

245  
2-21-78

Sh. 1856

COO/2948-1

**CULTIVATION OF MACROSCOPIC MARINE ALGAE  
AND FRESHWATER AQUATIC WEEDS**

**Progress Report, May 1—December 1, 1976**

**By  
John H. Ryther**

**MASTER**

**February 1977**

**Work Performed Under Contract No. EY-76-S-02-2948**

**Woods Hole Oceanographic Institution  
Woods Hole, Massachusetts**



**U.S. Department of Energy**



**Solar Energy**

**DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED**

## **DISCLAIMER**

**This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.**

## **DISCLAIMER**

**Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.**

## NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

This report has been reproduced directly from the best available copy.

Available from the National Technical Information Service, U. S. Department of Commerce, Springfield, Virginia 22161.

Price: Paper Copy \$4.50  
Microfiche \$3.00

CULTIVATION OF MACROSCOPIC MARINE ALGAE  
AND FRESHWATER AQUATIC WEEDS

Progress Report  
for Period May 1, 1976 - December 1, 1976

John H. Ryther

Woods Hole Oceanographic Institution  
Woods Hole, Massachusetts 02543

NOTICE  
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Department of Energy, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

February 1977

Prepared for

The U. S. Energy Research and Development Administration

Under Contract No. E(11-1)-2948

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

## ABSTRACT

The research carried out under ERDA Contract E(11-1) 2948 has been divided, scientifically and geographically, into two parts. The first, carried out at the Woods Hole Oceanographic Institution, consisted of studies of the basic biology, physiology, biochemistry, and nutrition of certain species of seaweeds that were already established in culture. These were the red algae (Rhodophyta), Neogardhiella baileyi and Gracilaria foliifera. These species are of existing or potential commercial value. The other phase of the work, carried out at the Harbor Branch Foundation Laboratory, Ft. Pierce, Florida, consisted of three parts:

1. As many species of seaweeds as possible were collected from local Florida waters and screened for their growth potential under natural sunlight and temperatures but in artificial culture systems. A standard growth assay procedure and physical system was developed. Species to be screened for their growth potential are being evaluated at different times of the year to determine whether they are suitable for cultivation throughout the year.
2. Cultures of several, if possible, but of a least one species of seaweed were maintained throughout the year to measure sustained, annual productivity so as to obtain a better understanding of the potential annual yield of seaweed biomass.
3. The development and evaluation of new, non-energy intensive and non-labor intensive seaweed culture methods that might find application in large-scale plantations, and that would be energy cost-effective, is the third phase.

Progress to date:

The research proposed under ERDA Contract E(11-1)-2948 was to be divided scientifically and geographically, into two parts. The first, to be carried out at the Woods Hole Oceanographic Institution, consisted of studies of the basic biology, physiology, biochemistry, and nutrition of certain species of seaweeds that were already established in culture. These were the red algae (Rhodophyta), Neogardhiella baileyi and Gracilaria foliifera. These species are of existing or potential commercial value, Neogardhiella for its content of the hydrocolloid, carrageenan, and Gracilaria as an agarophyte. Although both are well known, relatively little work has been done on their growth and nutritional physiology, as is generally true with most seaweeds. Such information was considered necessary if these, or other related species, were to be artificially cultivated on a large scale and on either a cost-effective or an energy-effective basis.

The other phase of the work, to be carried out at the Harbor Branch Foundation Laboratory (Ft. Pierce, Florida) consisted of three parts. First, as many species of seaweeds as possible were to be collected from local Florida waters and screened for their growth potential under natural sunlight and temperatures but in artificial culture systems. For this purpose, a standard growth assay procedure and physical system was developed. Species

to be screened for their growth potential would be evaluated at different times of the year to determine whether they would be suitable for cultivation throughout the year.

The second objective of the research in Florida was to maintain cultures of several, if possible, but of at least one species of seaweed throughout the year and to measure its sustained, annual productivity (as opposed to short-term yields) so as to obtain a better understanding of the potential annual yield of seaweed biomass.

The third objective was to develop and evaluate new, non-energy intensive and non-labor intensive seaweed culture methods that might find application in large-scale energy plantations and that would be energy cost-effective.

At the time this report was written, Contract E(11-1)-2948 had been in effect for seven months. Because part of that time was required for recruiting personnel, purchasing equipment and supplies, and fabricating experimental systems, progress during the period has been limited. Preliminary results obtained at Woods Hole are included in Woods Hole Oceanographic Institution's Technical Report WHOI-76-92 (October, 1976). Those obtained in Florida are included in Harbor Branch Foundation's Aquaculture Project Annual Progress Report (December, 1976). Both documents have been submitted separately to ERDA. The relevant sections of these reports are summarized below.



Research at Woods Hole, Mass.:

Seaweeds were initially cultured at Woods Hole as part of a combined waste recycling-aquaculture project, the objectives of which were to remove the nutrients from secondary sewage effluent at the same time producing commercially-valuable crops of marine organisms. The system that was developed for this purpose consisted of large cultures of unicellular marine algae (phytoplankton) grown on mixtures of sewage effluent and seawater and fed to oysters and other bivalve molluscs. The phytoplankton removed the nutrients from the wastewater and the oysters removed the algae, leaving a clean effluent and a crop of shellfish. However it was found that a final polishing step was needed to remove any nutrients not initially assimilated by the phytoplankton as well as those regenerated through the metabolism and excretion of the oysters and/or other cultured animals. This polishing step consisted of seaweeds grown in suspended culture by vigorous aeration. The species selected for this purpose were red algae (Rhodophyta) that have existing or potential value for their hydrocolloids, agar or carrageenan, that are used in the food, drug, and cosmetic industries, in bacteriological research, and, in the Orient, for food.

