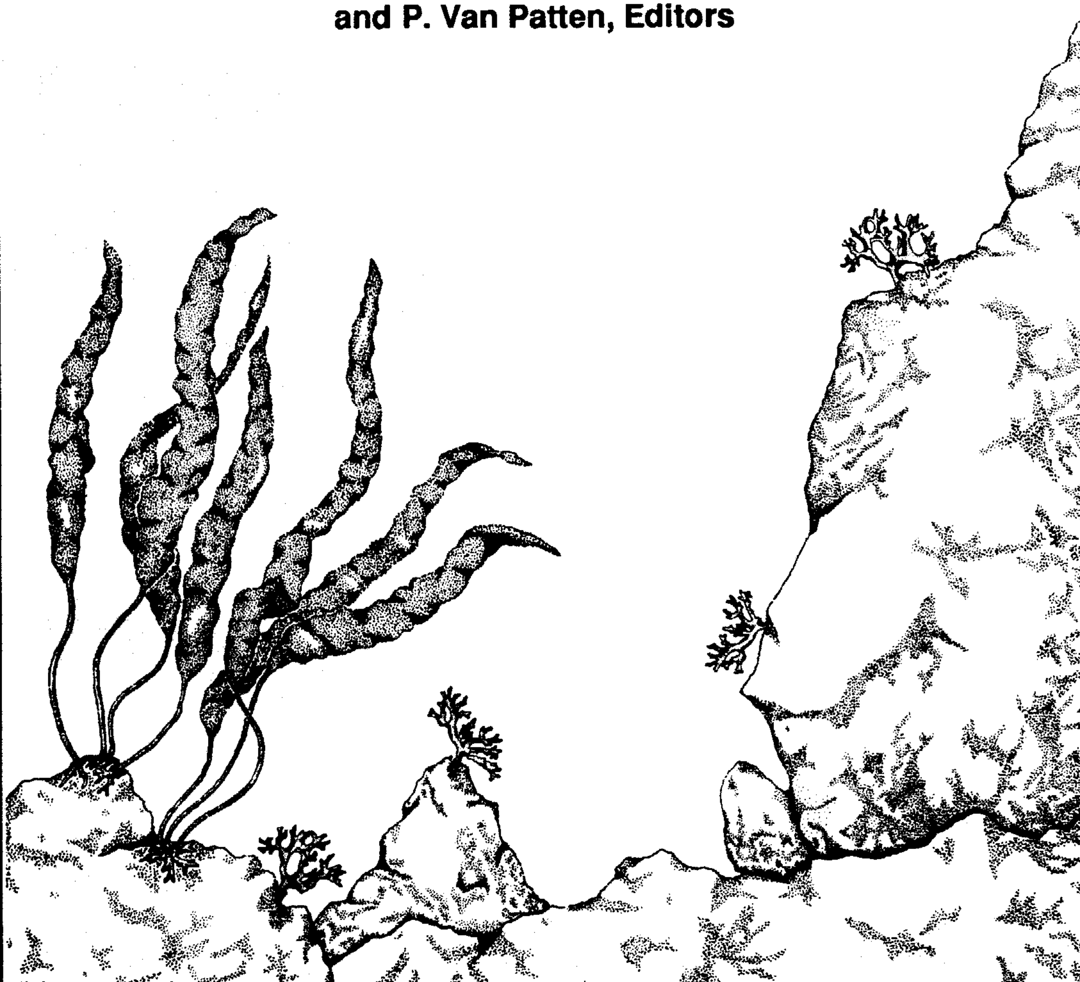
ECONOMICALLY IMPORTANT MARINE PLANTS OF THE ATLANTIC

Their Biology and Cultivation

C. Yarish, C. A. Penniman
and P. Van Patten, Editors



Economically Important Plants of the Atlantic:

Their Biology and Cultivation

Charles Yarish, Clayton A. Penniman
and
Peg Van Patten
Editors

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Preface

On October 2, 1988 a symposium was convened on "Economically Important Marine Plants of the Atlantic: Their Biology and Cultivation" to celebrate the designation of The University of Connecticut as a Sea Grant College. One of us, Professor C. Yarish, was asked by the Connecticut Sea Grant Program to organize a symposium on marine plant aquaculture and utilization which would bring together experts from around the North Atlantic. Since many of the advances in marine plant aquaculture have been supported by the U.S. Sea Grant Program, Professor Yarish decided to expand the organizing committee to include C. A. Penniman of the University of New Hampshire (a recipient of Sea Grant support) and Ms. Peg Van Patten, the Connecticut Sea Grant Communicator. The organizing committee then assembled a program of academic, government and industry representatives from Connecticut, Florida, Maine, Massachusetts, New Hampshire, New York, Washington, D.C., the Canadian Maritime Provinces, and the United Kingdom. The experts that were gathered presented their latest research endeavors to an audience interested in the recent advances in marine plant aquaculture. Representatives of the National Sea Grant Program, industries associated with phycocolloids and pharmaceuticals and scientists from the public and private sectors all participated in a stimulating two day symposium.

Professor C.J. Dawes (University of South Florida) presented an overview of ecophysiological studies for tropical seaweed aquaculture. Dr. J.P. van der Meer (Atlantic Regional Lab, National Research Council of Canada) followed with a presentation of recent advances in the genetics of commercially important seaweeds. Afterwards, Professor D.P. Cheney (Northeastern University) introduced the latest developments in protoplast fusion and somatic cell hybridization from his laboratory. Dr. J.S. Craigie (Atlantic Regional Lab, National Research Council of Canada) discussed the role of physiological studies and their importance in the development of commercially viable aquaculture systems. Following Dr. Craigie's presentation, Professor J.M. Kain (University of Liverpool) presented work by her laboratory concerning the successful cultivation of European Laminariales in the Irish Sea near the Isle of Man. Dr. Kain's aquaculture research had been supported by the European Economic Community. The late Professor B.H. Brinkhuis (State University of New York at Stony Brook) discussed the results of his and Professor C. Yarish's cooperative studies on the development of protocols needed for marine plant aquaculture that have been supported by the Connecticut Sea Grant College Program.

Following the basic work on the biology and cultivation of commercially important marine plants, Dr. J. Pringle (Canadian Dept. of Fisheries and Oceans) discussed management considerations for harvesting of natural seaweed beds in the Canadian Maritimes. After that stimulating presentation, Dr. K. Bird (Harbor Branch Oceanographic Institution) challenged the Symposium participants by presenting an economic analysis of potentially important marine plants for phycocolloid as well as for food production in the North Atlantic.

The final session of the Symposium was an invited panel discussion on the future of marine plant aquaculture and utilization in the North Atlantic. Dr. C. A. Penniman convened this session which included Dr. R. Wildman (U.S. National Sea Grant Program), D. Stancioff (Marine Colloids Division, FMC Corporation), Dr. G. Schulte (Pfizer, Inc.), and Dr. K. Bird (Harbor Branch Oceanographic Institution) as participants. Dr. Wildman described the role of the National Sea Grant Program in directing seaweed aquaculture research during the past ten years and the program's view towards efforts in the 1990's. D. Stancioff focused his discussion on the future research needs of the phycocolloid industry, e.g. understanding taxonomy and chemistry of carrageenophytes. Dr. G. Schulte gave an historical overview of the improvements in the recovery of biologically active compounds from fermentation culture of microorganisms. He then described how

The late Dr. Boudewijn H. Brinkhuis, to whom this volume is dedicated, examines a frond of kelp in the Flax Pond Greenhouse at the State University of New York at Stony Brook.

marine organisms may offer a rich suite of secondary metabolites. Dr. K.T. Bird discussed the need for aquaculturists to understand economic and market forces, i.e. the development of seaweed production for special target markets which would yield the highest possible prices. Live products (e.g. aquarium plants, high value labile biochemicals, or feeds for other organisms) and specialty foods to certain ethnic cuisines offer attractive areas for investment and research in the next decade. He challenged the symposium participants to concentrate on new value-added uses of seaweeds, not just the current state of commercial products.

While this volume was in press, it was with deep regret that we learned that Boudewijn H. Brinkhuis, professor at the State University of New York at Stony Brook, died suddenly and unexpectedly on July 10, 1989, a few months after his 43rd birthday.

Bud, as he was known to his friends and colleagues, was an active participant in the development of and advances in seaweed aquaculture in the North Atlantic, Mexico and China. His pioneering work on the ecophysiology of *Laminaria* and other commercially important marine plants has laid the foundation for future seaweed development.

Bud was an activist and enthusiast for his science. He was fervently devoted to his students and gave them his unreserved attention. At the time of his death, he was advising two graduate students from Mexico and one from Taiwan, and designing research programs for Chinese and other Mexican graduate students who had planned to work with him in the coming year. Bud always considered himself a researcher; from many of his colleagues' point of view he was one of the brightest and most energetic in the field. Bud always had a vision. He looked forward to the day that there would be commercial exploitation of seaweeds through aquaculture in North America. At the time of his death, he was eagerly working with Mexican colleagues on the mariculture of *Eucheuma* and *Gracilaria* in the Baja California region and the technology transfer of biochemistry techniques to China.

The loss of Bud Brinkhuis is widely and deeply felt, most certainly by his mother, sisters and brothers, his daughter Jennifer and his wife Joan. His colleagues in marine phycology will miss his wit and readiness to help. His friends will remember his smile when he proudly discussed his work on *Laminaria*. Although we are all greatly saddened by the abrupt and untimely end of a very productive scientific career, we should find a degree of solace in knowing that some of Bud Brinkhuis' scientific thought and inspiration has passed to his graduate students. The Editors and the Connecticut Sea Grant College Program wish to dedicate this volume to the memory of Professor Boudewijn H. Brinkhuis. His spirit will be a guiding force for researchers and students of phycology in the North Atlantic.

Charles Yarish
Clayton A. Penniman
Peg Van Patten

February, 1990
Groton, Connecticut

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1

Genetic Contributions to the Development of Marine Crops

John P. van der Meer
Atlantic Research Laboratory
National Research Council of Canada
Halifax, Nova Scotia, B3H 3Z1 Canada

Abstract

Genetic contributions to the development of marine crops are still limited. This is not surprising; genetic selection and plant breeding normally accompany commercial activities, which have begun only recently and only for a very few species. The act of establishing a cultivated crop from a wild population has genetic implications in itself through sampling, inbreeding, and unconscious selection. Organized selection and selective breeding have made important contributions to the development of some current marine crops, particularly *Eucheuma*, *Porphyra* and *Laminaria*. Basic genetic research on these and other algae has not led directly to improved cultivars because these studies are not linked directly to industry. Nevertheless, these studies are making important discoveries that will benefit future breeders. The most significant discoveries made thus far include: the discovery of a sexual cycle for *Palmaria,* the discovery of the correct position of meiosis in the life history of *Porphyra,* the discovery of extensive mitotic recombination and the resultant consequences on tetrasporophytes of *Gracilaria,* and the discovery of self-sterile mutants of monoecious

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Gelidium, which facilitate breeding. Current molecular genetic studies on marine algae have much potential for future application, but have not arrived at the point where they can be applied to strain improvement. Future application of molecular genetics will require a significant investment in research, not only in molecular biology, but also in the basic biochemistry and physiology of marine crop species.

Introduction

In historical terms, the emergence of marine crops is a very recent event. Although seaweeds have been part of the diet of Oriental peoples since antiquity, and have been cultivated by primitive means for centuries (Tseng, 1981), modern marine plant aquaculture emerged only a few decades ago with the development of the *Porphyra* industry. Lack of knowledge, and difficulties presented by an unfamiliar aquatic environment, remained major impediments for marine crop development into the middle of the present century. It was not until the past few decades that progress in phycological research laid the foundations for controlled studies of algae in culture, and opened the doors for a mariculture industry. On this occasion it is worth acknowledging that without these fundamental contributions of dedicated phycologists, past and present, we would not be in a position to discuss marine crop development today.

Genetic strain selection generally coincides with the advent of marine plant mariculture for a particular species. A significant strain development program is not easily justified unless a serious commitment to try to grow a species commercially has been made. Conversely, the success of commercial cultivation may be closely tied to the quality of the available cultivars. Thus it is obvious that cultivation and genetic selection are coupled processes.

Spontaneous Change

Genetic selection is partially a spontaneous process. Cultivation of a species has to be initiated from a limited sampling of individuals taken from a variable wild population. Hence, the genetic make-up of cultivated plants is always more uniform than the natural population. Over time, unconscious selection will cause the cultivated stocks to diverge even more from the wild plants, particularly when seed stock is taken repeatedly from the preceding cultivated crop.

Cultivated plants nurtured by man can tolerate wider phenotypic deviations from the normal "wild type" than those that must survive in nature. Phenotypic variants that would normally have died might be acceptable, even desirable, in the relatively protected environment of a cultivation tank. Even completely sterile mutants are acceptable if they can be propagated as clones. Repeated rounds of simple selection, coupled with spontaneous genetic mutation, can yield significantly improved cultivars, even without formal genetic understanding on the part of the grower.

Plant Selection and Breeding

Simple plant selection, i.e. isolating robust plants from wild populations, has already played a significant role in the development of marine crops. Indeed, for the near future, such selection is likely to remain the most effective method for rapidly obtaining improved clones of plants, especially for species only recently put into cultivation. For *Eucheuma*, such simple selection led to the discovery of very productive strains, in particular the "Tambalang" clone (Doty, 1985), which is now extensively cultivated in the south Pacific. Selection of high-yielding plants from wild populations of *Chondrus crispus* has improved the prospect for commercially successful, tank-based cultivation of this species (Neish and Fox, 1971; Cheney *et al.*, 1981; J. S. Craigie, unpubl.). Further selection of *Chondrus* clones is underway (J. S. Craigie, unpubl.), and organized selective breeding will likely begin as the cultivation industry becomes established. Robust clones have been isolated for several other species of seaweed considered to have commercial potential, but without supporting aquaculture industries, there has been no follow-up for these discoveries.

Plant breeding of marine crops is not yet an established activity of the sort associated with terrestrial crop species. Nevertheless, significant starts have been made in the Orient, particularly for commercially cultivated species of *Porphyra* and *Laminaria*.

Porphyra

Selection for length has significantly increased the average size of fronds for both *Porphyra yezoensis* and *P. tenera*. It appears that this selection favored plants with delayed fertility, permitting a longer growth period before the onset of spore release and accompanying frond erosion (Miura, 1978). Japanese growers have selected a large number of species and strains that are adapted to local conditions. There are about 30 recognized cultivars and numerous additional strains that have been selected informally (T. Mumford, pers. comm.). Some of these have also been farmed successfully in Puget Sound in the past year (J. Merrill, pers. comm.). High levels of nori production in recent years have led to a somewhat saturated marketplace for *Porphyra* products. As a consequence there is currently an emphasis on efficiency and quality, rather than quantity, of production. To help meet these objectives, breeding targets include monospore-producing strains which can "self-seed" onto the production nets, and disease-resistant strains that require less attention.

Laminaria japonica

During the 1960's Chinese scientists conducted a series of genetic experiments on *Laminaria japonica*, and demonstrated that several morphological characteristics and iodine concentration, (important because *Laminaria* is a major source of iodine in the Chinese diet), are under quantitative genetic control (Fang, 1983). Through a program of selection and inbreeding, a number of stable, productive lines were developed for aquaculture. This breeding program has continued at a modest level, with new improved strains reported at intervals (Anonymous, 1976; Fang *et al.*, 1983). Strains of *L. japonica* thought to have improved high-temperature tolerance have also been described by Chinese breeders but the documentation on these is not very convincing; the successful introduction of *Laminaria* to southern areas appears to be entirely due to the development of an effective transplantation program.

Recently, female gametophytes derived from commercial Chinese kelp lines were hybridized with a male plant of Japanese origin (Fang *et al.*, 1985). One of the resulting hybrids was described as the most luxuriously growing, high-iodine line obtained by breeders thus far. The suggestion that this luxuriance is due to "hybrid vigor" remains to be clarified. It may be due to additive genetic effects and not heterosis in the strict sense, i.e. superior growth from hybridity that cannot be fixed by selection and inbreeding.

Fang and his coworkers (1978) discovered that it was possible to obtain quite normal haploid sporophytes of *Laminaria japonica* through parthenogenetic development of the egg cell. The haploid sporelings exhibited a high frequency of spontaneous chromosome doubling, yielding fertile, diploid parthenosporophytes derived from a single sex. From a strain development perspective, these parthenosporophytes provide a method for assessing the blade-growth potential of individual haploid genotypes, a method analogous to monoploid breeding in higher plants.

Mendelian Contributions to Marine Crop Improvement

Mendelian studies of marine Chlorophyta were initiated in the early 1960's with the isolation of mutants of *Ulva mutabilis* (for a review see Fjeld and Løvlie, 1976). These studies continued for slightly more than a decade, contributing significantly to our understanding of reproduction in *Ulva*, but then ended.

There have been very few Mendelian genetic studies of marine Phaeophyta, with the exception of Yarish *et al.*, (this volume). Only one Mendelian mutation, which causes brown spots on *Laminaria japonica*, has been described (Fang *et al.*, 1982). Obviously Mendelian genetic studies have had no impact on the commercial development of Phaeophycean species thus far.

The situation is a little better for the Rhodophyta. Studies on the transmission and expression of Mendelian marker genes have contributed significantly to our understanding of reproduction in some species, and this new information has revealed some important implications for breeders.

Porphyra

Color mutants of *Porphyra* behaved in a totally unanticipated fashion when they were first studied. In crosses, sporelings from conchospores did not segregate into color classes in a Mendelian ratio; rather, the large majority of the sporelings were individually striped into two, three, or four sectors of different color (Ohme *et al.*, 1986). The reason for this outcome is that meiosis, and consequently genetic segregation, occurs in the germinating conchospores, (not in the conchosporangia as had been thought) with the first four cells of the germlings constituting the meiotic tetrads (Ma and Miura, 1984; Ohme *et al.* ,1986). Studies on other *Porphyra* species suggest that meiosis probably occurs during conchospore germination in most, if not all, sexually-reproducing species of *Porphyra* (Burzycki and Waaland, 1987; G. Mitman and J. van der Meer, unpubl.; C. K. Tseng, pers. comm.).

This discovery has significant consequences for the breeding of *Porphyra* strains because the breeder now has to recognize that all fronds are potentially genetic mosaics. In practice, however, past ignorance concerning this point does not appear to have been a very great impediment to selection. Since both of the commonly cultivated species, *P. tenera* and *P. yezoensis* are monoecious, repeated self-fertilization of selected haploid fronds has reduced the amount of genetic variability in cultivated strains. (It should be recalled in this connection that self-fertilization of a haploid plant yields 100% inbreeding, i.e. complete genetic homozygosity, in a single step.) Consequently, in crosses, there may not be much genetic variability in the different frond sectors except when deliberate efforts are made to hybridize lines with different ancestries.

Gracilaria tikvahiae

Studies on *Gracilaria tikvahiae* have established that the fundamental genetic characteristics of the Floridiophyceae are not significantly different from those of terrestrial plants, once differences in the life histories of the groups are taken into consideration. These studies add confidence that normal genetic and plant breeding practices will pertain to the red algae; however, they have not yet contributed to the actual commercial development of any species.

One outcome for the research on *Gracilaria tikvahiae* was the demonstration that mutant clones with radically changed morphology have altered agar content, and in some cases appear to be superior for cultivation. The *bu* (bushy) mutation not only grows well (Patwary and van der Meer, 1983), but has a harder-gelling agar as compared to the wild type (Craigie *et al.*, 1984). The ball-like growth habit of this mutant is attractive for tank-based cultivation because the fronds circulate more readily than those with the wild-type habit.

Although the *bu* clone has these attractive characteristics, it arose in a stock that has unremarkable growth, and thus the superior performance of the mutant is only relative to its parental stock. Under natural aquaculture conditions, its growth is actually no better than that of some wild clones (Fig. 1). Growth improvement of the *bu* mutant clone was attempted through hybridization to unrelated wild-type genetic backgrounds but little improvement was observed (Fig. 1), and the breeding experiment was discontinued after the first generation of hybrid offspring.

Perhaps the most significant observation to emerge from the *Gracilaria* studies is that mitotic recombination occurs frequently on fertile, diploid, tetrasporophytic fronds (van der Meer and Todd, 1977; van der Meer, 1981). As a consequence, small sectors with reduced heterozygosity are continuously produced. (In mitotic recombination all genes between the cross-over point and the end of the chromosome become completely homozygous.) New growing points (e.g. lateral branches) initiated within recombinant tissue give rise to fronds with a new genotype. Thus, for diploid *Gracilaria* clones, the grower could expect to see a high frequency of variant fronds and a gradual decrease in the heterozygosity of the clone unless the recombinant fronds are removed or prove to be less vigorous than the parent plant.

Another important consequence of mitotic recombination in

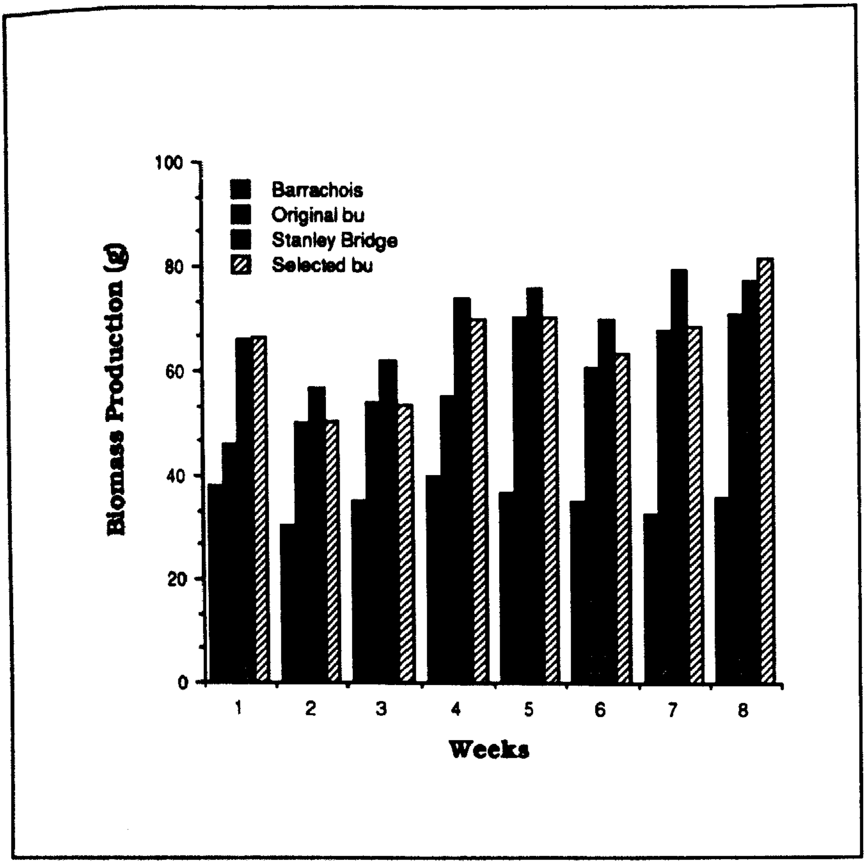

Figure 1. A comparison of the productivity of four strains of Gracilaria tikvahiae. *Barrachois is the wild-type line that gave rise to the original* bu *mutant line. Stanley Bridge is a second, more productive, wild-type line. The selected* bu *line was obtained by crossing the original* bu *mutant with a third wild-type line (=Grand River), collecting tetraspores from the mature hybrid tetrasporophyte, and selecting, in culture, the fastest-growing female of 250 gametophytes. This particular Barrachois-Grand River gametophyte was the most productive isolate obtained from several crosses involving 5 different wild-type backgrounds. The final productivity comparisons were made using unfertilized, mature, female clones adapted to greenhouse conditions for several weeks before the growth experiment. Plants were inoculated (30g) into small white buckets (10 L) and weighed weekly, with the starting biomass adjusted to 30g at the beginning of each week.*

Gracilaria is the production of functional diploid gametes which can be used to construct polyploids (van der Meer and Todd, 1977; van der Meer, 1981). Several autopolyploids of *G. tikvahiae* have been examined for their growth potential. Triploid and tetraploid tetrasporophytes grow well, but do not appear to have a significant advantage over diploids in growth characteristics (Patwary and van der Meer, 1984). Interestingly, triploid and tetraploid gametophytes are severely stunted and the tetraploids are genetically unstable as well (Zhang and van der Meer, 1988). The difference between sporophytes and gametophytes in this regard is interesting but unexplained.

Palmaria palmata

During culture studies on the potentially important food alga *Palmaria palmata* a few years ago, discovery of a recessive, green, mutant clone greatly facilitated the elucidation of the sexual life history of this species (van der Meer and Todd, 1980). The discovery has only been of academic interest thus far, but clearly this fundamental information will become important for anyone who might try commercial development of *Palmaria*. While a direct comparison is somewhat presumptuous, it may be recalled that the life-history observations by Drew (1949) connecting *Conchocelis* with *Porphyra* established the foundation of the modern nori industry.

Gelidium vagum

Species of the genus *Gelidium* are a traditional source of high quality agar. At the present time there is a developing interest in the aquaculture of various species of *Gelidium* due to the fact that natural beds cannot supply the commercial demand. For the past two years we have been experimenting with the monoecious species *G. vagum* (van der Meer, 1986) to develop techniques that will facilitate genetic manipulation. Because *G. vagum* is monoecious, with male and female gametes produced in close proximity, hybridization must compete with self-fertilization. A small number of Mendelian color mutants have been isolated and characterized, and are now used as marker genes to permit identification of hybrid offspring. When the female parent has a recessive color mutation, the two types of offspring can be distinguished readily; self progeny have the mutant color of the female while hybrid progeny are restored to wild-type color by the normal gene from the male.

To eliminate self-fertilization from breeding lines entirely, we are currently attempting to isolate self-sterile mutants that retain single-sex fertility in crosses. By screening a large number of mutagen-treated sporelings, several dozen such mutants have been isolated. Initial tests indicate that both male sterile and female sterile mutants are present in the collection along with a number of other reproductive anomalies. The inheritance of only a very few of these sterility mutations has been tested thus far, but some are transmitted in simple Mendelian fashion. The anatomical and histological phenotypes of the mutants remain to be characterized. In terms of commercial development of *Gelidium*, it is hoped that the availability of these sterile mutants will facilitate the future production of hybrid clones.

The Promise of Molecular Genetics

Although the tremendous potential of recombinant DNA techniques for biological and biochemical studies on algae is recognized by all, these techniques are still in the developmental stage for actual genetic transformation of marine plants. It will be some time before they are available for strain improvement. Little is known about algal genes and their regulation, and no reliable technique is yet available for introducing foreign genes into an algal cell so that their expression can be tested in transgenic plants. Research on tissue cultures and protoplasts has made a promising beginning in a few species, (Polne-Fuller and Gibor, 1984, 1987; Cheney *et al.*, 1986, 1987; Liu and Gordon, 1987; L.C.-M. Chen, pers. comm.) but a great deal more fundamental work is necessary before protoplasts can serve as host cells for molecular genetic experiments. Recent success in transferring genes into cells through "micro-ballistics", i.e. directly shooting DNA-coated, tungsten pellets into a cell, (Sanford *et al.*, 1987; Klein *et al.*,

1988) raises the interesting prospect that it may be possible to find a general method for algal transformation. Such a development would be most welcome since it would circumvent the difficult and tedious process of finding appropriate conditions for protoplast formation and regeneration in each individual species.

At present the precise targets for algal biotechnological development are still unclear. A few objectives, such as herbicide-resistant strains, have been suggested, but for the most part the possibilities are obscured by large gaps in our knowledge of algal biochemistry, including what useful natural products they might produce. Much serious thought will have to be given to the possible unique uses of algae and algal products in order to identify those objectives that are worth pursuing through a molecular genetic approach.

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2

Genetic Improvement of Seaweeds Through Protoplast Fusion

Donald P. Cheney
Department of Biology
Northeastern University
Boston, MA 02115

Abstract

Although plant regeneration from protoplasts still presents difficulties for many anatomically-complex seaweeds, genetic manipulation techniques such as protoplast fusion are being rapidly developed in a small number of cases. Protoplast fusion offers unique opportunities for strain improvement in red algae in particular, because of its ability to produce hybrids (somatic hybrids) between plants of the same or different species, regardless of their interfertility. This report provides a preliminary account of protoplast fusion in the commercially valuable, agar-producing seaweed *Gracilaria* using the chemical fusogen PEG.

Introduction

At the turn of this decade, many of us who study commercially-valuable seaweeds would have probably predicted that seaweed cultivation would be far more accomplished and widespread than it is today. Except for the recent introduction of nori

(*Porphyra*) farming in the state of Washington (Mumford, 1987) and that of *Chondrus* tank culture in southern Nova Scotia (see Craigie, this volume), there don't appear to be any significant new commercial programs in recent years, albeit some past programs have greatly expanded, e.g. *Eucheuma* farming in the Philippines and Indonesia (Adnan and Porse, 1987), and *Laminaria* farming in China (Tseng and Fei, 1987). The general lack of new programs is certainly not due to a lack of trying, for substantial efforts were made in connection with the marine biomass program in the late 1970's and early 1980's (see Bird and Benson, 1987). Rather, it appears to be largely a result of inherent economic constraints connected with seaweed cultivation (e.g. see Bird, this volume). In order for seaweed cultivation to become more commercially attractive to industry, especially in developed regions, it has been suggested (Cheney, 1984) that new cultivation attempts should be aimed at providing one (or both) of the following: (1) valuable new or improved products which are not available in existing wild plants, or (2) commercially-valuable, existing products at reduced costs, increased quality or greater dependability. To do so will likely require, if not benefit from, new and improved strains of seaweeds.

In the past, seaweed strain improvement techniques have generally been restricted to classical plant breeding approaches (for reviews, see Cheney, 1984; Lewis *et al.*, 1986; van der Meer, 1986). Briefly, these techniques have primarily consisted of simple strain selection of either wild plants or new genetic variants produced by such techniques as mutagenesis and colchicine treatment, or, to a lesser extent, the use of polyploid construction and sexual hybridization. Although there have been some notable successes (e.g. the tambalang strain of *Kappaphycus (=Eucheuma) alvarezii* and the Chinese *Laminaria japonica* strains), these remain few in number and generally required many years of research effort to develop. At present, there is a real need for a more efficient means of producing improved strains of commercially valuable seaweeds.

Compared to its important role in land plant crop improvement, the contribution of sexual hybridization to seaweed strain improvement has been limited, especially in red algae. Although this may be due in part to practical difficulties (e.g. obtaining both sexes of the strains desired), the principal problem appears to be one of a lack of interfertility between different species of red algae. Both intra- and interspecific incompatibility have been reported in several red algae, including *Chondrus* (Chen and Taylor, 1980), *Gigartina* (Guiry and West, 1983) and *Gracilaria*. Several interspecific crosses have been attempted in *Gracilaria* to date, but in all cases there has been no evidence of interfertility between species (e.g. Bird and McLachlan, 1982; Plastino and De Oliveira, 1988). Because of such interfertility constraints, it would appear unlikely that interspecific hybridization can be utilized as effectively in commercially valuable red algae as it has in land plants to develop new strains showing hybrid vigor. Tests for hybrid vigor or heterosis *sensu stricto* have been conducted using intraspecific hybridization in *Gracilaria tikvahiae*, however, evidence of such has not been detected (see Patwary and van der Meer, 1983; Zhang and van der Meer, 1987).

In order to provide a much needed new approach to strain improvement of commercially valuable red algae, my laboratory began efforts some years ago to develop genetic manipulation techniques. The potential benefits of genetic engineering and manipulation techniques to seaweed strain improvement have been described elsewhere (e.g. Cheney, 1986; Polne-Fuller and Gibor, 1986). Although not generally distinguished in the past, genetic engineering and manipulation refer to different techniques. Genetic engineering techniques *sensu stricto* refer to procedures involving recombinant DNA transfer and include, for example, direct DNA uptake via electroporation, as well as the use of vectors such as in *Agrobacterium tumefaciens*-mediated gene transfer. Genetic manipulation, on the other hand, refers to transformation methods which do not involve recombinant DNA, such as protoplast fusion to produce somatic hybrids. While this distinction may appear to be quite minor, it has important implications regarding current regulations controlling the release of such laboratory-produced organisms into the environment.

The principal value of protoplast fusion techniques is that they provide the opportunity to produce unique genomic combinations which are impossible or impractical by classical breeding techniques, such as the production of hybrids between sexually incompatible species. This paper presents a preliminary report of our recent efforts to produce somatic hybrids between two non-interfertile *Gracilaria* species through protoplast fusion. This research was conducted while on leave at the Department of Botany of the University of Nottingham (U.K.) Further details of this work, as well as of preliminary fusion experiments with *Gracilaria*, will be presented elsewhere (Cheney, in preparation; Duke and Cheney, in preparation.)

