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The MIT/Marine Industry Collegium
Opportunity Brief #24

Progress in Controlled Environment Aquaculture and Algae Husbandry

A Project of
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The MIT/Marine Industry Collegium

PROGRESS IN CONTROLLED ENVIRONMENT AQUACULTURE AND ALGAE HUSBANDRY

Opportunity Brief #24

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PREFACE

This Opportunity Brief and the accompanying Workshop held on March 25, 1981 were presented as part of the MIT/Marine Industry Collegium program, which is supported by the NOAA Office of Sea Grant, by MIT and by the more than 100 corporations and government agencies who are members of the Collegium.

Through Opportunity Briefs, Workshops, Symposia, and other interactions the Collegium provides a means for technology transfer among academia, industry and government for mutual profit. For more information, contact the Marine Industry Advisory Services, MIT Sea Grant, at 617-253-4434.

The underlying studies at the University of Delaware at Lewes were carried out under the leadership of Professor Ellis Bolton and Professor Garry Pruder, and while I drew heavily from their work I remain responsible for the conclusions presented herein and any errors which may appear.

Norman Doelling

July 1, 1981

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1.0 Business Perspective

This Brief summarizes two food-related topics being investigated at the University of Delaware: closed cycle aquaculture systems for growing oysters, and the potential for domesticating plants which grow in brackish water for commercial sources of fodder, grain or seed crops. In both areas results indicate that industrial involvement could be timely and profitable. Attendees at the Collegium Workshop (March 26, 1981 at Lewes, Delaware) will hear more detailed presentations on these topics and visit the University of Delaware's Otis H. Smith Laboratory - a well equipped, modern mariculture/halophyte facility with extensive research capabilities.

The basic system for growing oysters, summarized in an earlier Opportunity Brief (Closed-Cycle Aquaculture, May 1977), has been implemented on a pilot scale of operation. While the goal of economically raising oysters to full, marketable maturity remains unmet, the system may have significant value in growing seed oysters to sizes of up to about one cm. Current prices of seed oysters suggest that profitable business opportunity exists.

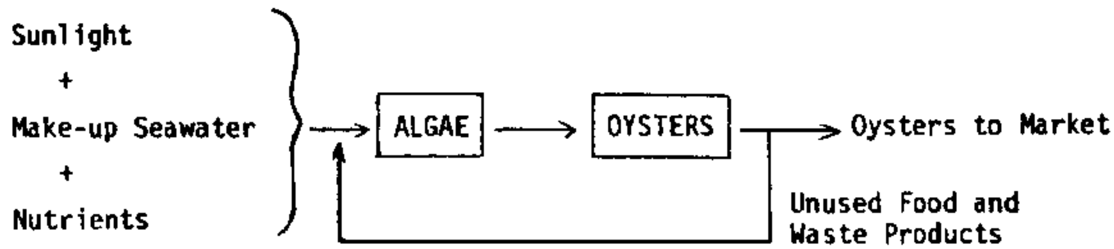
Of perhaps greater significance are the advances in understanding algae husbandry. Since the algae is grown to feed the oysters, these advances are, in effect, a by-product of the study. Algae is a potentially interesting food-stuff or source of organic chemicals because its high efficiency in photosynthetic conversion is many times that of common land-based plants. Delaware researchers have conducted important experiments that demonstrate success in increasing algal yield, sustaining its growth, and manipulating the physical/chemical characteristics.

Delaware researchers are also interested in domesticating naturally occuring salt tolerant plants, halophytes, as supplements to agricultural food crops for humans or animals. In many coastal areas of the world, fresh water supplies are not available for irrigation. The domestication of halophytes could conceivably open these arid regions to agriculture using sea water. In some of the most productive farm land in the United States, salt has accumulated through years of irrigation with water containing trace amounts of salt. This build-up is already reducing productivity and may ultimately destroy the agricultural value of the land. Development of salt tolerant crops could restore the productivity, as well as opening vast new areas for agriculture.

2.0 Mollusc Mariculture and a Hatchery Nursery Plan

2.1 General Outline

The basic concept for a closed-cycle oyster or clam aquaculture closed-cycle aquaculture system is depicted below.



Through photosynthesis, nutrients and the sun's energy are converted to algae which are fed to the oysters. The water returned to the algae block contains unused food and waste products of the oysters. Some of the oyster wastes, such as nitrogen, carbon dioxide, and phosphorous, are nutrients for the algal culture. Recycling keeps nutrient costs down, enhances the capacity of the oysters to utilize a larger fraction of the nutritional inputs, and reduces the amount of energy needed to heat water.