Initially, the species used was Chondrus crispus (Irish moss), the local red algae harvested in New England and the Canadian Maritimes as a source of carrageenan. There is a ready

market for Chondrus, it has been well studied, and it has been the subject of intensive culture research by A. C. Neish and his collaborators in Nova Scotia, who kindly supplied us with a fast-growing strain (T-4) isolated in their screening tests. We found, however, that Chondrus at best grew slowly in our system (both the Neish T-4 strain and local clones), it became heavily epiphytized with other species of undesirable algae, and it could not tolerate our high ( $> 20^{\circ}\text{C}$ ) summer temperatures. Chondrus was therefore replaced with two more-southern species that occur in the Woods Hole region as summer annuals. These are Neogardhiella baileyi, a carrageenan producer like Chondrus, and Gracilaria foliifera, an agar-containing plant.

Both N. baileyi and G. foliifera proved highly successful in our system throughout the year except for the winter months (December-March) when growth ceased entirely due presumably to a combination of low temperature and solar radiation. Despite that, annual dry weight yields averaged  $15 \text{ g/m}^2/\text{day}$  for Neogardhiella and  $9 \text{ g/m}^2/\text{day}$  for Gracilaria. Excluding the four winter months, when zero growth occurred, yields for the remaining 8 months averaged 22 and  $15 \text{ g/m}^2/\text{day}$  respectively for the two seaweeds. These are extremely high values for sustained photosynthetic yields over long periods of time, making these algae rank well among the most productive plant crops on earth.

One of the basic questions in seaweed culture is how dense a population should be maintained (i.e., what biomass per unit area and/or volume) to achieve maximum yields. This, however, cannot be simply answered, since it is dependent upon incident radiation and may differ from species to species. Our experiments have demonstrated that specific growth rate (growth per unit weight or percent increase per unit of time) is very high (20-30% per day) at very low densities of the plants, but that this value decreases rapidly with increasing densities to a value of zero at high biomass levels. Many people mistakenly confuse specific growth rates with yields (i.e., production per unit of area and time), the latter being the product of specific growth rate and density. Maximum yields are obtained at intermediate densities that differ, depending upon season, solar radiation, species, and perhaps other factors, but appear from our preliminary results to fall between two and four kg (wet weight)/m<sup>2</sup>, at which specific growth rates range from about 4-8%/day.

In a continuous flow system, an equilibrium becomes established, under steady-state conditions, between the concentration of nutrients in the incoming seawater and that in the volume of the culture itself (and the effluent from the culture). Nutrient uptake and, over long periods of time, growth of the algae are determined by the residual concentration in the culture. Preliminary experiments suggest that the residual nitrogen

concentration necessary for the maximum rate of uptake (and perhaps maximum growth rate) of Neoagardhiella is as high as 10  $\mu$  moles/l, ten times or more than that for most marine phytoplankton. In other words, rather high residual levels of nitrogen must be maintained in the culture medium and the effluent to achieve maximum rates of nutrient removal by the seaweeds.

There appear to be other areas in which the nutrient uptake kinetics of the seaweeds differ significantly from that of the microscopic algae. We have, for example, obtained evidence that nitrogen assimilation in the seaweeds is closely coupled with photosynthesis and that little or no uptake occurs in the dark. This is in direct contrast to the phytoplankton, in which there is no diel periodicity in nutrient removal. The seaweeds also show a strong preference for ammonium over nitrate as a nitrogen source. Although such is also the case with phytoplankton, most species of the latter can readily adapt to nitrate utilization, in the absence of ammonium, through induction of a nitrate reductase system. Such a shift appears to be much slower and more complex in the seaweeds, though there is evidence that it eventually does take place.

Some very preliminary studies have also been carried out on the production of carrageenan by Neoagardhiella baileyi and the relationship between the content of hydrocolloid, residual nitrogen content of the culture medium, nitrogen:carbon ratio

of the plant tissue, and the red pigment (phycoerythrin) content of the alga. By studying these relationships, it is hoped that the carrageenan content and hence the commercial value of the seaweeds can be quickly and easily assessed by visual inspection or by simple chemical tests.

As A. C. Neish and his collaborators found with Chondrus, the carrageenan content of N. baileyi was highest (about 36% of ash-free dry weight) in plants grown at very low concentration of nitrogen. However, the plants grew much faster at higher nutrient concentrations so that, although their specific weight content of the hydrocolloid was significantly lower, total production or yield of carrageenan per unit area and time was higher.

The seaweed cultures normally contain large numbers of herbivorous crustacea. Because all of the plants are never harvested at any one time, and because these small grazers reproduce rapidly, large numbers of the animals accumulate in the plant populations. Thus, there is the possibility that a significant fraction of the algal production may be consumed by these animals. A study was therefore undertaken to measure feeding rates and to determine algal food preferences by two of the herbivorous crustacea commonly found in the seaweed cultures, the large isopod, Idotea baltica and the smaller tube-building amphipod, Ampithoe valida. Although both of the cultured

seaweeds, Neogardhiella baileyi and Gracilaria foliifera are eaten by both animals, neither ranks high in preference among the several algal species tested. From measured feeding rates and the observed density of the animals in the seaweed cultures, estimates could be made of the impact of their grazing on algal productivity. This was generally low, of the order of 1% during most of the year, but was significant (ca. 8%) when productivity was very low. It could be concluded from these studies that herbivorous grazing is probably not normally a problem in seaweed culture, but that it should be guarded against in situations where non-growing stocks of the seaweeds are being held for extended periods of time.

#### Research at Ft. Pierce, Florida:

Seaweeds have also been grown at the Harbor Branch Foundation Aquaculture Project as part of a waste recycling-aquaculture system similar to that developed at Woods Hole. Initially, the plants were grown as a final polishing step in a polyculture system, receiving the wastes from shellfish cultures. Later, the seaweeds were grown directly on mixtures of sewage effluent and seawater, as a one-step waste recycling-aquaculture system.

