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CULTIVATION OF MACROSCOPIC MARINE ALGAE AND FRESHWATER AQUATIC WEEDS

Progress Report

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NOTICE

Abstract

Studies were continued during 1977-78 on the growth and yields in culture of the red seaweed Gracilaria tikvahiae. Partial control of epiphytes was achieved by nutrient removal, shading, and/or biological agents. For the first time, a single clone of the alga was grown continuously throughout the year without replacement. Yields in large (2600 l) aluminum tanks averaged 21.4 g dry weight/ m^2.day, equivalent to 31 tons/acre.year (15.5 ash-free dry wt tons/acre.year).

Growth of Gracilaria and other seaweeds in Vexar-mesh baskets in natural habitats and in the oceanic waters of a power plant cooling water intake canal were unsuccessful. Further studies were also conducted to determine the cause of the positive relationship between water exchange rate and growth of the seaweed, but results were inconclusive.

Productivity of the freshwater macrophytes Lemna minor (common duckweed), Eichhornia crassipes (water hyacinth), and Hydrilla verticillata have now been measured throughout the year with mean yields of 3.7, 24.2 and 4.2 g dry weight/m^2.day (5.4, 35.3, and 6.1 dry tons/acre.year) respectively. Yields of duckweed and water hyacinths in the Harbor Branch Foundation culture units have averaged roughly three times those of the same species growing in highly-eutrophic natural environments.

The yields of several other species of freshwater plants were investigated. Only the pennywort (Hydrocotyle umbellata) appears to approach the productivity of water hyacinth on the basis of preliminary measurements. Studies on the growth and water loss of duckweed, water hyacinth and Hydrilla in stagnant cultures have been initiated.

Chopped water hyacinths and unprocessed Gracilaria have both been successfully fermented to methane in anaerobic digesters and the liquid digester residues recycled to produce more of the same plants. A preliminary budget for recycled nitrogen has been determined for water hyacinths.

Productivity of both water hyacinths and Gracilaria has been calculated from nitrate-nitrogen assimilation and good agreement with measured yields was obtained.

Commercial Gracilaria culture in Taiwan and kelp culture in the People's Republic of China was observed by the Principal Investigator and yield data for both industries were obtained.

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I. Summary

The present report was prepared mid-way through the third year of research under D.O.E. Contract EY-76-S-02-2948, conducted at the Harbor Branch Foundation, Ft. Pierce, Florida, on the growth and biomass yields of macroscopic marine algae and freshwater weeds.

Over 40 species of marine algae (seaweeds) indigenous to Florida coastal waters were tested and evaluated for their growth potential in artificial culture systems. Of these, the most successful and suitable species to date has been *Gracilaria tikvahiae* (formerly *G. foliifera*), a macroscopic red alga.

Growth of *G. tikvahiae* in small (50 l), intensively-operated culture systems, with strong aeration and over 20 culture-volume exchanges per day of enriched seawater, resulted in biomass yields throughout the year that averaged 34.8 grams dry wt/m^2.day (equivalent to 127 dry metric tons/hectare.year, about half of which is organic). Yields were found to be directly proportional to seawater exchange rate, between one and 30 culture volumes/day, for reasons that are still not clear, but that are apparently not related to nutrient or CO_2 limitation at the slower exchange rates. Maximum yields occur at relatively low nutrient concentrations, 10-100 μmoles N/l as NO_3^- or NH_4^+ and 1-10 μmoles/l $PO_4^{\equiv}$-P together with essential trace metals, and a starting seaweed density of 2-4 kg wet weight/m^2 culture surface

area, harvested back to that density every one to two weeks. Growth of Gracilaria occurred throughout the year at Ft. Pierce, Florida, with a maximum mean weekly yield of 46 g dry wt/m^2.day at the end of July and a minimum of 12 g/m^2.day in late January, in a year when the water temperature in the culture fell to 12°C.

During the first two years, epiphytization, the overgrowth of the cultured seaweed with undesirable species of filamentous marine algae, was the major problem in the cultivation of seaweeds. That problem necessitated the periodic discard of the cultures when they became seriously infested, and their replacement with newly-collected material - an expedient that was clearly not feasible in any large-scale culture operation.

Various chemical control methods, including enzymes to prevent the attachment of the epiphytes and algicides that might prove selectively toxic to the epiphytes, were tried with limited or no success. The economic cost-benefits of such treatment, even if successful were judged to be unacceptable in any event.

However, limited but adequate epiphyte control was achieved during the past year (1977-78) with a combination of physical-biological control methods consisting of: (1) temporary (5-10 days) shading of the infested seaweed, (2) temporary (5-10 days) cessation of nutrient enrichment, and (3) introduction of the herbivorous snail Costoanachis avara, which feeds selectively on the epiphytes. It has also become apparent that healthy, rapidly-growing seaweeds do not normally become epiphytized, particularly when the seaweeds themselves assimilate all or most of the dissolved nutrients from the water. When growth and nutrient assimilation stops, even temporarily, and the nutrients accumulate in the culture, the growth of epiphytes is encouraged.

It is clear from the observed growth patterns of the seaweeds, as observed over the past 2 1/2 years, that growth is not continuous in these plants, but rather proceeds irregularly, with stops and starts of undetermined frequency. This phenomenon will be investigated further with the objective of understanding and defining the growth periodicity, because nutrient enrichment, water circulation, aeration and other costly operational factors may not only be wasteful during quiescent periods, but they may also encourage growth of epiphytes at such times.

In December, 1977, a specimen of _G. tikvahiae_ was collected from the Indian River near the Harbor Branch Foundation which has subsequently been designated "Oslo Road Clone A" or "ORCA". That clone has since been grown continuously in culture for one complete year, using the epiphyte control methods described above.

In a larger, less energy-intensive culture system than used to obtain the yields described earlier (an aluminum tank of 2.4 m^2 surface area and 2600-liter volume exchanged four times per day), a mean yield over the 12-month growth period of 21 g dry wt/m^2.day (76 dry tons/ha.year or 38 ash-free dry tons/ha.year) was obtained. This has been the first time that a single clone of seaweed has been maintained and grown continuously over an entire year, and this achievement is considered to represent a significant landmark in the seaweed culture efforts of this project.

Attempts have been made, beginning in the summer of 1978, to grow Gracilaria and several other species of seaweeds, including three species of the floating brown alga Sargassum, in floating Vexar-mesh trays suspended in several locations in the Indian River, in the Fort Pierce Inlet which connects the Indian River with the Atlantic Ocean, and in the intake canal of the Florida Light and Power Company's Hutchinson Island nuclear power plant. The latter receives its water from an intake line located 250 feet offshore at a depth of 35 feet in the Atlantic Ocean, and the seawater has a much higher, more constant salinity and more oceanic properties and better water quality in general than the more brackish and relatively polluted Indian River. Furthermore, seawater is pumped through the intake canal at a rate that provides water exchange through the seaweed cultures in the trays of the order of 10,000 times per day. Nutrient concentrations were, however, lower in the intake canal than in the Indian River or in the experimental systems at Harbor Branch Foundation, averaging about 1.0 μmole N/1 and about 0.3 μmole P/1.

Most of the new species inoculated in the trays failed to grow at all. G. tikvahiae and one species of Sargassum (S. filipendula, an estuarine form), grew initially at moderate to high rates (10-25 g/m^2.day) in the Inlet and in the intake canal, but after two to three weeks, growth declined and eventually stopped entirely and the plants became heavily epiphitized and necrotic. Ocean cage culture of these seaweeds without additional nutrient enrichment, even at very rapid water exchange rates, therefore does not appear to be feasible.

The freshwater macrophytes Eichhornia crassipes (water hyacinth), Lemna minor (common duckweed), and Hydrilla verticillata have now been grown for roughly 18 months at Harbor Branch Foundation. These plants are grown in 25,000-liter (30 m^2) PVC-lined earthen ponds and/or in 2.2 x 0.8 x 0.2 m concrete burial vaults through which enriched well water is passed at exchange rates that have ranged from 0.06 to 2.0 culture volumes per day. Water exchange is apparently much less important with the freshwater plants than with the seaweeds. Not only will they tolerate much slower circulation rates in general, but increasing the exchange rate does not improve yields, except for some slight enhancement with the more rapidly-growing water hyacinths.

Initially, only nitrogen and phosphorus were added, at concentrations that varied inversely with the water exchange rates from 100 to 1500 μmoles N/l and from 10 to 150 μmoles P/l. After approximately six months it was found that the most rapidly growing plant cultures (i.e., water hyacinths) became flaccid and chlorotic in appearance, followed by reduction and eventual cessation of growth. Subsequent experiments revealed that these plants required enrichment with nutrients other than N and P, and the culture medium was subsequently supplemented with a commercial trace metal mix[1]. The latter is now used routinely in all freshwater and marine plant culture experiments. Although the addition of trace metals did not affect the growth of the other freshwater plants, that of the water hyacinths significantly increased, so earlier yield data for that species were therefore disregarded and monitoring of the annual growth cycle of water hyacinth was re-started in December, 1977.

Culture experiments with the freshwater plants were similar in most respects to those with the seaweeds except that much less energy-intensive culture methods were used. The cultures were not aerated and, as mentioned above, water was circulated through them much more slowly.

Optimal densities for maximal yields were found to vary from species to species, from about 0.2 kg wet wt/m^2 for duckweed to 20 kg/m^2 for water hyacinth, presumably because the former is essentially a two-dimensional plant which has nowhere to go once it covers the water surface (i.e., at 0.2 kg/m^2), while the hyacinth is three-

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[1]Sunniland Nutri-Spray (Chase and Co., Sanford, Florida 32771) containing 2% Fe, 0.1% Cu, 0.75% Zn, 0.75% Mn, 0.02% B, 0.01% Mo, 1.5% S.

dimensional, growing upward out of the water to a height of as much as one meter above the surface.

As with the seaweeds, the water hyacinth and duckweed cultures were weighed every one-two weeks, depending upon season and growth rate, and the incremental biomass harvested, returning the cultures to the same initial starting densities each time. Both of these plants have been grown in large Vexar-mesh baskets that are immersed in the ponds or vaults. In weighing, the entire basket is hauled out of the pond by means of a vertical hoist to which the spring balance is attached, thereby providing as little physical disturbance as possible to the plants.

Mean annual production of duckweed was 3.7 g dry wt/m^2.day (range 0.1-7.0 g/m^2.day). A few comparative growth studies of _Lemna minor_ and the giant duckweed (_Spirodela polyrhiza_) indicated that yields of the two species were almost identical, so further work with the latter was not pursued. Problems with duckweed, in addition to its relatively low growth rate, were the difficulty in maintaining an even coverage of the small plants in the ponds during heavy winds, at which times they would pile up at the downwind side or be actually blown out of the water (a problem that would be exacerbated in large culture systems), and a tendency for the duckweed cultures to become heavily overgrown with filamentous algae such as _Hydrodictyon_ sp., _Rhizoclonium_ sp., and _Oedogonium_ sp.

Mean annual growth of water hyacinth (over the 11 months since the complete enrichment medium has been used) was 24.2 g dry wt/m^2.day, equivalent to 88 dry tons/ha.year. This plant contains about 17% ash, (in contrast to 50% ash in Gracilaria), so that the ash-free dry weight yield of hyacinths is equivalent to 73 tons/ha.year, somewhat better than that of Gracilaria (63.5 tons/ha.year for small, intensive culture systems).

Both duckweed and water hyacinth have their photosynthetic and gas exchange mechanisms exposed to the air and are not dependent upon the CO_2 dissolved in freshwater, a factor that may limit photosynthesis and growth in many submerged freshwater plants including Hydrilla. In addition, Hydrilla is a rooted plant that grows from meristematic tissue at the plant tip. While it was found that the plant would grow equally well if the root end were simply attached to Vexar screening, it would not grow if completely unattached. The only way to harvest the population, then, was to cut off the growing end some distance below the water surface, a procedure that was found to arrest growth for one to two weeks until new meristematic tissue was regenerated. In practice, this means that a harvested crop of Hydrilla must spend roughly half its time regenerating its growing tips without growing. In the present experiments, Hydrilla was not harvested at all between weighings but merely allowed to accumulate its biomass for the whole season, a practice that may and probably did carry the population well beyond its optimal density for best yield.

The other major problem with Hydrilla grown at the latitude of central Florida is that plants flower in the fall, begin to store organic matter in root tubers, and virtually cease vegetative growth throughout the winter. Our experiments demonstrated that some growth would occur in winter if water temperatures were maintained at an elevated temperature of 25°-30°C, but for practical purposes Hydrilla must be considered as a seasonal crop in most if not all of mainland United States.

The mean annual yield of Hydrilla was 4.2 g dry wt/m^2.day, slightly better than duckweed but far from that of water hyacinths. The low yields and attendant related problems of growing the duckweeds and Hydrilla would appear to eliminate them as viable candidates for a biomass-based energy plantation.

The only other freshwater macrophyte tested to date that would appear to approach water hyacinth as a biomass source is the pennywort (Hydrocotyle umbellata), a floating species that resembles water hyacinth in its growth habit and appearance. Pennywort, grown only over the past five months (July-November) has averaged 15.9 g dry wt/m^2.day during that time. Work will be continued with that species, which may have the advantage of better cold tolerance and better yields in winter than water hyacinths, possibly leading to better year-round productivity. A disadvantage of pennywort is that, as new plants bud off the parent stock, their root systems become inextricably inter-twined and must be cut apart for harvesting, in contrast to water hyacinths which bud off entirely separate new plants.

To determine whether growth of the freshwater plants in the artificial culture systems at Harbor Branch Foundation was at least comparable to that attained by the same species in nature, comparative growth studies were undertaken in the spring of 1978 of populations of water hyacinths and duckweed in well-enriched nearby natural habitats with specimens from the same natural populations transplanted to the Harbor Branch Foundation culture systems. Initially, growth of the two (natural and laboratory) populations were roughly the same for each species, but during summer and fall yields of the natural populations of both duckweed and hyacinths fell to about one-third that observed at the laboratory ponds, presumably due to the lack of controlled enrichment and water exchange in the field. These studies will be continued so as to obtain data for an entire year, but the results obtained to date suggest that production of the freshwater macrophytes observed at the Harbor Branch Foundation culture facility may closely approach the maximum potential for the species.

Good progress has been made during the current year in using nitrate uptake as an index of aquatic plant production. A home-made, continuously recording autoanalyzer measuring nitrate concentrations in the effluent from water hyacinth and <u>Gracilaria</u> cultures, together with known input concentrations, flow rates and elemental composition of the plants, has permitted calculation of ash-free dry weight yields over periods up to one week. These are compared with measured weight increases over the same period of time. Agreement between estimated and measured yields has averaged 88%. Such an indirect production index is considered to be indispensable in any large-scale aquatic biomass farm system, where it will be clearly impractical to weigh entire populations or even representative samples thereof.

With the assistance of an agricultural engineering consultant from the University of Florida (Gainesville), five 125-liter experimental digesters have been constructed and have been in operation since early spring, 1978, for anaerobic fermentation of water hyacinths and Gracilaria. After initial difficulties with the seaweeds (which eventually required anaerobic sediment from a natural seaweed bed as a starter culture), biomass of both species grown at the facility are currently undergoing anaerobic digestion with methane production.

Gas production from the fermenters is being monitored, both qualitatively and quantitatively. However, the main purpose of this work is to investigate the possibility of recycling the digester residues as a nutrient source for growing more of the same species of plants that produced them. Growth of the water hyacinths to date in water enriched with diluted digester residue has been as good as or better than that in the usual enrichment medium. Similar experiments with Gracilaria are still in the preliminary stage. To date, it appears that about 21% of the original nutrient content of hyacinths, following their digestion, is assimilated by new cultures of the same species.

Finally, studies are now in progress to determine the water balance of water hyacinths, duckweed and Hydrilla grown in stagnant cultures in large plastic tubs. Water losses from the three plant cultures are compared with evaporative losses from a control container with no plants. Water losses of all three plant cultures are greater than that of the control, due to evapo-transpiration in the emergent duckweed and water hyacinths. Greater water loss in the Hydrilla culture is probably the result of the plant biomass preventing or retarding convective circulation in that container with the result that the water surface becomes more heated and undergoes more evaporation than does the control.

Growth of all three species in the stagnant tub cultures was initially poor, due probably to inadequate nutrient enrichment, and they were therefore an inadequate test of water loss in healthy growing populations of the plants. That problem has now been corrected and growth rates for the three species appropriate for the time of year are now being attained. The water losses for all three species at their normal growth rates were 1.5 to 2.5 times as great as the controls, losses that are significantly less than those reported in the literature for some of the same species. However, these measurements were made in the fall of 1978 at which time growth was only about half of its summer maximum. These studies will now be continued for an entire year, during which time appropriate meteorological data will be recorded.

During September-October, 1978, the Principal Investigator had the opportunity to visit the People's Republic of China as a member of a U.S. Delegation of Oceanographers. During that visit, kelp (Laminaria japonica) culture was observed in the coastal waters and harbors of the northern Chinese cities of Tsingtao and Dalien. Kelp sporelings (3-5 cm long), reared in hatchery-greenhouses on shore during the summer are set out onto buoyed ropes in the fall, when the water temperature falls below 20°C. The young sporophyte plants, suspended just below the water surface, are hand-brushed to remove epiphytes, transplanted to larger ropes, fertilized daily by broadcasting liquid fertilizer over the kelp beds, and are harvested the following spring, when they reach maturity. Yields in the Tsingtao area are about 30 dry tons/ha.year and in the colder Dalien region, where the growing season is longer, about 50 dry tons/ha.year.

En route to the People's Republic, a stop-over was made in Taiwan, where commercial Gracilaria culture was observed at the southern end of the island, near Tainan. The Gracilaria, reported to be G. verrucosa, is grown in 5-15 hectare, shallow (0.5-1.0 meter) mud-bottom ponds orginally constructed for milkfish culture (now less profitable than seaweed culture). The Gracilaria is grown loosely on the bottom and is harvested by raking and netting, every 10-40 days depending upon the season, eight to ten times a year, each time leaving a seed population for the new growth. The herbivorous milkfish is introduced as needed to control epiphytes, primarily Enteromorpha, but must then be removed before they consume the Gracilaria. Crabs are sometimes grown in the same ponds a a secondary crop. Water is added from the adjacent estuary as needed to control salinity and temperature and to provide nutrients for the Gracilaria and oxygen for the animals. No other fertilization of the ponds is normally done.

At the Gracilaria farms visited, the same population of seaweed had been grown continuously since the practice was started some 5-10 years ago. Yield from one 15 hectare farm is currently some 16 dry tons/ha.year. That from the more than 135 hectares of seaweed ponds in Pingtung County was reported to be 14.8 dry tons/ha. year.

II. Seaweed culture

The major problem in seaweed culture up to the present has been that of ephphytes - undesirable species of filamentous marine algae that overgrow the cultivated plants, ultimately killing them. Pennate diatoms may also grow so heavily on the seaweeds as to smother them, and occasionally fouling animals (epizoans) such as bryophytes may also create serious problems. Several methods have been tried to eliminate or ameliorate this problem with various degrees of success.

Proteolytic enzymes (protease, trypsin, chymotrypsin) used with varying effectiveness in Great Britain to prevent the settling of zoospores of the green alga *Enteromorpha* spp. (one of the more common and troublesome epiphytes in seaweed culture) were not successful. *Gracilaria* plants, soaked for 16 hours in 0.1, 1.0 and 10.0 g/liter solutions of the enzymes and then returned to a running seawater culture system were no less prone to attachment of *Enteromorpha* than were untreated controls.

Experiments were then initiated with various commercial algicides in an attempt to find such a substance that might be selectively toxic to the fouling organism but relatively ineffective and harmless to the cultured seaweed. Various concentrations of chlorine, in the form of sodium hypochlorite, copper sulfate (3-15 ppm), 2-4-D (10-50 ppm), and the commercially available herbicide Aquathol Plus (3-15 ppm), Simazine (3-15 ppm), and Swimtrine (3-15 ppm) were tested.

The procedure was to immerse the seaweeds overnight (ca. 16 hours) in solutions of the algicides, wash them off, and return them to runnig seawater trays.