Methods

General Comments:

The development of protoplast fusion-somatic hybridization procedures in general involves five key steps (Table 1). All five steps are essential for success and should be developed more or less sequentially. Thus, one of the first requirements is to develop a reproducible protoplast regeneration system. This should be done before initiating a fusion program, since the protoplast fusion procedure itself (particularly with PEG fusion) substantially reduces plant regeneration. Similarly, since a typical fusion experiment might involve several hundred thousand protoplasts, it is essential to have a reliable method for isolating biparental fusion products or heterokaryons from unfused protoplasts and uniparental fusion products. Finally, it is essential that putative hybrids be analyzed in such a fashion that their hybrid nature can be verified. Generally, this is done by identifying gene products encoded by the genomes of both parental material using either isoenzyme analysis or recombinant DNA techniques (e.g. restriction fragment length polymorphism (RFLP) fingerprinting or species specific DNA-clone probes). Morphology and chromosome number are generally not sufficient indicators to provide unequivocal proof of hybridity, since they can vary after culture due to a variety of reasons other than hybridity (e.g. somaclonal variation and autopolyploidy). Furthermore, an increase in chromosome number or total DNA by itself can not distinguish between fusions that are uniparental vs biparental.

Table 1.

Essential Steps to
Protoplast Fusion - Somatic Hybridization

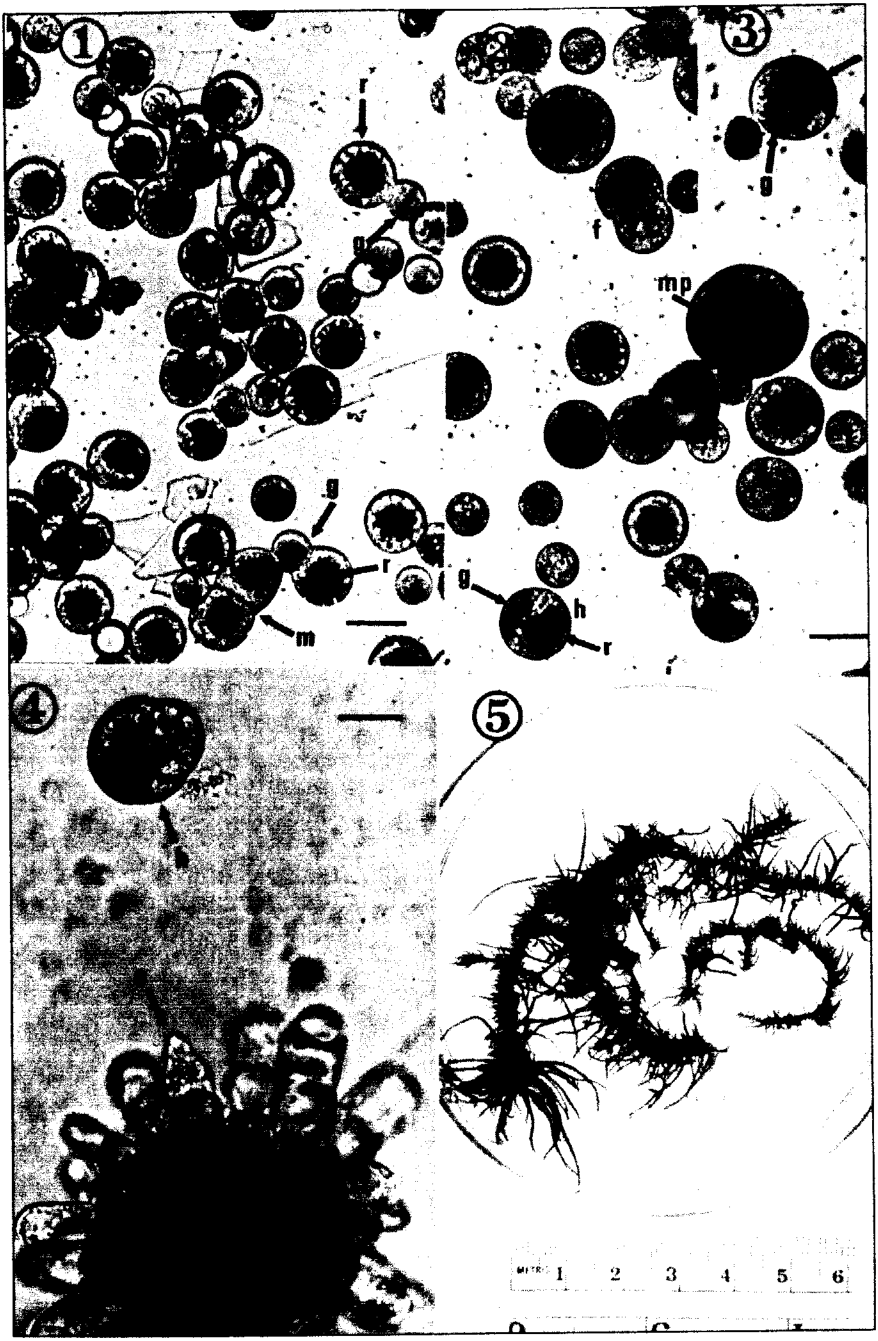
1). Isolation and culture of viable protoplasts
2). Regeneration of protoplasts into whole plants
3). Fusion of protoplasts
4). Selection of heterokaryons and regeneration of hybrid plants
5). Confirmation of hybridity of putative hybrids

Protoplast Preparation and Fusion:

Several different sources of protoplasts were tested for suitability in fusion, including haploid and diploid vegetative tissue and asexual reproductive cells (i.e. spores). Results of these experiments will be reported elsewhere (Cheney *et al.*, in prep.; Duke and Cheney, in prep.) Protoplasts for this study were prepared from a green mutant, non-Mendelian strain (NMG) of *Gracilaria tikvahiae*, kindly provided by John van der Meer, and a red, wild-type strain of *Gracilaria chilensis*. Use of such differently-pigmented strains provided a useful visual marker for the identification and isolation of biparental fusion products. Reciprocal crosses between these two species have proven unsuccessful, thus they appear to be non-interfertile (Cheney and Bradley, unpublished).

Protoplasts were prepared fresh before fusion using an enzyme mixture and methods similar to those described in Cheney *et al.* (1986), except that pectolyase and macerozyme were omitted from the enzyme mixture. Protoplasts were fused using the chemical fusogen polyethylene glycol (PEG) and methods modified after those developed for land plants (Kao and Michayluk, 1974). After isolation, equal numbers of both types of protoplasts were mixed together and fused in a culture dish using a 40% solution of high molecular weight (6000) PEG and a "high-calcium-high pH" fusion solution similar to that used in Kao and Michayluk (1974). Following fusion, protoplasts were cultured in a medium consisting of half-strength ESS (see Bradley *et al.*, 1988), 0.4 M mannitol and 12.5 mM $CaCl_2$ at a pH of 7.6. The culture media was diluted gradually over several days. Bacterial growth was controlled with an antibiotic mixture selected using the "one step antibiotic disc method" described by Bradley *et al.* (1988). The antibiotic mixture consisted of 30 μg/ml each of polymixin B, nalidixic acid, erythromicin, colisin, vancomycin, ampicillin, trimethoprin and chlorotetracycline; this stock was used at a concentration of 100 μl/ml of culture medium. Approximately five days after fusion, red and green, bicolor heterokaryons were isolated and transferred to a new culture dish containing a *Gracilaria* callus nurse culture (Fig.4). After two or three months of growth, putative hybrid cell colonies were regenerated to whole plants in aerated flasks.

Right, facing page: Plate 1, Figures1-5. *Protoplast fusion between* Gracilaria tikvahiae *and* G. chilensis *and development of fusion products. Scale bar=30 μm.*
Fig. 1. Protoplast clumping and agglutination after the addition of PEG. Protoplasts from G. tikvahiae *are smaller and green-pigmented (labeled "g"), while protoplasts from* G. chilensis *are larger and red-pigmented (labeled "r".) Note the two biparental fusions (labeled "g" and "r") and the uniparental fusion with a still intact plasma membrane ("m"). Fig. 2. Later stage in fusion caused by the addition of fusion solution. Note the example at the top ("f") where fusion and cytoplasmic mixing is nearly completed and the example of a just-formed, bicolor heterokaryon ("h") at the bottom. The large protoplast in the center ("mp") is the product of multiple fusions. Fig. 3. Close-up of a bicolor, red and green plastid-containing heterokaryon. Fig. 4. Heterokaryon cell colony development in callus ("c") nurse culture. Fig. 5. Regenerated tetrasporic whole plant from a protoplast fusion experiment. Note the many side shoots, which were produced by* in situ *germination of tetraspores.*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Results and Discussion

In general, this study confirms the ability of the chemical fusogen PEG to induce fusion in *Gracilaria* protoplasts. The addition of the PEG solution to the protoplast mixture caused immediate clumping and agglutination of protoplasts (Fig. 1). In the presence of PEG, the plasma membranes of adhering protoplasts became tightly compressed (Fig. 1, "m") but seldom completely disappeared. Fusion itself, or the cytoplasmic mixing of protoplasts, generally occurred only after the addition of the fusion solution (Fig. 2).

The addition of the fusion solution caused the protoplasts to immediately begin fusing (Fig. 2). Generally, fusion was completed within less than 1 hr. After fusion was completed, biparental fusion products called heterokaryons could be easily distinguished by their bicolor appearance; that is, they contained both red and green chloroplasts (Fig. 2 and 3). Thus, the process of protoplast fusion itself was confirmed by direct microscopic examination. Fusion products involving from 2 to 4 protoplasts were observed, with multiple fusions (3 or more; Fig. 2, "mp".) showing reduced viability.

In order to follow the development of putative hybrid plants from fusion products, it was generally necessary to isolate and culture the bicolor heterokaryons separate from the unfused protoplasts within one week after fusion. This was primarily needed because the bicolor nature of the heterokaryons usually disappeared within ten days after fusion. During this time, one or the other plastid type usually became dominant while the other would degenerate and disappear. Similar chloroplast incompatibility has also been reported in vascular plant protoplast fusions (e.g. Binding *et al.*, 1986), as well as for somatic cell fusion in the red alga *Griffithsia* (Koslowsky and Waaland, 1987).

More than 20 fusion products have been regenerated to whole plants to date, including several red and green chimeric plants and several plants with unusual morphological features, which suggests that they may be putative hybrids. Bicolor, red and green chimeric *Gracilaria* plants were also produced in a previous study that attempted spore fusions (Duke and Cheney, in prep.). Such chimeras were produced at too high a frequency to have likely been caused by either mutations or reversions. It appears more likely that they were caused either as a result of nuclear (or chloroplast) segregation prior to cell division or simply the adherance of red and green protoplasts after incomplete fusion. Regardless, the production of such bicolor chimeras is unusual, and has only been reported for one other red seaweed, *Porphyra* (see Fujita and Migita, 1987).

The putative hybrids exhibit several unusual features. In general, these include a branching morphology unlike either of the parental plants. Most are red pigmented; few are green pigmented. One such red plant, referred to as # 18-HVG2 (Fig. 5), has been cultured through two subsequent generations. Interestingly, this plant produced red tetraspores in relatively low numbers, which in turn developed into plants that were also tetrasporic. The original plant exhibited an abundance of *in situ* germination of tetraspores causing a brush-like appearance (Fig. 5). Since the parental green plant used in this fusion experiment was a non-Mendelian mutant, segregation of red and green tetraspores would not be expected. Determination of whether this plant and other putative hybrids are heterokaryons or homokaryons is in progress, using isoenzyme analysis.

Protoplast fusion has been attempted in several other marine algae, but to date evidence of having produced a somatic hybrid has been lacking. Saga *et al.* (1986) reported an attempt to fuse protoplasts from the green alga *Enteromorpha* with those of *Porphyra*, a red alga. Although a heterokaryon was apparently produced, the authors were unable to regenerate whole plants. The first report to my knowledge that presents convincing evidence of having achieved protoplast fusion and fusion product regeneration in a seaweed is that of Fujita and Migita (1987). In their study, Fujita and Migita attempted to fuse protoplasts from two different color morphs of the same *Porphyra* species, *P. yezoensis*. Although they were able to produce several green and red chimeric thalli, they were unable to demonstrate that true somatic hybrids had actually been produced. The current study is the first report of protoplast fusion and fusion product regeneration in either a phycocolloid-producing or anatomically-complex seaweed. Although the study has not proven somatic hybrid production, it has, I believe, demonstrated the feasibility of applying protoplast fusion techniques to commercially valuable, phycocolloid producing seaweeds.

Acknowledgments

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3

Observations on the Physiological Ecology of *Eucheuma isiforme* from the Western Atlantic: A Review

Clinton J. Dawes
Department of Biology
University of South Florida
Tampa, Florida 33620

Abstract

The red alga *Eucheuma isiforme* from the tropical western Atlantic occurs in a number of forms ranging from deep water, non-spinose to bushy, thick and spiny plants in 1 to 3 m depth. All forms yield a carrageenan consisting of iota (75%) and a mixture of nu and kappa (25%). There is no distinction in carrageenan chemistry between diploid and haploid plants. All forms show spring growth with a high protein to carbohydrate ratio, a low rate of growth in the summer with a low protein to carbohydrate ratio and a fall or early winter die-back during reproduction. Most forms have been found to have cystocarpic, spermatangial and tetrasporic phases except for a population at Bahia Honda Key in the Florida Keys. Laboratory culture of all forms has proven successful using SWMD-1 and ESS media. Wound healing has been followed in cultured *E. alvarezii* var. *tambalang* from the Philippines as well as of *E. isiforme* of the Florida west coast. Measurements of photosynthesis, respiration, and growth in culture indicate that all forms of *E. isiforme* are stenohaline (32 to 35 ppt salinities) and show highest responses at lower temperatures (20° to 24°C).

Introduction

Of almost 200 species of red algae that have been harvested in the world, three phycocolloid-producing genera account for about 90% of the commercial effort in the tropics: *Eucheuma, Hypnea,* and *Gracilaria* (Dawes, 1981; Dawes, 1987). The only tropical phycocolloid-producing brown algal species that is presently harvested is from the genus *Sargassum.*

Species of all four genera occur in the western tropical Atlantic and have been subjects of field and laboratory physiological studies. Because of recent and ongoing studies, this paper will review the information available on the red carrageenophyte, *Eucheuma.*

The tropical western Atlantic area includes the Caribbean region (coasts of Columbia and Venezuela in South America, the east coast of Central America from Panama north to the Yucatan Peninsula, the Caribbean Sea and eastern island arc), the Florida Keys, the Bahama Islands and Bermuda. The deeper waters (to 30 m) off the North Carolina coast and shallower waters off both coasts of south Florida are also included (Dawes *et al.*, in press).

Eucheuma

The red alga *Eucheuma* J. Agardh (Solieriaceae, Gigartinales), which is extensively farmed in the South China Sea and elsewhere in southeast Asia and Japan, occurs throughout the tropical western Atlantic (Taylor, 1960; Dawes *et al.*, 1974a). Doty and Norris (1985) have reviewed the taxonomy of *Eucheuma* species that are commercially important and Cheney and Dawes (1980) have discussed the taxonomic problems of Floridian and Caribbean species. All of the forms of Floridian *Eucheuma* considered in this paper can be assigned to the species *E. isiforme* (C. Agardh) *J. Agardh* (Cheney, 1975). The forms found in the tropical western Atlantic (see Dawes, 1974a; Cheney, 1975 for photographs) range from the typical, coarsely branched bush, which is characteristic throughout the Caribbean. Other forms include an elongated, loosely branched type found in the Florida Keys (Bahia Honda form) and two, smooth, non-spinose or "nudum" forms that occur on the east coast of Florida in shallow, high energy reefs and in deeper (10 to 40 m) waters off the west coast of Florida.

Growth and reproduction

Field studies of tagged plants placed on lines or attached to concrete pads have demonstrated that all three forms become reproductive in the fall (Dawes *et al.*, 1974a). Tagged plants showed growth rates of 1.2 to 2.5% grams dry weight per day (typical form) to 5% grams dry weight per day (Bahia Honda form) in the Florida Keys. All forms showed peak growth in the spring, a slow down during the summer, and die-back in the late fall to early winter months when the plants became reproductive. For the most part, all forms showed a triphasic life history, although male plants were more difficult to identify in the fall and the Bahia Honda form appeared to have a modified life history with few gametophytes evident.

The size class distribution of randomly collected plants from the west coast of Florida, Bahia Honda and Molasses Keys in the Florida Keys indicate the the typical form was perennial, while the other two forms (Bahia Honda, West Coast) were annuals. The plants regrow from perennial bases and spores in the spring (Dawes *et al.*, 1974a). Outdoor tank culture of the two Florida Key forms could not overcome the fall reproduction and die-back (Guist *et al.*, 1985) although growth rates improved to 7% grams dry weight per day from March through July and to 5% per day from August and September. In summary, the fall reproductive die-back in field or tank cultures has not been avoided and this has been a serious limitation to field mariculture of *Eucheuma isiforme*.

Laboratory culture studies were first used to determine whether nutrient addition under controlled abiotic conditions would result in increased plant productivity using the west coast "nudum" form of *Eucheuma isiforme* (Dawes *et al.*, 1976). The results indicated that plants gave highest photosynthetic responses when grown at 20° to 24°C and under relatively low (ca 100 $\mu E\ m^{-2}\ s^{-1}$) with 5.0

ppm nitrate and 3.0 ppm phosphate. However, these studies were short-term and attempts to maintain or grow any form for long periods of time were not successful.

More recently, culture procedures have been developed for long term maintenance and culture of *Eucheuma* species from the Philippines and Florida. These procedures included determination of initial cleaning and shipping of branches, use of antibiotics and culture media. One of the problems encountered in laboratory culture was the die-back of cut branches due to bacterial activity and slow wound healing. Meso-insoitol was found to be the key chemical in the ESS nutrient-enriched seawater of Saga (1986) that aids healing of cut branches. The rate of wound healing is markedly improved if meso-insoitol is added to other culture media. Both SWMD-1 (Chen and Taylor, 1978) and ESS media are effective in branch and segment culture for *Eucheuma* species.

The process of wound healing was studied in the kappa carrageenan-producing Philippine species *Eucheuma alvarezii* var. *tambalang* Doty (Azanza-Corrales and Dawes, 1989), using histochemical stains. During the first stage, 2-4 days after wounding, proteinaceous and phenolic substances concentrate on pit plugs of cells adjacent to the wounded surface. In the second stage, about the sixth day, cellular extensions are produced from the medullary and cortical cells of the sub-wound layer. During the third stage, about the eighth day, these cells divide several times and elongate towards the surface. A new cortical or wound tissue is formed during the fourth stage beginning on the twelfth day. A new cortex is continuous with the old cortex in about three weeks.

Proximate constituents

Seasonal changes in the levels of protein, carbohydrate, lipid and ash have been used to follow allocation of energy in marine plants (Dawes *et al.*, 1974b; Dawes, 1981). Protein levels were highest in spring while carbohydrate (and phycocolloid) levels were highest in the summer in the various forms of *Eucheuma* from Florida. The protein to carbohydrate ratios could be used to determine when the plant was actively growing (1:6) or not (1:15).

Because carrageenan levels parallel that of soluble carbohydrate, a mariculture/harvest strategy was proposed in which plants were allowed to grow rapidly in nutrient-enriched seawater and then transferred to seawater to allow conversion of photosynthates to phycocolloid (Dawes, 1974b; Dawes *et al.*, 1974b; Guist *et al.*, 1985).

In a comparative study of east and west coast *E. isiforme* ("nudum" forms), no differences in proximate constituents between gametophyte and tetrasporophyte plants were evident (Dawes, 1982). This was also true for 3, 6-anhydrogalactose, the primary residue in iota carrageenan found in these plants, and this suggested that there was no difference in carrageenan chemistry between reproductive phases.

Iota carrageenan extracted from the various forms of Floridian *Eucheuma isiforme* and *E. uncinatum* Setchell and Gardner from the Gulf of California was analyzed seasonally at the Marine Colloids facilities in Maine (Dawes *et al.*, 1977a, 1977b). Yield, viscosity, gel strength and molar ratios indicated that there were no differences between extracts obtained from diploid (tetrasporphytes) or haploid (spermatangial, cystocarpic) plants. Thus, all iota-producing forms of *Eucheuma*, including the Philippine species, *E. denticulatum* (N. L. Burman) Collins et Harvey do not show an alternation of carrageenan types between diploid and haploid plants as found in some other members of the red algal order Gigartinales (Dawes, 1979).

The Floridian forms were found to have a "deviant" type of iota carrageenan. Later, the typical iota carrageenan found in *E. denticulatum* and in *E. uncinatum* was compared with the carrageenan of *E. isiforme* and the "deviant" characteristics of the latter's carrageenan was again confirmed (Dawes, 1979). A collection of vegetative *E. isiforme* from Vero Beach was sent to the late Dr. Yaphe in 1981 who determined the "deviant" iota carrageenan to be a mixture of 75% iota and 25% nu and kappa using enzymatic and NMR analyses (Greer and Yaphe, 1984a, 1984b).

Photosynthetic responses

All three freshly collected forms of *Eucheuma isiforme* from the Florida Keys (Bahia Honda and typical form); and the west coast for Florida ("nudum") form showed surprisingly low photosynthetic responses to temperatures above 28° C with peak responses occurring at 20° to 24° C (Mathieson and Dawes, 1974). A similar set of narrow peak photosynthetic responses was found for freshly collected plants in relation to salinity (Mathieson and Dawes, 1974); all three forms showing peak responses at 35 to 40 ppt with the west coast "nudum" form showing a broader tolerance to salinity. The optimal temperature response of 21° C explains, in part, why the three forms showed maximum field growth in the spring and a slow-down in the summer when water temperatures exceeded 28° C. The high salinities at which peak photosynthetic responses occurred also correlated with the high salinities encountered in the Florida Keys while the west coast form showed high responses to a broader range of salinities at 20° C, conditions encountered along the west coast of Florida.

When photosynthetic responses were compared with other iota carrageenan-producing species of *Eucheuma*, similar results were obtained (Dawes, 1979). *Eucheuma uncinatum* from the Gulf of Mexico (Dawes *et al.*, 1977a) and *E. denticulatum* (formerly *E. spinosum*), as taxonomically described by Doty (1985), showed highest "net" photosynthetic and lowest respiratory rates with ambient, high salinities (30 to 35 ppt) and cooler temperatures (20° to 24° C). All three species showed increasing photosynthetic rates with increasing irradiance (max = 18,000 μw cm^{-2} white light) with no photoinhibition evident. Thus the iota carrageenophytes in the genus were similar in their physiological responses to light, salinity and temperature.

Recently physiological responses of the three forms of *Eucheuma isiforme* from the Florida Keys and west coast have been re-examined (unpublished data) with relation to shifts in irradiance, salinity, and temperature. All three populations initially showed no photoinhibition to irradiances up to 1200 μE m^{-2} s^{-1} with photosynthetic maximums of about 2 to 7 mg oxygen g $dwt^{-1}h^{-1}$. However, after culture for 5 to 7 weeks, the productivity dropped to about 1 mg oxygen g $dwt^{-1}h^{-1}$. All three cultured forms were intolerant to salinities of 20 ppt or less and to 10°C regardless of the growth in nutrient-enriched seawater. These findings of very limited tolerances of *E. isiforme* were in contrast to the broad tolerances and ease of culture of another iota carrageenophyte, *Solieria filiformis* (Kütz) Gabrielson, cultured with the various forms of *Eucheuma* from Florida.

The temperature acclimation ability of cultured west coast *Eucheuma isiforme* has been compared with the intensively farmed Philippine species, *E. alvarezii* var. *tambalang* (unpublished data). There was a strong acclimation in the west coast form when grown for two weeks each at 18° C, then 22° C and finally 25° C, but little acclimation if transferred from 18° C to 25° C directly. The higher photosynthetic response was at 25° C with gradual acclimation. This acclimation of the west coast plan parallels the increase in summer water temperatures.

In contrast, *Eucheuma alvarezii* var. *tambalang* did not show as strong an acclimation when grown two weeks each at 25° C, 22° C and 18° C. The plants almost died when transferred directly to 18° C from 25° C. This suggests that the Philippine material cannot grow in lower temperatures and is actually more "tropical" than the west coast Floridian species.

Continued studies on *Eucheuma isiforme* from the tropical western Atlantic will emphasize laboratory culture and selection of physiological forms that will acclimate to changing conditions. Forms must be found that will not die back every year during reproduction if field mariculture is to be successful. In this regard, the typical form appears to be the best choice. It is a perennial and also yields the highest amount of the "deviant" or iota-kappa-nu carrageenan extract. However, the typical form is also the most difficult to culture and grows slowly when compared with the west coast and Bahia Honda forms. Laboratory culture will also allow experiments to determine acclimation not only with relation to photosynthesis and respiration, but also in regard to carrageenan modification.

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4

Irish Moss Cultivation: Some Reflections

James S. Craigie
Atlantic Research Laboratory
National Research Council of Canada
1411 Oxford St.
Halifax, Nova Scotia
Canada B3H 3Z1

Abstract

A brief outline of the domestication of *Chondrus crispus* as a species for aquaculture in Nova Scotia is presented. Appropriate strain selection combined with research to optimize physical, chemical and biological variables has led to sustained high levels of productivity on an annual basis. The effects of temperature and irradiance on nitrate uptake are presented. Net production of *C. crispus* in outdoor tanks at commercial loading densities is a linear function of irradiance. Photosynthetic efficiencies measured over a one-year period were 1.73% of the total available solar irradiance. Approximately 3.4 ha. of culture tanks are now devoted to the commercial production of Irish moss.

Introduction

Irish moss of commerce has been defined as the dried and bleached plants of two red algae, *Chondrus crispus* and *Mastocarpus stellatus* (formerly *Gigartina mamillosa*). In the present

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context, I refer only to the former alga which bears the common name "carrageen" (carragheen or carageen), the hot water extract of which has been termed "carrageenin" (Stanford, 1862). The name carrageen is thought to derive from the Irish word "carraigeen," meaning rock moss (Mitchell and Guiry, 1983). The Irish moss extract consists of a variable mixture of the kappa and lambda families of carrageenans, the ratios of which reflect the proportion of gametophytes and sporophytes present in the sample when it was harvested (cf. Craigie, in press).

World sources of carrageenans include eight genera of red algae which yielded 43,000 metric tonnes of commercially dry seaweeds in 1984 (Lewis *et al.*, 1988). Only about 10% of the current world production (ca. 50,000 mt) of carrageenophytes originates in North America, principally as Irish moss from the Canadian Provinces of Prince Edward Island and Nova Scotia, and the States of Maine and Massachusetts. Approximately half of the world's carrageenophytes are produced in the Philippines and Indonesia where several species of *Eucheuma* are cultivated on a large scale in the sea. Much of the remaining supply comes from natural harvests in Europe and South America, chiefly Chile.

The world market for refined and semi-refined carrageenans was some 13,200 mt in 1982 with a compound annual growth rate of 2.2% between 1982 and 1986 (Lewis *et al.*, 1988). It is obvious that the industry, based upon present products, can be considered mature. Expansion will depend on the development of new applications for the existing carrageenans of commerce, iota and the kappa/lambda mixtures, or the utilization of the hitherto ignored carrageenans of the lambda and beta families. For example, the structure of lambda carrageenan has been known for more than 25 years, and its properties as a highly viscous, non-gelling, polyanionic hydrocolloid are well suited to applications in cold, dry-packaged, instant-mix food products (Witt, 1985).

The current world supply of ca. 200 mt of low quality lambda carrageenan is insufficient to permit development of the perceived market (P.S. Laite, *pers. comm.*, 1987). The problem of supply can be alleviated either by locating and developing a specific source of lambda carrageenan, or by separating the lambda-containing algae from the existing commercial harvest. Both approaches depend upon the knowledge that carrageenans of the kappa-iota family are biosynthesized by the gametophyte generation of members of the Gigartinaceae and Phyllophoraceae, while lambda carrageenans are formed by the sporophyte generation. The separation of commercially harvested *Chondrus crispus* fronds has been achieved by a combination of chemical and mechanical steps based upon the the differing properties of lambda and kappa carrageenans (Whitaker, 1988, 1989). However, this approach has the potential to increase the pressure on natural populations, and to divert the harvest from its traditional markets.

The farming of seaweeds, on the other hand, was recognized as of prime importance for the stabilization and future development of the phycocolloid industry (Woodward, 1966; Neish, 1968; Doty, 1979). Initially, the intention was to cultivate *Chondrus crispus* as a high grade source of kappa carrageenan (Neish and Fox, 1971). The discovery that lambda and kappa carrageenans were carried, respectively, in the sporophyte and gametophyte isomorphs (Chen *et al.*, 1973; McCandless *et al.*, 1973) led to the separate cultivation of the two generations to produce the desired carrageenan (Chen *et al.*, 1975).

The process of adapting a species to intensive cultivation is composed of several recognizable steps, which include three preconditions: availability, utilizability, and discovery; and four stages of domestication: collection from natural populations, resource management, husbandry, and full cultivation (Woessner, 1981). During the past one and a half centuries, the preconditions for Irish moss cultivation have been fully met, as have two (arguably three) of the four stages of domestication. The final stage of full cultivation of *Chondrus crispus* is only now being realized.

In his vision of the seaweed industry of the future, Woodward (1966) pointed out, by analogy with agriculture, several important topics that required research: (a) genetic studies to provide tailor-made algae and hybrids; (b) introduction of foreign species having specific desirable properties; (c) development of new growing techniques leading to large-scale culture; and (d) mechanization. Similar views were shared by the late Arthur C. Neish of the

Atlantic Research (then Regional) Laboratory, who concluded that the application of modern agricultural principles to seaweeds could result in new crops from the sea (Neish, 1968).

Farming *Chondrus Crispus*

The general principles of farming are widely known. The aim is to convert as many photons of light into product (crop) as possible by optimizing conditions for photosynthesis, growth and possibly reproduction of the desired species. In practice, the irradiance available and the annual temperature profile are "givens" so the problem reduces to one of how to best optimize the remaining controllable variables.

The Species And The Site:

The choice of alga depends on the product (i.e., food, chemical extracts, etc.) to be introduced into the market. Once the species, in our case *Chondrus crispus*, is identified, any screening or selection process must take into consideration the interactions of the alga with the local environment, as a wide gradient of responses exists when individuals from a wild population are exposed to parallel conditions in culture (Neish and Fox, 1971; Cheney *et al.*, 1981).

Selecting a site for seaweed cultivation necessitates compromises involving environmental quality, available infrastructure, the topography, the cost of land, as well as legal and regulatory aspects. The seawater should be relatively free of sediments, potential biological contaminants and chemical pollutants. The annual water temperature and salinity cycles must be compatible with survival of the alga. Adequate electrical power and communication facilities including year-round roads must be available to the site. The topography and surface geology must permit large tank construction with minimum difficulty. The beach should not be so energy intensive that seawater pipelines are jeopardized during storms. The cultivation of algae must be in accordance with local land use and zoning regulations. Finally, the cost of the property must fit the business plan. It is obvious from these considerations that each site will be unique and may necessitate the development of algal strains best suited for that site.

Domestication:

The first challenge was to obtain an isolate of *Chondrus crispus* that would grow satisfactorily in culture tanks. Successful domestication of the alga was achieved in early 1970 when clone T4 was selected from several hundred fronds taken from a beach near Halifax, Nova Scotia (Neish and Fox, 1971). Clone T4 was first assumed to be a vegetative plant, but was shown later to be a functional male (Guiry, 1981; van der Meer, 1981). For much of the decade, it served as a biological reference for experimental phycologists and aquaculturists. It was evaluated in Canada between 1973 and 1979 in small-scale cultivation trials by two commercial firms, Genu Canada Ltd. (Bidwell *et al.*, 1985), and Marine Colloids Ltd. The screening carried out by these companies and independently (Braud and Delepine, 1981; Cheney *et al.*, 1981; Craigie and Shacklock pers. obs.) revealed that other *C. crispus* gametophytes could be selected with growth rates equal to or better than clone T4.

The rapidly accelerating production of kappa carrageenan-containing *Eucheuma* spp., from seaweed farms in the Philippines during the late 1970's (Ricohermoso and Deveau, 1979) made it imperative to develop *Chondrus crispus* as a source of lambda carrageenan. The search for sporophytes with high growth rates produced two isolates, no. 750128C and ARL 16 (Chen *et al.*, 1982) which were tested in small (1.45 m x 0.75 m deep) outdoor tanks. The productivity-per-unit area for both isolates was inferior to that of clone T4 in parallel trials. A third sporophyte isolate, BH-D, raised from spores in 1982 from Basin Head; P.E.I., stock was clearly superior in productivity to T4 and was adopted for commercial production (Craigie and Shacklock, 1989). By October of 1987, BH-D had produced approximately 300,000 kg live weight. The growth rates of both life history phases of the BH line are indistinguishable from each other.

Overwintering:

The next challenge was to devise a strategy that would permit an operator to hold a large quantity of *Chondrus crispus* simply and inexpensively during winter months in the Canadian Maritimes. Initial trials conducted in 1972 established that the alga would grow throughout the winter in an unheated plastic covered shed if supplied with running seawater and agitation. Further experiments proved that even these minimal precautions were unnecessary as the fronds would survive in unprotected, undisturbed outdoor tanks of seawater (Shacklock and Craigie, 1986). As the water temperature approaches 0°C, both agitation and the seawater supply are interrupted and the tanks are allowed to freeze. *C crispus* at the bottom of the tanks survives the low temperatures of (<-1.9° C) and high salinities (58 ppt) when up to half of the water in the tanks has frozen. To ensure consistent success the stocking densities must not exceed ca. 10 kg m^{-3}. The nutrient status of the alga must be sufficient to avoid bleaching should mild conditions during the winter encourage growth. These simple steps are being applied routinely on a commercial scale to carry inoculum into the next growing season.

