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CULTIVATION OF MACROSCOPIC MARINE ALGAE AND FRESHWATER AQUATIC WEEDS

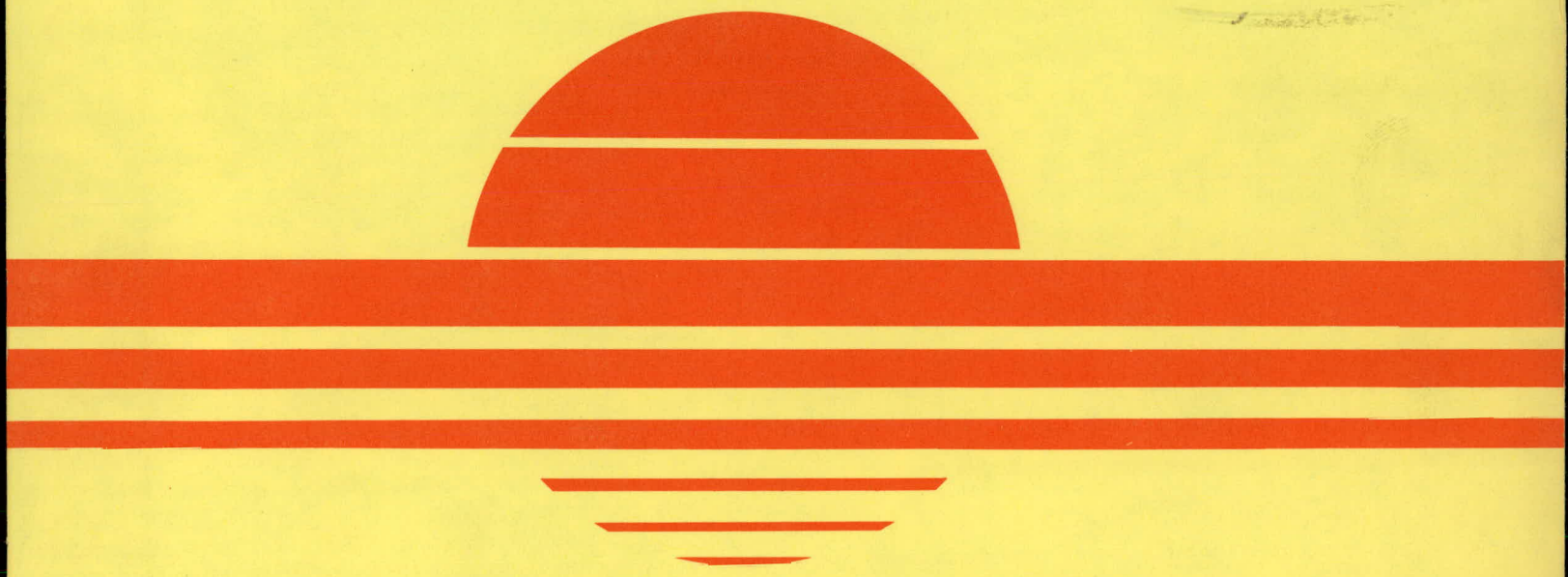
Progress Report, May 1—December 31, 1976

By
John H. Ryther

January 1977

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

Woods Hole Oceanographic Institution
Woods Hole, Massachusetts



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John H. Ryther

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ABSTRACT

Research during the 1977-78 Contract year was divided between basic physiological studies of the growth and nutrient-uptake kinetics of macroscopic marine algae and the more applied problems involved in the selecting of species and the development of inexpensive, non-energy intensive culture methods for growing seaweeds and freshwater plants as a biomass source for conversion to energy.

Best growth of the seaweeds occurs at low (0.1-1.0 μmolar) concentration of major nutrients, with ammonia as a nitrogen source, with rapid exchange of the culture medium (residence time of 0.05 days or less).

Of 43 species of seaweeds evaluated, representatives of the large red alga genus Gracilaria appear most promising with potential yields, in a highly intensive culture system under optimal conditions, of some 129 metric dry tons per hectare per year (about half of which is organic). Non-intensive culture methods have yielded one-third to one-half that figure. Unexplained periodicity of growth and overgrowth by epiphytes remain the most critical constraint to large-scale seaweed culture.

Freshwater weed species in culture include water hyacinth (Eichhornia crassipes), duckweed (Lemna minor), and Hydrilla verticillata, with yields to date averaging 15, 4, and 8 g dry wt/m^2/day respectively. However, these plants have not yet been grown through the winter, so average annual yields are expected to be lower. In contrast to the seaweeds, the freshwater plants grow well at high nutrient concentrations and slow culture volume exchange rates (residence time ca. 20 days or more).

Experiments were initiated on the recycling of digester residues from the fermentation of the freshwater and marine plants as a possible nutrient source for growth of the same species.

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A

Cultivation of macroscopic marine algae and freshwater aquatic weeds

Summary

John H. Ryther

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Summary

During the 1977-78 Contract year, research on the cultivation of seaweeds and freshwater weeds has been about evenly divided between rather basic physiological and biochemical studies of growth and nutrient-uptake kinetics and related subjects and the more applied problems of screening large numbers of plant species for their growth potential, determination of both short- and long-term (e.g., annual) yields of organic matter, effects of environmental variability on such yields, and the development and improvement of culture methods. The latter studies were carried out entirely at the Harbor Branch Foundation, Fort Pierce, Florida and were partially supported by that organization. The more basic studies were divided between Florida and the Woods Hole Oceanographic Institution, Woods Hole, Mass. where, during the first part of the year, studies were carried out by two Postdoctoral Fellows (J. A. DeBoer and C. F. D'Elia), who were supported by the Jessie Smith Noyes Foundation.

The laboratory studies at Woods Hole concentrated upon the kinetics of nutrient, specifically nitrogen, uptake and growth of the red algae, Gracilaria foliifera and Neoagardhiella baileyi. It was demonstrated that growth of these seaweeds follow saturation (e.g., Michaelis-Menton)-type nutrient uptake kinetics for plants grown in ammonia, nitrate, urea, or treated sewage effluent (in

which the nitrogen was predominantly present as nitrate). Growth was best in ammonia-N, followed by sewage effluent, nitrate, and urea.

A most significant finding was that the half-saturation coefficient (K) of nitrogen for growth of these algae is remarkably low, in the range of 0.2-0.4 μmolar N, similar to that of many species of phytoplankton, indicating that non-nutrient limited growth of these plants can occur at very low levels of nitrogen, concentrations that are essentially unpolluted environmental levels, provided, of course, that there is rapid enough water exchange to satisfy the absolute nitrogen requirement of the plants.

Nitrogen uptake by Gracilaria and Neoagardhiella also follows Michaelis-Menton-type rate saturation kinetics for nitrate, but appears to be diphasic for ammonium uptake, with an active transport component that becomes rate saturated and what is apparently a diffusive component that does not become saturated at concentrations as high as 50 μmoles, one hundred times the concentration at which growth becomes rate saturated.

A much more rapid uptake of nitrogen, in any form, was observed in plants that were nitrogen starved, as indicated by high C:N molar ratios of the plant tissue (10-30) than in those that had been grown in a nitrogen-enriched medium and had more normal C:N ratios of 5-10. Nitrogen-starved alage also assimilated nitrogen in the dark at the same rate as in daylight, while those grown in a nitrogen

surplus assimilated the nutrient at a markedly lower though still measurable rate at night.

A strong preference was shown by both species of red algae for ammonia over nitrate. Uptake rates of the former were much greater and, when ammonia was present at concentrations greater than 5 μmolar, nitrate uptake was completely surpressed. Below 5 μmolar concentrations, normally the situation in nature and well above the half-saturation concentration for growth, the two forms of nitrogen are taken up simultaneously.

The correlation between phycocolloid content of the algae and their nutritional condition, again as exemplified by their C:N ratio, has been further studied and placed on a more quantitative basis. Highest concentrations of agar in Gracilaria (45% of ash-free dry weight) were found in N-starved algae (C:N > 10), while plants that were not nitrogen-limited had an agar content of only 25% of ash-free dry weight. Similarly, the carrageenan content of Neoagardhiella was found to range from 36% to 29% of ash-free dry weight in plants grown at nitrogen concentrations of < 1 and 25 μmoles/l respectively.

The availability of nitrogen was also found to influence the ratio of wet to dry weight, the percentage of ash, and the caloric content of Gracilaria. Nitrogen enriched cultures (C:N $\leq$ 10) were about 90% water, 5% ash, 5% volatile solids, and had a heat of combustion of 4500 cal per gram volatile solids. Nitrogen starved

cultures (C:N $\geq$30), on the other hand, contained about 94% water, 2% ash, 4% volatile solids and 4100 calories/g volatile solids. In other words, seaweeds grown in artificial culture in non-growth-limiting concentrations of nutrients may contain over one-third as much additional energy per unit of fresh weight as nutrient-deficient plants.

The experimental culture system that was developed in Florida for screening the growth potential of different species of seaweeds was also used for physiological studies that required a large number of experiments to be conducted simultaneously, since the apparatus involved, consisting of longitudinally-sectioned and lengthwise partitioned 18" PVC pipes, provided 32 50-liter experimental chambers each with separate and controllable inputs of seawater and nutrients. Three separate series of experiments were conducted with G. foliifera in which there were (a) four different rates of flow of water through the cultures all containing the same concentration of nutrients, (b) four identical rates of flow through the culture each containing a different concentration of nutrients, and (c) four different rates of flow through the culture each containing a different concentration of nutrients so that the mass flow of nutrients (concentration x flow) were all the same. In this way, it was possible to separate the effects of nutrient concentration per se, total daily nutrient loading or availability, and flow rate (culture volume exchange rate).

From those experiments, it was clear that a given biomass of Gracilaria would remove a relatively constant amount of nitrogen from solution which was independent of concentration or flow rate as long as the product of the two (mass flow of nutrients) was adequate. However, growth of the seaweed was strongly dependent upon and directly proportional to flow rate per se and never reached a saturation level at the highest flow rate tested (30 volumes per day or a residence time of 0.8 hours).

The fact that the same amount of nitrogen was removed from solution independent of flow rate while the latter markedly affected growth was subsequently explained when it was found that a substantial amount of the assimilated nitrogen was stored in the algal tissue in the inorganic form and that this amount was roughly ten times greater at the lower flow rates (1 volume/day) than at the highest flow rate (30 volumes/day). In other words, algae that are unable to grow will still assimilate and store nitrogen, presumably against the day when other conditions for growth may be favorable and nutrients are limiting, the uptake itself being constant or at least independent of growth.

The reason that growth increased with the rate of flow or water exchange is not known. It is unlikely to have resulted from water movement per se, because the seaweed was continually and rather violently agitated and rotated in the culture volume by vigorous

aeration. It would seem more likely that the more rapid exchange of seawater either brought more of some limiting nutrient (other than N and P, which were added separately) or prevented the accumulation of some toxic or inhibitory substance.

Forty three species of seaweeds were screened for their growth potential in the experimental system described above, including representatives of the brown, green, and red algae. Over half of these, including all of the brown algae, failed to grow or subsist in culture. Some of the green algae (Ulva, Enteromorpha, Chaetomorpha) grew well for short periods of time, but periodically distintegrated into reproductive spores. The most successful were the red algae, particularly several species of the large genus Gracilaria, but these assay experiments were admittedly biased by the fact that the culture system employed had been developed empirically, by trail and error over some considerable time, specifically for the growing of the commercially-valuable red seaweeds such as Gracilaria, Neoagardhiella, and Hypnea.

The only species that could be grown throughout the entire year in Florida was G. foliifera V. angustissima, the mean production of which was 35.5 g dry wt/m^2.day, a yield that is equivalent to 129 dry metric tons/hectare.year, considerably greater than that of any photosynthetic plant for which records are available. The high value is, however, misleading in that roughly half of the dry weight of Gracilaria is ash (salt). The yield of organic matter

(ca. 65 t/ha.yr), the figure that is of significance in considering the value of the plant as a potential energy source, is still comparable to the best agriculture yields (i.e., sugar cane, corn, etc.) and is therefore still impressive.

However, although plants freshly collected from culture stocks did grow throughout the year in the screening tanks, a given culture would persist for no more than several weeks to, at most, a few months before becoming overgrown with epiphytes (filimentous red or green algae or diatoms), eventually requiring replacement with new material. This remains the single most serious problem and constraint to large-scale, commercial seaweed culture and must be resolved before these plants may be considered as serious candidates for an energy plantation.

The method of growing seaweeds in suspended culture, involving vigorous aeration and rapid circulation and exchange of water, was recognized as being impractical for large-scale commercial culture both economically and with respect to the energy input: output balance. Several other less energy-intensive and less costly culture methods for growing Gracilaria and a few other species of red algae were therefore investigated. These included growing the plants loosely on the bottoms of small, plastic-lined ponds and cement tanks through which enriched seawater was passed at different rates, holding the seaweeds in plastic-mesh (Vexar) baskets which were either floated at the surface or fastened to the

bottoms of the same PVC-lined ponds or in natural estuarine areas in the Indian River, and spraying enriched seawater from conventional shower heads on trays of the seaweeds held out of water. In every case, growth proceeded initially at rates that averaged from about one-third (for the first two methods) to roughly two-thirds (for the spray cultures) of the yields obtained by the more intensive, suspended culture method described earlier. However, growth in the cultures that were loose in the ponds and tanks or in the trays gradually decreased and after a few weeks eventually stopped, for reasons that were not apparent. Some but not all of those cultures become epiphytized. In some, the algae become necrotic and gradually disintegrated, while in still others the plants remained viable and apparently healthy but simply did not grow. In all of these methods, in which water was simply circulated through the container with no aeration or mixing, an obvious problem was the lack of circulation of the enriched influent through the plant material itself.

The spray-irrigated, out-of-water cultures appeared more promising. Growth of these algae, held in 24" x 24" x 3" plastic trays designed for shellfish culture, proceeded upwards towards the light, resulting in a thick mat of algae that assumed a strikingly different habit and morphology from the normal, water-grown plants. However, these cultures, too, become heavily epiphytized after several weeks, usually with the green alga Entermorpha, and the cultures eventually had to be discarded and replaced.

An evaluation of the economics and energy requirements of the different culture systems has not been made, but it is believed that some modification of the spray metnod may prove most attractive with respect to cost:benefit considerations, when and if the common problem of epiphytization is resolved.

Experiments were initiated in Florida in the spring of 1977 on the cultivation of three species of freshwater weeds, the floating plants water hyacinth (Eichhornia crassipes) and duckweed (Lemna minor) and the submerged, rooted plant Hydrilla verticellata. These studies have been made in shallow (1 m) PVC-lined ponds of approximately 30 m^2 area and in concrete burial vaults, through both of which well water enriched with sewage effluent and/or sodium nitrate and sodium phosphate was passed at various flow rates and nutrient concentrations.

Unlike the seaweeds, growth of the freshwater plants appears to be independent of flow (residence time), the weeds growing well at relatively slow exchange rates of two weeks or more. Difficultly was encountered with measurement of the yield of the rooted Hydrilla, measurement of which involved sacrifice of the culture, until it was found that the plant would grow equally well if its basal end were fastened to plastic mesh (Vexar) screening, around which a root system quickly developed. In this way, the entire culture with its Vexar base could be periodically removed from the water, drained, and weighed. Harvesting Hydrilla by clipping off the tops

of the plants, as would be required in any large-scale cultivation of this rooted species, is followed by a hiatus of one to two weeks in its growth while the plants develop new terminal meristematic tissue. This is a factor that needs to be considered in estimating annual yields of such plants, in contrast to floating species such as hyacinths and duckweeds that may be harvested by removing entire plants that are produced by budding off from the parent stocks.

Over the period of approximately eight months that these freshwater plants have been grown, dry weight yields have averaged approximately 16 g/m^2/day for the water hyacinths and 5 g/m^2/day for the Hydrilla, and the duckweed. However, production of all three species has declined sharply with the onset of winter, when this report was written, and significantly lower mean yields over the entire year are therefore expected.

Hydrilla, in particular, appears to be seasonal in its activity. The plants were observed to flower in October after which little or no growth appears to have occurred. Although the flowers themselves are minute and could have required an insignificant fraction of the plants biomass and energy for their development, there is apparently a physiological change accompanying florescence in the course of which growth is arrested, perhaps for an extended period of time.

Whether the water hyacinths and duckweeds will follow a similar seasonality in their growth remains to be seen, although individual hyacinth plants have bloomed throughout the spring, summer and fall, apparently without influencing the productivity of the culture as a whole. Whether or not the plants have a natural intrinsic periodicity in their growth, their yields must still be significantly reduced in winter as a result of decreased temperature and solar radiation, even in central Florida, and they may be severely set back or even killed in extreme winters such as that of 1976-77.

Attempts to measure the organic production of the freshwater plants from their rate of uptake of nitrogen (as nitrate) have thus far been unsuccessful. Estimates by this method are always higher, often by a factor of twofold, than direct measurements made by weighing the plants. Further research will be done on this problem, including a greatly refined method of measuring the input and output of both nitrogen and phosphorus throughout the diel cycle, for this approach would represent a most valuable tool for measuring aquatic plant production, particularly in situations where it is impractical to determine growth by weighing, as in the case of rooted plants.

It is now generally agreed that to supply the nutrients for any large-scale (hundreds of square miles), land-based energy plantation at an acceptable economic and energy cost and without

seriously depleting the natural reserves of fertilizers, it will be necessary to recycle the nutrients contained in the organic matter and remaining in the residue from anaerobic fermentation, feeding them back to the plant growth system. Little is known, however, about the quantitative recovery and the chemical state of the nutrients in such fermentation residues, their availability to plants as they come out of the digester, or the nature and extent of any additional processing that may be needed to make them available to plant assimilation.

Preliminary research in this area was initiated during the present contract year under the terms of a sub-contract with Dynatech R/D Company (Cambridge, MA). Dried cultured plant material consisting of the seaweeds Gracilaria and Ulva and the freshwater weeds Hydrilla and Lemna (duckweed), in quantities of 80 kg per species, were shipped to Dynatech for fermentation in their digesters. The fermenation residues, as they became available, were then shipped back to Harbor Branch Foundation for analysis and experimentation on their use as a nutrient source for growing the plants that produced them.

Most of the effort during the present year has been devoted to problems associated with the fermentation of these plants, particularly the seaweeds which, because of their contained sulfonated organic polymers or their salt content in general, have proved difficult to digest, in some cases producing hydrogen sulfide.

Towards the end of the experimental period, three of the four plants (excluding the seaweed Gracilaria) had been successfully digested with the production of methane at encouraging rates and efficiencies. However, it was found necessary to add daily to the stirred tank digesters sewage sludge suspensions in volumes equal to the plant biomass suspension and to add ammonium-nitrogen at approximately weekly intervals in quantities roughly equal to the nitrogen contained in the plants in order to facilitate the fermentation. These extraneous sustances so completely masked the qualitative and quantitative characteristics of the plant wastes themselves that we have not yet been able to determine their nature and ability to be recycled. Future research in this area will concentrate on simpler and less efficient fermentation processes that may be accomplished without addition of other material, with emphasis on the residues rather than the fermentation process itself and its gaseous products.

Studies on the cultivation of macroscopic red algae. I. Growth rates in cultures supplied with nitrate, ammonium, urea, or sewage effluent[1]

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SUMMARY

Gracilaria foliifera (Forsskal) Børgensen and *Neoagardhiella baileyi* (Harvey ex Kutzing) Wynne and Taylor were grown in continuous-flow culture under controlled environmental conditions in 15-liter experimental chambers. Growth rate was related to the source and concentration of nitrogen enrichment supplied to the plants. Growth rate appeared to follow saturation-type nutrient uptake kinetics for plants receiving ammonium, nitrate, urea, or sewage effluent enrichments. Ammonium enrichment produced higher growth rates than nitrate or sewage enrichment. The lowest growth rates occurred in the chambers receiving unenriched seawater or urea enrichment. Half saturation constants (K) for growth were in the range of 0.2 to 0.4 μM nitrogen for all nitrogen enrichments examined. The low values of K measured compare closely to those found for microalgae and indicate that *G. foliifera* and *N. baileyi* possess the ability to utilize very low concentrations of nitrogen.

INTRODUCTION

Macroscopic marine algae are among the most productive plants in nature (19) and in cultivation (5,18); moreover, a number of species contain commercially valuable phycocolloids such as agar and carrageenan (20). Thus it is not surprizing that recently there has been increasing interest in seaweed cultivation for "fuel from biomass production" (29) and for phycocolloid yield (4,6).

In contrast to the attention paid to the relationship between nutrient concentration and phytoplanktonic growth (2,3,8,9,10), there is a paucity of quantitative information concerning the nutrient-growth relationship for macroscopic algae. Such information would be most useful for purposes of seaweed cultivation and pollution assessment. Accordingly, we initiated the present study to examine the qualitative and quantitative aspects of nutrient utilization by two species of macroscopic marine red algae, Gracilaria foliifera (Forsskal) Børgesen and Neoagardhiella baileyi (Harvey ex Kutzing) Wynne and Taylor. In this paper we relate growth rate of these algae with nitrogen concentration and source.

MATERIALS AND METHODS

Preliminary experiments demonstrated that G. foliifera and N. baileyi have exceedingly high growth rates and are able to utilize very low concentrations of nitrogen (6). It is essential, therefore,

to use extremely large-volume batch cultures or continuous-flow cultures to study the nitrogen-growth kinetics of these plants. We chose the better continuous-flow culture method even though it is very demanding, time consuming, and is susceptible to equipment failures. Practical considerations precluded the use of axenic cultures in these studies.

G. foliifera, obtained from Long Island Sound during August, 1974, and N. baileyi, obtained from Waquoit Bay, Massachusetts during August, 1976, were grown in suspended culture in waste recycling-polyculture or seaweed monoculture systems (6) prior to the present study. The unattached, free floating plants reproduced only by vegetative means in these culture systems and in the experimental chambers used in the present study.

Growth experiments were conducted in continuous-flow chambers. These chambers were constructed from a section of 40.6 cm diameter PVC pipe, 90 cm in length, which was cut longitudinally into two equal sections. Each section or "trough" was divided by fiberglass partitions into chambers 30 cm long, 34 cm wide, and 18.5 cm deep. An overflow was inserted 2 cm below the top of each chamber to give a functional volume of 15 liters. An air line cemented to the bottom of each chamber provided the vigorous agitation needed to keep the plants in suspension and to provide mixing and gas exchange.

Artificial illumination on a 16-8 h LD photoperiod was provided by Luxor^{R} fluorescent tubes suspended 12 cm above the chambers. Extraneous light was eliminated by the use of black curtains placed around the entire culture area. Photosynthetically active radiation (400-700 nm), measured 1 cm below the water surface, using an RP-90 Digital Radiometer-Photometer (International Light, Inc., Newburyport, MA), was 0.052 langleys·min^{-1}. Both species have been shown to be light saturated at this irradiance level (11, Olhoeft, unpublished manuscript).

Seawater, obtained from Vineyard Sound, was filtered through a 1-µm polypropylene cartridge filter and pumped to a constant level device above the chambers. Flow rates of 140 liters·day^{-1} (9.3 culture volumes·day^{-1}) were controlled by means of needle valves for each chamber. Water temperatures were maintained at 22.1 ± 1.4°C. Salinities ranged between 29 and 31‰.

A total of fifteen different nutrient regimes were provided to G. foliifera (Table 1) and seventeen to N. baileyi (Table 2) by enriching the seawater flowing through each chamber. Nutrient enrichment media were provided continuously to the chambers by peristaltic pumps. One enrichment medium was unchlorinated, secondary sewage effluent from the Town of Wareham, Massachusetts. The influent to the chambers receiving sewage effluent contained mixtures of 0.20 to 1.80% secondary effluent in seawater. Sewage effluent was

utilized in the study to eliminate the possibility that some trace element, instead of nitrogen, was limiting growth. Mixtures of seawater and secondary sewage effluent have been shown to be a complete medium for several species of microscopic and macroscopic marine algae (5,13, DeBoer, unpublished). The other enrichment media were concentrated solutions of reagent grade chemicals (NH_4Cl, $NaNO_3$, or urea for nitrogen source, and $NaH_2PO_4 \cdot H_2O$ for phosphorus source) in distilled water. An N:P ratio (molar) of 7:1 was used to simulate that of sewage effluent and to insure that the media contained an excess of phosphorus over nitrogen with respect to the requirements of algae (14).

Each growth chamber was stocked with 25.0 g fresh weight (approximately 15 small thalli) of G. foliifera and N. baileyi. Growth was measured by fresh weight determinations of the chamber contents three times per week. Fresh weights were obtained after removing the plants from the water and draining them in a polyethylene sieve for 5 minutes. Control experiments showed that this procedure did not affect their growth rate. After each weighing the biomass in each chamber was adjusted to the initial stocking density by harvesting the incremental growth. The specific growth rate (μ), reported here as the percentage increase in fresh weight per day, was calculated by the equation.

$$\mu = \frac{100\ (\ln\ (N_t/N_o))}{t}$$

where N_o is the initial biomass and N_t is the biomass on day t. This equation assumes steady-state, exponential growth. All experiments, which were conducted between October 15 and December 15, 1976, lasted for two weeks and were preceded by a two-week preconditioning period under experimental conditions. This was to ensure steady state response during the experimental period itself.

In the absence of a proven mathematical model which relates the growth rate of macroscopic algae to the concentration of a limiting nutrient we have used the Michaelis-Menten model. The relationship between specific growth rate of phytoplankton and nutrient concentration is often experessed by this equation:

$$\mu = \mu_{max} \frac{K}{K + S}$$

where μ is the specific growth rate, μ_{max} is the maximum growth rate under prevailing environmental conditions, and K is the concentration of S (the limiting nutrient) at which $\mu = 1/2\ \mu_{max}$. Half-saturation constants (K and μ_{max}) for G. foliifera and N. baileyi were calculated by regression analysis of S on (S/μ).

Ammonia, nitrate, nitrite, and phosphate were determined on filtered water samples using the methods given by Strickland and Parsons (27). Urea was determined by the method of Newell et al. (23).

Total nitrogen (TN) determinations were by the method of D'Elia et al. (7). Organic nitrogen (ON) was calculated as the difference between TN and inorganic nitrogen (IN), the latter being the sum of NH_4^+ + NO_3^- + NO_2^- values.

RESULTS AND DISCUSSION

The residual (effluent) nutrient concentrations in the G. foliifera and N. baileyi experiments are shown in Tables 1 and 2, respectively. The nitrite levels (not shown) in the influents and effluents of the experimental chambers were negligible (less than 0.12 μM). The dissolved organic nitrogen content of the effluents from the experimental chambers tended to increase with increasing influent nitrogen concentration, possibly as the result of a loss of dissolved organic nitrogen from the plants in response to high external nitrogen concentrations. A comparison of the influent and effluent nitrogen concentrations indicates no net utilization by the seaweeds of the dissolved organic nitrogen in the influent seawater. Therefore, total inorganic nitrogen (IN) appears to be the best parameter to use when comparing the effects of the different nutrient enrichments on the growth rate of the plants.

There was little difference between the influent and effluent nitrogen concentrations in the control chamber which did not contain algae (Table 1). The pH values in all chambers remained within

a range of 7.9 to 8.4. These observations suggest that changes in the form and concentration of inorganic nitrogen due to microbial activity or physical factors (i.e., loss of ammonia at high pH) were negligible.

The specific growth rate of G. foliifera increased with increasing nitrogen concentration for all sources of nitrogen enrichment up to an inorganic nitrogen concentration of approximately 1.5 μM (Fig. 1). Growth rate did not increase substantially at higher nitrogen concentrations. G. foliifera receiving ammonium enrichment exhibited substantially higher growth rates than plants receiving nitrate enrichment, particularly at lower nitrogen concentrations. Maximum growth rate was approximately 12% day^{-1}.

Growth rates of N. baileyi increased in proportion to nitrogen concentration, regardless of the type of enrichment, up to nitrogen concentrations of approximately 0.7 μM (Fig. 2). Little increase in the growth rates occurred at higher nitrogen concentrations. Growth rates of plants receiving ammonium enrichment averaged 17% day^{-1}, at nitrogen concentrations in excess of 0.7 μM, while those plants receiving urea enrichment averaged only 7% day^{-1}. Growth rates under nitrate or sewage enrichment were intermediate, averaging 9 and 12% day^{-1}, respectively.

The ability of these red algae to utilize very low nitrogen concentrations can be observed from their growth curves (Fig. 1, 2) and is also reflected in the low values of K measured (0.2 - 0.4 μM

nitrogen). While the values for K (Table 3) were similar for all nitrogen sources in both species, μ_{max} values were dependent on nitrogen source.