Several species of red seaweeds containing the hydrocolloids carrageenan or agar were cultured in preliminary experiments of varying but usually rather short (1-3 weeks) duration during

1974 and 1975, employing rectangular, 1.2 x 2.4 m (2000 liter) plywood tanks with sloping bottoms and vigorous aeration. Only one of those species has subsequently been found to subsist and grow throughout the entire year at the climate and under the environmental conditions existing at the Harbor Branch Foundation aquaculture facility. This was Gracilaria foliifera (initially identified and reported as G. cylindrica). This alga was consequently maintained in culture in two of the rectangular tanks throughout 1976, during which the seaweed was periodically harvested, weighed, and the incremental growth since the previous harvest removed, maintaining a starting biomass each time of approximately 2 kg (wet wt)/m<sup>2</sup>. There were, however, three interruptions in this annual record, twice when the Gracilaria became heavily epiphytized by other algae and had to be replaced with new specimens collected from nature, and once when the culture tanks had to be rebuilt. The yields of G. foliifera, expressed as dry wt/m<sup>2</sup>/day, are shown for the individual harvest intervals in Figure 1, which also includes the annual yield of the same species grown in Woods Hole, Mass. under similar culture conditions. The total annual production of this seaweed at the two locations is shown on Table 2, where it may be seen that yields in Florida are almost exactly twice those in Massachusetts, despite the use of heated water

in the latter case. The difference is presumably due largely to the difference in incident solar radiation between the two locations (Figure 1).

One of the stated objectives of the ERDA-sponsored seaweed research at Harbor Branch Foundation was to screen as many species of algae as possible for their potential use as a cultured source of biomass for energy conversion. Such a screening process includes evaluation of maximum sustained growth rate throughout the year as well as consideration of such factors as viability, availability of replacement stocks, susceptibility to disease and epiphytization by other, undesirable algae, and the ability to control reproduction and prevent the culture from disintegrating into reproductive spores or gametes (all of which affect annual yields).

For the screening program, a standard assay system was developed which consists of four 6 m-long, longitudinally-sectioned, .4 m diameter PVC pipes divided into .75 m (50 liter) compartments by means of plywood sections. Each section is provided with a calibrated flow of enriched seawater and a overflow drain. Compressed air is fed into the bottom of each compartment through holes drilled along the bottom of the pipe connecting to an air line cemented to its outside. Thirty-two individual assay chambers were produced in this way (see Figures 2 and 3). These are operated so as to provide as nearly as possible optimal



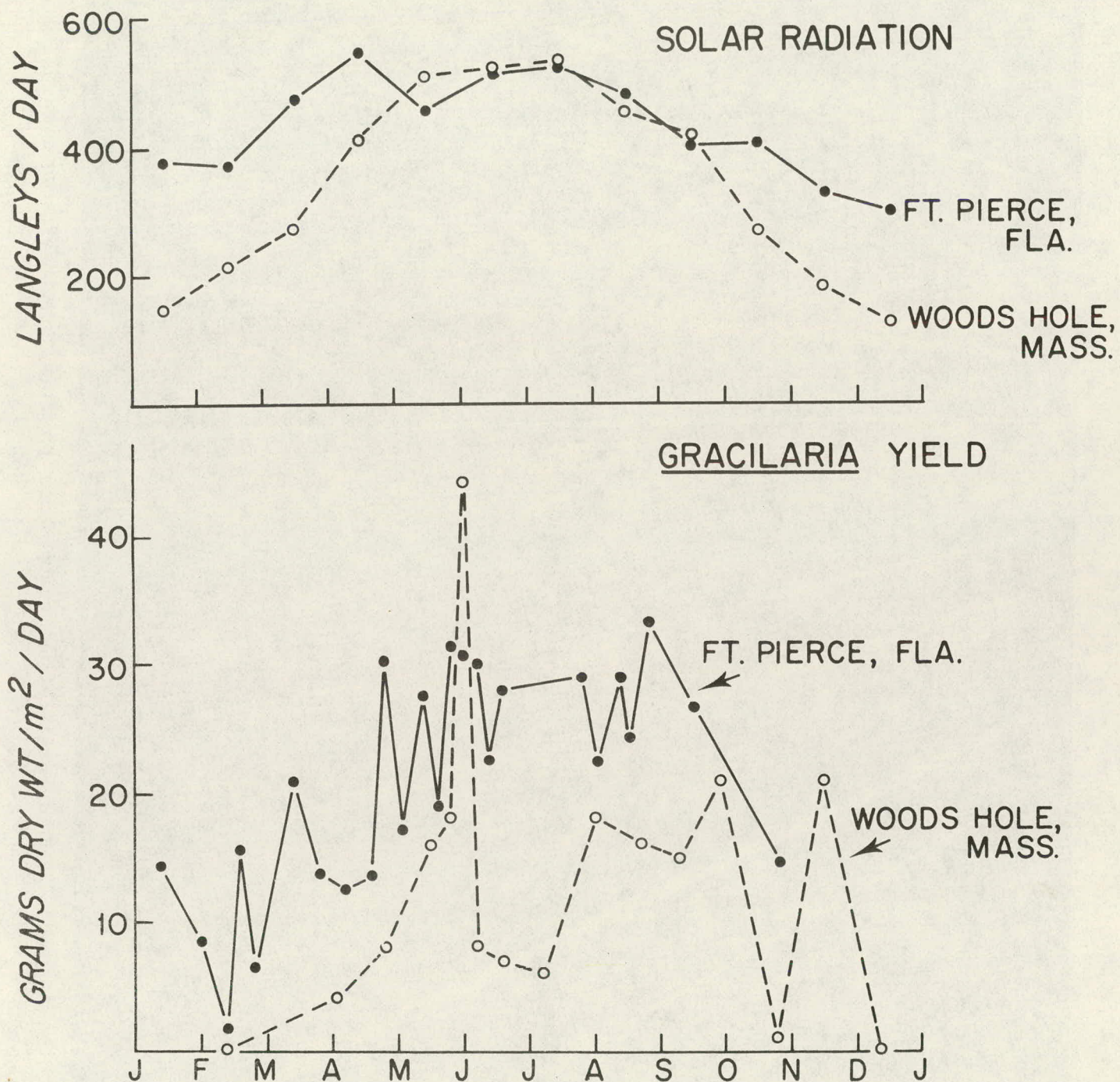


Figure 1. Solar radiation and yields of Gracilaria foliifera at Ft. Pierce, Florida and Woods Hole, Massachusetts.



