCK CULTURES

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I) Outline of Gametophytes in N.M.I. Seedstock Collection

II) Origin and Number of Female and Male Gametophytes in the N.M.I. Collection

III) Collection Data
I) OUTLINE OF GAMETOPHYES IN N.M.I. SEEDSTOCK COLLECTION

Genus/species

A) WILD STOCK

Macrocystis pyrifera (Mp)
Macrocystis angustifolia (Ma)
Macrocystis integrifolia (Mi)
Pelagophycus porra (Pp)
Nereocystis luetkeana (Nl)
Dictyoneurum californica (Dc)
Pterogophora californica (Pc)
Alaria Marginata (Am)
Dictyoneuropsis reticulata (Dr)
Laminaria setchellii (Ls)
Laminaria saccharina (Lsa)
Laminaria japonica (Lj)

B) CULTIVATED STOCK

II-10
AA
AI
IA
IQ
QA
QI

C) E.M.S. STOCK

E.M.S.-0
E.M.S.-10
E.M.S.-20
E.M.S.-60
II) ORIGIN AND NUMBER OF FEMALE AND MALE GAMETOPHYES

A) WILD STOCK (gametophytes came from spores obtained from wild sporophytes)

Macrocystis pyrifera

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<tr>
<th>Parent</th>
<th>Origin</th>
<th>#Isolates (total)</th>
<th>#Females</th>
<th>#Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Monterey (Stillwater Cove)</td>
<td>15</td>
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### Alaria marginata

<table>
<thead>
<tr>
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<th>Origin</th>
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<th>#Females</th>
<th>#Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Monterey (Stillwater Cove)</td>
<td>19</td>
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</table>

### Dictyoneuropsis reticulata

<table>
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<tr>
<th>Parent</th>
<th>Origin</th>
<th>#Isolates (total)</th>
<th>#Females</th>
<th>#Males</th>
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<tbody>
<tr>
<td>A</td>
<td>Monterey*</td>
<td>40</td>
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### Laminaria setchellii

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<th>#Females</th>
<th>#Males</th>
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<tbody>
<tr>
<td>A</td>
<td>Oregon (Boiler Bay)</td>
<td>20</td>
<td>10</td>
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### Laminaria saccharina

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<th>#Females</th>
<th>#Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Oregon (Yaquina Bay)</td>
<td>14</td>
<td>6</td>
<td>8</td>
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</table>

### Laminaria japonica

<table>
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<th>Parent</th>
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<th>#Females</th>
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<tbody>
<tr>
<td>A</td>
<td>?</td>
<td>2</td>
<td>1</td>
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* Duplicates from the University of California, Santa Barbara Collection

### B) CULTIVATED STOCK (gametophytes came from spores obtained from cultivated sporophytes i.e., f/2 stock)

<table>
<thead>
<tr>
<th>Gametophyte</th>
<th>Parents</th>
<th>#Isolates (total)</th>
<th>#Females</th>
<th>#Males</th>
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<tbody>
<tr>
<td>II-10</td>
<td>Ma X Mp</td>
<td>20</td>
<td>8</td>
<td>12</td>
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<tr>
<td>AA-10</td>
<td>Mp X Mp</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>AI-4</td>
<td>Mp X Ma</td>
<td>13</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>AI-27</td>
<td>Mp X Ma</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>IA-15</td>
<td>Ma X Mp</td>
<td>20</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
C) E.M.S. GAMETOPHYTES (Macrocystis gametophytes that were treated with the mutagen Ethyl Methyl Sulfonate (E.M.S.). The gametophytes were treated for 0, 10, 20, or 60 minutes).