There is insufficient information available at the present time to determine if these "maximum" growth rates were actually the highest possible for these species or if some other factor (s) was limiting their growth. As discussed previously, it is not likely that light was limiting. The pH values in the chambers were never higher than 8.40 so it is doubtful that inorganic carbon was limiting. Although we took precautions to minimize the possibility that a micronutrient would become growth limiting, by using a low biomass/culture volume ratio and comparison cultures using a presumably complete medium (secondary sewage effluent in seawater), we cannot rule out the possibility of micronutrient limitation. Photosynthesis studies indicate that growth rates might be slightly higher at higher temperatures (11, Olhoeft, unpublished manuscript).

Although there have been a few recent nitrogen uptake studies (15,16), there are no previous published reports describing the nitrogen growth kinetics for macroscopic marine algae. Both nitrogen uptake kinetics and nitrogen growth kinetics have been widely used in studies of microscopic marine algae. Half-saturation constants for nitrogen uptake and growth (K) are usually in the 0.1 to 5.0 μM range for nitrate and ammonium (8,9,10) Evidence exists that the species distribution of phytoplankton may be influenced

by differential nitrogen assimilatory capacities between coastal and offshore species and by differences in nutrient concentrations in those environments (3,8,9). Insufficient data are available at this time to indicate whether differential nitrogen assimilatory capacities may also be important in the distribution of macroscopic marine algae.

At nitrogen concentrations greater than 5 μM, ammonium, nitrate, and sewage effluent were of almost equivalent value as nitrogen sources for G. foliifera (Fig. 1). In contrast, the growth rate of N. baileyi receiving ammonium enrichment was nearly double that of the plants receiving nitrate enrichment. Urea proved to be a comparatively poor nitrogen source for N. baileyi, producing a growth rate only one-third of that for ammonium. Growth rates of G. foliifera and N. baileyi receiving sewage enrichment were intermediate between ammonium and nitrate enriched plants. This may have been expected as the sewage effluent contained a mixture of the two. Thus, high ammonium content sewage would be expected to be superior to high nitrate content sewage as a nitrogen source for G. foliifera and N. baileyi.

Nitrate and ammonium have been shown to support similar growth rates in Chondrus (21,22,24) and Fucus (28). Growth rates of Goniotrichium and Nemalion (12) were greater when the plants were supplied with nitrate as compared to ammonium. Yamada (30,31) found ammonium to be the best nitrogen source for Gelidium. Nitrate

and ammonium have been shown to support higher rates of growth than urea in Chondrus (21) and Gelidium (30). At low nitrogen concentrations, nitrate, ammonium, and urea were equivalent nitrogen sources for Porphyra, but at higher concentrations the highest growth rates were obtained with nitrate (17).

Previous studies have reported the "optimum" nitrogen concentrations of macroscopic marine algae to be in the range of 120-2140 μM (1,12,17,26). Similarly, the culture media most frequently used in the cultivation of marine algae contain 500-2350 μM nitrate (25). These concentrations are based on maintenance of algae in small batch cultures and are obviously far in excess of the concentration of nitrogen required for maximum growth of G. foliifera and N. baileyi in continuous-flow culture systems or in the natural environment.

A better understanding of the nutrient requirements and the growth and uptake kinetics of macroscopic marine algae will be useful in improving the success of seaweed mariculture and will enable more precise predictions regarding the possible effects of sewage effluents and other nutrient sources on the growth of natural populations of macroscopic marine algae.

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Table 1. Influent and effluent nutrient concentrations in the Gracilaria experiment.

Chamber	Influent nutrient concentrations (μM N or P)				Effluent nutrient concentrations (μM N or P)				
	NH_4^+	NO_3^-	TN	PO_4^{-3}	NH_4^+	NO_3^-	TN	ON	PO_4^{-3}
Control									
0	9.81±0.16	9.92±0.21	30.73±1.2	3.40±0.21	9.72±0.48	9.76±0.61	30.28±3.1	10.8±1.1	3.42±0.28
Unenriched									
1	0.26±0.08	0.42±0.18	11.6±0.9	0.55±0.14	0.09±0.02	0.11±0.03	11.4±1.2	11.2±1.3	0.55±0.17
NH_4^+-enriched									
2	2.64±0.09	0.42±0.19	-	0.90±0.15	0.19±0.03	0.12±0.03	10.8±0.9	10.4±1.0	0.76±0.20
3	5.01±0.15	0.44±0.17	-	1.26±0.16	0.57±0.08	0.32±0.09	12.4±1.0	11.4±1.2	0.69±0.18
4	9.76±0.32	0.43±0.20	-	1.97±0.18	1.83±0.18	0.41±0.12	13.8±1.1	11.5±1.3	1.48±0.18
5	19.21±0.64	0.45±0.19	-	3.40±0.22	4.79±0.39	0.47±0.08	21.0±1.8	15.7±1.9	2.90±0.29
6	38.16±1.26	0.46±0.20	-	6.25±0.31	25.74±1.44	0.58±0.08	41.9±2.5	15.5±2.7	5.76±0.20
NO_3^--enriched									
7	0.26±0.08	2.69±0.22	-	0.91±0.14	0.10±0.02	0.38±0.06	11.4±0.8	10.9±1.0	0.73±0.14
8	0.25±0.09	5.01±0.34	-	1.27±0.14	0.15±0.07	1.61±0.16	12.7±1.2	10.9±1.4	
9	0.26±0.09	9.84±0.46	-	1.98±0.15	0.15±0.08	3.88±0.22	16.6±1.8	12.5±2.0	1.54±0.15
10	0.27±0.10	19.31±0.80	-	3.41±0.22	0.19±0.07	12.34±0.53	24.8±2.1	12.2±2.4	2.80±0.17
11	0.28±0.10	38.14±1.43	-	6.26±0.32	0.21±0.08	31.26±1.57	44.5±3.2	13.0±3.8	5.76±0.20
Sewage enriched									
12	1.08±0.08	4.15±0.20	16.6±0.8	1.10±0.16	0.25±0.06	0.87±0.06	12.8±1.1	11.6±1.4	0.91±0.18
13	1.88±0.09	7.78±0.21	21.6±1.0	1.68±0.17	1.05±0.51	2.57±0.13	16.1±1.7	12.4±2.0	1.38±0.18
14	4.15±0.09	17.25±0.21	32.1±0.9	2.82±0.21	1.13±0.15	14.60±0.65	27.0±2.8	11.2±3.1	2.53±0.20
15	7.18±0.10	29.02±0.24	52.6±1.2	4.92±0.30	2.15±0.28	25.40±1.19	40.9±2.9	13.2±3.3	4.61±0.22

Table 2. Influent and effluent nutrient concentrations in the Neoagardhiella experiment.

Chamber	Influent nutrient concentrations (μM N or P)					Effluent nutrient concentrations (μM N or P)				
	NH_4^+	NO_3^-	Urea	TN	PO_4^{-3}	NH_4^+	NO_3^-	Urea	TN	ON
Unenriched										
1	0.16±0.03	0.20±0.03	-	11.1±0.9	0.36±0.02	0.03±0.01	0.03±0.00	-	11.2±0.6	11.1
NH_4^+ enriched										
2	2.64±0.07	0.20±0.03	-	-	0.67±0.02	0.12±0.02	0.03±0.01	-	11.2±0.4	11.0
3	5.12±0.15	0.20±0.03	-	-	0.98±0.03	0.26±0.02	0.04±0.01	-	11.1±0.4	10.7
4	10.10±0.29	0.20±0.03	-	-	1.60±0.05	1.56±0.26	0.06±0.01	-	12.8±0.5	10.6
5	39.08±1.16	0.22±0.04	-	-	5.34±0.15	21.26±1.95	0.14±0.01	-	39.4±2.0	17.9
NO_3^- enriched										
6	0.16±0.03	2.67±0.09	-	-	0.67±0.02	0.02±0.00	0.23±0.03	-	10.6±0.4	10.3
7	0.16±0.03	5.16±0.19	-	-	0.98±0.04	0.04±0.00	0.64±0.06	-	12.8±0.5	12.1
8	0.16±0.03	10.13±0.32	-	-	1.61±0.05	0.04±0.01	3.93±0.33	-	16.4±0.7	12.4
9	0.17±0.04	38.21±1.22	-	-	5.35±0.17	0.01±0.01	24.60±0.52	-	43.1±3.0	18.5
Urea enriched										
10	0.24±0.03	0.20±0.04	2.0±0.1	-	0.61±0.03	0.02±0.01	0.02±0.00	0.60±0.04	12.3±0.5	11.7
11	0.33±0.03	0.20±0.03	4.0±0.2	-	0.88±0.06	0.03±0.01	0.03±0.00	0.98±0.08	14.1±0.8	13.0
12	0.52±0.04	0.22±0.04	7.9±0.4	-	1.37±0.07	0.03±0.01	0.02±0.01	2.24±0.18	17.8±0.6	15.5
13	1.67±0.05	0.28±0.04	31.9±1.2	-	4.44±0.17	0.07±0.02	0.03±0.01	17.83±1.82	41.0±2.7	23.1
Sewage enriched										
14	0.99±0.05	3.43±0.08	-	4.6±0.1	0.86±0.03	0.08±0.01	0.26±0.04	-	18.4±0.5	13.0
15	1.82±0.07	6.66±0.19	-	9.2±0.3	1.36±0.04	0.15±0.01	1.55±0.26	-	14.8±0.7	13.1
16	4.90±0.15	18.61±0.47	-	26.1±0.7	3.22±0.09	0.43±0.06	10.14±0.80	-	30.2±2.1	19.5
17	7.56±0.24	28.95±0.85	-	38.4±1.2	4.73±0.15	0.81±0.08	23.12±0.85	-	41.8±3.2	17.8

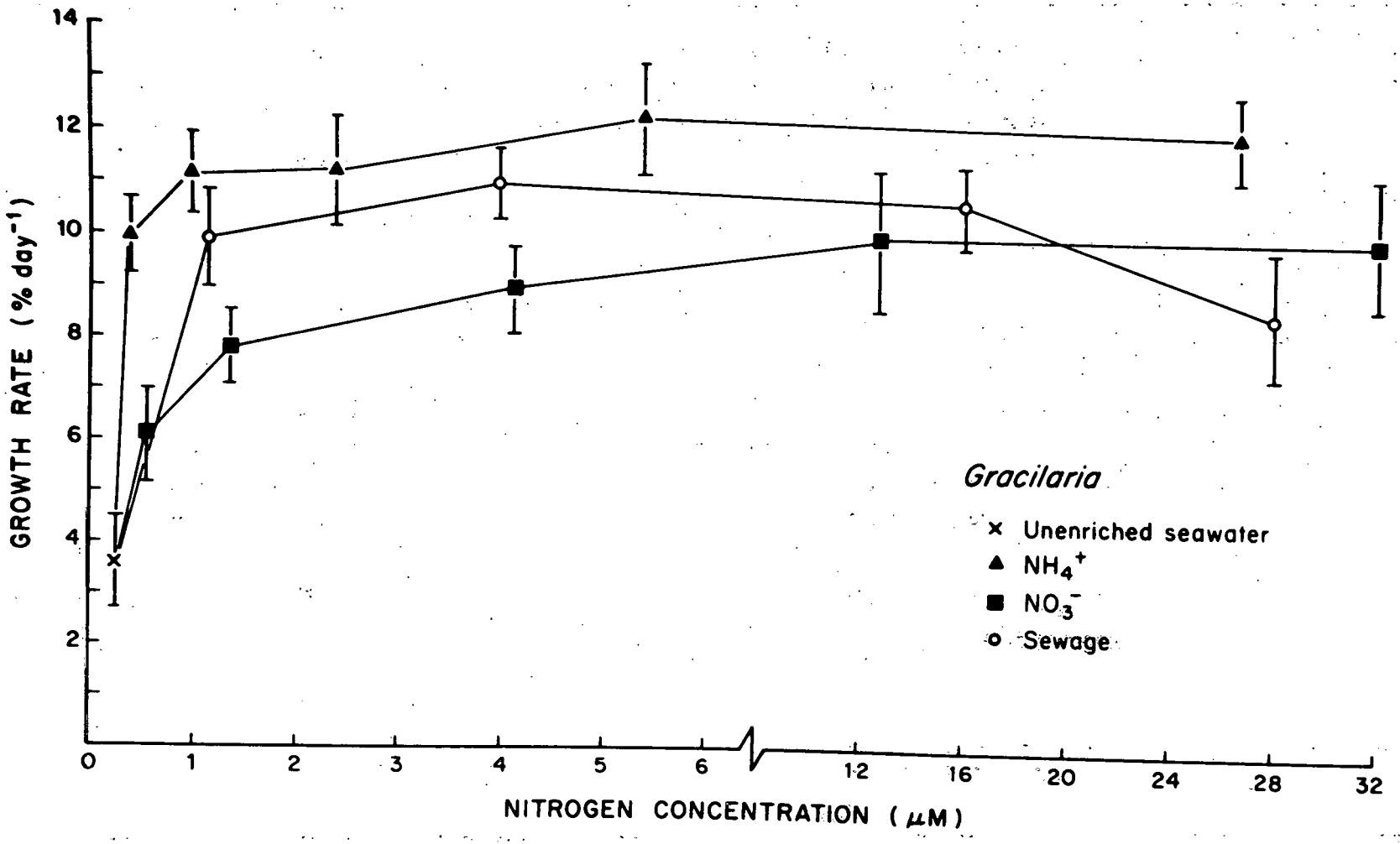

Figure 1. Growth rates (± 1SE) of Gracilaria foliifera as a function of residual inorganic nitrogen concentration ($NH_4^+ + NO_3^- + NO_2^-$) for various nitrogen enrichment sources.

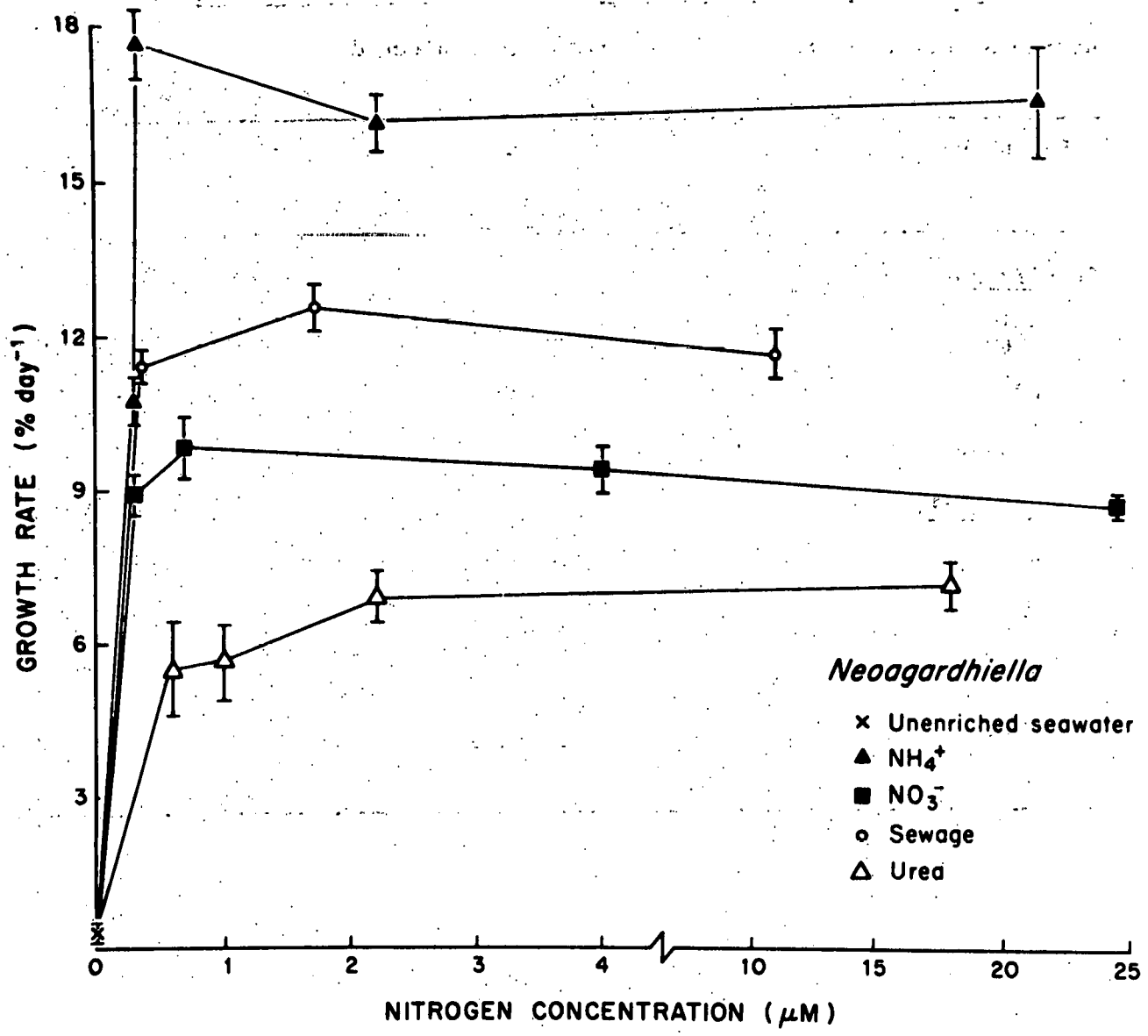

Figure 2. Growth rates (± 1 SE) of Neoagardhiella baileyi as a function of residual nitrogen concentration for various nitrogen enrichment sources. Nitrogen concentrations shown are $NH_4^+ + NO_3^- + NO_2^-$ ($NH_4^+ + NO_3^- + NO_2^-$ + urea for urea enriched cultures).

Table 3. Half saturation constants for growth (K) and maximum growth rates (μ_{max}) of Gracilaria foliifera and Neoagardhiella baileyi for various nitrogen enrichments. Standard errors are indicated.

Enrichment	K μM N*	μ_{max} % day^{-1}
Gracilaria		
NH_4^+	0.2±0.1	12.8±1.4
NO_3^-	0.4±0.1	9.9±1.2
Sewage	0.3±0.1	11.3±1.6
Neoagardhiella		
NH_4^+	0.2±0.1	17.0±1.3
NO_3^-	0.2±0.2	10.5±1.5
Sewage	0.2±0.1	13.1±1.4
Urea	0.2±0.2	6.9±0.8

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Studies on the cultivation of macroscopic red algae.

II. Kinetics of ammonium and nitrate uptake[1]

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SUMMARY

The ammonium and nitrate uptake kinetics of Neoagardhiella baileyi (Harvey ex Kutzing) Wynne and Taylor and Gracilaria foliifera (Forsskal) Børgesen were studied. Similar kinetic patterns were observed for both Rhodophyceans. Nitrate is taken up in a rate-saturating fashion that can be described by the Michaelis-Menten equation. Ammonium uptake is multiphasic: in addition to a saturable component, there is a diffusive or a high K component which shows no evidence of saturation at the highest concentrations tested (ca. 50 μM).

Nitrogen starved plants, i.e., those with C/N ratios (by atoms) exceeding 10-11, show much higher transient rates of ammonium uptake at a given concentration than do plants which are not nitrogen limited. Only plants with high nitrogen content exhibit diel changes in ammonium uptake rates, and show transient rates of ammonium accumulation which do not far exceed the capacity to incorporate nitrogen in steady-state growth.

There is a strong preference for ammonium over nitrate, even in plants preconditioned on nitrate as the sole nitrogen source. Nitrate uptake is suppressed at ammonium concentrations exceeding 5 μM, but below that concentration simultaneous uptake of both is possible at unsurpressed rates. At higher substrate concentrations increasingly more nitrogen can be accumulated via ammonium uptake than can be accumulated via nitrate uptake.

Changing capacity for ammonium uptake with nitrogen content appears to be a mechanism whereby excessive assimilation of nitrogen is avoided by the plants under conditions of nitrogen satiation, or whereby large amounts of ammonium-N can be rapidly assimilated under conditions of nitrogen privation.

Seaweeds offer great economic potential both as sources of commercially important products (20) and as agents of biological tertiary sewage treatment (10). Despite a growing interest in such potential, little attention has yet been paid to the relationships between nutrient supply and growth rate of these algae or to factors affecting their transient rates of nutrient uptake. This basic knowledge will be essential for assessing growth potential of macroscopic algae in nature or for effective managing of seaweed culture systems in which optimum productivity and efficient nutrient utilization is desired.

This study, undertaken as part of a program dealing with the growth and culture of macroscopic marine algae (27), focuses on factors affecting the short-term uptake of fixed, inorganic nitrogen by two rhodophycean algae, Neoagardhiella baileyi (Harvey ex Kutzing) Wynne and Taylor and Gracilaria foliifera (Forsskal) Børgesen. Of particular interest here are the kinetics of nitrate and ammonium uptake, and the effect of the nitrogen content of the seaweed and of photoperiod on nitrogen assimilation by these macrophytes.

MATERIALS AND METHODS

G. foliifera and N. baileyi were collected locally and maintained in suspended culture for over a year at the Environmental Systems Laboratory, Woods Hole Oceanographic Institution. Several days before an uptake experiment began, epiphyte-free seaweeds were preconditioned in continuous flow growth chambers which have been described elsewhere (9). Seawater medium containing desired levels of ammonium or nitrate was then supplied at appropriate rates so that a steady-state nitrogen content

could be established in the plants. It has been shown elsewhere (DeBoer, in preparation), that when both species used in this study are so preconditioned, their growth becomes severely nitrogen-limited once the C/N ratio (by atoms) exceeds 11.

Ammonium determination, using 10 or 25 ml samples, incorporated the Solórzano (28) reagents. To avoid photochemical effects (18), color development (2 h) was in the dark. Experiments in the concentration range exceeding the upper limit of the method's use on undiluted samples (ca. 60 μM) were avoided. Semi-automated methodology was used for NO_2^-- & NO_3^--N determination (12): a peristaltic pump controlled flow rate through a cadmium reduction column (34) and added reagents (29). Reacted samples were read manually with a Beckman DU spectrophotometer incorporating Gilford model 252-1 modifications. CHN analysis on freshwater rinsed, dried and powdered samples, was conducted with a Perkin-Elmer model 240 Elemental Analyzer.

All incubations were performed in either of two 11 cm radius cylindrical plexiglas chambers which were fitted with airtight tops to prevent gas exchange. The capacities of these chambers (one of which is pictured by D'Elia and Webb (12)) were 6.4 and 8.3 liters. Each chamber was fitted with a rheostat-controlled external pump for mixing; by placing the submersible pump in a cold water bath, it was also possible to maintain the temperature in the chamber at $20\pm1^{\circ}$ C. Intake and outflow of the pump was channeled through small holes closely spaced down the side of the chamber; their number, size, and orientation were designed so that the plants were kept in suspension by water flow. The angular velocity of

the vortex in the chamber was approximately one radian. sec^{-1}. Dye studies showed that mixing was complete and virtually instantaneous. Light was provided by a bank of cool white fluorescent tubes. The irradiance at the chamber periphery was 3.0-3.5 x 10^{-3} watts.cm^{-2}. Seawater for the incubations was collected from the Environmental Systems Laboratory seawater system just prior to experiments; it was passed through cartridge (nominal pore size, 1 μm) or Millipore (nominal pore size, 0.45 μm) filters. Depending on the experiment, between 2 to 7 g fresh weight of seaweed per liter were placed in the chamber and allowed to acclimate for 15 min before the addition of nutrients (reagent grade $(NH_4)_2HPO_4$ or $NaNO_3$) at the start of the experiment. Water for ammonium or nitrate determination was removed on a continuous basis by means of a peristaltic pump; in all experiments, discrete samples were collected by a fraction collector at ca. 5 min intervals.

In diel flux experiments, medium was supplied as required continuously or semi-continuously by a peristaltic pump. The supply medium containing 20-60 μM NH_4^+- or NO_3^--N and 4-10 μM PO_4^{3-}-P was made fresh every 6-12 h. The chamber was provided with an overflow so that a constant volume of water was maintained. Samples removed from the chamber to be processed for ammonium determination were "spiked" with the first Solórzano reagent (ethanol-phenol) to preserve the ammonium content of the sample until the other two reagents were added and the color developed (11). Samples preserved in this manner were analyzed within 12 h. Samples for NO_2^- & NO_3^--N, which were passed through the reduction column and reacted as they were taken, were read spectrophotometrically within 6 h.

This delay did not appreciably affect color yield.

Uptake kinetics experiments were all conducted as "batch" or "static" incubations. After nutrient addition, the time course of nutrient depletion was obtained from the fraction-collected samples. Since these experiments were short (usually less than 3 h), nitrogen determinations were made shortly after the samples were taken.

The effect of substrate concentration, S, on the rate of substrate uptake, V, has been traditionally modeled by the Michaelis-Menten expression

$$V = V_{max}S/(K + S) \qquad (1)$$

where V_{max} and K are constants respectively representing maximum uptake rate and the value of S at which $V = \frac{1}{2} V_{max}$. As this mathematical relationship does not invariably apply, one must avoid invoking it without first verifying its applicability. To do this, V vs S curves must be obtained initially. We chose to obtain such curves by mathematically differentiating S vs time, t, curves from substrate depletion experiments. To avoid erratic values of V resulting from the distorting effect of the analytical error inherent in the measurement of S (4), we did not calculate V simply by using the difference in substrate concentration between consecutive sample pairs. Instead, least squares linear regressions were performed on small segments (4-7 points) of the depletion curve from which data obviously in error were omitted. For such small segments and with the frequent sampling and small concentration change involved, correlation coefficients (r) are typically very high (>0.95), and the regression coefficient (m, i.e. the slope), obtained can be used to calculate V at the mean S value

over the segment ($V = m \cdot volume \cdot biomass^{-1}$). Such regressions were performed stepwise down the depletion curve, each consecutive step omitting the first data point of the previous step, and adding the next unused point in the sequence. If S/V vs S linear transformations (13) of the data were rectilinear, the Michaelis-Menten expression was used to model the data. If not, alternate expressions were sought as kinetic models (23).

When it was apparent from representative experiments that Michaelis-Menten kinetics were substantially followed, it was possible to simplify data reduction of subsequent experiments by calculating kinetic constants directly from depletion data by the method of Caperon and Meyer (4).

RESULTS

Nitrate uptake kinetics. Gracilaria foliifera, when grown with nitrate as its sole nitrogen source and incubated in the light in seawater containing elevated concentrations of nitrate, produces a depletion curve (i.e. S vs t) characteristic of Michaelis-Menten uptake. Figure 1 shows a typical curve in which the nearly linear depletion of nitrate with time (i.e. dS/dt) at higher concentrations reflects "zero order kinetics" or concentration-independent, rate-saturated transport, and at lower concentrations, dS/dt reflects "first order kinetics" or concentration-dependent, rate-unsaturated transport. At near saturating concentrations, there is no discernable increase in dS/dt as the experiment progressed. This means that growth, which could not have exceeded several percent during the experiment, did not appreciably affect uptake activity. By the end of this and most other experiments like it, no measurable amount of substrate remained in the medium; in a few of the other experiments (not shown),

a small but measurable amount of substrate (generally less than 100 nM) could be detected when uptake stopped. This unexplained cessation in uptake has been reported elsewhere for other nitrate transport systems (4 ,12). In such cases kinetic constants were calculated by the method of Caperon and Meyer (4): the small remaining amount of substrate, "S_o," when present, was subtracted from all S values in the depletion curve. This minor empirical correction is mainly to simplify treatment of the data and does not alter appreciably the kinetic values obtained (the S_o value is small relative to that of K in any case; V_{max} is unaffected). The results of kinetic analyses of all nitrate depletion experiments are summarized in Table 1. There was no correlation between C/N ratio and K (r^2 = 0.043; t = 0.672) or V_{max} (r^2 = 0.031; t = 0.567) in this C/N range. Mean values of K and V_{max} respectively, were 2.48±0.51 (SE) μM and 162±19 nmoles N.(g dry weight.min)$^{-1}$.

The kinetics of Neoagardhiella baileyi compared closely with those of Gracilaria foliifera: K was 2.40±0.30 μM and V_{max} was 195±16 nmoles N·(g dry weight.min)$^{-1}$ (Table 1).

Preferential uptake of ammonium. Figure 2 illustrates a number of aspects of nitrate and ammonium uptake by Gracilaria foliifera. Initially, when nitrate alone was present in the medium, it was depleted at a constant, essentially concentration independent rate. Addition of approximately 18 μM ammonium to the medium slowed nitrate removal; but once ammonium levels were reduced below ca. 5 μM, nitrate depletion again resumed at its original rate to accompany ammonium uptake.

Figure 2 demonstrates also that ammonium depletion showed no indication of concentration independence at the highest ammonium concentrations

attained, and that the maximum rate of ammonium depletion far exceeded the maximum rate of nitrate depletion during the experiment. These aspects of nitrogen assimilation will be considered in greater depth below.