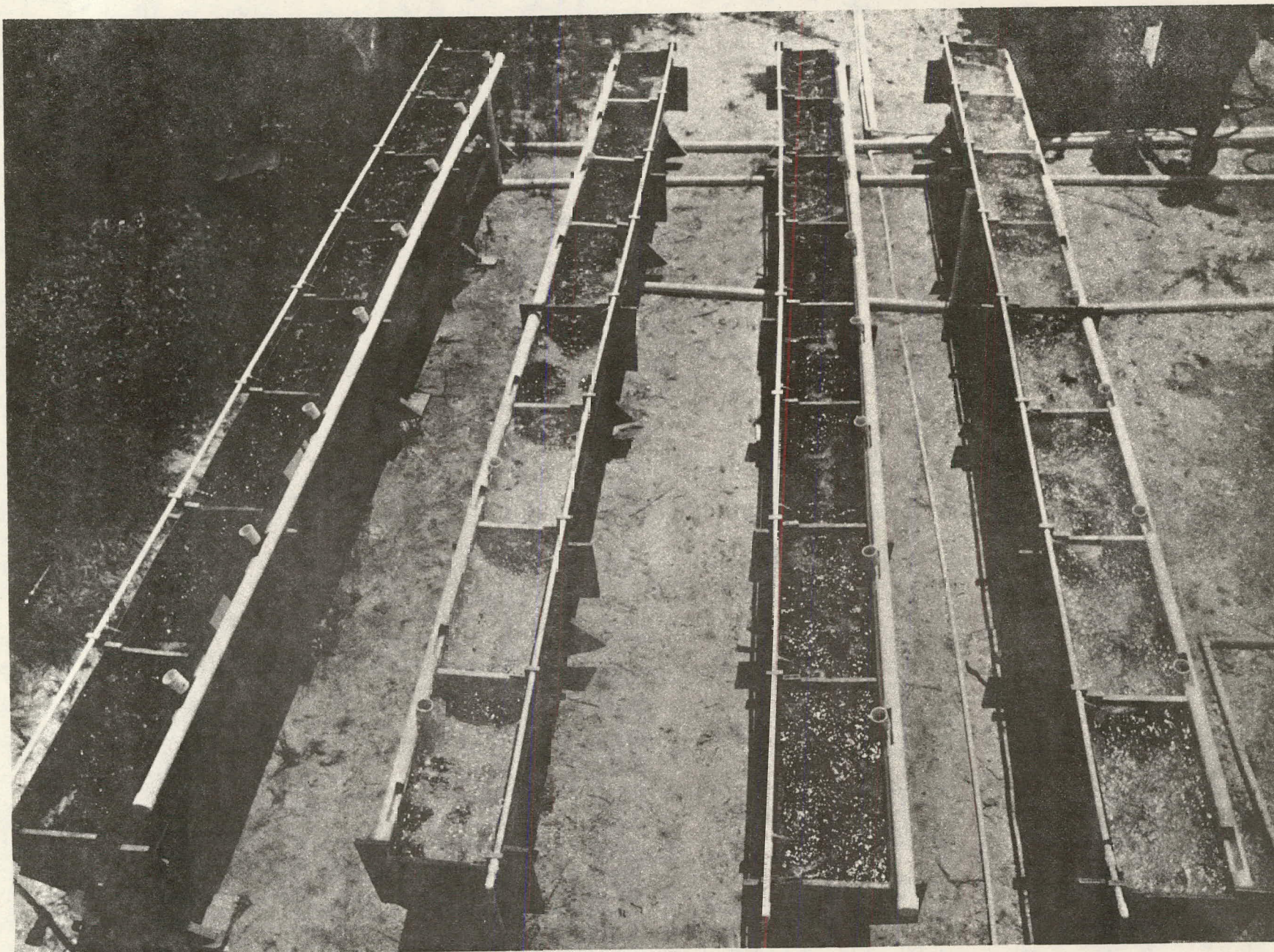


Figure 2. Experimental culture troughs for screening growth of seaweeds.