<table>
<thead>
<tr>
<th>Gametphyte</th>
<th>#Isolates (total)</th>
<th>#Females</th>
<th>#Males</th>
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<tbody>
<tr>
<td>EMS-0</td>
<td>18</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>EMS-10</td>
<td>24</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>EMS-20</td>
<td>25</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>EMS-60</td>
<td>17</td>
<td>7</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>26</td>
<td>58</td>
</tr>
</tbody>
</table>

D) TOTAL NUMBER OF GAMETOPHYTES IN NMI CULTURE COLLECTION

<table>
<thead>
<tr>
<th></th>
<th>#Isolates (total)</th>
<th>#Females</th>
<th>#Males</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild Stock (f/1)</td>
<td>625</td>
<td>316</td>
<td>309</td>
</tr>
<tr>
<td>Cultivated Stock (f/2)</td>
<td>132</td>
<td>67</td>
<td>65</td>
</tr>
<tr>
<td>E.M.S.</td>
<td>84</td>
<td>26</td>
<td>58</td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>841</td>
<td>409</td>
<td>432</td>
</tr>
</tbody>
</table>
III) COLLECTION DATA

A) Wild Sources

M. pyrifera


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-K Sporophylls collected from a wild sporophyte from Campus Pt. by B. Harger on 10/3/75. Spores released 10/6/75. Gametophytes isolated 10/10/75 by Y. Sangonsuga.


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.


**M. integrifolia**


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.

Sporophylls collected from a wild sporophyte in the intertidal zone at Stillwater Cove, Monterey Peninsula by S. Clabeusch and S. Fain on 10/9/80. The sporophyte was discarded. Spores released 10/10/80.

Sporophylls collected from a wild sporophyte collected by Jill Thayer at Baranof Island, Sitka Sound, Alaska in 10/83. Sporophylls received in Santa Barbara on 10/14/83 in good condition. Gametophytes isolated 11/29-30/83.

**Pelagophycus porra**

**Nereocystis luetkeana**

Donor sporophyte collected from drift in Stillwater Cove, Monterey Peninsula, CA by S. Fain on 10-9-80. At the collection site, fertile blades were sampled from the donor sporophyte which was then discarded.

Donor sporophyte was collected at Boiler Bay, Oregon by Ray Lewis on 6-22-86. Sori washed with 10% Betadine for 10 minutes and allowed to dessiccate for a short time. Many spores released on 6-22-86.

**Dictyoneurum californica**

Donor sporophyte collected from drift in Stillwater Cove, Monterey Peninsula, CA. by S. Fain on 10-9-80. Donor sporophyte was vouchered as an herbarium specimen and stored at the NMI Goleta facility.
Pterogophora californica

Pc-A Donor sporophyte was collected from drift in Stillwater Cove, Monterey Peninsula, CA by S. Fain on 10-9-80. Fertile blades were sampled from the donor sporophyte at the collection site. The donor sporophyte was then discarded.

Alaria marginata

Am-A Donor sporophyte collected from drift in Stillwater Cove, Monterey CA by S. Fain on 10-9-80. The sporophyte was then pressed and mounted on herbarium paper.

Dictyoneropsis reticulata

Laminaria setchellii

Ls-A Donor sporophyte collected from Boiler Bay, Oregon on 6-22-86 by Ray Lewis. Spore release was obtained on 6-23-86.

Laminaria saccharina

Lsa-A Collected at the breakwater inside Yaquina Bay, Oregon on 6-21-86 by Ray Lewis. Sorus excised, treated with 10% Betadine for 10 minutes and allowed to dessicate overnight. Spore release obtained on 6-22-86.

Laminaria japonica

B) Cultivated Sources

M. pyrifera X M. pyrifera

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


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. pyrifera

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.
A HISTORY OF KELP UTILIZATION IN CALIFORNIA

ABSTRACT

The History of California's Kelp Industry, 1911-1986

by

Peter Neushul

This paper investigates the technology developed by the early California kelp industry 1911-1919, and its continuation from 1927 to present. Re-examination of the large scale harvesting and processing techniques used by the early industry's potash, acetone, algin, and animal feeds businesses provides information which may be vital to the proposed large scale extraction of methane gas from kelp biomass. Harvesting is the most difficult and expensive stage in the kelp-to-methane conversion program. Between 1913 and 1919, California's World War I industry harvested 400-500 thousand tons of kelp per year, a quantity which has never again been duplicated by contemporary harvesters. The early industry's fermentation processing technique produced many valuable by-products, some of which might also be derived from the kelp-to-methane conversion process. A review of the large scale harvesting and processing technology developed by the early California kelp industries is important if kelp is to be seriously considered as a new source of biomass for methane production.
LIST OF MEETINGS ATTENDED BY NMI STAFF AND VISITORS TO NMI 1980-1986