The prototype system is of course much more complex. The growth system is outlined in schematic form in Figure 1. This figure shows some of the hydraulic flows that take place among the several algae production systems (the 1200 liter tubes

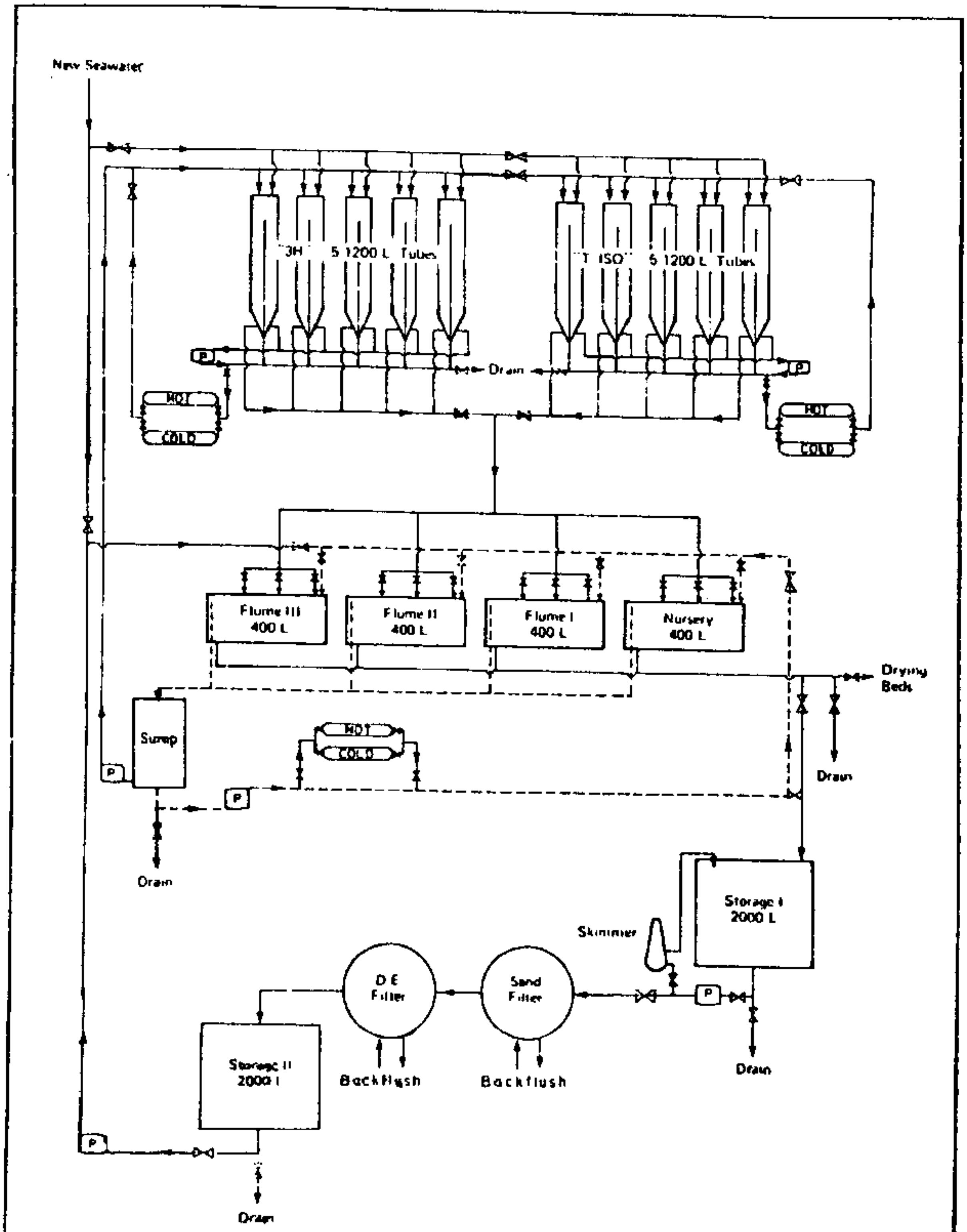


Figure 1. Schematic of prototype system.

shown at the top), the flumes in which the oysters are fed and grown (in the middle), and the filtering and temperature control systems.

In addition, algae seed supplies must be maintained for axenic (single species) cultures and a hatchery must provide brood stock to spawn sexually mature adults, to rear larvae, and carry out setting and growth of juveniles.