Ammonium uptake kinetics. The ammonium uptake kinetics for both seaweed species were distinctly different from their nitrate uptake kinetics. Figure 3A shows the results of duplicate ammonium depletion experiments using Neoagardhiella baileyi; Figure 3B shows the V vs S curves derived. Saturation of uptake was not evident at concentrations near 50 μM (Fig. 3B), the highest concentration during the experiment (chosen because it was just below the upper limit of determination of the unmodified Solórzano method). Instead of reaching saturation, V increased with S in an essentially linear fashion at S values greater than ca. 10 μM. Such data suggest a multiphasic uptake system: below 10 μM a high ammonium affinity (i.e. low K) component predominates, at higher concentrations a low affinity (high K), high V_{max} or a strong diffusive component predominates. The curve fitted to the data in Fig. 3B is drawn assuming that the simplest case is accurate, i.e., that there is a strong diffusive component predominating. By extrapolating from the linear portion of the curve to the intercept on the ordinate, an estimate of the value of V_{max} is obtained for the high ammonium affinity uptake component; the slope of the extrapolated line gives the value of the diffusive component. To calculate K for the high ammonium affinity component, the contribution to uptake represented by the linear portion of the curve was subtracted from all V data. The resulting corrected uptake rates (V'), could then be transformed in the S/V vs S form (Fig. 3C) and K obtained as the absolute value of the intercept on the abscissa.

Although the absolute rate of ammonium assimilation at a given concentration varies with the nitrogen content (reflected by the plant's C/N ratio) of Neoagardhiella baileyi, the non-saturating form of the V vs S curves does not (Fig. 7). The kinetic constants calculated for the curves in Figure 7 are shown in Table 2. Values for K are approximately the same regardless of C/N ratio, whereas values for the diffusion coefficient, K_D, and V_{max} at the lower C/N ratio are considerably lower than at the higher C/N ratio. Evidence will be adduced later indicating that experimental variability account for much of the differences between constants obtained for the two higher C/N plants.

The ammonium V vs S curves for Gracilaria mirrored closely those for Neoagardhiella (Fig. 5). The curve indicated by the dashed line is a composite fit to data from four separate depletion experiments, starting at varying initial ammonium concentrations. As the data from different experiments superimpose well on each other, the non-saturating shape of the curve seems to be verified, and is not the result of a decrease in uptake rate during an individual experiment in response to nitrogen accumulation by the plant. Kinetic constants calculated for this composite of four experiments are shown in Table 2. The value of K is reasonably close to those calculated for Neoagardhiella.

Relationship between nitrogen content and ammonium uptake rate. DeBoer (in preparation) shows that the growth rates of N. baileyi and G. foliifera are severely nitrogen-limited once the plants' C/N ratios exceed ca. 11. There is also a relationship between C/N ratio of the seaweeds preconditioned with varying supplies of ammonium and their rate of ammonium uptake (Fig. 6). Due to the complicated, non-saturating uptake

kinetics for both species, the simplest approach to assessing this relationship was to compare uptake rates at an arbitrarily chosen concentration. Standard errors on given uptake rates were small relative to the variation in rates measured at a particular C/N ratio. Hence, most error is between-experiment rather than within-experiment. Biological variation as well as errors in measurements of biomass and water present in an individual experiment probably account for most of this variability. Clearly, at C/N ratios greater than 10, the uptake of ammonium for both species varies little on the average, if at all, at the substrate concentrations tested. Below that C/N ratio, for both species, the uptake rates drops off precipitously with decreasing C/N ratio and increasing nitrogen content. At the lowest ratios obtained by supplying a substantial excess of ammonium (relative to growth requirements) to the seaweeds over a several day period, a net efflux of ammonium was observed (Fig. 6), suggesting that such preconditioning creates an internal ammonium pool from which exchange to the external medium occurs. The curvilinear relationship we have visually fitted for C/N ratio vs uptake rate appears to indicate a varying uptake ability dependent on the nitrogen content of the algae.

Diel rhythmicity in nitrogen uptake rate. Figure 7 illustrates the difficulties inherent in designing experiments to detect diel changes in nutrient uptake rates. When medium containing ammonium was supplied on a continuous basis at the rate of two exchanges (turnovers) per day during an incubation of Gracilaria, the residual (effluent) concentration in the chamber medium remained low and did not fluctuate appreciably. This indicates that capacity for ammonium uptake kept apace with or

exceeded resupply of ammonium to the chamber via fresh medium. However, this did not rule out undetected diel changes in uptake. When the resupply rate of ammonium was increased to four exchanges per day, such changes in uptake became evident (Fig. 7). During the day, uptake exceeded resupply and the residual concentration in the chamber medium dropped; during the night, resupply exceeded uptake and the residual ammonium concentration increased, but at a slower rate than it would have if the seaweed were not present in the chamber.

In view of the difficulties encountered in interpreting results of experiments such as the one given in Fig. 7, it is preferable to design experiments so that uptake measurements are made directly at a single concentration without the complication imposed when assessing the differential between uptake and resupply during continuous flow experiments. Accordingly, we chose to run repeated depletion experiments over the diel period, only flushing the chamber with fresh medium between experimental runs (Fig. 8). For Gracilaria with a C/N ratio at which growth was clearly nitrogen-limited (here, 12.3 by atoms), no statistically significant diel periodicity could be detected in ammonium uptake rate at 20 μM substrate concentration ($V^{20}_{NH_4^+}$). However, when nitrogen content was adequate to sustain maximum growth (C/N = 7.5), not only was the absolute rate of ammonium uptake less, but also statistically significant diel variation was observed.

No diel changes in the uptake rate of nitrate at a substrate concentration of 20 μM ($V^{20}_{NO_3^-}$), when the C/N ratio was 17.2 (Fig. 8). We did not however, attempt to measure diel effects using nitrate preconditioned seaweeds at lower C/N ratios, so we cannot say whether diel effects occur under such conditions.

DISCUSSION

One interesting result of this study is the remarkable similarities we have encountered in the nitrogen uptake kinetic patterns for the two seaweed species. Thus, the general discussion here has applicability to both.

Although the Michaelis-Menten expression has proved very useful to relate uptake rate to substrate concentration for many organisms and many substrates, there has probably been too great a tendency to accept the validity of that model in a given situation without rigorous verification. The uptake of ammonium by Neoagardhiella and Gracilaria seems to provide a good example of why such verification is necessary. A proper kinetic model for ammonium uptake by these macrophytes should include both a term for a Michaelis-Menten, rate-saturating component, and an additional one for diffusive uptake or for a high K, high V_{max} component.

There have been other reports of analogous kinetic patterns for inorganic nutrient uptake by other organisms. For example, Chisholm and Stross (6) found that phosphate uptake by Euglena gracilis followed non-saturating kinetics. Their discussion is relevant to this work. Likewise, Muscatine and D'Elia (21) have similarly described the uptake of ammonium by corals containing endosymbiotic algae. Their mathematical treatment of the kinetics seems applicable in the current situation, hence we will not elaborate on that further here.

This study was not directed at elucidating the specific biochemical mechanisms whereby ammonium is incorporated by the macroalgae studied, the kinetic patterns we observed do suggest that more than one transport

system exists. If that is true, at low concentrations more typical of the natural environment, a high affinity component with a K of less than 5 μM predominates; at higher concentrations, the other component with low substrate affinity predominates. It has been suggested (15) that in phytoplankton, more than one pathway exists for ammonium incorporation, with one predominating at low natural concentrations.

Our research has not included possible effects of water movement (shear) on the rate of nutrient uptake (3,17,25,32,33). Boundary layers, which form in response to reduced shear, reduce nutrient influx. This reduction has been termed "diffusion transport limitation" (17,25). In this paper, we use "diffusion" to indicate a membrane transport component, and have chosen to avoid "diffusion transport limitation" experimentally by providing vigorous mixing to reduce boundary layers. Future studies will be necessary to assess such effects for seaweeds.

Preferential ammonium uptake by the seaweeds was indicated both by the suppression of nitrate uptake by ammonium (Fig. 2), and by the differences in kinetic patterns for the two nutrients. Preferential assimilation of ammonium over nitrate has often been shown for marine algae (4 , 5 , 8,14,24, 26, 30). The ammonium concentration required to suppress nitrate uptake by phytoplankton typically is in the 0.5-1.0 μM range. That concentration seems to be higher for G. foliifera (and presumably N. baileyi), as we found that unsupressed nitrate uptake occurred even at 5 μM ammonium. Thus, simultaneous uptake of ammonium and nitrate appears possible at quite high ammonium concentration. This is consistent with the report of Bird (1) who found that Gelidium nudifrons, also a rhodophycean, was capable of simultaneous assimilation of both forms of nitrogen.

As a result of the difference between kinetic patterns for ammonium and nitrate transport for both species, far more nitrogen can be assimilated via ammonium transport than via nitrate transport when the nutritional state of the algae is such that maximum transport rates occur (i.e. at C/N ratios greater than 10). This is illustrated by Fig. 9 which gives idealized V vs S curves for Gracilaria based on the results shown in Fig. 5 and Table 1. At a nitrate concentration of 15 μM, the rate of nitrogen assimilation via nitrate uptake for G. foliifera could only support a growth rate of 9.3% per day (i.e. g N assimilated . g N content . 100 . day^{-1}), assuming a 3% nitrogen content by dry weight at maximum growth rate (DeBoer, in preparation). In contrast, the rate of ammonium assimilation at 15 μM substrate concentration, for example, if sustained, could support a growth rate of nearly 50% per day. This is far in excess of the maximum growth rate of 12.3% measured at this nitrogen concentration (9). At higher ammonium concentrations, this discrepancy would be even greater.

The impressive transient uptake capacity demonstrated for ammonium may reflect an interesting strategy for nitrogen acquisition by these algae. Thus, as quickly as ammonium is locally regenerated (excreted) by sporadic sources such as epifauna or passing fish, it is sequestered by the seaweeds. There is some evidence that microalgal ammonium uptake ability may also exceed nitrate uptake ability (5 , 19) and far exceed that necessary to sustain maximum growth rate (2 , 5 , 22).

The effect of nitrogen content (as indicated by C/N ratios) on both the absolute rate of ammonium uptake and the diel fluctuations in that rate underscores the need to consider the nutritional status of the

organism in nutrient flux studies. Variations in the capacity to take up ammonium have been shown to reflect nitrogen content in other algae (2, 4, 16, 31); of the authors cited, only Caperon and Meyer (4) reported increasing uptake capacity (as increasing V_{max}, with K remaining constant) with increasing nitrogen content and growth rate, the others reported results more consistent with our observations of decreasing uptake rate with decreasing C/N ratios and increasing growth rate. It appears that this and the nocturnal decrease in uptake rate may be in response to nitrogen satiation.

CONCLUSIONS

The seaweeds studied take up nitrate in a rate-saturating fashion: V vs S curves yielded can be fitted with a rectangular hyperbola described by the Michaelis-Menten equation. At substrate saturation, plants with widely varying nitrogen content exhibit maximum rates of uptake which compare closely to the daily nitrogen accumulation at steady-state, maximum growth rate on that substrate. No diel effects are detected in rate of uptake, nor does there appear to be a decrease in uptake ability at maximum growth rates.

For ammonium, the kinetic pattern is different. Uptake does not saturate. The capacity for incorporation of nitrogen by N-starved seaweed (i.e. when C/N ratio exceeds 11) far exceeds the capacities of unstarved seaweeds to do so. N-starved seaweeds exhibit no diel periodicity in ammonium uptake rate, but once the plants have assimilated enough nitrogen that they are no longer N-starved, diel effects become apparent, with the rate of uptake declining substantially in the dark.

Thus there appear to be mechanisms by which excessive assimilation of nitrogen is avoided by the plants and by which they quickly overcome substantial nutritional deficits in nitrogen, once ammonium becomes available.

This study provides an example of why one should use caution in the analysis of kinetic patterns of nutrient uptake and in ascribing ecological significance to half-saturation constants: Michaelis-Menten uptake does not invariably apply. Even if it does, although a low value of K may indicate a high affinity of a transport system for a substrate, one must think also in terms of the significance of V_{max} as well (4, 7).

ACKNOWLEDGMENTS

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Table 1. Summary of nitrate uptake kinetics, G. foliifera and N. baileyi. Constants were obtained by fitting the integrated form of the Michaelis-Menten equation by least aquares to nitrate depletion curves (4). C/N given by atoms, K in μM, and V_{max} in nmoles N. (g dry weight)$^{-1}$. min^{-1}.

Species	C/N	K	V_{max}
G. foliifera	26.6	1.8	125
	22.0	4.1	181
	21.7	7.1	242
	20.1	0.2	56
	16.1	1.0	126
	15.6	1.2	106
	13.2	3.6	271
	11.8	2.0	126
	11.8	1.6	164
	11.1	2.5	194
	10.7	3.8	248
	10.6	0.9	104
Mean (SE)		2.48 (0.51)	161.9 (19.2)
N. baileyi	11.1	2.7	210
	10.9	2.1	179
Mean (SE)		2.40 (0.30)	194.5 (15.5)

Table 2. Summary of kinetic constants calculated for ammonium uptake. These data are calculated from the curves shown in Figures 3, 4, and 5.

Species	C/N [a]	K [b]	V_{max} [c]	K_D [d]
N. baileyi	17.06	4.9	199	0.0341
	12.45	4.5	500	0.0370
	6.60	2.3	94	0.0056
G. foliifera	10.22-16.26	1.6	397	0.0232

[a] By atoms.
[b] In μM.
[c] In nmoles N.(g dry weight.min)$^{-1}$
[d] In liters.(g dry weight.min)$^{-1}$

FIGURE LEGENDS

Fig. 1. Typical nitrate depletion curve for G. foliifera.

Fig. 2. Effect of ammonium addition on the depletion of nitrate by G. foliifera (C/N = 17.6) preconditioned on nitrate.

Fig. 3. A, Replicate ammonium depletion experiments (i.e. S vs t) using N. baileyi (C/N = 6.7) preconditioned on ammonium. B, Dotted line--rate of uptake of ammonium (V) vs ammonium concentration (S); solid line--diffusive component calculated as the regression coefficient (slope) using all V points with corresponding S values greater than 4.5 μM. C, S/V' vs S linear transformation of data in B, using V' values obtained by subtracting the diffusive component from all V values.

Fig. 4. Ammonium V vs S curves for N. baileyi with different nitrogen content--(●) C/N = 6.7 (from Fig. 3B), (▼)C/N = 12.45, (▲)C/N = 17.06.

Fig. 5. Ammonium V vs S curves obtained from 4 separate depletion experiments using G. foliifera of similar nitrogen content--(□) C/N = 10.22, (○) C/N = 11.32, (■) C/N = 11.66, (●) C/N = 16.26.

Fig. 6. Effect of C/N ratio on ammonium uptake rates ($V_{NH_4^+}$) of seaweeds at a given mean concentration. A, $V^{25}_{NH_4^+}$ for G. foliifera. B, $V^{30}_{NH_4^+}$ of N. baileyi. Standard errors given, obtained from linear regressions of small portions of depletion curves in the appropriate concentration range. Curves fitted visually.

Fig. 7. Diel changes in ammonium concentration during a continuous flow incubation of G. foliifera (C/N = 6.89), at two different exchange

rates of fresh influent medium consisting of filtered seawater with 60 μM ammonium addition. Shaded area indicates period of darkness.

Fig. 8. Diel variations in uptake rates (± 1SE) of G. foliifera with different nitrogen content. A, Ammonium uptake rate at 20 μM ($V^{20}_{NH_4^+}$): (o) C/N = 12.3, (•) C/N = 10.0, (▲) C/N = 7.5. B, Nitrate uptake rate at 20 μM ($V^{20}_{NO_3^-}$): C/N = 17.2.

Fig. 9. Idealized uptake curves for G. foliifera using kinetic constants from Table 1 and Figure 5.

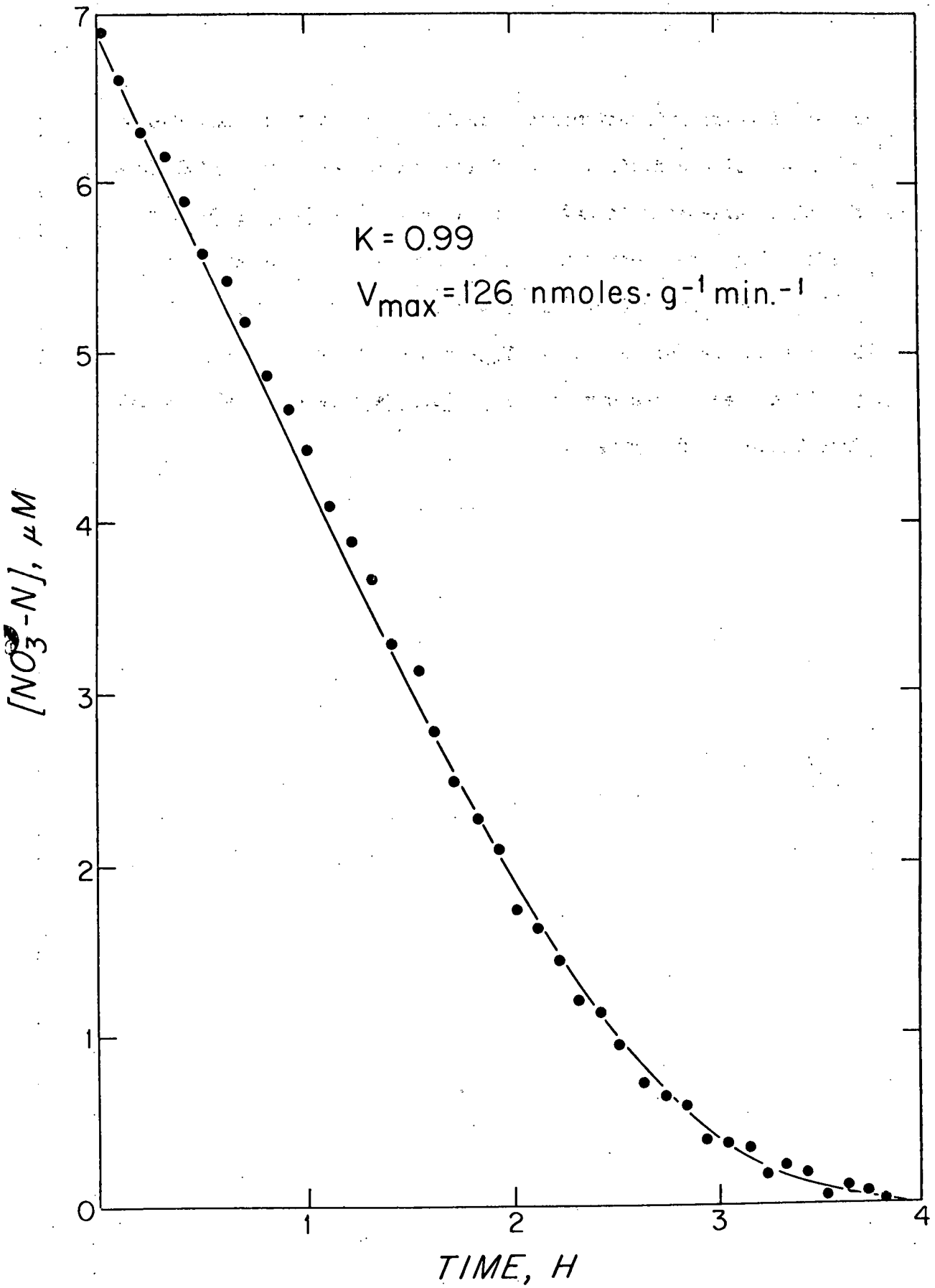

Fig. 1

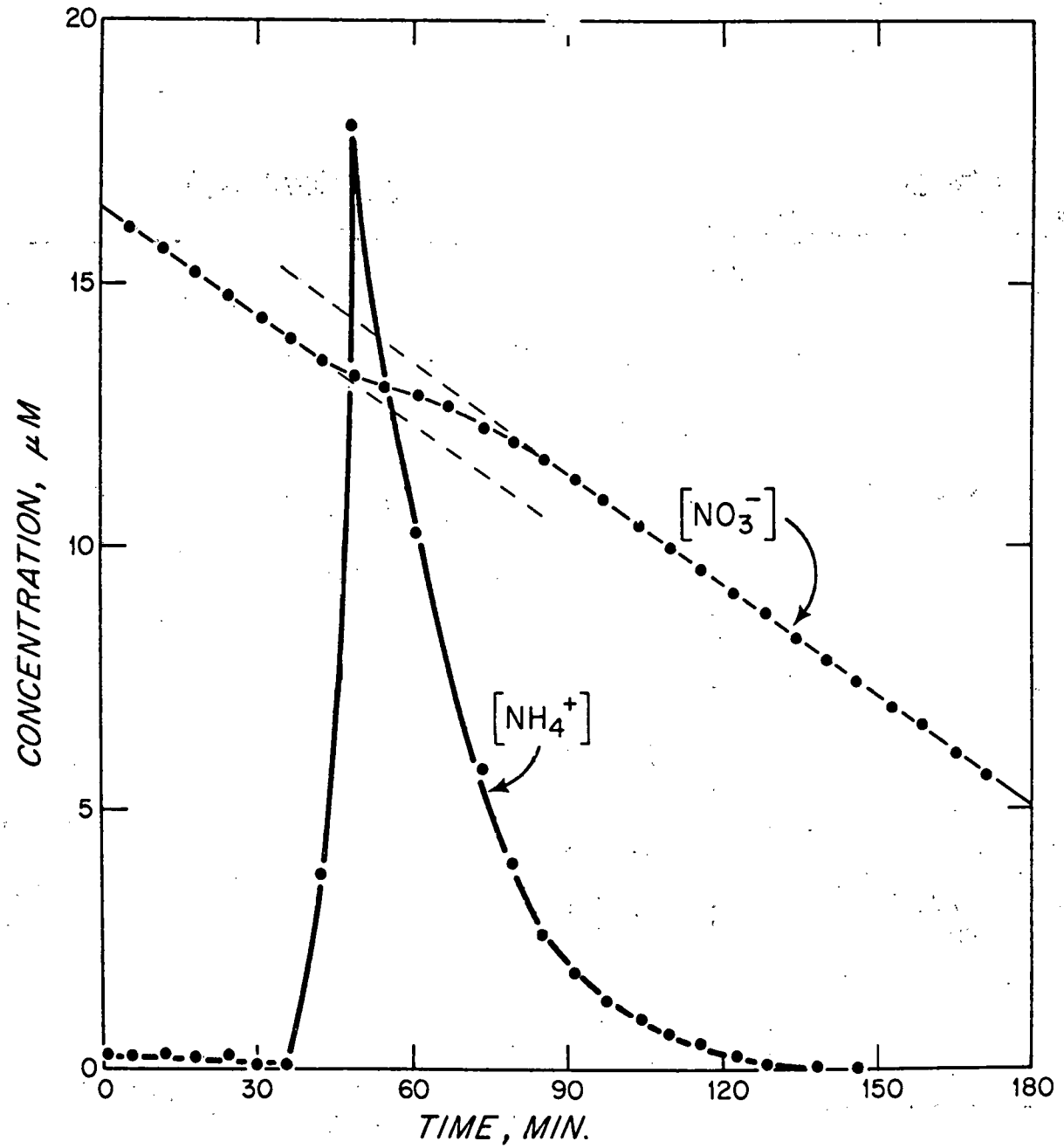
20
15
10
5
0
0
30
60
90
120
150
180
CONCENTRATION, μM
TIME, MIN.
[NO3-]
[NH4+]

Fig. 2

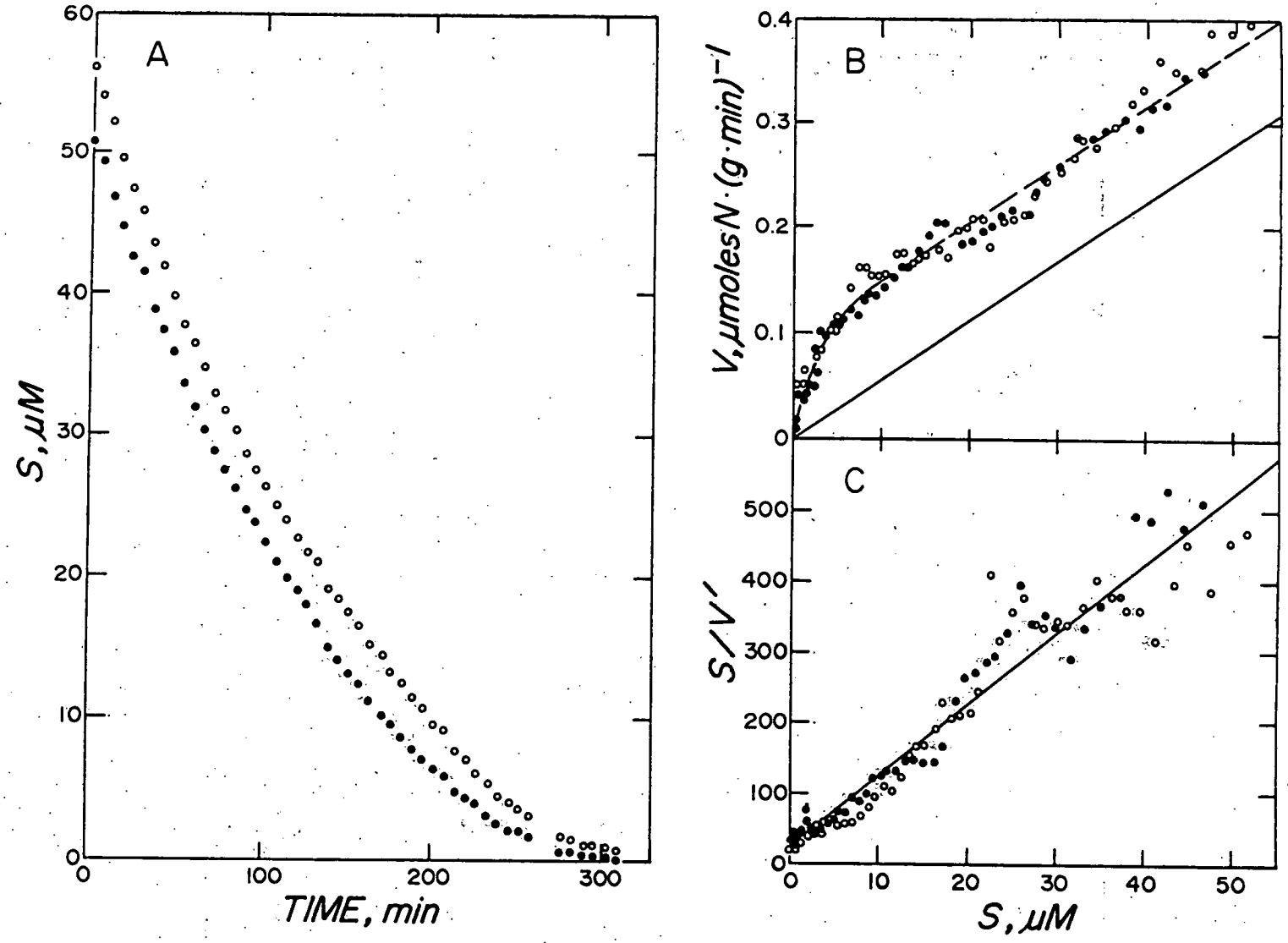
A
S, μM
TIME, min
0
10
20
30
40
50
60
0
100
200
300
B
V, μmoles N·(g·min)⁻¹
0
0.1
0.2
0.3
0.4
C
S/V′
S, μM
0
100
200
300
400
500
0
10
20
30
40
50