Of those tested, only Swimtrine, the active ingredient of which is chelated copper, proved to be selectively toxic to Enteromorpha, a 15 ppm concentration killing it completely and eliminating it from cultured Gracilaria. Growth of the latter was also set back by about 36% for two weeks after exposure to the algicide, following which it appeared to recover completely. Because of its limited but significant toxicity to Gracilaria and its high cost (ca. $25/gal), Swimtrine was not considered to be an adequate solution to the epiphyte problem, and other approaches were pursued.

While no final cure to epiphytization has yet been found, several reasonably effective deterrents have been discovered. One is to simply stop the addition of nutrients to the seawater when the epiphytes first begin to appear. Interestingly, this does not affect the growth of the cultivated seaweed for one to two weeks, since Gracilaria has the ability to draw upon stored nutrients and continue normal growth for at least that length of time with little or no dissolved nitrogen or phosphorus in the ambient seawater. Enteromorpha, however, and other, similar opportunistic species that are able to respond quickly to an enriched environment with rapid and prolific growth, are apparently quickly affected by nutrient depletion and may completely disappear if maintained in unenriched seawater for as long as two weeks.

Similarly, Gracilaria is much more resistent to low light intensity and even complete darkness than is Enteromorpha and other fouling algal species. A more drastic measure that arrests the growth of the Gracilaria completely and could incur a considerable capital and labor cost if done on a large scale, dark treatment for one to two weeks does have the advantage of being even more effective than nutrient depletion in controlling epiphytic growth.

Third, biological control methods, used alone or in combination with nutrient depletion and/or darkness, are surprisingly effective in keeping Gracilaria clean of epiphytic algae. Moderate numbers of the small omnivorous snail (Costoanachis avara), of the order of 10-20 animals per kg wet weight of seaweed, keep the latter remarkably clean and free from Enteromorpha, Giffordia (a filamentous brown alga), pennate diatoms and apparently small fouling animals as well, while the Gracilaria itself apparently remains unaffected by the antifouling browsers.

The small snails unfortunately have no commercial value, but larger invertebrates of considerable market value such as conch, penaeid shrimp, and even juvenile spiny lobsters, may prove equally effective as biological antifouling agents and will be assessed in future experiments. In that connection, it may be pointed out that in the highly successful commercial pond culture of Gracilaria in Taiwan, the herbivorous milkfish is used to control Enteromorpha and other epiphytes that grow there on the cultured seaweed.

Finally, with respect to the epiphyte problem, it has gradually become apparent in the course of the past three years of research, that the fouling of the cultured seaweeds is often, perhaps always a secondary affliction that seriously affects seaweeds only when they have already slowed or stopped their growth for some other reason, environmental or internal. Several possible reasons for such a correlation may be conceived. The healthy plants may simply grow too fast to permit the epiphytes to smother them, or they may excrete or coat themselves with a substance that is inhibitory to the growth, attachment, or development of the fouling organisms. Certainly, the slowing or cessation of growth will result in the reduced assimilation of nutrients by the cultured species which, as noted above, encourages the growth of the epiphytes. Again, maintaining ambient nutrient levels to the lowest concentration possible that is consistent with optimal growth of the cultured species appears to be essential to epiphyte control.

A few other simple measures have proven to be effective deterrents to epiphytes. Culture tanks or containers of whatever design should be constructed with a lip several inches wide around the top to prevent the growth of *Enteromorpha* at the air-surface interface, a favorite area of initial invasion and establishment of the green alga. Pennate diatoms and other loosely-attached epiphytes may be easily removed by draining the water and hosing the seaweed for a few minutes with a strong stream of freshwater, another treatment that has no harmful effect on *Gracilaria*.

Prior to 1978, neither Gracilaria nor any other species of seaweed had been grown continuously for more than two-three months without becoming so heavily infested with epiphytes that the cultures had to be discarded and started anew with freshly collected material. Such restocking was recognized as impractical for any large-scale, commercial seaweed farming operation. In December, 1977, a new collection of Gracilaria was made in the Indian River, a short distance from the Harbor Branch Foundation. A single specimen from that collection has since produced a vegetative clone, designated Oslo Road Clone A (ORCA), that has now been maintained in culture for one complete year, using the epiphyte-control methods described above. That accomplishment is considered to be a significant landmark in the seaweed culture efforts of this project.

"ORCA" clone of Gracilaria has now been grown continuously since January, 1978, in four 2600-liter (2.4 m^2) aluminum tanks, each provided with four exchanges of enriched seawater per day (Figure 1). The cultures are maintained in suspension by vigorous aeration from a air manifold extending across the bottom of the tank. All of the seaweed is weighed biweekly and harvested back to a starting density that has ranged from 0.83 to 8.04 and averaged 3.53 kg wet weight/m^2. Average yield during the one year period has been 21.4 g dry wt/m^2.day, equivalent to 78 dry tons/ha.year (31 tons/acre year). Mean weekly yields are shown graphically in Figure 2. Rather close correlation between yield and solar radiation

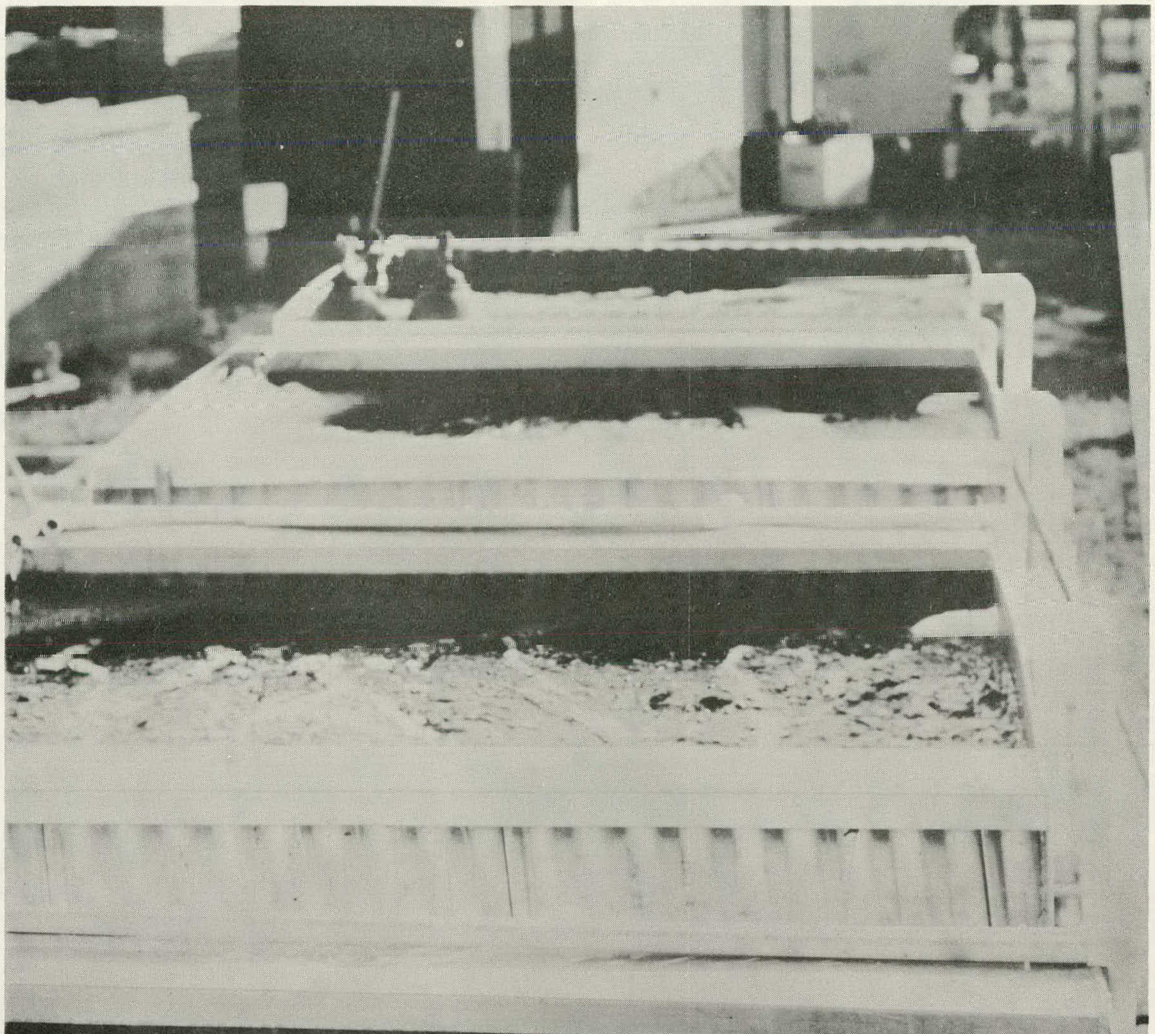

Figure 1. 2600 liter (2.4 m^2) aluminum tanks used to grow Gracilaria tikvahiae ORCA clone.

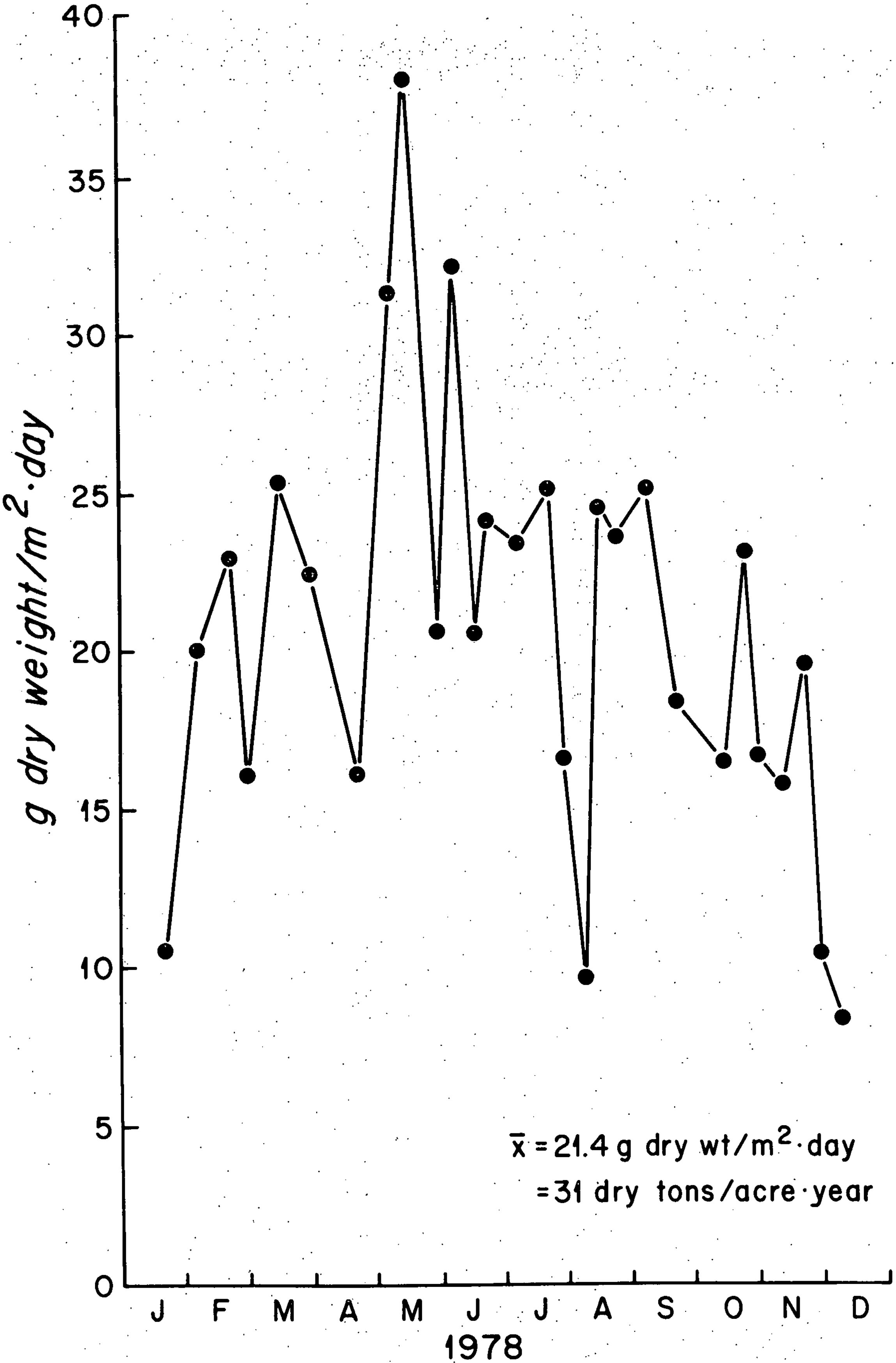

Figure 2. Mean weekly yields of Gracilaria tikvahiae ORCA clone during 1978.

could be seen, particularly with respect to seasonal effects and major peaks and depressions, but there is also variability in the growth of seaweed that could not be attributed to solar radiation or any other known environmental factor. Such growth irregularity creates difficulty with yield prediction as well as operational procedure. As discussed above, excessive enrichment appears to favor the growth of epiphytes, a problem that is exacerbated during the unpredictable periods of slower growth.

Nutrients were initially added at concentrations of 50 μmoles/l NO_3-N, 5 μmoles/l PO_4-P, and 0.1 ml/l trace element mixture[1]. The nitrogen and phosphorus levels were doubled during the period June-September to provide for the anticipated higher summer growth rates. In October it was found that a significant fraction of the nitrogen and phosphorus were not being used. At the same time, it was also noted, in looking at ambient nutrient concentrations of the incoming seawater, that nitrogen and phosphorus levels had gradually increased over the time since the project started to concentration ranging from 6 to 13 μmoles/l NO_3-N and 1-3 μmoles/l PO_4-P. Beginning in October, therefore, no nutrients were added to the seawater passing through the _Gracilaria_ culture, an omission

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[1] Sunniland Nutri-Spray (Chase and Co., Sanford, Florida 32771) containing 2% Fe, 0.1% Cu, 0.75% Zn, 0.75% Mn, 0.02% B, 0.01% Mo, 1.5% S.

that produced no obvious effect on subsequent yields of the seaweed and has reduced the incidence of epiphytization.

It had been found earlier that the growth and yield of Gracilaria is directly proportional to the rate at which seawater is circulated through the culture between one and 30 volume exchanges per day. That is presumably why the mean seaweed yield in the large aluminum tank cultures described above, at four exchanges per day ($21.4\ g/m^2 \cdot day$) was lower than that obtained earlier in the small (50 l) trough cultures flushed at 22 exchanges per day ($34.8\ g/m^2 \cdot day$).

The correlation between growth rate and water exchange was not related to the supply or concentration of either nitrogen or phosphorus. In separate experiments in which volume exchange was varied from one to 30 per day but in which first the concentration and then the daily loading of N and P were held constant, the same linear relationship between growth and water exchange was observed. A possible explanation was that some other essential nutrient was growth limiting and was supplied in the seawater in amounts proportional to the water flow. The same experiments described above were therefore repeated but using the trace element mixture described above (Sunniland Nutri-Spray) in addition to N and P enrichment.

The experiment was carried out in the 50-liter culture units constructed from sectioned 0.4 m diameter PVC pipe (see 1977-78 Progress Report). Gracilaria was stocked at a starting density of 1.21 kg wet wt/m^2 and grown in suspension by aeration. Seawater was circulated through duplicate cultures at exchange rates of 1, 7.5, 15, and 30 volumes/day, but N, P and trace element mix were added separately at the same rate in all the cultures giving concentrations that ranged from 300 μmoles/l N, 30 μmoles P and 0.1 ml/l trace element mix at one exchange/day to 10 μmoles/l N, 1 μmole/l P and 0.003 ml/l trace elements at 30 exchanges per day. Gracilaria was weighed at one-week intervals and harvested back to its initial starting density, the experiment lasting for four weeks. Mean daily yields for the four-week growth period are shown in Table 1. As in the earlier experiments, the more rapid the water exchange, the higher the yield of seaweed. Limitation of trace elements, at least those contained in commercial mix, was therefore apparently not the explanation.

Another possible reason for the enhanced growth with more rapid water exchange was thought to be CO_2 limitation, the more rapid exchange of seawater bringing more dissolved CO_2 to the cultures. Even at one exchange per day, there is sufficient CO_2 normally present in the seawater to provide for a yield of over 20 g dry wt/m^2.day, but at high pH, much of that CO_2 may be unavailable in the form of carbonate. The same experiment as described above was therefore repeated at one and 30 exchanges per day in duplicate cultures with and without aeration with pure, compressed CO_2 at a rate sufficient to decrease the seawater pH in the cultures from the normal daytime range of 8.0 to 9.5 to a level of 6.5-7.5. The added CO_2 provided

Table 1. Effect of culture exchange rate on the yield of _Gracilaria tikvahiae_ with daily input of nitrogen, phosphorus, and trace element mix held constant, and with aeration with pure CO_2.

Water exchange rate (volumes/day)	_Gracilaria_ yield (g dry wt/m^2.day)
1	5.6
7.5	17.5
15	19.8
30	28.7
With CO_2 addition	
1	1.6
30	29.6

no enhancement of growth, depressing the yield at one exchange/day and not significantly affecting that at 30 exchanges/day during the course of a 10-week experiment.

The reason that yields increase with water flow thus remains unsolved. Future experiments will investigate further the possibility that some essential micro-nutrient, limiting in seawater and not present in the commercial trace element mixture, is the answer. The other remaining possibility, of those conceived to date, is that the seaweeds excrete a growth auto-inhibitor that rapid water exchange helps to dissipate. That possibility, too, will be investigated by recirculating the water in a static culture through charcoal and/or other absorbent material to see if that removes the hypothetical growth inhibitor.

During the first two years of the contract, attempts were made to grow Gracilaria and other seaweeds in floating Vexar-mesh baskets suspended in the 25,000 liter (30 m^2) PVC-lined ponds at the Harbor Branch Foundation and at various locations in the Indian River. In all of those experiments there was initial fair to good growth of the seaweeds for the first few weeks, followed by a gradual decline and ultimate death of the plants, usually accompanied by severe epiphytization.

In 1978, the opportunity developed to attempt seaweed culture in the intake canal of the nearby Florida Power and Light Co. Hutchinson Island nuclear power plant. The intake water is drawn from approximately 250 feet offshore in the Atlantic Ocean, at a mean depth of about 35 feet. It is of higher salinity and of genrally much more oceanic and more constant composition than the more brackish and highly variable Indian River seawater. It was felt, therefore, that species of seaweeds could be grown in the intake water that could not be grown in Indian River seawater. Although nutrient concentrations were low (1-3 μmoles NO_3-N/1, 0.1-0.5 μmoles PO_4-P/1) the water flows through the canal rapidly providing a water exchange in the baskets equivalent to some 10,000 times per day or more. Such a flow would appear from earlier experimental results to favor good growth of seaweeds.