2.2 Facilities

The Otis H. Smith Laboratory began full operations in February 1980 when the sea water system was installed. The funding came from the Economic Development Administration, the University of Delaware and private benefactors. The laboratory was designed to accommodate intensive controlled mariculture and includes research laboratories for salt-tolerant plant investigations. A 6,000 square foot greenhouse to house the shellfish prototype has also been erected.

The building, exclusive of greenhouses, covers 11,000 square feet. One wing houses algae culturing, hatchery, chemical and microbiological service activities, a wet laboratory, offices, and conference and reception areas while the other wing includes a common room and research laboratories. In addition to the usual pH and specific ion meters, fluorimeters, spectrophotometers, balances and centrifuges, the laboratory equipment includes a Perkin Elmer Carbon-hydrogen-nitrogen analyzer, atomic absorption equipment, and walk-in programmable environmental chambers, one of which provides humidity control.

The design provides for area and room environmental control by a heating-ventilating-air-conditioning system that takes advantage of the local ground water thermal energy mass through eighteen locally programmable heat recovery (heat pump) devices. Future solar or geothermal energy sources and sinks can also be readily incorporated into the laboratory energy balance scheme. Sea water from the Indian River, Delaware inlet, will be supplied by a fiberglass tank truck. Heat exchangers and chillers are available to provide each service room or laboratory with hot, cold, and ambient sea water under pressure from a building head tank. Greenhouses can be supplied with building sea water and also through independent systems.

Space is available for more than 12,000 additional square feet of plastic greenhouse with or without interior partitioning. A mobile laboratory port and dock connects to the research wing, so specialized laboratories can be equipped for land or sea field operations. Traffic patterns and interior fenestration allow visitors to view the operations on educational and demonstration tours, while maintaining privacy for research and preserving sanitation necessary to the culture of marine organisms.

2.3 Algae Seed Supply

The algae laboratory culture facility provides single-celled marine algae for the hatchery, for research needs, and for stock to seed mass cultures. Axenic cultures for experimentation can be provided. The algae supply is systematically maintained though continuous subculture in nutrient supplemented sea water. Mass culture of the diatom Thalassiosira pseudonana and the flagellate Isochrysis galbana for broodstock maintenance and conditioning and for hatchery production is carried out in tubular reactors, illuminated by fluorescent lights in a temperature controlled room. Seed algae for the prototype can be supplied from this facility on a regular schedule and to meet research needs.

2.3.1 Algae Supply Procedure

The laboratory maintains axenic cultures of 24 isolates representing 16 marine algal species of interest to researchers and industrial concerns. Three parallel axenic lines are maintained for each algal species in use at the facility. These axenic cultures are maintained in a clean room with controlled access and constant temperature. The clean room also contains equipment to isolate new algal strains and reisolate contaminated cultures.

Algae for seeding larger vessels are supplied from the clean room. These large cultures are used, in turn, as seed for the greenhouse algal reactors, and as a food source for broodstock, hatchery and research needs. Algae intended for seed cultures are batch harvested, while algae for direct use as food are maintained as semi-continuous cultures. The latter cultures are not axenic; however, strict sanitation procedures are followed to lessen possible contamination problems.

2.4 Bivalve Hatchery

The bivalve hatchery maintains and conditions broodstock, spawns sexually mature adults, rears larvae, carries out setting and grows juveniles for entry into the prototype and for research and other development activities. The hatchery operation is routine, reliable and generally trouble-free.

The principal function of the hatchery is to supply seed oysters (*Crussostrea virginica*) to the prototype system on a regular monthly schedule. Larvae and juveniles are also supplied for research and the hatchery can spawn and rear larvae and juveniles of other bivalve species, especially the clam *Mercenaria mercenaria*.

2.4.1 Hatchery Procedures

The principal resident brood stock consists of adult oysters (*Crassostrea virginica*) obtained from Delaware's Broadkill River, yearling oysters, descendants of Delaware Bay, stocks which have reached sexual maturity in 16-20 weeks after setting in the laboratory; and a group of the northern quahog (*Mecenaria mercenaria*). Other broodstock, including other bivalve species, can be introduced according to need.

Broodstock are conditioned at $18^{\circ} \pm 2^{\circ}$ and fed a two-part algal diet consisting of *Thalassiosira pseudonana* (3H) and *Isochrysis* , Tahitian strain ("T-ISO"), twice daily.