1980
June 17-18  Gas Research Institute, Chicago (program planning)
July 28-29  Kelco company, San Diego (seedstock collection)
August 11-15  International Seaweed Symposium, Gothenberg, Sweden
Sept. 16-18  GE Budgetary meeting, Goleta
Nov. 5  GRI, GE, (program planning) Newport
Nov. 6-7  GRI Advisors Meeting and Tour, Goleta
Nov. 17-20  Biosaline Resources Meeting, La Paz, Mexico

1981
Jan. 28-29  GE/GRI Seminar on Biomass Program, Goleta
Mar. 13-31  GE/GRI Visit to Japan and China
Apr. 7-9  GRI Energy Farm Impacts, Napa, Calif.
July 15  GRI site visit, Goleta
July 29  X.G. Fei, arrives from China
Aug. 10  NMI/Caltech cooperative research seminar, Goleta
Aug. 28  R. Spencer, GRI-program review
Sept. 23-4  GRI Advisors, Newport
Nov. 11-Dec 8  National Academy of Sciences, Study Trip to China

1982
Jan. 27-8  GRI visit by J. Frank, K. Bird, Goleta
Feb. 10  UCSD Seminar on Marine Farm Engineering
Feb. 15-17  Seminar on Marine Farming, Southampton Col. New York
Mar. 2  Visit by Pete Benson, Kermit Woodcock, Goleta
Mar. 10-11  GE Engineers, consulting, Goleta
Mar. 23-24  R. Hoppman, G.E., visit to biomass farm, Goleta
April 16  U.C. Riverside, Lecture on Marine Farming
June 10  Cooperative research, Caltech, Newport Beach
June 17  GRI/GE, K. Bird, A. Bryce, Harvester Review
June 22-23  GRI Advisors, Goleta
June 25  GE Division Manager, R. Tharpe, Visit to Farm
Aug. 3-5  GE, Parsons visit, GE Budgetary Review
Aug. 18-19  M. Miura visits from Japan
Aug. 21-22  C. K. Tseng visits from China
Nov. 7-8  California Coastal Commission, Monterey
Nov. 10  A. Tompkins, GE, Goleta
Nov. 20-22  Dr. Uki, visit from Japan
Nov. 30  Louise Burden and party, Dayton Power and Light
Dec. 5-7  K. Bird, G.E. Meeting, Newport
Dec. 9  T. Arzee, visitor from Israel, Goleta
1983
Jan. 24-25 Kelco symposium on kelp beds, San Diego
Feb. 1-2 GRI, Japanese Study Tour, Goleta
Feb. 3 GRI, Judy Mueller, Toni Storto, Budget Review
Feb. 10 GE visit, A. Bryce, R. Hoppman
Mar. 9-10 SERI program review, San Diego
Mar. 17 GE/GRI, co-products and by-products meeting, Goleta
Mar. 22-23 Univ. Arizona, Arizona State, Seminars on Marine Farming
April 7-8 Dr. Fujita, visit from Japan
April 9-13 Philip Morris Symposium, Marine Farming
April 20 Cao Shuli, visits from Yellow Seas Fisheries Inst, China
June 10 R. Show, U.C. Davis, Visits re: Breeding plan
June 15-29 International Seaweed Symposium, Qingdao, China
July 19-20 GRI Advisors, San Diego
Aug. 8-9 Kelco visitors (L. Whitney, R. Pettit), Goleta
Sept. 14 Meeting with K. Anderson, So.Cal.Gas., re Test Farm
Oct. 3 Meeting with K. Wilson, Cal. Fish & Game, Goleta
Oct. 8-28 Y. Sanbonsuga visits from Japan, Kelp Cytogenetics
Nov. 2-4 Artificial Reef Symposium, Newport
Nov. 23-Dec 9 U.S.A.I.D.-sponsored trip to Senegal, Marine Biomass