Nine species including the ORCA clone of Gracilaria tikvahiae and three species of Sargassum were experimentally grown in PVC-frame, Vexar-mesh basket (Figure 3). Most of the nine species did not grow or survive. Gracilaria grew well (mean 33.5 g/m^2.day) for one month (8/10-9/3) and then died. A second stocking grew at a mean rate of 30.3 g/m^2.day for three weeks (9/22-10/11) and has remained senescent and heavily epiphytized with no significant growth since that time. One species of Sargassum (S. fluitans) grew from 8/24-10/20 at a mean daily rate of 8.9 g/m^2.day. By late October, with the onset of heavy fall winds and much turbulence in the coastal water at the location of the intake pipe, the waters of the intake canal became heavily loaded with suspended sediment and very turbid, a condition that has persited up to the time of this writing. No

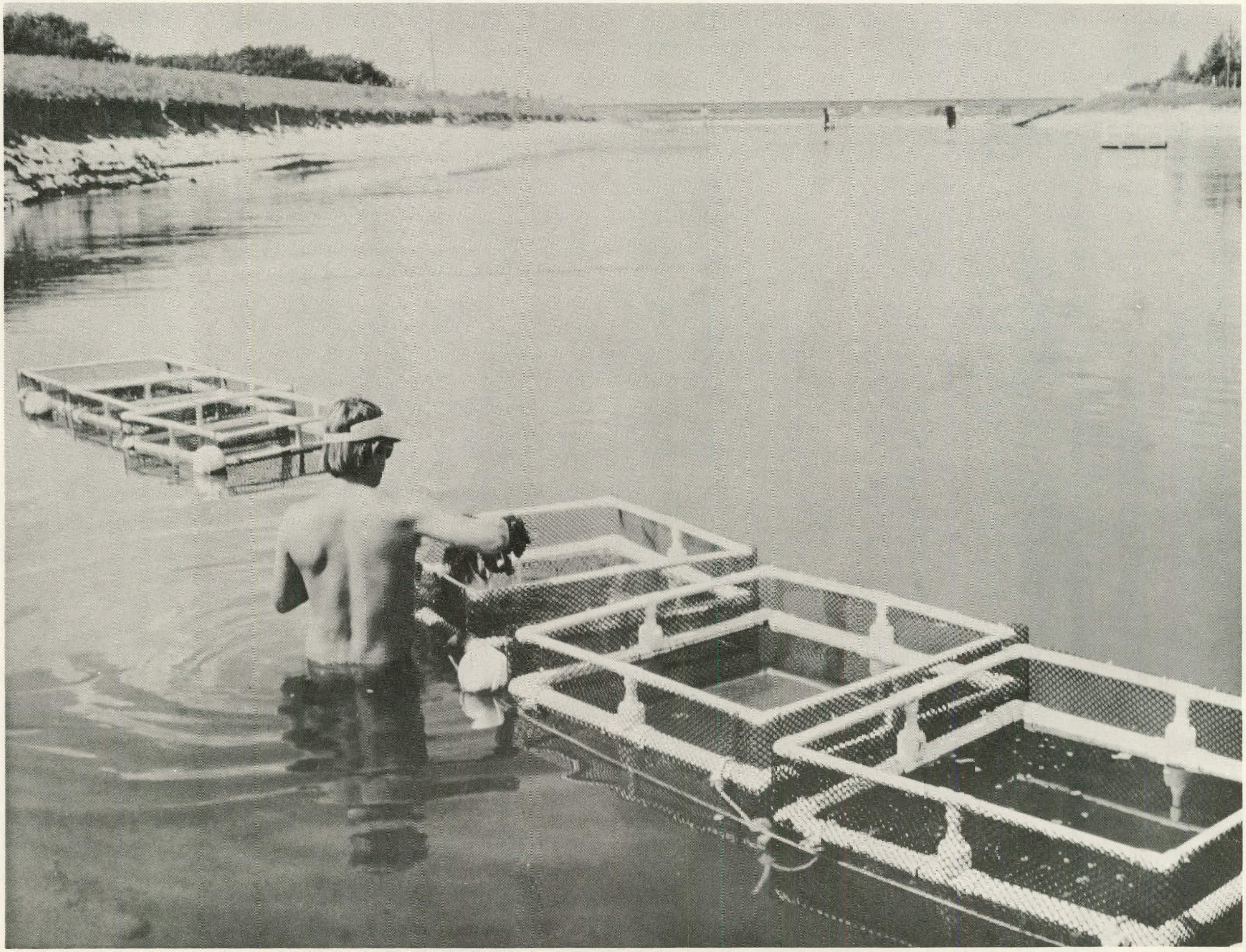

Figure 3. Vexar-mesh baskets used for growing seaweeds in the FPL Hutchinson Island Power Plant intake canal.

significant seaweed growth has occurred in the intake canal since the turbid condition became established. However, even before the turbidity problem became severe, yields of the few species that did grow in the intake canal were erratic and undependable, for reasons that are not understood, and essentually no better than the results of tray culture in the Indian River or at the Harbor Branch Foundation.

Samples of all of the seaweed grown at Harbor Branch Foundation and in the field are routinely analyzed for their percent composition of ash, volatile solids, carbon and nitrogen. Table 2 shows some of these analyses of Gracilaria grown in trays at Harbor Branch Foundation (in ponds), in the Indian River adjacent to the Harbor Branch Foundation, at Fort Pierce Inlet (connecting the Indian River to the Atlantic Ocean approximately six miles south of Harbor Branch Foundation), and in the Florida Light and Power Company's Hutchinson Island nuclear power plant seawater intake canal, which takes its water from the open coastal Atlantic Ocean. The data show remarkable consistency in the composition of the seaweed grown in the same way from all locations. There is, for example, no indication of nitrogen starvation in the seaweed grown in the nutrient-poor and unenriched FPL intake canal water.

There is, however, an interesting trend in all of the cultured seaweed, which tend to increase both their ash and nitrogen content in culture following their collection from wild stocks. This is

Table 2. Mean composition of *Gracilaria tikvahiae* grown in different areas (% of dry weight).

	Ash	Volatile solids	Carbon	Nitrogen	C:N
Harbor Branch Foundation					
5/78-7/78	41.5	58.5	26.9	1.7	16
8/78-11/78	43.9	56.1	24.9	2.7	9
FPL Intake Canal	52.5	47.5	18.8	1.8	10
Fort Pierce Inlet	47.4	52.6	23.2	1.8	13
Indian River (near HBF)	46.8	53.1	23.8	1.7	16

illustrated in Table 3, showing an increase in ash and a corresponding decrease in organic content of about 13% while nitrogen increased by about 25% after three months in culture.

Studies have been underway for the past year to relate quantitatively the uptake of nutrients, specifically nitrate, to the growth of aquatic plants. From the change in concentration of nitrate in the water passing through the cultures, the daily flow of water, and the chemical composition of the plant in question (i.e., % N of total dry weight), it should be possible to calculate total daily dry weight production of plants, if there is no other source or sink of nitrogen in the system. The purpose of making such growth estimates is that routine and automated nutrient analyses would represent a much simpler and more practical method of monitoring growth in a large aquatic plant energy farm than would weighing representative samples of the plants or any other direct method of measuring growth.

More progress has been made in the past year in using the above approach for estimating the growth of water hyacinths than that of seaweeds. A separate chapter of this report is devoted to the water hyacinth growth estimates from nutrient uptake. The techniques and calculations used in the method are described there and will, therefore, not be repeated here.

Nevertheless, some promising progress has also been made in estimating the yield of <u>Gracilaria</u> from nitrate uptake from its medium.

Table 3. Changes in the composition of *Gracilaria tikvahiae* grown in culture at Harbor Branch Foundation (% dry weight).

Days in culture	Ash	Volatile solids	C	N
0	37	63	28	2.3
8	37	63	28	2.5
14	38	62	27	2.0
20	39	61	27	2.4
28	43	57	26	2.8
34	43	57	25	2.7
43	45	55	24	2.8
48	44	56	23	2.9
57	44	56	23	2.8
62	48	52	22	2.7
71	48	52	24	3.2
77	49	51	20	2.9
85	51	49	23	2.9
92	49	51	26	3.0

Table 4 shows growth estimated from nitrate uptake and measured by weight increase for six one-day periods. The problem is complicated by the presence of nitrogen in the seawater in the form of ammonium that is not considered in these estimates. Utilization of that nitrogen source should result in an underestimation of growth from nitrate uptake alone. In fact the reverse happens and estimates from nitrate uptake were, in all but one case, higher than measured growth. With the exception of the first value, however, agreement is quite good, averaging better than 80%.

Table 4. Growth of *Gracilaria tikvahiae* estimated from nitrate uptake and measured by weight increase (g dry wt/m^2.day).

Time	Estimated	Measured	M/E (%)
9/14-9/15/78	49.15	20.05	41
9/26-9/27	25.21	20.05	80
9/27-9/28	23.42	20.05	85
11/7-11/8	16.94	18.80	111
11/21-11/22	18.25	16.17	89
11/30-12/1	23.78	16.17	69

III. Freshwater macrophyte culture

Studies were continued through 1978 on the growth and yields of freshwater macrophytes grown in culture at the Harbor Branch Foundation and of natural stands of several of the same species in nearby habitats. Primary emphasis has been devoted to the water hyacinth (Eichhornia crassipes), the common duckweed (Lemna minor) and the submerged aquatic weed Hydrilla verticillata. Some additional studies were carried out with the giant duckweed (Spirodella polyrhiza), the pennywort (Hydrocotyle umbellata), the water ferns (Azolla caroliniana and Salvinia rotundifolia) and the filamentous algae (Rhizoclonium sp., Hydiodictyon sp., Spirozyra sp., Pithophora kewensis, and Oedogonium sp.

The plants at the Harbor Branch Foundation were grown in 2 types of culture systems: (1) PVC-lined earthen ponds (15,000-20,000 l in volume, roughly 12 x 2.4 x 1.2 m and 30 m^2 in water area), and (2) concrete burial vaults (700-900 l in volume, 2.2 x .8 x .45 m and 1.7 m^2 in water area).

In the initial experiments, secondarily treated sewage effluent enriched with $NaNO_3$ and $NaHPO_4$ was passed through the culture units at varying residence times (0.5 to 16.5 days) and varying concentrations of N (50-150 μm) and P (5-15 μm). However, in the fall of 1977, the water hyacinths became flaccid and chlorotic and it was apparent that the N and P enrichment of the extremely dilute Harbor

Branch Foundation sewage was an insufficient growth medium. Thereafter, the water was also enriched with a commercial trace metal mix[1].

Growth of the duckweeds, Hydrilla and water hyacinths was measured by weighing the wet plants and noting the increase in biomass. Consistent wet weight values were obtained by draining the plants for specific time intervals. For each weighing, a plant sample was removed, weighed, dried and then reweighed, providing a ratio of dry matter in the plant to wet or fresh weight. All productivity measurements were calculated as the product of these ratios and the increase in fresh weight per unit time and area.

Vexar cages ranging in size from 1-2.3 m^2 were filled with water hyacinths and placed in ponds and vaults. Approximately once a week, these cages were weighed by lifting them from the water, using a winch and rope suspended over the culture to which a spring scale was attached. The plants in each cage were allowed to drain for 4 minutes, weighed, and returned to the water (Figure 1).

The duckweeds grew in ponds and vaults with the plants covering the entire surface area. Each week, the plants were netted from the water, placed in a bucket, and then transferred a handful

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[1] Sunniland Nutri-Spray (Chase and Co., Sanford, Florida 32771) containing 2% Fe, 0.1% Cu, 0.75% Zn, 0.75% Mn, 0.02% B, 0.01% Mo, 1.5% S.

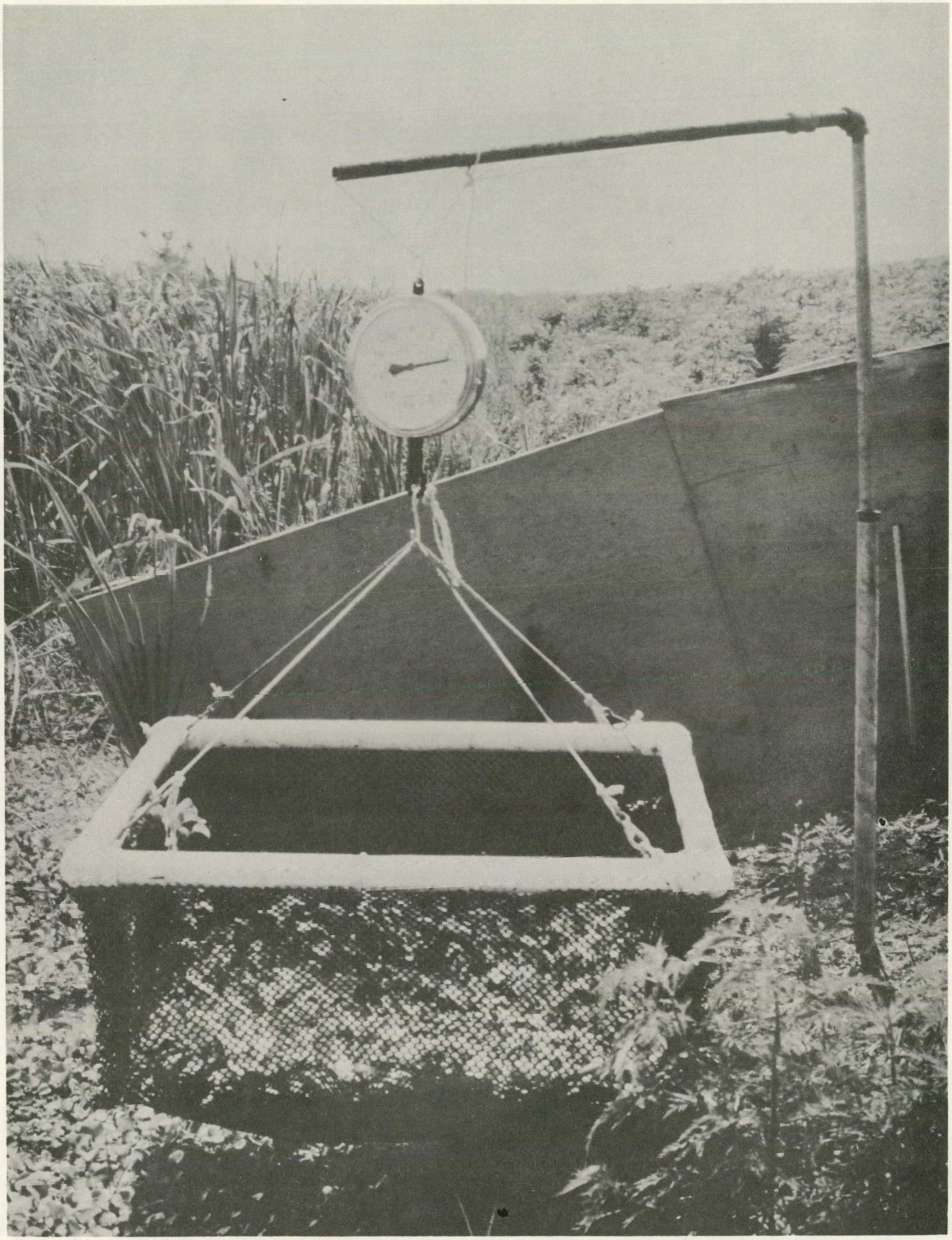

Figure 1. Vexar-mesh basket culture of water hyacinth (*Eichhornia crassipes*) being weighed to determine growth.

at a time into another bucket, allowing the excess water to drain off. The duckweed was then weighed on a balance or spring scale and restocked in the water. Hydrilla in natural stands is found rooted to the bottom, so it initially appeared impossible to weigh the plants without destroying the culture. After unsuccessful attempts at growing Hydrilla in a free-floating fashion, it was discovered that apical sections of the plants would grow when woven through a Vexar-mesh screen, suspended 25 cm above the bottom of the pond (Figure 2). The Vexar screens and attached plants were then periodically removed from the pond and weighed, allowing 10 minutes for the plants to drain.

In the cultures of water hyacinth and duckweeds, it was necessary to determine the densities at which maximum growth and yield occur. At lower densities, all of the incident sunlight is not absorbed by the plants. At excessively high densities, however, self-shading of the plants and other adverse effects of overcrowding occur.

Table 1 contains the results of a series of experiments in which yield of Eichhornia crassipes was measured as a function of starting density of the culture. The data points for October 1977 represent the mean daily productivity obtained from five one-week growth periods, and those for June and July 1978 represent the mean productivity from three one-week growth periods. Yields for October 1977 were significantly lower than those recorded in summer, 1978,

Figure 2. Hydrilla verticillata grown with root ends woven in Vexar mesh screens

Table 1. Effect of culture density on the yield of *Eichhornia crassipes* harvested weekly and maintained at the indicated starting density.

Culture density kg wet wt/m^2	Yield (g dry wt/m^2.day) Oct. 1977*	June 1978**	July 1978**
2	6.0	15.5	
5	9.0	25.7	
7			28.4
8	9.7		
10		32.2	
11	9.0		
12.5			30.7
14	4.1	37.2	
17	4.0	37.9	32.1
19.5			30.4
20		23.3	
22.5			27.9

*Each data point represents the mean of 5 replicates.

**Each data point represents the mean of 3 replicates.

in part due to the insufficient growth medium the plants were receiving at that time as well as seasonal effects. The summer density experiments reveal that optimum yields under these conditions were at starting densities of around 14-17 kg/m^2. Most of the yearly productivity experiments were started prior to this time, however, and thus were conducted at the October optimum of 10 kg/m^2.

Table 2 shows a similar experiment with both the common duckweed (Lemna minor) and the giant duckweed (Spirodela polyrhiza), with each data point representing the mean of five one-week growth periods. The best yields for both of these species were obtained at the stocking density of 0.24 kg wet wt/m^2, a density roughly 60 times lower than the hyacinth optimum.

The possibility that greater yields of Lemna could be achieved if the plants were maintained at a higher starting density and then harvested back more frequently was investigated, as seen in Part I, Table 3. The optimum stocking density for the duckweed when harvested every 3 or 4 days was found to be between 0.25 and 0.5 kg/m^2. Growth at these optimum densities, harvesting both at 3 and 4 day intervals and 7 day intervals, was not significantly different (Part II, Table 3).

Pond cultures of Lemna minor and Eichhornia crassipes have been maintained at Harbor Branch Foundation since May 1977, and Hydrilla has been grown since August 1977. In the case of Eichhornia, however, the culture has not been monitored continuously. In

Table 2. Effect of culture density on the yields of _Lemna minor_ and _Spirodela polyrhiza_, harvested weekly and maintained at the indicated starting density.

Culture density (kg wet wt/m^2)	Yield* (g dry wt/m^2.day)	
	Lemna minor	_Spirodela polyrhiza_
.05	1.5	
0.10	2.6	2.6
0.20	2.9	
0.25	4.9	3.2
0.40		3.0
0.50	3.9	
0.80		2.6
1.00	2.9	
1.20		0.5
1.50	1.8	
2.00	2.0	
3.00	0.6	

*Each data point represents the mean of 3 replicates.

Table 3. Effect of culture density and harvest frequency on the yield of *Lemna minor*, maintained at the indicated starting density.

	Culture density (kg wet wt/m^2)	Yield* (g dry wt/m^2.day) Plants harvested at intervals of:	
		3 days	4 days
I.	0.25	6.5	4.8
	0.5	6.1	5.2
	1.0	3.7	3.5
	1.5	3.4	3.5
	2.0	2.3	2.8
		Avg. of 3-4 days	7 days
II.	0.25	4.7	6.8
	0.5	5.7	

*Each data point represents the mean of 3 replicates.

September, 1977, the hyacinths became chlorotic and flaccid, and in this weakened condition were devastated by red spider mites. The plants were revived by spraying a commercial nutrient mix onto the foilage, but growth measurements had to be suspended until the plants recovered.

Table 4 shows the mean weekly yields of hyacinths grown from 12/21/77, the date complete nutrient enrichment was begun, to 12/13/78, a complete year. The mean productivity for this period was 24.2 g/m^2.day, with a range of 5.3 to 34.9 g/m^2.day. Water hyacinth is by far the most productive, trouble-free, and generally the most successful and suitable freshwater macrophyte grown to date as a candidate species for a biomass plantation. Its mean annual production of 24.2 dry wt g/m^2.day is equivalent to 88 dry metric tons/ha.year or 35 dry tons/acre.year, 82% of which (28 tons/acre.year) is organic matter.

The mean weekly yields of Lemna minor for the period 5/18/77-6/20/78 are shown in Table 5. However, these cultures were not continually maintained; at five times during the year, the duckweed was overgrown by the filamentous alga Hydrodictyon, necessitating replacement of the culture with additional plants. The culture also had to be replaced once because heavy winds literally blew the duckweed out the pond. The productivity for the year showed a range of 0.1 to 7.0 g/m^2.day and an annual mean of 3.7 g/m^2.day.

Table 4. Yields of <u>Eichhornia crassipes</u> grown in ponds at a water exchange rate (residence time) of 0.5 days in enriched medium, harvested and maintained at a constant starting density of 10 kg wet wt/m^2.