Physical Variables

Temperature and Salinity:

Chondrus crispus is a cold temperate species (Bird *et al.*, 1979; Simpson and Shacklock, 1979). It survives -1.9°C, grows slowly at 0° to 2°C with an optimum between 14° and 20°C depending on the available irradiance, and is impaired at 23°C (Enright and Craigie, 1981). Long term survival does not occur above ca. 26°C (Prince and Kingsbury, 1973).

The salinity for optimum growth of *Chondrus crispus* lies near 30 ppt although a range (10 to 58 ppt) may be tolerated (Bird *et al.*, 1979; Shacklock and Craigie, 1986).

Agitation:

High productivity can only be sustained if mechanical agitation is provided. Water jets, paddles, and air bubbles are useful in various applications. The first two, while suitable for laboratory cultures, do not appear feasible on a commercial scale. Agitation serves several important functions: It (i) suspends the alga, (ii) reduces boundary layers thus facilitating nutrient/metabolite movements, (iii) breaks up larger plants, (iv) causes abrasion of the fronds thereby aiding epiphyte removal, (v) suspends debris to be flushed away, and (vi) air agitation minimizes oxygen supersaturation (Craigie and Shacklock, 1989).

Water Exchange:

The flushing rate of a tank usually is expressed in tank volume changes per day. Initially, high exchange rates (up to 80 per day) were used, but these were insufficient to maintain a productive population so nutrients were added and flushing rates reduced. The lowest rate tested at commercial loading densities was one tank volume in three weeks. Current usage is three to seven tank volumes of new seawater per week to maintain adequate water quality.

Chemical Variables

Nutrient Requirements:

The productivity of any marine alga is intimately associated with the pH-dependent bicarbonate equilibrium system. Natural seawater (ca. pH 8.0) is near the equivalence point for the equilibrium between atmospheric CO_2 and the dissolved inorganic carbon species (DIC) in the sea:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow HCO_3^- + H^+ \rightarrow CO_3^{2-} + H^+$$

Photosynthesis by the alga rapidly depletes the DIC raising the pH towards a limit near pH 9.5, under which conditions photosynthesis of *Chondrus crispus* is negligible. Restoration of photosynthesis requires that the pH be lowered and that a supply of inorganic carbon be available. This is conveniently achieved by supplying CO_2 via the air agitation system or by direct injection into the seawater. Efficiencies based on carbon recovered as biomass range up to 85% with direct

CO_2 injection through a basswood diffuser. Stoichiometry can be achieved by using a solution of H_2CO_3 in seawater to titrate the culture medium. In all cases the additions are regulated by pH-activated solenoid valves.

The second and third nutrients to become limiting in tank culture are N and P, respectively. Nitrogen depletion becomes evident within three to five days, while phosphorus deficiency requires up to a week to be measurable as reduced growth. Increasing N depletion results in a paler colored frond until, in extreme cases, a greenish-yellow color is attained. The nitrogen content of *Chondrus crispus* can range from about 0.8% to 4.5% of the dry weight. The dramatic inverse relationship between the nitrogen and carrageenan contents of Irish moss is called the Neish Effect (Neish *et al.*, 1977). Nitrate uptake by *C. crispus* is influenced by its availability (generally, high levels of nitrogen are applied in commercial systems), the water temperature, irradiance, and the internal NO_3 content of the thallus. When millimolar concentrations of NO_3 are supplied, the fronds reach a saturation level of approximately 100 mM g^{-1} fresh weight in 10 days at 15°C (Fig. 1). The uptake rates are highest when the nitrate concentration in the fronds is low and they decline abruptly as the fronds reach saturation (Fig. 2). Nitrate uptake is strongly influenced by temperature with a Q_{10} of 2.23 between 5°C and 15°C (Table 1). Uptake rates in light are approximately 47%greater than those in darkness (Table 2). Nitrate accumulated in the frond, while significant, is less important than the dipeptide, citrullinyl-arginine, as a nitrogen reserve (Laycock *et al.*, 1981).

Agricultural fertilizers, NH_4NO_3 and $(NH_4)_2HPO_4$, are normally dissolved in water to give a 10:1 molar ratio of N:P. This concentrate is added one to three times per week in summer to give 1.0 mM and 0.1 mM of N and P, respectively, in the culture tanks. Pulse fertilization has been used continuously since 1970 as it seems to be beneficial in minimizing epiphyte contamination and it takes advantage of the nutrient storage capabilities of the alga. Phosphorus occurs in *Chondrus crispus* to the extent of ca. 0.4% of the dry weight and is thought to accumulate in an organic form (Chopin, 1986).

Attempts to induce nutrient deficiencies of Co, Cu, Fe, Mn, and

Zn in actively growing *Chondrus crispus* cultures were successful only in the case of iron (Craigie and Shacklock, 1988). At commercial loading densities and seawater flushing rates of three tank volumes per week, the apices of the rapidly growing fronds became chlorotic and productivity declined. These symptoms were completely alleviated within one week after adding Fe-EDTA to the cultures. None of the other metals were effective.

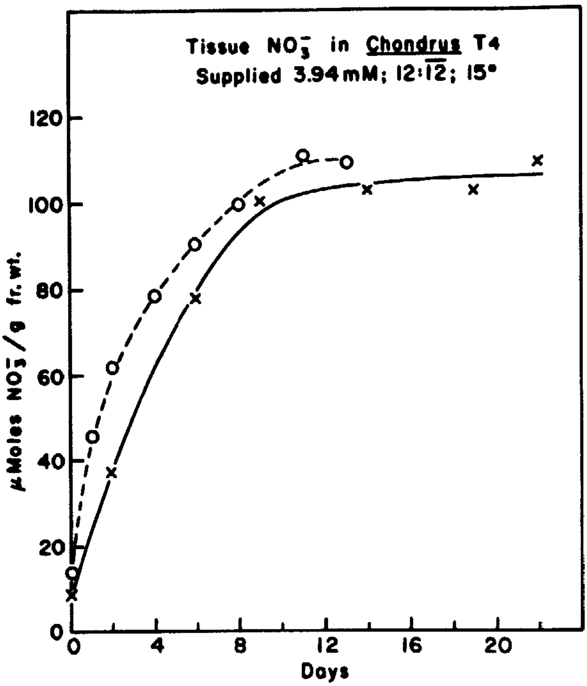

Figure 1. Time course for the accumulation of nitrate internally by Chondrus crispus *T4 at 15°C. Symbols represent independent experiments.*

Biological Variables

Weed Control:

Among the various weed species of algae that may foul *Chondrus crispus* (cf. Craigie and Shacklock, 1989) few are as persistent as *Enteromorpha* spp., (Shacklock *et al.*, 1973) and *Ulva* spp., (Enright, 1979). Control strategies such as increased loading densities in tanks, pulse feeding of fertilizers (Neish and Fox, 1971), the use of mesoherbivores (Shacklock and Croft, 1981;

Shacklock and Doyle, 1983), and the application of herbicides (Bidwell *et al.*, 1985) have all been used with varying degrees of success. Management of a population of suitable grazers (e.g. *Gammarus* spp., *Idotea* spp.) appears to offer the most satisfactory solution to the problem of weed control in commercial cultures.

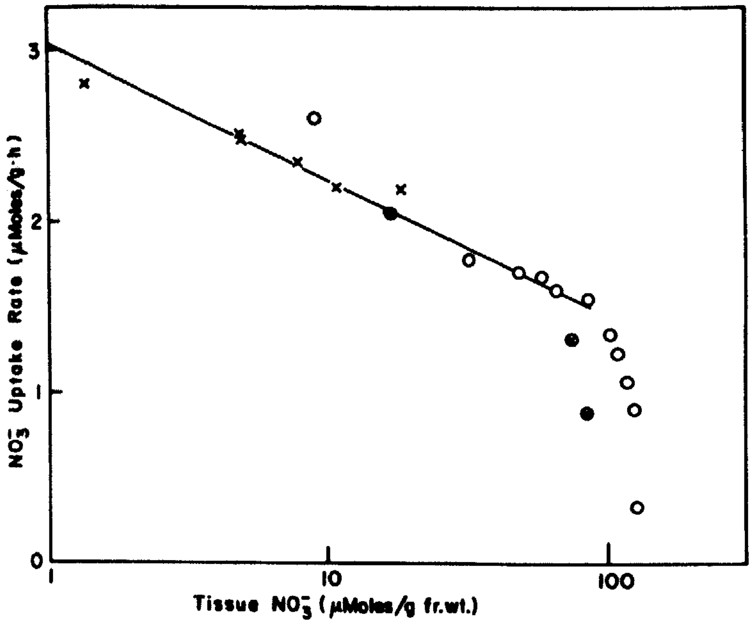

Figure 2. Uptake rates for nitrate by Chondrus crispus *at 15° C as a function of internal nitrate concentration. Symbols represent independent experiments.*

Diseases:

The development of *Chondrus crispus* aquaculture has been hampered by the outbreak of two serious diseases. The causal agent for the first was a fungus, *Petersenia pollagaster*, which, appearing in 1980, attacked and destroyed the apical regions of the alga. The disease was not lethal but it completely prevented algal growth for several months. The application of low levels (ppm) of sodium dodecyl sulfate to the cultures prevented reinfection of the alga by the fungal zoospores (Craigie, 1984).

Table 1. Influence of temperature on nitrate uptake rates (μmol · g⁻¹ · h⁻¹ fr. wt. basis) by *Chondrus crispus* T4 in light.

0°C	15°C	0°:15°	5.5°C	15.5°C	Q_{10}
0.12	1.18	10.2	0.88	2.08	2.36
0.07	1.34	5.2	0.67	1.41	2.11
0.41	1.78	23.0	0.66	1.47	2.23
0.44	1.93	22.8	0.60	1.20	1.97
					Mean = 2.23

Data are from separate experiments using paired trials, 0°C with 15°C; 5.5°C with 15.5°C.

Table 2. Uptake rates for nitrate (μmol · g^{-1} · h^{-1} fr. wt. basis) by *Chondrus crispus* T4 in light and in darkness at 15° C.

Dark	Light	Ratio D:L
1.20	1.87	0.64
0.84	1.19	0.71
0.77	1.27	0.61
1.07	1.76	0.61
1.40	1.72	0.81
		Mean =0.68

Data are for independent paired experiments conducted at various times.

The second disease appeared initially as small greenish spots on the older parts of the thallus. These developed into lesions which resulted in holes of two to three millimeters in diameter. If the holes become sufficiently numerous the weakened thallus breaks into small fragments that are lost from the system. The disease is now known to be of bacterial origin and control strategies are under development.

Productivity:

The net productivity of *Chondrus crispus* in tank culture is a linear function of irradiance (Fig. 3) over the temperature range of 10° to 20°C when nutrients are not limiting and the pH is controlled. The relationship between net productivity and irradiance is improved considerably when early morning and late afternoon irradiances (sun elevation less than 28° from the horizon) are excluded from the calculation. Presumably irradiance losses by reflection become important at low sun angles.

The net production (dry weight basis) over a consecutive 365 day period has been measured at 6.27 kg m^{-2} in Nova Scotia (Craigie and Shacklock, 1989). The overall annual conversion of solar radiation is, therefore, ca. 1.76% (= 4.0% on a PAR basis). Comparable photosynthetic efficiencies are 0.5% to 1.3% for plants of the temperate regions, while in the subtropics efficiencies range between 0.5% and 2.5% (Hall, 1980). A maximum photosynthetic efficiency for *Chondrus crispus* of 2.3% (= 5.3% on a PAR basis) was measured in summer over a 92 day period. This value compares favorably with the theoretical maximum of 6.0% (PAR basis) calculated for the species (Craigie and Shacklock, 1989). It may be concluded that *C. crispus* is a highly productive alga and that the tank culture technology is well optimized for maximum production.

The principles outlined above are being implemented on a commercial scale at Charlesville, Nova Scotia by Acadian Seaplants Ltd. The production tanks are 2000 m^3 and 3.4 hectares of tanks have been developed. The first 100 tonnes of cultivated dry Irish moss were marketed in 1987.

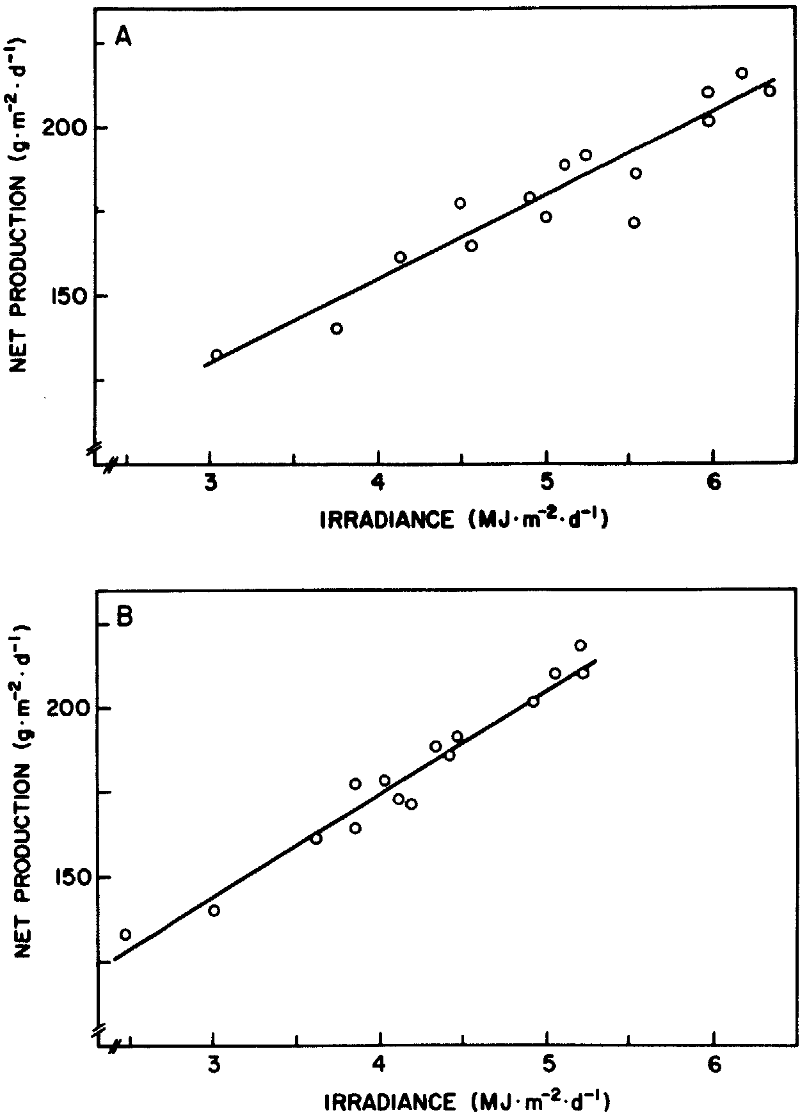

Figure 3a, b. Relationship between net productivity and irradiance (PAR = 400 to 700 nm) for Chondrus crispus *T4 grown in outdoor tanks from May 3 to September 1. Temperatures varied between 10° and 20° C. Each datum point is the mean value for 12 culture tanks. A) Mean daily irradiance (PAR) from sunrise to sunset. Linear regression: $y = 24.5x + 56.4$; $r^2 = 0.88$. B) Mean daily irradiance (PAR) for the period when the sun elevation exceeded 28° from the horizon. Linear regression: $y = 30.7x + 51.5$; $r^2 = 0.96$.*

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5

Morphological and Physiological Bases for *Laminaria* Selection Protocols in Long Island Sound Aquaculture

C. Yarish, B. H. Brinkhuis[†*], B. Egan, and Z. Garcia-Ezquivel[*]
Dept. of Ecology and Evolutionary Biology
University of Connecticut
641 Scofieldtown Rd,
Stamford, CT 06903

Abstract

Considerable progress has been made over the last five years toward development of genetic selection procedures for the kelp, *Laminaria*, in a collaborative research program between the State University of New York and the University of Connecticut. We review here the current status of taxonomy and our investigations into genetics and breeding of *Laminaria*. Growth and reproduction characteristics of *L. saccharina* from Long Island and *L. longicruris* from eastern Connecticut are compared. We emphasize application of gradient plate studies to development and growth characteristics from *L. saccharina* and *L. longicruris* populations in Long Island Sound. Such studies have yielded important insights into the possibility of selecting strains that are superior for given combinations of light and temperature. Preliminary crossing experiments between these local entities indicate a sex-linkage for certain morphological characters.

[†] Deceased

[*] Marine Sciences Research Center, State University of New York, Stony Brook NY 11794

Introduction

Geographic variation among seaweed populations has resulted in considerable taxonomic confusion where morphological or physiological characters have been used to delineate species (Mathieson *et al.*, 1981; Rice and Chapman, 1985). Certainly, the definition of what constitutes a species lies at the heart of taxonomic disputes. The classically accepted definition states that true biological species are reproductively isolated. Where "species" cannot be demonstrated due to a lack of sterility barriers, ecotypic status is frequently assigned to the taxon. By definition, an ecotype is the product of a genotypic response of a species to a particular environmental condition of a habitat, and it implies interfertility with other members of the species (Turesson, 1922). Both physiological and morphological ecotypes have been described for various algae (e.g., Gerard and Mann, 1979; Yarish *et al.*, 1979; Mathieson *et al.*, 1981; Russell and Fielding, 1981; Espinoza and Chapman, 1983; Bolton, 1983; Innes, 1984; Gerard, 1988; Gerard and Du Bois, 1988). The occurrence of ecotypic variation in seaweeds has been taken for evidence of genetic differentiation among populations, whose members are perhaps in the process of speciation (Innes, 1984). It is this premise that facilitates efforts directed at selection of strains with both morphologically and physiologically desirable qualities.

Taxonomic delineation in brown seaweeds has been conducted mainly on the basis of phenotypic characters observed in the field and in culture (Russell, 1978). The developmental state of plants and their responses to environmental conditions have caused the greatest consternation among brown algal taxonomists using phenotypic characters to delineate species. With the advent of computers, hierarchical analysis in numerical taxonomy has been attempted as a means of simplifying taxonomic criteria and identification of species (e.g., Widdowson, 1971; Russell and Fletcher, 1975; Prud'homme van Reine, 1978; Pankhurst and Tittley, 1978, Marsden *et al.*, 1983; Rice and Chapman, 1985). These methods have had limited success in natural groups, but have failed where phenotypic variability is poorly understood. Many of the criteria used are based on gut feeling or intuitive weighting. Russell (1978) cited numerous instances where the role of genotype has been inadequately separated from phenotype in dictating morphological form. The influence of common environmental factors on morphological characters of seaweeds has recently been reviewed by Norton *et al.*, (1981, 1982). Frequently, attempts to culture representatives compound the problem since responses in culture are not analogous to responses to variable environmental conditions in the field. The lack of appreciation of phenotypic vs. genotypic variability makes attempts to describe phytogeographic distribution difficult. It also hampers selection of new strains for use in mariculture. To date, there has been only one attempt at using numerical taxonomic methods in the study of laminarian populations, namely *Alaria* (Widdowson, 1971).

The genetic basis of population differentiation in the Laminariales has been inferred from observations after one or more generations reared under similar laboratory conditions, reciprocal transplants between field sites, outplants of germlings to a common "garden", and reciprocal crosses between geographically separated populations (Lüning, 1975; Lüning *et al.*, 1978; Bolton *et al.*, 1983; Espinoza and Chapman, 1983). The most extensive kelp genetics experiments have been conducted by Chinese workers (Fang *et al.*, 1962a/b, 1963, 1965, 1966, 1978, 1979; Fang and Li 1963, 1965; Fang and Dai, 1980; Fang, 1984) in attempts to generate new strains with higher temperature tolerance and increased iodine content. Only attempts at the latter were successful (see Brinkhuis *et al.*, 1987 for review).

Crossing experiments offer a means of assessing differentiation when correlated to specific environmental differences. However, interpretation of such experiments is not straightforward. Genetic differentiation may evolve without affecting compatibility, and intersterility does not necessarily indicate accumulation of a large number of genetic differences (Innes, 1984). Recent studies have found that greater incompatibility is associated with greater geographical separation (Rueness, 1973; Müller, 1979; West *et al.*, 1978; Guiry and West, 1983), but there are numerous examples that have demonstrated genetic differentiation among populations separated by only short distances (e.g., Russell and Morris, 1970; DeSilva and Burrows, 1973; Espinoza and Chapman, 1983; Innes, 1983).

In this paper, we review aspects of morphological variation in certain *Laminaria* species, variation that has complicated the species concept in this genus. This variation also permits a basis for selection protocols, as certain morphological attributes are considered desirable for particular mariculture objectives. For example, plants producing wider or longer blades might be required if the objective is to maximize biomass production. Similarly, plants with longer stipes might be desirable if the objective is to maximize biomass of stipe material that generally has higher alginate content. Selection criteria also apply to determining the most appropriate conditions for obtaining the best growing materials. Our review, then, is followed by experiments we have conducted following these guidelines.

Taxonomic characteristics of North Atlantic *Laminaria* species

The genus *Laminaria* inhabits temperate and polar waters in the northern and temperate waters in the southern hemispheres. It is presently believed that the genus originated in the northern Pacific (Lüning, 1985). The Pacific is characterized by greater numbers of genera and species of Laminariales than is the Atlantic. This is probably due to repeated displacements of the marine flora towards the south during the cold periods of the Pleistocene (Hoek, 1984; Yarish *et al.*, 1984; Lüning, 1985). Development of cold water-resistant algae probably occurred during the Miocene, 21 million years ago. Cooling of the earth in the late Tertiary forced the newly-formed cold water species in a circumpolar route along the still ice-free coasts of the Arctic into the North Atlantic (Simmons, 1906; Lüning, 1985). This paleo-history has been linked to plate tectonic patterns occurring during the Tertiary (Lüning, 1985).

The laminarian sporophyte is in three distinct parts: holdfast, stipe and lamina. The lamina, or blade, of *Laminaria* may be simple and undivided (Section Simplices), or digitate (Section Digitatae). The primary growth is intercalary, occurring in the transition zone between stipe and lamina. Some morphological characteristics used to distinguish species within the genus (mucilage duct anatomy, stipe anatomy and bullations in lamina) are now known, or suspected to be highly variable and subject to change in response to environmental differences (see Kain, 1979 for review). In the Section Digitatae from the North Atlantic, Sundene (1959, 1964) found that the taxa *Laminaria stenophylla, L. intermedia, L. platymeris,* and *L. cucullata* were merely environmentally induced forms of the highly variable *L. digitata.* Similarly, Wilce (1959) found *L. cuneifolia* and *L. groenlandica* in the Section Simplices were identical. Our detailed analysis of pertinent *Laminaria* literature is described below.

Mucilage ducts as a taxonomic character

The presence or absence of mucilage ducts was proposed by Fritsch (1945) as a character to delineate *Laminaria groenlandica* Rosenv. (ducts in laminae and stipes), *L. saccharina* (L.) Lamour. (ducts only in laminae) and *L. agardhii* Kjellm. (no ducts). Wilce (1965) doubted the validity of mucilage ducts as a taxonomic character, particularly because of the tendency for duct occurrence and size to increase with latitude (decreasing temperature). In contrast, Burrows (1964) found that duct occurrence and size increased with increasing temperature (implying decreasing latitude) after a comparative culture study of *L. saccharina* and *L. agardhii* from Europe at 5 and 10°C. However, Wilce (1965) argued that the ductless plants (*L. agardhii*) Burrows found at 5°C were, in fact, a ductless form of *L. saccharina,* because Burrows' use of 5°C did not approach the cold temperatures of Arctic waters, and because the west and northwest coasts of Norway (Burrows obtained plants from Spitzbergen) are still somewhat under the influence Gulf Stream water, an environment no more severe than that of some areas of North America where *L. saccharina* (=*L. agardhii*) is dominant.

Thus, *Laminaria saccharina* has three ecotypes (Wilce, 1965) based on mucilage duct anatomy. Wilce (1965) concluded that there were only three species in the Section Simplices from the North Atlantic. These are *L. solidungula* J. Ag. (with a distinct discoid holdfast, whereas all other species have haptera), *L. saccharina* and *L. longicruris* de la Pylaie. According to Wilce (1965), *Laminaria* at its southern distribution should not have ducts. We have recently determined that plants in Long Island Sound may exhibit ducts at various times, but there is no apparent relationship to taxonomic affinity (Levine, Collantes and Brinkhuis, unpublished data).

Laminaria longicruris is characterized by being the only species in the genus having a long and hollow stipe (Wilce, 1965). This species was also described as having three ecotypes based on duct anatomy: *L. longicruris* with ducts in laminae and stipes from the Northwestern Atlantic, *L. faeroensis* Børgesen from the Northeastern Atlantic with ducts only in laminae, and an unnamed ecotype without ducts described from the Western Atlantic. Based on the invalidity of duct anatomy as a taxonomic character, Kain (1976) suggested that *L. faeroensis* be placed within *L. longicruris*. Wilce (1965) proposed that all hollow-stiped plants be referred to as *L. longicruris* and all solid-stiped as *L. saccharina* in his revision of the northern Atlantic Simplices section, since mucilage ducts could be found in either species.

Chapman (1975) conducted crossing experiments with three "species" and examined the mucilage duct heritability component using materials from Nova Scotia (no mucilage ducts present) and Newfoundland (both with and without ducts in blade or stipes). Various combinations of ducts were present in stipes and blades of Newfoundland populations. There were no discontinuities in the series of biotypes. There was a large environmental component in the expression of phenotypic variance, and there were no internal reproductive barriers between any of the anatomical types. Thus, he concluded that this character does not conform to the biological species concept. Nova Scotian plants never exhibit mucilage ducts, and this character breeds true. These breed freely with plants from Newfoundland showing canals, but the heritability component of the phenotypic variance was low. The regression of offspring measurements on parental mean measurements gives an estimate of heritability. Heritability is the ratio of additive gene variance to total phenotypic variance- a low heritability indicates high environmental component in phenotypic variance observed. Chapman suggests that the two "species" be given ecotypic status. An ecotype is the product arising as the result of genotypic response of a species to its particular habitat (Turesson, 1922) and this status implies interfertility. Recently, Müller *et al.*, (1985) have found that pheromones in the Laminariaceae are identical, suggesting a possible mechanism for interfertility in *Laminaria*. Chapman concluded that *L. longicruris*, and *L. saccharina* (=*L. agardhii*) are synonymous and should be placed within the taxon *L. saccharina*. Interestingly, some workers from Nova Scotia still refer to their local form as *L. longicruris*.

In crosses between European (with ducts) and Nova Scotian (ductless) strains, Lüning *et al.* (1978) found that hybrids all lacked ducts. However, Bolton *et al.* (1983) reported that hybrids produced from crosses of ductless Nova Scotian and ducted Brittany (France) plants exhibited ducts on two separate occasions. They also reported that hybrids from crosses of Helgoland (Germany) and Nova Scotia material exhibited ducts in 1980 experiments, but not in 1981. They suggested that these latter inconsistencies may have been due to differences in maturity of the plants, but came to the same conclusion as Chapman (1975) that the presence or absence of mucilage ducts is largely environmentally induced. Which environmental variables effect exhibition of ducts and their functional role is unknown (Lüning *et al.*, 1978).

Stipe anatomy as a taxonomic character

Stipe anatomy and morphology in kelps are important characters, not only for taxonomic criteria, but for commercial applications. For example, Chinese mariculturists have bred lines of *Laminaria japonica* with shorter stipes to minimize stipe biomass and increase blade production (they eat blade tissues, not the stipe). On the other hand, the alginate content of stipes is roughly twice that of laminae on a dry weight basis (Brinkhuis and Pabst, unpublished data) and use of *Laminaria* for the alginate industry could exploit strains or species with longer or more solid stipes. The increasing emphasis of Chinese kelp cultivation for the production of alginates (1.6 million wet tons of kelp and 7,000 tons of alginate were produced in 1986- Tseng, personal communication) may redirect efforts to producing varieties with longer, more valuable stipes.

The published distribution of hollow-stiped plants (in Kain, 1976) is from Cape Cod to Ellesmere Island (Taylor, 1957), west Greenland (Lund, 1959), Iceland (Jonsson, 1912), the Faeroes (Børgesen, 1903-1908; Irvine, 1982; Price and Farnham, 1982; Tittley *et al.*, 1982) and the Shetland Isles (Irvine *et al.*, 1975). However, Parke (1948) encountered plants with hollow stipes in Argyll, Scotland. Kain (1976) noted Parke's observation, but Parke (1948) attributes the finding to the species *Laminaria saccharina*. Kain did not compare any of the features between populations at Devon (short, solid stipe) and Argyll (long hollow stipe). Was Parke's record *L. longicruris*? It appears so. Parke (1948) concluded that the hollow stipe is produced in summer- or autumn-developed plants because of rapid growth in length and thickness during the first growth period and, as a result, the medullary tissue does not keep pace with that of the cortical tissue so that air pockets develop in the central portion of the stipe. If this is the case, then why don't *L. saccharina* plants produce long hollow stipes? What other environmental conditions could induce this phenomenon?

The use of the hollow stipe as a taxonomic character was first questioned by Mann (1971), who had difficulty separating *Laminaria longicruris* from *L. agardhii*. Chapman (1973) shared similar doubts when he found a clinal relationship of stipe length and hollow condition with environmental conditions. According to Chapman (1974), these two taxa are conspecific by the following reasoning:

1. no discontinuities in the variation of phenotypic characters previously used to separate the taxa;

2. the two species are fully interfertile and produced fertile hybrids; and

3. parents of each species produced at least some offspring resembling the other.

However, our analysis of Chapman's (1974) results found the following causes for concern regarding his interpretations:

1. both stipe length and hollow diameter exhibit considerable variation at both exposed and sheltered sites that would obscure statistically significant differences. Some of the data also suggest seasonal and substratum effects on stipe length;

2. there is a strong genetic component controlling stipe length and hollowness, explaining the lack of change in specimens transplanted from exposed (normally short, solid stipes) to sheltered (normally long, hollow stipes) locations;

3. no transplants or outplants of materials were conducted before the critical stipe length increase period (spring);

4. six parents were selected from an exposed and seven from a sheltered site resulting in production of 13 full-sibling families. There is no indication as to how crosses were made (i.e., random matings, self- crosses, or reciprocal male / female crosses);

5. pooling all 13 families showed that there was significant among-family variation for the stipe length character. However, there was also significant variation among families from the sheltered and exposed sites. The latter means one can not determine the genetic component of variability;

6. stipe morphology and measurements were not presented.

Further, there is apparent confusion concerning short solid, short hollow, and long hollow stipes in Chapman (1973, 1974).

No short, hollow-stiped mature forms occur in the *L. saccharina* or *L. longicruris*. Børgesen's materials from the Faeroes did include short (10-15 cm), hollow stipes in *L. faeroensis*. Kain (1976) stated "inflation of the stipe is not solely the result of continued growth in length and thus diameter, but a particular event which occurs after the stipe is about 0.4 m long". She also presents three hypotheses with regard to stipe morphology:

1. *L. saccharina* and *L. longicruris* are distinct species;

2. hollow stipes are produced by sheltered conditions and low temperatures, but similar conditions exist in the fjords of Norway where the species has not been recorded; and

3. one species is involved as in (2) above, but there has been an inflow of genes into the area resulting in long hollow stipes under fairly sheltered conditions.

A number of studies have reported crossing *Laminaria saccharina* with *L. longicruris* (Lüning *et al.*, 1978; Bolton *et al.*, 1983). These crosses have been made between European and Pacific representatives of *L. saccharina* and Canadian specimens of *L. longicruris* using both male and female gametophytes of each species for reciprocal crosses (Table 1). Lüning *et al.* (1978) produced hybrids from *L. saccharina* (Helgoland, Isle of Man) X *L. longicruris* (Nova Scotia) crosses, with the exception of a female *L. longicruris* X male *L. saccharina* (Isle of Man) cross. Maximum stipe lengths of all crosses conducted in the above experiments were 10 cm. Bolton *et al.* (1983) produced successful hybrids from *L. longicruris* X *L. saccharina* (Brittany and Helgoland) crosses with stipe lengths similar to those reported by Lüning *et al.* (1978). *L. longicruris* X *L. longicruris* also produced short solid stipes. All of these experiments were conducted in holding tanks or in the sea at Helgoland, Germany. Are conditions there not conducive to stipe elongation and hollow development? Lüning (pers. comm.) suggested that perhaps the outplants were too young to exhibit hollow or long stipes. Interestingly, no crosses between *L. saccharina* and *L. longicruris* from the western Atlantic Ocean have been reported by Lüning and Bolton.

Table 1. Origin of parent *Laminaria saccharina* (L.s.) and *L. longicruris* (L.l.) sporophytes.

Locality	Species	Morphology
Nova Scotia	L.l.	Bullate wide blade, long hollow stipe, no mucilage ducts
Helgoland	L.s.	Smooth narrow blade, short solid stipe, mucilage ducts in blade
Isle of Man	L.s.	Bullate narrow blade, solid short stipe, mucilage ducts not examined
Brittany	L.s.	same as for Isle of Man
Vancouver	L.s.	same as for Isle of Man

Chapman (1974) indicates he made crosses between 3 hollow-stiped and 3 solid-stiped plants, but states "plants with long, hollow stipes and those with short, hollow stipes belong to the same biological species". No data from these crosses are presented. Furthermore, all of the crosses reported thus far have been made with materials collected from near the middle of the latitudinal distribution ranges for the genus. Lüning (pers. comm.) has urged that crosses between *Laminaria longicruris* and *L. saccharina* from the northwest Atlantic be performed to clear up the picture for the eastern North America coast.