Fig. 3

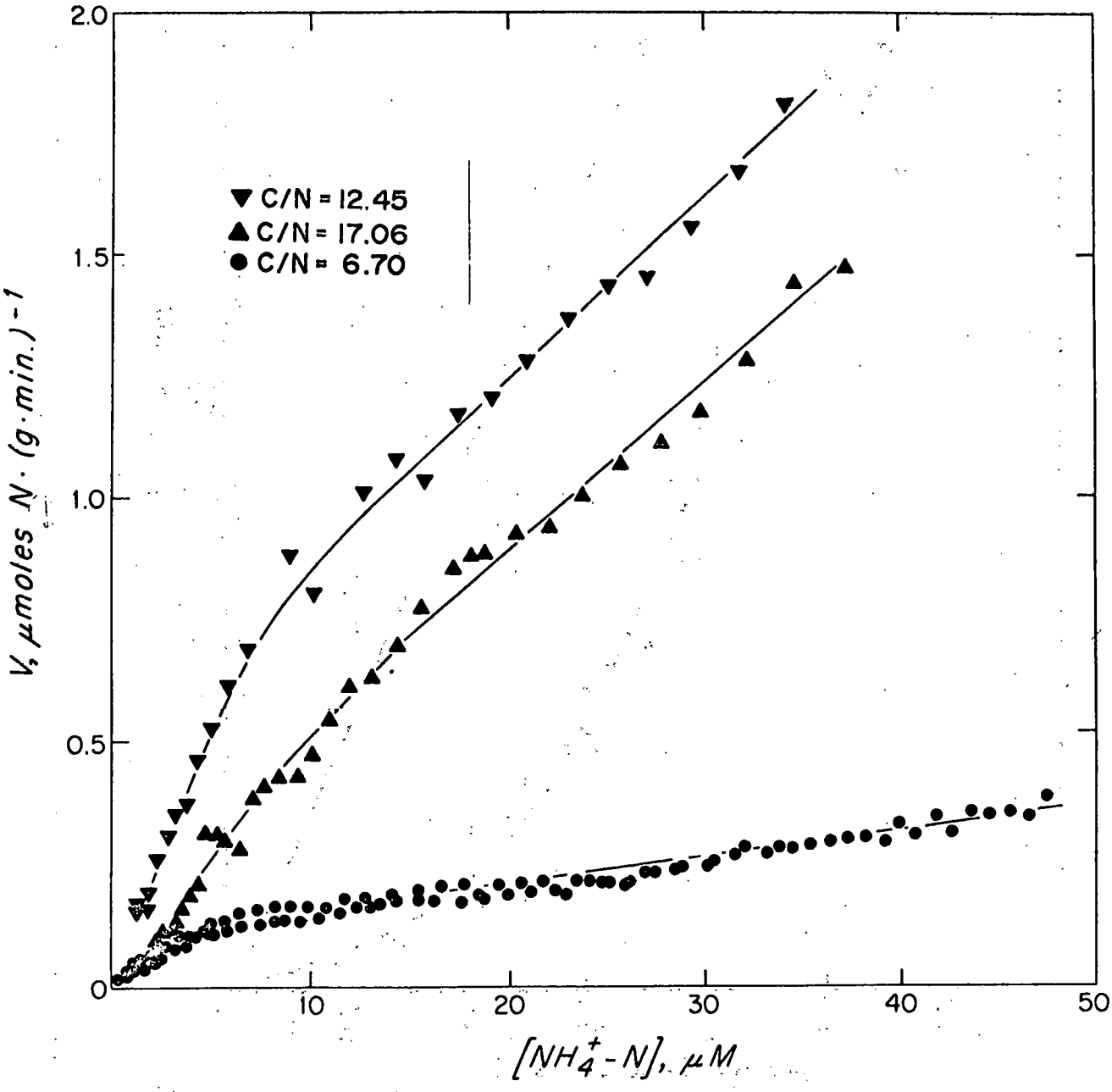
2.0
1.5
1.0
0.5
0
10
20
30
40
50
▼ C/N = 12.45
▲ C/N = 17.06
● C/N = 6.70
V, μmoles N · (g · min.) - 1
[NH4+ - N], μM

Fig. 4

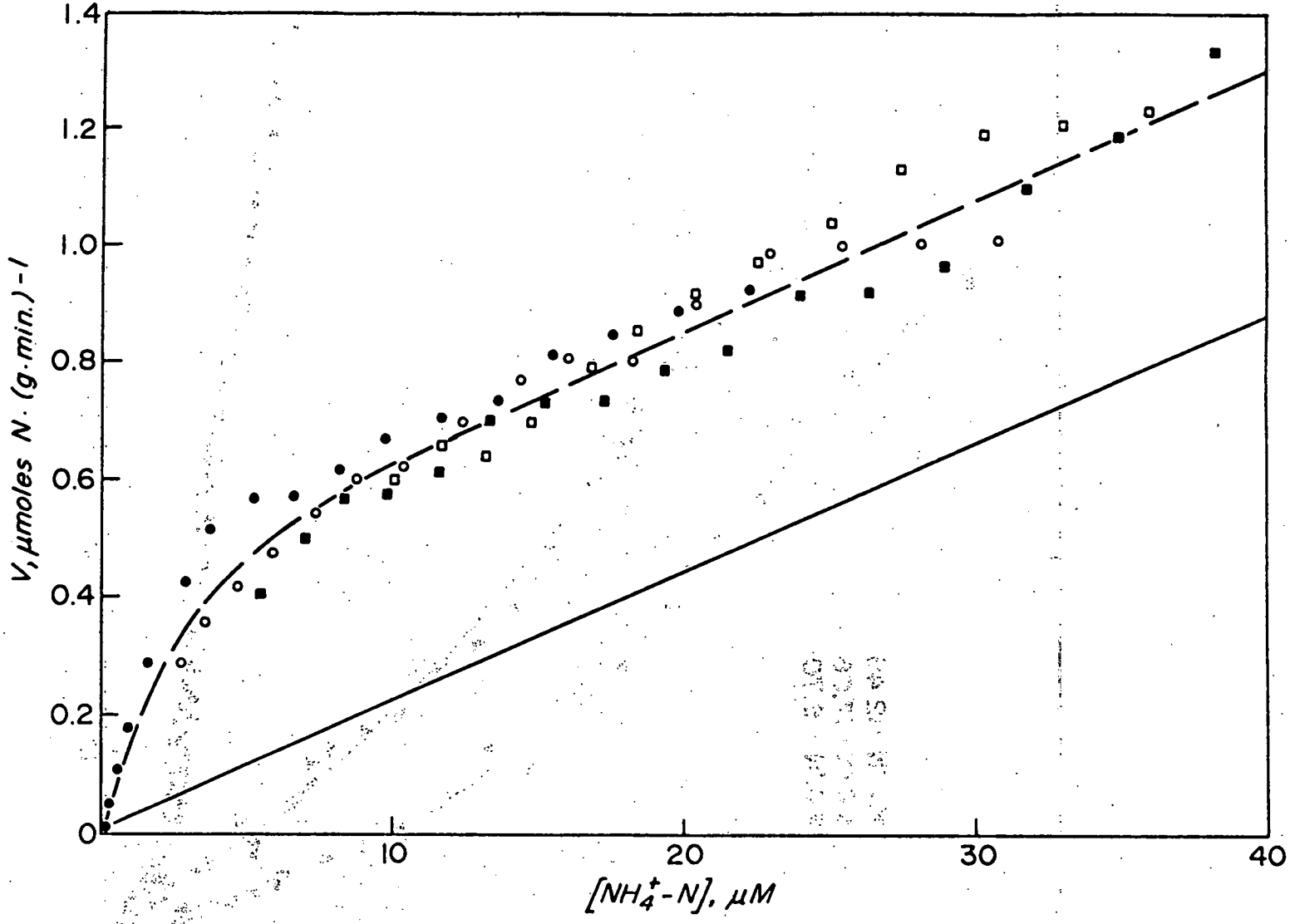

Fig. 5

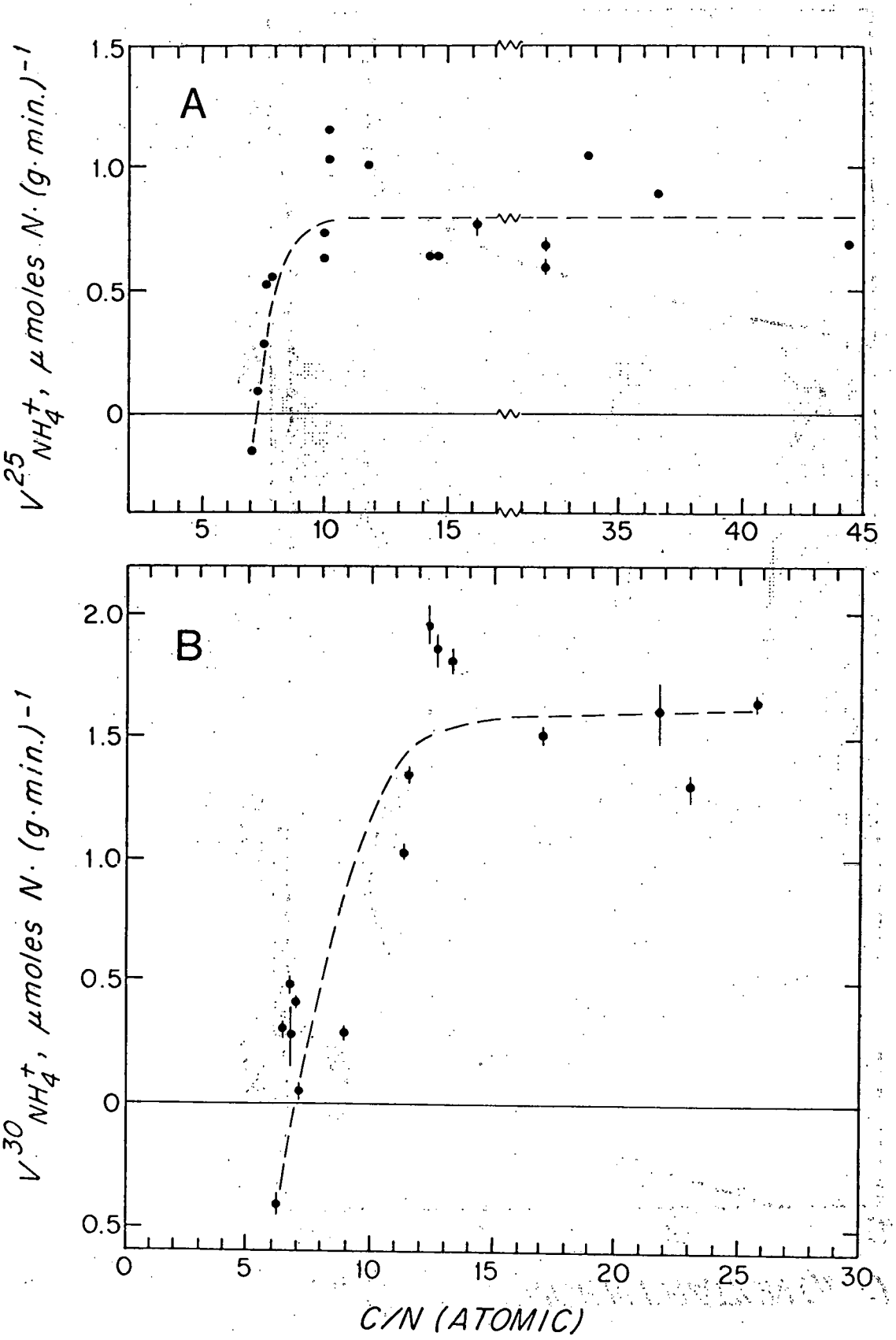
A
B
$V^{25}_{NH_4^+}$, μmoles N·(g·min.)$^{-1}$
$V^{30}_{NH_4^+}$, μmoles N·(g·min.)$^{-1}$
C/N (ATOMIC)
1.5
1.0
0.5
0
5
10
15
35
40
45
2.0
1.5
1.0
0.5
0
0.5
5
10
15
20
25
30

Fig. 6

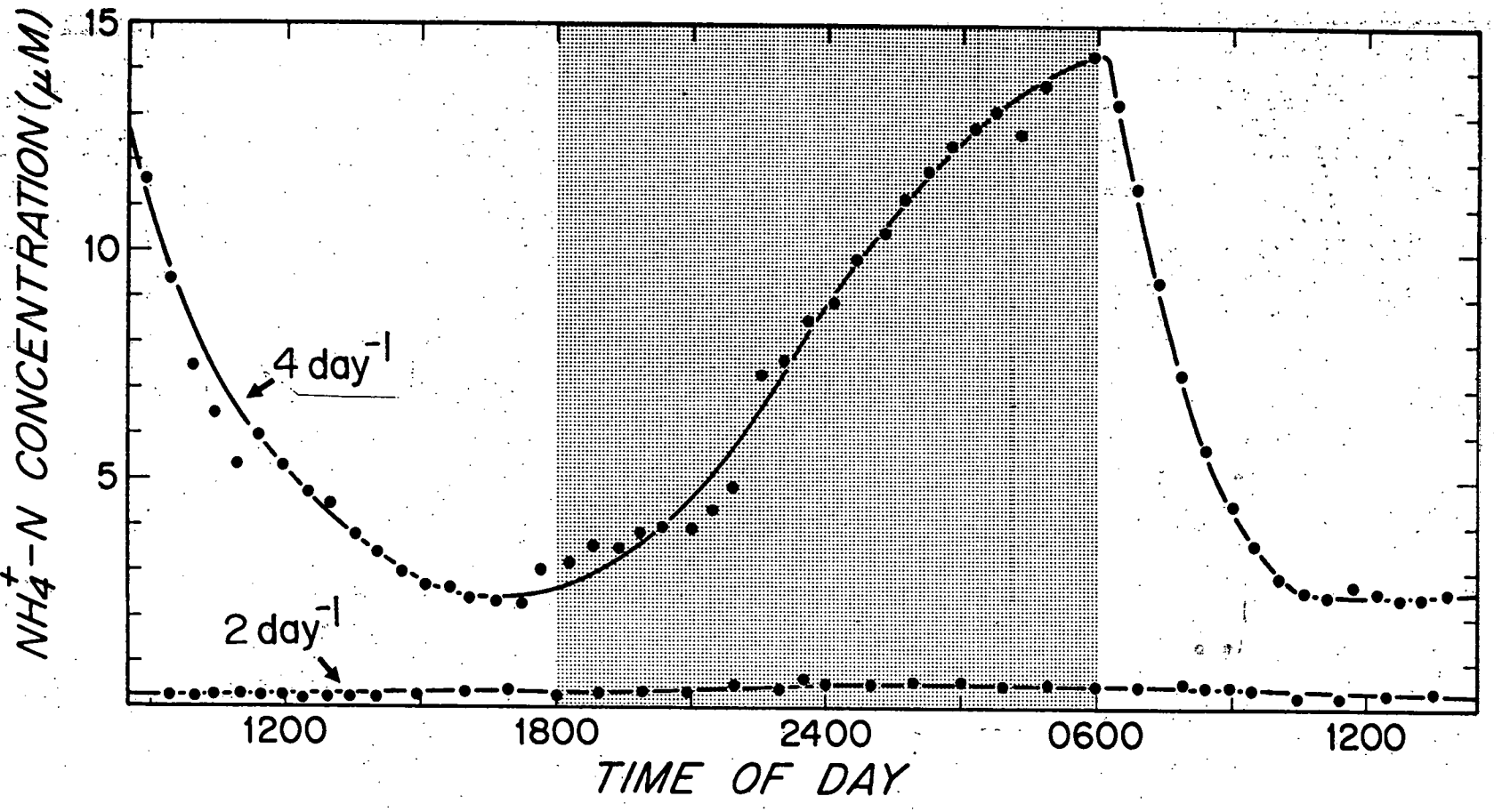
15
10
5
NH_4^+-N CONCENTRATION (μM)
4 day^{-1}
2 day^{-1}
1200
1800
2400
0600
1200
TIME OF DAY

Fig.

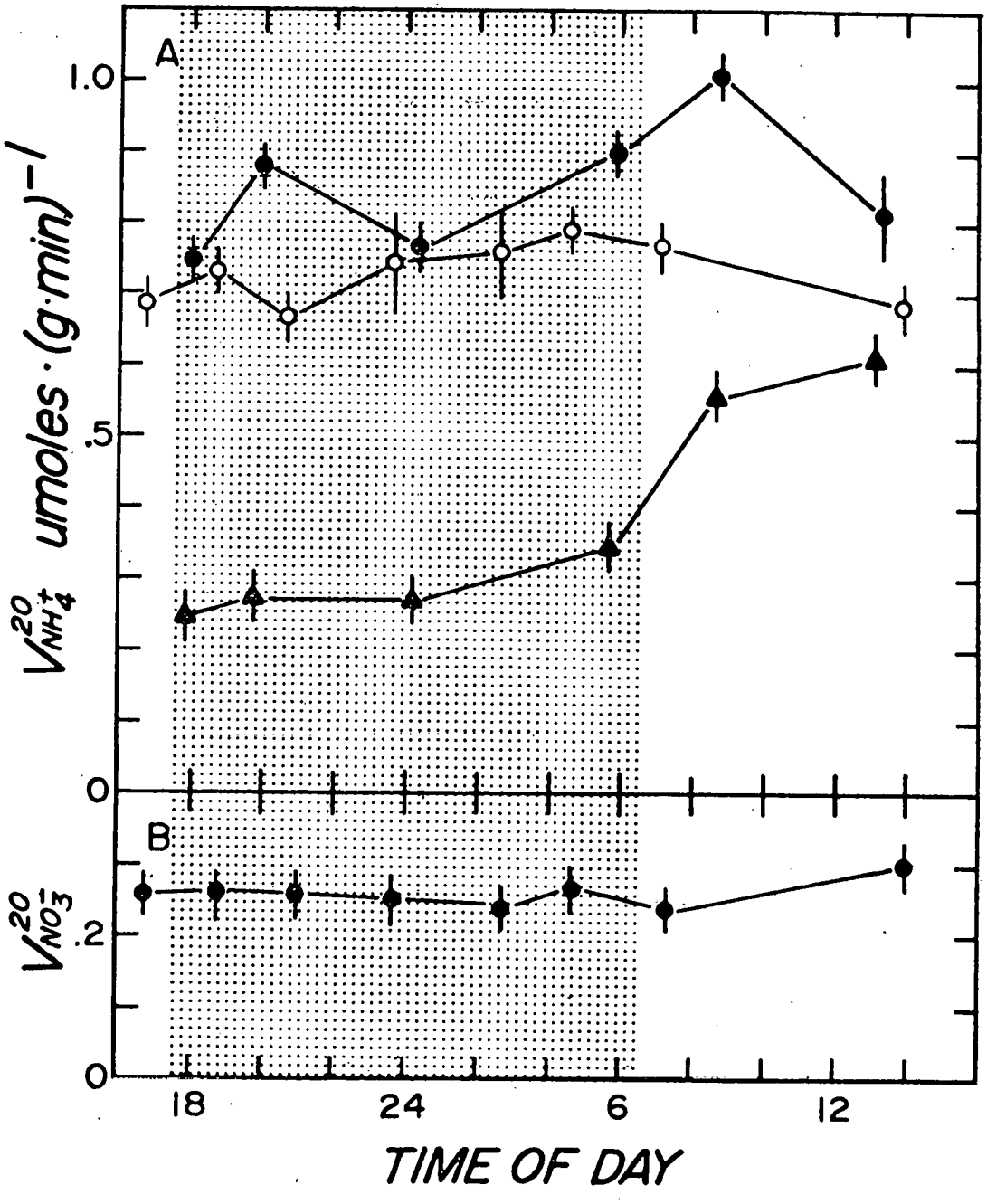

Fig. 8

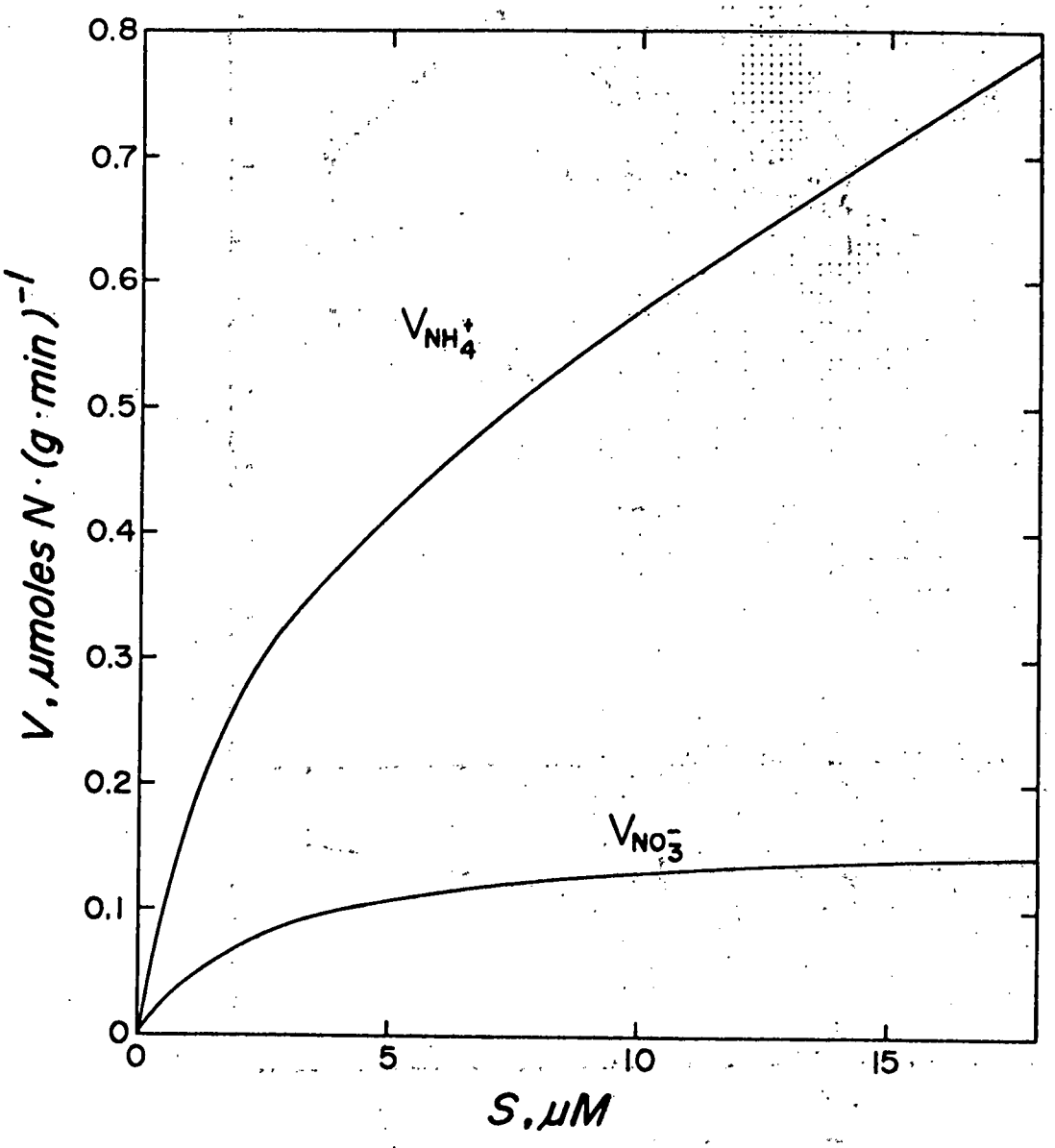

Fig. 9

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The effects of nitrogen and flow rate on the growth and composition of *Gracilaria foliifera* V. *angustissima* (Harvey) Taylor in mass outdoor cultures*

by

Brian E. Lapointe

and

John H. Ryther

Woods Hole Oceanographic Institution

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*Contribution No. 4044 from the Woods Hole Oceanographic Institution.

Contribution No. from the Harbor Branch Foundation, Inc.

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Abstract

A series of outdoor, continuous-flow seawater cultures (50-1) were used to investigate the effects of nitrogen form (NO_3^- vs. NH_4^+, nitrogen loading (total nitrogen input per day) nitrogen concentration and water turnover rate on the growth and composition of Gracilaria foliifera v. angustissima (Harvey) Taylor (Gigartinales). Both nitrogen assimilation and algal growth were related to nitrogen loading and found independent of nutrient concentration. Above a minimum nitrogen loading of between 9 and 15 m moles/day algal growth was highly dependent upon turnover rate. Levels of stored inorganic nitrogen ranged from 0.5 μ mole/g dry wt in slow growing, nitrogen limited plants to 5 μ mole/g dry wt in slow growing, nitrogen-enriched plants. Growth of Gracilaria was better with NH_4^+ - N than NO_3^- - N at nitrogen loadings that were just adequate to support exponential growth. However, at higher levels of nitrogen loading, the greatest yields (up to 44 g/dry wt/m^2/day) occurred with NO_3^- - N rather than NH_4^+ - N possibly due to toxic effects of the latter. Percent of dry weight and ash of Gracilaria was highly correlated with nitrogen content of the plant and affected the heat of combustion of the alga in a similar fashion. Although levels of both phycoerythrin and chlorophyll increased with increasing nitrogen content of the plant, phycoerthrin/chlorophyll ratios did not change substantially and suggests that nitrogen-related pigment changes in Gracilaria are light intensity and not light quality adaptations. The use of carbon/nitrogen ratio as an index of the nutritional state of Gracilaria

(and probably other seaweeds) appears to be a useful tool in understanding its growth and composition and should be considered in ecological investigations concerning seasonal patterns of seaweed biochemistry and their relation to herbivore and detritavore food chains.

Introduction

Seaweeds may contribute significantly to the autotrophic production of the coastal marine environment. Mann (1973) has shown that in the Margaret's Bay, Nova Scotia, seaweeds may fix as much as three times the annual production of the phytoplankton. Ryther (1963) suggested that even though the macroscopic algae are restricted to an area 1% that of the phytoplankton, seaweeds may contribute as much as 10% of the total productivity of the oceans on an annual world basis. Clearly, these larger algae make a substantial contribution to marine food chains via direct grazing by herbivores or, more importantly, by decomposing and forming one of the major sources to the detrital pool (Mann, 1972). Khailov and Burlakova (1969) estimate that 30% of the gross seaweed production becomes available for detritavore secondary production inthe Barents Sea, whereas only 10% is consumed directly by herbivores.

Yet, studies concerning the growth and nutrition of seaweeds even in relation to aquaculture procedures are few. Other than some "farming" practices carried out in the Orient (Bardach et al., 1972) controlled seaweed culture is a relatively new field.

Recently, as a result of the increasing demand for seaweed extracts (carrageenan and agar) for the food, drug and cosmetic industries (Silverthorne and Sorenson, 1971), investigations in the culture of commercially important species (i.e., Irish moss, Chondrus crispus) were begun both by the National Research Council of Canada (Neish and Fox, 1971; Neish and Shacklock, 1971) and by several industries involved in the processing of seaweeds. Their hope was to supplement the limited natural resources by intensive aquaculture of select seaweed species. This stimulated a number of laboratory studies in which short term photosynthetic rates as a function of light and temperature (Mathieson and Dawes, 1974), growth and reproduction (Dawes et al., 1974a) and carrageenan content (Fuller and Mathieson, 1972; Dawes et al., 1974b) of a number of seaweed species were examined. However, none of these studies was concerned with specific effects of inorganic nitrogen on the growth dynamics and related composition of the algae in long term culture experiments.

While the summer decline in biomass and production of phytoplankton has been related to the depletion of nutrients and is well known (Harvey, 1926; Ryther and Dunstan, 1971), the role of nutrients in the seasonal growth of seaweeds is not well understood. The kelp, Laminaria longicruris (Chapman and Craigie, 1977) actually stores nitrogen internally to support its growth at times of the year when levels of dissolved inorganic nutrients are low. Seasonal studies with seaweeds have indicated distinct seasonal variations in caloric content and percent dry weight (Himmelman and Carefoot, 1975), protein and

carbohydrate (Dawes et al., 1977) and pigment content (Moon and Dawes, 1976). These studies showed that seaweeds have the ability to store nitrogen and undergo biochemical changes related to seasonal environmental changes. Again, however, none has related the specific effects of inorganic nitrogen levels on the seasonal growth and composition of the algae.

As part of our efforts in developing wastewater recycling aquaculture systems, we have used continuous cultures to investigate the effect of nitrogen on the growth of the red alga, Gracilaria foliifera v. angustissima. Because Gracilaria can have a rapid growth rate with high yields over long time periods (Lapointe and Ryther, 1977) this alga is an excellent assay organism for such culture experiments. In the previous experiments, the two operating parameters of turnover rate (flow rate/culture volume) and nutrient loading (total input per day) were held constant so that the effects of these parameters on algal growth were not known. In the present study, we have simultaneously investigated the effects of turnover rate, total daily nitrogen loading and nitrogen form on algal growth and composition.

Materials and Methods

Experimental Culture System: Design and Operation

The culture system consisted of two six meter long, 0.4 m diameter PVC pipes, longitudinally sectioned and further divided by

means of fiberglassed plywood partitions into eight 0.75 m long (50 l; 0.23 m^2 surface area) compartments (Figs. 1a, 1b). Compressed air (5 psi) entered along the bottom of each chamber through small drilled holes from an airline -- a half section of a one inch PVC pipe cemented to the outside bottom of the chamber. Each compartment also had a non-clogging overflow drain. The growth chambers were located out-of-doors in full sunlight on a 6.2 m x 12.4 m concrete platform (Fig. 2). Total radiant energy incident to the growth chambers with an Epply pyroheliometer.