Dates	Mean yield (g dry wt/m^2.day)
12/21/77-1/4/78	5.3
1/4-1/16	6.4
1/16-1/23	10.1
1/23-1/31	8.9
1/31-2/7	10.1
2/7-2/15	10.0
2/15-2/24	8.4
2/24-3/6	11.5
3/6-3/13	22.2
3/13-3/20	20.4
3/20-3/27	20.9
3/27-4/3	31.7
4/3-4/11	34.4
4/11-4/18	31.7
4/18-4/25	33.0
4/25-5/2	33.0
5/2-5/9	28.5
5/9-5/16	27.9
5/16-5/23	33.0
5/23-5/30	31.7
5/30-6/6	29.2
6/6-6/13	27.9
6/13-6/20	28.5
6/20-6/27	29.8
6/27-7/5	30.0
7/5-7/12	31.7
7/12-7/19	26.6
7/19-7/26	27.2
7/26-8/2	27.3
8/2-8/9	30.4
8/9-8/16	34.9
8/16-8/23	29.2
8/23-8/30	30.5
8/30-9/5	32.0
9/5-9/14	24.7
9/14-9/20	20.0
9/20-9/28	26.6
9/28-10/6	24.4
10/6-10/13	31.7
10/13-10/23	19.5
10/23-10/27	24.4
10/27-11/3	20.3
11/3-11/16	17.1
11/16-11/27	15.3
11/27-12/4	14.6
12/4-12/13	12.3
Mean (12/21/77-12/13/78)	24.2

Table 5. Yields of duckweed (Lemna minor) grown in ponds at a water exchange rate (residence time) of 5 days in enriched medium harvested and maintained at a constant starting density of 0.5 kg wet wt/m^2.

Dates	Mean yield (g dry wt/m^2.day)
5/18-6/6/77	3.9
6/6-6/14	4.9
6/14-6/21	3.7
6/21-7/1	5.6
7/1-7/8	5.7
7/8-7/15	7.0
7/15-7/22	6.1
7/22-7/29	6.0
7/25-8/3	Culture overgrown with epiphytes; restocked
8/3-8/18	2.1
8/18-9/6	Culture blown out of pond, restocked
9/6-9/12	4.9
9/12-9/19	3.7
9/19-9/26	4.6
9/26-10/3	4.5
10/3-10/20	4.3
10/20-11/2	4.5
11/2-11/9	5.3
11/9-11/16	3.3
11/16-11/23	3.6
11/23-11/30	3.5
11/30-12/15	Culture overgrown, restocked
12/15-12/29*	1.7
12/29-1/6/78	1.5
1/6-1/17	1.6
1/17-1/25	2.1
1/25-2/2	1.2
2/2-2/13	1.5
2/13-2/16	Culture overgrown, restocked
2/16-2/24	0.1
2/24-3/6	2.6
3/6-3/13	2.3
3/13-3/20	2.5
3/20-3/27	3.6
3/27-4/3	3.7
4/3-4/11	5.5
4/11-4/18	5.3
4/18-4/25	4.8
4/25-5/2	5.2
5/2-5/8	Culture overgrown, restocked
5/8-5/16	1.9
5/16-5/23	2.1
5/23-5/30	1.9
5/30-6/7	2.7
6/7-6/13	6.4
6/13-6/20	3.6
Mean (5/18/77-6/20/78)	3.7

*Complete nutrient enrichment begun.

A growth study was carried out early in 1978 in the concrete vaults to compare the growth of the 2 duckweed species, Lemna and Spirodela. The results, shown in Table 6, indicated that both species were equally productive during this time period. Because of the difficulty in finding Spirodela in natural stands near Harbor Branch Foundation, most subsequent duckweed studies were with Lemna.

The orientation of Hydrilla when grown attached to vexar screens is similar to that of rooted plants. Hydrilla cultured in this manner was allowed to grow unharvested and to spread over the water surface, since growth was found to be interrupted for as long as one to two weeks after harvesting while the plants regenerated new meristematic growing tips. Weighings of the Hydrilla were conducted infrequently during the winter months, because the plants appeared unhealty and their growth was visibly poor. Each data point in Table 7 represents the mean of the productivities of plants attached to three 0.6 m^2 Vexar screens. The time-weighted mean daily yield for 8/24/77-8/28/78 was 4.2 g/m^2.day.

The attachment of the Hydrilla to Vexar screens raised some questions as to whether growth obtained with this culture technique was equivalent to that of plants rooted in the hydrosoil. Two experiments were therefore conducted to compare the growth of plants attached to the screening with those rooted in sand and mud (Table 8). Growth was found to be roughly the same in all three culture units.

Table 6. Yields of *Lemna minor* (common duckweed) and *Spirodela polyrhiza* (giant duckweed) in concrete vaults at a water exchange rate (residence time) of 0.5 days in enriched medium, harvested and maintained at a constant starting density of 0.5 kg wet wt/m^2.

Dates (1978)	Mean yield (g dry wt/m^2.day) *L. minor*	*S. polyrhiza*
1/17-1/25	2.4	3.3
1/25-2/1	2.3	1.5
2/1-2/8	1.9	1.8
2/8-2/21	2.0	2.3
2/21-3/1	2.2	3.4
3/1-3/14	1.8	3.2
3/14-3/28	6.1	5.8
3/28-4/14	3.6	3.8
4/14-4/24	7.9	5.9
	3.3	3.4

Table 7. Yields of *Hydrilla verticillata* attached to Vexar screens grown in ponds at a water exchange rate (residence time) of 5 days in enriched medium. Plants weighed but not harvested during growth period.

Dates	Mean yield (g dry wt/m^2.day)
8/24-9/13/77	8.4
9/13-10/5	7.7
10/5-11/2	3.1
11/2-11/28	0.84
11/28-1/19/78	2.6
1/19-2/24	0.0
2/24-3/14	0.75
3/14-4/4	1.1
4/4-4/24	4.0
4/24-5/8	0.80
5/8-5/22	7.4
5/22-6/5	8.1
6/5-6/19	6.9
6/19-7/3	5.8
7/3-7/17	6.9
7/17-8/1	10.4
8/1-8/14	5.8
8/14-8/28	5.5
Mean (time weighted)	4.2

Table 8. Comparative yields of Hydrilla verticillata in ponds attached to Vexar screening and rooted in sand and mud, with water exchange rate (residence time) of 5 days in enriched medium well water. Cultures all started at a density of 2.0 kg wet wt/m^2.

Substrate	Mean yield (g dry wt/m^2.day)		Nitrogen (% dry wt)*
	10/14-11/7/77	4/7-5/9/78	
Vexar screening	2.9	14.9	3.5
Mud	3.8	11.2	4.3
Sand	2.5	11.3	4.2

*Apical growth tips (2.2 cm long) from plants at end of 4/7-5/9/78 experiment.

Analyses of the growing tips of the plants at the end of the second experiment also showed similar nitrogen content, indicating that the plants suspended in the water were as successful in obtaining nutrients as were those rooted.

Experiments were initiated in July, 1978 with the cultivation of the pennywort (Hydrocotyle umbellata). This is an emergent plant similar in its habit and appearance to water hyacinth, and it has been grown in Vexar-mesh baskets immersed in the concrete vaults exactly as with the hyacinths (Figure 3). Yields from July 3 through November 14, 1978 averaged 15.9 g dry wt/m^2.day (Table 9). During the five months it has been cultured, pennywort has been found to be considerably more productive than the other freshwater macrophytes tested with the exception of water hyacinths. Its advantage may lie in the fact that it is reputedly more cold tolerant than the tropical water hyacinth and may therefore be adaptable to a more temperate climate. Conceivably, through better growth performance in winter, it could have a better annual yield than hyacinths in Central Florida. It will be grown for an entire year at Harbor Branch Foundation to evaluate its annual yield. A disadvantage of pennywort is the fact that it does not bud off separate plants, as does the water hyacinth and the duckweeds, but the new plants remain firmly attached to the parent stock through an intricately connected and interwoven root system. Harvesting therefore would require virtually cutting the plants apart, a practice that has not yet been attempted in the culture of the species to date.

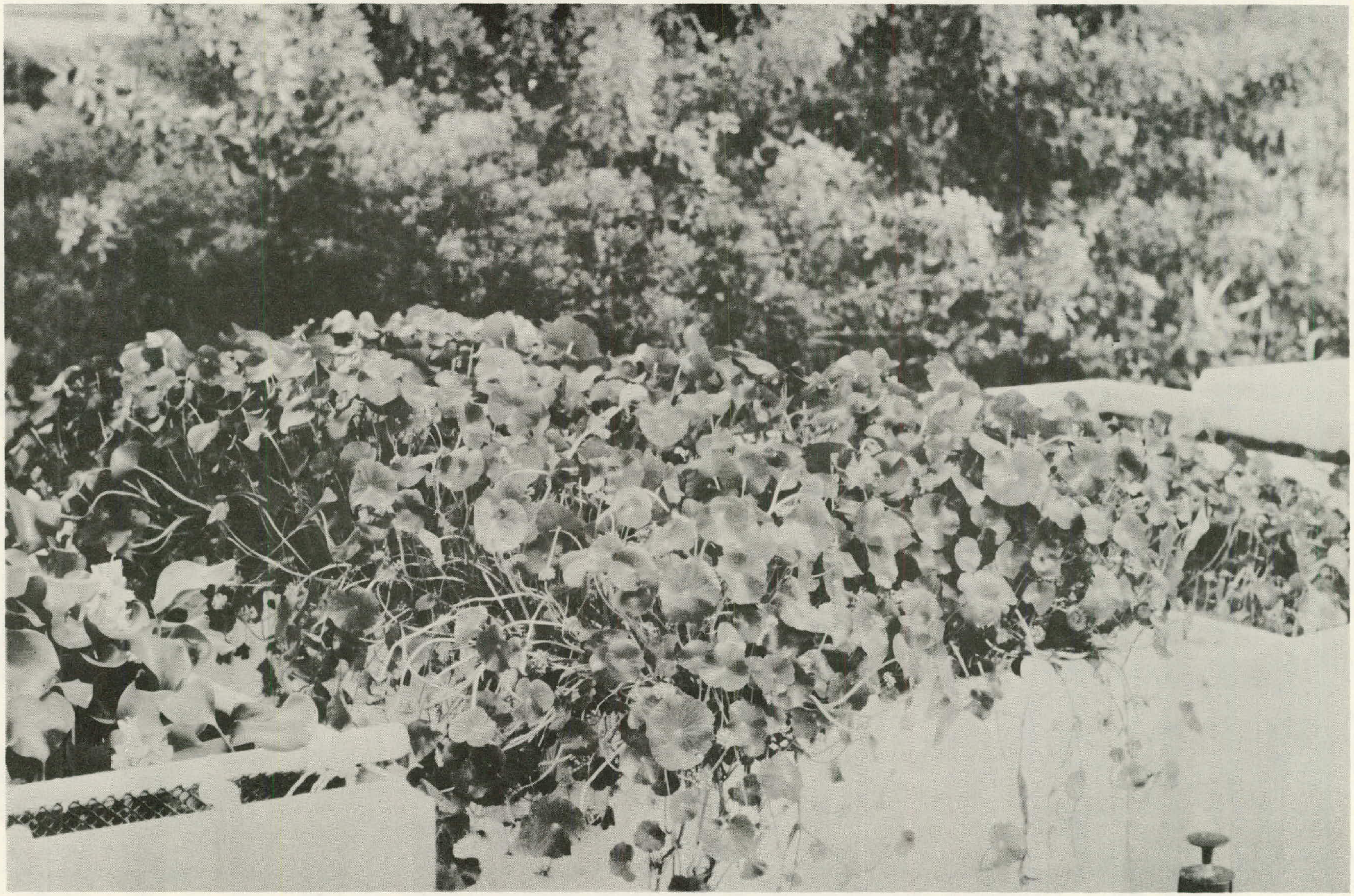

Figure 3. Culture of pennywort (Hydrocotyle umbellata) grown in concrete vault.

Table 9. Yields of pennywort (Hydrocotyle umbellata) grown in vaults at a water exchange rate (residence time) of 0.5 days with influent NO_3-N of 50 μmoles/l and $PO_4^{\equiv}$-P of 5 μmoles/l.

Dates	Mean yield* (g dry wt/m^2.day)
7/3-7/14/78	9.3
7/14-7/21	14.6
7/21-7/28	21.6
7/28-8/11	16.9
8/11-8/18	20.8
8/18-8/25	16.7
8/25-9/1	15.4
9/1-9/6	29.7
9/6-9/15	22.3
9/15-9/22	9.7
9/22-10/6	5.4
10/6-10/17	4.6
10/17-10/25	12.7
10/25-10/31	23.2
10/31-11/14	15.4
Mean (7/3-11/14/78)	15.9

*Constant starting density not maintained.

The possibility of growing the water fern (Azolla caroliniana) has been intriguing because of its symbiotic association with the filamentous blue-green alga Oscillatoria sp. Since the latter can fix atmospheric nitrogen, the symbiotic association of alga-fern can reputedly live in an inorganic nitrogen-free environment, a situation that could result in significant economy in a biomass production system. Azolla, however, is difficult to grow and apparently not very productive. Table 10 shows yields at two densities of Azolla during a one-month experiment that was terminated when the plant culture became overgrown with filamentous algae.

Because the smaller duckweeds and water ferns consistently became overgrown with filamentous algae, some preliminary experiments with growing the latter were undertaken in the hope that they might prove both productive and easy to grow. Neither has proved true to date.

Small batch cultures, in 10-liter polyethylene cylinders, were attempted with Hydrodictyon sp. Spirogyra sp., Pithophora kowensis, and Oedogonium sp., but yields were extremely low (1-2 g dry wt/m^2.day) and the cultures lasted for no more than one to two weeks.

More success was obtained with growing Hydrodictyon sp. and Rhizoclonium sp. in the concrete vaults with circulating, enriched well water (Residence time 0.5 days, 50 μmoles/l NO_3-N, 5 μmoles/l $PO_4^{\equiv}$-P, .1 ml/l trace element mix). Yields of the two species

Table 10. Yields of <u>Azolla</u> <u>caroliniana</u> (water fern) grown in concrete vaults at a water exchange rate of 0.5 days in well water enriched with 50 μmoles/l NO_3-N and 5 μmoles/l $PO_4^{\equiv}$-P. Plants were harvested back to the given starting densities.

Dates (1978)	Yield (g dry wt/m^2.day) Density: 0.41 kg wet wt/m^2	Density: 0.71 kg wet wt/m^2
2/7-2/14	3.4	3.5
2/14-2/21	1.5	1.8
2/21-3/6	2.7	1.6
Mean	2.5	2.3

averaged about 7.0 g dry wt/m^2.day while they grew, but the cultures again deteriorated after about two weeks.

There being little or no available data on the natural productivity of freshwater macrophytes, there was no way to assess the significance of the yield data that was being obtained in the artificial culture systems that had been developed at the Harbor Branch Foundation. For that reason, measurements were begun in the spring of 1978 of the yields of natural populations of water hyacinth, duckweed, and the floating fern _Salvinia rotundifolia_, comparing these with yields of the same species taken from the same populations and transplanted in the Harbor Branch Foundation culture systems.

Natural stands of water hyacinths and _Salvinia_ growing in a fire ditch adjacent to the Kissimmee River near Okeechobee, Florida, and a pond containing _Lemna minor_ north of Ft. Pierce, Florida, were the sites chosen for field studies. Water hyacinths and ferns from the fire ditch location were collected and placed in 1 m^2 Vexar cages. Three such cages of each species were left with the natural population in the fire ditch, and another three were returned to the Harbor Branch Foundation and placed in a pond through which enriched sewage was passed with a residence time of 0.5 days (Figure 4). _Lemna_ was collected from the pond north of Ft. Pierce and placed in 1 m^2 PVC enclosures having nylon screened sides. Three of these

Figure 4. Monitcring the growth of a natural population of water hyacinth (Eichhornia crassipes).

enclosures were left in the field, and three were returned to the Harbor Branch Foundation and cultured in a fashion similar to that of the other two species.

The yields of all three species at their respective field locations have been much lower than those obtained with similar plants grown in nutrient enriched ponds at Harbor Branch Foundation (Table 11). The productivity of water hyacinths for the initial two weeks at Harbor Branch Foundation was approximately the same as that of the field plants, but following this short adjustment period, the hyacinths cultured at Harbor Branch Foundation consistently outgrew the plants remaining in the fire ditch. The mean productivities from 3/30-11/3/78 for Eichhornia at the Harbor Branch Foundation and fire ditch locations were 36.3 and 10.4, respectively. Lemna exhibited mean daily yields from 5/11-11/3/78 of 4.8 g/m^2.day at Harbor Branch Foundation and 1.4 g/m^2.day at the field pond. Salvinia could never be grown very successfully in culture and, after becoming overgrown with algae, had to be replaced twice at Harbor Branch Foundation with new cultures from the field. Nevertheless, it grew at nearly twice the rate there (2.6 g/m^2.day) than in the field (1.5 g/m^2.day) during the period 3/30-11/3/78.

A major constraint to the possible use of freshwater macrophytes in a large-scale energy plantation could be the consumption of water, in short supply over much of the earth's surface. It was found earlier that the freshwater plants, unlike the seaweeds, do

Table 11. Yields of Eichhornia crassipes, Lemna minor and Salvinia rotundifolia natural populations and in aquaculture ponds at the Harbor Branch Foundation receiving two exchanges per day of enriched nutrient medium. Cultures harvested and maintained at a density of 10 kg/m^2 for Eichhornia and 0.5 kg/m^2 for Lemna and Salvinia. (All data points represent mean of three 1 m^2 enclosures.)

Dates	Yields ($g\ dry\ wt/m^2.day$)					
	Eichhornia crassipes		Lemna minor		Salvinia rotundifolia	
	HBF	Field	HBF	Field	HBF	Field
3/30-4/6/78	22.8	19.0				4.8
4/6-4/13	20.1	20.7				1.5
4/13-4/20	34.4	20.8				3.2
4/20-4/27	34.1	18.9			5.4	1.4
4/27-5/4	39.4	18.9			3.4	0.9
5/4-5/11	30.5	11.5			2.4	1.1
5/11-5/18	40.2	15.2	5.1	2.5	2.8	1.4
5/18-5/25	43.1	11.1	7.0	2.8	0	2.2
5/25-6/1	41.8	11.4	3.1	1.2	0	1.6
6/1-6/8	44.7	8.8	3.5	0.5	(++)	0.2
6/8-6/15	44.5	10.3	6.2	1.1	3.6	0.8
6/15-6/22	55.0	9.5	5.7	1.5	7.9	0.4
6/22-6/29	40.9	10.3	4.8	1.6	1.4	0
6/29-7/6	50.1	8.7	2.6	0.9	(++)	1.2
7/6-7/13	45.3	9.0	4.8	2.5	2.2	0.6
7/13-7/20	41.9	2.7	(+)	0.7	---	1.0
7/20-7/27	41.1	6.8	3.0	0.7	2.4	1.0
7/27-8/3	37.0	11.3	4.2	1.9	3.1	2.8
8/3-8/10	37.6	7.8	4.2	1.9	5.8	4.5
8/10-8/17	40.0	4.9	6.7	1.2	3.0	1.4
8/17-8/23	37.4	6.2	5.0	0.3	0.3	1.8
8/23-8/30	37.5	9.2	5.2	0.2	1.7	0.2
8/30-9/6	38.3	7.9			2.5	1.5
9/6-9/14	31.9	9.4			2.3	0.7
9/14-9/20	23.7	11.5	6.4	---	---	---
9/20-9/27	36.2	11.5	5.1	---	1.3	0.1
9/27-10/5	25.9	10.0	4.7	---	0	0
10/5-10/12	30.1	9.0	1.3	1.3	2.1	0.3
10/12-10/19	24.3	1.6	---	0.3	2.9	0.7
10/19-10/26	31.6	1.8	3.9	3.5	6.1	1.0
10/26-11/3	25.6	6.3	---	4.2	---	5.9
Mean	36.3	10.4	4.8	1.4	2.6	1.5

+Plants blown out of cages.

++Cultures replaced.

not require a rapid exchange of water to insure their maximum growth. Experiments had not previously been conducted, however, to determine the relative growth of these plants in completely stagnant cultures. Such an experiment was therefore begun in July, 1978.

Both duckweed (Lemna minor) and water hyacinths (Eichhornia crassipes) have been grown in 1000-l polyethylene cylinders, maintaining densities of 0.5 and 10.0 kg wet wt/m^2 respectively (Figure 5). One culture of each species received freshwater enriched with 100 μmoles/l NO_3-N, 10 μmoles/l PO_4-P and 0.1 ml/l trace element mixture at a volume residence time of 0.5 days. The duplicate cultures of each species received no flow of water but were enriched once a week with a concentrated solution that provided the same weekly ration of N, P, and trace elements as did the flow-through systems. The weeds were weighed weekly and harvested back to their starting densities. The results are shown in Table 12. For the first part of the experiment, the stagnant cultures did very poorly. The duckweed had to be replaced three times and the water hyacinths once during that period. The plants appeared flaccid and chlorotic and were apparently suffering from nutrient impoverishment in spite of the weekly ration of highly concentrated nutrients. It was noted, however, that the latter precipitated when it was added and the precipitate sunk to the bottom of the vessel, presumably making at least some of the trace elements unavailable to the plants. Beginning on September 26, therefore, additional trace element mix

Figure 5. Stagnent cultures of freshwater macrophytes for measuring evapotranspiration water loss.