Chapman (1978) summarized a variety of hybridization studies with kelps and suggested that crossability tests are an effective way to evaluate genetic and taxonomic affinities of seaweeds. The primary conclusion from these studies is that where species are easily distinguished morphologically, there is usually reproductive isolation while morphologically confused species are interfertile (Mathieson *et al.*, 1981). However, Bolton *et al.* (1983) suggested that great care should be exercised in assessing the value of hybridization experiments in the taxonomy of the Phaeophyta. The experiments of Lüning *et al.* (1978) and Bolton *et al.* (1983) have shown that isolates of *Laminaria* from different geographical regions and different oceans hybridize both within and between species, whereas a sterility barrier has been demonstrated between some trans-Atlantic members of the Simplices (as noted above). The fact that Laminariaceae share a common sex pheromone (Müller *et al.*, 1985) is further reason to expect interfertility. Sanbonsuga and Neushul (1978) demonstrated interfertility in float-bearing kelps between genera, resulting in intermediate morphological forms. Stace (1980) remarks "in a great many groups of plants morphologically distinct species capable of hybridizing with others are the usual situation, and cannot be considered in any way abnormal".

While Chapman (1978) suggests that crossability tests are effective, one must have some reservations about results reported in Chapman (1974), Lüning *et al.* (1978) and Bolton *et al.* (1983) due to the failure of some crossability experiments, some of which were not complete designs. Percent fertility of the offspring was not assessed, F_2 generation crosses were not conducted, and no backcrosses with parents were carried out.

While phenetic variation in stipe morphology has been documented in *Laminaria longicruris* by Chapman (1973), Bolton *et al.* (1983) found no evidence that *L. saccharina* from the Isle of Man, Brittany, Helgoland, or the northeast Pacific can be environmentally induced to produce long hollow stipes. Thus, they concluded that the ability to produce these characters under particular conditions appears to be genetically determined. This is in contrast to data in Gerard and Mann (1979) suggesting that stipe length is inversely related to exposure and water movement. However, the relationship of stipe length to exposure is not discussed and is far from clear. Druehl (1967) noted that *L. groenlandica* stipe length was shorter in more exposed sites, and distinguishes this species from *L. saccharina* in the Pacific. However, Wilce (1965) placed the British Columbian *L. groenlandica* in synonymy with *L. saccharina*. No one has reported crossing *L. saccharina* with this

"*L. groenlandica*", but Bolton *et al.* (1983) did use material from the Pacific where the latter is presumed to occur, according to Druehl (1967). Bolton *et al.* (1983) consider this species as synonymous with *L. saccharina*, citing Wilce (1965). Wilce (pers. comm.) doubts that the Vancouver *L. groenlandica* has any relationship to the material from the West Greenland area described by Rosenvinge. The Vancouver material reportedly can produce a digitate blade (not caused by physical ripping of the blade, Druehl 1967) and its taxonomic position still needs to be established (Wilce, pers. comm.). Setchell and Gardner (1925), unlike Druehl, recognized that their *L. cuneifolia* were different from the Greenland type-material. They state that *L. cuneifolia* from Greenland seems to be a different species and is closely related to *L. groenlandica* described by Rosenvinge. Recall that Wilce (1959) placed *L. groenlandica* and *L. cuneifolia* from the Atlantic in synonymy, and subsequently combined *L. groenlandica* and *L. agardhii* into *L. saccharina* (Wilce, 1965).

Bullations as a taxonomic character

Bullations are indentations that appear seasonally in some species of *Laminaria*. Their physiological significance has been suggested to be related to increasing lamina surface area for absorption of nutrients, aiding in the disruption of boundary layers to enhance nutrient uptake under conditions of low water flow over lamina. From a maricultural standpoint, production of increased blade area through bullation formation means more biomass for harvesting.

What conditions induce the development of bullations on the laminae of some Simplices laminarians? Setchell (1900) wrote, "In *Laminaria agardhii* and *L. saccharina* of the New England coast, the writer has found that the summer form is usually ample, with ruffles and rows of indentations fully developed; but in August, a change takes place and this summer blade is replaced by a winter blade which is perfectly plane and devoid of both these features. Again, in the spring, this plane blade is replaced by the ruffled and indented form".

This same phenomenon was noted by Lüning *et al.* (1978) with crosses involving *Laminaria saccharina* from Helgoland and *L. longicruris* from Nova Scotia, and in hybrids (Bolton *et al.*, 1983) produced by crosses between *L. saccharina* from Vancouver and Helgoland and between *L. longicruris* (Nova Scotia) and *L. saccharina* (Helgoland). In the latter studies, pure strains from Vancouver and Nova Scotia produced bullations year-round. In European *L. saccharina*, the presence or absence of bullations appears to be a fixed genetic trait with the occurrence of this character being dominant (Lüning, 1975; Lüning *et al.*, 1978). Bolton *et al.* (1983) point out that in other species of non-digitate *Laminaria* the inheritance of this trait is more complicated than that found in Europe. These authors all suggest the ability to produce bullae seasonally is controlled by some environmental aspect. Our own work (Brinkhuis and Yarish, unpublished data) further supports that contention. Bullae are initiated in December and initiation ceases in late May.

Biogeography of *Laminaria*

To characterize potential genetic morphological differentiation in *Laminaria*, we can first examine its biogeography over a large scale (Egan and Yarish, 1988). We have synthesized the stipe morphology observations and plotted the biogeographic distribution of *L. saccharina* (Fig. 1) and *L. longicruris* (Fig. 2) in the North Atlantic and Arctic Oceans. The figures also include mean sea surface temperature (°C) in February (F) and August (A). The distribution data is based on the following sources: Batters (1889), Børgesen (1903-08), Christensen and Thomsen (1974), Guiry (1978), Hoek (1982a,b), Hylander (1928), Irvine (1974), Jonsson (1912), Kain (1976), Kylin (1947), Lee (1980), Lund (1959), Lüning (1975, 1985), Lüning *et al* (1978), Parke (1948), Parke and Dixon (1976), Taylor (1957), and Turner (1802). Ocean isotherms were derived from Sverdrup *et al.* (1942). Wilce (1965) indicated that the southern limit of *Laminaria* distribution in the western Atlantic is Long Island, New York. However, Egan and Yarish (1988) have recently reported discovery of a deep-water population of *L. saccharina* off the coast of New Jersey.

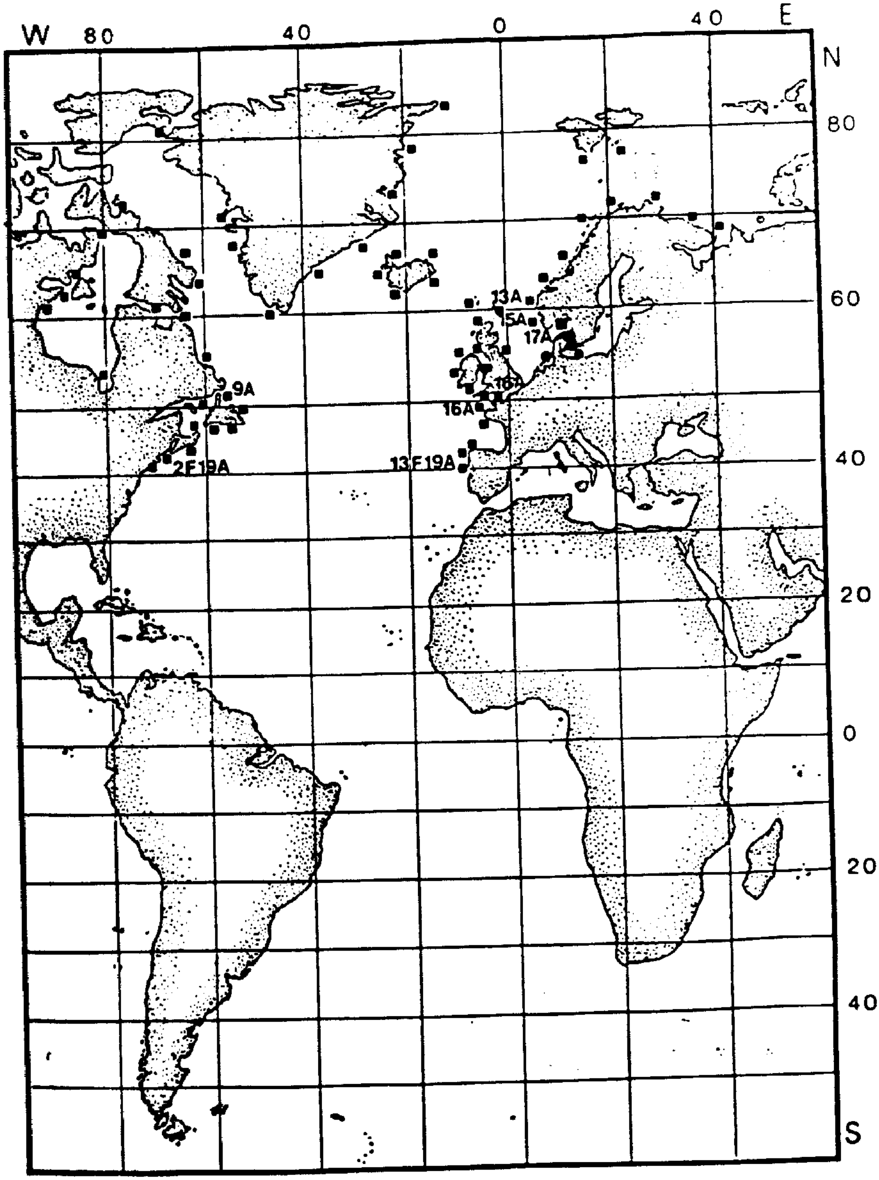


Figure 1. The reported distribution of Laminaria saccharina *in the North Atlantic. Sources are those listed in Egan and Yarish (1988). Additional sources are referenced in the text.*

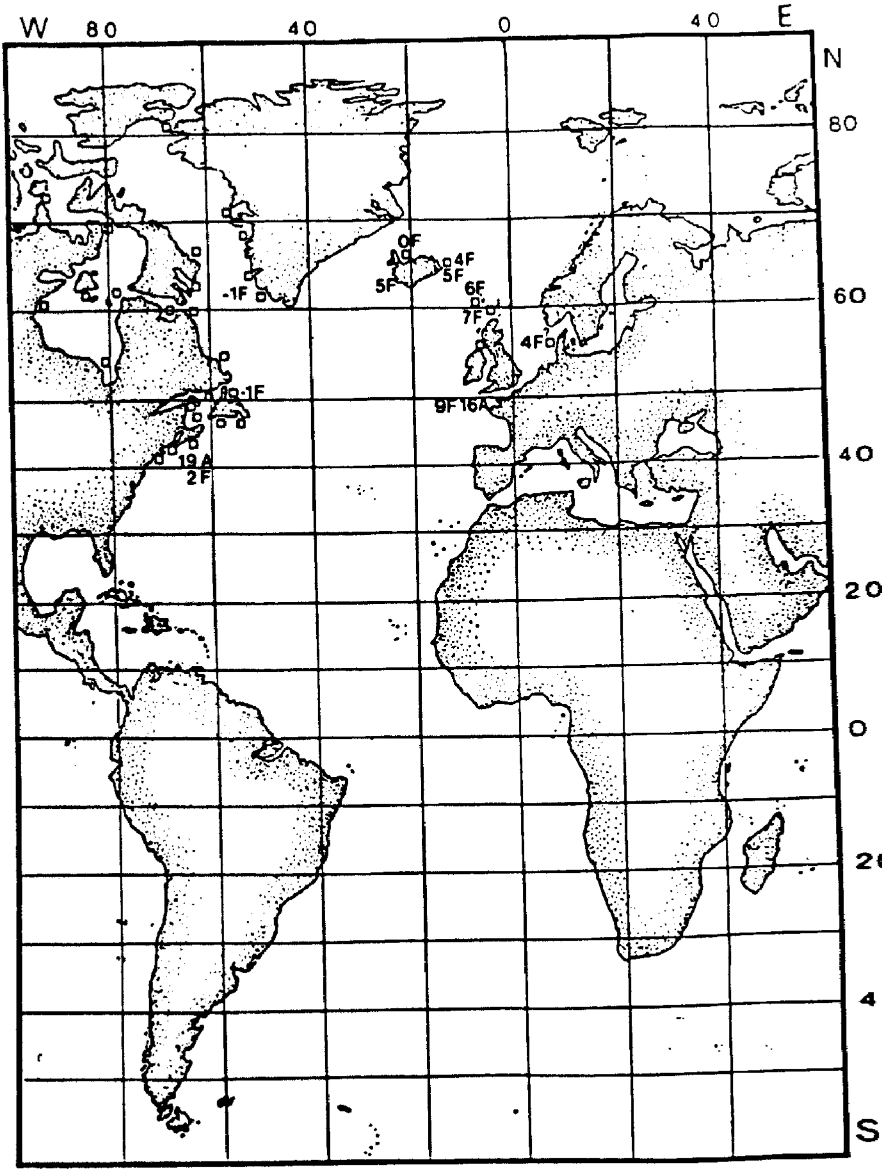


Figure 2. The reported distribution of Laminaria longicruris *in the North Atlantic. Sources are those listed in Egan and Yarish (1988). Additional sources are referenced in the text.*

On a small scale, we can examine the distribution of *Laminaria* in Long Island Sound. The importance of Long Island Sound to an analysis of taxonomic variability is that it is the only documented location where clearly identifiable and apparently segregated populations exist near each other. Our observations on distribution of these two entities in Long Island Sound, using stipe morphology, are shown in Fig. 3. Two species of *Laminaria* are found on Long Island, *L. saccharina* and *L. digitata*. *L. digitata* is found at Montauk Point only (see Fig. 3). *L. saccharina* is found primarily along the north shore of the Island wherever cobble or rock deposited during the last glaciation occur; it also occurs at Montauk Point. Plants were found in 1969 by Yarish as far west on the south shore as the jetty at Shinnecock Inlet (unpublished data); Liddle (pers. comm.) reported its continued presence there in the winter of 1985. Populations are rarely found at depths greater than 5 m along the north shore. All of the plants have stipe lengths and morphology typical of *L. saccharina*. The period of maximum stipe elongation is spring and stipes reach a mean length of 4.5 cm by July (Lee and Brinkhuis, 1986). Occasionally, longer-stiped plants can be found at Montauk Point, a more exposed environment than other north shore locations. Some plants with stipes up to 60 cm long have been collected, but these have not been observed to be hollow.

Figure 3 shows that *Laminaria longicruris*-type plants have been observed as far west as the Thimble Islands (Branford, Connecticut) in water 5 m deep. *L. digitata* only penetrates as far west as New Haven, CT, or, in depths of 7 m. Significant stands of *L. saccharina* type plants occur as far west as Stamford, Connecticut, in water depths rarely exceeding 4 m.

Physiological differentiation

Several studies have pointed out that physiological ecotypes of *Laminaria* exist along the Atlantic coast of North America. Gagné *et al.* (1982) reported that there are seasonal differences between some kelp populations, i.e. ones from nutrient-poor and others from nutrient-rich environments. If nitrate was available in high concentrations during winter and spring, maximum growth occurred at that time of year. Findings of these workers suggested ecotypic differentiation in nutrient/growth interactions for *L. longicruris*. These authors reported that accumulation of large tissue nitrate reserves was unnecessary in nitrate-rich environments.

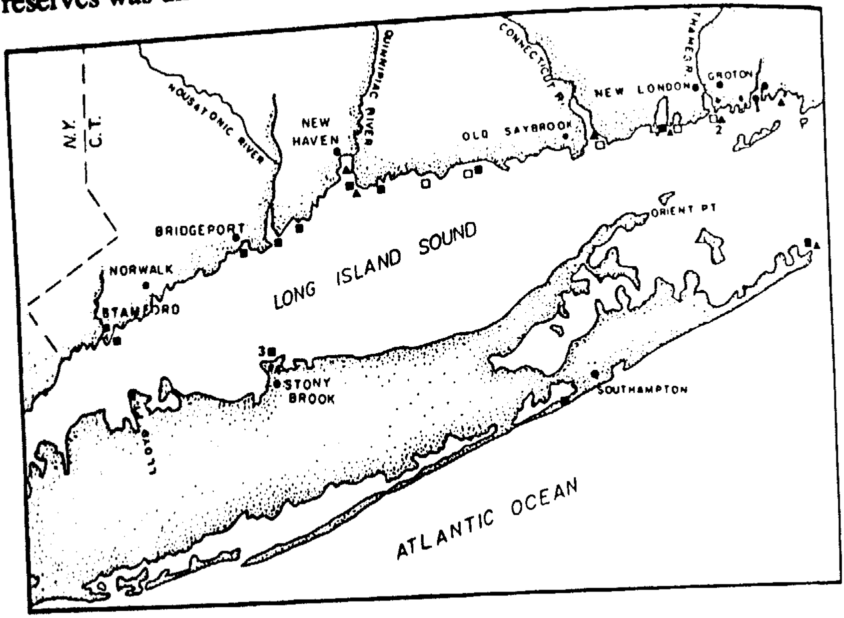


Figure 3. The reported distribution of Laminaria saccharina *(solid squares),* L. longicruris *(open squares) and* L. digitata *(solid triangles) in Long Island Sound. Sources are those listed in Egan and Yarish (1988).*

Other types of differentiation in *Laminaria* have been reported as well. Gerard and co-workers have described ecotypic differentiation in light/photosynthesis interactions (Gerard, 1988) and photosynthesis/ temperature interactions (Gerard and Du Bois, 1988) of sporophytes. Yarish and Egan (1987) compared growth of nine microsporophytic strains at 15° C and noticed significant differences in growth rate after 43 days. Subsequently, Yarish and Egan (1989) demonstrated that self-crosses from 15 additional gametophytic isolates produced microsporophytes with significantly different thermal tolerances. A thermal gradient was created by utilizing temperature gradient plates (Yarish *et al.*, 1979; Yarish and Edwards, 1982; Lee and Brinkhuis, 1988) at an irradiance optimal for microsporophyte production. Sporophyte growth was optimal at 17°C for 12 strains and at 19°C for the remaining three strains after 38 days. Lüning *et al.* (1978) reported that there are thermal differences among populations of *L. saccharina*.

Materials and Methods

A unique combination of culture facilities at the University of Connecticut at Stamford (UCONN) and the State University of New York at Stony Brook (SUSB) exists. At UCONN, several environmental growth chambers were used to conduct culture studies of gametophytic and juvenile sporophytic plants. SUSB also has growth chambers for gametophyte cultivation and a flow-through marine greenhouse for maintaining large sporophytes (see Brinkhuis and Hanisak, 1982).

Field observations on *Laminaria* populations have been conducted for several years on the Connecticut and Long Island sides of Long Island Sound. For the sake of brevity, the reader is referred to several recent publications that describe the approaches taken to studying growth and reproduction in these populations (Brinkhuis *et al.*, 1983, 1984; Lee and Brinkhuis, 1986; Yarish and Egan, 1987; Egan and Yarish 1988; Yarish and Egan, 1989).

Gradient plate studies have been conducted on *Laminaria saccharina* (Lee and Brinkhuis, 1986, 1988) and on *L. longicruris* (Yarish and Egan, 1988; Egan *et al.*, 1989). For a review of methods, see Lee and Brinkhuis (1988) and Egan *et al.* (1989).

We also conducted reciprocal crossing experiments between *Laminaria saccharina* from Long Island and *L. longicruris* from Connecticut. Meiospores from a single isolate of each species were raised to the immature gametophytic stage, at which time cultures were placed under red light to inhibit sexual development and reproduction. Gametophytes were then sexed and separated for continued vegetative growth under red light, producing masses of male and female gametophytes. In February (1988), these masses were gently ground in a mortar and pestle, and crosses between males and females from each species were placed under white light, which stimulated reproductive development. When sporophytes became evident, cultures were transferred to 4-liter glass jars and maintained in an incubator at 15°C until mid-March, 1988. At that time, sporophytes were transferred to the greenhouse at SUSB. We recorded growth rates by measuring blade length changes over two week intervals, as well as using the hole-punch technique (Parke, 1948). Measurements of stipe length and blade width were also recorded bi-weekly.

Results and Discussion

Field cultivation observations

Growth and reproduction of *Laminaria saccharina* has been extensively studied at one site, Crane Neck, NY, over a several-year period (Fig. 4a). The period of maximum blade elongation occurs in April-May when water temperatures range from 10 - 15°C. Meristematic growth virtually ceases during July as water temperatures reach 18 - 20°C. Severe distal erosion follows, and most of the adult population disappears during August (Brinkhuis *et al.*, 1983, 1984; Lee and Brinkhuis, 1986). Warm summer sea-water temperatures result in growth reduction before the plants exhaust internal nitrogen reserves (Brinkhuis and Charnon, unpublished data), unlike further north where either limiting irradiance or nitrogen supply limits growth (e.g., Chapman and Craigie, 1977; 1978; Chapman and Lindley, 1980).

Laminaria saccharina on Long Island primarily behaves as an annual plant. Reproductive activity in sporophytes is maximal in autumn and April-July, when the greatest number of plants are fertile (Fig. 5a). However, fertile plants may be found throughout the year, except August and September when few adults are present. There is some year-to-year variability in the proportion of the population that becomes reproductive, but the pattern from one year to another is similar. Meiospore release and reproductive success are maximum in the spring (Lee and Brinkhuis, 1986). During late September, small sporophytes may be found at Crane Neck. Thus, Lee and Brinkhuis (1986) believed that the superior reproductive activity period in April-May was responsible for the major recruitment in the population. During the April-May period,

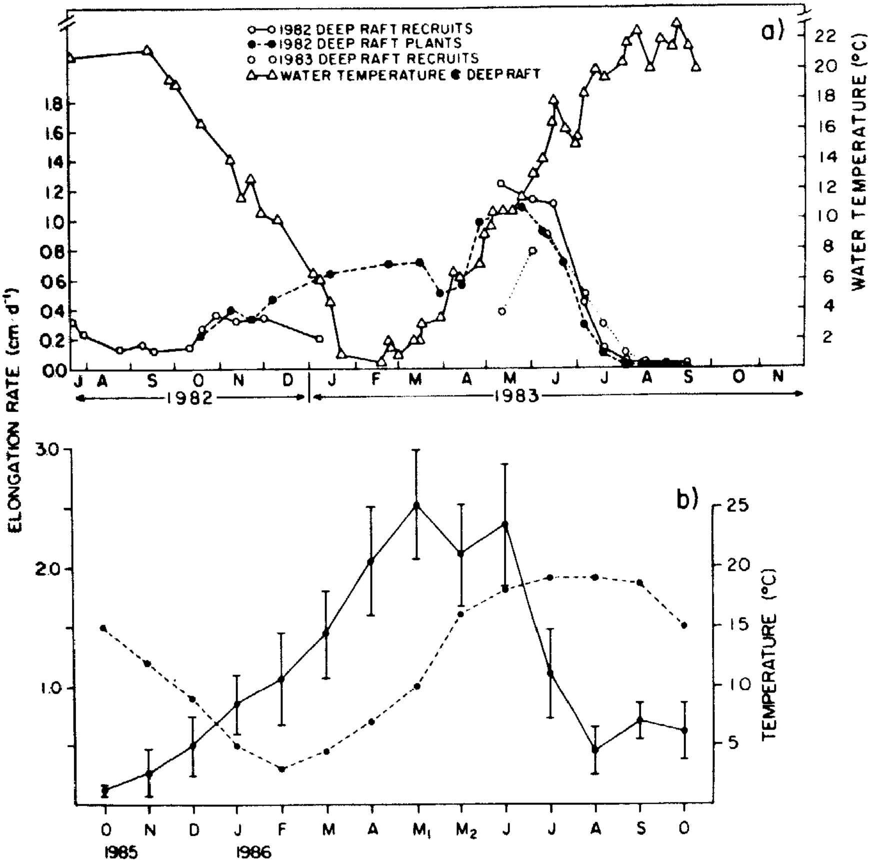


Figure 4. Seasonal variations in meristematic growth rates (means ± S.D., cm d⁻¹) and water temperatures in Long Island Sound for Laminaria saccharina *(a) and* L. longicruris *(b).*

juvenile sporophytes resulting from earlier reproduction may be found, but these don't survive summer conditions. Thus, it was believed that the population "over summers" in the gametophytic stage. Other workers have found that kelp gametophytes have higher temperature survival limits than sporophytes (e.g., Bolton *et al.*, 1983).

Recent studies (Lee and Brinkhuis, 1988) have shown, however, that the annual recruitment pattern does not involve an over-summering phenomenon. The autumn sporulation results in the

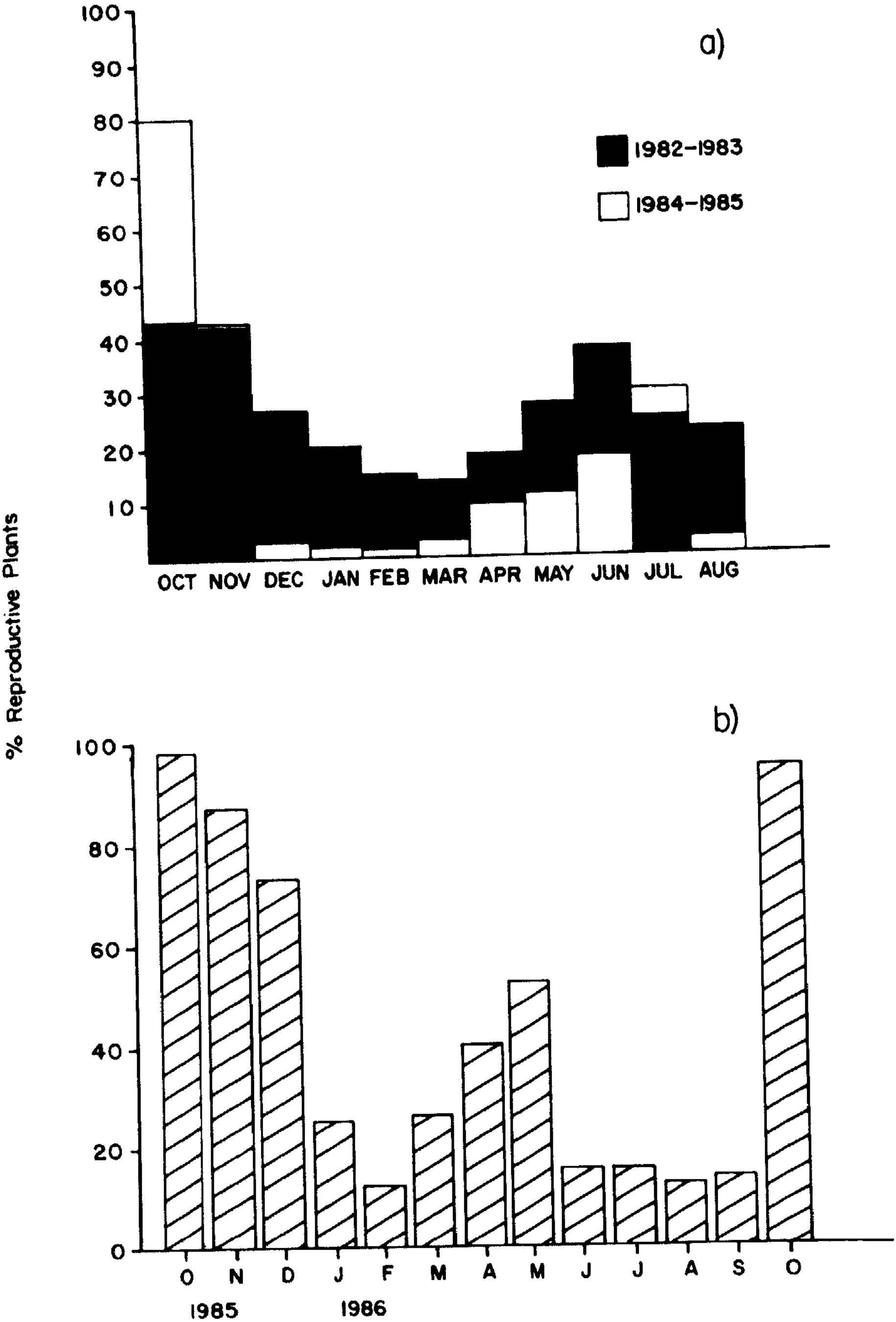


Figure 5. Per cent reproductive sporophytes with blades > 60 g possessing sorus tissue for Laminaria saccharina *(a),* L. longicruris *(b). Data derived from tagged populations in Long Island Sound.*

only offspring that survive the summer conditions as mature sporophytes. While tissue erosion is significant and the majority of the mature plants succumb to high water temperatures, some sporophytes survive. These now small sporophytes (due to blade erosion) have a small meristematic region that begins to grow again in September when water temperatures fall. The blades that form when growth initiates have a wide, short segment of new tissue, to which are attached remnants of the old blades. These older tissues rapidly become reproductive in early-October, perpetuating the annual pattern. Interestingly, the superior characteristics of the spring sporulation (fecundity, growth, etc.) are of little consequence to the survival of the species in Long Island populations.

Growth and reproduction of *Laminaria longicruris* has been intensively studied at one site, Black Ledge (Groton, CT) over the last two years (Fig. 4b). The period of maximum stipe elongation is spring and stipes reach a mean length of 120 cm by June (Egan and Yarish, 1988). These plants all exhibit hollow stipes by spring, in an area of moderate exposure. The period of maximum blade elongation is April-May when water temperatures range from 8 to 12°C. Meristematic growth decreases during July when water temperatures reach 16 - 17°C (Yarish and Egan, 1987). Sporophyte reproductive activity is maximum in October, when the greatest number of fertile plants are found; another peak occurs in April-May (Fig. 5b). Meiospore release and subsequent reproductive success following gametogenesis also is maximum at this time. *Laminaria longicruris* in eastern Connecticut (Black Ledge) behaves as a perennial plant, whereas *L. saccharina* in western Connecticut (Stamford) behaves as an annual in a manner similar to that on Long Island (New York).

The annual patterns of growth and reproduction are very similar for both *Laminaria saccharina* in New York and *L. longicruris* in Connecticut. Seawater temperatures at the eastern Connecticut site appear to be cooler, and this probably accounts for the perennial life history of *L. longicruris*. Here, greater numbers of adult sporophytes survive during summer (Yarish and Egan, 1989).

Tank cultivation observations

At Stony Brook, we conducted experiments aimed at identifying fast-growing individuals that could be used to establish new strains. An extensive data base exists for measurements of *Laminaria* plants from many areas of the world, and this could be used to make comparisons. However, size-dependent growth rates complicate such comparisons. It is, therefore, essential that plants which are growing fast relative to other members of their size class be identified. Attempts to describe size-dependent growth rates have resulted in several biomass-growth models for the Laminariales (e.g. Mann and Mann, 1981; Gendron, 1985). A major assumption of these models is that size-dependent growth remains the same throughout the year. In other words, the size-growth relationship for a given size class remains constant.

We maintained a population of 150 plants in a greenhouse and monitored growth and morphology between December, 1984 and April, 1985. The plants were harvested from a natural population at Crane Neck, NY. Figure 6 depicts a time-series of growth rate plotted against total blade length. During December, there is a strong linearity of size-dependent growth rates. In January, the relationship is still linear, but the slope has decreased dramatically. Growth rate decreased uniformly across all sizes. At this time, water temperature is near 0°C, while for December it was 6 - 10°C. Irradiance also decreases to its yearly minimum in January. By early February, the slope of the relationship had increased again. Growth rate had reached a maximum at the end of April. These data show that the size-dependent relationship is not constant over time and the previous models are not applicable to Long Island Sound populations. The examples shown provide some indication of non-linear relationships between blade length and growth rate; a point is reached beyond which increased size is not accompanied by an increase in growth rate. Gendron's (1985) data also exhibit non-linear relationships, but her treatment ignored this.