Conditioned broodstock are temperature-shocked (27-29°C) to induce spawning. When necessary, stripped sperm are added to further stimulate the broodstock animals to spawn. Larvae are reared in 400 l conical tanks held at 25-28°C and fed a two-part diet of T.pseudonana (3H) and either Isochrysis (T-ISO) or Isochrysis galbana (ISO). Mature pediveliger oyster larvae are then set. Mylar sheets are used as the setting substrate for small groups of experimental animals, while crushed oyster shell of approximately 2-4 mm in size is used for larger groups. Both methods produce individual cultchless oyster spat, which are fed the two-part diet (3H/T-ISO or ISO) and achieve a mean shell height of approximately 10 mm 4 to 5 weeks after setting. These seed oysters are then moved to growout systems to be raised to adult size. Juveniles and larvae are made available to graduate students as well as other intra- and inter-university cooperative research efforts as part of hatchery services.

The basic system is now in operation in the new Otis H. Smith Laboratory. The shellfish life support subsystem is operating satisfactorily and the algae production subsystem has been in continuous operation for many months. Thalassiosira pseudonana (3H) and Isochrysis galbana (T-ISO) are grown in fiberglass reinforced polyester rigid wall tubes, 3 m by 76 cm in diameter, which transmit about 85% of the ambient solar radiation. They are connected through a manifold to a single circulation pump that provides mixing within and between the tubes and drives venturis mounted at the base of each tube for gas injection. The venturis introduce nitrogen to help purge oxygen from the medium. The oxygen problems will be discussed at more length during the Workshop.

Since our last Workshop at Delaware (MIT Sea Grant Report No 77-15), the economic problems of raising oysters on a purely algal diet have become better defined. While the goal of growing oysters on a commercially viable basis has not been met, considerable progress in spawning and raising juvenile oysters has been made using Delaware's techniques and algal diets.

A primary reason for the high cost is that the oysters consume more algae as they grow larger, and also require more space in the flumes. Four approaches are being pursued to make the total mariculture operation more cost effective:

1. Efforts are being devoted to reduce cost through automation, elimination or alteration of ineffective process steps and/or selection of less costly components;

2. All aspects of mollusc nutrition are being carefully studied to find less costly, more biologically efficient feeds to augment the algae diets;

3. Algae husbandry is being studied to raise yields, stability and nutrition of the algae while lowering costs;

4. We are continuing the techniques to grow oysters to an optimum size for sale as seed.

Many factors are involved in the fourth approach. However, current prices of seed oysters in dollars per pound suggest that large (5mm or perhaps even 1 cm) oysters might prove to be saleable at a few hundred dollars per pound.

Whether the market would bear the same or higher prices for larger 1 or 2 cm juveniles is pure speculation. However, if survival rates were shown to improve with size, a market niche may very well be found.

3.0 Mass Cultivation of Algae: Implications of Environmentally Induced Unbalanced Experimental Growth

3.1 Rationale

University of Delaware researchers estimate that more than 80% of oyster mariculture system costs are related to raising the diatom *Thalassiosira pseudonana* clone 3H and the flagellate *Isochrysis galbana* (T-ISO)to feed the oysters. Such photosynthetic single-cell proteins are the main foods for filter-feeding bivalve molluscs. The photosynthetic efficiencies of converting sunlight into stored food energy can reach 4%, compared to between 1/10th of 1% and 1% for the most intensely cultivated land crops. Thus the implied potential for algae husbandry is very high and is one motivation for continuing research. If such photosynthetic efficiencies can be obtained continuously in very large systems, a real contribution might be made to some of the world's food problems.

It is comparatively simple to sustain high rates of photsynthesis provided that cell densities are comparatively low. However, real progress in algal husbandry depends upon maintaining high rates of photosynthesis at very high cell densities. In the case of algae being fed to oysters, for example, high density means less water has to be treated, heated, stored, and circulated to provide a fixed amount of nutrients to a given amount of oysters. Also, more oysters can be raised in a given space. Thus higher densities of algae culture imply lower costs.

3.2 The Economic Problem

Algal feed materials are presently projected, with considerable uncertainty, to cost 3 or 4 times as much as non-photosynthetic single-cell feeds. With the latter estimated at about $800 per ton, the implied price of algae feed becomes roughly $3,000 per ton. At the same time agriculturally based formulated feeds for non-filter feeding organisms, such as shrimp and lobster, cost about $300 per ton or only 10% as much. These figures provide a strong motivation to lower the feed cost. It might be possible to supplement algae with modified shrimp and lobster feeds; however, relatively little is known about the nutritional needs of oysters.