Table 12. Yields of *Lemna minor* and *Eichhornia crassipes* in stagnant and flow-through cultures receiving the same enrichment.

Dates (1978)	Mean yield (g wet wt/m^2.day)			
	Lemna minor		*Eichhornia crassipes*	
	Stagnant	Flow-through	Stagnant	Flow-through
7/18-7/25	3.4	2.3	---	7.8
7/25-8/1	0.1	4.3	7.7	10.5
8/1-8/8	0	6.7	4.3	19.2
8/8-8/14	3.5	6.0	0*	22.8
8/14-8/22	1.3	8.4	23.2	28.4
8/22-8/29	0*	8.9	21.3	34.3
8/29-9/5	3.8	8.0	7.7	40.8
9/5-9/12	0	6.9	0.6	32.4
9/12-9/19	0	6.3	0	32.4
9/19-9/26**	0*	6.8	0	25.9
9/26-10/4	7.3	6.9	14.3	30.3
10/4-10/11	0*	2.6	0.9	15.1
10/11-10/18	3.7	3.0	14.2	19.4
10/18-10/25	4.1	4.3	2.2	18.9
10/25-10/31	2.4	3.4	15.4	21.4
10/31-11/7	1.2	3.6	17.3	22.7
11/7-11/14	2.6	2.8	19.4	21.6
11/14-11/21	0.9	3.8	17.3	32.4

*Cultures restocked.

**Spray - addition of trace metals begun.

was sprayed on all the cultures once a week. Recovery of the stagnant cultures was rapid and their yields were beginning to approach those of the flow-through cultures at the time this report was written.

Even stagnant cultures of freshwater plants may lose significant quantities of water through evapo-transpiration, reported to be several times as great for several aquatic weed species including water hyacinths as the evaporation rate from water alone. The same stagnant duckweed and water hyacinth cultures referred to above and an additional polyethylene cylinder-culture of Hydrilla were monitored for water loss, adding weekly the measured amount lost the previous week by evapo-transpiration (straight evaporation for the submerged Hydrilla culture and a control cylinder containing only water). The absolute water losses from the three plant cultures and the control cylinder are shown in Table 13. The relative water losses (losses from the plant cultures/losses from the control) are shown in Table 14, together with data on rainfall, solar radiation, and air temperature for the periods in question.

Of the three species, water hyacinth lost the most water, as would be expected from its greater exposure to the air and relatively greater metabolic activity. Duckweed behaved essentially like water alone. The slightly greater loss in the Hydrilla culture compared to that of the control probably resulted from greater light absorption and/or restriction of vertical convective circulation in the dense plant culture. Little correlation can be seen

Table 13. Water loss (l/m^2.day) from cultures of *Eichhornia crassipes*, *Lemna minor*, and *Hydrilla verticillata* and from a control container of water.

Dates	*Eichhornia*	*Lemna*	*Hydrilla*	Control
7/18-7/25/78	6.86	4.14	--	3.99
7/25-8/1	2.94	2.29	--	2.37
8/1-8/8	5.60	3.64	4.20	3.49
8/8-8/14	5.77	3.84	4.10	3.88
8/14-8/22	9.00	5.60	6.90	6.50
8/22-8/29	9.35	5.17	5.27	5.27
8/29-9/5	7.97	6.29	7.94	6.09
9/5-9/12	6.80	3.90	4.65	4.22
9/12-9/19	6.76	4.90	6.19	5.33
9/19-9/26	--	--	--	--
9/26-10/4	7.18	7.24	4.57	--
10/4-10/11	7.34	--	4.56	4.13
10/11-10/18	5.98	2.16	3.18	2.32
10/18-10/25	7.58	4.38	5.12	4.98
10/25-10/31	3.77	--	1.82	1.48
10/31-11/7	5.69	4.81	3.94	4.08
11/7-11/14	3.34	0.97	--	--
11/14-11/21	6.75	3.50	3.80	4.22
11/21-11/28	5.41	3.12	3.57	4.28
11/28-12/5	4.25	2.16	1.88	3.31

Table 14. Water loss from cultures of Eichhornia crassipes, Lemna minor, and Hydrilla verticillata relative to water loss from a control container of water (plant culture/control) and related meteorological data.

Dates (1978)	Eichhornia	Lemna	Hydrilla	Total Rainfall (mm)	Mean Sunlight (langleys/d.)	Air Temperature (°C) Mean	Max	Min
7/18-7/25	1.72	1.04	--	62.7	553	29	34	24
7/25-8/1	1.24	0.97	--	91.4	417	27	33	21
8/1-8/8	1.60	1.04	1.20	43.2	499	28	33	23
8/8-8/14	1.49	0.99	1.06	57.2	551	28	34	22
8/14-8/22	1.38	0.86	1.06	0	536	29	32	26
8/22-8/29	1.77	0.98	1.00	36.8	547	28	34	22
8/29-9/5	1.31	1.03	1.30	2.5	488	27	33	22
9/5-9/12	1.61	0.92	1.10	7.6	422	28	34	22
9/12-9/19	1.27	0.92	1.16	6.3	454	27	33	22
9/19-9/26	--	--	--	15.2	409	27	33	22
9/26-10/4	--	--	--	21.6	389	27	33	22
10/4-10/11	1.78	--	1.10	27.9	375	26	--	--
10/11-10/18	2.58	0.93	1.37	53.3	308	24	30	16
10/18-10/25	1.52	0.88	1.03	27.9	343	22	27	17
10/25-10/31	2.55	--	1.23	55.9	388	25	30	21
10/31-11/7	1.39	1.18	0.97	35.6	380	21	28	11
11/7-11/14	--	--	--	91.9	--	24	29	18
11/14-11/21	1.78	0.92	1.11	0.8	--	24	29	18
11/21-11/28	1.51	0.87	1.20	0	--	22	29	14
11/28-12/5	2.26	1.15	1.76	10.2	--	23	31	14
Mean	1.69	0.98	1.18					

visually between water loss and productivity of the plants (i.e. Tables 12 vs. 13) or between water loss and the various meteorological factors measured (Table 14). However, the data have not yet been subject to statistical analysis.

Evapo-transpiration loss from the water hyacinths has been surprisingly low - 1.69 times on the average and 2.58 times at most the loss from evaporation alone. As explained earlier, however, the hyacinths were not growing well in the stagnant culture during the first part of the experiment and, by the time the problem had been corrected, the season for the best growth of the species had passed. The experiment will therefore be continued so as to obtain valid data for water loss of water hyacinth throughout the year at the Harbor Branch Foundation location, and relevant meteorological data will be collected at the same time in an attempt to identify the factor(s) that control water balance in the species.

All of the freshwater macrophytes cultured at Harbor Branch Foundation and in the field are routinely analyzed for their content of ash, volatile solids, carbon and nitrogen. Mean compositions of six species are shown in Table 15. Note that the percent volatile solids in the enriched laboratory cultures of duckweed and <u>Hydrilla</u> is significantly lower than in the natural stands, water hyacinths are the same at both locations, and cultured pennywort

Table 15. Percent of ash, volatile solids, carbon and nitrogen in freshwater macrophytes grown at Harbor Branch Foundation (HBF) and in natural populations of the same species.

Species	Ash		Volatile Solids		Carbon		Nitrogen	
	HBF	Field	HBF	Field	HBF	Field	HBF	Field
Eichhornia crassipes	19	19	81	81	35	35	2.2	1.5
Lemna minor	33	22	67	78	31	34	1.8	2.0
Hydrocotyle umbellata	15	22	85	78	39	39	2.1	2.4
Hydrilla verticillata	28	19	72	81	37	37	3.4	3.6
Salvinia rotundifolia	37	28	63	72	31	34	1.9	2.4
Azolla caroliniana	13	--	87	--	42	--	3.0	--

(*Hydrocotyle*) has a higher volatile solids content than the natural population, though the latter is based on an inadequate number of two samples.

IV. Recycling the nutrients from methane digester residues

One of the major potential costs, both in terms of economics and energy, of operating an energy farm is that of the nutrients (N, P, Fe, trace elements, etc.) required to grow the plant biomass. For example, a 100 square mile energy plantation producing 10 dry tons of plant biomass acre.year would require of the order of 1500 tons per year of commercial fertilizer or its equivalent. However, methane production by anaerobic digestion of plant biomass does not either consume nor dissipate the plant nutrients, which remain in the digester residue. Recycling of those nutrients would therefore seem a logical procedure. Experiments were therefore initiated to ferment water hyacinths and seaweeds (Gracilaria tikvahiae) by anaerobic digestion and to recycle the nutrients remaining in the digester residues to support further growth of the same two plant species.

Five 125-liter digesters measuring approximately 45 x 45 x 80 cm were constructed from 0.6 cm sheet plastic. Filling and emptying ports were made of 13 cm PVC pipe with screw-cap ends. One side of each digester contained a plexiglass window for visual observation (see Figure 1). Gas lines lead from the tops of the digesters to inverted, submerged 50-gallon drum monometers, where the gas is collected and its volume monitored.

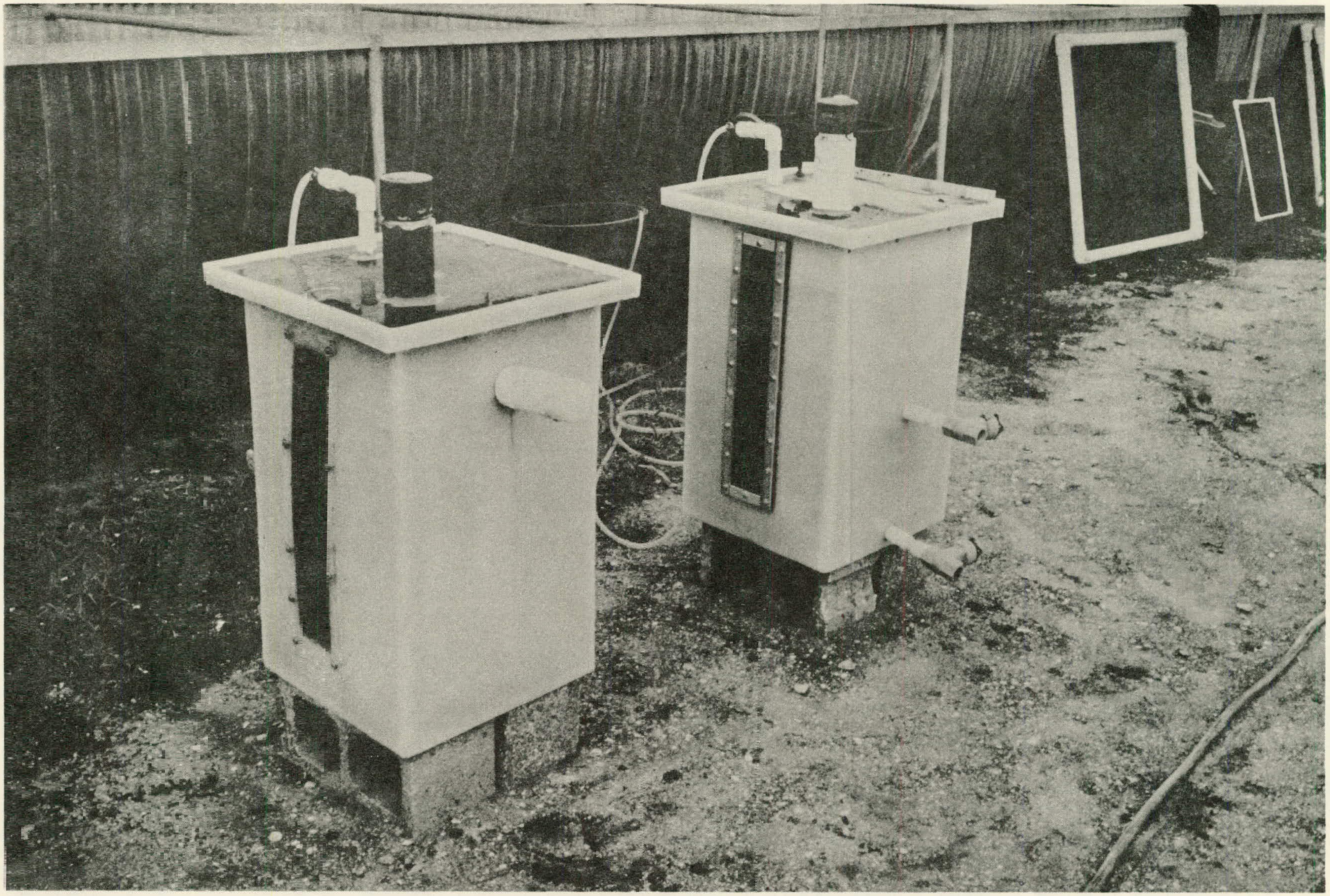

Figure 1. Anaerobic digesters used for fermenting water hyacinths and Gracilaria.

Water hyacinths:

Two of the digesters have been used for water hyacinth fermentation, two for Gracilaria fermentation, and the fifth is kept in reserve in case of failure of any of the other four cultures. Beginning in November, 1978, the digesters have been kept partially submerged in a large circular water tank, the water of which has been kept at 30°C by an immersion heater.

The water hyacinth digesters were started with dairy manure. With the establishment of anaerobic fermentation and methane production, the manure was gradually replaced with water hyacinths.

The water hyacinths require fine shredding or chopping prior to digestion. A Sears-Roebuck electric yard and garden shredder is used for that purpose, producing a greenish-black slurry that has the consistency of thick mud. The water hyacinths must be chopped immediately after harvesting and removal from the water, at least with the chopper presently in use. Even partial drying renders the plant material tough and fiberous and resistent to shredding, and soaking in water does not reconstitute the plant flesh to a suitable form for chopping.

The hyacinth digesters are loaded three times a week at 0.8-1.0 g volatile solids/liter digester volume (2.4-3.0 kg chopped hyacinths/digester/ day). At the time they are loaded, an equivalent volume of liquid residue is removed from the digester. The digester contents are not stirred, and the solid fraction floats at the surface above the liquid fraction. To prevent the solids from plugging the discharge port, a screen is inserted above which the solids are trapped. Stratification of the solid phase is, however, probably a deterrent to more complete digestion of the organic matter and some change in design, perhaps including gentle agitation, would appear desirable.

The water hyacinth digesters have now been in operation for over eight months, producing on the average 0.4 l gas/g volatile solids (24 g wet wt plant material), at 60% methane[1]. The pH has been maintained at 7.0 to 7.3. Higher digester loadings were found to depress pH and reduce methane content of the gas.

The liquid residue withdrawn from the hyacinth digesters contains, on the average, approximately 384 mg nitrogen and 28 mg phosphorus per liter. Roughly half the nitrogen is NH_4^+-N, the remaining half is dissolved organic nitrogen of unknown exact identity.

To investigate the suitability of the digester residue as a nutrient source for growing water hyacinths, three cultures of the plants, maintained at a density of 10 kg wet wt/m^2, were established in 750 l, 2.28 x 78 x 64 cm (1.8 m^2) concrete vaults. Two of the three cultures were operated in a "batch" mode with one complete

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[1]Analysis performed by Mr. Daniel Young, Dept. Agricultural Engineering, University of Florida.

exchange of well water per week. One of these cultures received no enrichment. The second received enrichment with the chemical nutrient medium normally used to grow water hyacinths (Table 1). Approximately half the nitrogen and phosphorus was assimilated each week from the enriched culture. However, after one month, both of the above cultures became chlorotic and unhealthy in appearance, so both were thereafter sprayed weekly with additional trace-element mixture (Nutri-Spray, Table 1) which partially restored them to a normal appearance.

The third culture received five liters of liquid digester residue from the water hyacinth digester three times a week, a total of approximately 5760 mg N and 420 mg P per week. The water in that culture was not exchanged nor did the plants receive the trace-element spray or any other form of enrichment.

The water hyacinths were contained in a Vexar-mesh basket in the vaults and the basket containing the entire culture was hauled out of the vault once a week, allowed to drain for four minutes, and weighed. Incremental growth was removed from the cultue and it was returned to the water. Growth, expressed as mean daily yield of dry weight/m^2, was calculated for each one-week interval assuming dry weight = 5% of wet weight. Data for the period 8/11-11/24/78 indicate that growth in the digester residue has averaged 37% more than in the chemically-enriched culture and three times that of the unenriched control (Table 2).

A sample of each harvest from each of the three cultures was also analyzed for ash, carbon and nitrogen content. The mean compositions for each culture over the three-month experimental period are shown in Table 3.

Table 1. Final composition of enrichment medium used for growing water hyacinths (mg/l).

$NaNO_3$	2.55
KNO_3	2.02
$CaCl_2 \cdot 2H_2O$	1.87
$NaHPO_4$	0.80
$MnSO_4 \cdot 7H_2O$	0.79
Trace metal mix*:	
S	1.50
Fe	2.00
Mn	0.75
Zn	0.75
B	0.02
Cu	0.10
Mo	0.01

*Sunniland Nutri-Spray (Chase and Co., Sanford, Florida 32771).

Table 2. Yields of water hyacinth (*Eichhornia crassipes*) grown in unenriched water, in chemically-defined enrichment medium, and in the liquid residue from anaerobic digestion of water hyacinths.

Dates 1978	Mean yield (g dry weight/m^2.day) Unenriched	Chemical medium	Digester residue
8/11-8/17	27	35	40
8/17-8/25	17	28	30
8/25-9/1	8	10	22
9/1-9/8	12	9	21
9/8-9/15	8*	11*	24
9/15-9/22	7	24	30
9/22-9/29	5	14	22
9/29-10/6	2	21	23
10/6-10/13	2	8	17
10/13-10/20	5	15	20
10/20-10/27	1	7	14
10/27-11/3	< 1	12	17
11/3-11/10	6	20	23
11/10-11/17	< 1	9	16
11/17-11/24	1	11	12
Mean	7	16	22

*Began spraying foliage with trace-element mix.

Table 3. Composition of water hyacinths (*Eichhornia crassipes*) grown in unenriched water, in chemically-defined enrichment medium, and in the liquid residue from anaerobic digestion of water hyacinths (percent dry weight). Each value is the mean of 15 measurements over growth period of 8/11-11/24/78.

	Unenriched	Chemical medium	Digester residue
Ash	15.46	21.48	20.69
Volatile solids	85.54	78.52	79.31
Carbon	39.00	35.80	36.64
Nitrogen	1.04	3.21	2.32

A rough mass balance of the nitrogen recycled through the culture-digestion-culture system can now be made. Over the three-month experimental period, the digester was loaded 43 times with a total of 215 kg wet weight of water hyacinths. This is equivalent to 10.75 kg dry weight (at 5% of wet weight) or 249 g N (at 2.32%, see Table 2). Since one kilogram of water hyacinths added was replaced by one liter of liquid residue, a total of 215 l of residue were removed containing 384 mg N/l or 82 g N. Addition of that amount of nitrogen to the culture produced 2310 g dry weight of water hyacinths containing 53 g N.

In summary, of the 249 g nitrogen loaded in the digester, 82 g (33%) were recovered in the residue, of which 53 g (65%) were reassimilated by the water hyacinths, an over-all efficiency of 21% (Figure 2). Attempts will be made in the continuing research to improve that efficiency.