The relationship between blade length and width also is non-linear. Figure 7 shows this relationship in December for blade width 10 cm above the stipe and widest blade dimension. Note the

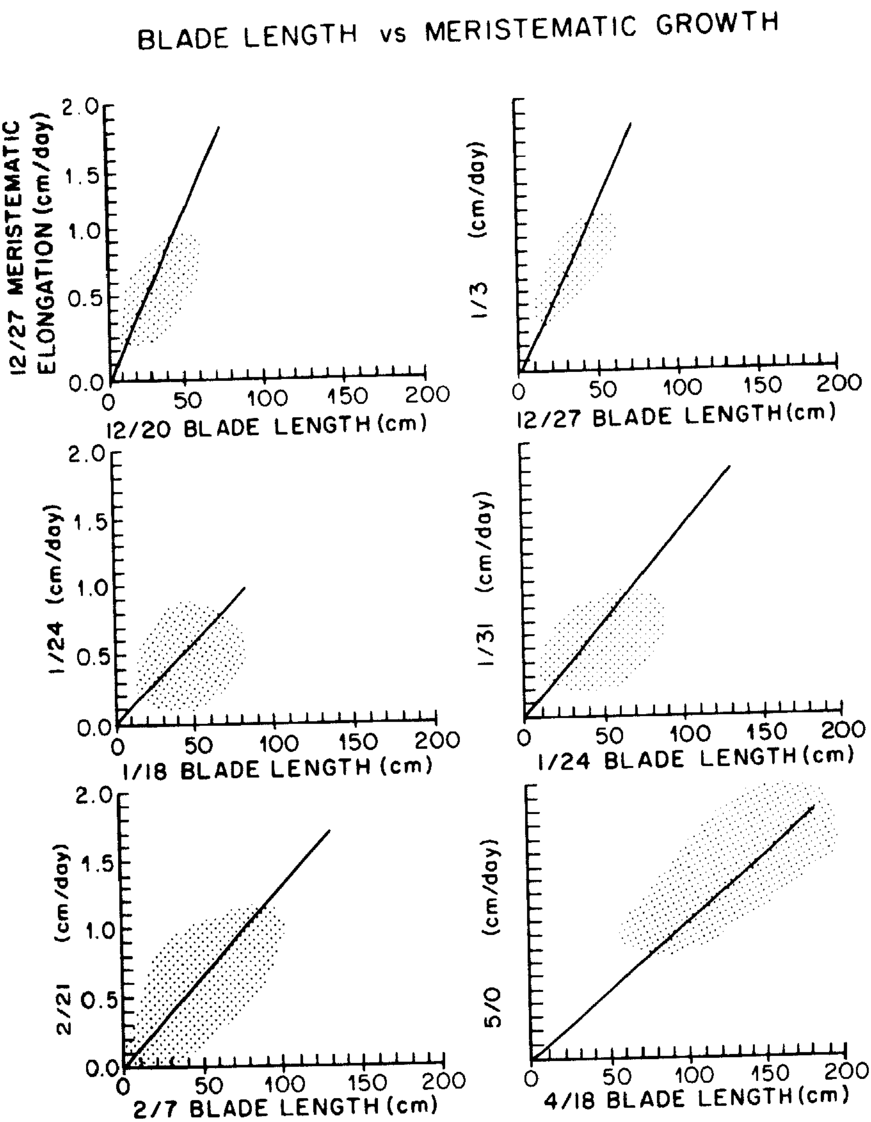
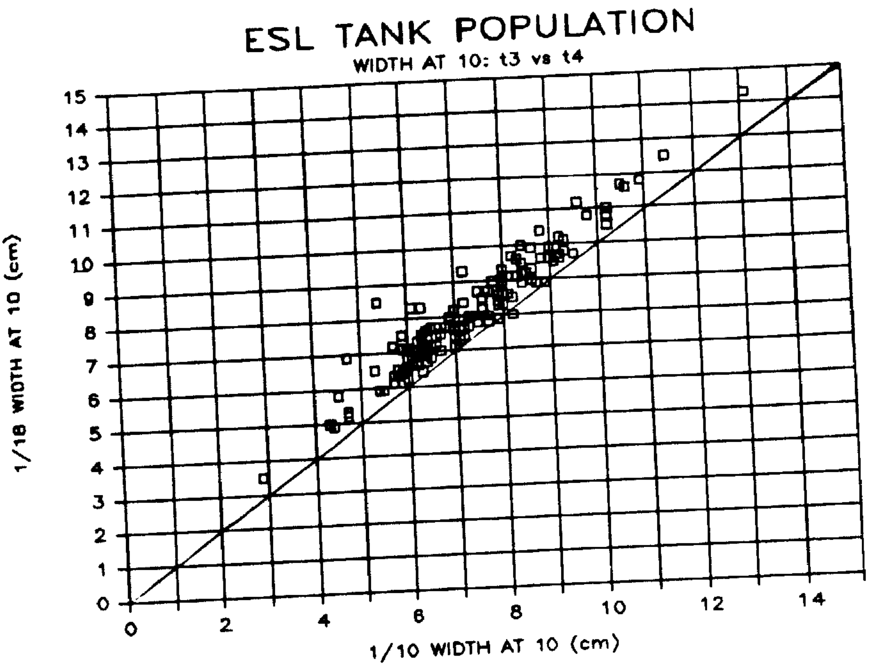


Figure 6. Time series of plots of meristematic growth rates of Laminaria saccharina *versus total lamina length from a population of 150 plants maintained in the greenhouse culture. Plants were harvested from a natural population at Crane Neck, New York. Note the size-dependent relationship is not constant over time.*



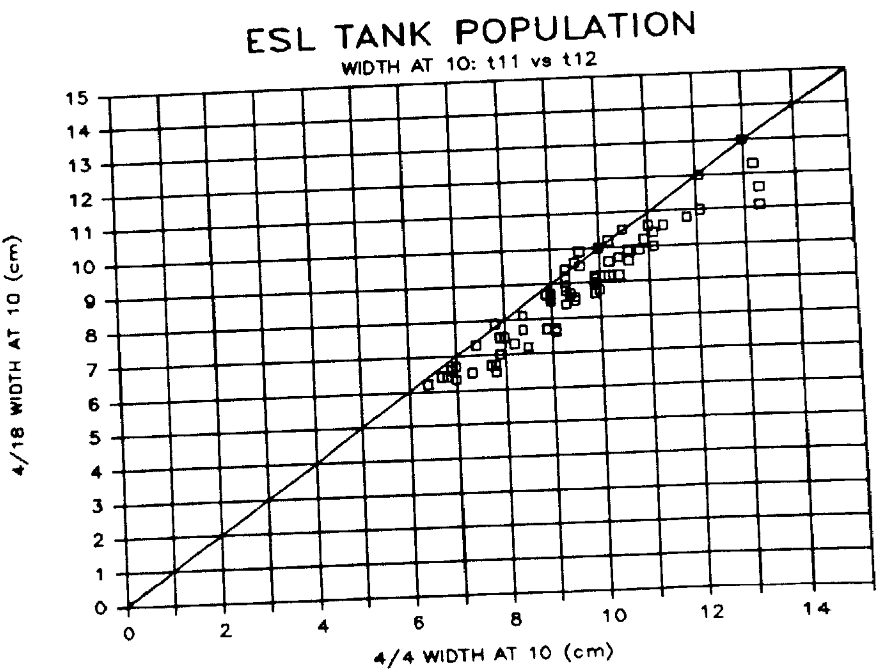


Figure 7. Plot of blade length (cm) versus lamina width (cm) of Laminaria saccharina *from January and April greenhouse reared plants. Note the line transecting the plot and that the s-axis is blade width at the beginning of a growth interval and the y-axis is width at the end of the interval.*

line transecting the plot and that the x-axis is blade width at the beginning of a growth interval and the y-axis is width at the end of the interval. Here, the width at 10 cm provides an indication of what happened during growth in the most recently laid down tissue. This "unity" line indicates that in early January the population was expanding in width in a uniformly linear fashion, i.e., as a function of original blade width. During April, widths at 10 cm seem to be decreasing.

In summary, the previous growth models overestimate production because they measured the relationship only during the period of maximum growth rate and/or size and extrapolated this relationship over the year. The types of plots depicted provide a tool for selecting the consistently best-growing individuals whose spore crop can be harvested for subsequent breeding experiments. Careful record keeping is essential in assessing inconsistencies. For example, we have noted the appearance of blade damage can explain subsequent decreases in growth rate.

Gradient plate observations

The interaction of irradiance and temperature on meiospore germination, gametophyte development and growth, and juvenile sporophyte growth in *Laminaria saccharina* from New York was recently reported by Lee and Brinkhuis (1988). That study utilized crossed light:temperature gradient plates to examine seasonal differences during 1985. Similar studies on *L. longicruris* were conducted by the UCONN group (Egan *et al.*, 1989). For comparison, we illustrate juvenile sporophyte growth in the two species (Figs. 8 and 9).

Laminaria saccharina embryonic sporophyte growth was a function of irradiance and temperature throughout the year (Fig. 8). However, temperature was the single most important determining factor. Most often, low irradiances were not conducive to juvenile sporophyte growth. Sporophytes exhibited a much narrower range of survival temperature than gametophytic stages (Lee and Brinkhuis, 1988). Growth exhibited seasonal differences. From November to March, the optimal temperature for growth was 11°C.

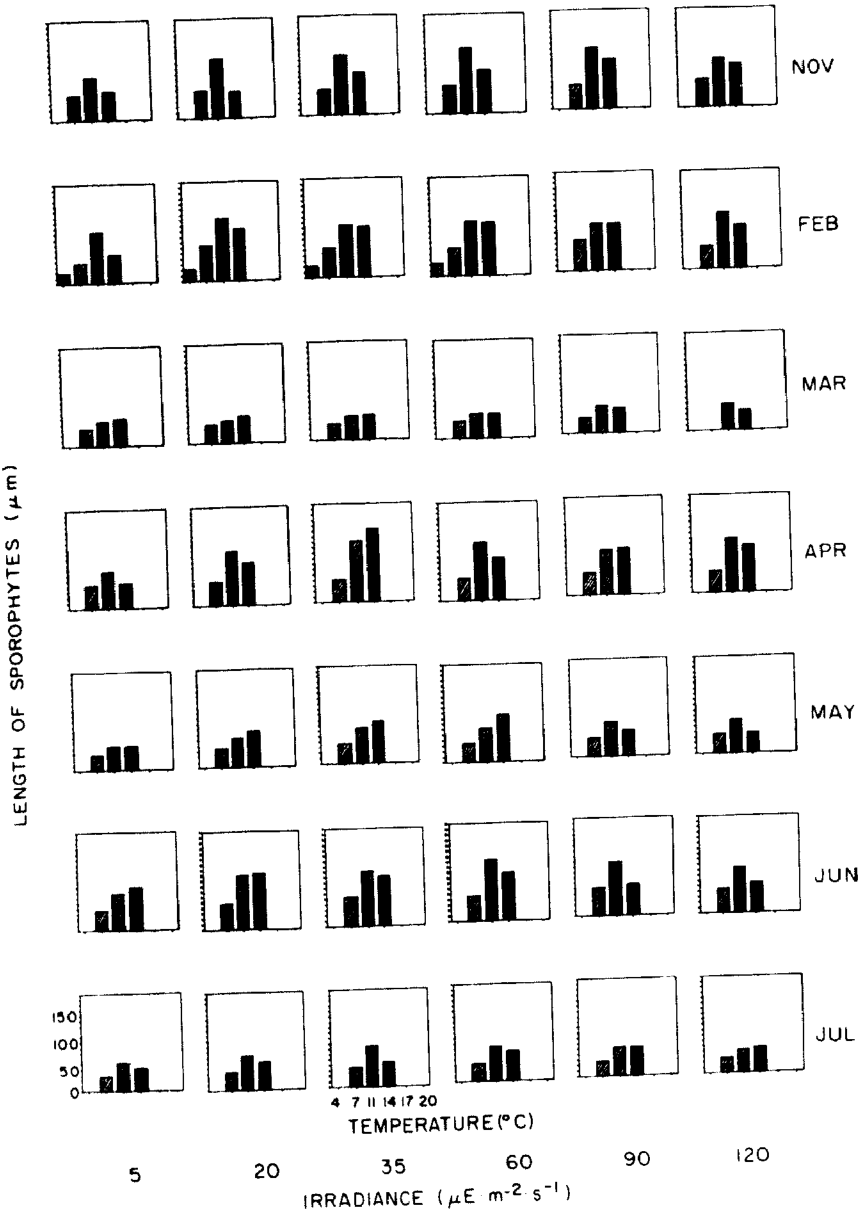


Figure 8. Growth (length μm) of 23-day-old Laminaria saccharina *sporophytes from gradient plate studies.*

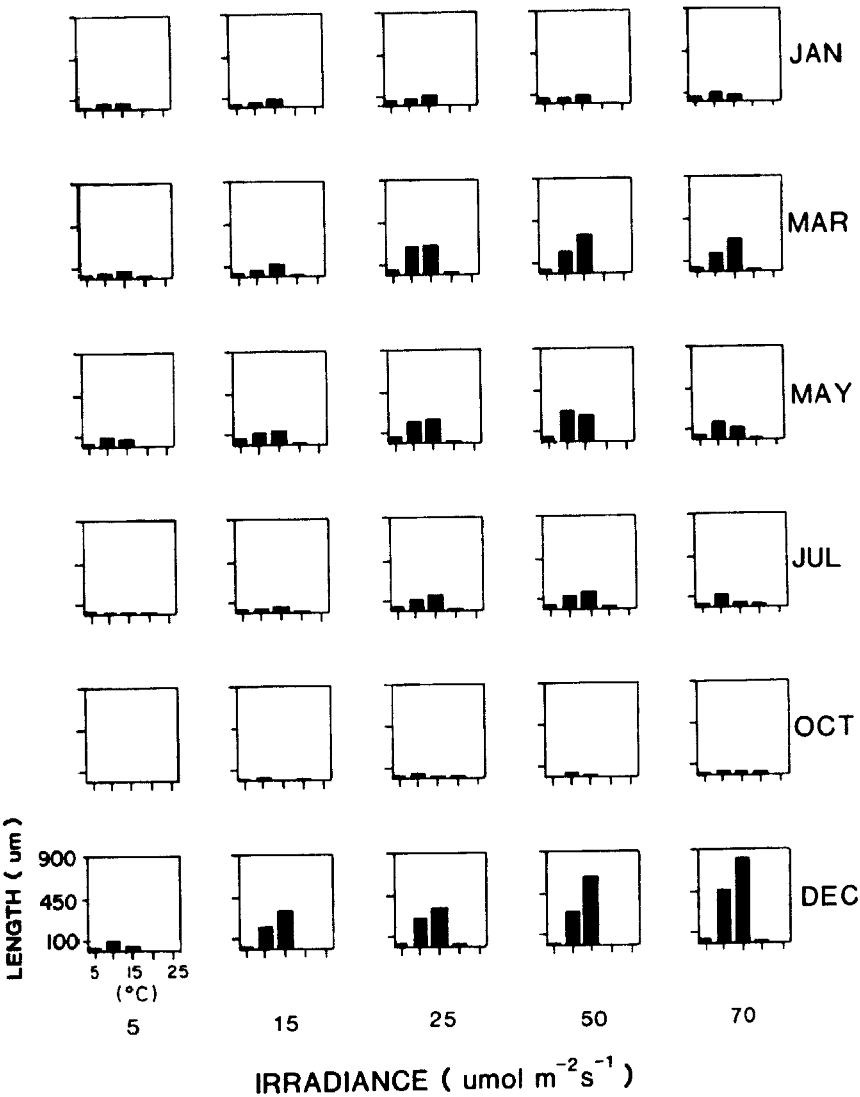


Figure 9. Growth (length µm) of 23-day-old Laminaria longicruris *sporophytes from gradient plate studies. Courtesy of Elsevier Science Publishers B.V.*

From April to July, 14° C was the optimum growing temperature. The best growth was observed in April at 14° C and 35 µE m^{-2} s^{-1}, resulting in sporophytes 133.4 µm long two weeks after meiospores were seeded. This possibly is an adaptation response to seasonally increasing ambient temperatures. Temperatures of 17 - 20° C always totally suppressed sporophyte development and growth. There appeared to be a case of adaptation to cold temperatures in February, as this was the only month in which sporophyte development and growth was seen at 4° C.

Seasonal growth responses of *Laminaria longicruris* to light and temperature (Fig. 9) exhibit differences in comparison to *L. saccharina*. The most rapid development and growth of *L. longicruris* sporophytes occurred during December. At the time of meiospore production, developing sporophytes exhibited a clear response to light, as well as to temperature. Optimal growth was seen at 15°C and 70 µE m^{-2} s^{-1}. Poorest development and growth in *L. longicruris* was seen in October and January.

To better illustrate differences between the two species, the months of March, May and July can be compared directly. Growth of *Laminaria saccharina* juvenile sporophytes was independent of light, and temperatures between 7 and 14° C supported growth equally well (except at 120 µE m^{-2} s^{-1} and 7° C). However, growth in March was the poorest of all months. In contrast, growth of *L. longicruris* sporophytes was quite good in March. Above 15 µE m^{-2} s^{-1}, growth was independent of light, and there is a clear relationship to temperature. Also, *L. longicruris* sporophytes grew at a temperature of 20° C; this was never observed in *L. saccharina*. Development and growth of *L. longicruris* in May was comparable to that of *L. saccharina*, except that the former's sporophytes grew to a larger size, i.e. more quickly. Responses to light and temperature are quite similar. However, some growth at 5° C was seen in *L. longicruris* sporophytes and growth was not observed in *L. saccharina* at 4° C. In July, *L. longicruris* sporophytes grew best at irradiances above 15 µE m^{-2} s^{-1} and the best growth was observed at 15° C (except at 70 µE m^{-2} s^{-1}). In the other hand, *L. saccharina* sporophytes grew equally well at all irradiances and best at a temperature of 11° C. Sporophytes in both species reached approximately the same lengths under their respective optimum growth conditions.

We also examined differences between the two species in the sex ratio of gametophytes with respect to incubation temperature on the gradient plate. The ratio of female:male gametophytes in *L. saccharina* was the same at temperatures between 4 and 14° C (Fig. 10a). At higher temperatures, males were more prevalent. In contrast, male gametophytes in *L. longicruris* became more prevalent as temperature increased from 5 to 15° C (Fig. 10b). There were significantly more female gametophytes at 5° C. At 20° C, the number of males and females was similar.

In summary, these data illustrate species differences in when to obtain meiospore material for culture of gametophytic and sporophytic material for seeding in mariculture. These differences are probably due to ecotypic differentiation, as are other physiological traits. It appears anomalous that the species that experiences a warmer summer (*Laminaria saccharina*) does not exhibit survival at warmer temperatures, i.e. 20° C, and that *L. longicruris* living in cooler waters does. The gradient plate approach has not only provided a means of selecting the best seed harvest time and the optimal growth conditions, but also is a tool that can be used to screen for ecotypic differentiation and selection of superior strains.

Crossing experiments and taxonomic resolutions

During the spring of 1985, we conducted preliminary experiments in which juvenile sporophytes from Black Ledge, CT and New York populations were grown side by side in the same greenhouse culture tank. The range in blade lengths of measured plants on June 24, 1985 was 17-74 cm for *Laminaria saccharina* and 18-99 cm for *L. longicruris*. Thus, many size classes were sampled. Stipe elongation was significantly greater in *L. longicruris*, but blade elongation rates were statistically similar (Egan and Yarish, 1988).

Crossing experiments provided further insight into differences in growth rates (Fig. 11), blade width (Fig. 12), and stipe length (Fig. 13). Mean growth rates in March (n=54 for each cross) were

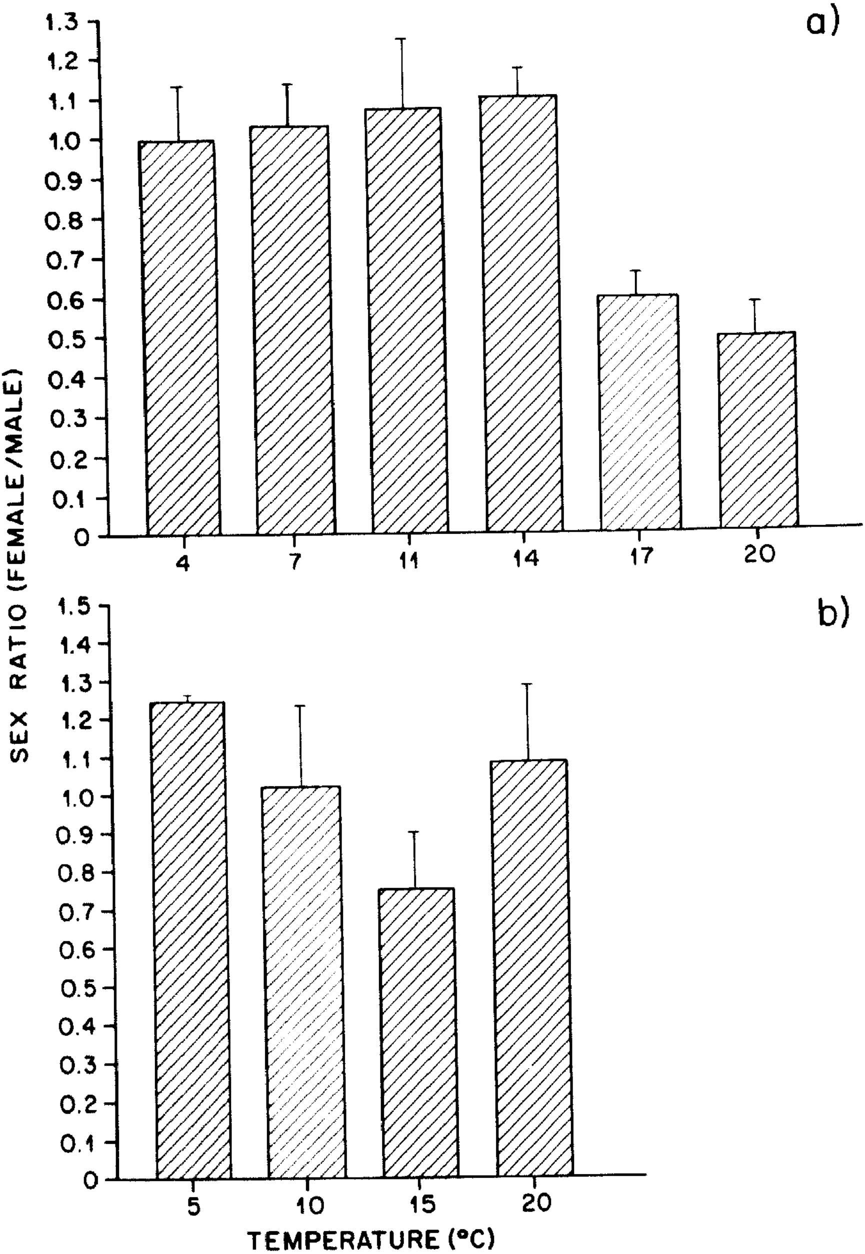


Figure 10. The ratio of females:male gametophytes versus temperature for Laminaria saccharina *(a) and* L. longicruris *(b) from May, 1985 gradient plate studies.*

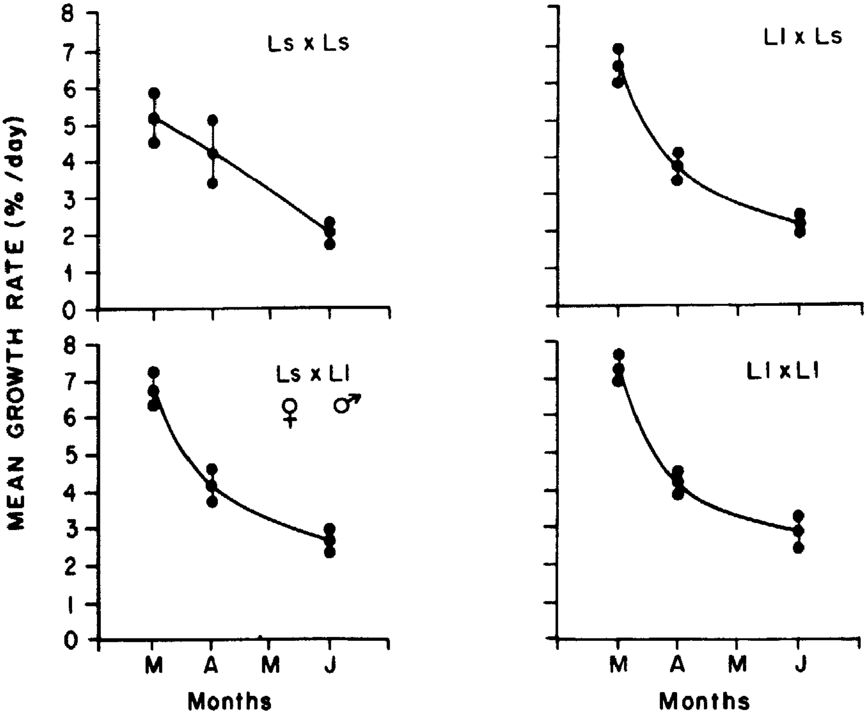


Figure 11. Mean growth rate (% day) of F_1 *progenies from crosses of* Laminaria saccharina *and* L. longicruris *in greenhouse culture. First species code listed for each cross represents the female.*

highest for the *Laminaria longicruris* self-cross and lowest for the *L. saccharina* self-cross; this difference was significant. Growth rates for interspecific crosses were intermediate, and the difference between the male *L. longicruris:* female *L. saccharina* and the *L. longicruris* self-cross was not significant. On the other hand, growth rates of the alternate inter-cross were significantly different from both self-crosses. Generally, these patterns were observed in April and June measurement intervals as well. The data suggests growth rate inheritance is linked to the *L. longicruris* plants. Further, these data contradict the finding of Egan and Yarish (1988) that growth rates of *L. saccharina* and *L. longicruris* are similar.

Mean maximum blade widths recorded on 1 July, 1988 for each of the crosses (Fig. 12) also indicate differences. Blade

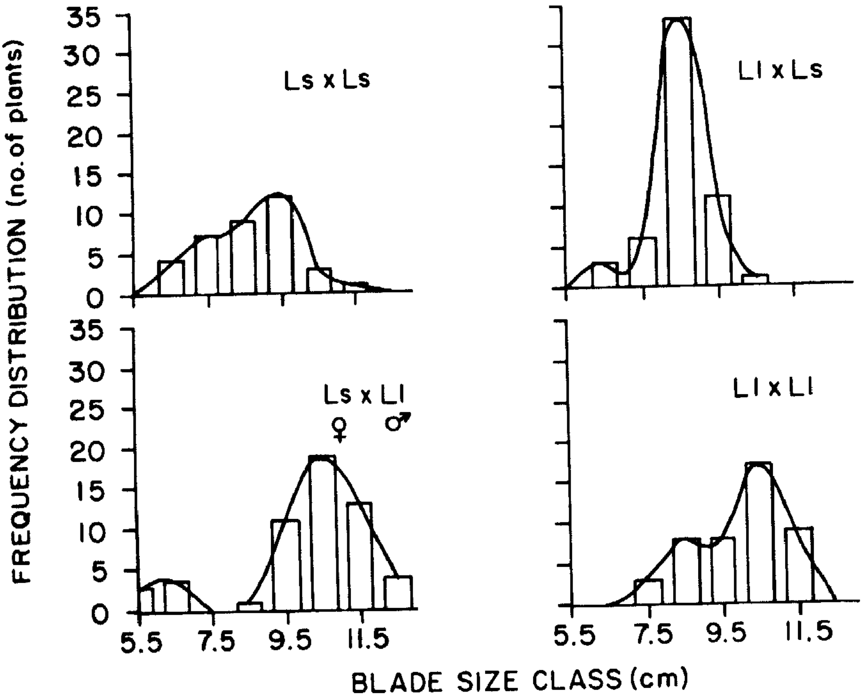


Figure 12. Frequency distributions for maximum blade width (cm) of F_1 progenies from crosses of Laminaria saccharina *and* L. longicruris *in greenhouse cultured plants. Data from 1 July, 1988. First species code listed for each cross represents the female.*

widths for self-crossed *Laminaria saccharina* were narrower than those of the *L. longicruris* self-cross. The male *L. longicruris:* female *L. saccharina* inter-cross exhibited blade widths similar to those of the *L. longicruris* self-cross, and the alternate inter-cross blade widths were most similar to the *L. saccharina* self-cross. The data suggest sex-linkage to the male gametophyte.

Mean stipe lengths shown for 1 July, 1988 (Fig. 13) indicate self-crosses of *Laminaria saccharina* and *L. longicruris* were significantly different; the range of stipe lengths for *L. saccharina* is smaller. On the other hand, stipe lengths of reciprocal crosses between male and female gametophytes of each species were intermediate, with both exhibiting a wider range of lengths than either self-cross. The data suggest stipe length is linked to the female gametophyte.

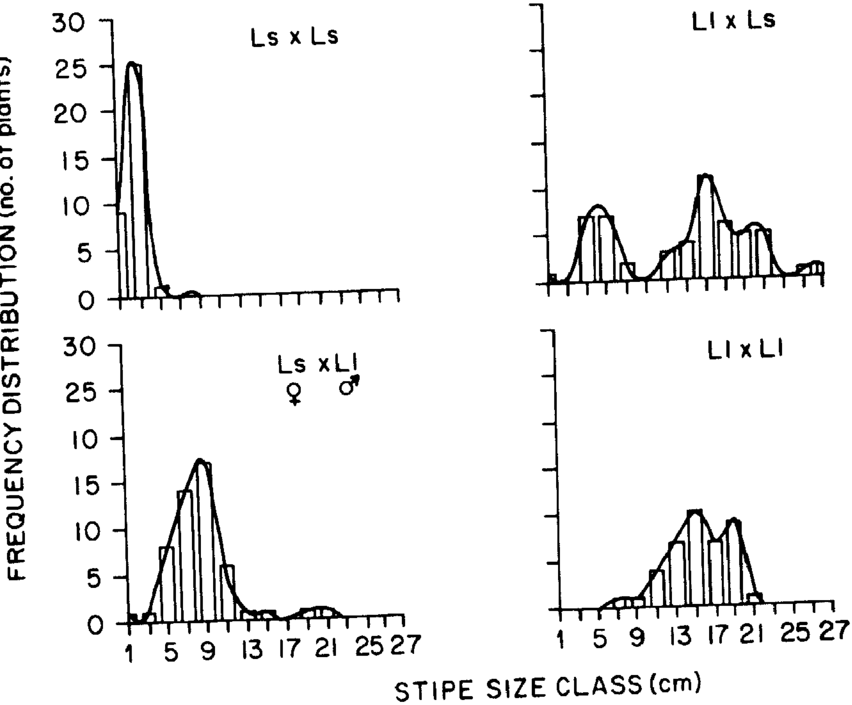


Figure 13. Frequency distributions for stipe length (cm) of F_1 *progenies from crosses of* Laminaria saccharina *and* L. longicruris *in greenhouse cultures plants. Data from 1 July, 1988. First species code listed for each cross represents the female.*

Conclusions

Laminaria longicruris and *L. saccharina* are dominant members of productive subtidal communities in Long Island Sound and throughout the western North Atlantic basin. Long Island Sound is a unique area in which to study morphological and growth variability in these two entities. This communication has reviewed the taxonomic problems separating these species. Through an integrated, experimental field and laboratory culture approach, our preliminary crossing and morphometric studies of *L. longicruris* and *L. saccharina* have enabled us to determine some degree of genetic differentiation with respect to genotypic and phenotypic characters. This study has illustrated how protocols should be developed to determine which morphological characters have a genetic basis and, therefore, hold promise for selection and use in mariculture applications.

Acknowledgments

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6

European Laminariales and Their Cultivation

Joanna M. Kain (Jones), T.J. Holt and C.P. Dawes
Port Erin Marine Laboratory
Isle of Man
British Isles

Abstract

The life history of the Laminariales allows convenient manipulations by cultivators although the cultivated species cannot reproduce vegetatively. *Laminaria saccharina* and *Alaria esculenta* have been cultivated off the Isle of Man in the Irish Sea for eight years. At first, string was seeded, cut and the pieces applied to horizontal ropes (long lines) in the sea. Later, seeded 6 mm cord was used directly as the substratum for growth of the crop. The currently used rope structure bearing the cords is in the form of a grid. It is clearly feasible to cultivate native species of Laminariales in Europe and for human food this may be economically successful.

Introduction

Laminaria has been cultivated on a very large scale in China for decades (Tseng, 1981) but different techniques had to be found for use in Britain partly because the native species are different (and it would be wrong to import exotic species) and partly because the environmental conditions are different. An advantage at the start of this work was that a considerable amount was known about the European species (reviewed by Kain, 1979). An essential preliminary to successful cultivation is adequate knowledge of the biology of the crop plants.

Important aspects of the biology of the Laminariales

The basic life history is well known but a version slightly different from that usually presented is shown in Figure 1. The large sporophyte produces many spores: around 50 million per cm^2 of blade (Parke, 1948; Kain, 1975). The development of spores into gametophytes can take two routes (Fig. 1). Under certain, apparently 'good', laboratory conditions, they become mature in one to two weeks, but each female gametophyte produces only one egg. Under apparently less favorable conditions they are prevented from becoming fertile, with the result that they grow vegetatively as filaments. When these eventually become fertile their fecundity is greatly increased; each female can produce as many eggs as there are cells.

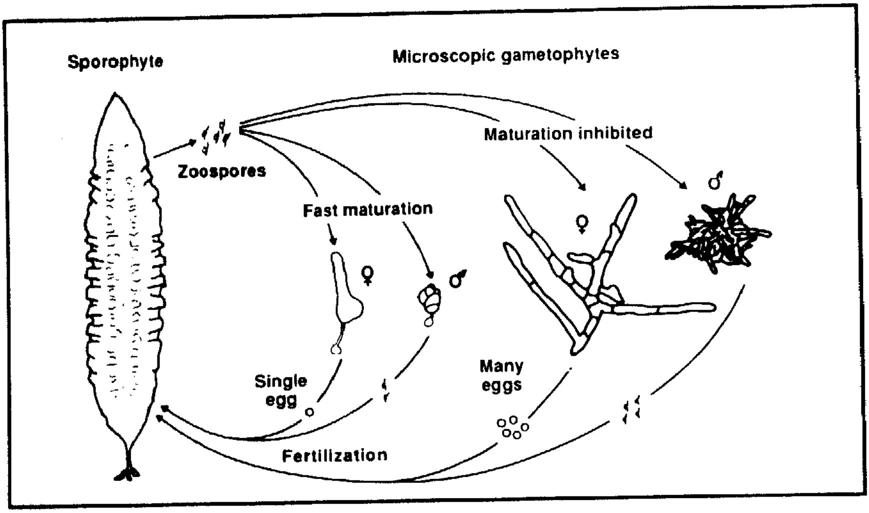

Figure 1. A diagram of the life history of Laminaria.