1984
Jan. 23 K. Bird, GRI, visits
April 3 Ventura Reef Installation, Dulah, Calif.
April 4-6 SERI review meeting, Boulder, Colorado
April 25-27 GRI contractors meeting, Key West, Florida
May 10 UCSB, Dept of Engineering, Marine Farm Design
Aug. 5-10 Phycological Society Meeting, Ft. Collins, Colorado
Aug. 13 T.C. Fang arrives, cooperative genetic studies
Aug. 22 GRI Program Advisors, San Diego
Sept. 10-11 Field Trip, Piedras Blancas, with T.C. Fang
Oct. 1 Fish and Game, Kelp Leasing, Sacramento
Oct. 8 Drs. Chen & Suo, visit from China, with W.J. North
Oct. 12 W. Smith, IFAS, research review visit
Nov. 29 Dr. Kusumi, visit from Japan
Dec. 27-8 J. Benson, attends Western Soc.
Dec. 28 W. Wheeler visits from Canada

1985
Jan 14-15 P. Benson, K. Bird, visit from GRI
Feb. 13 R. Isaacson, P. Benson, Program Review, Goleta
Mar. 20 GRI Advisors visit to test farm, and review
Mar. 21-22 SERI review, Golden Colorado
April 13 Hercules Chemical, W. Cottle, Goleta
April 19 H. Williamson, Danish Biomass Production
July 26-Aug.10 International Phycological Congress, Copenhagen
Aug. 10-15 Phycological Society Meetings, Gainesville, Florida
Sept. 25 Dr. Kaikuchi, Hokkaido, Japan
Sept. 27 Seminar, U.C. Berkeley, Macroalgal genetics
Oct. 11 A. Mayer, Argentina
<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
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<tr>
<td>Jan. 5-8</td>
<td>N. Yamamoto, visit from Japan</td>
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<tr>
<td>Mar. 15</td>
<td>F. Cattarino, visit from Portugal</td>
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<tr>
<td>Mar. 18</td>
<td>R. Hoshaw, visit from Univ. Arizona</td>
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<tr>
<td>Mar. 27</td>
<td>R. Issacson, GRI</td>
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<tr>
<td>April 5-10</td>
<td>IGT Symposium on Bio-energy, Washington, D.C.</td>
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<td>May 2</td>
<td>J. Fernandez, Midland Bank, Mexico city, re: Kelp Beds.</td>
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<td>May 13-14</td>
<td>Computer Graphics Trade Show, Los Angeles</td>
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<tr>
<td>June 3-7</td>
<td>M. Blakeslee, visit from Alaska</td>
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<td>June 18</td>
<td>Gavino Trono, Philippines, Seedstock Program</td>
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<td>June 22-26</td>
<td>American Society for Virology, U.C.S.B.</td>
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<tr>
<td>July 1</td>
<td>Dr. M. Stekoll, visit from Univ. of Alaska</td>
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<td>Aug. 1</td>
<td>Dr. B. Goldstein, visit from New Mexico</td>
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<tr>
<td>Sept. 23-25</td>
<td>SERI meeting, and Presentation, Golden, Colorado</td>
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<tr>
<td>Dec. 3</td>
<td>Kelco visit, L. Whitney, D. Pettit</td>
</tr>
<tr>
<td>Dec. 31</td>
<td>GRI Final Draft Report Completed</td>
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LIST OF PUBLICATIONS - 1980 to 1986

1980


1981


1982


1983


1984


1985


1986