Gracilaria:

Several attempts were made to convert an anaerobic digestion substrate from dairy manure to Gracilaria, but these were all unsuccessful. Extremely low pH was reached and maintained in the digestion mixture, indicating the presence of persistent organic acids that presumably destroyed the relatively sensitive methanogenic bacteria. The conclusion was reached that successful digestion

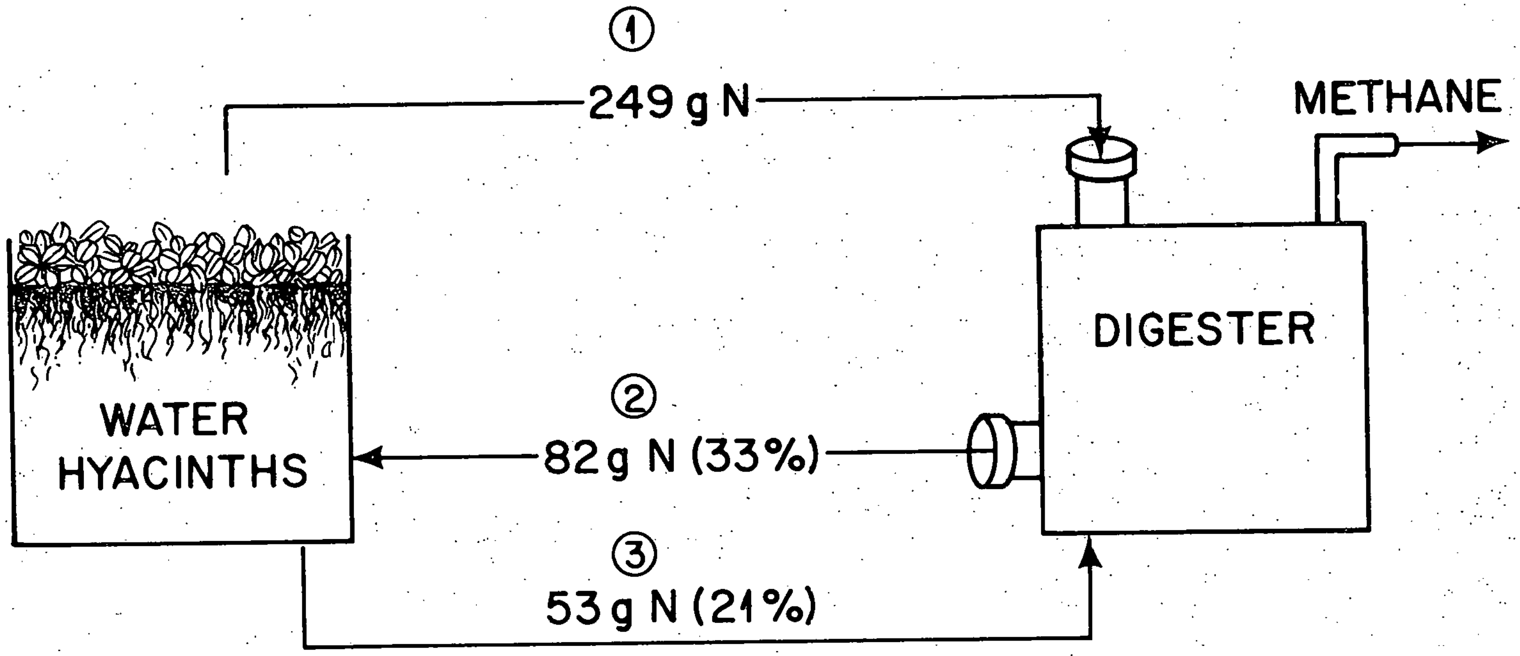

Figure 2. Efficiencies in the recycling of nitrogen from water hyacinths through anaerobic digestion and reassimilation by the water hyacinths.

of *Gracilaria* would require a bacterial culture acclimated to the marine environment and to the tissues of marine organisms. Accordingly, a new attempt was made, using highly organic, anaerobic marine sediment collected from an area where *Gracilaria* and other seaweeds had been observed to collect and accumulate on the bottom.

The 125-liter digester was loaded with 20 kg of wet sediment, 5 kg of fresh *Gracilaria*, and 80 liters of seawater. The digestion mixture was maintained at approximately 30°C and received no agitation. Within two days, the pH dropped from 7.4 to 6.6 and thereafter remained in the 6.3-6.6 range. After six days, gas evolution began, and on the seventh day, the evolved gas became combustible, indicating the presence of methane and the commencement of complete digestion.

Two *Gracilaria* digesters have now been in operation for approximately two months, loaded three times a week at the rate of 0.75 g volatile solids per liter of digester volume (ca. 2.0 kg wet wt *Gracilaria*/digester) per day. Gas production is in the range of 0.2-0.4 liters/g volatile solids at 60% methane. The *Gracilaria* digesters appear to be particularly sensitive to temperature, particularly below 25°C. However, gas production by the seaweed has improved steadily since the successful digestion commenced and was exceeding that of the water hyacinths (in liters/g volatile solids) at the time of this writing. A major advantage in the digestion of *Gracilaria* is that the seaweeds do not need to be chopped, shredded, or otherwise processed prior to loading - a factor that could substantially reduce operating costs relative to water hyacinths and other species that do require such processing.

Because of the initial difficulties in fermenting Gracilaria, experiments have only recently been started on recycling the digester residues as a nutrient source for growing the seaweeds. Cultures have been started in 50-liter containers (sectioned 0.4 m dia. PVC pipes), that are aerated to maintain the seaweed in suspension. There is no flow of seawater through the cultures, but the water is exchanged once a week. One culture is enriched with 1500/μmoles/l NO_3-N, 150 μmoles/l PO_4-P, and 5 ml/l trace element mix (i.e., the seawater enrichment normally used to grow Gracilaria). The other culture receives one liter of liquid Gracilaria digester effluent three times per week. The analysis of the Gracilaria digester residue is not yet available, so comparable nutrient loading of the two cultures is not yet possible.

The experiment had been in progress for two weeks at the time this report was written. At that time, mean yield of Gracilaria in the chemically-enriched seawater culture was 2.5 g dry wt/m^2.day, that in the digester-residue enriched seawater was 7.1 g dry wt/m^2.day, almost three times as great. That experiment will be continued and the recycling efficiency determined, as in the water hyacinth experiment described above.

V. Estimation of primary productivity from diel nitrate uptake measurements with the water hyacinth *Eichhornia crassipes* in an aquaculture system

M. Dennis Hanisak

ABSTRACT

The primary productivity of the water hyacinth <u>Eichhornia crassipes</u> was estimated from diel measurements of NO_3-N uptake in an aquaculture system using a continuous, automated nutrient analyzer apparatus. Productivity values obtained by this method agree favorably with those made by directly harvesting the biomass. Since productivity measurements based on harvesting methods would be impractical on a large scale, the use of diel nutrient uptake measurements would be a significant improvement for proper management of aquatic macrophytes in an aquaculture system. There were little diel changes in NO_3-N uptake by <u>Eichhornia</u>, a fact which is potentially beneficial to this species not only to its ecological success in its natural ecosystem, but also to its ability to remove nutrients as a component in tertiary sewage treatment.

INTRODUCTION

Large-scale cultivation of freshwater macrophytes is a promising new development in aquaculture (Ryther et al. 1977, 1978; Hillman and Culley 1978). Biomass produced in an aquaculture system can be directly consumed by herbivores in the same system (Ryther et al. 1977) or harvested and used as fodder, compost, mulch, fertilizer, or other useful products (Boyd 1974, Tourbier and Pierson 1976).

Although primary productivity is a fundamental process in such an aquaculture system as well as in natural ecosystems, there are difficulties involved in accurately measuring productivity. Different methodologies result in different estimates of productivity that may not be readily interconverted (Vollenweider 1974, Cooper 1975). Thus, the investigator attempts to utilize the method which is most suitable to his particular research need. For aquaculture of freshwater macrophytes, this would ideally be obtained by directly harvesting and weighing the plant biomass produced during a given time period, but this method would be impractical on a large-scale. However, since maximal production probably depends upon the maintenance of an optimal plant density, some estimates of primary productivity must be made in order to properly harvest and manage an aquafarm. Thus, other methods of estimating primary productivity need to be developed. This communication demonstrates the use of diel nitrogen uptake measurements to estimate the primary productivity of the water hyacinth, *Eichhornia crassipes*.

The earliest attempts to relate primary productivity to nutrient uptake were by oceanographers who calculated _in situ_ productivity during spring phytoplankton blooms in temperate seas (Atkins 1923, Kreps and Verjbinskaya 1930, 1932, and Cooper 1938). Such measurements were probably underestimates because they did not consider nutrient regeneration or replenishment of nutrients due to mixing (Ketchum et al. 1958). Despite this, phytoplankton productivity models have often included terms for nutrients (Steele 1962, Steele and Menzel 1962, Riley 1963, 1965), and it has been suggested that productivity could be measured with the use of ^{15}N tracers (Dugdale 1967, Dugdale and Goering 1967).

Although the approach of using nutrient uptake measurements to estimate primary productivity has been mainly confined to phytoplankton studies (and these with limited success), this approach merits attention for possible use in an aquaculture system with aquatic macrophytes. Nitrate was used in this study because, unlike other nutrients, it will not volatilize (like NH_3) or readily precipitate out (like PO_4). In addition, nitrogen is a key nutrient on which to focus because it can be readily measured and is often the limiting nutrient in the system, particularly on whose nutrients are derived from secondary sewage (Ryther et al. 1977). Primary productivity estimates based on nutrient uptake measurements are more valid in a controlled aquaculture system where nitrogen levels are relatively high, mixing is sufficient to assure homogeneity of the water column, and nutrient regeneration is minimal than in an uncontrolled natural system.

MATERIALS AND METHODS

This research took place at the Aquaculture facility at the Harbor Branch Foundation located near Fort Pierce, Florida, where there is presently a project to produce aquatic plant biomass that is used as substrate in anaerobic fermentation to produce methane gas. For these particular experiments, the water hyacinth, Eichhornia crassipes (Mart.) Solms was grown in Vexar plastic mesh cages (1.2 m^2) that had been placed in concrete burial vaults (2.20 x 0.80 x 0.45 and 1.70 m^2 in water area) containing approximately 750 ℓ of water. These vaults received 2 volume turnovers each day of enriched nutrient medium (Table 1) which contained approximately 50 µM NO_3-N.

The stocking density of water hyacinths was usually 10 Kg/m^2. At approximately weekly intervals, the cages containing the water hyacinths were lifted out of the vaults, drained for four minutes, weighed with a spring scale, and, after removing the incremental growth, returned to the vaults. Primary productivity by this harvest technique was calculated as the increase in dry plant weight per unit area and time. The dry weight of Eichhornia was considered to be 5% of its wet weight. A more complete discussion of these methods are found in Ryther (1978).

In order to estimate primary productivity from diel measurements of nitrate removal, the effluent from one of these vaults was analyzed for its NO_3-N content in a continuous basis over a period of 24 hours with an automated

Table 1. Approximate composition of enriched medium flowing into cultures of *Eichhornia crassipes*.

	Concentration (μM)
$NaNO_3$	30.00
KNO_3	20.00
$CaCl_2 \cdot 2H_2O$	12.56
$Na_2HPO_4 \cdot H_2O$	5.00
$MgSO_4 \cdot 7H_2O$	3.21
S[a]	1.56
Fe[a]	1.19
Mn[a]	0.46
Zn[a]	0.38
B[a]	0.062
Cu[a]	0.052
Mo[a]	0.003

[a]Added as commercial liquid fertilizer (Nutri-Spray, Chase & Company, Sanford, Florida).

nutrient analyzer system. An Autoanalyzer Model I proportionating pump pumped samples from the effluent of the vault through an in-line reagent filter and then through a copper-cadmium column (Strickland and Parsons 1972, Stainton 1974) which reduced the NO_3-N in the sample to NO_2-N. The resulting solution was pumped through a flow-through cell in a Bausch and Lomb Spectronic 100 spectrophotometer which transmitted an output signal that was recorded on a OmniScribe B-5000 recorder. The entire apparatus was enclosed in a wooden box (1.28 x 0.65 x 0.50 m) which could be readily moved around from one sampling location to another. Input and output tubing went through an opening in the box by means of a PVC pipe (2.54 cm in diameter) that was fitted with an elbow to prevent precipitation from entering into the apparatus. A 60 watt light bulb was kept on inside the box to facilitate monitoring the apparatus at night and also to aid in humidity control. The vault was aerated with compressed air through holes drilled in a PVC pipe (2.54 cm in diameter) placed on the bottom of the vault in order to insure complete mixing within the vault and to prevent any time lag in observing changes in NO_3-N concentration. Although it was not necessary, a new cadmium column was prepared for each run. Standards, reagent blanks, and influent NO_3-N levels were monitored at the beginning and end of each diel run. For each run, a composite sample was made from three plants for an analysis of the internal nitrogen content of the plants with a Perkin-Elmer Model 240 Elemental Analyzer.

From the continuous 24 hour record of the effluent NO_3-N concentration, primary productivity, based on diel nitrate uptake by the plants was calculated from the equation:

$$P = \frac{1.4 \times 10^{-5} (C_i - C_o)(V)\ (T)}{(A)\ (N)} \quad (1)$$

where C_i = the NO_3-N concentration (μM) in the influent into the vault, C_o = the NO_3-N concentration (μM) in the effluent from the vault, V = the number of volumes (liters) per turnover, T = the number of turnovers per day, A = the area occupied by the cage of plants, N = the internal nitrogen content of the plants (g N/g dry weight), 1.4×10^{-5} converts μ mole NO_3-N to g N. The differences between influent and effluent NO_3-N levels were obtained by integrating the area under the diel curves (e.g. Fig. 1) and determining the amount of NO_3-N removed from the water over 24 hours. All diel graphs were plotted on a scale from 0000 to 2400 to facilitate comparison of experiments that began at different times of the day.

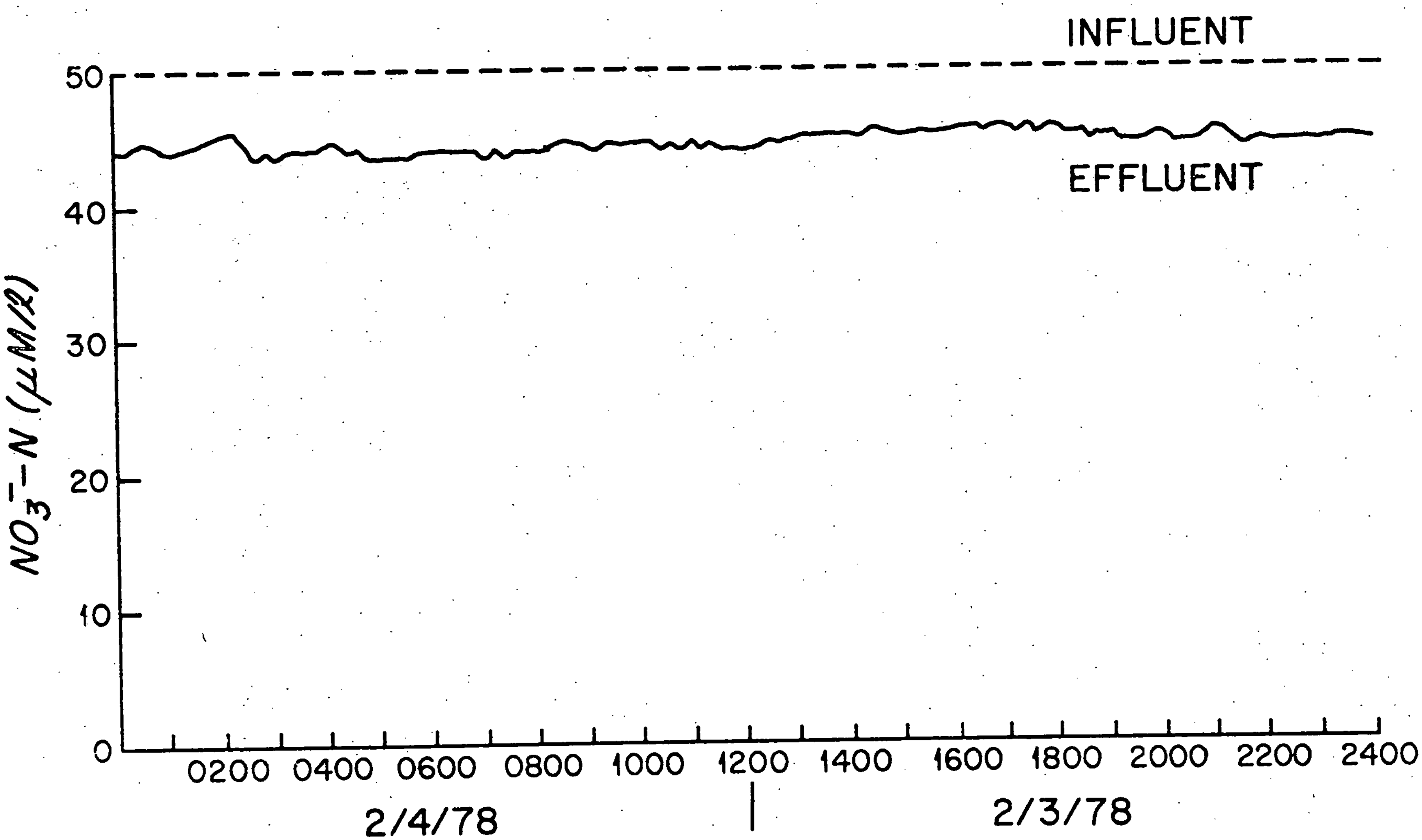

Figure 1. Diel pattern of effluent nitrate-nitrogen concentration from water hyacinth culture. Influent concentration shown as broken line.

RESULTS

Eleven diel experiments were conducted from January to July 1978 for *Eichhornia crassipes* (an example is illustrated in Fig. 1). Based on these data, there was little evidence of a diel cycle in NO_3-N uptake. The NO_3-N concentration for any particular diel period was similar during the day and night although slightly lower NO_3-N levels often occurred at night. Maximal concentrations (i.e. minimal uptake) tended to be in the afternoon.

From these data, primary productivity rates were estimated using equation (1). These estimates (Table 2) agreed favorably with those obtained with the harvest method, being on the average 12.33% less. Interestingly, the best agreement between the two methods was in July when productivity was the highest of the study. At that time, estimates of primary productivity from diel NO_3-N uptake measurements were slightly higher than those made with the harvest method. The worst agreement was on April 6-7, 1978, which was an exceptionally inclement day. That day was the last of four consecutive days in which diel studies were conducted, the first three of which demonstrated good day-to-day reproducibility although the individual diel patterns did vary somewhat.

Primary productivity estimates were also made from NO_3-N uptake measurements at different specific times throughout the day, as well as from the entire, continuous diel record. Calculated primary productivity rates for six times during each diel period (Table 3) indicated that there was better agreement

Table 2. Comparison of estimates of primary productivity for *Eichhornia crassipes* as calculated from measurements of diel NO_3-N uptake (P_D) and from direct harvesting (P_H).

Dates of diel 1978	Primary productivity (g dry weight.m^{-2}.day^{-1})		
	P_D	P_H	P_D/P_H
1/5-1/6	7.00	7.26	96.42
1/7-1/8	5.75	7.26	79.20
1/24-1/25	7.01	7.47	93.84
2/3-2/4	3.18	4.85	65.57
4/3-4/4	12.24	16.77	72.99
4/4-4/5	12.84	16.77	76.57
4/5-4/6	13.47	16.77	80.32
4/6-4/7	5.89	16.77	35.12
7/14-7/15	29.49	28.01	105.28
7/15-7/16	28.32	28.01	101.11
7/27-7/28	36.57	34.37	106.40
Average	14.71	16.76	87.77

Table 3. Diel variation in primary productivity values (g dry weight$\cdot m^{-2} \cdot day^{-1}$) calculated from NO_3-N uptake by *Eichhornia crassipes*.

Date of Diel	0400	0800	1200	1600	2000	2400
	Time of Day					
1/5-1/6/78	9.45	7.61	2.54	6.22	8.99	8.99
1/7-1/8/78	5.08	6.05	5.57	3.63	4.12	4.84
1/24-1/25/78	6.81	7.86	3.64	8.13	4.17	7.33
2/3-2/4/78	3.17	3.44	3.44	2.63	3.17	3.44
4/3-4/4/78	12.40	12.40	8.92	15.51	13.59	13.59
4/4-4/5/78	16.28	16.28	11.63	5.43	8.91	16.28
4/5-4/6/78	15.82	15.82	9.10	10.28	14.63	5.82
4/6-4/7/78	6.59	5.43	5.43	2.32	6.59	6.59
7/14-7/15/78	32.16	30.00	26.74	26.02	26.74	33.25
7/15-7/16/78	30.36	25.30	24.21	27.47	29.64	30.36
7/27-7/28/78	36.87	38.81	35.90	38.81	36.87	37.84
Average	15.91	15.36	12.47	13.31	14.31	16.21

with the harvest method at night and in the early morning (2400, 0400, 0800). Thus, if a continuous record of NO_3-N concentration is not possible, single samples taken during this period of time rather than later in the day may suffice to obtain a good approximation of primary productivity as measured by the harvest method. Estimates made from the 0800 samples were most often closest to the estimates obtained by the harvest method, although the most comparable overall average for all 11 diel experiments was with the 2400 samples.