Various factors control which route development takes. For fast maturation a certain dose of blue light is necessary (Lüning and Dring, 1975), low temperature is stimulatory (Schreiber, 1930), changing the culture medium may be necessary (Kain, 1964), and it may also be preferable not to autoclave the medium (Walton, 1986). Thus this stage can be manipulated. In the next stage, gamete release and fusion, the plants have four devices for increasing the chances of fertilization: (1) the fact that fertility is under environmental control means that at one site males and females will probably be triggered simultaneously; (2) eggs are released in the first half-hour of the night, concentrating their appearance to one-fiftieth of the time (Lüning, 1981); (3) released eggs exude a substance which stimulates the release of male gametes (Lüning and Müller, 1978); and (4) this same substance attracts the males to the eggs (Lüning and Müller, 1978).

Thus the Laminariales is a group of plants that produce prodigious numbers of spores and although a gametophyte phase is obligatory, this can be easily manipulated. It therefore poses little problem because fertilization and progression to the sporophyte phase is easily achieved. This is fortunate for cultivators because in these plants, unlike the red algae, regeneration and vegetative reproduction are poor.

Development of cultivation techniques in Britain

Cultivation work on Laminariales off the coast of the Isle of Man in the Irish Sea started in 1980 (Jones and Holt, 1981). At that time three species were used: *Laminaria saccharina, Alaria esculenta* and *Saccorhiza polyschides*. The last has since been dropped.

It seemed that the most suitable substratum for attachment of the plants was rope, but it was assumed that this was too clumsy to be seeded directly. The gametophytes were therefore grown on string (Holt, 1984; Jones and Holt, 1985). Various types were tried; some natural fibres were found to be toxic. The best was polypropylene fibre film.

This was wound around small frames which were immersed in seawater (Holt, 1984; Jones and Holt, 1985). A spore suspension was produced from fertile plants from the sea. This suspension was poured over the frames and the spores allowed to settle. The frames of string were then held in small tanks of enriched seawater at controlled temperature in the laboratory. Contaminants were discouraged by the following means: (1) the seawater was fine-filtered; (2) germanium dioxide (a diatom poison) was included in the medium for the first week; and (3) green light was used at a relatively low level, reducing the growth of unicellular green algae.

After some time the string was transferred to ropes in the sea. The first method of doing this was to wind the string around the rope but water movement converted the neat helix to one big loop with all the turns at the ends. The method was therefore revised and the string was cut into short lengths, each of which was passed through the lay of the rope. This was successful though many of the plants grew only on the loose ends of the string and failed to attach to the rope.

The rope structures each consisted of a horizontal rope held in place by an anchored vertical rope at each end and at a given depth by a series of buoys and weights (Fig. 2). Because of the tidal range (up to 6 m) the so-called vertical ropes allowed considerable looping of the system at low water.

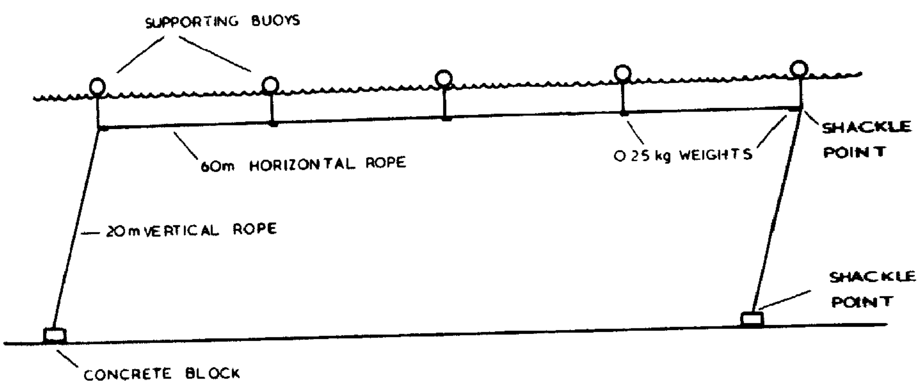

Figure 2. The horizontal rope structure used in early experiments off the Isle of Man.

Various experiments were made on suitable cultivation techniques. Varying the length of time in culture gave unpredictable results (Fig. 3). A period of about a week could result in complete failure or be quite successful. It is probable that the conditions on the days of transfer are very important, particularly before

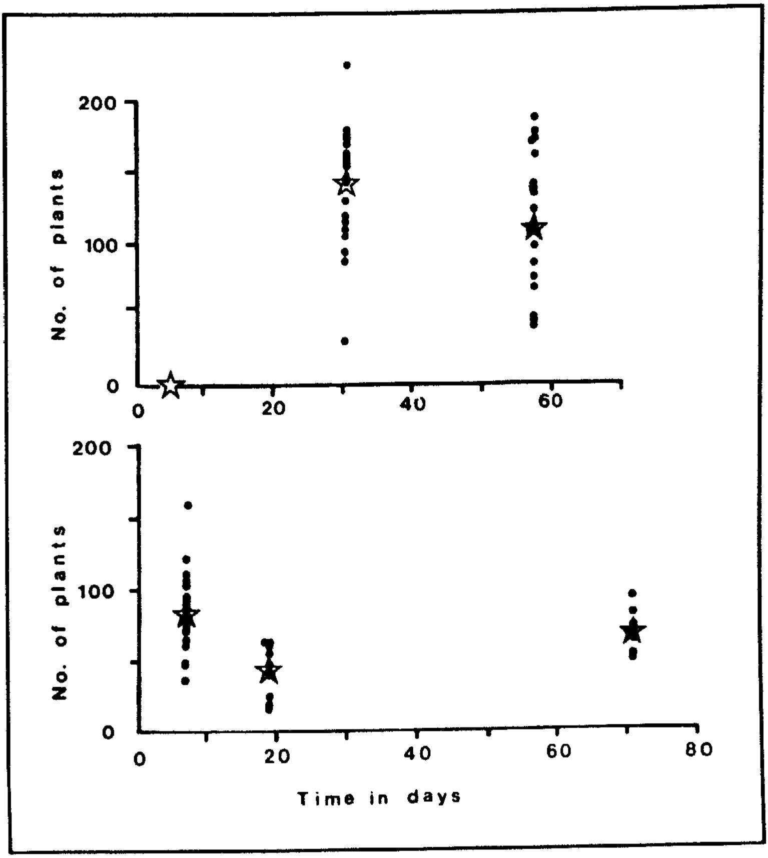

Figure 3. The effect of different lengths of time that the seeding strings remained in laboratory culture upon the number of plants of Alaria *which arose from each string. Upper, cultures started on 24 Feb. 1982; lower, cultures started on 10 Feb. 1982.*

sporophytes are formed. Longer periods usually resulted in good yield. The distance between the strings had a marked effect on *Alaria* (Fig. 4). There was no difference between the sizes of groups of plants growing from strings placed at one metre and half a metre apart. There was therefore no direct shading or other interference between these groups of plants. At intervals of less than a quarter of a metre, however, there was a significant reduction in biomass per group. At 5 cm intervals the biomass was less than a quarter of unimpeded groups. In spite of this the biomass per metre of rope rose dramatically as the seeding interval

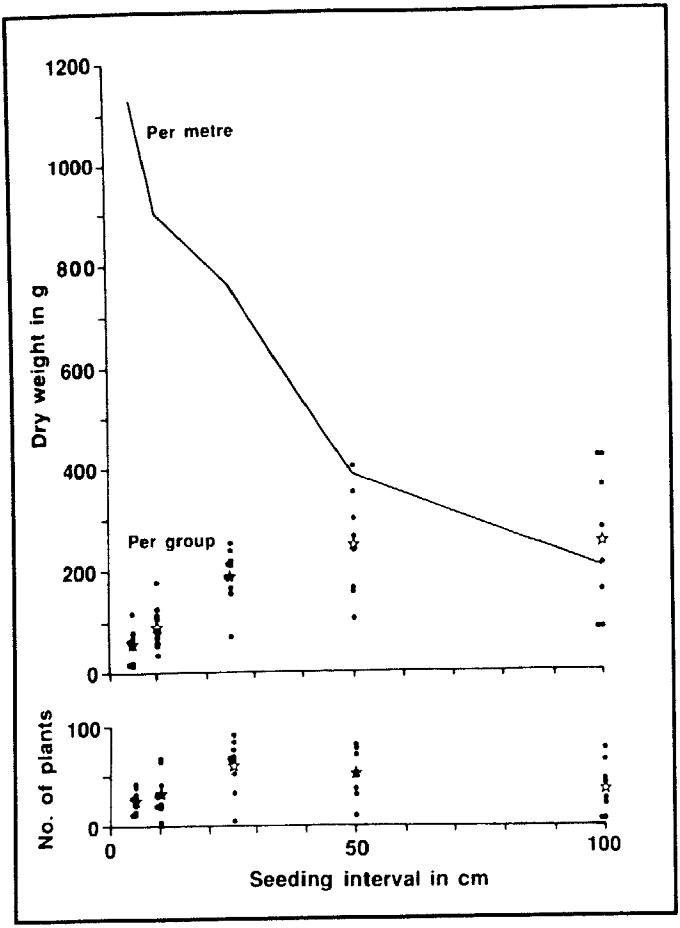

Figure 4. The effect of the interval between seeding strings upon the number of plants and the biomass of Alaria *arising on a horizontal rope.*

decreased. With this plant an interval of 25 cm was chosen as being economical of labor.

The time of year that seeding took place was found to be important (Fig. 5). With both *Alaria* and *Laminaria*, November was probably too early while December and February produced similar biomasses though numbers were higher for February. April seeding produced a reasonable number of plants but their biomass was relatively low. This was a consistent result through several years and was due mainly to smothering by diatoms which flourished at this time of year.

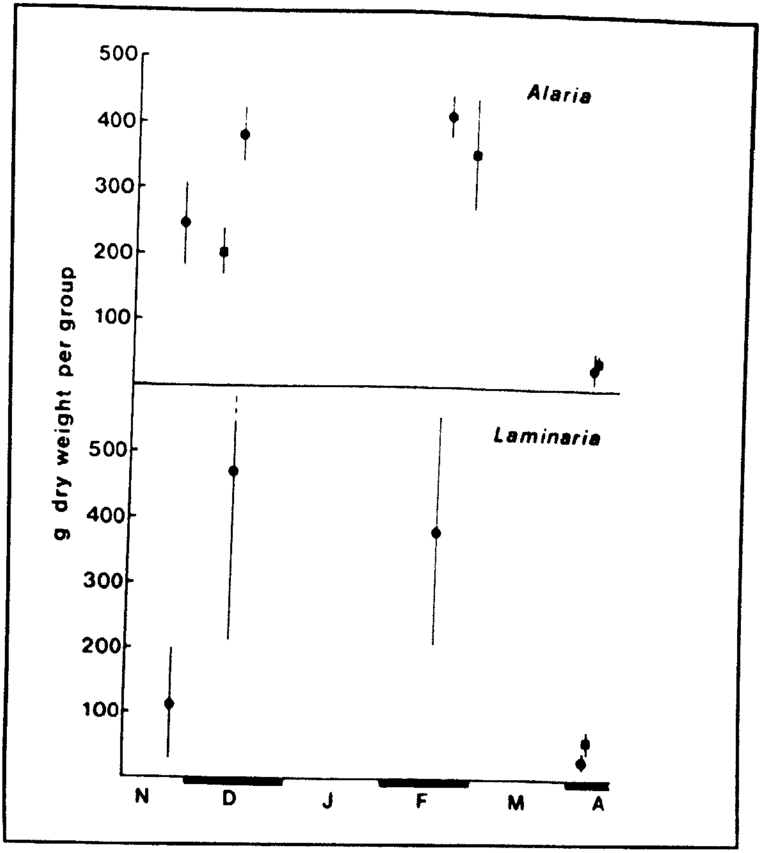

Figure 5. The biomass arising from seeding strings outplanted at different times of year on ropes at 2m below the sea surface. ● harvested in 1983; ■ harvested in 1984. Bars, 95% confidence limits. From Kain and Dawes (1987), courtesy of Dr W Junk Publishers

The depth of the horizontal rope was also varied (Fig. 6). Ropes have a singular advantage over the natural environment here. It might be presumed that there is an optimum depth for the growth of a subtidal alga at any particular season. For a fast-growing laminarian this is probably fairly shallow, unless the water is very clear, but even a shallow site will have an extra 6 m of tidal water over it on the coast of the Isle of Man, whereas the optimum depth can be maintained with a cultivating system. The depth experiments were with April-seeded ropes, consequently the biomass was low. It was found that 2 m was less favorable than

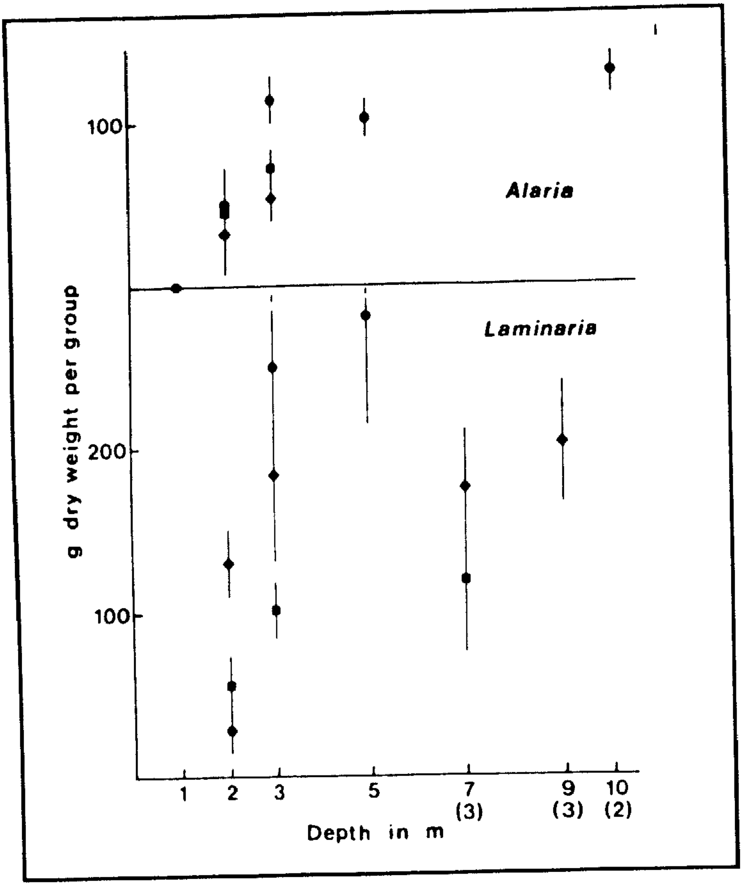

Figure 6. The biomass arising from seeding strings outplanted in April on ropes at different depths below the sea surface. Gametophytes grown in: ●, continuous light, 1983; ■, continuous light, 1984; ◆, 12:12 h L:D, 1984. Bars, 95% confidence limits. From Kain and Dawes (1987), courtesy of Dr W Junk Publishers.

3 m, possibly due to diatoms. The deepest ropes (7-10 m) were only maintained for 6 weeks, after which they were raised to 2 or 3 m. This was to prevent diatom smothering before the laminarians grew to a substantial size. The technique seemed to be favorable. Into this experiment another variable was introduced: a discontinuous photoperiod during the preliminary culturing (Fig. 6). With *Laminaria* this gave a significantly higher biomass than did continuous light. The effect on plant numbers was even more pronounced (Fig. 7).

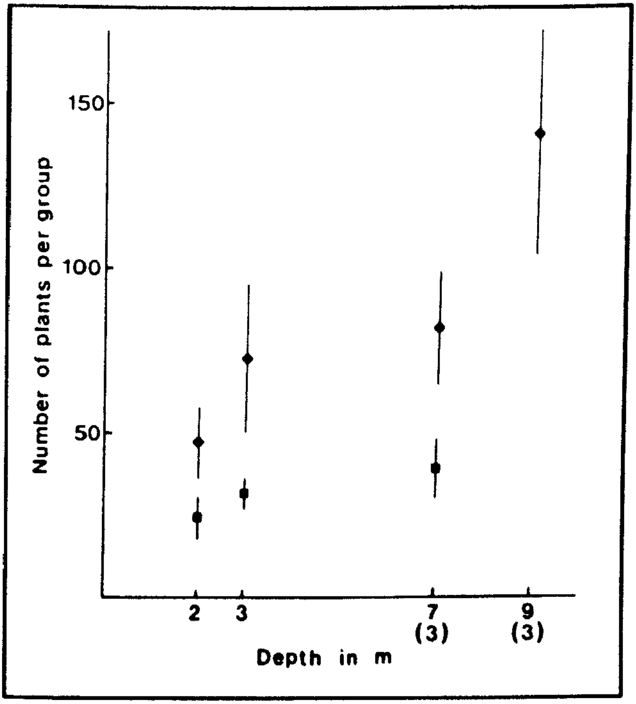

Figure 7. The mean final number of Laminaria *plants arising from each seeding string outplanted in April. Symbols as in Figure 6. From Kain and Dawes (1987), courtesy of Dr W. Junk Publishers.*

Harvesting time is very important; it is not advisable to harvest actively growing plants which could give a greater yield if left in the sea for a longer time; nor does it improve a crop to remain in the sea to collect epiphytes. In order to time harvesting correctly it was necessary to measure growth rates. This was done using the punched hole method (Parke, 1948) and the relative growth rate near the transition zone was found. *Alaria* growth slowed quite rapidly in May and stopped by the beginning of June, except for the April-seeded plants which continued for some time (Fig. 8). *Laminaria* continued growth for longer, albeit slowly, and again the April-seeded plants were growing faster than the winter-seeded ones near the end of the season (Fig. 9). These results provide evidence that cessation of growth is not primarily determined by an external factor such as nutrient level. The difference between species would be useful to farmers attempting to spread the work load at harvest time.

Most kelp farmers seed string: gametophytes are attached to string while they are cultured. This is labor intensive; it is necessary for string to be applied to rope by hand. It also carries some

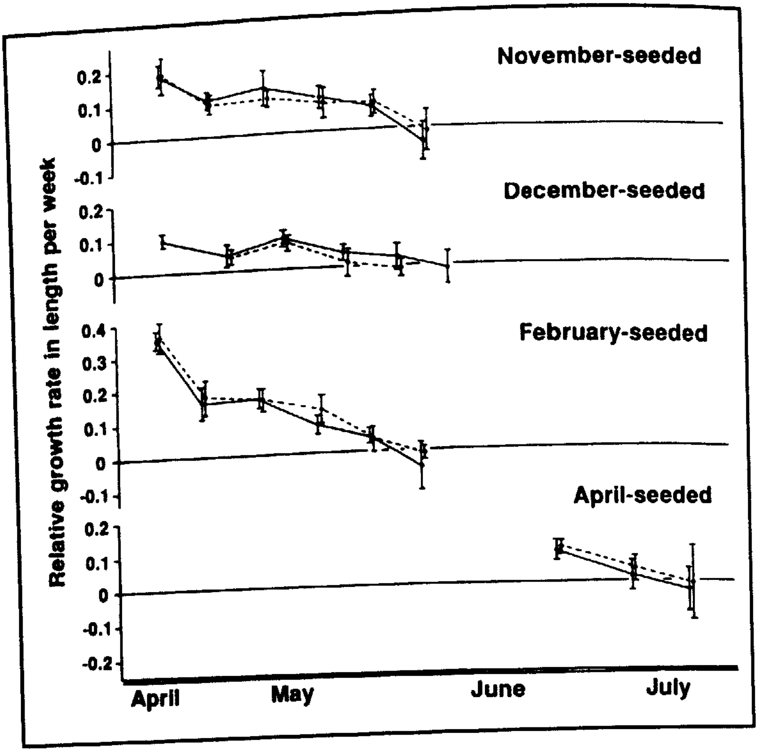

Figure 8. The relative growth rate in length of Alaria *plants seeded at different times on ropes at 2 m depth, during the period approaching the suitable harvesting time. Bars, 95% confidence limits.*

risk; the delicate young plants have to be exposed to the air which may be considerably colder or warmer than the sea. There may also be a danger of desiccation. However, rope is too clumsy to seed directly. A compromise was therefore sought and the possibility of seeding intermediate-sized cord capable of bearing the weight of the algae and attached to a rope framework was investigated (Dawes, 1987; Kain and Dawes, 1987).

Polyethylene line of 3 or 6 mm diameter was wound around half metre square wooden frames and placed horizontally in seawater tanks. The spore suspension was poured in twice, turning the frames over between each pouring. After the spores had settled, the frames were held vertically in tanks of enriched seawater in natural light (providing a suitable photoperiod). When the cords were placed in the sea, they were cut into 8 m lengths and

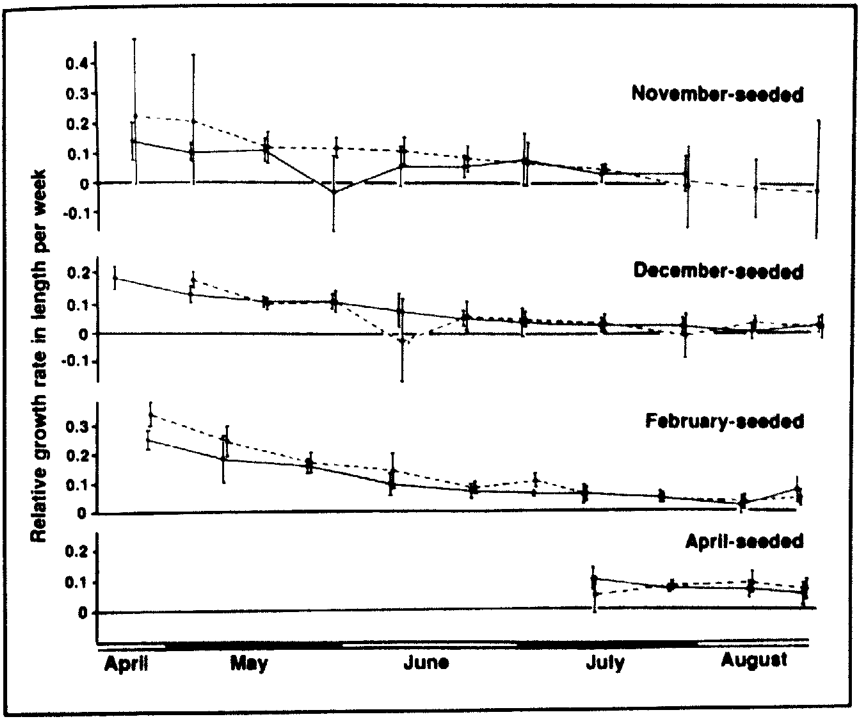

Figure 9. The relative growth rate in length of Laminaria *plants seeded at different times on ropes at 2 m depth, during the period approaching the suitable harvesting time. Bars, 95% confidence limits.*

attached to the thick rope used before. Several different designs were tried, hanging the cords vertically, based on the horizontal rope system (Fig. 10). The frequency of the cords was varied from half to 2 m and 1 m intervals were found best. A 1 kg weight used at the lower end of each cord was found to be adequate. Of cords free or joined at the bottom, free cords were preferable, avoiding a domino effect if one broke. A cord thickness of 6 mm was preferable to 3 mm. The system worked quite well with buoyed anchor ropes keeping the system taut (Fig. 11).

The biomass of *Alaria* per metre of cord was always low on the uppermost metre (Fig. 12). This could be due to inhibition by high light near the sea surface, the effect of wave action or damage by the boat. On the other hand there was little falloff in biomass with depth except near the base of the cord. This was probably

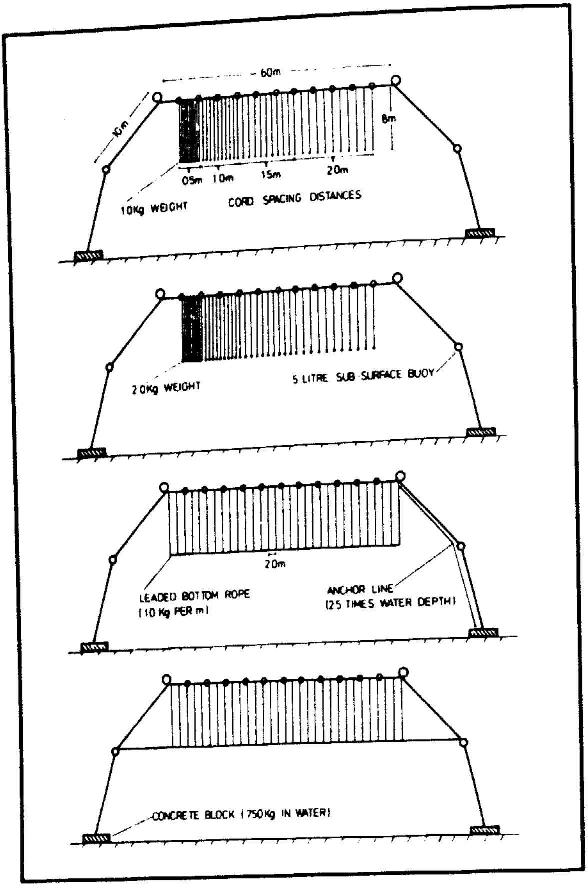

Figure 10. Experimental rope systems.

because the tidal currents lifted the cords and brought the lower parts into better illumination.

The most recent development is of a farm that should yield a profitable crop. The rope system has been further redesigned; instead of long lines it consists of a grid which remains in the sea throughout the year with seeded cords being added for the growing season (Fig. 13). The grid is actually suspended 2 m below the surface, each supporting buoy has 2 m of rope below it attaching it to the horizontal rope. The grid measures 250 x 200 metres, being 5 hectares. Seeded 6 mm diameter cords are produced as before, except that the frames are hung in the seeding tanks and not laid on the bottom. Instead of being hung vertically in the sea the cords are attached at each end to 16 mm diameter grid ropes. Because of weather problems, only about a third of it was seeded in 1988.

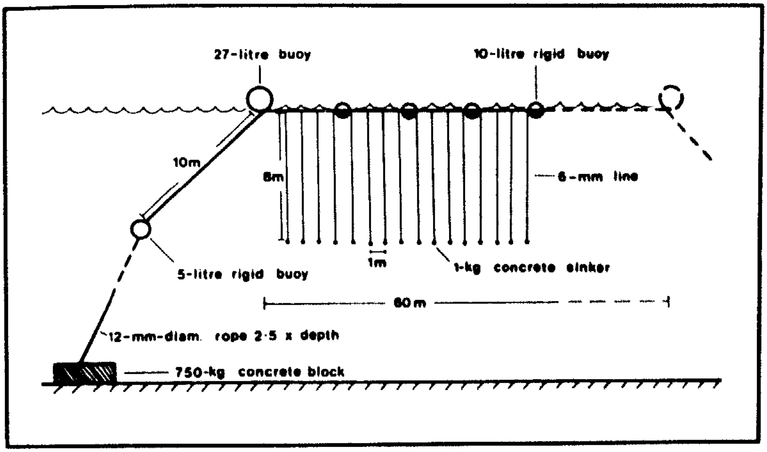

Figure 11. Successful rope system used for deploying directly seeded cords (6 mm lines). From Kain and Dawes (1987), courtesy of Dr W Junk Publishers.

The yield was about 6 wet tonnes of *Alaria* and 12 wet tonnes of *Laminaria saccharina*.

There have been many practical difficulties. Some of these could have been avoided if we had had first hand experience of the Chinese method at the beginning. Most of the problems have been associated with water movement. The winter weather pattern involves a series of depressions which sweep across the British Isles. Though the Irish Sea is not subjected to oceanic swells there are many gale force winds which produce steep seas. Concrete blocks never seem to be sufficiently heavy and either are dragged or turn over and chafe the ropes. It is really necessary to add anchors to the blocks. Shackles become loose even when the pins are tied; splicing the ropes is a preferable method of joining them. It is most important to keep ropes taut at all times.

The system lags very much behind that developed in China, both in scale and technique but European cultivation is unlikely to approach that scale. In technique the main lack in Europe is in experiments on genetic improvement. There is, however, less urgency for that: the endemic species are well adapted for their conditions, unlike *Laminaria japonica* which does not grow naturally on most of the coast of China. There is another advantage in terms of environment: the Yellow Sea becomes devoid of nitrogen

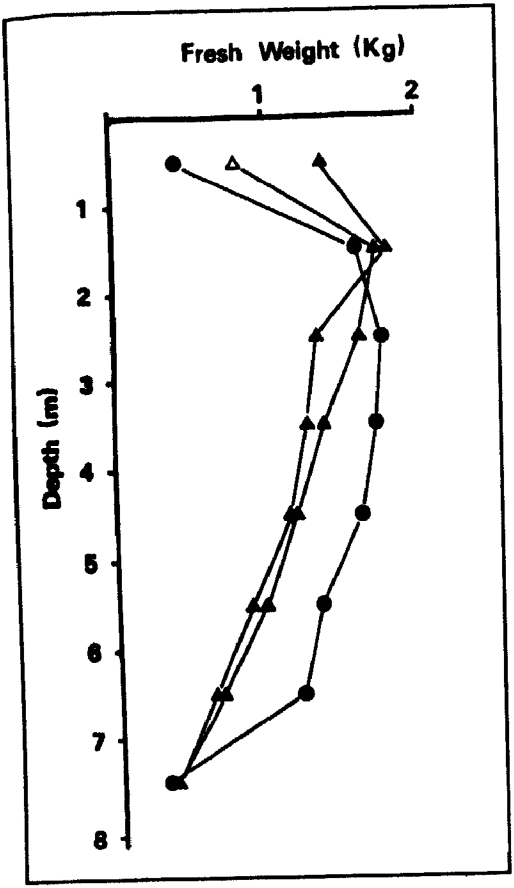

Figure 12. Mean final fresh weight of Alaria *per metre of vertical cord.* ●, *6 mm cord;* ▲, *3 mm cord.*

in the summer so it is necessary to fertilize almost all the farms off China artificially (Tseng, 1981). In the Irish Sea there is always some nitrate even though it drops quite low (Slinn and Eastham, 1984). The low level is compensated for by strong tidal streams, the water next to each plant being continuously replenished.

The potential of kelp cultivation

In considering kelp farming the choice of site is of prime importance. The following points should be borne in mind:

(1) wave action should not be excessive;

(2) tidal streams are beneficial, particularly in sheltered water;

(3) for these partly winter-growing plants there is probably a latitude limit;

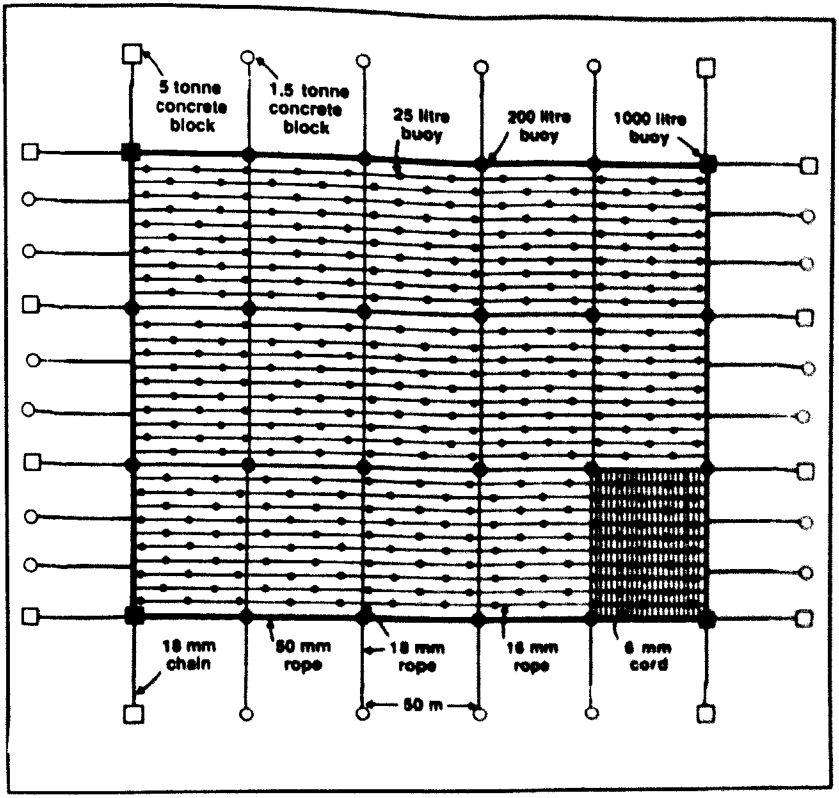

Figure 13. Grid rope system supported at 2 m below the sea surface to which directly seeded cords are attached (only shown in the bottom right rectangle). Anchor lines are not to scale.