3.3 Research Advances and New Directions for Delaware Research

Algae cultivation at high photosynthetic rates has frequently resulted in unstable algal cultures that precipitously die off or "crash." Environmental conditions such as temperature, pH and carbon dioxide concentration, and light intensity are all suspected of contributing to unbalanced growth. This growth evidences itself in a variety of ways: algal cell size change does not occur in a predictable manner through succeeding generations; cell division rates decrease in successive batch sub-cultures; some species survive but not others; and a variety of other phenomena including "crashes."

These problems occur in ponds as well as laboratory cultures.

Thalassiosira Pseudonana, an important food for shellfish, is one of the most studied marine algal species. Despite considerable progress, the practical mass culture of this species has not proven to be simple. This species, like single-celled algae in general, apparently cannot respond biochemically to coordinate all its biosynthetic activities at the same rate when environmental conditions change beyond certain, unknown limits.

One major contribution at the University of Delaware has been to provide very important insights into the relationships among environmental variables and the resulting growth rate for algal cultures.

Unbalanced growth can be illustrated by considering the growth phase of a batch culture where cell numbers increase exponentially. This condition is mathematically expressed as follows:

$$CC = CC_o \, e^{(k_{cc}t)}$$

where CC (or PC or PN) is the number of cells (or the Particulate Carbon or Particulate Nitrogen content, respectively) at time "t"

CC_o (or PC_o or PN_o) is the number of cells (or the amount of cellular carbon or nitrogen) at time zero

k_{cc} (or k_{pc}, or k_{pn}) is the specific cell division (or net carbon or net nitrogen depostion) rate constant

t is elapsed time

As cell numbers (CC) increase there is generally a corresponding increase in cell mass (PC, PN), including cell wall structure, cytoplasm, chloroplasts, etc. It is obvious that for cell growth to continue on an indefinite basis, the <u>rate</u> of change of these many components must equal the <u>rate</u> of change in cell number. Particulate carbon (PC) and particulate nitrogen (PN) are useful approximations of cell mass. Balanced or steady-state growth is a special case where:

$$k_{cc} = k_{pc} = k_{pn} = k ....$$

Research at the University of Delaware has shown that algal cultures do not in general meet this condition. In fact

$$k_{cc} = k_{pc} = k_{pn} = k ....$$

except under certain favorable combinations of environmental conditions.

When the various rate constants (whatever biosynthesized component is measured, providing it is not lost from the system) are not equal, a condition of unbalanced growth may exist. Examples of unbalanced growth of <u>Thalassiosira pseudonana</u> are shown in Figure 1 for two O_2 concentrations at $25^{o}C$. At a low light intensity (350 uW/cm^{-2}) and high O_2 concentrations (1250 umole/l) the cells divide nearly 1.4 times more rapidly than they deposit carbon into particulate form. Clearly such a condition cannot be sustained long before the cells become deficient in carbon. It can also be seen in Figure 1 that only at a low O_2 level (15 umole/l) and moderate light intensity (1500uW/cm^{-2}) are cell division and carbon deposition rates in balance.

The results discussed above are simply the results of a typical set of experiments. A large array of experiments involved different species of algae grown under a very wide range of experimental conditions with carefully controlled light intensity, temperature, carbon dioxide, pH, and other environmental variables. These unpublished results have been submitted for peer review and will be discussed in some detail at the Workshop. While the results cannot be considered entirely complete, they do suggest the following important implications for algae husbandry.

First, controlling a range of environmental variables creates conditions in which rapid quasi-balanced growth can take place for a specific algal species but not for other species. Thus, manipulating environmental variables can encourage a single species while discouraging "weed" species.

Second, the growth environment might be able to "design" the properties of a final end product. For example, to reduce the size of the individual algae one could select conditions that would tend to maximize the rate of cell division (k_{cc}) while minimizing the rate of cellular carbon deposition (k_{pc}). Alternatively engineering an environment that minimizes k_{cc} while maximizing k_{pc} might encourage large algae cells. These particular proposals to change cell size may be entirely incorrect. However, the potential to control algae properties by manipulating environmental variables is strongly suggested by experimental results.

Dr. Pruder will report on these experiments and discuss the possible general implications for algae husbandry in detail at the Workshop.