DISCUSSION

The results of this study indicate that measurements of nutrient uptake can be used to estimate the primary productivity of aquatic macrophytes in an aquaculture system. This method also appears to be applicable to seaweeds (Hanisak, unpublished data). While our study was limited to NO_3-N, it is reasonable to assume that the uptake of other nutrients (such as NH_4-N and PO_4-P) could also be employed.

The applicability of this technique to measuring the primary productivity of natural systems is unclear due to potential problems, such as reduced ambient nutrient levels, lack of a steady state, a less homogeneous plant population, greater nutrient regeneration, and lack of clearly defined and measurable nutrient inputs and outputs of the system. However, this technique would probably be appropriate in circumstance similar to those that permit the use of diurnal oxygen or "upstream-downstream" methods of measuring productivity (Odum and Hoskin 1958, Vollenweider 1974).

There were some variations in and between most of the diel experiments, but the causes of these are unclear. However, productivity estimates obtained from other methods can vary tremendously throughout the diel period (e.g. Doty and Oguri 1957, Verduin 1957). Because of these variations, productivity measurements over a complete diel period should be preferred to those made over a smaller sampling interval (Wetzel 1965).

The limited diel variation in NO_3-N uptake is surprising; NO_3-N uptake is generally believed to be greater in the light than in the dark for aquatic macrophytes (Toetz 1971) and algae (Morris 1974), although D'Elia and DeBoer (1978) did not observe diel changes in the uptake rate of NO_3-N in the red seaweed Gracilaria foliifera growing in an aquaculture system. The ability of Eichhornia crassipes to assimilate NO_3-N just as rapidly, or more so, at night as in the day is probably of considerable ecological significance to this very weedy species that is widely distributed in tropical and subtropical areas throughout the world. This ability also indicates the potential utility of this species to remove nutrients as a component in a tertiary sewage treatment system.

At this time, it appears that nutrient uptake measurements can be used to estimate primary productivity and would be particularly useful in managing aquatic macrophytes in an aquaculture system. Additional refinements in the technique may be required as the size of the cultures is increased. Besides estimating productivity, these measurements can help monitor the effectiveness of nutrient removal either as part of a tertiary sewage treatment or to prevent potential eutrophication problems near outfalls of aquaculture systems. In this work, no attempt was made to maximize nutrient removal; quite to the contrary, NO_3-N concentration and the turnover rate were kept high enough to insure a plentiful supply of nitrogen. This method can be used with phytoplankton and seaweed cultures as well as with that of aquatic macrophytes.

ACKNOWLEDGEMENTS

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VI. Kelp farming in the People's Republic of China.

A crude form of seaweed culture has been practiced in the Orient for several hundred years, but since the life cycles of many species of marine algae have begun to be understood and controlled in the laboratory, a more sophisticated and much more successful industry has opened up. This development occurred first in Japan in the years immediately following World War II and later spread to China, following its revolution.

Seaweed culture in Japan is well known and documented. Principal emphasis is devoted to the culture of Porphyra or "nori", a luxury food that is used extensively as a flavoring agent or condiment and brings a price of $2.00 to $4.00 per dry ounce. Quality of the cultured seaweed is more important than quantity, and its high price helps to justify the intensive labor that is required to achieve very modest yields - of the order of 1-2 dry tons/acre·year.

Much less is known of the seaweed industry in the People's Republic of China, where Porphyra is also grown but in smaller quantities than the kelp, Laminaria japonica. In many parts of China, the inhabitants are subject to a chronic problem of goiter, a disease caused by iodine deficiency. The consumption of brown seaweeds rich in iodine is a prophylactic measure to prevent goiter, and Laminaria has therefore become an important item in the Chinese diet.

As its name implies, Laminaria japonica is indigenous to the cold-water environment of Hokkaido, the northern island of Japan, from which some 3000 tons/year were formerly exported to China. Now it is grown in over 750 acres of China's northern coastal water, with a production of more than 10,000 dry tons per year. This annual rate of production has far exceeded the local demand of the product for direct use as food. Over 1000 dry tons per year are now exported back to Japan, whose production has declined, and approximately half the annual production is now extracted in China for its contained hydrocolloid, alginic acid, that is used for many purposes in the food, medical and other industries.

During September-October, 1978, the Principal Investigator visited the People's Republic of China as a memeber of a U.S. Delegation of Oceanographers, and had the opportunity to observe Laminaria culture and to discuss the industry with scientists and culturists in the northern coastal cities of Tsingtao and Dalien. A brief account of Laminaria culture in China follows.

Figure 1 shows one of the 15 kelp "nurseries" in northern China, this one consisting of two large greenhouses (5200 m^2) containing shallow tanks through which fertilized, refrigerated (5-8°C) seawater is circulated. Roughly a half million gallons per day is passed through each greenhouse, three quarters of which is recirculated and one quarter discarded and replaced. As the seawater passes through the chiller, it is enriched with 4 mg/l nitrate-nitrogen and 2 mg/l phosphate-phosphorus. The greenhouse glass is painted white to permit a maximum solar intensity of no more than 4000 lux.

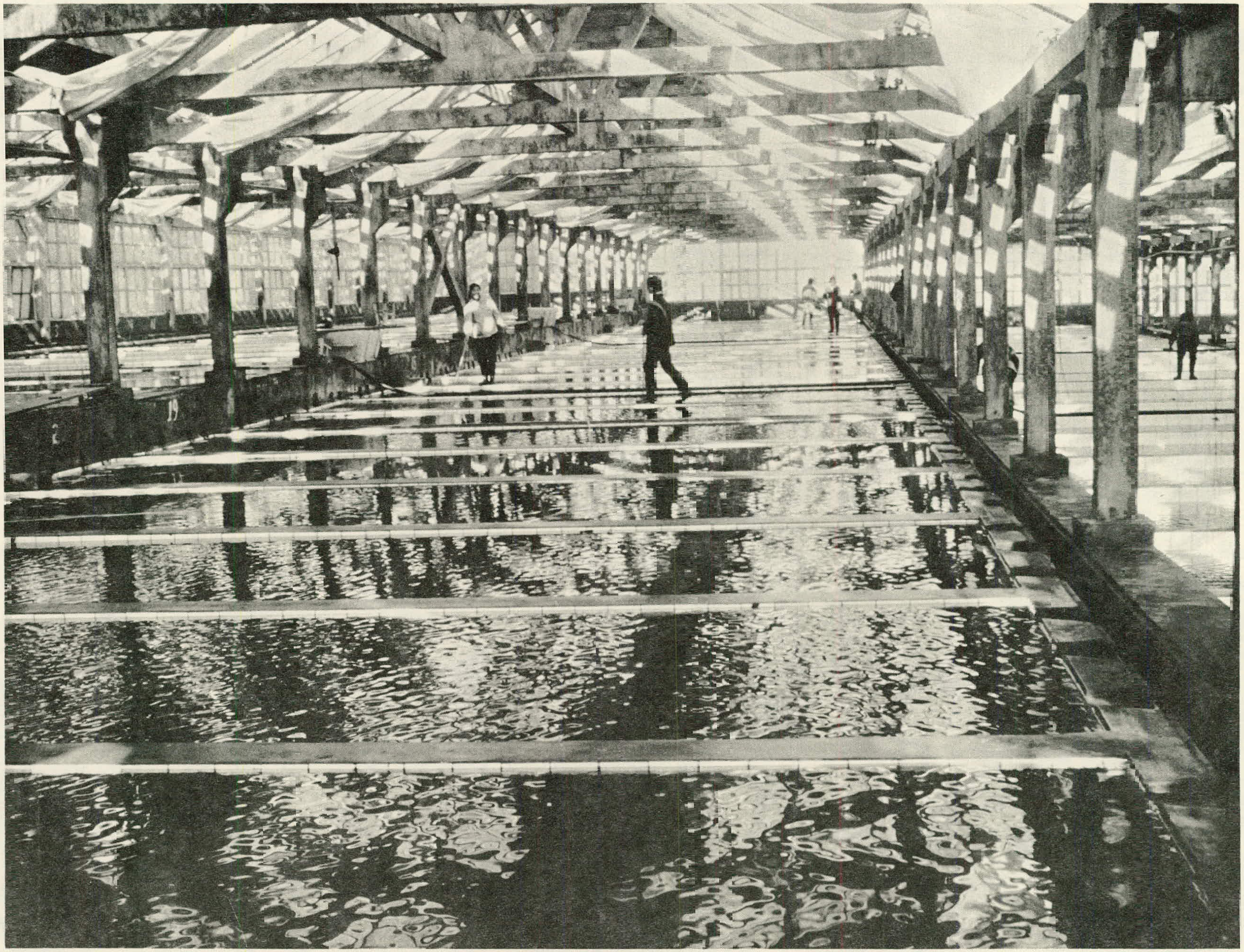

Figure 1. Kelp nursery-greenhouse in Tsingtao, China.

In spring, the shallow (ca. 10 cm deep) tanks in each nursery are filled with 10,000 wooden frames, each roughly 40 x 60 cm around which 50 meters of rough, ca. 0.5 cm diameter string is wound. Mature sporophyte plants of Laminaria are briefly sun dried to stimulate the release of zoospores and are then spread over the wooden frames, which are laid out flat in the nursery tanks. The zoospores are shed from the sporophyte plants and attach to the string frames ("spore curtains") within two hours. There the complex life cycle of the Laminaria is completed. The spores develop into microscopic male and female gametophytes (the sexual form of the alga), which quickly mature to produce sperm and eggs, the motile sperm swims to and fertilizes the egg which germinates to produce the sporeling that eventually grows into the large mature, asexual sporophyte - the familiar, obvious seaweed plant.

All of the above stages in the life cycle, from the shedding of the zoospores to the development of the young sporelings, take place in the nursery during the period June-October. When the outside water temperature falls below 20°C, in mid- to late October in Tsingtao but in late September to early October in the more northern Dalien area, the "spore curtains" are taken off their wooden frames and moved to the ocean, where they are suspended between parallel rows of large buoyed and moored ropes (Figure 2). At this point, the sporelings are 2-4 cm long and there are some 50,000 of them per 50-meter "spore curtain" (Figure 3).

When they are small, the sporelings are tended daily, lifting each "spore curtain" from the water, meticulously brushing off the sediment and attached plants and animals from each plant, and immersing the entire curtain into a tub of concentrated liquid fertilizer. When they reach the size of about 10 cm, after 25-30 days in the ocean, the entire young crop is harvested, manually stripped off the strings to which they are attached, and bundles of four sporlings each are inserted into the weave of larger, 5 cm diameter, coarse, loosely woven ropes that are again tied across the parallel suspending lines. There they remain until they are harvested over a six-week period beginning in early June.

The plants are no longer individually tended after they are transplanted, but the crop is usually fertilized. Formerly, this was done by attaching to the ropes ceramic containers of fertilizer through which the nutrients could slowly diffuse. Now it is found more expedient and effective to broadcast or spray liquid fertilizer daily over the kelp beds (Figure 4).

A section of a typical kelp farm in Dalien is shown diagrammatically in Figure 5. In Tsingtao, where the growing season is some 230 days, the kelp reach a length of about three meters at the time of harvest. Yields average 12 dry tons/acre·year. In the colder

Figure 2. Moored, buoyed lines (sets) to which kelp "spore curtains" are attached.

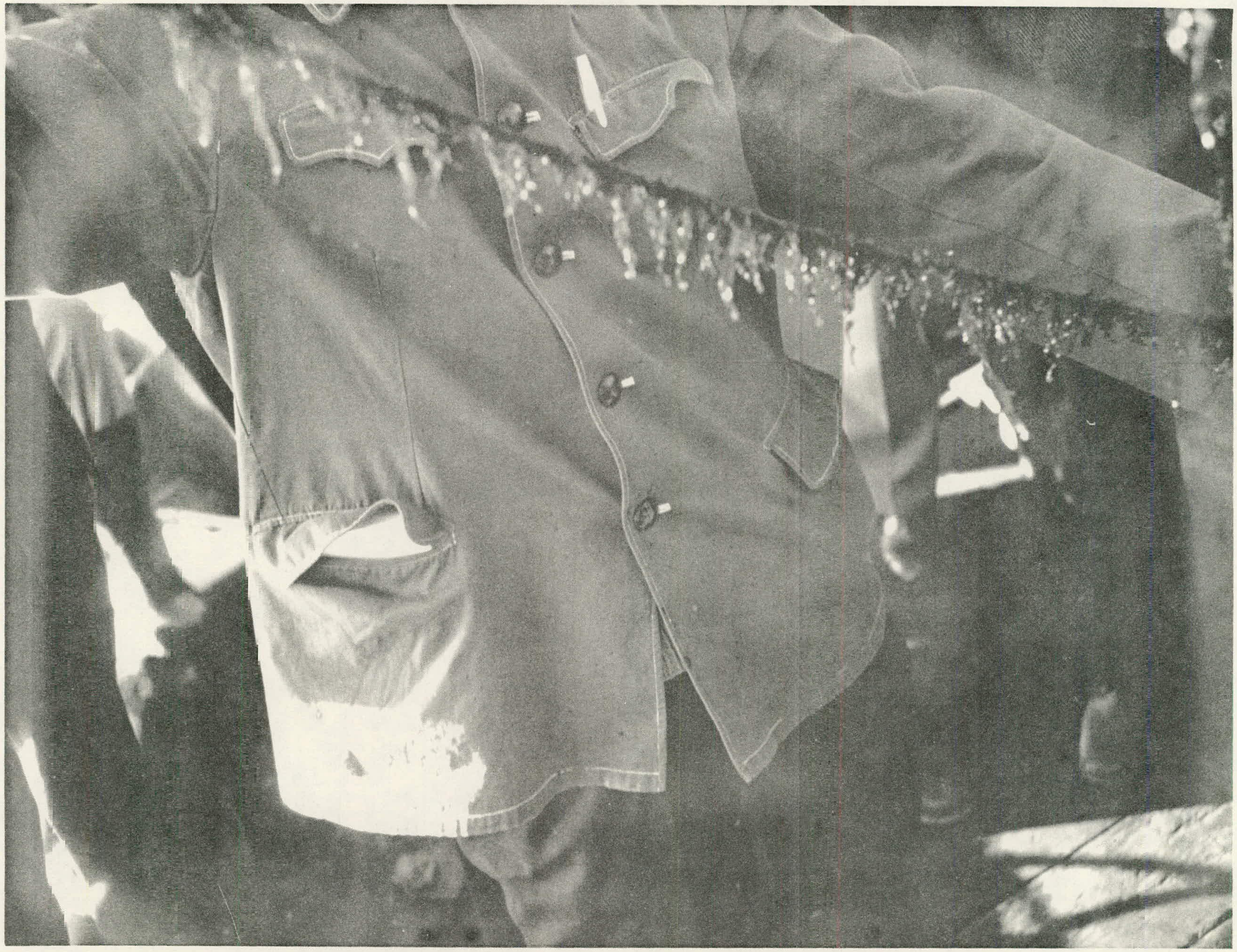

Figure 3. "Spore curtain" of kelp sporelings soon after transfer to the ocean from the nursery.

Figure 4. Spraying liquid fertilizer on kelp farm.

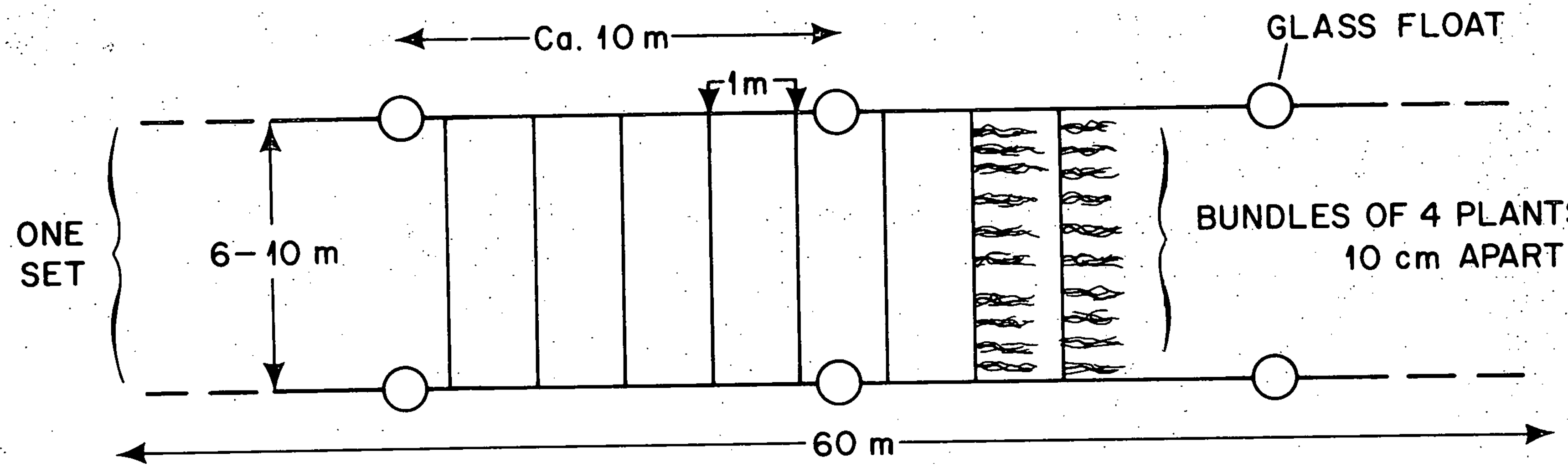

Figure 5. Diagrammatic section of a typical kelp farm in Dalien.

Dalien region, the season is perhaps one month longer. Because of that and/or for other reasons, the kelp plants there reach a length that may exceed 5 meters and yields of 20 dry tons/acre.year are reported. The wholesale value of dried kelp in China is $0.60 U.S. per pound.

The City of Tsingtao is China's main center for marine research, with its Institute of Oceanography (Chinese Academy of Sciences), Stantung College of Oceanography, and Yellow Sea Fisheries Institute (National Bureau of Fisheries). The Deputy Director of the Institute, C. K. Tseng, is himself a phycologist who received his doctorate with W. R. Taylor at the University of Michigan and worked at Scripps Institution of Oceanography (La Jolla, CA) before returning to China. T. C. Fang, Chairman of the Biology Department at Shantung College, is also a algal specialist of note. Understandably, then, much emphasis within the Tsingtao scientific community is devoted to research on seaweed culture and related subjects.

Scientists at the Institute of Oceanography have had considerable success, through X-ray-induced mutation and selective breeding, in developing pure strains of Laminaria that grow more rapidly than the wild populations, contain more iodine, and can tolerate higher temperatures. The latter feature is an important attribute that allows extension of the southern range of the species and a corresponding expansion of the Laminaria culture industry.

T. C. Fang is carrying out interesting basic genetic studies with Laminaria. By treatment with colchicine at low temperatures, Fang has been able to induce the microscopic female gametophyte of Laminaria to develop parthenogenetically into a large, undifferentiated cell mass (callus). Each callus may be considered as a genetically pure clone which may be maintained indefinitely and each cell of which, when isolated and returned to its normal environment, will develop into a normal sporophyte (i.e., the commercially-valuable seaweed plant). This pioneer work in seaweed genetics opens the door to the development of pure-breeding, improved stocks of this important seaweed, following in the footsteps of modern higher plant genetics.