(4) salinity should remain within reasonable limits;
(5) the same applies to temperature;
(6) nutrients should not drop too low in summer; the best sites are those where the water column does not become stratified, because in mixed water nutrients are not completely removed by phytoplankton;
(7) the water depth must be within reasonable limits or too much rope is required for anchoring;
(8) the type of bottom determines the ease of anchoring; and
(9) in many cases the most difficult consideration is the necessity to obtain the water rights for a farm. This is a new development for the many authorities and they are slow to make

decisions. In European countries this step varies from being easy to being virtually impossible.

In contemplating the future of kelp farming in Europe, one should consider first the availibility of suitable sites. There is plenty of coastline with a wide variety of conditions and many sites have all the right attributes. It must be stressed, however, that until a site has actually been tried there is no certainty that it is suitable.

Kelp is already being farmed commercially in France (Pérez *et al.*, 1984). Unfortunately, the species farmed is *Undaria*, which is exotic. We cannot condone any encouragement of the spreading of exotic species. However, the seeding method is interesting: the life history is manipulated, the gametophytes being held at the vegetative stage and used as broken up filaments to seed the strings (Pérez *et al.*, 1984). The ability to control this stage is thus being used to keep and seed an exotic species. There is another use for this control of the life history: the creation of gametophyte banks. Such a bank could provide a permanent stock of known parents allowing genetic manipulations.

The most important consideration in cultivation is whether it will pay. At present it seems that the human food industry is likely to be sufficiently profitable. In Britain there is now an increased awareness of seaweed as food. There are potential niches for seaweed in new products.

There is considerable potential for rope or net farming in the sea. Looking at algal cultivation worldwide it is apparent that almost all the commercially successful operations have been in the sea or lagoons, not in tanks. It seems that a product has to fetch a very high price indeed for it to pay the expense of equipping tanks.

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A Canadian Fishery Update and Advice to Policymakers and the Stock Assessment Phycologist

J.D. Pringle and G.J. Sharp
Biological Sciences Branch
Halifax Fisheries Research Laboratory
Fisheries and Oceans Canada
P.O. Box 550
Halifax, Nova Scotia
Canada B3J 2S7

Abstract

The five marine plant species harvested commercially in eastern Canada are *Chondrus crispus* (Irish moss), *Ascophyllum nodosum* (rockweed), *Furcellaria lumbricalis* (horsetail), *Laminaria* spp. (kelp) and *Palmaria palmata* (dulse). A brief update of each fishery is presented. Irish moss demand increased markedly in the mid-1980's, evidenced by a three-fold increase in dockside prices. Annual yields, however, have only increased marginally. Rockweed annual yields are up five-fold since 1983. Harvesting technology has changed from an inefficient, reciprocating blade harvester to a versatile, modern suction cutter harvester. A renewed commercial interest in β-carrageenan has prompted a move from a passive (storm-toss gathering) collection of horsetail to a directed, dragrake harvest. Kelp and dulse annual production have remained steady for many years, at low yields. There is an interest

in enhancing local sodium alginate production using kelp. Progress has been made in adapting Norwegian harvesting technology to the Canadian habitat.

Experiences gathered from involvement with both the Canadian fishery and that of other countries permitted the formulation and presentation of the six major ingredients required for a successful industry. They range from resource abundance to resource management agency structure. Biological advice is important to resource management plans. Prior to designing a research/stock assessment program, it is important to understand both the agencies' approach to resource management, and to choose wisely the models to be used to generate the advice.

Introduction

Marine macroalgae are commercially harvested along portions of the Atlantic coasts of Argentina, Brazil, the United States of America, Canada, Morocco, Portugal, Spain, France, the United Kingdom, Ireland, Norway, and Denmark. Attempts to manage these commercial resources are rare. The Governments of France and Canada appear to have done the most to ensure a successful marine plant industry. The Canadian Federal Government has jurisdiction over renewable marine resources. The Department of Fisheries and Oceans assisted the development of the eastern Canadian seaweed industry in the mid 1960's (Pringle, 1986). By 1974 it was deemed important to provide biological advice to resource managers (Pringle, 1976).

We have been intimately involved with the eastern Canadian marine plant industry for 14 years. During this time we have developed a perception of what is required to permit the development of a successful marine plant industry, and the rationale for a research program designed to provide the pertinent biological advice for the resource management of such an industry. Prior to discussing these two topics, an update of the eastern Canadian industry is presented.

Industry Update

Chondrus crispus

The *Chondrus crispus* Stackhouse (Irish moss) industry of Maritime Canada was fully developed by the 1970's. Landings peaked in 1974 (Fig. 1) following a period of growing market demand, expanding harvesting efforts and technological improve-

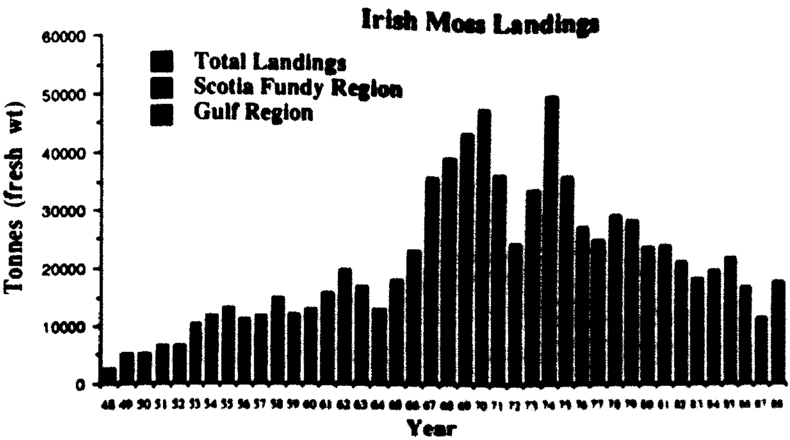

Figure 1: Maritime (Nova Scotia, New Brunswick and Prince Edward Island) Irish moss annual landings between 1948 and 1988 inclusive. See Figure 2 for Regional locations.

ments. All commercially significant *Chondrus* resources were being exploited or were surveyed for potential exploitation (Fig. 2). Four major companies purchased wet and dried *Chondrus* at numerous buying stations (Ffrench, 1971); Genu Canada Ltd., Marine Colloids Ltd., Stauffer Ltd., and Litex Ltd. Trends in annual landings and industry development were different between the southern Gulf of St. Lawrence and Atlantic Nova Scotia as a result of species distribution and local economics. Gulf *Chondrus* occurs subtidally and is harvestable as "storm toss" or by towed dragrakes over a bottom of low relief. The dragrake fishery is limited (no new licenses since 1977); thus a core of 150 harvesters have become dependent upon it for a significant portion of yearly income (Pringle and Mathieson, 1986). *Chondrus* is accessible in the lower intertidal/upper subtidal (< -2m depth) along Atlantic Nova Scotia. Long handled rakes are used from 5m outboard powered skiffs to remove *Chondrus* from rock and boulder substrata (Pringle and Mathieson, 1986). The number of licenses are

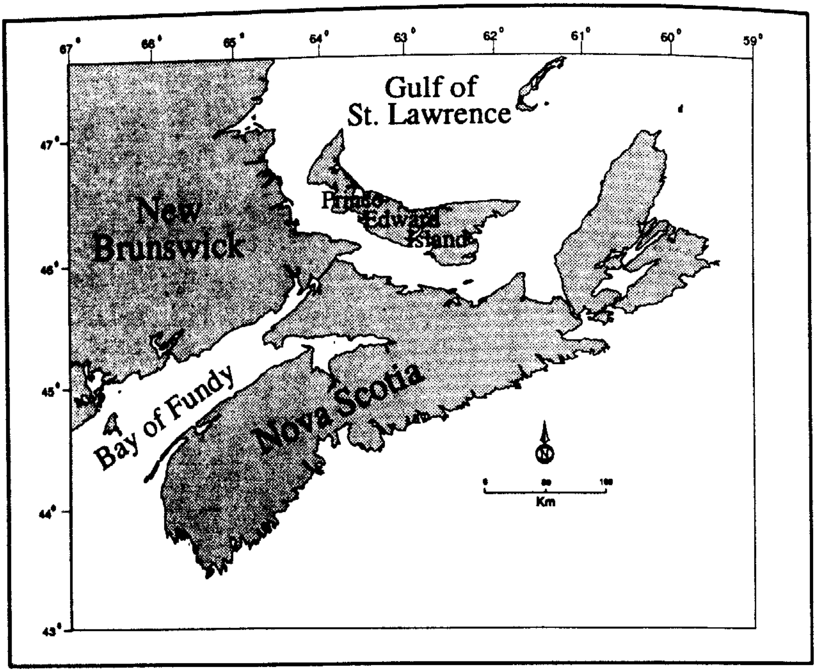

Figure 2: The southern Gulf of St. Lawrence (Gulf region) and Scotia- Fundy region (Atlantic Coast of Nova Scotia and Bay of Fundy Waters)

unlimited and the level of economic dependence low. Oscillations of landings in the Gulf from 1972 to 1979 were due to annual changes in abundance (Fig. 1). Between 1979 and 1986 Gulf landings have remained below 20,000 t. The weakness of market demand was reflected in a stagnant price and a reduced buying effort. Only two major buyers were active on P.E.I.; Genu and Acadian Seaplants, Ltd. A succession of poor standing crops and a voluntary reduction in effort led to a shortened harvest season. Since 1978, the landings of *Chondrus* in Southwestern Nova Scotia (sole area for landings from Atlantic Nova Scotia since the 1970's) have declined steadily to an all time low of 1,600 t in 1987. Number of licenses purchased during the season declined from about 900 in 1978 to 350 in 1987. Dockside price was stagnant and buying effort reduced. Although there was an observed decrease in annual abundance, and resource accessibility, sometimes limited by long-term tidal patterns and weather, these factors alone cannot account for the large drop in annual yield (Fig. 1). An attempt was made by buyers to enhance harvesting efforts by doubling dock-side prices between 1985 ($0.143 kg^{-1}, fresh weight) and 1988 ($0.287 kg^{-1}). Landings increased by 1200 t to 2800 t. Interestingly, the bulk of the yield came, not from the nearshore islands, but further offshore. Reasons for this are being investigated.

Furcellaria lumbricalis

Furcellaria is the only commercial source of β-carrageenan, beyond the small crop of *Eucheuma fragile* taken from the eastern Pacific. It is gathered, as a mixture with *Chondrus,* from the northeastern shore of Prince Edward Island (Fig. 2). Landings (Fig. 3) are extremely variable, being dependent not only on harvesting effort, but on storm frequency. Wave surge must be of sufficient force to remove the fronds, and from the appropriate direction to drive the fronds onshore. Until recently the *Chondrus/Furcellaria* mixture was purchased by Litex Ltd. The Litex operation was suspended in 1984. The provincial government stockpiled material until the company was purchased by Acadian Seaplants. An experimental dragrake harvesting operation was performed in 1988 in an attempt to promote an active harvest.

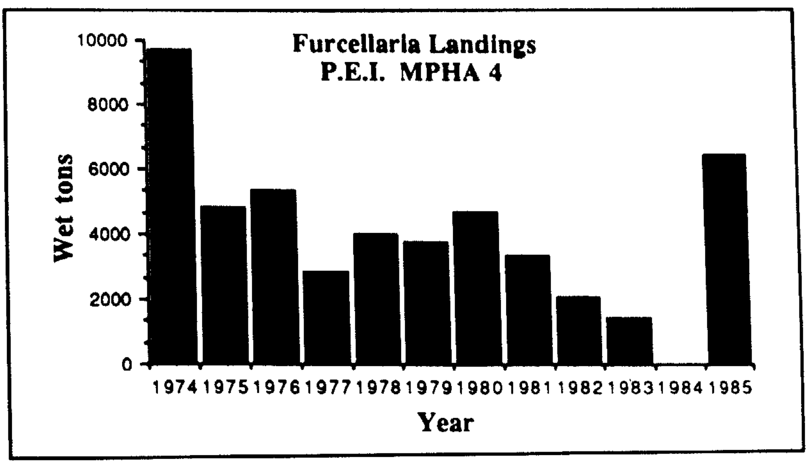

Figure 3: Annual landings of mixture (Furcellaria *and* Chondrus) *from northeastern Prince Edward Island, between 1974 and 1985.*

Ascophyllum nodosum

Although *Ascophyllum nodosum* is the dominant intertidal seaweed in Atlantic Canada, it is only commercially harvested in southwestern and western Nova Scotia (Fig. 2). Harvesting of *Ascophyllum* as a raw material for alginate extraction began in 1959 when a subsidiary of Kelco Ltd., Scotia Marine Products Ltd., was established in Lower Woods Harbour. Hand-cut rockweed was purchased from local fishermen. The company introduced mechanical harvesters in the early 1970's. Each had reciprocating cutter heads attached to a conveyer belt system (Sharp, 1987a). Due to gear and processing limitations the harvest was seasonal and landings only ranged between 4,000 to 8,000 t (fresh weight) per year. The low level harvests (<1000 t), using hand methods, were processed for meal or fertilizer products (Digby Seaweeds, R&K Murphy, Acadian Seaplants, and Bonda Foods). The purchase of Scotia Marine Products by the western world's second largest alginate producer, Protan A/S, brought Norwegian harvesting technology (Fig. 4) to Canada (Sharp, 1987a). This machine

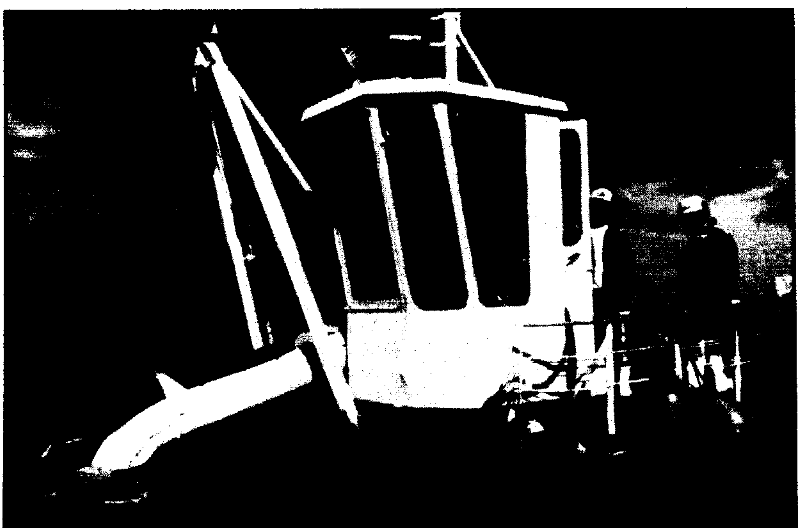

Figure 4: The Norwegian hydraulically operated Ascophyllum *harvester. Fronds are sucked into the funnel and cut with a rotating blade (head below water).*

crops plants using a combination of water suction and a rotary cutting blade. The year round harvesting capability of this new equipment, and greater harvesting efficiency, has factorially increased landings over the past 3 years (Fig. 5).

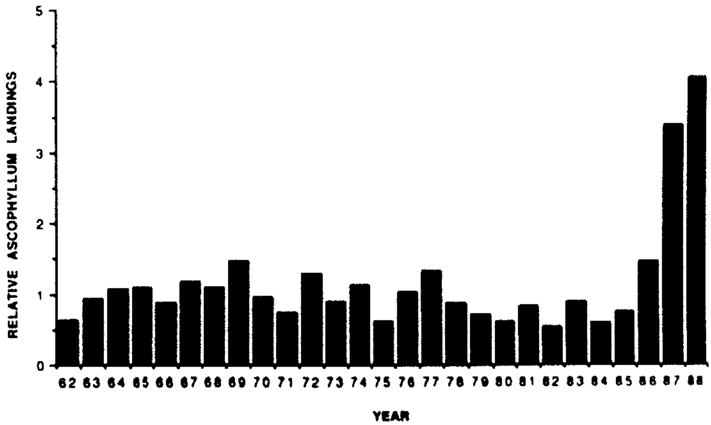

Figure 5: Relative annual landings of Ascophyllum *from southwestern Nova Scotian waters between 1962 and 1988. Annual yields are confidential. The annual data presented are the yearly yield as a proportion of the average yield between 1962 and 1971.*

Hand harvesting (Fig. 6) in the same area, during the past 2 years has increased dramatically to supply a new market. However, the annual sustainable yield for southwestern Nova Scotia has been exceeded without a rational resource management plan (Sharp, unpublished). Areas which were scheduled to remain fallow over 3 years were harvested in 2 years. Unless jurisdictional questions between federal and provincial governments are resolved and new management strategies implemented, it is predicted that, due to recruitment overharvesting, landings in southwestern Nova Scotia will decline dramatically over the next five years.

Figure 6: Ascophyllum *harvester in southwestern Nova Scotia deploying a cutter rake.*

Palmaria palmata

Palmaria (dulse) has been a locally popular food product for well over 100 years. Commercial harvests (50-100 t annually) are concentrated on Grand Manan Island (southwestern New Brunswick - Fig. 2) where 20-50 individuals hand-pick the crop at the most extreme low tides, spring through fall. *Palmaria* is ubiquitous on Nova Scotia's Atlantic and Fundy coasts, but only the Minas Basin area supports a commercial harvest. Market demand has strengthened with the development of a wider health food identity for seaweeds in general. However, the labor-intensive, hand-gathering technique with its irregular hours (based on tide patterns) does not appeal to the non-traditional harvester.

Laminaria *spp.*

Laminaria spp. were harvested between 1940 and 1949 as a raw material for alginate production by the American Alginate Co. (Sharp, 1980). Intertidal populations were cut with sickles, and subtidal kelp was drag-raked. Annual landings peaked at 3,000 t. Subsequently, small harvests of kelp for specialty food stuffs (<500 t) were made by a variety of companies. Recently, a renewed interest in kelp for alginate production stimulated a review of the resource base, resulting in a proposed pilot scale harvest of 10,000 t.

Requirements for a Successful Marine Plant Industry

A successful industry could have a variety of definitions. We define it as one that has had long-term (at least five years) financial viability, where both the harvesters and processors attain reasonable returns for their efforts and where government receives resource rent. We have drawn on our personal experiences from both the Eastern Canadian industry and elsewhere, to develop the following list of six essential requirements.

1. A large biomass of commercially important seaweeds that are accessible to harvesters and markets.

The marine plant density and biomass required for a successful industry is in part a function of the unit value of the crop. Obviously lower priced alginophytes and carrageenophytes such as *Ascophyllum nodosum* and *Chondrus crispus*, respectively, must have a higher standing crop than more highly valued agarophyte taxa such as *Gelidium* spp. and *Pterocladia* spp. Paine (1984) used the term monoculture to describe the occurrence of a wild species at a biomass level ≥ 80% of the total biomass. The bulk of the Canadian commerical *Chondrus* and *Ascophyllum* could be classed as monocultures (Pringle and Semple, 1980, 1983; Sharp, 1987a) as could that along coastal Maine (USA) (Vadas and Wright, 1986). There is a commercial harvesting district in southern Prince Edward Island where *Chondrus* occurs at a lesser standing crop (about 50% of the total standing crop). However, harvesting only occurs there when demand is high and industries' quality standards are low.

Proximity of the beds to both harvesters and markets is important. There are large quantities of *Macrocystis* spp. in both the southwest Atlantic (Argentina and the Falkland Islands) and southwest Pacific (southern Chile). Ventures aimed at cropping these resources were not cost effective given combined costs of harvesting and transport to both processing sites and markets. Protan A/S provides sufficient financial remuneration to Norwegian rockweed harvesters to encourage them to live on small boats in isolated areas for a week at a time. A cargo vessel picks up the crop weekly and transports it to the processing site.

2. A commercially productive harvesting technique that has a low-level ecological impact.

Development of efficient, ecologically sound, mechanical seaweed harvesters is no small task. Organizations as different as the Massachusettes Institute of Technology to independent inventors (Pace, 1982) have been frustrated in their quest to improve the harvester-developed Irish moss (*Chondrus crispus*) handrake and dragrake. The former tool is perfectly good for cropping *Chondrus* from Gulf of Maine granite substratum (MacFarlane, 1956). The latter, however, has a high adverse impact on recruitment (Pringle and Semple, 1978) when employed on southern Gulf of St. Lawrence sandstone. Numerous attempts to develop an equally efficient harvester, but with a reduced impact, have failed. To maximize yields one must factor this impact into production models and resource management strategies.

Commercial beds of *Gracilaria* occur on intertidal/subtidal, mud/sand flats in Chile. Cropping by either hand-pulling or raking (Fig. 7) has resulted in severe recruitment overharvesting (the concept is dealt with in the last section) in many areas (Fig. 8). Similarly, the dredging for unattached fronds of *Furcellaria lumbricalis* in Denmark resulted again, in recruitment overharvesting (Austin, 1960). Raking for unattached or weakly attached species

Figure 7: Drag-raking Gracilaria *in the Valdivia, Chile estuary on January 16, 1985. The season was closed to* Gracilaria *harvesting and the use of a rake is illegal (Santelices, pers. comm.).*

Figure 8: A recruitment—overharvested Gracilaria *bed near Puerto Monde, Chile, in January, 1985. A row of temporary dwellings, (see Fig. 10) used by* Gracilaria *harvesters, are located along the sand bar.*

is not recommended, or for species such as *Ascophyllum nodosum* (Fig. 6), where annual reproductive capacity is low in relation to competitors, and where fronds do not recruit from cropped holdfasts.

There are huge standing crops of *Ascophyllum nodosum* along coastal Norway (Baardseth, 1958) and Nova Scotia, Canada (Sharp, 1987a). Personnel of Protan A/S have developed, over many years, an efficient, productive and sophisticated mechanical harvester (Fig. 4). This capital-intensive, yet non-labor-intensive technique, has allowed the company to take large annual harvests. If they were using the traditional labor-intensive sickle technique, the yields would be insignificant. Fishermen would likely be unwilling to harvest for the economic returns using manual techniques.

3. *A labor force sufficiently large and with adequate skills and incentive to harvest the crop.*

The traditional methods of harvesting seaweeds, for example, the handraking of *Chondrus* in eastern Canada and handpicking in France, are labor intensive. Fishermen of these modern, western societies would generally not perform such tasks, and yet here they do. First, the fisheries have been part of the respective economic fabric for generations. Certain of the offspring grow up with the expectation of earning a portion of their livelihood in this way. Secondly, the economic returns must keep pace with alternate sources of income. The latter is important, for once the tradition is broken it may be difficult to re-involve former harvesters and attract new harvesters. For example, prices paid for Irish moss to harvesters in eastern Canada were soft in the mid and early 1980's. Recently demand increased. Dockside price more than doubled, and yet the harvesting effort has been disappointingly low. The New England states (USA) Irish moss fishery ceased in the 1970's; it may have been for this reason.

Iridaea spp. are a commerical source of iota carrageenan. Commercial quantities were present in two eastern Pacific nations, Chile (Westermeier and Rivera, 1986), and British Columbia, Canada (Austin and Adams, 1978). Chilean harvesters (Fig. 9) in 1985 attained a similar dockside price for handraked *Iridaea* that Canadian harvesters received for handraked *Chondrus*. By contrast, industry and government could not induce British Columbia fishermen to harvest *Iridaea*. Likely the lifestyle and socio-economic climate in Western Canada in the early 1970's was not the appropriate background to successfully introduce a labor-intensive fishery to coastal communities.

Figure 9: Handrakers and dragrakers harvesting Iridaea, *near Puerto Monde, Chile in January, 1985.*

The Chilean socio-economic climate was appropriate during the late 1970's early-1980's when manual, marine plant harvesting flourished. A large (about 10,000 harvesters; B. Santelices[1], pers. comm.), and mobile group of harvesters move along the coast, from bay to bay, content to live in temporary dwellings (Fig. 10) during these forays.

[1] Dr. B. Santelices, phycologist and professor, Catholic University of Santiago, Santiago, Chile.

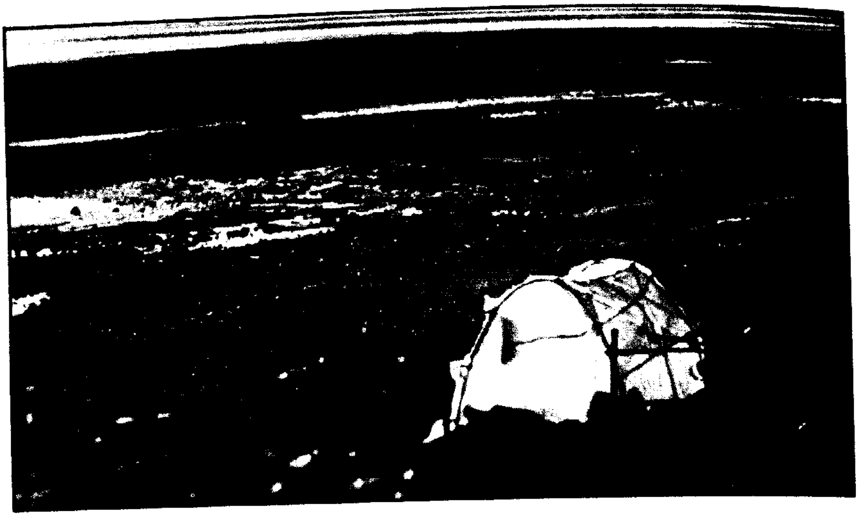

Figure 10: A temporary dwelling of an itinerant Gracilaria *harvester, near Puerto Monde, Chile, in January,1985.*

4. Adequate solar radiation to permit sun-drying of the fresh harvest and/ or sufficiently inexpensive fossil fuel to permit mechanical drying.

Under certain circumstances extractors tend to be concerned about the distance to ship a dried crop. For example, *Eucheuma* is routinely transported from the southwestern Pacific to Europe and North America. Given that water is about 80% of fresh weight, drying is important.

Drying seaweed must be done soon after harvest and prior to significant rainfall. Fresh water induces cell rupture; leakage of colloids follows. Solar energy is the preferred method. Here road verges (Fig. 11), fields, driveways, yards, etc., are used as the drying platform. *Chondrus* can be sun-dried to about 20% moisture content in <24 h along the coastal southern Gulf of St. Lawrence. This is the common method of drying here. The alternative method is a fossil-fueled drum drier, a number of which are strategically placed in eastern Canada (Ffrench, 1971). This is the common form of "moss" drying in the Gulf of Maine, where consecutive days of summer sun can be rare. This method was not consecutive days of summer sun can be rare. This method was not cost effective in the mid-1970's when fossil fuels cost increased.

Figure 11: Iridaea/Gigartina *harvesters solar drying their crop near Puerto Monde, Chile, in January, 1985.*

An efficient and cost effective method of drying marine plants is a concern throughout the international industry. The Chinese and Icelandic industries have problems in drying *Laminaria* and *Ascophyllum*, respectively. The drying of cultured *Eucheuma* in Micronesia is disrupted daily by tropical showers. The buyers of *Ascophyllum* and *Laminaria* along rain-soaked coastal Norway have avoided the drying problem by shipping the crop fresh to extraction facilities.

5. Scientific personnel willing and able to provide the scientific advice (biological, economic and social) necessary for resource management.

Research in the fields of biology, economics, and to a lesser extent sociology, are required in the development of species-specific, resource management plans. Ideally, stock assessment-related research will be carried out within a research wing of the resource management agency (Pringle, 1986), and, in particular, by those responsible for stock assessment. However, research programs in support of resource management can be carried out by non-aligned agencies such as universities, other government laboratories, private consulting firms, etc. France and Canada are possibly the only two western countries that provide civil service research support for marine plant resource management, which, in its broadest meaning, includes resource development. Canadian science in support of marine plant resource management is carried out at universities, National Research Council (NRC) Laboratories, and Department of Fisheries and Oceans (DFO) laboratories; the mandate for resource management resides in the latter department. Interaction between Canadian government scientists and universities is encouraged through DFO-sponsored grants to universities to carry out fisheries oriented-research. NRC scientists are encouraged to perform science in aid of resource development. This is evidenced by the large and long-term effort on Irish moss aquaculture.

Of interest is the long list of marine plant harvesting countries where little or no government sponsored research is carried out. In spite of a large Norwegian kelp and rockweed harvest, there has been little recently published research related to stock assessment/resource management. Similar situations exist in Portugal, Spain, Ireland, the Phillipines, Argentina and elsewhere.

By contrast, the People's Republic of China (PRC) has developed a large and sound phycological research team centered in Qingdao. A number of research stations dot the coast south to Hainan Island. Coastal universities often specialize in fisheries science and phycology. It appears the emphasis has been placed on resource development and, in particular, aquaculture. In fact, certain species such as *Eucheuma gelatinae* have been overharvested in southern China.

Chile has recently become a significant supplier of seaweeds to the international gum industry. Biological research in support of both resource management and development occurs in numerous universities led by B. Santelices[1]. Research quality is high. Annual national, phycological conferences are held to report research progress (Westermeier, 1986), which is often at the "cutting edge" of the international field. There is a keen interest by most researchers to carry out research on applied problems.

The eastern U.S.A. has a small, commercial marine algal harvest, but considerable monies are funded to university science researchers to study commerially important species. The Universities of Maine (Professor R.L. Vadas), New Hampshire (Professor A.C. Mathieson) and South Florida (Professor C. Dawes) have led the way in performing excellent research in support of wild harvests and/or aquaculture.

6. A government agency that has the mandate to manage renewable marine resources and the human, physical and monetary resources to perform the task.

There are few countries that have assigned to a government agency the responsibilty for marine plant resource management, and then attempted to actually manage the resource on an optimal yield basis. The often-cited failure to maintain a sustained yield (examples given above) may be the end result of neglecting resource management.

The Department of Fisheries and Oceans in Canada took up the challenge to manage marine plant resources in 1974 (Pringle, 1976). One Director, H.D. Johnston, had the foresight to invest in a marine plants program within a new fisheries research initiative. A phycologist was hired in this unique position and a research program initiated (Pringle, 1976). Marine plants are administered in the same manner as finfish and invertebrate resources. France may be the only other western nation that manages marine plant resources as animal resources are managed.

Jurisdictional problems within a country often prevent resource management (Pringle, 1986). A paradoxical situation exists in Chile where good resource science wants for sound resource management plans. This may be due to a suite of agencies involved in resource management. The national government appears to recognize the need for resource management; it funds research and has a biological advisor in place. However, each of Chile's twelve regions actually has jurisdiction over nearshore renewable resources (B. Santelices[1], pers. comm.). The regional bureaucrats appear not to have the will, the ability, and/or the resources to institute resource management. Also, the socio-economic situation is not conducive to resource management. Ironically, economic stress prevents long-term financial gains that might accrue from sound resource management. Commercial *Gracilaria* beds have become extinct in many regions (see Fig. 8) and national annual landings have fallen dramatically (R.Ugarte[2], pers. comm.).

The failure of the Danes to manage their *Furcellaria* crop, and the Norwegians their brown algal crops (details above), are enigmas. They both play active roles in finfish management and, in fact, are among the world leaders in fisheries science. As well, each have had (ICES) marine biological research programs for years. Similarly, a large annual crop of rockweed (about 40,000 t) is taken from the shores of southern Ireland. The government in 1979 had one civil servant (non-scientist) dedicated to this industry. As near as could be discerned, there appeared no attempt to manage the resource. Portugal, on the other hand, attempts to manage the harvest of *Gelidium,* but with little scientific advice. There is only a small coordinating group in the fishery laboratory and no research teams in the universities (R. Santos[3], pers. comm.).

Thus it appears Canada should be leading the way, but here there are problems as well. An active program on Gulf of St. Lawrence Irish moss, between 1974 and 1982 was lost through bureaucratic reorganization. As well, a provincial government demand for resource jurisdiction may end a successful 13-year program in the northern Gulf of Maine.

[2] Mr. R. Ugarte, research associate, Catholic University of Santiago, Santiago, Chile.

[3] Mr. Rui Santos, graduate student, Dalhousie University, Halifax, Nova Scotia, Canada.

A Brief Review of the Biological Requirements for Resource Management

To date there is no handbook available to guide an applied phycologist, when given the task of providing biological advice to a resource manager. Based on experience with the Canadian fishery we suggest three major areas of involvement. Obviously, questions involving resource abundance will be paramount, followed by concern over the ecological impact of harvesting tools. The third task of the applied phycologist is communication. Without considerable effort expended here in the latter area, brilliant research will be for naught (Pringle, 1985). There is insufficient space in the short exercise here to cover all these concerns. Thus, only the concern dealing with resource will be dealt with, and this not in complete detail. However, the following should give the fledgling stock assessment/research phycologist some direction.

Resource Questions

1. Resource Distribution: (Where is the resource?)

The distribution of the commercial resource in an established marine plant industry can be determined simply by discerning the pattern and distribution of fishing effort and industry catch rates. Level and location of effort can be obtained directly from fishermen-kept logbooks or systematic interviews with harvesters. Regular counts of active harvesters via aerial, on water or shore reconnaisance was used to define the location and yield of commercial beds (Pringle and Semple, 1980). Gear and economic limitations prevent these assessments from being comprehensive descriptors of distribution and abundance. The resolution of early surveys of aerial extent were dependent on the degree of effort in a ground survey (see below). Aerial photography and other remote sensing technology, e.g., multispectral scanning, have been effective means of rapidly surveying entire coastlines (Mouchot *et al.*, 1986). Cost effectiveness is related to the level of resolution, type of data collected, and degree of analysis. The precise planimetry of remote sensing is dependent on the ability to separate species. However, unless algal zones are monocultures, even the use of spectral signatures may prevent the separation of mixed species assemblages.