Seawater was pumped from the Harbor Branch Foundation ship channel which connects to the Indian River, a shallow lagoon on the Atlantic Ocean. No attempt was made to control water temperature, which ranged from 18 to 34°C, or salinity, which ranged from 26% to 33% over the experimental period from May 11 to June 24, 1977. Seawater, after being pumped to an elevated headbox, was gravity fed to the sixteen culture chambers at the desired flow rates -- four chambers receiving each of the desired 1, 7.5, 15 and 30 turnovers per day.

Different media made with seawater were held in 4000 l reservoirs from which they were pumped to elevated headboxes and distributed to the appropriate chambers in Experiment I. The three media compositions (NH_4 - N plus PO_4^3 - P; NO_3^- - N plus PO_4^3 - P; and NO_3^- - N enriched sewage plus PO_4^3 - P) were maintained at a concentration of 300 µ mole/l of inorganic - N (ΣIN) and 30 µ mole/l of PO_4^3 - P; an atomic N/P ratio of 10/1. Flow rates of these media were distributed to the twelve chambers such that the chambers receiving the three

different media types at each of the 1, 7.5, 15 and 30 turnovers per day all maintained the same daily total nitrogen loading. In effect, the instantaneous influent nutrient concentration was inversely proportional to turnover rates, and resulted in influent nutrient concentrations of 300, 40, 20 and 10 μ mole/l of ΣIN, respectively. The four remaining "control" chambers were given seawater without nutrient enrichment at the 1, 7.5, 15 and 30 turnovers per day.

In Experiment II, NH_4^+ - N plus PO_4^3 - P and NO_3^- - N plus PO_4^3 - P media were each maintained at a concentration of 50 μ moles/l ΣIN and 5 μ mole/l PO_4^3 - P. These two media were then distributed to eight culture chambers at 1, 7.5, 15 and 30 turnovers per day in such a way as to maintain equal influent nutrient concentrations. In this case, the daily nitrogen loading was directly proportional to turnover rate.

Gracilaria foliifera v. angustissima used in these experiments had been actively growing and all of the plants used had the same C/N ratio at the beginning of the experiments. In each experiment, 280 g of the Gracilaria stock were added to each of the culture chambers. This gave a starting density of 1.2 kg wet wt./m^2, a somewhat lower than optimal density for maximum Gracilaria yield in this culture system (Lapointe and Ryther, 1977). Experience with previous Gracilaria cultures indicated that growth rates with the different treatments would be large. The lower biomass levels were an attempt to maintain more nearly constant conditions (i.e., self-shading) in the cultures than would have occurred at the higher densities and yields.

At intervals of 4 to 6 days, the plants were removed from the chambers, shaken vigorously in the air to remove water, and weighed. A dry weight conversion factor was used and growth calculated in dry wt/ m^2/day (Table 1).

Water Analysis

Water samples were taken at midday for chemical analyses of PO_4^3 - P (Murphy and Riley, 1962), NH_4^+ - N (Solarzano, 1969) and NO_3^+ - N (Wood et al., 1967). Daytime pH values were measured triweekly while diurnal pH values were recorded at 4-h intervals twice during the experimental period. Extreme culture temperatures were measured daily at each turnover rate with Taylor maximum-minimum thermometers.

Plant Analysis

Percent dry weight was determined by oven-drying the seaweed at 90°C for 48 hours. Ash content was determined by combusting samples (originally dried at 60°C) at 475°C for 8 hours.

Phycoerythrin, an accessory phycobilin protein pigment present in Gracilaria, was determined using a Ten Broeck homogenizer for cold extraction from plant material (∿500 mg) in 5 ml of 0.1 m aqueous phosphate buffer (pH 7.0). The homogenate was then centrifuged at 25,000 xg at 4°C for 30 minutes. The supernatant was decanted into a cuvette and a visible scan (700 to 380 nm) of this extract yielded three absorption peaks at 498, 540 and 565 nm (Vander Velde, 1973; O'hEocha, 1971). Phycoerythrin content was determined by measuring

the optical density at 565 nm. An absorption coefficient of 8.10 (O'hEocha, 1955) was used to calculate mg of phycoerthrin.

Chlorophyll content was determined by resuspending the pellet from the previous phycoerythrin extraction in 5 ml of cold 80% spectro-analyzed acetone. The suspension was then centrifuged at 25,000 xg for 30 minutes at 4°C. The supernatant was scanned between 700 and 380 nm and a sharp peak observed at 665 nm used to measure optical density. The absorption coefficient of 12.3 was used to calculate mg chlorophyll a (Vernon and Seely, 1966).

Total plant nitrogen (organic and inorganic fractions) were determined from dried Gracilaria samples (60°C) on a Perkin-Elmer 240 Elemental Analyzer. Reactive NO_3^- - N was measured with 100 mg samples of ground Gracilaria by three separate extractions with 8 ml of hot 80% ETOH. The supernatant was then extracted once with absolute ETOH, twice with ETOET and the combined extracts heat evaporated. The resulting salts were redissolved in NO_3^- - N free seawater with heat, brought to 50 ml and 0.45 µ filtered. Samples were then analyzed for reactive NO_3^- - N by the cadmium wire reduction method (Stainton, 1974). NH_4^+ - N was determined by the blue indophenol method (Solarzano, 1969). Caloric content of dried Gracilaria was determined using a Parr microbomb calorimeter.

Results and Discussion

Effects of Nitrogen Form:

Nitrogen is introduced to coastal and estuarine waters mainly as ammonia from domestic sewage while surface drainage contains predominately

nitrate. Due to the need for plants to reduce nitrate to ammonia for assimilation (Nicholas, 1959) the latter is usually the preferred form of nitrogen for plant growth (Syrett, 1962). In Experiment I of the current study, NH_4^+ - N enhanced the growth of Gracilaria in comparison to that obtained with NO_3^- - N enriched seawater or with sewage effluent that contained nitrogen primarily as NO_3^- - N.

These differences appeared to be independent of turnover rate. Maximum yields of 36 g dry wt/m^2/day were attained with the high turnover (30/day) NH_4^+ - N culture whereas maximum yields were only 27 and 21 g dry wt/m^2/day for the similar sewage and NO_3^- - N cultures, respectively (Fig. 3). However, this trend changed during Experiment II when, at the end of the experiment, the NO_3^- - N culture attained yields of 44 g dry wt/m^2/day in contrast to 33 g dry wt/m^2/day for the NH_4^+ - N culture (Fig. 4). Because the cultures in this experiment were receiving amounts of nitrogen that were proportional to the turnover rate in contrast to Experiment I, it is possible that the reduced growth in the NH_4^+ - N cultures may have reflected a toxic effect. Waite and Mitchell (1972) found concentrations beyond 57 µ mole/l to be inhibitory to the growth of Ulva lactuca at low phosphate levels. Although influent concentrations in this experiment were constant at all turnover rates at only 50 µ mole/l, it may be possible for NH_4^+ - N to be toxic with large daily loads in well mixed cultures, particularly if a significant fraction of the assimilated NH_4^+ - N is stored in the inorganic form. Because the

NH_4^+ - N plants grew better than the NO_3^- - N plants at the beginning of Experiment II (Fig. 4), NH_4^+ - N may have accumulated in the plant tissues to high and perhaps toxic levels over time.

Effects of Nitrogen Loading and Turnover Rate:

Experiment I

Nitrogen is usually the limiting factor to phytoplankton production in coastal areas of the world's oceans (Ryther and Dunstan, 1971; Thomas and Owen, 1971). In the current culture experiments, nitrogen, at least below a minimum level, also appeared to limit growth. Yield of Gracilaria in all of the seawater controls was less than that attained in any of the respective enriched cultures (Fig. 3). Available nitrogen in the seawater controls was directly proportional to flow rate. Even at the highest turnover rate of 30, algal production decreased over the experimental period and the C/N ratio increased from 10 to 16 (Fig. 5). In the most extreme case of 1 turnover/day of unenriched seawater, growth decreased linearly after the first six days of the experiment with a concomitant rise from 10 to 30 in the C/N ratio by the 22nd day of the experiment. This shift in the C/N ratio can be attributed to limiting amounts of nitrogen, which ranged from 0.3 m moles/day ΣIN at 1 turnover/day to 8.6 m moles/day ΣIN at 30 turnovers/day (Fig. 6). In contrast, the constant C/N ratios of ca. 10 in the three nutrient enriched treatment over the duration of Experiment I reflected the constant nutrient loading of 15 m moles/day of ΣIN. Nitrogen assimilation was nearly complete in all the cultures, regardless of differences in

influent concentrations, and thus was identical to the daily ΣIN loading. This suggests that nitrogen uptake is determined by total daily input of nitrogen and not by concentration or flow rate per se.

The fact that it was nitrogen that limited growth in the unenriched seawater cultures is further substantiated by their low N/P ratios relative to those of the enriched cultures. Although the enriched cultures received 1.5 m moles/day of PO_4^3 - P, assimilation of that nutrient was not complete but ranged from 0.3 to 0.7 m moles/day (Fig. 6). The N/P ratios varied from 20 to 33 by atoms in the enriched cultures, whereas they decreased linearly from 19 to 9 in the four seawater controls (Fig. 5).

Internal Inorganic Nitrogen Reserves:

Continuous culture techniques with phytoplankton are designed to circumvent problems with batch culture experiments in which nutrient concentrations are continuously changing. However, even when continuous culture experiments have been used growth rates cannot always be directly related to nutrient concentration (Caperon, 1968; Williams, 1971). Williams (1965) and Droop (1968) have independently postulated that inorganic nutrient storage reserves were the most likely explanation of their variable results.

In this experiment, levels of stored inorganic nitrogen (ΣIN) in the algal tissue of the enriched cultures decreased linearly with increasing turnover rate. Concentrations of up to 5 μ moles ΣIN/g dry wt in the NH_4^+ - N - and $NO_3^=$ N - enriched cultures at 1 turnover/day decreased to ca. 1 μ mole ΣIN/g dry wt at 30 turnovers/day,

although the C/N ratio remained constant (Fig. 7). In the unenriched seawater controls, however, ΣIN levels in the algal tissue increased from ca. 0.5 μ mole/g dry wt at 1 turnover/day to 2 μ mole/g dry wt at 30 turnover/day while the C/N ratio decreased linearly from 30 to 16, respectively. This increase in absolute levels of stored ΣIN with increasing turnover rate in these unenriched culturescould be expected since nitrogen loading was directly proportional to turnover rate. However, the opposite trend was observed in the enriched cultures, in which ΣIN storage was inversely correlated with turnover rate. In the latter case, the relatively slow growth rate of the algae at low turnover rates was apparently caused by some limiting factor(s) other than nitrogen and phosphorus, and there were relatively high concentrations of stored inorganic nitrogen in the plants, while at high turnover and growth rates, most of the internal nitrogen pool was organic. This phenomenon seems to be a mechanism by which, if certain controlling factors for growth are limiting, plants can still take up and store nitrogen in the inorganic form until the limiting factor(s) is removed. For example, Laminaria longicruris stores inorganic nitrogen in the winter, when the nutrient is available but growth of the alga is light-limited, for its rapid late spring-early summer growth when nutrient levels in the water are low but other conditions are favorable for growth (Chapman and Craigie, 1977). This ability of seaweeds to store inorganic nitrogen in their thalli gives them

a distinct advantage over phytoplankton for continuous seasonal growth. The high levels of inorganic nitrogen contained in the thallus of Gracilaria may also explain the sometimes lower affinity for and irregular removal of inorganic nitrogen by the seaweed as compared to phytoplankton (D'Elia et al., 1976).

Effects of Nitrogen Loading and Turnover Rate:
Experiment II

In this experiment nutrient loading and turnover rate both increased simultaneously. At 1 turnover/day, in both the NH_4^+ - N and NO_3^- - N enriched media, growth decreased over the duration of the experiment (Fig. 4). In those cultures, which received 2.5 m moles/day of nitrogen, the C/N ratio of the algae increased from 10 to 24 (NH_4^+ - N) and 22 (NO_3^- - N) respectively (Fig. 8). At the higher turnover rates of 7.5, 15 and 30, the algae received approximately 19, 38 and 76 m moles/day of nitrogen respectively, and both the NH_4^+ - N and the NO_3^- - N enriched algae maintained low C/N ratios of 8-10. Because yield did not increase with increasing nitrogen loading above the 19 m mole/day level (7.5 turnovers/day) other than that attributed to increased turnover rate (Experiment I), a minimal nitrogen level between 8 and 15 m moles/day of nitrogen was required to support sustained high growth rates without reduction of plant nitrogen.

Thus, the growth of Gracilaria, above this minimum nitrogen level, appeared to be highly dependent upon turnover rate but independent of concentration. Yields increased in both Experiments I and II with increasing turnover rate. In the first case, nutrient

concentration varied inversely with turnover rate while in the second, concentrations remained constant at all turnover rates.

The reason that yields increase with culture turnover or exchange rate is not yet known. CO_2 availability does, of course, increase with exchange rate and diurnal changes in pH in the enriched cultures at the lowest turnover (1/day) of 8.3 in early morning to 9.3 at midday may reflect carbon limitation (Fig. 9).

If CO_2 assimilation (photosynthesis) outstrips CO_2 addition to the culture, the aqueous bicarbonate reservoir will be depleted and the pH will rise accordingly. Such inorganic carbon limitations are more common in freshwater natural systems and mass culture (Goldman et al, 1972), but may also affect growth of seaweeds in marine systems even though they are well buffered (Jackson, 1977). The unenriched seawater cultures had relative low pH values compared to the enriched cultures, were a result of lower rates of photosynthesis (Fig. 9). Alternatively, nutrients other than nitrogen and phosphorus may be present in the seawater at concentrations that could be growth-limiting to an extent inversely proportional to exchange rate. The same could be true of toxic or growth-inhibiting metabolites of the algae that might concentrate in the medium. Lastly, culture mixing, which is important in breaking down diffusion gradients of essential mineral nutrients in intense cultures (Gavis, 1976), could also be affected by turnover rate, though this is unlikely in view of the bigorous aeration that was provided to all cultures.

Effect of Nitrogen on Plant Composition

The results of the present study indicate that nutrient availability, in addition to limiting growth, can also cause variations in the present dry weight and ash of the algae. In the seawater controls and enriched cultures in Experiment I the percent dry weight and ash of Gracilaria varied as a function of culture turnover rate and hence, nitrogen loading and the C/N ratio of the plants. Dry weight ranged from 7% to 10% of wet weight in the algae and correlated well with C/N ratios which ranged from 33 to 10, respectively ($r = 9.5$, Fig. 10). Similarly, ash content ranged from 35% to 50% of dry weight and was highly correlated with C/N over the same range ($r = .97$, Fig. 10). These variations appear to be largely a function of increased salt content (ash) in rapidly growing, nutrient enriched plants as opposed to slow growing nutrient-limited plants (unpublished information). This variable ash content influences the organic content and heat of combustion of the Gracilaria when these parameters are expressed per unit of dry weight. Table 1 illustrates that nitrogen-starved algae contain less ash and their heat of combustion is therefore greater per unit dry weight. However, the heat of combustion of the organic matter per se (ash-free dry weight) is highly correlated with nitrogen content ($r = .98$) and is higher in nitrogen-rich plants, indicating a difference in the organic storage products (Fig. 11, Table 1).

Effect of Nitrogen on Pigment Content

The ability of seaweeds to adapt to spectral changes in light quality (Englemann, 1883) and changes in light intensity (Oltmann, 1891) have been studied for nearly a century. Changes in the ratio of accessory pigments (phycoerythrin) to chlorophyll a have been used as an indicator of complimentary chromatic adaptation to algae growing at different water depths and exposed to light of different spectral composition (Ramus et al., 1976; Moon and Dawes, 1976). In contrast, increases in pigment concentrations (absolute levels) have been used to signify intensity-related pigment changes, which are analogous to the "sun-shade" adaptation by land plants (Björkman, 1973). Critical experiments which distinguish chromatic from intensity adaptation have been convincingly demonstrated (Halldal, 1970; Bogorod, 1975).

Previous experiments (Lapointe and Ryther, 1977) have indicated that nitrogen starvation causes pigment depletion in Gracilaria; however, nitrogen content and pigment levels have not been quantified. In Experiment I, absolute levels of both phycoerythrin and chlorophyll were highly correlated (r = .94 and .96 respectively) with increasing nitrogen content of the plants (Fig. 12). Visually, nitrogen starved plants (C/N = 30) appeared straw colored whereas nitrogen enriched plants (C/N = 10) appeared dark brown. These distinct color differences could be used as a quick field method for estimating the relative nitrogen content of the plants. The phycoerthrin/chlorophyll ratio (P/C) varied from 7.2 to 8.5 over this C/N range and is not a substantial shift when compared to P/C ratios from 1

to 20 which have been reported for Eucheuma isiforme chromatically adapting in a seasonal manner (Moon and Dawes, 1976). Hence, our P/C ratio change probably did not indicate an adaption according to Engelman's theory, which is not surprising since the spectral composition of the natural light available to the cultures was constant over all treatments. Rather, these pigment changes indicate an increase in the absolute levels of both phycoerythrin and chlorophyll which is better described by Oltmann's theory of "intensity" adaptation. This increase could be an adaptation to non-nutrient limiting conditions during which the plants increase their photon gathering "antennae" thus achieving the high yields which were recorded (in nutrient enriched, high turnover cultures).

In summary, nitrogen plays an important role not only in the growth of Gracilaria but also in its composition and hence, the nutritional quality of the plant. During nitrogen-enriched exponential growth, protein synthesis predominates and is reflected by the high protein pigment levels (phycoerthrin). When nitrogen limits growth, carbohydrate synthesis predominates and probably accounts for the increased polysaccharide content at this time (Neish and Shacklock, 1971; Dawes et al., 1974b). These nitrogen related growth phenonmenon are general biochemical principles (Fogg, 1964) that should be considered in studying both the basic ecology of seaweeds and their relation to herbivore and detritovore food chains (Mann, 1969). Although research in fish (Gerking, 1962)

and polychaete worms (Tenore, 1977) culture and agriculture has demonstrated the regulatory role of nitrogen, both energy and carbon budgets have often been used in marine ecology and aquaculture with questionalbe results. Carnivore food chains are rarely nitrogen limited and can be budgeted in caloric units; however, as pointed out, nitrogen regulates growth not only in autotrophic production but also in detritovore and herbivore secondary production. This, combined with the fact that nitrogen affects other nutritional qualities of plants (i.e., caloric value, protein, carbohydrate), may indicate that nitrogen budgeting may be ecologically more significant in studying algal-herbivore-detritovore interactions.

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Figure legend

Fig. 1. Schematic diagram of seaweed culture system (A) and individual culture chambers (B).

Fig. 2. Photo of experimental seaweed culture system at the Harbor Branch Foundation, Ft. Pierce, FL.

Fig. 3. Yields of Gracilaria during Experiment I cultured with three media (NO_3^-, NO_3^--enriched sewage, NH_4^-) and unenriched seawater at four turnover rates. The three enriched cultures received equal daily loadings of nitrogen (15 m mole/day) whereas the unenriched seawater cultures received only the nitrogen present in the seawater.

Fig. 4 Yields of Gracilaria during Experiment II cultured with two media (NH_4^- and NO_3^-) at four turnover rates. Influent nutrient concentrations were held constant so that total daily nitrogen loading was directly proportional to turnover rate.

Fig. 5. Carbon/nitrogen ratios of Gracilaria during Experiment I.

Fig. 6. Daily assimilation of nitrogen and phosphorus by Gracilaria during Experiment I.

Fig. 7. Total dissolved inorganic nitrogen (ΣIN) and carbon/nitrogen ratio of Gracilaria tissue during Experiment II.

Fig. 8. Carbon/nitrogen ratios of Gracilaria during Experiment II.

Fig. 9. Diurnal variation of ph during Experiment I.

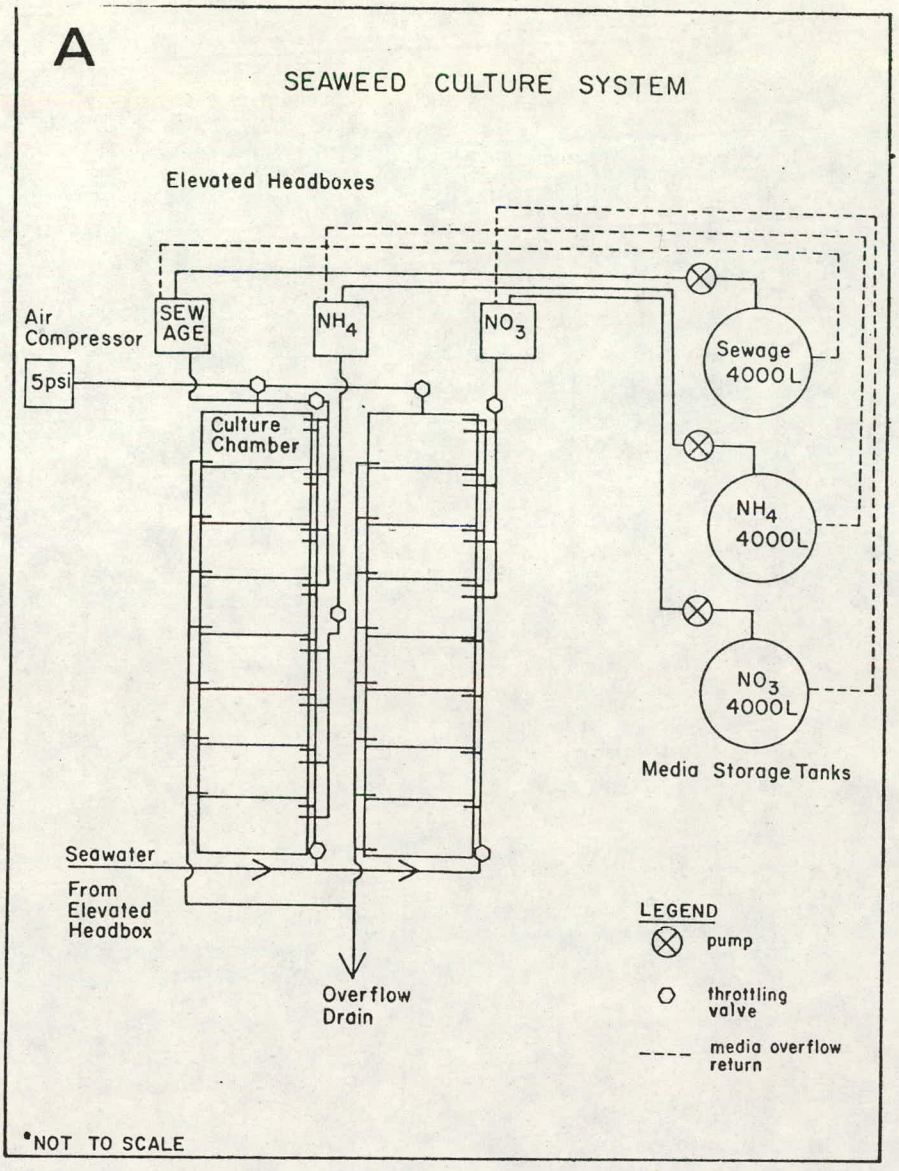
Percent ash and dry weight of Gracilaria as a function of carbon/nitrogen ratio.

Fig. 11. Caloric value of Gracilaria expressed as calories per gram ash-free dry wt (AFDW) as a function of carbon/nitrogen ratio.

Fig. 12. Pigment content (phycoerythrin and chlorophyll a) of Gracilaria as a function of carbon/nitrogen ratio.

Table 1. Caloric value per gram dry weight and per gram ash-free dry weight, carbon/nitrogen ratio, percent dry weight and percent ash weight of Gracilaria cultured in enriched (A) and unenriched (B) media.

	Calories/g dry weight	Calories/g ash free dry weight	C/N	% Dry weight	% sh weight
Gracilaria A	2656 ± 52	4618 ± 96	9.9	10.2	48
Gracilaria A	2619 ± 24	4595 ± 42	11.0	10.3	47
Gracilaria B	2855 ± 38	4326 ± 12	35.0	7.8	34
Gracilaria B	3106 ± 37	4314 ± 51	39.0	7.5	31


A
SEAWEED CULTURE SYSTEM
Elevated Headboxes
Air Compressor
5psi
SEW AGE
NH_4
NO_3
Culture Chamber
Sewage 4000 L
NH_4 4000L
NO_3 4000L
Media Storage Tanks
Seawater From Elevated Headbox
Overflow Drain
LEGEND
pump
throttling valve
media overflow return
*NOT TO SCALE

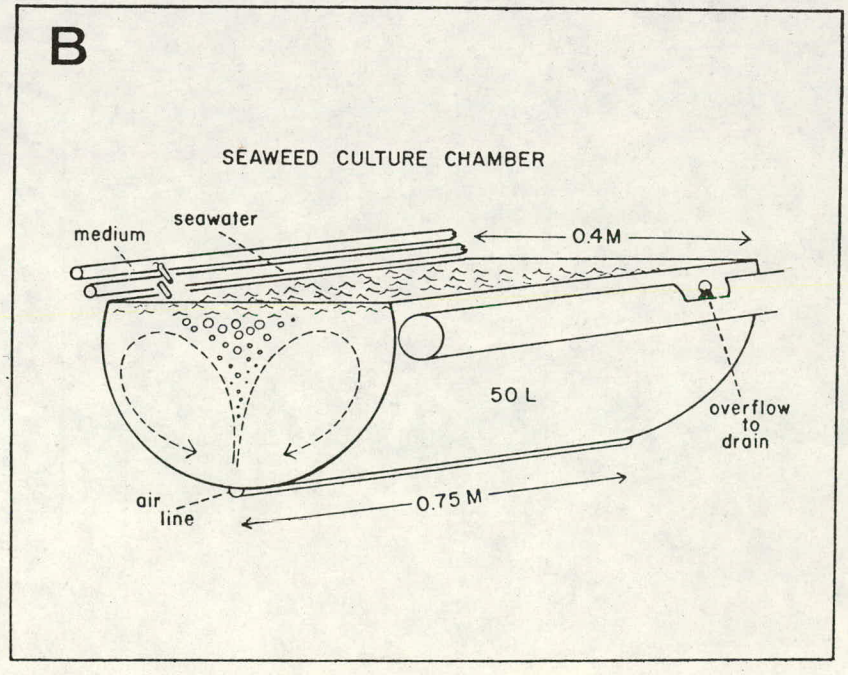

B
SEAWEED CULTURE CHAMBER
medium
seawater
0.4M
50 L
overflow to drain
air line
0.75 M