The cultivation of the red seaweed, Porphyra, in the People's Republic of China is a more recent introduction and is still undergoing development. Essentially the same methods are used for growing this alga in China as are employed in Japan. Since the latter have been thoroughly documented elsewhere, the practice will not be described here except where it differs significantly. A recent innovation in the Chinese system is to spread the nets (to which the spores and later the mature plants are attached) to floating bamboo rafts (Figure 6), in contrast to the fixed nets, attached to poles driven into the bottom, which are used in Japan and initially in China. The floating rafts keep the plants permanently at or just below the sea surface and this has reputedly greatly enhanced yields. Another

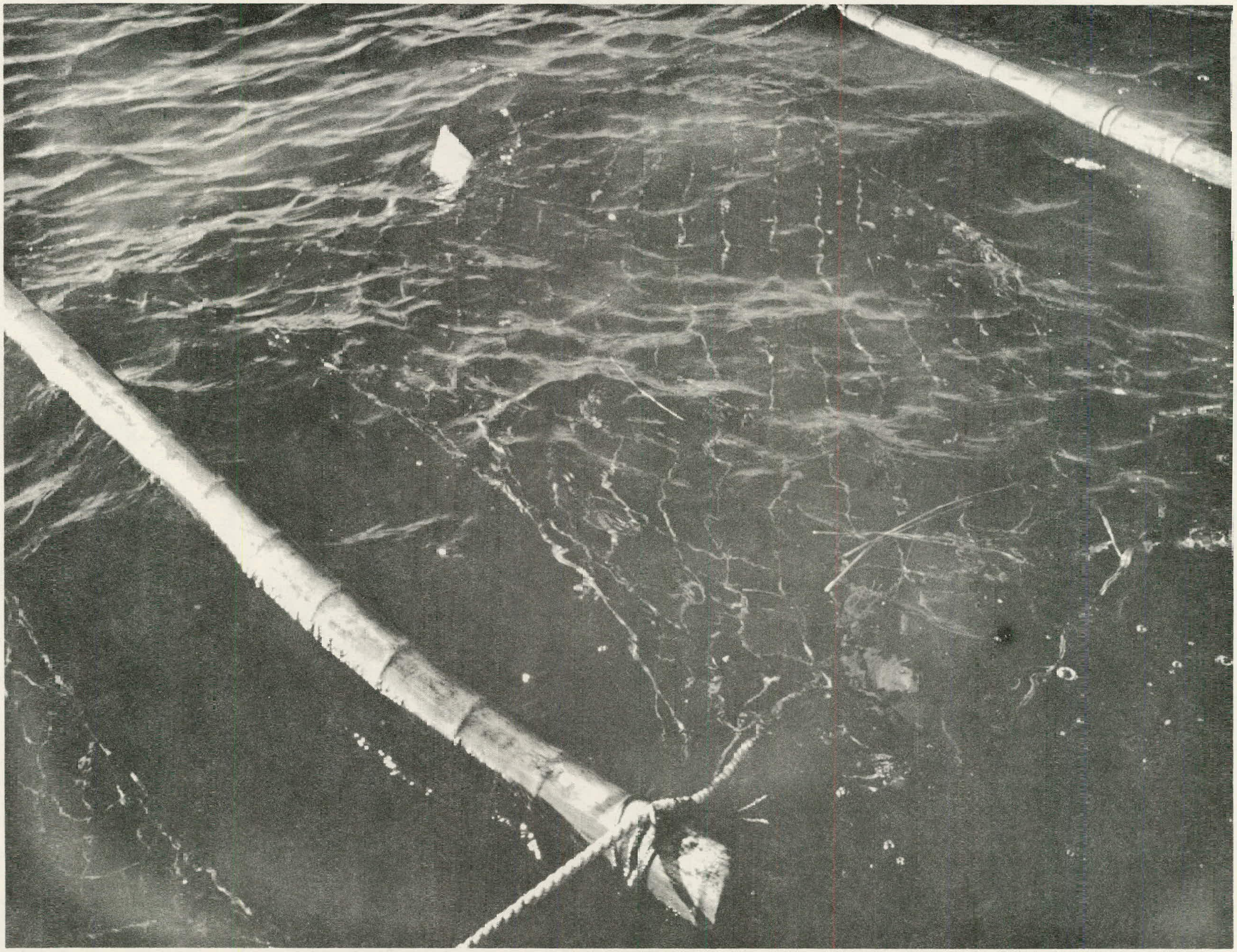

Figure 6. Floating bamboo raft for <u>Porphyra</u> culture in Dalien. Floating white object is plastic bag of fertilizer.

departure from Japanese "nori" culture is the fertilization of the *Porphyra* beds, accomplished by attaching small plastic bags of fertilizer to each bamboo raft (Figure 6), through which the nutrients slowly diffuse as they dissolve.

The small, cold water species of *Porphyra* (*P. yezoensis*), introduced from Japan, is grown in northern China, where yields of about 0.25 dry tons/acre.year are obtained. In the South China Sea region, the more tropical *P. haitanensis* is grown. That species reportedly reaches a length of more than nine meters in southern China, in contrast to *P. yezoensis*, which grows to only about one meter in the north. Yields of *P. haitanensis* from large production units are of the order of 3.5 dry tons/acre.year, and in small, experimental plots, as high as 8.0 dry tons/acre.year. Yields of *Porphyra*, though significantly higher than those in Japan, are thus much less than Chinese yields of *Laminaria*, but the higher price of *Porphyra*, over $5.00 U.S./dry lb, makes its cultivation popular in the People's Republic.

VII. *Gracilaria* farming in Taiwan

Agar-agar, the hydrocolloid contained in certain red algae (Rhodophyceae), is traditionaly manufactured in Japan, where it is extracted from one of the several species of *Gelidium* that are indigenous to that country. Faced with limited or complete loss of supply from Japan during World War II, many countries started agar industries of their own. Beginning in 1944, Taiwan began to collect *Gelidium* from natural stands along its northern and eastern coastline. However, *Gelidium* stocks were limited and difficult to harvest, and the industry never became very well developed.

In 1962, members of the Chilou Fishermen's Association first demonstrated the feasibility of culturing *Gracilaria*, another genus of the red algae that produces agar. Since then, the practice has grown steadily, largely utilizing ponds that were originally constructed for milkfish farming (Figure 1). The latter is carried out with some difficulty in Taiwan. Milkfish is a tropical species that does not easily survive the winters of Taiwan. The fish must be held in protected deep channels over the winter months (Figure 2) and do not grow at that time of year. Milkfish has also never been successfully spawned in captivity and the industry is dependent upon fry captured along the coastline at restricted times and places. The young fish are often both scarce and expensive.

Gracilaria may be grown in the same ponds as milkfish. The practice is more labor intensive than the fish farming, but when

Figure 1. Gracilaria pond near Tainan, Taiwan formerly used for milkfish farming.

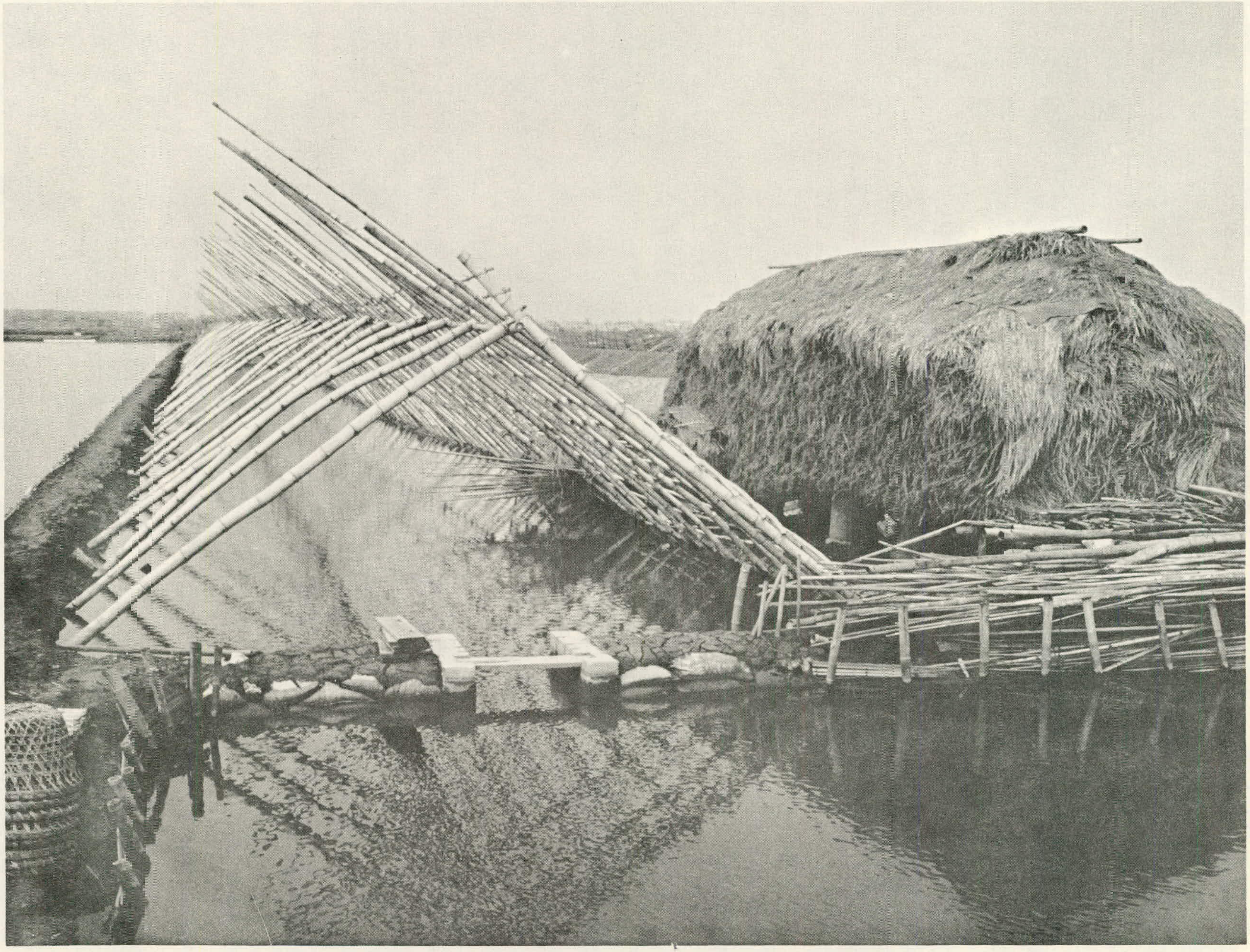

Figure 2 Deep, over-wintering channel in Gracilaria-milkfish pond with bamboo frame. Hay at right is used to cover frame and provide shelter from wind.

labor is available and cheap, the farmer can gross five to ten times the income from the seaweed as he can from milkfish. From its beginnings in 1962, the Gracilaria industry of Taiwan has grown to a production of 2500 dry metric tons in 1973 and 6800 metric tons in 1977, the latter harvested from a total culture area of 400 hectares.

A portion of the Gracilaria grown in Taiwan is processed and the agar extracted at one of the six Taiwanese agar plants. However, a growing proportion of the harvest is put through its initial alkaline processing in Taiwan and then shipped to Japan for agar extraction, where good quality material (> 30% agar with gel strength of > 500 g/cm^2) brings a price as high as $2000/metric ton.

The old milkfish ponds where Gracilaria is now grown are usually rectangular, between one and ten hectares in size, and about one meter deep when filled to capacity. The bottom is hard, sandy loam (soft mud bottom is considered undesirable both for growth and harvesting). The ponds are located adjacent to estuaries or tidal rivers so that they may be filled or replenished by pumping from the estuary.

Maintenance of a salinity ranging between 22 and 35‰ is one of the most critical aspects of pond management. The Gracilaria farmer continually monitors salinity with a hydrometer, and must add as much as 10 cm per day of freshwater (pumped from the estuary during low tide) during the winter, dry season to compensate for evaporation.

In the wet season (summer), the opposite problem of excessive freshening of the pond is encountered. This is countered by pumping out a fraction of the pond contents and replacing it with saline water. However, most of the ponds are situated far enough upstream that highly saline water can be obtained only during extremely high (spring) tides. Thus management is summer is more difficult than in winter, and is particularly difficult during an excessively rainy wet season. The *Gracilaria* farmer's dilemna is that proper pond management is most difficult during the best growing season for the seaweed and easiest in winter, when growth is minimal.

Addition of water from the estuary also provides the other important function of fertilizing the *Gracilaria* with essential nutrients. The farmer has no way of measuring nutrients, but judges the current state of the pond's fertility by its clarity, since the addition of nutrient-rich water also stimulates the growth of phytoplankton suspended in the pond water. With the pond stocked and maintained with the proper density of seaweed, the phytoplankton cannot grow to excessive "bloom" proportions, a situation that is undesirable because it shades the *Gracilaria* and retards its growth. The farmer must be careful to leave a sufficient biomass of seaweed when he harvests to prevent such phytoplankton dominance as well as to maintain optimal production from the *Gracilaria* itself. But when the water becomes perfectly clear and transparent, due to the absence of phytoplankton, the farmer also realizes that nutrients are exhausted from the water, a signal to add more fertile estuarine water.

Fertilization practices appear to vary. When the ponds are first prepared for Gracilaria culture, the newly-introduced water may be enriched with fermented pig manure at a rate of about 100 kg/hectare. Some farmers add no further enrichment other than the practice, referred to above, of adding nutrient-rich estuarine water as needed. Others use either inorganic or organic fertilizers routinely, in one example 3 kg urea/hectare/week.

The best growth of the seaweed occurs in the temperature range of 20°-25°C. Growth stops below about 12°C, but the plants can tolerate temperatures as low as 8°C. In Southern Taiwan, where virtually all of the Gracilaria is grown, the normal water temperature range in the ponds is from about 10°C in winter to about 30°C in summer. Pond depth is carefully regulated seasonally, partly to control temperature and partly to control the intensity of sunlight that penetrates to the seaweed on the pond bottom. In summer, the ponds are maintained at 60-80 cm depth and in winter, at only 30 cm depth.

Several species of Gracilaria are cultured in Taiwan, often together in the same pond. The most popular appears to be that identified by the government biologists as G. confervoides. Cuttings or torn fragments of the seaweed, purchased from other farmers, are used for seed stock and are introduced to a new farm at a density of 3-5 kg wet wt/m^2. The plants are evenly spread over the pond bottom and grow there vegetatively throughout the year. When the population has roughly doubled in density and biomass, as estimated by eye, half the crop is harvested by a crew of 10-20 women, half of whom rake the seaweed into piled rows on the pond bottom, and the other half of whom dip-net the plants out of the water and into large bamboo baskets on wooden barges (Figures 3,4). The remaining half of the crop is then spread evenly over the pond bottom.

The netted seaweeds are shaken in the water to remove sediments, epiphytic diatoms, snails, and other animals that live in the plants. They are then spread out on flat earthen or concrete surfaces and allowed to sun-dry, turning the plants once to facilitate and hasten the drying process (Figure 5).

There are usually seven to eight harvests per year, each of one to three dry tons/hectare, mostly occurring from June through December. Little if any growth occurs during the late winter and very early spring and the stocks are sometimes held in deep, protected areas during the coldest part of winter, using the same covered shelters as were originally designed for milkfish culture (Figure 1).

Some farmers harvest smaller crops more frequently during the growing season (i.e., every 10 days or so) but annual yields are approximately the same whatever the harvest routine. Yields range from 10 to 20 dry tons/hectare·year and average about 14 t/ha·year.

Figure 3. Raking the Gracilaria into rows on bottom prior to harvesting.

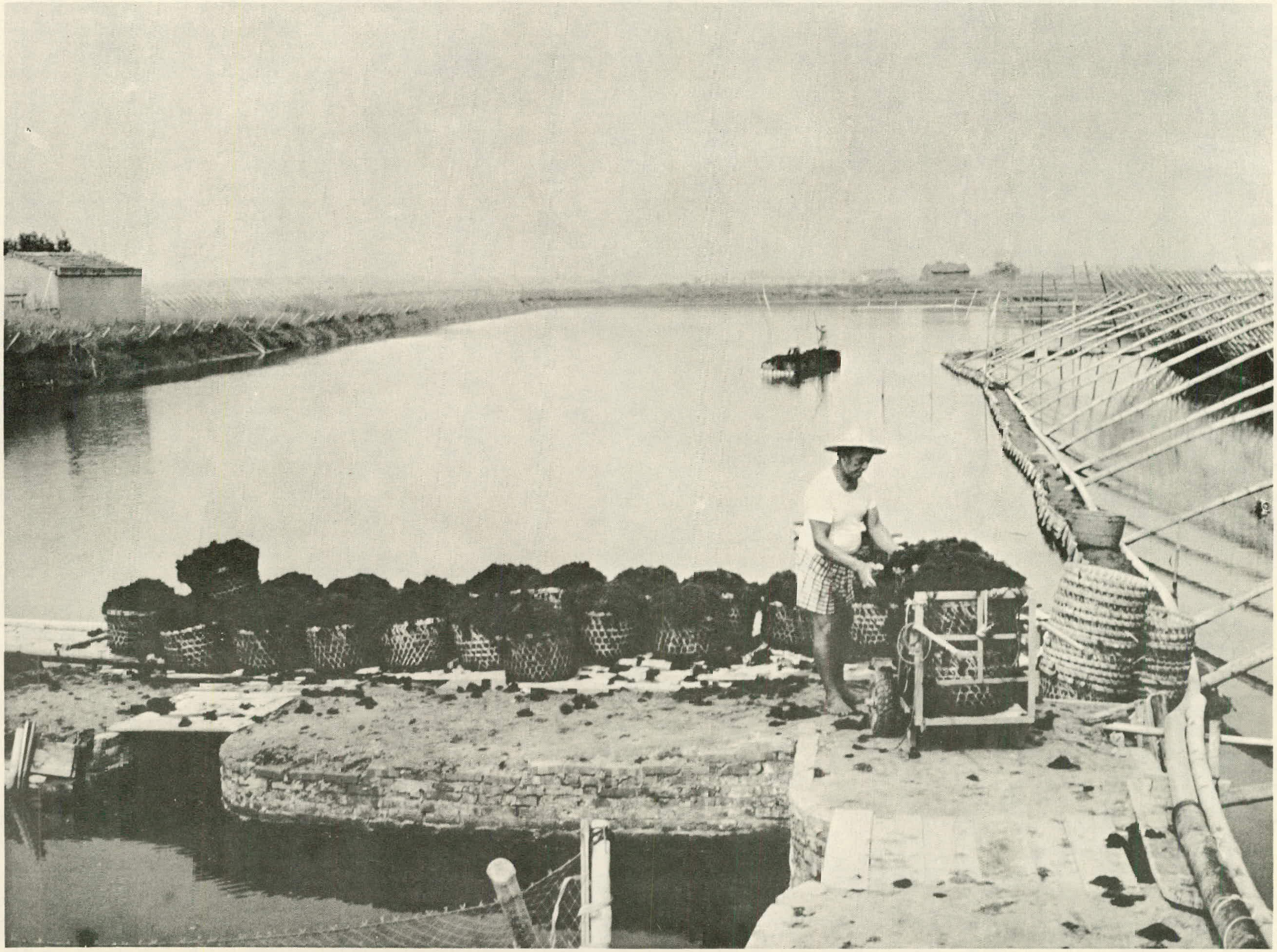

Figure 4. Baskets of <u>Gracilaria</u> harvested from pond.

Figure 5. *Gracilaria* spread on hard earthen surface for sun-drying.

There is some problem with epiphytes, principally the filamentous green algae Enteromorpha and Chaetomorpha, growing on the Gracilaria. This is controlled by stocking 500-1000 herbivorous milkfish/hectare (150 gram or larger fish), which preferably graze upon the epiphytic algae. After the seaweeds are thoroughly cleaned of the epiphytes, however, the milkfish will turn to the Gracilaria itself for nourishment, so the fish must be netted out after they have performed their cleaning service.

The other chronic problem in Gracilaria culture is maintaining the culture evenly distributed on the bottom. Ponds are usually oriented with their long axis perpendicular to the prevailing wind, and there is often a wind-break of trees or other vegetation planted on the up-wind side of the pond. Despite this, strong winds will often pile up the loose plants along the downwind side of the pond, and considerable hand labor is required to spread them out evenly again by raking.

Additional income is often obtained by the Gracilaria farmer through the simultaneous rearing of shrimp and/or crabs together with the seaweeds. These animals are not fed, but obtain their nutrition from animals that occur naturally in the ponds. Some of the food organisms grow as epizoa on the Gracilaria, so the secondary crop also functions as cleaners of the seaweeds. The farmers may realize up to 10-20% of the income of his pond from such ancillary crops.