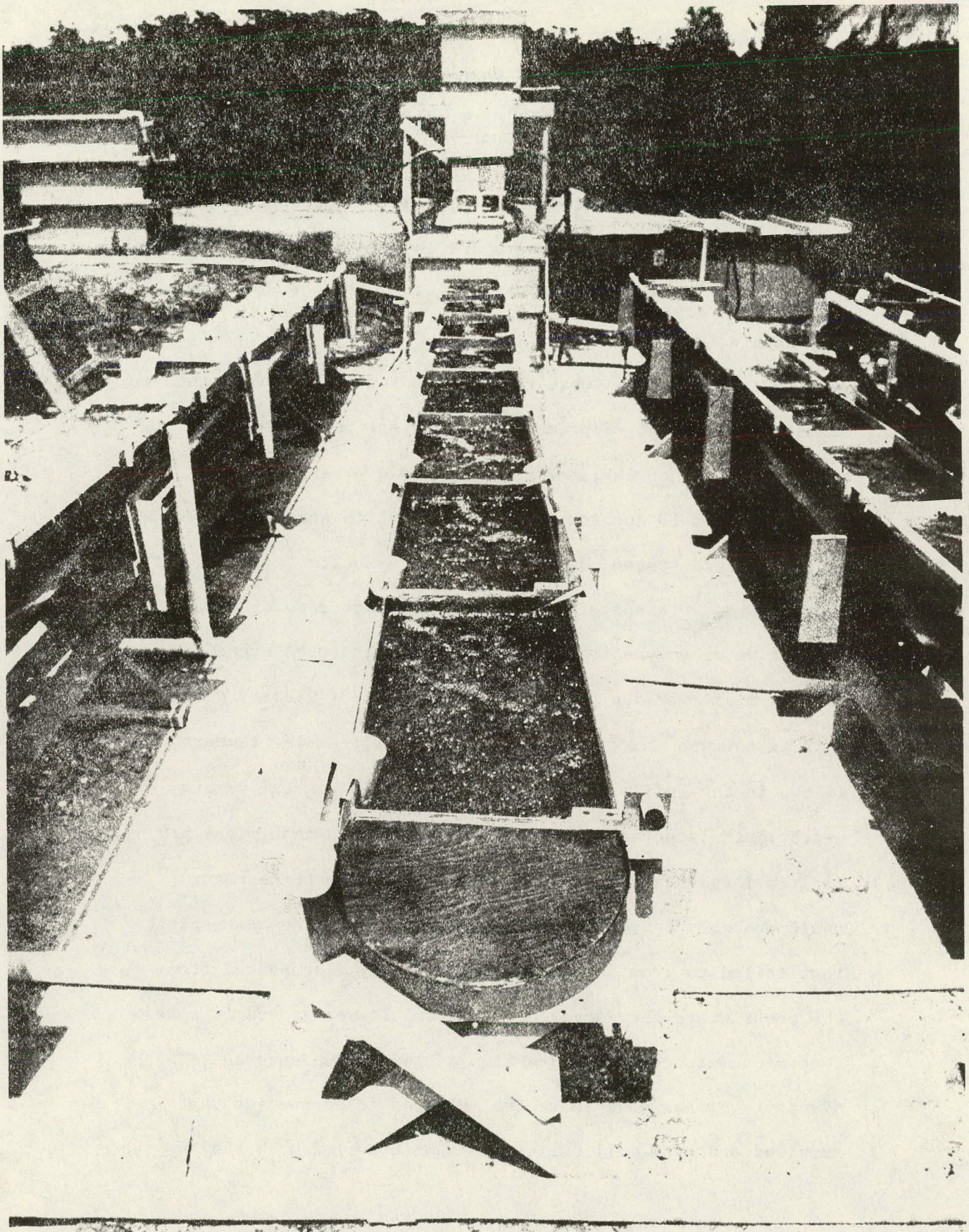


Figure 3. Close-up of seaweed screening troughs showing headbox for medium supply.



growth conditions for the algae: a high turnover rate of 22 volume exchanges per day at relatively low nutrient concentrations of 14  $\mu$ moles/l sodium nitrate and 3  $\mu$ moles/l sodium phosphate. The culture chambers are located out-of-doors in full sunlight so as to simulate natural conditions as closely as possible.

Table 3 shows the results of screening tests during the summer of 1976 (July-September). Each test period lasted approximately one week, for which period dry weight yields are shown. Of 13 species tested, 8 failed to survive or grow in the culture system, due primarily it is believed to high temperatures. In spite of the rapid turnover rate of the ambient water volume in the cultures, temperatures exceeded 35°C in midsummer due to the shallow depths of water. While this is somewhat more extreme than ambient seawater temperatures (i.e., in the Indian River or Atlantic Ocean coastal waters nearby), it is perhaps close to the conditions that might prevail in large-scale culture systems in which water exchange would probably be much less rapid. Several of the same species that failed to grow in culture subsequently disappeared from the areas where they were collected and it was generally noted that midsummer represented a time of least abundance and diversity of seaweeds in central Florida. The species that survived and grew well during the summer period were the two red

Table 3. Marine algae screened for growth potential at Harbor Branch Foundation, 1976.

| Species                         | Dates                 | Yield (g dry wt/m <sup>2</sup> /day) |
|---------------------------------|-----------------------|--------------------------------------|
| Summer (July-September)         |                       |                                      |
| <b>Chlorophyta</b>              |                       |                                      |
| <u>Enteromorpha clathrata</u>   | 7/20-7/26             | 55.8                                 |
|                                 | 7/26-8/2              | 47.8                                 |
|                                 | 8/2-8/9               | 27.2                                 |
|                                 | Mean, all experiments | 42.3                                 |
| <u>Chaetomorpha linum</u>       | 7/20-7/26             | 36.7                                 |
|                                 | 7/26-8/2              | 38.1                                 |
|                                 | 8/2-8/9               | 24.6                                 |
|                                 | 8/11-8/16             | 36.4                                 |
|                                 | Mean, all experiments | 34.0                                 |
| <b>Rhodophyta</b>               |                       |                                      |
| <u>Gracilaria foliifera</u>     | 7/20-7/26             | 48.0                                 |
| V. angustissima                 | 7/26-8/2              | 49.7                                 |
|                                 | 8/2-8/9               | 45.6                                 |
|                                 | 8/11-8/16             | 49.5                                 |
|                                 | 8/16-8/23             | 43.3                                 |
|                                 | 8/27-9/2              | 45.6                                 |
|                                 | 9/2-9/7               | 48.0                                 |
|                                 | 9/8-9/14              | 40.0                                 |
|                                 | 9/14-9/21             | 35.2                                 |
|                                 | 9/21-9/28             | 48.9                                 |
|                                 | Mean, all experiments | 45.4                                 |
| <u>Hypnea musciformis</u>       | 8/11-8/16             | 28.6                                 |
|                                 | 8/16-8/23             | 23.7                                 |
|                                 | 8/27-9/7              | 14.4                                 |
|                                 | Mean, all experiments | 22.2                                 |
| <u>Solieria tenera</u>          |                       | 0                                    |
| <u>Acanthophora spicifera</u>   |                       | 0                                    |
| <u>Acanthophora muscoides</u>   |                       | 0                                    |
| <u>Chondria</u> sp.             |                       | 0                                    |
| <u>Bryothamnion triguetrum</u>  |                       | 0                                    |
| <u>Halymenia agardhii</u>       |                       | 0                                    |
| <u>Chrysomenia halymeniodes</u> |                       | 0                                    |
| <b>Phaeophyta</b>               |                       |                                      |
| <u>Dictyota dichotoma</u>       |                       | 0                                    |

algae, Gracilaria foliifera and Hypnea musciformis and the green algae Enteromorpha clathrata and Chaetomorpha linum. Of these, it is now known that the last three do not grow well in culture under Florida winter conditions, so that it already appears that G. foliifera, the species that has to date been most extensively cultured at Harbor Branch Foundation, may in fact be the best indigenous candidate species for year-round cultivation in Florida.