Fig. 1

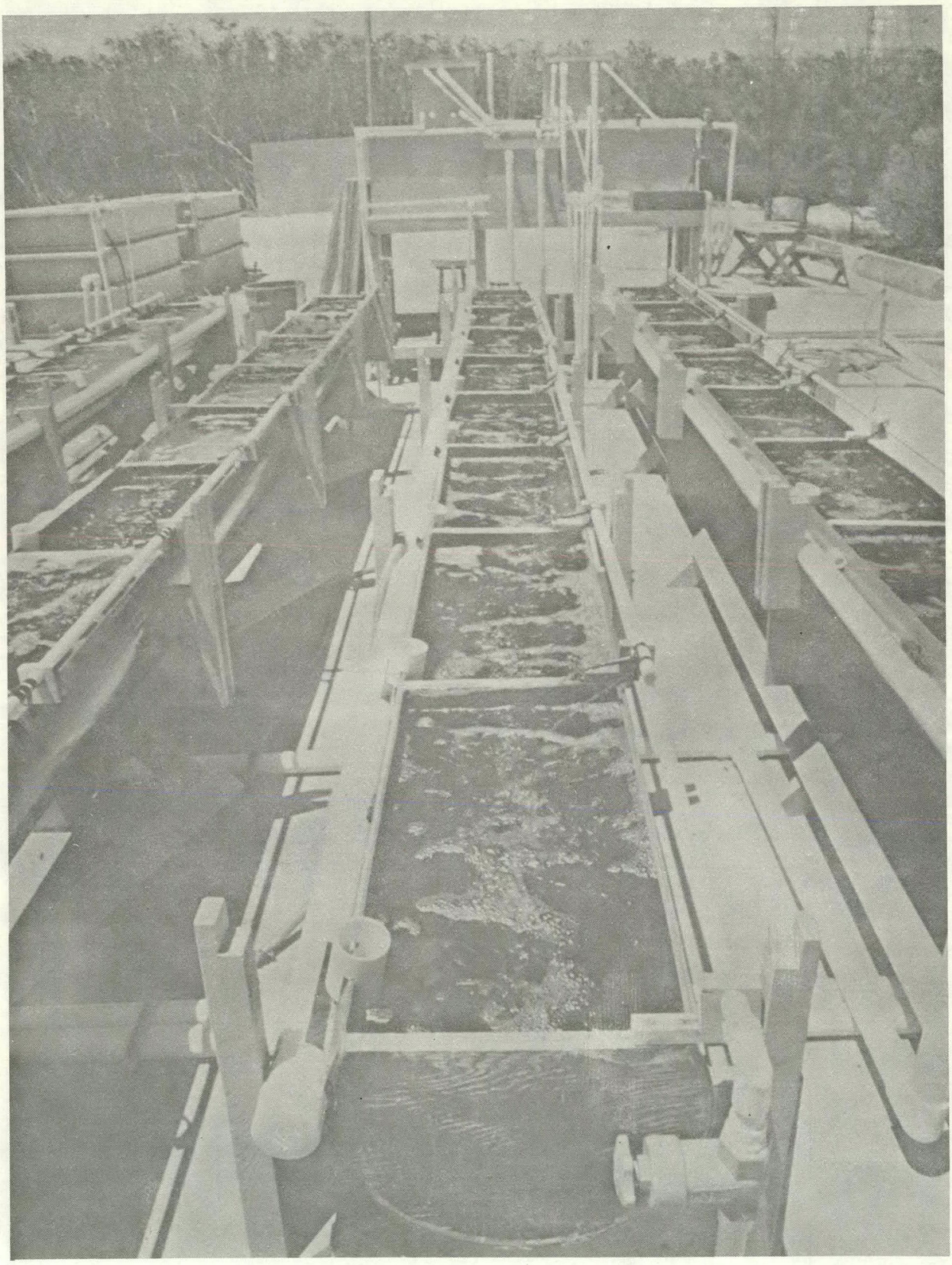


Fig. 2

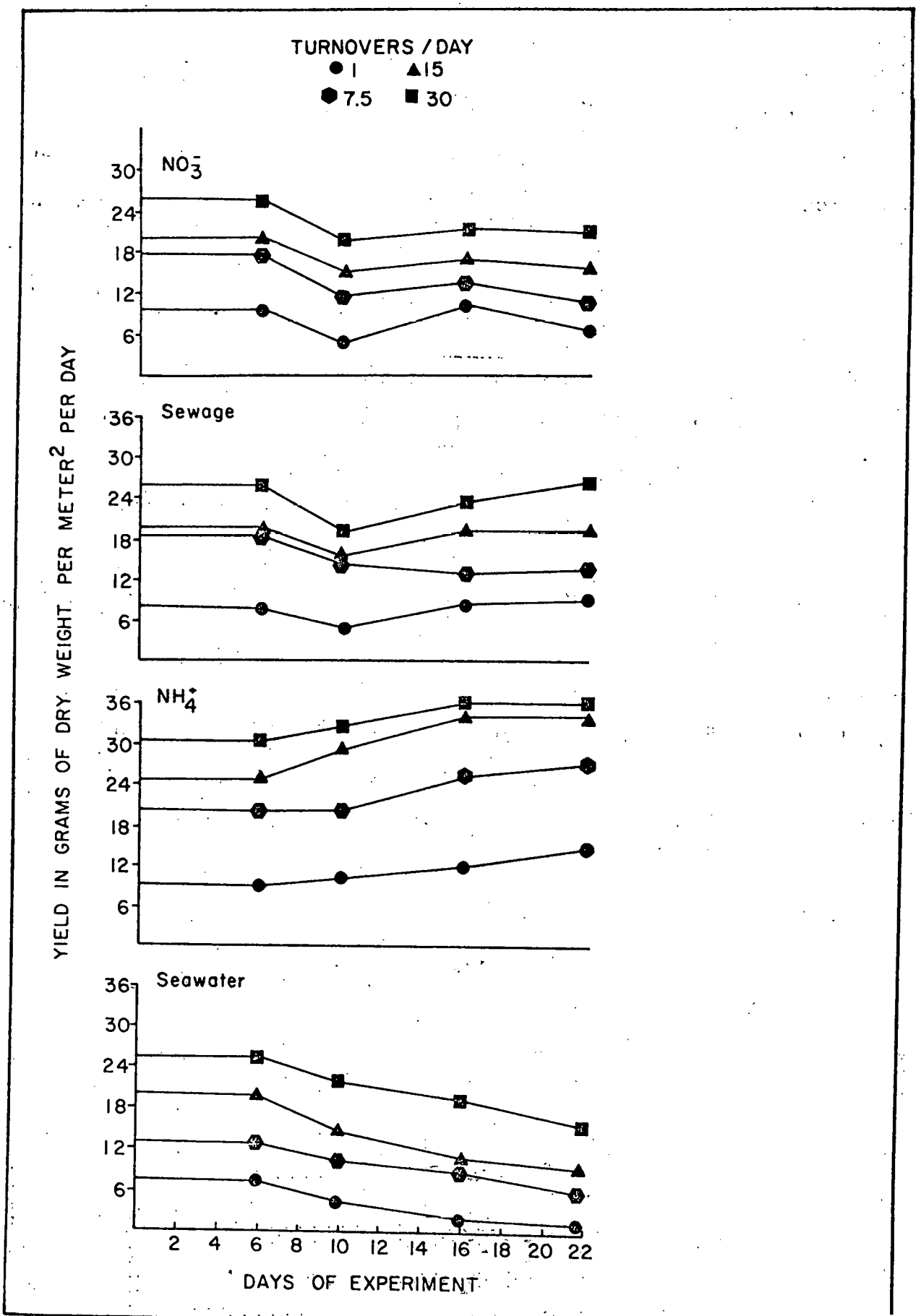

TURNOVERS / DAY
1
15
7.5
30
NO_3^-
Sewage
NH_4^+
Seawater
YIELD IN GRAMS OF DRY WEIGHT PER METER^2 PER DAY
DAYS OF EXPERIMENT
2 4 6 8 10 12 14 16 18 20 22
6 12 18 24 30 36

Fig. 3

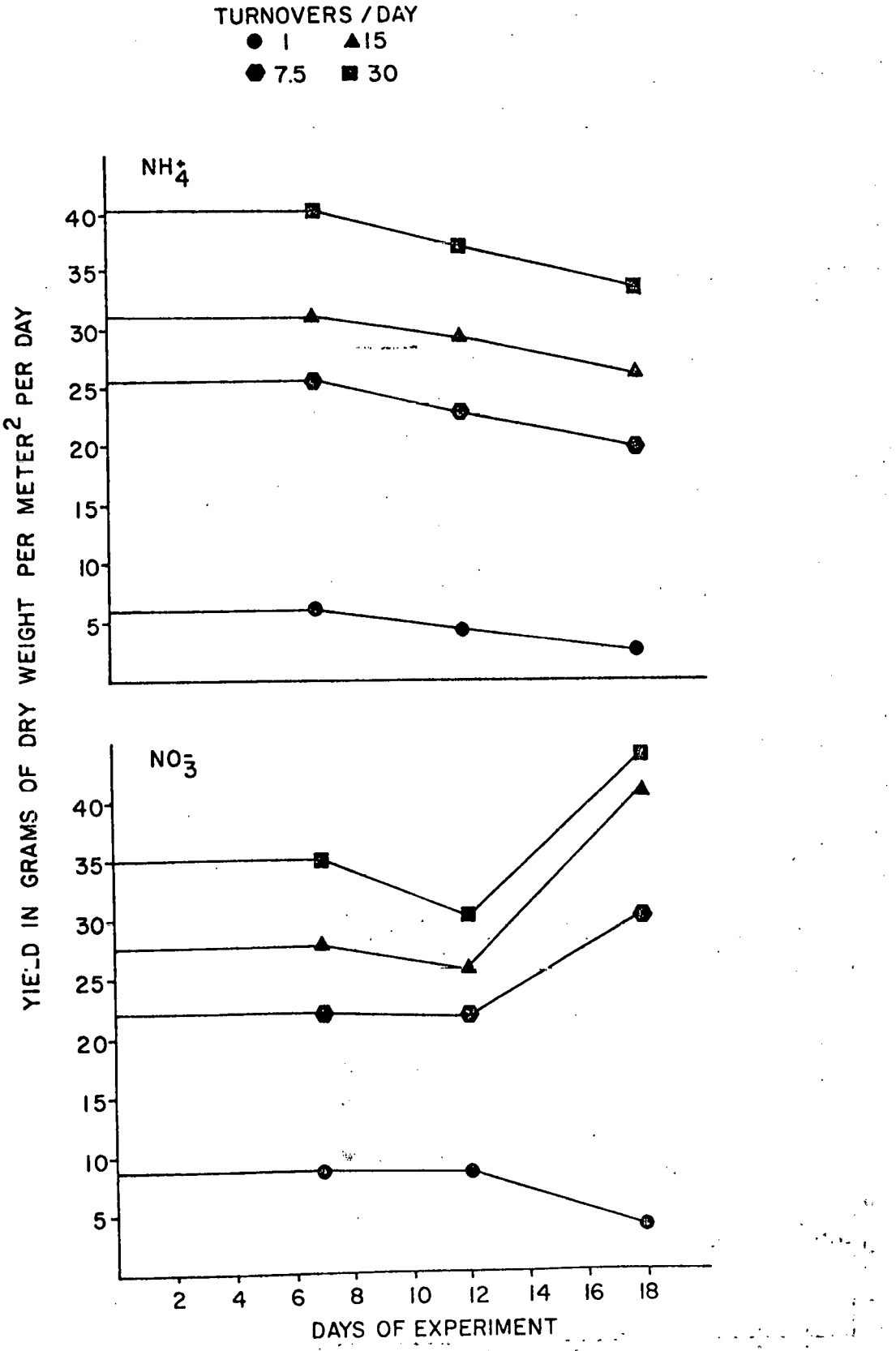

TURNOVERS / DAY
1
15
7.5
30
NH4+
NO3-
40
35
30
25
20
15
10
5
YIELD IN GRAMS OF DRY WEIGHT PER METER2 PER DAY
2
4
6
8
10
12
14
16
18
DAYS OF EXPERIMENT

Fig. 4

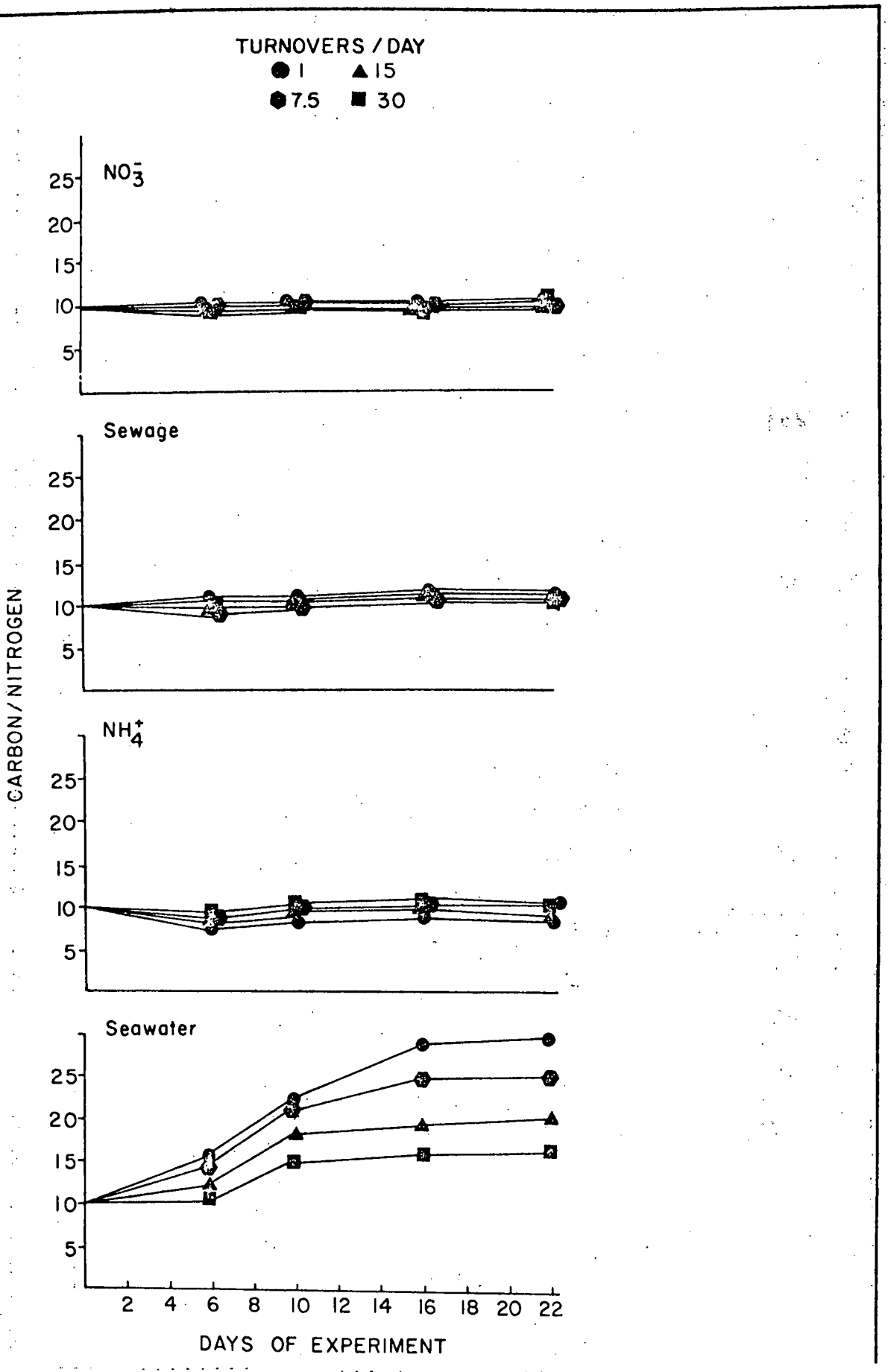

TURNOVERS / DAY
1
15
7.5
30
NO3-
Sewage
NH4+
Seawater
CARBON / NITROGEN
25
20
15
10
5
2
4
6
8
10
12
14
16
18
20
22
DAYS OF EXPERIMENT

Fig. 5

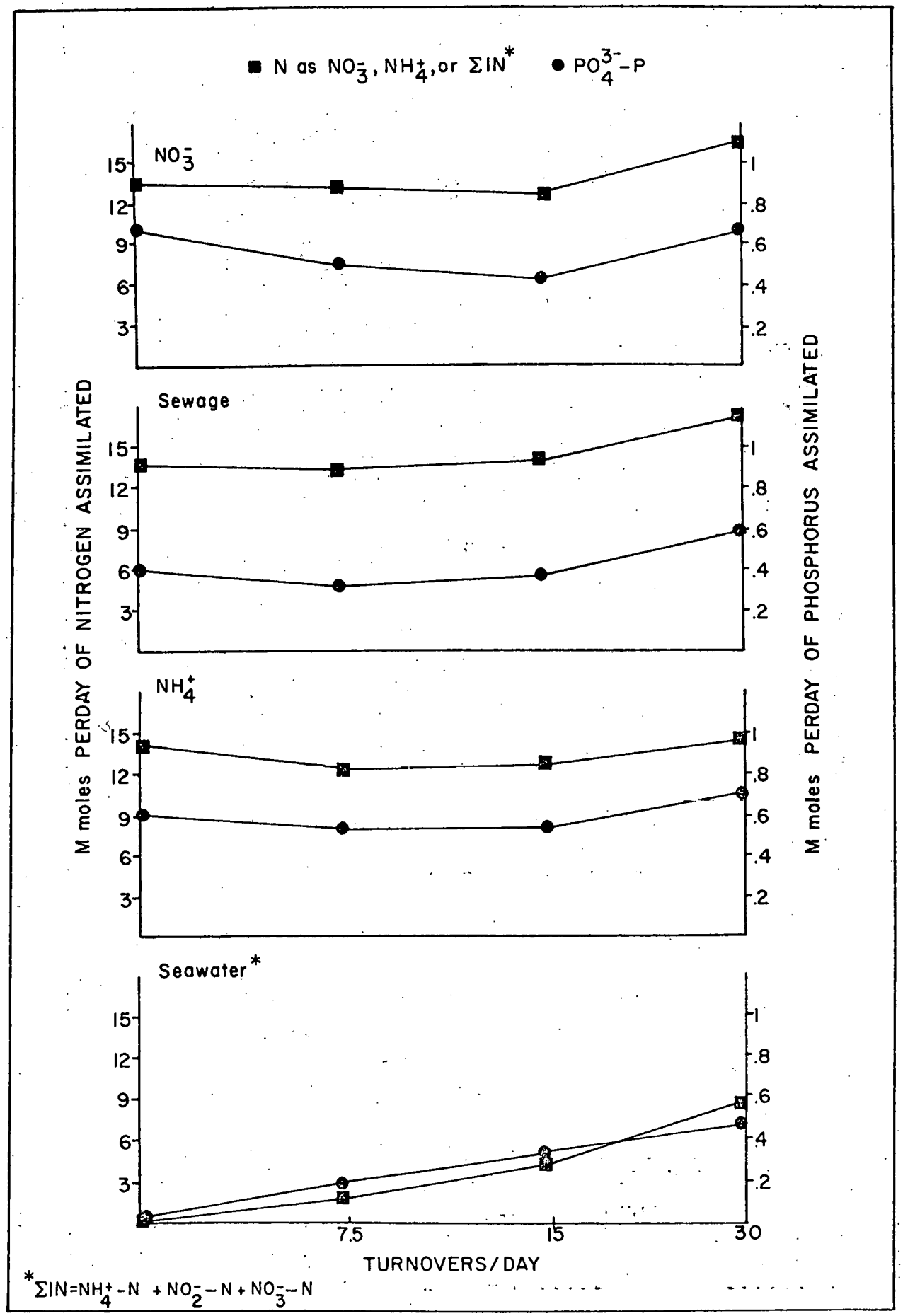

■ N as NO_3^-, NH_4^+, or ΣIN*
● PO_4^{3-}-P
NO_3^-
Sewage
NH_4^+
Seawater*
M moles PERDAY OF NITROGEN ASSIMILATED
M moles PERDAY OF PHOSPHORUS ASSIMILATED
TURNOVERS/DAY
7.5
15
30
*ΣIN=NH_4^+-N + NO_2^--N + NO_3^--N

Fig. 6

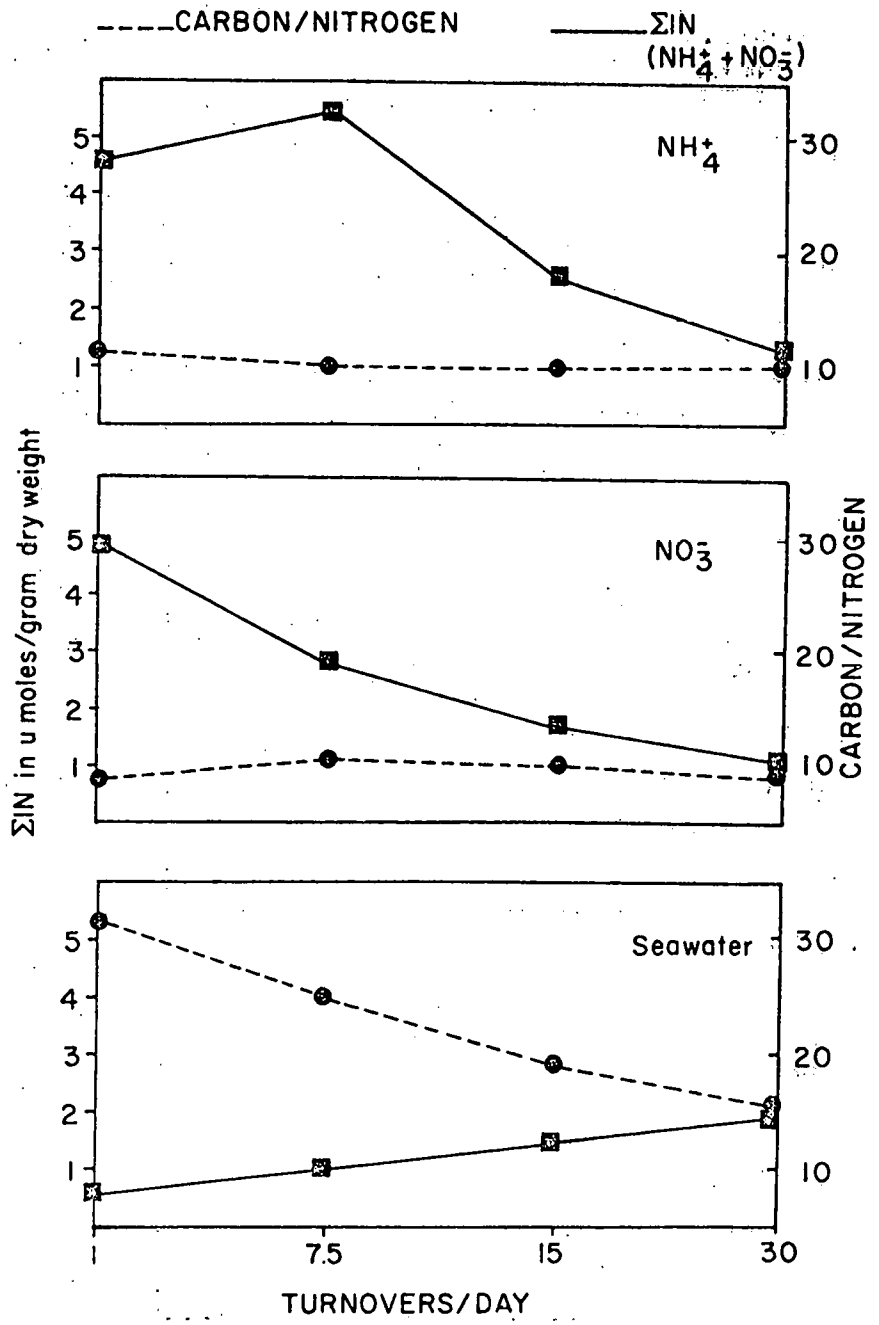

CARBON/NITROGEN
ΣIN
(NH4+ + NO3-)
NH4+
NO3-
Seawater
ΣIN in u moles/gram dry weight
CARBON/NITROGEN
TURNOVERS/DAY
1
7.5
15
30

Fig. 7

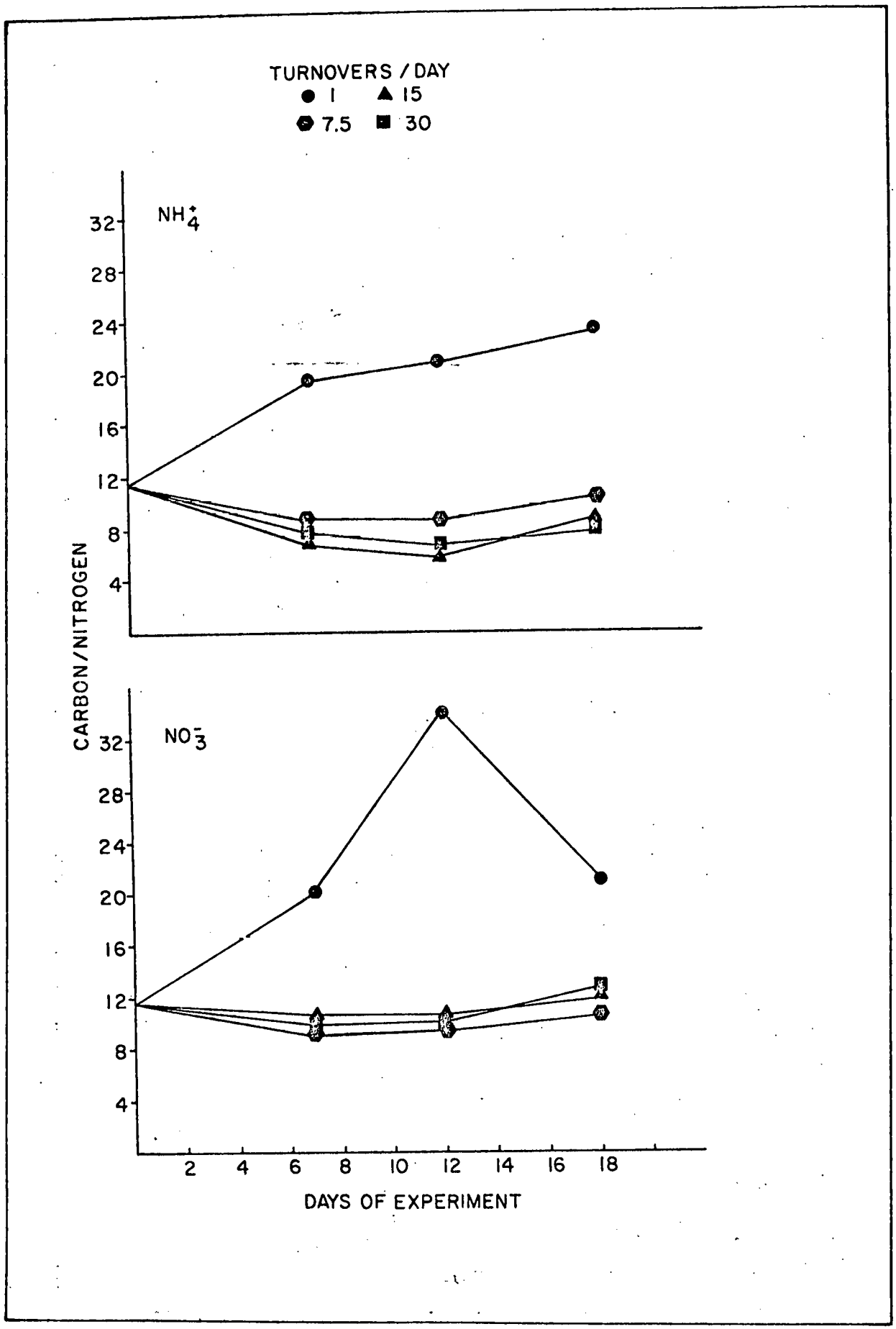

TURNOVERS / DAY
1
15
7.5
30
NH4+
NO3-
CARBON/NITROGEN
32
28
24
20
16
12
8
4
2
4
6
8
10
12
14
16
18
DAYS OF EXPERIMENT

Fig. 8

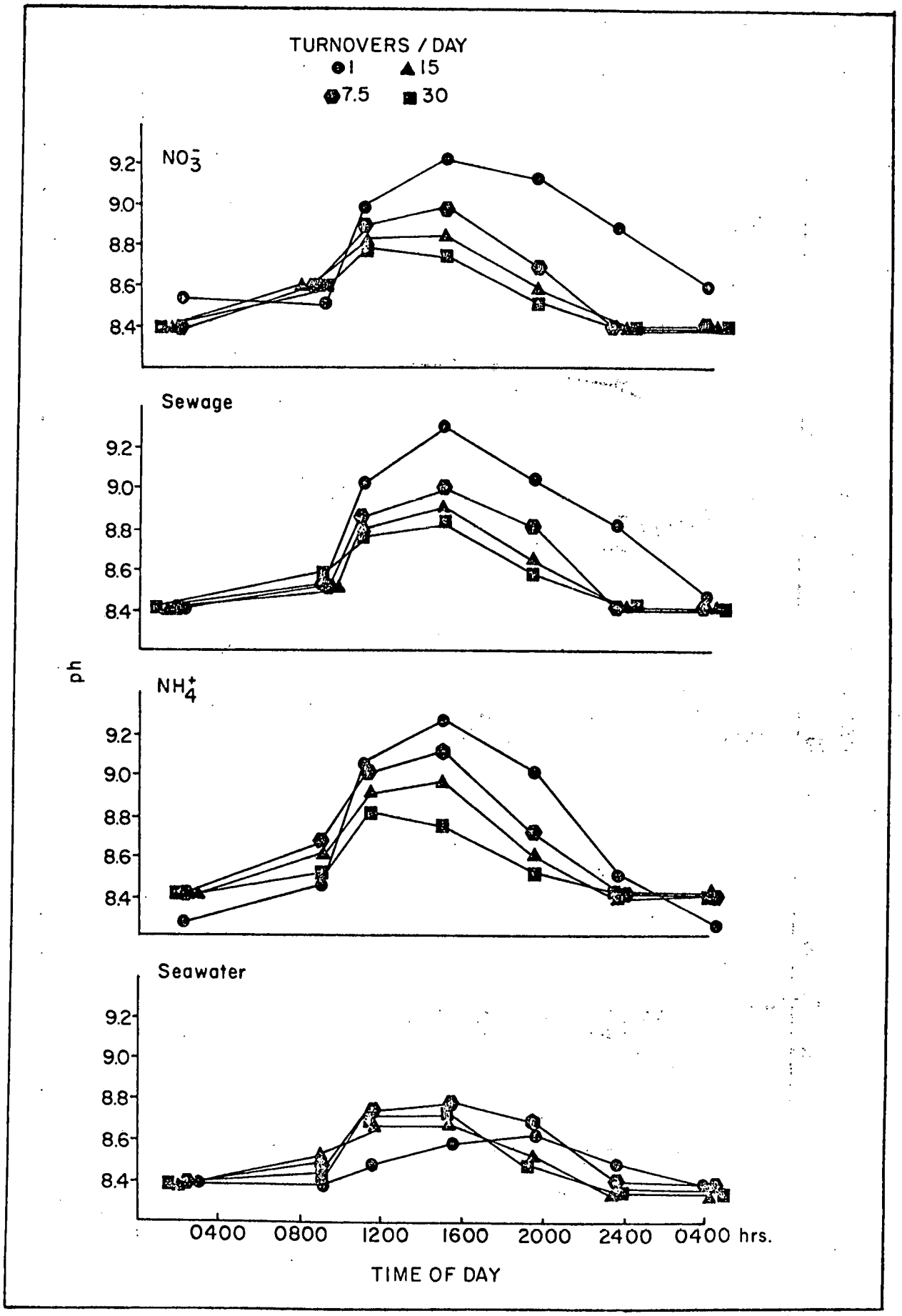

TURNOVERS / DAY
1
1.5
7.5
30
NO3-
Sewage
NH4+
Seawater
ph
9.2
9.0
8.8
8.6
8.4
0400
0800
1200
1600
2000
2400
0400 hrs.
TIME OF DAY