4.0 Domestication of Halophyte Plants

In many areas of the world the soil quality is capable of supporting intensive cultivation of food crops, but supplies of fresh water are inadequate. Some of this land lies adjacent to estuaries or sea shores where virtually unlimited amounts of brackish or salt water are available. If salt tolerant plants (halophytes) edible by humans or animals could be cultivated, such land could be put into agriculture to alleviate growing food shortages. Researchers at the University of Delaware have begun an investigation of the technical prospects to identify and develop halophytic plant species suitable for cultivation as food crops using saline water for irrigation. A 1974 conference of specialists in the development of new crop species and halophytic plants recommended that:

- Identification and development of salt tolerant plants for human or animal food was a worthwhile research objective and should be pursued.
- The objective was achievable in a reasonable amount of time.
- The program should focus on the study of existing salt-tolerant plant species, rather than attempt to develop salt tolerance in plants now cultivated with fresh water.

- The research program should identify species which tolerate moderate salinity as well as full strength seawater.

A number of plants now cultivated with fresh water were originally developed from salt water plants, including beetroot, asparagus, radish and cabbage. Also, many halophytic plants were once utilized as foods by humans. Eelgrass (Zostera marina) yields seeds containing 13% protein, 50.9% starch and 1% crude fats which were dried and ground into flour by the Seri Indians on the Gulf of California. Seed harvesting was from wild species, never from cultivated plants.

To date, more than 100 lots of 50 species of seed from locations all over the world have been collected for the initial screening experiments. Each was tested in the laboratory and subsequently in the field for germination performance.

From this first round of initial screening tests, three candidates were selected for more intensive investigation:

Cordgrass (Spartina alterniflora)

Orach (Atriplex patula, var. hastata (L.) Gray)

Seashore mallow (Kosteletskya virginica)

Several other species are still under investigation, but with somewhat lesser intensity. In addition, new samples are being obtained from locations all over the world and are being submitted to the preliminary screening trials.

Testing of the three primary candidates mentioned above is now in progress. Each candidate is grown in outdoor plots in sufficient volume to provide preliminary data on growth rate, seed productivity, nutritive value and resistance to disease and pests. These test plots also provide the opportunity to improve the strain by selecting seed stock from plants showing above average growth or tolerance to salinity.

These evaluations have given considerable information on plant growth performance variations associated with environmental conditions. For example, cordgrass grew best in areas where standing water was present almost continuously; orach grew poorly under similar conditions, since this plant required well drained soil.

The current in-depth screening of halophytic plants measures plant performance against the following standards:

- Does it grow naturally and vigorously in a saline habitat?
- Does it produce abundant seeds for new plantings as well as possible food or forage for domestic animals?
- Are the seeds large enough for easy harvesting?
- Does the plant show promise as a human or animal foodstuff and is its nutritive value attractive?
- Can the plant be intensively cultivated to produce good yields?

The final answers to all these questions are not expected in the near future. However, those plants which fall short in screening trials will be passed over in favor of more promising candidates. The ultimate answer on the plant's yield under intensive cultivation will require the cooperative effort of a commercial seed producer and agriculturist who can provide the manpower and facilities required to obtain a definitive answer to this question. Cordgrass (Spartina alterniflora) has produced up to 6.5 tons per acre in the wild. Though this yield compares poorly to alfalfa, which yields 13-14 tons/acre under intensive cultivation, it is probable that a combination of selective breeding, fertilization and pest control could produce harvests approaching that for alfalfa.

5.0 Additional Reading

The Proceedings of the annual meeting of the World Mariculture Society from 1975 to date contain additional references. The early MIT Sea Grant Opportunity Brief #11 may be of interest as well as the extensive Pruder and Bolton papers listed below.

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6.0 Appendix

MIT/Marine Industry Collegium

Workshop #24

PROGRESS IN CONTROLLED ENVIRONMENT AQUACULTURE
AND ALGAE HUSBANDRY

March 25, 1981

Virden Conference Center
University of Delaware Marine Studies Campus
Lewes, Delaware

10:00 Welcoming Remarks

Norman Doelling

10:10 Overview of Marine Research at the College of Marine Studies

Dean W.S. Gaither

11:00 Mollusc Mariculture and Proposed Program for a Hatchery/Nursery Plant

Professor E. Bolton

12:00 Lunch

1:00 Mass Cultivation of Algae: Implications of Environmentally Induced Experimental Growth

Professor G. Pruder

2:00 Domestication of Halophytic Plants as Human Foods or Animal Forage

Professor J. Gallagher

3:00 Tour of the Otis B. Smith Mariculture Lab

3:45 Departure for Georgetown Airport (for those arriving by air)