Several additional seaweed species which were not available in summer have been collected during the fall of 1976 and were in the process of screening evaluation at the time this report was in preparation. These include other species of Gracilaria (blodgettii, debilis, verrucosa) as well as another variety of G. foliifera.

The short-term yields measured in the screening chambers for all four species evaluated during the summer of 1976 (Tables 1,3) are significantly higher, by about twofold on an average, than the yields of G. foliifera and the other species grown for sustained periods in the larger culture tanks (e.g., Table 2, Figure 1). Apparently growth conditions in the small screening tanks more closely approximates optimal conditions for these seaweeds. Care must be taken, however, not to extrapolate the results of small, short-term

experiments which may represent growth under idealized conditions that cannot be either scaled up or sustained throughout the year. Another caution in considering seaweeds as a biomass source for energy conversion is that only the organic content of the plant represent a potential energy source. In most higher plants, the mineral or ash content is normally not more than 10-15% of total dry weight (e.g., Westlake, 1973). In the seaweeds, however, the ash fraction is commonly as much as 50% of total dry weight. This mineral content includes not only structural material and salts contained in the cell sap, etc. but also salt that has evaporated onto and adhered to the dried seaweeds. The latter can be removed by careful washing of the plants in freshwater, but it is generally easier to establish the relationship between drained wet weight, dry weight, and ash-free dry weight with a few careful measurements and thereafter monitor seaweed growth by incremental changes in wet weight. The wet weight, dry weight and ash-free dry weight relationships of the four species screened during the summer of 1976 are shown in Table 4, which also gives the standard procedure (time and temperature) for obtaining the two dry fractions.

Another method of estimating the growth of seaweeds and other aquatic plants grown in a flow-through system is by the amount of nutrients removed from the water during its passage through the culture. The validity of such an approach depends upon several conditions: (1) flow rate must be constant or carefully monitored,

Table 4. Relationship between wet weight, dry weight, and volatile solids in four species of algae.

| Species                       | Dry weight <sup>*</sup> | Volatile solids <sup>**</sup> |
|-------------------------------|-------------------------|-------------------------------|
|                               | % wet weight            | % dry weight                  |
| <u>Gracilaria foliifera</u>   | 11                      | 51                            |
| <u>Hypnea musciformis</u>     | 12                      | 60                            |
| <u>Chaetomorpha linum</u>     | 10                      | 52                            |
| <u>Enteromorpha clathrata</u> | 16                      | 65                            |

\* Dried @ 90°C for 48 hours.

\*\* Weight loss after combustion @ 550°C for 3 hours.



(2) nutrient concentration in the input and discharge must be constant or carefully monitored, (3) the nutrient: total ash-free dry weight ratio in the plant must be constant and known, and (4) nutrient removal from the water must be by plant assimilation only. Neither phosphate nor ammonium-nitrogen satisfy the fourth condition, since at high pH levels (which frequently occur in dense plant cultures at midday) the former may precipitate while the latter may be lost to the atmosphere as  $\text{NH}_3$ . Nitrate-nitrogen, however, cannot be lost by any means other than plant assimilation, provided that anaerobic conditions and bacterial denitrification are avoided. Since the Harbor Branch Foundation waste treatment plant effluent contains nitrogen almost exclusively in the form of nitrate, its removal from the water may be used to estimate plant growth if the other conditions are met. This is now being investigated, since nitrate removal would not only be a fast and easy method of determining growth but would also avoid the possible damage to the more fragile plant species caused by weighing the entire population. Furthermore, in any truly large-scale culture system it would be economically and probably also logistically impossible to weigh frequently the entire cultures.

One of the complications in using nitrate removal as an index of productivity that has already come to light is that nitrate uptake in seaweeds is apparently not constant, but varies diurnally (see above). The nature and consistency of such diurnal

periodicity must be determined by further experimentation for any seaweed or other plant for which nitrate removal is to be used to estimate production, so that periodic monitoring of influent and effluent nitrate concentrations may be normalized to daily uptake rates.

Another controllable factor that may influence seaweed production is the turnover or exchange rate of water in the cultures (i.e., the reciprocal of residence time as used in sanitary engineering terminology). It was mentioned above that, in designing the standard assay method for screening different seaweed species for their growth potential, a rapid water exchange (22 culture volumes/day) was employed on the assumption that such is beneficial and may approach optimal conditions for the seaweeds. This assumption, however, required verification. Furthermore, the minimal rate of water exchange needed to produce maximal growth must be determined, since the pumping of water would have to represent one of the major operational costs in any large-scale, commercial seaweed culture system.

In determining this parameter, however, it is difficult to separate the effects of water movement per se and those of nutrient availability. If the concentration of nitrogen or phosphorus in the water remains constant, the more rapid the flow of water, the greater their availability to the plants. If, on the other hand, the input and flux of nutrients is kept constant (i.e., by adding

them separately at a constant rate while varying the flow rate of water), the slower the flow and exchange rate, the higher will be the concentration of nutrients present at any instant. In the latter case, the plants may be affected by inhibitory or toxic effects of high nutrient concentrations at very low flow rates or by concentrations too low to be effectively assimilated at very high flow rates.

On the assumption that nitrate and phosphate are non-toxic and equally available to seaweeds over a wide range of concentrations (an assumption that is not without basis but that needs further verification), an experiment was initiated in which Gracilaria foliifera was grown at five different exchange rates (1, 5, 10, 15, and 25 culture volume exchanges per day), in each case with a constant input of sodium nitrate and sodium phosphate so that nutrient concentrations were inversely correlated with exchange rates, with the exception of two unenriched seawater control cultures at the highest flow rates (25/day).

The poorest growth in the experiment occurred at the lowest turnover rate of one volume/day. These plants not only grew slowly but were clearly unhealthy and became heavily epiphytized by Enteromorpha and other algae by the end of the experiment. In general, the productivity of the seaweed appeared to increase with the exchange rate of the medium from one to 15 volumes per day,

but growth at 15 and 25 volumes/day did not appear to be significantly different.