Fig. 9

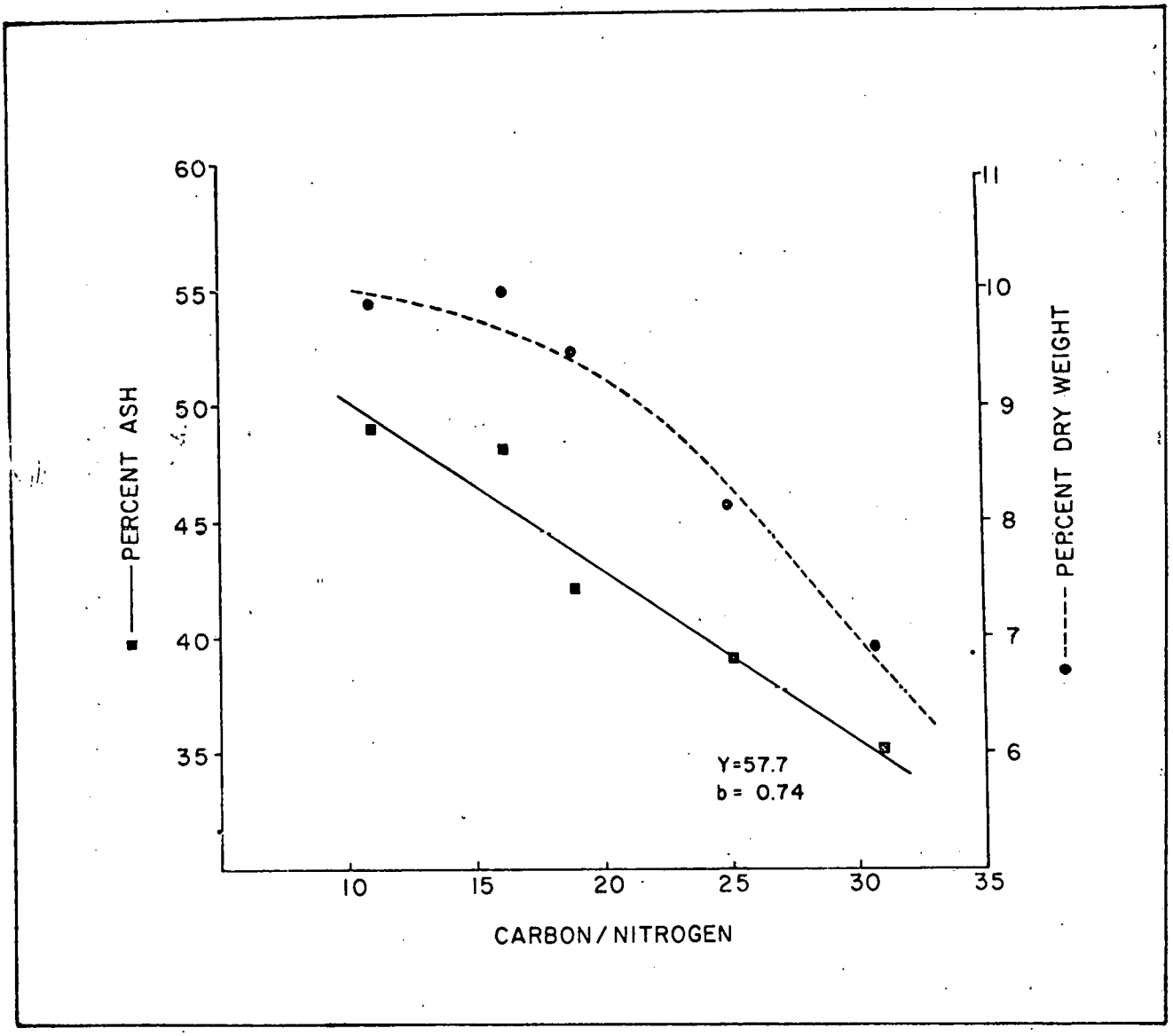

60
55
50
45
40
35
11
10
9
8
7
6
PERCENT ASH
PERCENT DRY WEIGHT
Y=57.7
b= 0.74
10
15
20
25
30
35
CARBON/NITROGEN

Fig. 10

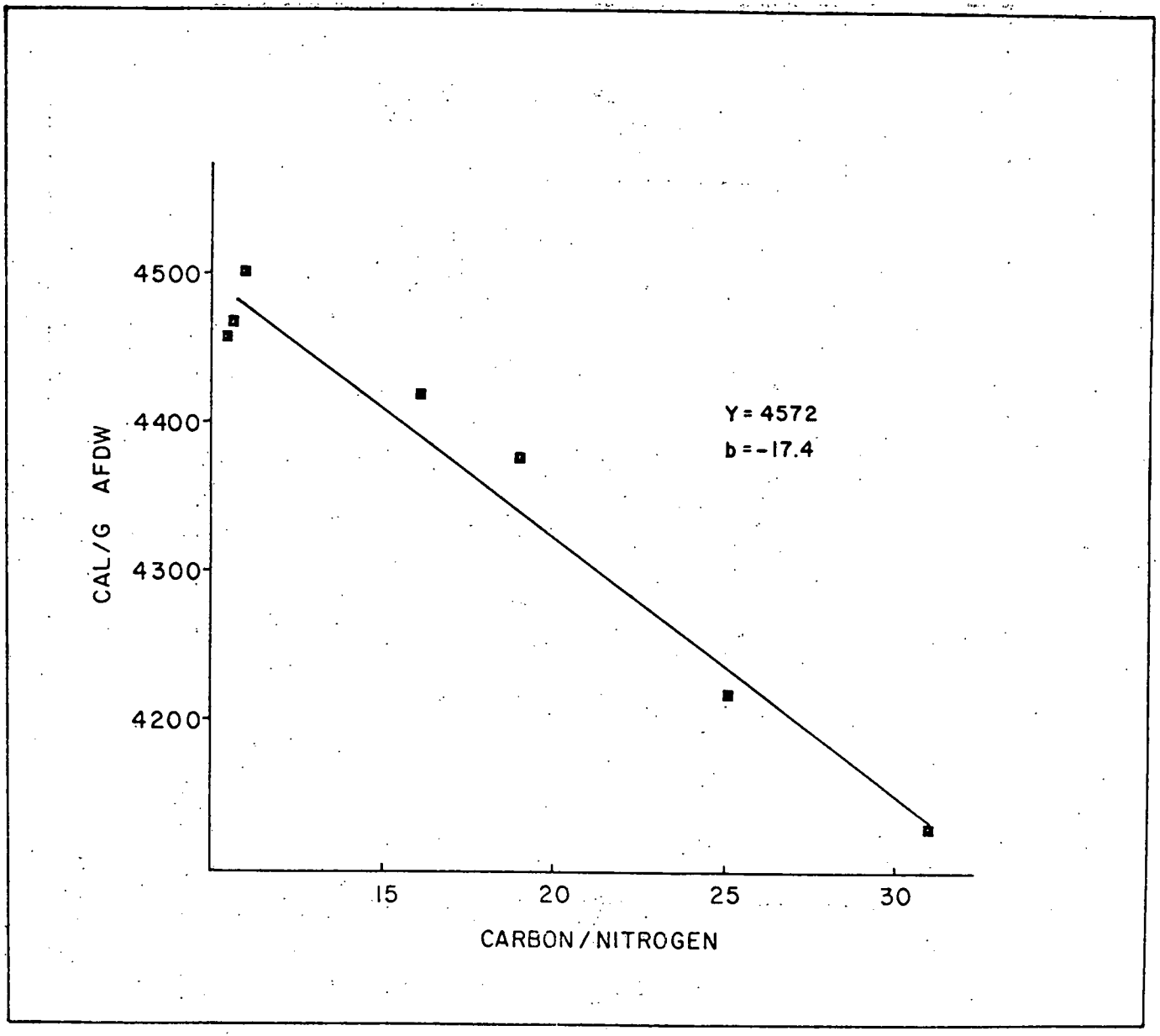

4500
4400
4300
4200
CAL/G AFDW
Y = 4572
b = -17.4
15
20
25
30
CARBON / NITROGEN

Fig. 11

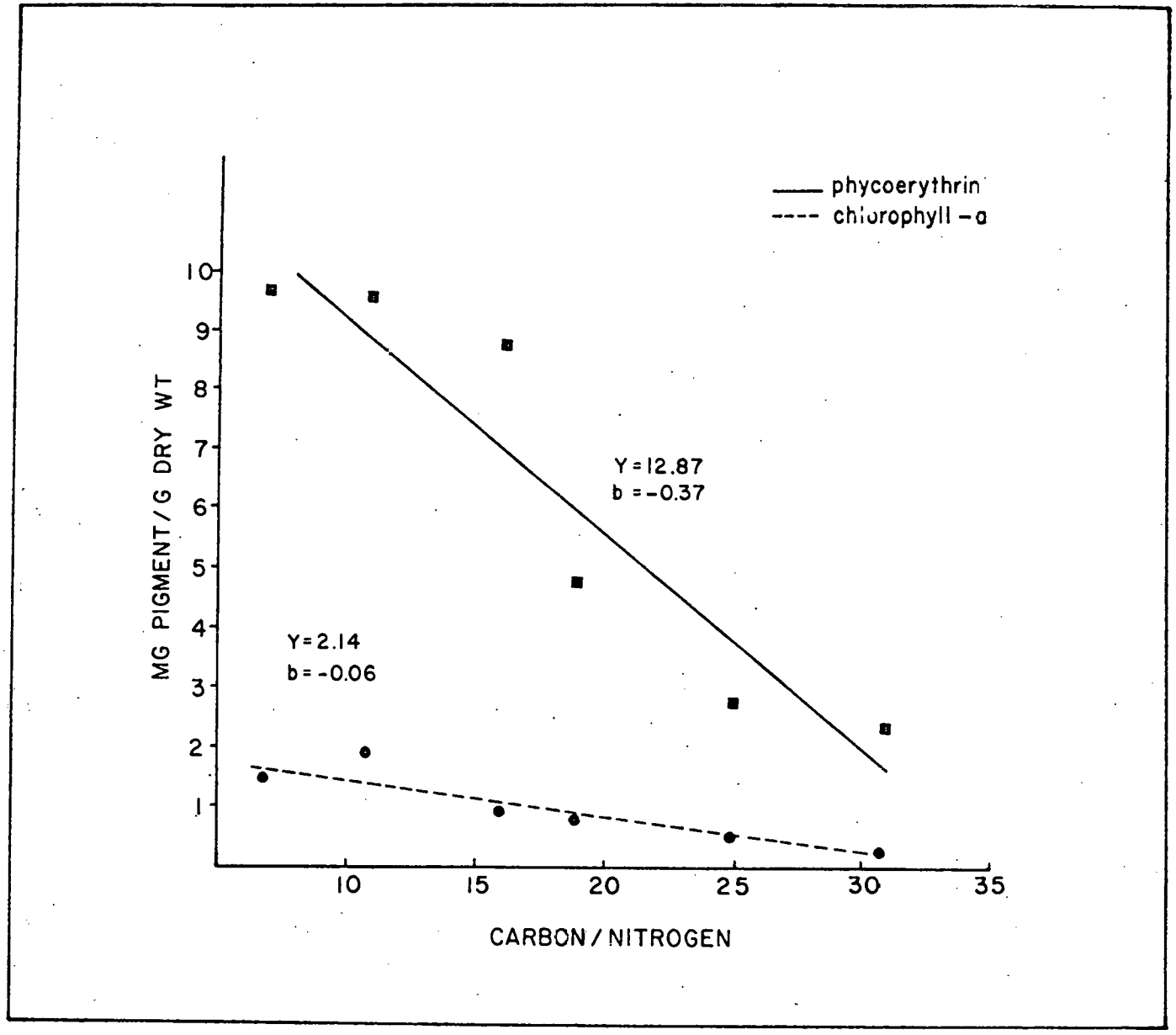

phycoerythrin
chlorophyll - a
Y = 12.87
b = -0.37
Y = 2.14
b = -0.06
MG PIGMENT / G DRY WT
CARBON / NITROGEN
1
2
3
4
5
6
7
8
9
10
10
15
20
25
30
35

Fig. 12

Effects of nitrogen enrichment on growth rate and phycocolloid content in *Gracilaria foliifera* and *Neoagardhiella baileyi*[1]

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ABSTRACT

Unattached, free-floating plants, were grown in continuous flow culture systems which received seawater enriched with 0-70 µM ammonium and 0-14 µM phosphate. Growth rate was measured every 3 days, and agar and carrageenan content, at the termination of experiments lasting 3-4 weeks. Agar content in Gracilaria foliifera (Forsskal) Børgesen ranged between 25% of the salt-free dry weight, in the plants receiving the highest concentration of nitrogen fertilizer, and 45%, in the plants receiving unenriched seawater. Carrageenan content in Neoagardhiella baileyi (Harvey ex Kutzing) Wynne and Taylor was highest (36% of the salt-free dry weight) at nitrogen concentrations in the range of 0.5-1.0 µM ammonium and was lower at both higher ammonium concentrations and in unenriched seawater. The application of these results to the farming and harvesting of agarophytes and carrageenophytes is discussed.

INTRODUCTION

The demand for agar and carrageenan has steadily increased during the past few years, while the supply of seaweed phycocolloids from natural populations has not substantially increased. Consequently, cultivation of various agarophytes and carrageenophytes has begun to supplement harvests from natural populations (7). The prime objective of this type of seaweed mariculture should be to obtain the highest production rate of high quality phycocolloid at the least cost by maximizing phycocolloid content and biomass production. Previous studies (2,3,4,9) have indicated that the supply of inorganic nitrogen in the seawater may have a significant effect on both the phycocolloid content and the biomass production of macroscopic red algae. To gain a better understanding of this relationship, the effects of nitrogen enrichment on the growth rate, phycocolloid content and phycocolloid production in Gracilaria foliifera and Neoagardhiella baileyi are examined in the present study.

MATERIALS AND METHODS

Gracilaria foliifera (Forsskal) Børgesen and Neoagardhiella baileyi (Harvey ex Kutzing) Wynne and Taylor were grown in suspended culture in tertiary waste treatment-polyculture or monoculture systems (4) prior to the present study. Both species remained vegetative throughout the study.

The Neoagardhiella experiment, conducted during March and April, 1976, utilized six plywood tanks (4) located in a heated greenhouse. The tanks, fitted with sloping bottoms, had a depth ranging from

1.14 m to 0.28 m and a surface area of 2.48 m^2. Each tank was stocked with approximately 1500 g fresh weight (approximately 100 g salt-free dry weight) of seaweed. Filtered seawater heated to 18-21° C and enriched with 4-70 µM ammonium (as NH_4Cl) and 1-14 µM phosphate (as NaH_2PO_4) was circulated on a continuous flow basis at the rate of three tank volumes per day (5475 L) in five experimental tanks. Unenriched seawater having an average inorganic nitrogen concentration of 1.1 ± 0.5 µM was circulated at the same rate in the sixth tank.

The Gracilaria experiment (Jan.-Feb., 1977) was conducted in six 1080 L fiberglass tanks (4), stocked with 750 g fresh weight (approximately 50 g salt-free dry weight) of seaweed. These tanks, located in a greenhouse, had a depth of 1.10 m and a surface area of 0.98 m^2. Filtered seawater heated to 20°C and enriched with 2-60 µM ammonium and 0.25-7.50 µM phosphate was circulated on a continuous-flow basis at the rate of five tank volumes per day (5400 L) in five experimental tanks. Unenriched seawater having an average dissolved inorganic nitrogen concentration of 0.7 ± 0.4 µM was circulated at the same rate in the sixth tank.

Following twelve days of preconditioning in each experiment, the plants were weighed every three days during a three-week period. After weighing, the biomass in each tank was adjusted to the initial stocking density. Samples of the harvested plants were dried at 60°C to obtain dry weight:fresh weight ratios. At the end of the experiment the entire contents of each tank were dried at 60°C for carrageenan or agar analysis. Ammonium, nitrate, and nitrite were determined (12) twice weekly on the effluents from the experimental

tanks.

Carrageenan content was determined using an alkaline extraction/alcohol precipitation procedure (3). Two different methods were used for the quantitative determination of the Gracilaria agar. In the acid method 50 g of dried weed were placed in a beaker containing 750 ml hot water. The pH was then adjusted to 5.5 and the mixture heated and stirred for 75 minutes at 90°C. The hot mixture was filtered using a pressurized filtration device; and the filtrate purified by quick freezing of the gel, followed by thawing and drying at 60°C. The agar content, determined gravimetrically, is expressed as a percentage of the salt-free dry weight. The latter was determined by washing a known amount of dried sample in cold water for 2 minutes, drying and then reweighing. In the alkaline method of agar analysis 50 g of weed were soaked in 200 ml of 5% NaOH for 30 minutes, and then placed in a covered beaker and baked at 85° for 3 hours. The weed was then washed well in cold water, drained and subsequently treated in the same manner as in the acid method.

RESULTS AND DISCUSSION

The nitrogen concentrations shown in Fig. 1 and 2 are the average inorganic nitrogen ($NH_4^+ + NO_3^- + NO_2^-$) concentrations measured in the tanks during the experiment. In the Neoagardhiella experiment (Fig. 1) growth rate increased with increasing nitrogen concentration up to approximately 0.5 µM, with no further increase occurring at higher concentrations. Carrageenan content was highest (36%) at a concentration of approximately 0.5 µM and decreased slightly at higher nitrogen concentrations.

The growth rate of Gracilaria (Fig. 2) was proportional to nitrogen concentration up to approximately 1.0 µM with little change in growth rate occurring at higher nitrogen concentrations. Agar content was highest (45%) in the plants receiving unenriched seawater, and decreased exponentially with increasing nitrogen enrichment. Gracilaria cultured at nitrogen concentrations ranging from 2-45 µM contained approximately 25% agar. Agar production increased with increasing nitrogen concentration up to approximately 1 µM with a slight decrease at 2.5 µM.

The rates of production of agar and carrageenan reported should not be interpreted as maximum phycocolloid production rates. The density of seaweeds used in these experiments was intentionally maintained at suboptimal levels (4) to reduce the variability in growth rates and nutrient uptake caused by fluctuating solar radiation.

Seasonal quantitative differences in phycocolloid content have frequently been reported for seaweeds harvested from natural populations (1,2,5,6,7,8,10,11). These variations have been attributed to plant size or age (8,11), rainfall (6), rate of growth (2,10), or nutrient levels (2,5,8). Neish and Shacklock (9) have shown that Chondrus cultured in unenriched seawater has a higher carrageenan content than Chondrus grown in nitrogen-enriched media. In addition, they demonstrated an increase in carrageenan content of Chondrus grown in nitrogen-enriched seawater following its transfer to unenriched seawater. Results of the present study are in general agreement with the findings of Neish and Shacklock (9) with the exception that the highest carrageenan content was observed in plants continuously receiving approximately 0.5 µM nitrogen rather than unenriched seawater.

This apparent discrepancy presumably can be attributed to the greater range of nitrogen concentrations examined in the present study and to differences in the concentrations of inorganic nitrogen in the unenriched seawater utilized in the two studies.

The inorganic nitrogen content of the seawater, which varies seasonally, appears to be a major factor influencing the phycocolloid content and growth rate of natural seaweed populations. It is important to note that the nitrogen concentration range for maximum biomass production and for the highest phycocolloid content coincide. Intensive seaweed mariculture facilities in which the plants are fertilized continuously, allow the inorganic nitrogen to be maintained within this optimum range of approximately 0.4-1.5 μM to obtain maximum phycocolloid production. As a result, plants may be harvested at any time without transfer to unenriched seawater. Determination of the optimum harvest time for seaweeds in a mariculture system receiving intermittent fertilization is more difficult. Results of this investigation indicate that, for a period prior to harvesting, inorganic nitrogen should be reduced to concentrations less than 0.5 μM in order to increase the phycocolloid content. The length of time necessary to accomplish this increase is dependent upon several factors including solar radiation, temperature, stocking density of the seaweeds, and the concentration of nutrients stored in the plant tissue and contained in the seawater. Preliminary studies have shown that it may be possible to use the N/C ratio of the plants or their pigment composition as an indicator of the nutrient status and phycocolloid content of the seaweeds (4). The capability of increasing phycocolloid production through regulation of the nitrogen concentration and the

utilization of indicators to monitor the nutrient status of seaweeds should increase the efficiency and success of seaweed mariculture.

ACKNOWLEDGEMENTS

This research was supported in part under Contract E (11-1)-2948 from the U. S. Energy Research and Development Administration, the Jessie Smith Noyes Foundation, Inc., and the University of Texas Marine Science Institute. The author appreciates the assistance received from Dr. J. H. Ryther, F. G. Whoriskey, and B. E. Lapointe. Particular thanks are extended to Mr. Charles Allen of Marine Colloids, Inc., Rockland, Maine, for his assistance in development of the phycocolloid extraction techniques.

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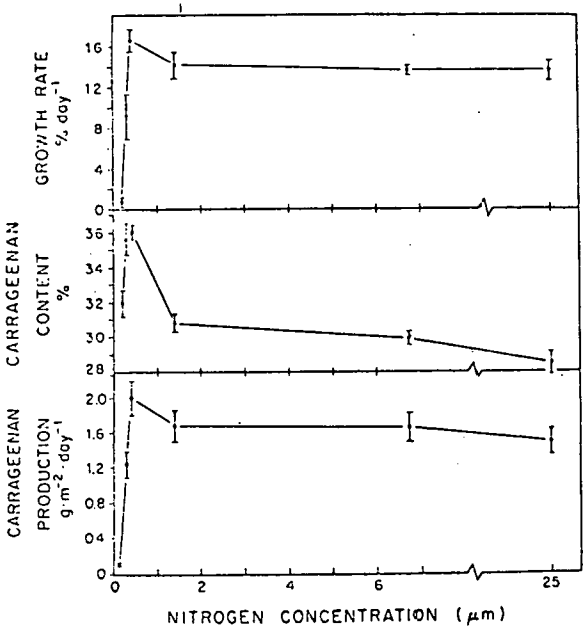

Fig. 1. Effects of nitrogen (effluent NH_4^++ NO_3^-+NO_2^-) concentration on the growth rate, carrageenan content and carrageenan production in Neogardhiella baileyi. Standard error indicated about each mean: Growth, n=7; Carrageenan, n=3.

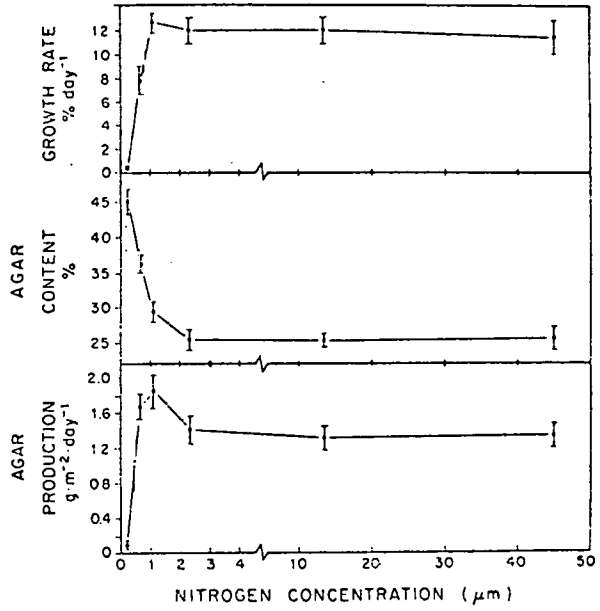

Fig. 2. Effects of nitrogen (effluent NH_4^++ NO_3^-+ NO_2^-) concentration on the growth rate, agar content and agar production in Gracilaria foliifera. Standard error indicated about each mean. Growth, n=7; Agar, mean of two acid and two alkaline extractions

PRELIMINARY STUDIES ON A COMBINED SEAWEED MARICULTURE-TERTIARY WASTE TREATMENT SYSTEM[1]

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ABSTRACT

A pilot-plant scale waste recycling-mariculture system was developed at the Woods Hole Oceanographic Institution's Environmental Systems Laboratory in 1973. Basically, the concept of this system is to grow unicellular marine algae in continuous flow cultures on mixtures of seawater and secondarily treated sewage effluent, and feed the algae to bivalve molluscs, maintained in a separate culture system. A final polishing step in the system consists of macroscopic algae (seaweeds) which remove the dissolved nutrients regenerated by the animal culture prior to discharge of the final effluent. In addition to serving as final polishing step in the phytoplankton-shellfish waste recycling system, seaweeds are also used as a one-step waste recycling-mariculture system.

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Currently, in the seaweed systems, red alage are being grown which contain either agar or carrageenan both of which are in short supply and therefore have a high economic value. Yields from these seaweed mariculture systems are notably high compared to other maricultural and agricultural crops. Results indicate that seaweed farms will be an economically profitable enterprise especially for developing countries in warm climates.

INTRODUCTION

A combined tertiary sewage treatment-marine aquaculture system has been developed which has the capacity for removing the inorganic nutrients from treated sewage effluent, prior to discharge of the latter to the environment, recycling these nutrients into commercially valuable crops of marine organisms (Ryther, 1977). The concept of this system is to grow unicellular marine algae in continuous flow cultures on mixtures of seawater and treated sewage effluent, and feed the alga to bivalve molluscs, maintained in trays in a separate culture system. The algae remove the nutrients from the wastewater and the shellfish remove the algae from suspension. Other animals, including lobsters and flatfish, grown together with the bivalves utilize their solid wastes and the fauna of small invertebrates that feed on the wastes. A final polishing step in the system consists of macroscopic algae (seaweeds) through which the effluent from the animal culture passes. The seaweeds remove the dissolved nutrients regenerated by the animal culture prior to discharge of the final effluent. The objective is to achieve a low nutrient final effluent that will meet the standards of tertiary treatment while at the same time producing commercially valuable crops of marine organisms.

There are numerous ways in which the seaweeds harvested from this system may be utilized. They may serve as food for herbivorous or omnivorous finfish or invertebrates or as a food supplement for cattle, swine or chickens (Chapman, 1970). Alternatively, some species of seaweeds can be sold for the commercially valuable products that they contain such as agar and carrageenan (Mathieson, 1975). The U. S. Energy Resource and Development Administration (ERDA) is currently supporting this study and others (Wilcox, 1976) to determine the feasibility of growing seaweeds as biomass to produce a source of energy through pyrolysis or fermentation.

In addition to using seaweeds as a polishing step in the waste recycling-polyculture system, they may also be grown in a one-step waste recycling system to remove the nutrients directly from a mixture of seawater and secondary sewage effluent. Instead of domestic sewage effluent, agricultural or food processing wastes or artificial fertilizers may be used as alternative nutrient sources. Similar growth rates are obtained using either secondary sewage effluent or chemical nutrients as a nutrient source (DeBoer, unpublished). Seaweeds may also be incorporated into a closed animal mariculture system (e.g. salmon culture) in which the algae serve as a biological filter, removing the toxic metabolic wastes of the animals.

Two species of macroscopic red algae (Gracilaria foliifera and Neoagardhiella baileyi) were grown in the Woods Hole Oceanographic Institution's waste recycling-seaweed mariculture project. Several species of Gracilaria are currently harvested as a source of agar. Neoagardhiella, which contains iota carrageenan (DeBoer et al., 1976), is not yet harvested commercially but has potential commerical value. A closely related genus (Eucheuma) which contains the same phycocolloid, is cultivated and harvested commercially in the Philippines (Parker, 1974). Agar and carrageenan are used extensively in food, pharmaceutical, textile, cosmetic, and other industries in such products as diet foods, toothpaste, pharmaceutical capsules, and infant formulas (Mathieson, 1975). Because the demand for agar and carrageenan exceeds the supply and because the industry is resource limited (Silverthorne and Sorenson, 1971; Mathieson, 1975), there has been a recent interest in the mass cultivation of seaweeds containing these phycocolloids.