2. Resource Abundance: (How much resource is there?)

Ground surveys are still required to provide all relevant population data including biomass, population structure, reproductive condition, etc. The level of resolution required in biomass surveys is related to the development stage of the industry. Exploratory surveys are used to identify commercial potential, and thus require only identification of areas with a minimum level of biomass. Management of the resource requires an understanding of the degree of biomass and population structure variability with wave exposure substrate type and biotic factors (Sharp and Carter, 1986; Pringle and Semple, 1983). Sampling design must deal with variation of biomass with depth as well as horizontal changes along the shore. Randomization of sampling is difficult in large geographical expanses of subtidal because of the need to grid an area and then randomly select each sample unit. To date, a purely random sampling scheme for large stretches of coastline has only been used for intertidal seaweeds (Baardseth, 1970). Aerial photos assist the resolution of these methods (Pielou, 1981). Volume to biomass correlation and eyeball estimates eliminate some survey steps, but calibration steps are required (Pielou, 1981). Subtidal surveys present greater logistical problems, particularly in regard to positioning. Marking systems were used to randomize the selection of samples in a one-half hectare area of *Chondrus* beds, but the time was great to set up each area (Semple and Pringle, unpub. data). If a high degree of correlation of biomass with a physical factor can be found, then these typical values can be applied extensively. However, the variability is rarely one dimensional and a geographic value based upon samples from a bed or shoal is more accurate.

Absolute values for abundance are the baseline for resource assessment, but are meaningless for determination of sustained yield unless placed in the context of the availability or vulnerability to harvest by the proposed harvesting gear or regime. Factors affecting availability include spatial access, seasonal access and, most critically, the selectivity of the gear and intensity of harvest. The efficiency of a *Chondrus* rake, in terms of density is, 11%; the efficiency of an *Ascophyllum* harvester is 20 to 95% in terms of biomass density (Sharp *et al.*, 1986; Sharp, 1987a). Distribution of biomass, relative to height above the substratum, is relevant to calculation of exploitation rates if the characteristics of harvesting technology are known. If mechanical harvesters are used, such as the dragrakes of western Prince Edward Island, their catch-per-unit-effort should be calibrated to biomass. Pringle (unpub. data), found this to be a suitable and rapid technique.

3. Resource Yields: (How much can be taken?)

The answer to how much can be taken requires the integration of biological data with the objectives of the resource manager. Productivity measured directly as successive levels of standing crop must also be translated to yield under a specific harvesting regime. Indirect measures of productivity as a function of growth and recruitment component, minus losses due to whole plant mortality, fragmentation and extracellular exudates are possible with most seaweeds.

Growth

Species such as *Ascophyllum* may be given a minimum age, but early growth (first two years) must be monitored by tagging and measurement of some change in weight, length or branching. Increments of length or branching can be misleading if not correlated with weight. Changes in length may be linear but weight changes are frequently exponential (Bhattacharya, 1985). For example, the *Chondrus* growth form adds growing points in an exponential function with length, which results in similar biomass increment (Sharp, 1987b). *Laminaria longicruris* has a meristem located at the juncture of the stipe and blade. Monitoring movement of tissue from this point (the "hole-punch" method of Parke,

1948) will give a gross growth measure only, as tissue erodes from the distal end at varying rates with wave exposure (Gérard and Mann, 1979). As well, conversion of this measure to biomass cannot be assumed as a direct length-to-weight relationship. Changes in blade thickness and width are more accurately portrayed by biomass models which relect the changes in growth along the blade and erosion at the tip (Gendron, 1985). Growth pattern may vary dramatically, nutrient regimes, for example, can affect seasonality of blade formation and erosion (Chapman and Gagné, 1980). *Ascophyllum nodosum* has both apical and lateral meristems; hence the development of lateral and basal shoots. This combined with stimulation of lateralization when fronds are truncated, requires shoot classification prior to converting linear measurements to biomass (Cousens, 1984).

Mortality

Natural mortality rates of marine plants can be empirically obtained, which is a distinct advantage over finfish stock assessment analysis. Successive sampling of population structure or monitoring survival rates of tagged cohorts is possible. However, the definition of mortality as "deaths from all causes" (Ricker, 1975), is inadequate for marine plants. First, it is not always possible to define the organism as a unit and holdfasts can remain following the loss of uprights. Furthermore, it may regenerate new shoots.

Reproduction/Recruitment

Vegetative reproduction can be the dominant means of recovery in harvested populations. *Chondrus* populations regenerate from holdfast material. Annual harvests are largely dependent on this form of recruitment, particularly where hard granite substratum permits better holdfast adherence, and hence a better chance for retention during the harvesting process. Recruitment from spore settlement to a harvestable frond size requires about 3 to 4 years (Pringle and Semple, 1980). Intensely dragraked beds can contain "barren" areas of prime substratum of 1.2% to 21.1% of total bed area (Pringle and Semple, 1984). Sexual reproduction is required to repopulate these areas (Chopin *et al.*, 1988). Total plant removal occurs in the harvest of bladed *Laminaria*, therefore, recruitment is dependent upon spore production. Spore rain, successful settlement and germination have proved to be functions of residual adult plant density and/or spore sources within dispersal range (Chapman, 1984). The rate of spore release from seaweeds can be high. Both spore phases of *Chondrus* can deliver more than 10^6 spores m^{-2} mo^{-1} (Bhattacharya, 1985). A *Laminaria longicruris* frond produces up to nine million microscopic fronds m^{-2}, but only one reaches recruitment size (Chapman, 1984). Despite this high level of reproduction, reproductive capacity can be further reduced by intense harvesting. A reduction in the largest *Laminaria* frond-size class reduces spore rain and directly the recruitment of macroscopic plants (Chapman, 1984). There is greater year-round carposporangial reproductive capacity in non-dragraked beds of Gulf of St. Lawrence *Chondrus crispus* than in an intensely dragraked bed (Chopin *et al.*, 1988).

Population sampling of macroscopic stages, or culturing of settled stages, can be used to fully quantify the seasonality of recruitment and the success of each stage leading to recruitment. The peak yield per recruit (see below) is one of two harvest management strategies.

Ideally, harvesters will crop only those fronds that have attained peak size; to take any others will result in yield overharvesting (see below). Pringle and Semple (1988) demonstrated the marked change in *Chondrus* size class structure with intense harvesting. They also demonstrated that, although the *Chondrus* dragrake tends to remove larger fronds, there is yet a large number of suboptimal fronds in the harvest (Pringle *et al.*, 1987). Thus, the lower the selectivity of the harvest tool, the more improbable that optimal yield-per-recruit will be attained.

Overharvesting

Fisheries science defines two forms of overharvesting - recruitment and yield. The first occurs when exploitation rates are at a level where reproductive capacity has been reduced to a point where recruitment does not sustain the fishery at the level deemed optimal by the resource manager. Recruitment overharvesting is most difficult to demonstrate. Although it is likely the prime cause of most fisheries collapses it has rarely been shown. The reasons for difficulty in demonstrating recruitment overharvesting are the myriad of factors impinging on the prerecruit between the time of formation (spore production in the case of seaweeds) and time of recruit to the fishery. What one is ultimately attempting to do is relate recruitment level as biomass, to past stock reproductive effort (to show a stock-recruit relationship).

Chopin *et al.*, (1988) carefully calculated spore production in two western Prince Edward Island Irish moss beds; one had undergone years of intense harvest pressure, the other with no recent history of exploitation. There was significantly less reproductive capacity in the former than in the latter. However, they failed to demonstrate the level of spore production required to maintain optimal recruitment to the fishery. Consequently, the biological advice to the resource manager could not state explicitly that recruitment overharvesting had occurred (Chopin *et al.*, 1987).

Yield overharvesting, due to exploitation, occurs when yield-per-recruit decreases below that level defined to be optimal by the resource manager. Yield overharvesting is much easier to demonstrate than recruitment overharvesting, and hence is much more frequently implicated in fisheries advice to resource managers. A fisheries science research team, over a period of years, monitored an intensely dragrak-harvested bed, on western Prince Edward Island. They demonstrated high harvest intensity (Pringle and Semple, 1984) and low harvest yield (Pringle *et al.*, 1987). They failed, however, to assess a density dependency in growth, which is an important factor in yield-per-recruit analysis. Thus again, advice (Pringle and Semple, 1987) to the resource manager could not be explicit. Although yield overharvesting appeared obvious, it had not been demonstrated and a further experiment is required.

Prior to undertaking a program of biological research in support of resource management, it is most important that the researcher understand the management agencies' approach to resource management. Once defined, the research program can then be designed to yield the quantitative data required by the most appropriate model. Aquatic research is difficult and expensive. The "shotgun" approach as advocated by Pringle (1976) cannot be trusted to yield the data required "down-the-road", to fulfill the management strategy and the ultimate model's requirement. The first step should be model definition. Research is then carried out in a systematic way to meet the data requirements of the model. We are not aware of this having been done for a seaweed resource.

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8

Economics of Seaweed Culture: Projection for the Northeast U.S.

Kimon T. Bird*
Division of Applied Biology
Harbor Branch Oceanographic Institution
5600 Old Dixie Highway
Ft. Pierce, FL 34946

Abstract

Commercialization of seaweed culture in the Northeast can occur only if the technologies can produce crops at competitive costs for sufficient markets to warrant investment. Two crops already have potential as food crops in the Northeast, *Chondrus crispus* and *Laminaria* spp. *Porphyra* (nori) could provide a third crop. Cost economics of crop production generally point to the need to target high value crops such as food rather than seaweed as a chemical feedstock such as production of hydrocolloids. Strategies for more cost effective seaweed cultivation include multiple crop production or higher value uses.

Introduction

Seaweed cultivation is widely practiced in many parts of the world for production of food, livestock feed, and sources of chemicals such as the hydrocolloids. The overall market for seaweed appears to be on the order of $1-2 billion (Jensen, 1979). Parts of the market such as agarose are growing in use. In the United

*Current address: Center for Marine Science Research, Univ. of North Carolina at Wilmington, Wilmington, NC 28403

States, *Gracilaria* cultivation provides "ogo" which is widely eaten in Hawaii. Some production of *Gracilaria* occurs in Florida to provide plants for the marine aquarium market or a small but developing restaurant garnish market. On the West U.S. Coast, kelp (*Macrocystis*) bed restoration activities are being undertaken to provide kelp as a chemical feedstock for alginates. Also, a number of nori farms have started in Puget Sound. In the northeast region, the largest known operation is Acadia Sea Farms in Nova Scotia, Canada, which grows *Chondrus* for carrageenan.

The growing interest in seaweed culture in the Northeast U.S. may be in part a reflection of successful aquaculture with clams, mussels, and salmon. However, commercial success will require more than technology development. Production costs must be competitive and markets of sufficient size to warrant interest. The economic returns must also be attractive enough to induce investors to consider these crops rather than others.

Procedures

Two algal genera, *Chondrus* and *Laminaria*, are already commercially exploited in the Northeast or other parts of the world. The success of nori farming in Puget Sound also suggests that this crop might be suitable for cultivation in the Northeast. Dr. Tom Mumford, who helped initiate nori culture in Puget Sound, has suggested that eastern Long Island would be highly suitable for these operations. On this basis, these three crops were selected for making production cost estimates.

These estimates were based on published analyses and projected for the Northeast, with suitable modifications where appropriate. For nori culture, the market and economic analysis conducted for the Puget Sound area was used (Anon., 1982). Cost economics for *Laminaria* culture was made by substituting the costs of commercial Japanese *Laminaria* farm structures for the *Porphyra* culture system (Bird, 1987). The intensive cultivation system was based on analyses by Huguenin (1976), Ryther *et al.* (1984), and Guerin (1986) which included a number of modifications based on improvements to previous culture systems (Bidwell *et al.*, 1985). Revenues were based on "average" prices quoted by wholesalers, or where noted, the wholesaler's price. Many of these quotations were confidential, hence sources are not noted. Production estimates were reduced by 5% to account for shrinkage. Profitability is based on the discounted cost flow method (IRR). The production costs were estimated using the capital recovery method (Mayer, 1978). Taxes were based on the 1987 United States Tax Code for either individuals or corporations, using the calculations which led to the least taxes. No allowance was made for state taxes. Fringe benefits for both salaried and hourly workers were set at 20%. No allowance was made for working capital, and it was assumed that the investment was at 100% equity. First year net income was assumed to be zero, and project life 20 years.

Results and Discussion

Projected revenues for different crops vary based on use as food versus chemical feedstocks. Prices vary from \$6 to \$40 kg^{-1} (dry weight) as a food, to \$300 to \$3000 t^{-1} (\$0.30 to \$3 kg^{-1}) for chemical feedstocks (Table 1). The market for food quality crops in the U.S. appears to be in the order of 247,000 kg, which at a price of \$20 kg^{-1} is ca. \$5 million. The Northeastern U.S. market is around 49,000 kg or approximately \$1 million per year. This market is sufficient to consider seaweed culture for food (Anon., 1982).

Table 1. Projected prices for seaweed crops.

		Food (\$ kg^{-1})	Chemical feedstock \$ t^{-1}
Laminaria	(Kombu)	6-30	400
Porphyra	(Nori)	40	----
Chondrus/Gracilaria	(Sea moss)	10-30	500-1500

Nori and kombu culture are obvious candidates. It might be possible to target *Gracilaria* or *Chondrus* for sea moss production which is widely used in Caribbean type drinks.

Porphyra

The basic unit for nori production is the saku, a system of nets seeded with nori and placed in the water column. Production of nori requires a hatchery for the conchocelis stage, a field based nursery, and production areas. These have been described in detail for the Puget Sound culture systems. Mechanical harvesting systems already exist, as well as the drying and processing systems (Anon., 1982). Key assumptions are noted in Table 2.

Production costs were made at several economics of scale. Detailed costs are shown in the appendix. Costs are based on a single product firm and on the basis of determining an economic profit (charges made on all capital goods).

Table 2. Production assumptions for nori culture.

500 sheets/net/harvest	200 d harvest cycle, Oct. 1 to June 30.
7 nets/saku	
12 saku/acre	3 harvests/net
10 day rotating harvest cycle	40 d growth/net

The 200 and 300 saku systems, assumed to be owned by single operators show a slight economy of scale (Table 3). The 100 saku farm is below the economics of scale (see Anon., 1982). Increasing scale to 2000 saku had little effect on per unit production costs. Production costs ranged from \$24-\$27 kg^{-1} and the internal rates of return yielded 30% to 40%.

Table 3. Cost economics of nori culture.

Size (saku)	200	300	2,000
Capital	298,000	348,000	2,208,000
O&M	112,000	148,000	1,070,000
Depreciation	42,000	58,000	287,000
$/kg @ 10%	27	24	24
IRR %	29	40	38
Net Revenues	46,000	81,000	547,000

It is clear from the analyses that nori culture had economic potential as a successful seaweed aquaculture crop. The internal rate of return is acceptable and the high value of nori makes it attractive in generating sufficient cash flow to warrant investment.

Major questions need to be resolved in terms of its feasibility for the Northeastern U.S. These include annual production rates, an appropriate cultivar, and the infrastructure concerns such as marketing, wholesaling, distribution, and so forth.

Laminaria

Another popular edible seaweed is kombu. Analysis of a kombu system was performed using the same costs as those for *Porphyra*, with the exception of the production system. Production costs were made using a figure of $54,340 ha provided by Ishikawajima - Harima Heavy Industries (Bird, 1987). It was assumed for this system that a mechnical harvester of identical cost and performance to the *Porphyra* system would be available. Production figures of 12 dry metric tons ha-y^{-1}, were assumed based on previous yield experiments in Long Island Sound (Brinkhuis *et al.*, 1987). For a *Laminaria* culture system, there was a considerable economy of scale, with production costs dropping from $31 kg^{-1} to $28 kg^{-1} when scale was increased from 0.4 ha (single farmer) to 4 ha for a larger firm (Table 4). It was assumed that a larger firm could act as a wholesaler rather than selling to one, hence it could receive $30 kg^{-1}.

A cooperative approach (coop) was also investigated for the *Laminaria* system. In this case, 10 farmers would work together and pay themselves a salary, but have no hourly workers. They would run a 4 ha operation. Under this system, each farmer would have his own 0.4 ha farm, but other resources would be pooled, and costs/investments allocated equally. This system resulted in the lowest production costs and may explain why so much of Japanese kombu culture is done through cooperatives.

Table 4. Production costs for *Laminaria*.

Size (ha)	0.4	4.0	Co-op*
Capital	231,900	1,892,800	154,000
O&M	111,800	1,069,890	71,290
Depreciation	27,700	216,800	21,680
$/kg @ 10%	31	28	20
IRR%	0	16	37
Net Revenue	0	91,300	36,500

*Based on costs per individual farmer

The *Laminaria* system is an excellent basis for performing sensitivity analyses. In these cases an increase/decrease in some factor is assumed and its associated impacts on costs are modified. These data can then be used to determine improvements in cost performance. Such analyses can help prioritize research. As an example, improvements in *Laminaria* yield were assumed possible. Such yield improvements would lead to increased harvesting costs due to the greater amount of material handled, but have only small effects on capital costs. A doubling of *Laminaria* yields provides a net revenue for even small farms, and points to the significance given to improving crop production rates (Table 5).

Table 5. Effects of increasing *Laminaria* yield on a 0.4 ha farm.

Yield (t $ha\text{-}y^{-1}$)	Net Revenue
12	0
18	$47,000
24	$71,000

It is clear from these analyses, however, that *Laminaria* culture in the Northeast U.S. for alginate production is unfeasible. The costs of bulk alginate weed is $0.40 kg^{-1}, far below the production costs. While this analysis did not investigate operations with large economics of scale, it is doubtful that corporations would undertake such operations given the availability of kelps from other sources.

Intensive Cultivation for Chondrus *and* Gracilaria

By far, less is known about the costs of intensive air suspension culture systems than other seaweed culture technologies. Depending on assumptions, estimates range from $200-4000 t^{-1} (Ryther *et al.*, 1984; Huguenin, 1976). A more recent analysis of a small 0.45 ha operation suggested costs of $2000 t^{-1} (Guerin, 1986). Some improvements based on economics of scale are possible, but in general, a range of production costs from $1500 to $2700 t^{-1} at production levels of 30 to 34 t $ha\text{-}y^{-1}$ seem reasonable, with land costs factored in at $100,000 ha^{-1}.

These costs are economically feasible for higher value products such as sea moss or restaurant garnishes. They may represent economically feasible systems for production of strains which produce high value agaroses. It must be emphasized, however, that these higher value agaroses are priced to recover both higher raw material cost based on scarcity *and* on high investments in quality control.

The current *Chondrus* operations in Nova Scotia are financially profitable due to several proprietary factors which favorably impact that facility. Other operations would have to find favorable situations in order to make financial profits. While this may seem unrealistic, it could range from facilities which receive revenue for waste-water treatment or as additional revenue streams at power plants which own land and pump seawater.

In general, production of algae as a source of chemical feed-stock does not look promising in the Northeast. The low costs of these raw materials from less developed countries that enjoy cheap labor make it difficult to compete for these high volume, low priced markets. It is conceivably possible that production of high priced, low volume specialty products might be possible, especially when these products have a labile nature such as pigments or lectins.

It appears that there is a reasonable certainty that algal cultivation in the Northeastern U.S. could provide high value crops. The food crops should be a priority as the market for them appears reasonable. These crops could be produced by single product firms, but attention should also be given to developing multicrop systems. It may be possible to place nori farms over bottom planted clam farms and produce two crops without significantly increasing overall costs. Seaweed crops would provide an annual cost flow while clams are going through their 3-5 year growth cycle. Other high value uses of seaweed would also facilitate aquaculture. Seaweed should be investigated for their role in marine/wetland habitat restoration, as well as their ability to detoxify pollutants. Strains with improved detoxification activities could be sold for habitat restoration and water treatment. Seaweeds may also be grown as feeds for other aquacultured organisms. More emphasis needs to be placed on obtaining such high value uses for marine algae.

Acknowledgments

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Appendix

Costs For Nori Culture

Size (Saku)	200	300		2000	
Capital					
Building	63000	63000	$35/sq ft	390000	$26/sq ft
Site	10000	10000	0.5 acre	150000	1 acre waterfront
Utilities	3150	3150		19500	5% of building
Mechanical	9450	9450		58500	20% of building
Motor pool	5000	5000	1 used truck	12000	2 trucks, fork lift
Cold storage	0	0		360000	
Seawater System	0	0		50000	
Subtotal	90600	90600		1040000	

Farming Equipment			
Hatchery	1050	1050	19900
Production	87460	137470	533690
Harvesting	11520	11520	88150
Processing	92820	92820	420500
Subtotal	192850	242860	1062240
Installation	14100	14100	106230
Total Equipment	297550	347560	2208470
Annual costs			
Fixed			
Salaries + 20%	28880	28800	108000
Bottom lease	16000	24000	160000
Acct. & legal	1200	1200	18000
Utilities & truck	720	720	1800
Transportation	3960	3960	7920
Utilities-bldg.	840	840	7050
Insurance	600	600	60000
Fixed annual total	52200	60120	362770
Variable			
Labor	38020	53210	491520
Dryer oil	5940	8910	55880
Boat fuel	2910	7020	29140
Electricity-equip.	450	390	2480
Water-sewer	1410	2090	18000
Packaging	3600	5400	14100
Cold storage	1270	1910	36000
Miscellaneous	6000	9000	60000
Total var. costs	59600	87930	707120
Depreciation	42300	58000	286910
Total operating costs	154100	206050	1356800
Revenues			
Sheets	2000000	3000000	20000000
$0.115/sheet	230000	345000	2300000
5% shrinkage	218500	327750	2185000
Pretax profit	64400	121700	828200
Net income after taxes	45642	80882 indiv. tax	546612 corporate tax

9

The Future of Seaweed Utilization and Aquaculture: Summary of Panel Discussion

Clayton A. Penniman
Jackson Estuarine Laboratory
University of New Hampshire
Durham, New Hampshire 03824

Introduction

On the last day of the symposium, a panel discussion by government and industry representatives addressed the "Future of Seaweed Utilization and Aquaculture." The panel participants included, Kimon Bird, Director of the Division of Applied Biology, Harbor Branch Oceanographic Institution (Ft. Pierce, Florida), Gary Schulte, Research Chemist, Immunology Group, Pfizer Pharmaceuticals (Groton, Connecticut); Dimitri Stancioff, Senior Research Scientist, Phycocolloid Products Group, Marine Colloids Division of FMC Corporation (Rockland, Maine), and Robert Wildman, Director, National Sea Grant College Program (Washington, D.C.). Clayton Penniman acted as moderator for the panel discussion. Each of the participants submitted a short summary of his/her views on the future prospects for seaweed utilization and aquaculture. The following are these summary statements.

Panelists' Statements

Dimitri Stancioff, Marine Colloids Division, FMC Corporation

The following is an outline of some of the carrageenan industry areas of interest.

1) Taxonomy.

Do we need to know more? Yes. There is great confusion in naming and separating many species of the Gigartinaceae especially in that large complex commercially known as *"Gigartina radula."*

2) Physiology and biochemistry - composition.

What is the role of carrageenan in plant biology? What is the composition of insoluble materials in red seaweeds? Is carrageenan bound to these? Structure and composition affect the biology of the living plant, but also affect industrial processing. Tissue of plants containing lambda carrageenan appears to be held together by a different mechanism than that of kappa-plants, K^+/Na^+ ratio varies with species. K^+ is especially high in tropical species.

3) Chemistry.

Is there need for more chemical structure studies? Yes, these should be continued but should also be correlated with physical properties. Extracts of gametophytes and sporophytes should be studied separately. One should use polysaccharide structure, and chemical composition for classification.

4) Raw material improvement and increased resources.

Quality, yield. How can quality be preserved? Large quality losses occur during harvest and storage. These could be bacterial, autolytic, oxidative temperature and moisture sensitive. What mechanisms are involved? What are the kinetics? How does weather affect the harvest? Year-to-year variations in crop yield and quality occur. Light, temperature, fertility, etc. correlation. Can they be correlated with weather?

Can harvest be timed for maximum quality and yield? Carrageenan viscosity and yield from *Chondrus crispus* improves as the plant matures and also correlates with high light intensity, low fertility and low nitrogen in the plant. Is this also the case with other seaweeds? Is there a best harvest time?

5) Cultivation.

Which direction should it take? Improved growth rate and yield through strain selection, hybridization or tissue culture and cloning by protoplast fusion. Which is most likely to be successful? When?

Is quality lost when growth rate and yield are improved? It is rumored that quality of cultivated weed is not as good as wild harvest. Is this physiological or due to plant age or poor drying? Cultivation sites, harvest time, techniques need optimization.

Gary Schulte, Pfizer Pharmaceuticals

Currently, most drug therapies for infectious and chronic diseases in humans are orally administered. A large percentage of these drugs are small organic compounds obtained either by chemical synthesis or by isolation from natural sources. The influence of World War II on the successful development and use of natural penicillins for previously life- threatening infections profoundly changed the pharmaceutical industry. Development of the penicillins required improvements in fermentation of microorganisms and recovery of biologically active compounds. The newer fermentation technology led pharmaceutical companies to screen soil microorganisms as potential new sources for useful drugs. The screening, however, was limited to antibacterial and antifungal agents because of the general simplicity of the bioassays. Most pharmaceutical companies can now screen 1,000 to 7,000 samples per week for these activities. However, since the early 1950s, an increasing proportion of drug research is being carried out in chronic disease areas such as diabetes, arthritis, cancer, and others. Until recently, bioassays for these areas were often too complicated and sensitive to screen crude fermentation broths at a useful capacity. As a result, synthetic chemistry has been the primary route for discovering drugs in these disease areas; Advances in molecular biology, biochemistry, and handling automation allow some chronic disease assays to be configured for fermentation screening. New screens will help identify new leads for drug research in chronic disease areas, but to increase the success for this approach, companies need to expand and diversify their natural products sources. This expansion is already taking place in some pharmaceutical companies. Marine organisms represent an area which pharmaceutical companies are eager to investigate. Micro- and macroalgae are especially appealing because of the species diversity and known richness of secondary metabolites. An added benefit is that algal culturing also provides a consistent source of natural products if biological activity is found. Since most companies are inexperienced in algal culturing, close collaborations with other researchers are essential. Once these collaborations are established, I believe marine natural products, and especially those isolated from algae, will have a significant impact on drug research.

Kimon Bird, Harbor Branch Oceanographic Institution

Increased economic utilization and cultivation of seaweeds requires some re-evaluation by phycologists and marine resource planners in terms of commercial strategies. Too often, wrong decisions are made due to lack of economic or market information. For example, it might be readily concluded that seaweed cultivation for food is infeasible due to competition from the Far East or lack of markets. However, experience in both Hawaii and Washington has shown a sufficient cultural diversity exists in Oriental or health food markets that fresh, local seaweed production finds a ready market. These same market characteristics are also available in the Northeast U.S., with its large Oriental populations or health-conscious consumers. They represent a substantial "niche" market. On the other hand, arguments for seaweed as a chemical feedstock (hydrocolloids) often ignore the fragmentary nature of the U.S. market, lack of U.S. processors, and very high U.S. labor costs (where one hour of minimum wage pays for a full man-day in other countries).

The U.S. is a highly value-added society, and such a society has high wages. The key to economic seaweed production is to develop or target markets with the highest possible prices. These include food (especially specialty foods) and live products. Live products, such as aquarium plants, plants used for wastewater treatment, sources of high value labile biochemicals, or feeds for other organisms may find the fastest niches, as it would be more difficult to import these live plants into the U.S.

Also, seaweed should be regarded in possible product mixes. They may find uses and perform as revenue streams in multiple product businesses such as polyculture. These may be small revenue streams; yet if they can be readily marketed, they represent improved profits. Examples might include sale of seaweed resulting from their use in wastewater treatment from aquaculture facilities such as fish farms.

The economic importance of seaweeds as a diffuse resource should also be considered. Artificial reefs planted with seaweed may be more attractive diving spots. If it is possible to establish causal links between certain algal species and successful fish/invertebrate recruitment of fisheries species, then deliberate introduction of these species on artificial reefs may be warranted. Such analyses could support an economic rationale for more seaweed production (and research).

The major task facing scientists interested in improving economic utilization of seaweed is to recognize that we have had too narrow an interpretation as to what seaweed can contribute economically. Phycologists need to concentrate on new value added uses for seaweed, not just the current state of commercial products. In addition, scientists need to elaborate on the indirect role that seaweed contributes to regional economics such as improved fisheries or recreational activities.

Robert D. Wildman, National Sea Grant Program

Sea Grant has supported research and demonstration programs on seaweed culture and utilization for nearly 20 years. Much of that work has been directed toward developing the information and techniques needed to grow and use the attached seaweeds which serve as raw material sources for the phycocolloids agar, algin, and carrageenan. A lesser amount of research was conducted on macroalgae used as human food, and, more recently, on marine flowering plants. In our view, seaweed research in the U.S. in the 1990's must recognize several developments that have taken place in the past 10 years.

• Raw material supplies for U.S. seaweed processors rely increasingly on imports, primarily for economic reasons.

• U.S. scientists have developed or adapted techniques for growing nearly all genera of commercially important marine macroalgae.

• Other uses of marine algae are being identified which may lead to large-scale cultivation operations in the U.S. in the 1990's.

• Submerged and emergent vascular plants are being recognized increasingly as valuable for a variety of purposes most of which require their cultivation.

• The marine biomass programs demonstrated that algae could be used as energy sources in systems that are approaching economic feasibility. However, the present situation of low price and availability of proven energy sources coupled with the shortage of R&D funds has curtailed this effort.

Thus, it would appear from the Sea Grant perspective that:

• Our past involvement with phycologists and seaweed culture will become broader; i.e., more one of involvement with marine botanists/agronomists and macrophyte culture.

• Microalgae and their culture may become of increasing importance.

• Marine botanists will benefit from joint research with other scientists such as chemists, pharmacologists, soil scientists, ecologists, etc.

• Opportunities for developing and using biotechnological tools will deserve emphasis due to their long-term, high-payoff potential.

Summary

While most applied phycological research around the Atlantic has been directed at seaweeds producing phycocolloids, e.g., carrageenan, agar, algin, there are few "success stories" in its aquaculture. At least within the United States, many of the impediments to successful seaweed aquaculture are institutional, as well as economic. Governmental restrictions on water-based aquaculture operations in the coastal zone are not limited to seaweed ventures. Furthermore, the high costs of coastal land generally precludes on-shore aquaculture operations.

As described above by Kimon Bird, it is necessary for phycologists to diversify their end-goals of applied research. The increasing ethnic and health food markets in the Northeast provide potential opportunities for seaweed aquaculture for direct consumption. Examples of positive applications of research to seaweed aquaculture include *Porphyra* aquaculture in the Pacific Northwest, as well as *Laminaria* and *Alaria* described by Kain *et al.* (this volume). Other uses for aquacultured seaweeds include marine aquaria plants (Bird, this volume). Thus, a diversification of the goals of applied phycological research will provide greater potential for success (both in terms of frequency and time frame). The ability of applied phycological research, i.e., seaweed aquaculture, to produce marketable products is critical to both the short-term expansion and maintenance of funding support, as well as longer-term research expansion.

While raw materials for carrageenan and agar production currently arise from both natural harvest and aquaculture, no extensive seaweed culture for phycocolloids exists around the North Atlantic. However, as described by Craigie (this volume), the production of novel or specific phycocolloids by aquaculture is nearing economic success in the Canadian Maritimes.

Phycocolloids are not the only seaweed chemicals that have potential for commercial exploitation. As described above by Gary Schulte there is great promise for macroalgae to become sources of new pharmaceuticals. Indeed, lectins from *Codium fragile* are already commercially available.

Other potential commercial uses of seaweeds include biomass to produce methane, as sinks for sewage-borne nutrients, or as food for other commercially aquacultured species (e.g., urchins or nudibranchs).

A major conclusion of the two-day symposium is that the future for seaweed aquaculture and utilization, and associated research, is a bright one. Diversification of the goals for applied seaweed research is an important step towards renewing and enhancing the vitality of the field. Research support from federal (i.e., Sea Grant) as well as industrial and entrepreneurial sources will continue to determine the primary directions of applied phycological research. However, initiatives to incorporate innovative techniques, particularly from agricultural biotechnology (Cheney, this volume) may allow seaweed aquaculture to leap-frog the slow, steady advance of terrestrial agricultural methods. Furthermore, as described by Dimitri Stancioff, there is still a substantial need by the phycocolloid industry for "basic" seaweed research. It should be emphasized that many of the successes in seaweed utilization have come about only because basic research provided the necessary knowledge of macroalgal ecology, biochemistry, physiology, or genetics.

The Connecticut Sea Grant College Program

This volume represents the Proceedings of an international symposium sponsored by the Connecticut Sea Grant Program on October 4-5, 1988, at the University of Connecticut Avery Point Campus.

The Connecticut Sea Grant College Program is a partnership between the National Sea Grant College Program of the National Oceanic and Atmospheric Administration and The University of Connecticut, supported by both federal and State funds. It is one of 30 university-based programs along the East, West, and Gulf coasts and the shores of the Great Lakes. Each program serves national and local constituencies through educational programs. The federal program, established in 1966, is modeled after the Land Grant College Programs of 1862.

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