Growth in the unenriched seawater controls was initially as great as that in the enriched cultures at high turnover rates (15-25/day), the plants presumably utilizing stored nutrients during that period. After three weeks, however, growth ceased in the unenriched cultures and the Gracilaria became pale, straw-yellow colored, typical of nitrogen-starved plants.

Nitrogen assimilation at the different exchange rates and nutrient concentrations was surprisingly constant at 11-17 m moles nitrate/day, differences being apparently random and probably attributable to sampling or analytical error. Although nutrient samples of the influent and effluent media were taken only at the times the seaweed were weighed for growth, they were usually taken at the same time of day to eliminate or at least standardize any effects of diurnal nutrient uptake variability (see above). The only significant difference in daily nitrogen assimilation that could be seen was in the unenriched control, from which the plants could remove only 3 m moles nitrate/day, one-third or less that taken from the enriched seawater.

As mentioned above, there is considerable risk in extrapolating the growth of seaweed obtained from small-scale experiments to anticipated yields from large-scale, commercial operations. In an attempt to study some of the effects of increasing the size

of the culture unit, a pair of tanks or raceways were constructed from 12 m long, longitudinal sections of 2.4 m-diameter aluminum culvert pipe (Figures 4,5). These raceways, 12 m long x 2.4 m wide x 1.2 m deep at the center, contain 25,000 liters when full and represent a culture area of 30 square meters. A perforated pipe cemented to the bottom of the raceway provides aeration and mixing of the water.

On completion of the first of these larger raceways, it was stocked with 30 kg of Gracilaria foliifera and supplied with sewage-enriched seawater at a concentration of 35  $\mu$ moles nitrate/liter and at an exchange rate of 2 volumes (50,000 liters) per day. Higher flow rates were found to move the air-suspended seaweed to the far end of the tank, while the plants remained evenly suspended at 2 exchanges/day. The Gracilaria was stocked on October 19, 1976 and harvested and weighed for the first time on November 9, 1976. During that period, growth averaged 10 grams dry weight/m<sup>2</sup>/day. While this yield is considerably lower than that obtained in the smaller culture units, the difference may be misleading. The inoculation of Gracilaria was of nutrient-starved, highly bleached plants that had been held without feeding for an extended period of time. Experience has shown that such algae require a lag time of some two weeks after restoring nutrients before they begin normal growth. The



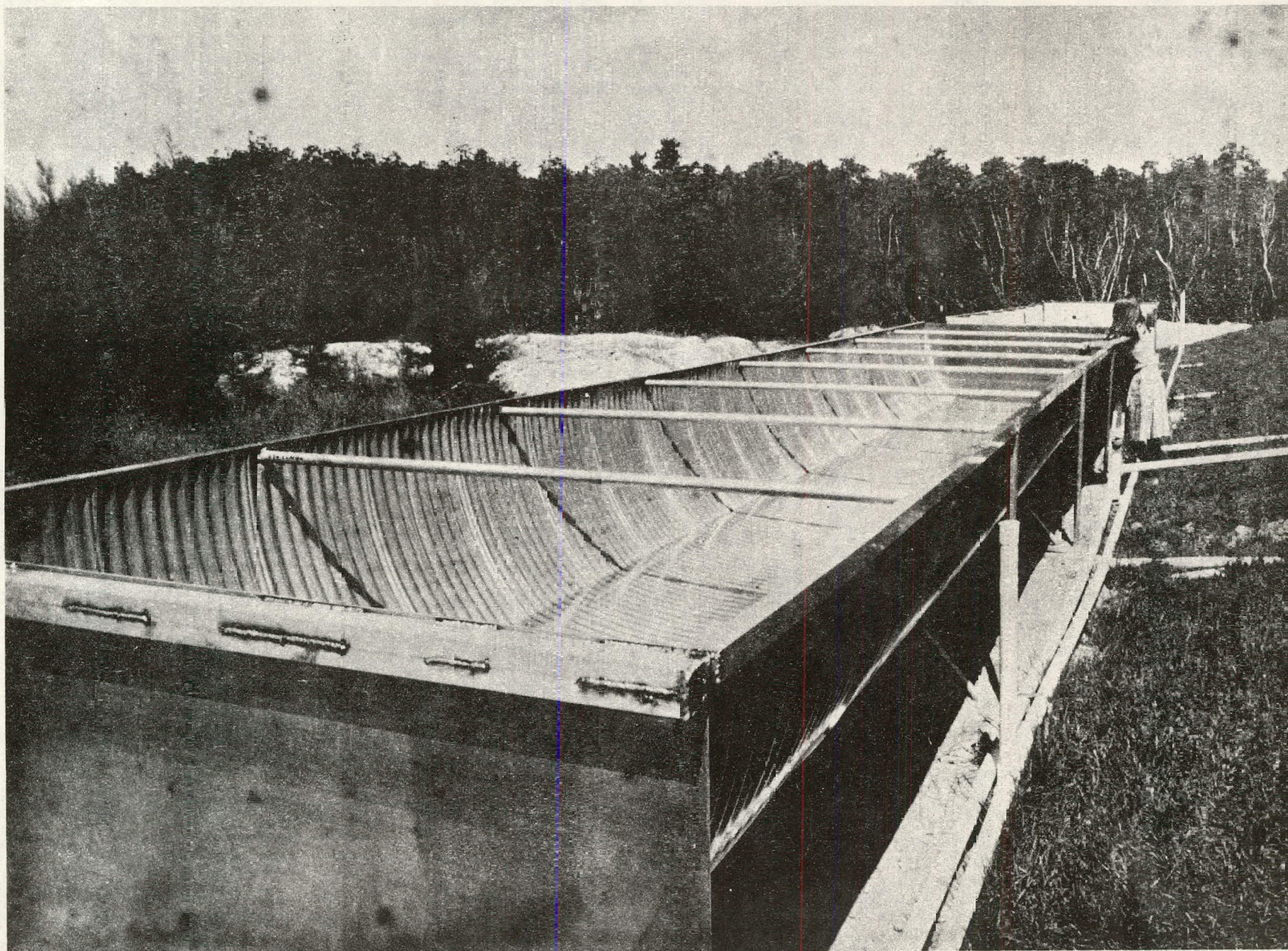


Figure 4. 25,000-liter aluminum tanks constructed from culvert pipe for seaweed culture.



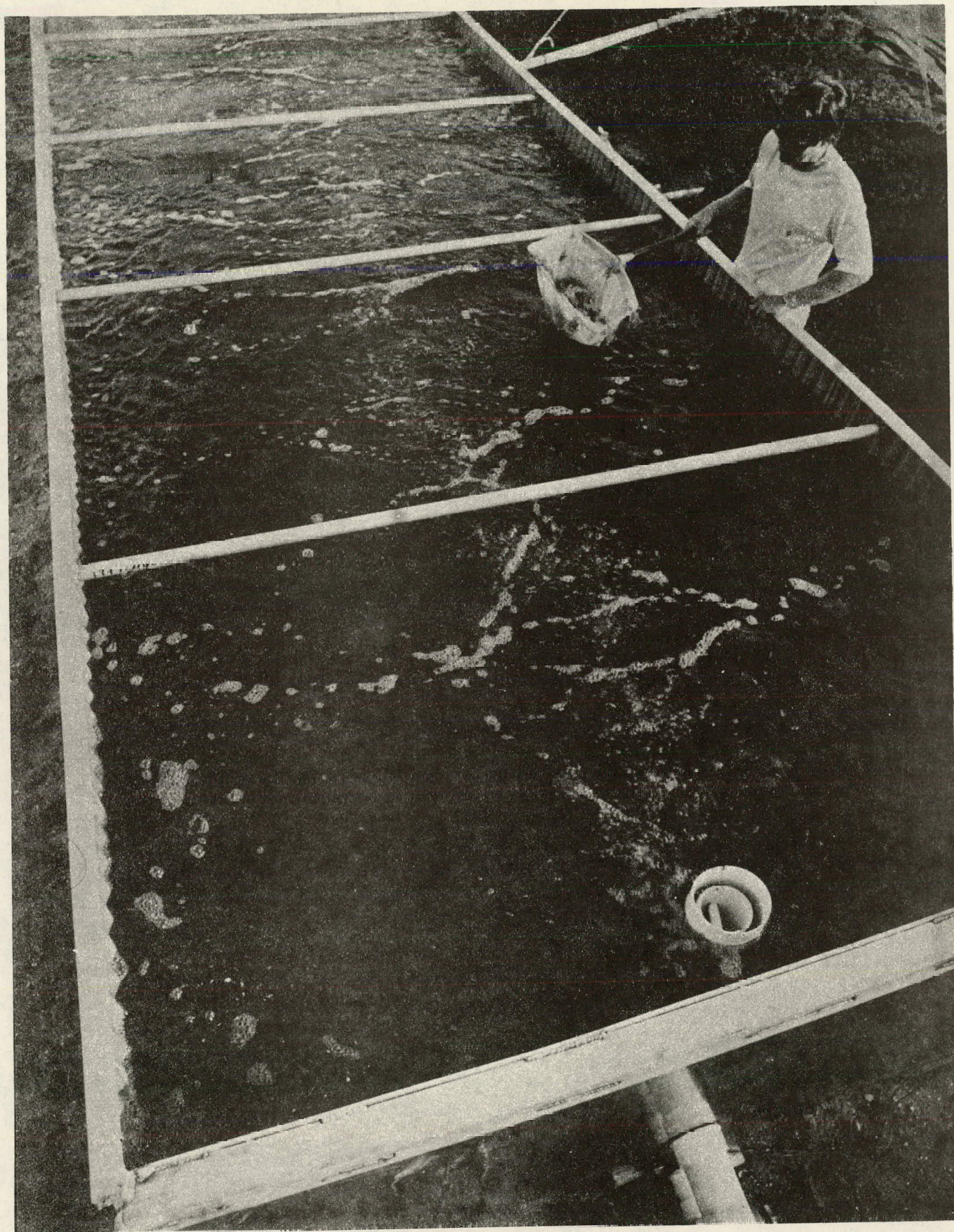


Figure 5. 25,000-liter aluminum tanks constructed from culvert pipe for seaweed culture.



seaweed was healthy in appearance and of normal coloration at the time of the first harvest (November 9, 1976) and was presumed to have been growing actively at that time, but it is believed that the observed growth probably took place almost entirely during the last week or 10 days of the period at a rate that is as much as double that indicated above. This experiment will be continued throughout the winter of 1976-77.

Finally, it is a matter of major concern that the seaweed culture methods that have been developed and evaluated to date may not be either economically cost-effective with respect to both initial investment and operation or energy-effective with respect to the ratio of input to return of energy. Experiments have therefore been initiated late in 1976 and will be continued in the future to develop much simpler, less costly, and less energy-intensive seaweed culture systems. The first such effort was to grow, without aeration or mixing, Gracilaria foliifera in shallow PVC-lined earthen ponds approximately 12 x 2.4 x 1.2 m deep (23,000 liter capacity). Preliminary results from these experiments appear promising, but insufficient data were available to be included in this report.



Manuscripts based upon work wholly or partially supported by ERDA Contract E(11-1)-2948 that have been prepared to date are listed below.

DeBoer, J. A., J. H. Ryther, and B. E. Lapointe. Yields of the seaweeds Neoagardhiella baileyi and Gracilaria foliifera in a waste recycling-polyculture system.

DeBoer, J. A. and B. E. Lapointe. Effects of culture density and temperature on the growth rate and yield of Neoagardhiella baileyi.

DeBoer, J. A., B. E. Lapointe, and C. F. D'Elia. Effects of nitrogen concentration on growth rate and carrageenan production in Neoagardhiella baileyi.

D'Elia, C. F., J. A. DeBoer, and J. H. Ryther. Some aspects of the nutrient uptake kinetics of the macroscopic red alga Neoagardhiella baileyi.

Nicotri, M. E. The impact of crustacean herbivores on cultured seaweed production.