This report describes the annual yields of Gracilaria foliifera and Neoagardhiella baileyi grown in culture at Woods Hole, Massachusetts.

MATERIALS AND METHODS

The unattached, free-floating seaweeds were grown in concrete raceways (Fig. 1) 12.2 m (L) x 1.2 m (W) x 1.5 m (D), which have sloping plywood bottoms with a depth ranging from 0.6 m on the shallow side to 1.5 m on the deep side. The seaweeds were kept in suspension by aeration from an air line located at the bottom of the raceway and extending its entire length. The aeration provided for mixing, gas exchange and uniform exposure of the plants to sunlight.

The seaweed cultures received the effluent from similar raceways containing various species of bivalve molluscs. Because of major problems with the mollusc cultures, a considerable research effort was devoted to that area including experimentation with flow rates, temperatures, shellfish stocking densities, and other factors. For that reason, the effluent from the shellfish raceways, which was utilized as a nutrient source for the seaweed raceway cultures, was highly variable throughout the year in its chemical and physical characteristics (Table 1). In spite of this, the flow of water and the supply of nutrients were not believed to have been limiting to seaweed growth. Rather, the primary factors influencing growth of the plants are believed to have been incident solar radiation and water temperature. The seaweed stocking density was varied experimentally during the year (Table 1) as the optimum denisty for the maximum rate of production in the raceways had not been previously determined. Production rates of Gracilaria and Neoagardhiella were monitored from March 20, 1975 to March 19, 1976. Production was measured by dipnetting all of the algae from the raceways and draining the plants in nylon mesh bags prior to weighing. Samples of the algae were oven dried at 60°C to determine the relationship between fresh and dry weight. Production rates or yields are expressed in terms of dry weight which ranged between 10-15% of the fresh weight. The productivity of seaweeds cultivated in tanks 0.6 m to 1.8 m in depth has been shown to be primarily a function

of the surface area and not the depth of the tanks (Shacklock et al., 1975; DeBoer, unpublished). As a result, the productivity values are reported here as the mass of dry algal material produced per unit area per unit time.

RESULTS AND DISCUSSION

The seaweed production rates are shown in Figure 2. Neoagardhiella had surprisingly high rates of production in the spring (22-41 g dry weight/m^2·day) and summer 20-36 g/m^2·day). Gracilaria production rates were quite variable, increasing from 4 g/m^2·day in April to 43 g/m^2·day in early June and decreasing again during the latter part of the summer to 7-18 g/m^2·day. Production rates of both species declined during the fall and early winter. By mid-December the Gracilaria production rate had fallen to zero and the plants had deteriorated, while the Neoagardhiella remained viable (although stunted) with a production rate of 6 g/m^2·day.

The gross seasonal changes in production appear to be correlated with both water temperature and incident solar radiation. From June 1 to October 1, however, when the water temperatures and solar radiation were presumably optimal for the seaweeds, production showed little correlation with those variables. Low production rates during that period were probably due to the high densities of seaweeds maintained in the raceways (as high as 12 and 10 kg fresh weight/m^2 for Neoagardhiella and Gracilaria respectively). DeBoer and Lapointe (1976) found a decrease in the rate of production of both species at densities above 5 kg fresh weight/m^2.

The mean annual dry weight production rate was 17 g/m^2.day or 63 metric tons/hectare.year for Neoagardhiella and 9 g/m^2.day or 33 t/ha.year for Gracilaria. These rates may be higher than would be realized in a commercial enterprise because the raceways were maintained at elevated temperatures for approximately six months of the year. Annual production rates based on a 5 1/2 month growing season when the raceways were not heated (May 8, 1975 to October 20, 1975) were 46 t/ha·year and 28 t/ha.year for Neoahardhiella and Gracilaria, respectively. Given favorable growing conditions it does not seem unrealistic to expect production rates to exceed 50 t/ha·year based on a five-six month growing season at this latitude. Even higher yields should be possible in warmer climates nearer the equator.

The yields obtained in this study were similar to those recently reported for Gracilaria sp. and Hypnea musciformis grown in essentially the same way but for shorter periods of time at the Harbor Branch Foundation in Florida (Lapointe et al., 1976). The yields obtained in both studies are considerably greater than literature values reported for other highly profitable seaweed crops (Table 2). Doty (1973) calculated that Eucheuma farming can "provide more than three times the dollar return that sugar [cane] produces". Shang (1976) estimates an annual profit of $1,399-2,413/ha for Gracilaria cultured in ponds (at yields 1/3 or less than those obtained in the present study), whereas

the same ponds employed for milkfish culture brought profits of only $250-$500/ha.

Most species of seaweeds currently cultivated are grown attached to ropes, nets or poles (Bardach et al., 1972). These methods are very labor intensive and are, therefore, best suited to countries having low labor costs. The method used in the present study, growing the unattached plants in raceways (ponds could also be used), is a more versatile means of culture and one that could easily be mechanized if desired.

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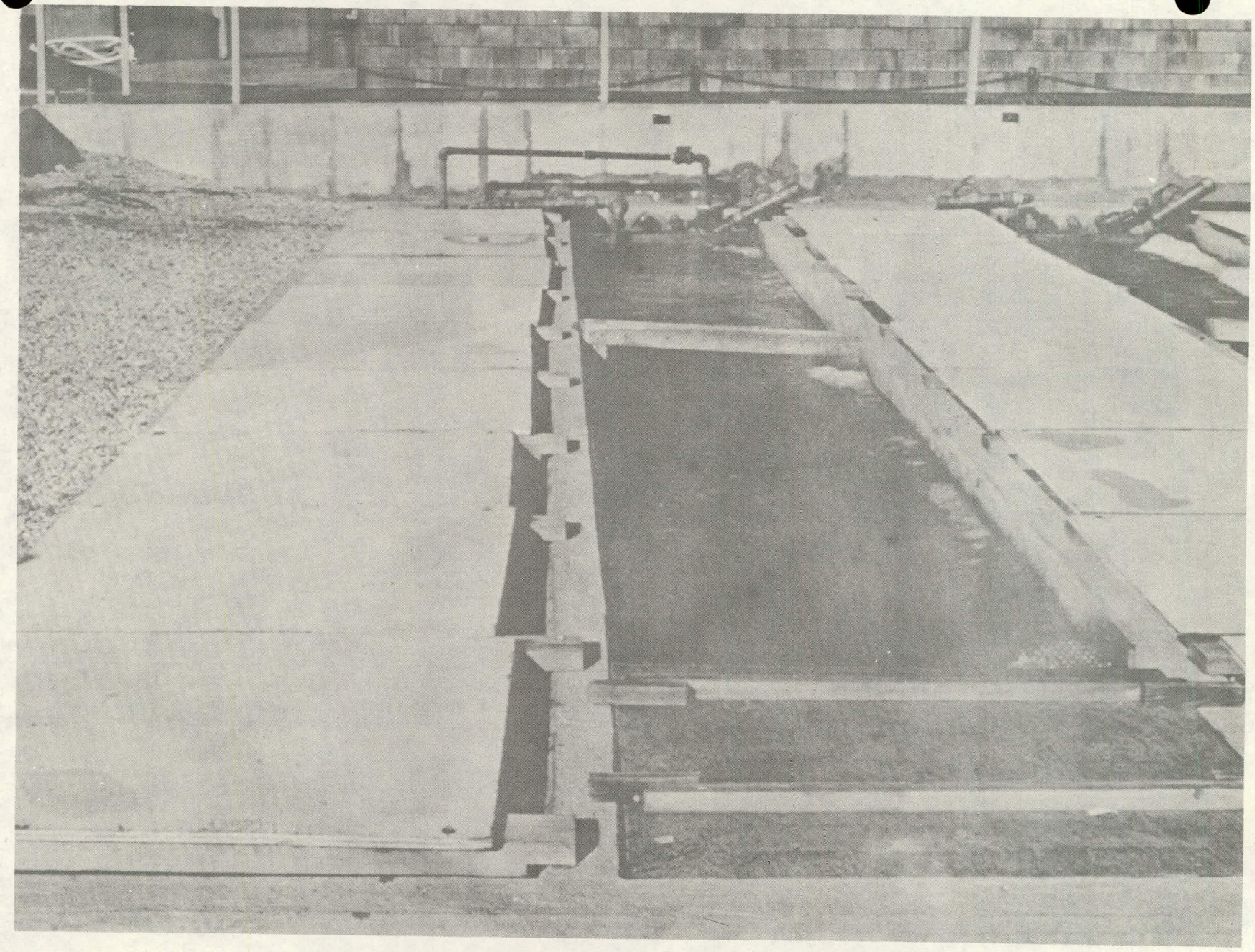

Figure 1. Raceways used to culture seaweeds (uncovered) and bivalve molluscs (covered).

Table 1. - Culture conditions in the seaweed raceways during 1975-1976

Dates	Radiation langleys/day	Nutrients (μmoles/l) $NO_2^- + NO_3^- + NH_4^+$	Nutrients (μmoles/l) $PO_4^{\equiv}$	Influent water temperature °C	Water flow liters/min[1]	Density (kg wet wt/m^2) *N. baileyi*	Density (kg wet wt/m^2) *G. foliifera*
Mar 20-Apr 15	366	37	12	15	48	3.9	2.8
Apr 15-May 6	400	14	8	17	48	9.5	3,9
May 6-May 20	543	21	7	18	72	8.9	5.4
May 20-May 28	548	11	2	18	72	12.3	6.4
May 28-Jun 5	519	27	7	19	72	10.1	8.1
Jun 5-Jun 12	430	16	7	20	72	11.5	8.4
Jun 12-Jun 27	566	34	9	21	96	8.2	10.2
Jun 27-Jul 16	518	75	13	23	138	5.5	5.9
Jul 16-Aug 14	496	58	10	26	96	6.6	6.3
Aug 14-Aug 27	467	81	14	26	48-144	5.9	6.5
Aug 27-Sep 18	423	--	--	21	48-144	4.2	4.0
Sep 18-Oct 6	361	--	--	19	48-144	3.1	2.6
Oct 6-Nov 7	246	--	--	16	70	2.6	1.7
Nov 7-Nov 21	181	44	8	14	72	3.1	2.8
Nov 21-Dec 19	140	37	5	13	60- 80	2.0	2.2
Dec 19-Feb 20	141	40	6	14	60- 80	3.0	---
Feb 20-Mar 19	202	--	--	15	60- 80	2.9	---

[1]48 liters/min = 3 exchanges/day

Table 2. Comparative dry weight productivity values for cultivated seaweed and terrestrial crops

Crop	Location	Annual production t/ha	Reference
Neoagardhiella	Massachusetts, USA	46	This study
Gracilaria	Massachusetts, USA	28	This study
Gracilaria	Florida, USA	46	Lapointe et al. (1976)
Gracilaria	Taiwan	7-12	Shang (1976)
Gracilaria	Taiwan	2-10	Parker (1974)
Hypnea	Florida, USA	39	Lapointe et al. (1976)
Eucheuma	The Philippines	13	Parker (1974)
Porphyra	Japan	3	Parker (1974)
Gelidium	Taiwan	2	Parker (1974)
Sugar cane	Hawaii, USA	67	Loomis and Gerakis (1975)
Corn	Colorado, USA	27	Loomis and Gerakis (1975)
Wheat	Washington, USA	30	Loomis and Gerakis (1975)

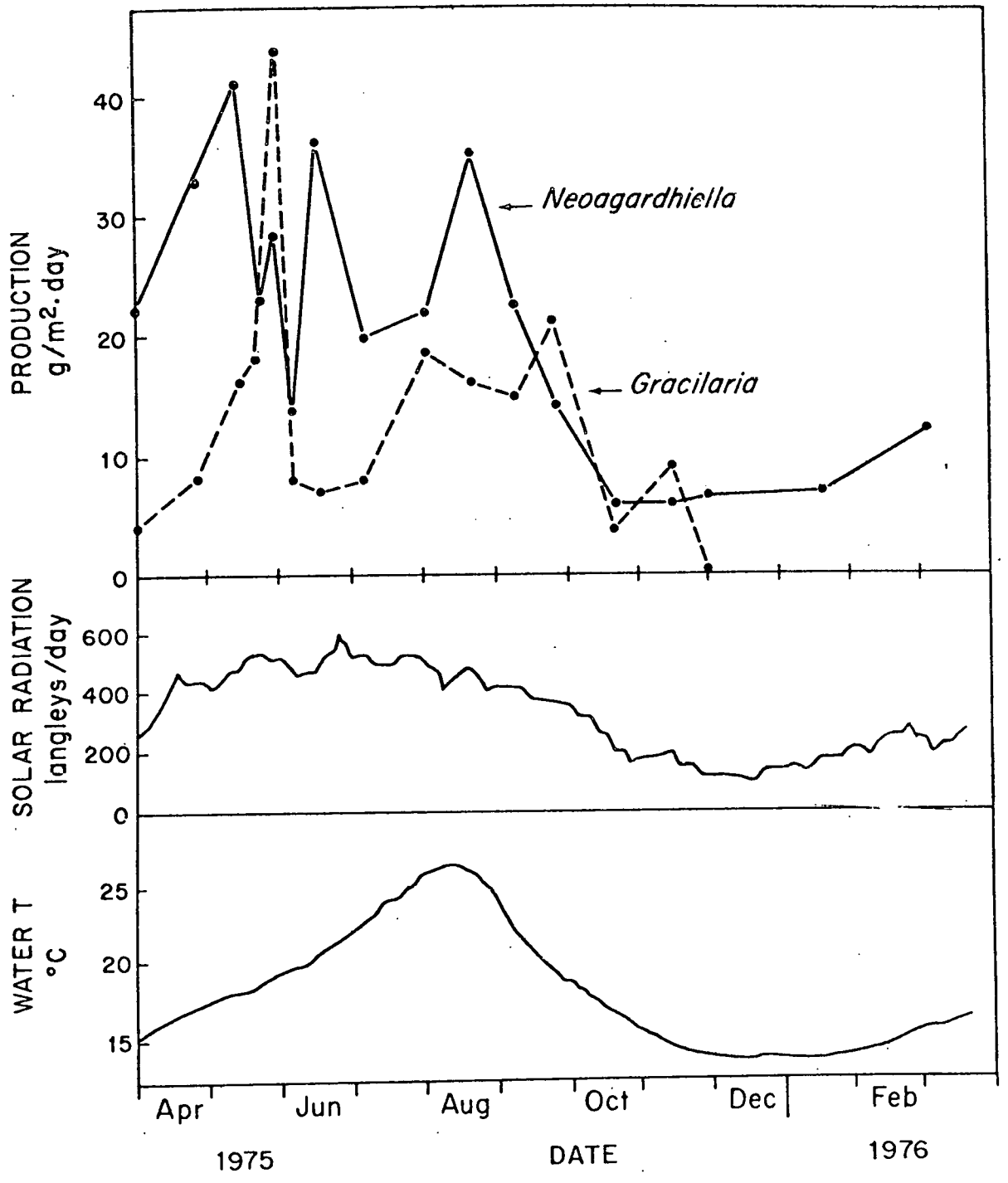

Figure 2. Production rates of seaweeds grown in raceways during 1975-76.

Potential Yields from a Waste Recycling-Algal Mariculture System

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INTRODUCTION

For centuries seaweeds have been an integral part of the Oriental diet, but only since World War I have they become an important commodity in the Western World. In recent years they have been used in the United States primarily for their phycocolloids (alginates, agar and carrageenan). Agar and carrageenan, cell wall polysaccharides produced by various red algal species, are widely used in the food, pharmaceutical, textile, cosmetic and other industries as suspending, thickening, stabilizing, and emulsifying agents (13, 29). The principal source of agar has been *Gelidium* spp., which is harvested in Japan where the agar is extracted and exported to the rest of the world. Carrageenophytes have been harvested from natural populations throughout the world, dried, and shipped to factories in North America or Western Europe, where the phycocolloid is extracted and refined for sale. Most of the world's supply of carrageenan

comes from *Chondrus crispus* (Irish Moss) populations in Eastern Canada and to a lesser extent New England and Northern Europe.

These seaweed resources are limited in area and are now heavily exploited. At the same time, the demand for phycocolloids has steadily increased. The discovery that different algal species or blends of phycocolloids from different algal species have dissimilar gelling or emulsifying properties has led to a large number of new applications of these products. These factors together have led to screening of various species and world-wide surveys of seaweed resources by the industry over the past two decades, in an attempt to expand the base of its operation. One example of such expansion is the relatively new exploitation of the red alga *Eucheuma* in the Philippines and other parts of Southeast Asia. These resources, old and new, have recently been decreasing in abundance due to overharvesting (2, 8, 30) pollution (20,30, 33) and storm damage (21) to the extent that the industry is resource limited and attention has become focused on cultivation as the only viable long-term solution.

Most studies with seaweeds have been concerned with their taxonomy, anatomy, life history or distribution. Unfortunately, there is very little known about the physiology

and autecology of most of these algae and even less concerning their cultivation.

CULTIVATION OF AGAROPHYTES AND CARRAGEENOPHYTES

To supplement insufficient natural supplies of agarophytes, the Japanese initiated a seaweed cultivation program several decades ago. One method involves scattering small fragments of *Gelidium* or *Gracilaria* (12) in bays where the plants are allowed to regenerate vegetatively. More recently the Japanese have propagated *Gracilaria* and *Gelidium* (14) on ropes in shallow bays. *Gracilaria* culture in Taiwan (28) has undergone a rapid expansion since its initiation in 1962. The unattached *Gracilaria* plants are grown in shallow ponds, approximately 1 ha in area, which formerly were used for milkfish culture. *Eucheuma* farming developed in the Philippines (7, 8, 9, 21) utilizes a net-culture technique that is similar to the cultivation of edible seaweeds in Japan. There are no commercial seaweed cultivation farms presently in existence in North America[1],

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[1] Atlantic Mariculture Ltd. of Grand Manan Island, New Brunswick, Canada uses V-shaped, air agitated ponds as part of a commercial *Rhodymenia* operation (19). Their primary objective is not cultivation, but rather to keep the plants alive until they can be processed.

but several research projects involving the cultivation of red algae have evolved in the past decade.

Beginning in the late 1960's a group, headed by A. C. Neish, at the Canadian National Research Council Atlantic Regional Laboratory near Halifax, initiated studies on the culture of unattached *Chondrus crispus* in tanks containing flowing seawater. One of their early observations was that plants of different origin grew at considerably different rates in the same tank. One clone (T-4) grew much faster than others and, in addition, was less susceptible to epiphytization by undesired algal species (17). Another important finding was that the chemical composition of the plants could be altered by manipulation of the culture environment. Neish and his co-workers discovered that plants grown in unenriched seawater have a higher carrageenan content than those grown similarly in nitrogen-enriched seawater. If *Chondrus* grown in nitrogen-enriched seawater was transferred to unenriched seawater, its carrageenan content increased. The effects of several other operating parameters were also investigated (17, 18, 26, 27).

Following Neish's lead, several research groups in the U. S. have experimented with growing unattached seaweeds in suspended culture. For example, Ryther's group (3, 4, 5, 11, 22, 23, 25) in Woods Hole and in Florida has grown

Gracilaria sp., *Neoagardhiella baileyi, Chondrus crispus, Gracilariopsis sjoestedtii, Hypnea musciformis* and other species in tanks (Figs. 1A-B) raceways (Figs. 1C & 2) and ponds (Fig. 2). Other research teams in the U. S. have used similar tank culture methods to grow *Hypnea* (10), *Iridaea* (31) and *Gigartina* (31, 32).

Two commercial seaweed companies, Marine Colloids, Ltd. and GENU Products, Ltd. have started pilot *Chondrus* products in Nova Scotia, with partial support from the Canadian Government, using different modifications of Neish's basic technique. Problems delaying full scale production in both projects include (1) control of algal contaminants (e.g., *Ulva, Enteromorpha, Ectocarpus)* in the culture system and (2) slow growth of *Chondrus*.

A WASTE RECYCLING - MARINE POLYCULTURE SYSTEM

Beginning about 1970, a project was started at the Woods Hole Oceanographic Institution in which a waste recycling-marine aquaculture system was developed. This system has the capacity of removing the inorganic nutrients from treated sewage effluent, prior to its discharge to the environment, recycling these nutrients into commercially valuable crops of marine organisms.

The concept of this system is to grow unicellular marine algae in ponds in continuous flow cultures on mixtures of seawater and secondarily treated sewage effluent (Fig. 3). The algae are fed to bivalve molluscs, maintained in Nestier® trays in raceways. The algae remove the nutrients from the wastewater and the shellfish remove the algae from suspension. A final polishing step in the system consists of macroscopic algae (seaweeds) through which the effluent from the animal culture passes. The seaweeds remove the dissolved nutrients regenerated by the animal culture prior to discharge of the final effluent. The objective of this polyculture system is to achieve a low nutrient final effluent that will meet the standards of tertiary treatment while at the same time produing commerically valuable crops of marine organisms.

Two species of macroscopic red algae, *Gracilaria* sp. and *Neoagardhiella baileyi*, were grown in the Woods Hole waste recycling-seaweed mariculture projects. Several species of *Gracilaria* are currently harvested as a source of agar. *Neoagardhiella*, which contains iota-carrageenan (4) is not yet harvested commercially but has potential commercial value. Species of a closely related genus, *Eucheuma*, which contain the same phycocolloid, are cultivated and harvested commercially in the Philippines (8).

The unattached, free-floating seaweeds were grown in concrete raceways 12.2 m (L) x 1.2 m (W) x 1.5 m (D), which have sloping plywood bottoms with a depth ranging from 0.6 m to the bottom (1.5 m). The seaweeds were kept in suspension by aeration from an airline located at the bottom of the raceway and extending its entire length. The aeration provided for mixing, gas exchange and uniform exposure of the plants to sunlight. The seaweed cultures received the effluent from similar raceways containing various species of bivalve molluscs.

Because of major problems with the mollusc cultures during the first year of operation a considerable research effort was devoted to that area including experimentation with flow rates, temperatures, shellfish stocking densities, and other factors. For that reason, the effluent from the shellfish raceways was highly variable throughout the year in its chemical and physical characteristics (Table I). In spite of these variations, the flow of water and the supply of nutrients were not believed to have been limiting to seaweed growth. The seaweed stocking density was varied experimentally during the year because the optimum density for the maximum rate of production in the raceways had not been previously determined. *Gracilaria* and *Neoagardhiella* were

monitored from March 20, 1975 to March 19, 1976.

Production was measured by dip-netting the algae from the raceways and draining the plants in nylon mesh bags prior to weighing. After weighing, the biomass was cut back to its starting value or allowed to accumulate. The populations were weighed at intervals of 7-60 days. Generally, the plants were weighed at intervals of about 1 week during the period of most rapid growth in spring and early summer and at longer intervals during the remainder of the year. Samples of the algae were oven dried at 60° C to determine the relationship between fresh and dry weight. Production rates or yields are expressed in terms of dry weight which ranged between 10-15% of the fresh weight.

The seaweed production rates are shown in Figure 4. *Neoagardhiella* had surprisingly high rates of production in the spring (22-41 g dry weight/m^2·day) and summer (20-36 g/m^2·day). *Gracilaria* production rates were quite variable, increasing from 4 g/m^2·day in April to 43 g/m^2·day in early June and decreasing again during the latter part of the summer to 7-18 g/m^2·day. Production rates of both species declined during the fall and early winter. By mid December the *Gracilaria* production rate had fallen to zero and the plants had deteriorated while *Neoagardhiella* remained viable (although stunted) with a production rate of 6 g/m^2·day.

The gross seasonal changes in production appear to be correlated with both water temperature and incident solar radiation. Although the seawater entering the bivalve mollusc cultures was heated in winter to enable the shellfish to feed and grow, it had cooled to as low as 13° C by the time it entered the seaweed cultures and as low as 8° C on leaving the cultures at times during mid-winter. From June 1 to October 1, however, when the water temperatures and solar radiation were presumably optimal for the seaweeds (3), production showed little correlation with those variables. Low production rates during that period were probably due to the high densities of seaweeds maintained in the raceways (as high as 12.3 and 10.2 kg fresh weight/m^2 for *Neoagardhiella* and *Gracilaria*, respectively.

SEAWEED MARICULTURE - OPERATIONAL CONSIDERATIONS

In addition to using seaweeds as a "polishing" step in a waste recycling-polyculture system, they may also be grown in a one-step waste recycling system to directly remove the nutrients from mixtures of seawater and secondary sewage effluent. This one-step system has been more successful than the polyculture system because of its simplicity. Even so, there are several operational parameters which need examination to ensure success and to optimize yields in a

seaweed mariculture system.

It was observed in the polyculture experiment that one very important operational consideration is the biomass of seaweeds to be maintained in the culture system to provide maximum yield per unit area. Two experiments were conducted to determine the relationship between seaweed density, growth rate and production for *Gracilaria* sp. One experiment was conducted during June 2-28, 1976 and the other, during November 3-30. Each of six plywood tanks (Fig. 1A) was stocked with an initial stocking density ranging from 180-4000 g fresh weight/ m^2. These tanks, measuring 2.4 m (L) x 1.0 m (W) x 1.2 m (D), were designed with sloping bottoms. An airline on the bottom along the deep side of the tank enabled the plants to be maintained in suspension and circulated by aeration. The tanks were located in a geodesic dome fitted during the winter with a vinyl cover to retain heat. Filtered seawater heated to 18.5-21.5° C was enriched with ammonium chloride and sodium phosphate to give a concentration of 50 μM NH_4^+ and 10 μM PO_4^{-3}. The heated, enriched seawater was circulated through the tanks on a continuous-flow basis at a rate of 2 tank volumes (3650 liters) per day. Three times per week the plants were weighed and the density in each tank adjusted to the initial stocking density by harvesting the incremental growth. The

specific growth rate (μ), the percent increase per day, was calculated by the equation:

$$\mu = \frac{100(\ln N/No)}{t}$$

where No is the initial biomass and N is the biomass on day t.

The mean growth and production rates at the different mean densities are shown in Fig. 5. The average solar radiation was 549 langleys/day during the late spring experiment and 152 langleys/day during the late fall experiment. The growth rate decreased exponentially with increasing culture density from 14%/day to 4%/day during the spring and from 9%/day to 1%/day in the fall. Production, or yield, which is a function of both specific growth rate and density, was highest at an intermediate density of 3000-4000 g fresh weight/m^2 in spring. In the fall, maximum production was achieved at densities of 2000-2500 g fresh weight/m^2.

In another experiment using similar methods (3) we investigated the relationships between growth rate, production rate and density in *Neoagardhiella baileyi*. The results, shown in Fig. 6, are averages over the entire 40 day experiment (Nov-Dec, 1975), during which time the mean incident solar radiation was 136 langleys/day. Maximum production

occurred at densities of 2000-2500 g fresh weight/m^2. During brief periods (ca. 1 week) of sunny weather when the solar radiation averaged 160 ly/day, productivity increased markedly to a peak of 22 g dry wt/m^2/day at a density of 2900 g fresh wt/m^2. During cloudy periods (85 ly/day) production decreased to less than 5 g dw/m^2/day at densities of 500-1500 g fresh wt/m^2.

It may be concluded from these experiments that to obtain maximum yields of *Gracilaria* and *Neoagardhiella*, the density should be maintained in the range of 1800-2800 g/m^2 during the winter, 2800-4500 g/m^2 during late spring and summer, and at intermediate densities during the remainder of the year. These relationships between production and seaweed density for *Gracilaria* and *Neoagardhiella* are similar to those reported for other red algae. For example, the maximum rate of production for *Chondrus* during the summer was obtained at a population density of 5800 g fresh weight/m^2 (18). The optimum density for *Iridaea* in April was approximately 2100 g fresh weight/m^2 (31).

The optimum depth of the culture system is another variable which is best determined empirically. Shacklock *et al.* (27) found that the growth of *Chondrus* was greater at a depth of 91 cm than it was at either 46 or 1800 cm. From experience

with various types of culture enclosures and modes of circulation of the plants and water we have found that the optimum depth for the suspended cultures of *Gracilaria* and *Neoagardhiella* is 60-110 cm.

Another critical operating parameter in seaweed cultivation is the concentration of nutrients necessary to sustain maximum rate of growth. Ryther and Dunstan (24) found that nitrogen is the chemical nutrient most likely to be limiting algal growth in marine waters. Our seaweed studies have also indicated nitrogen to be the critical limiting factor in the Woods Hole seaweed mariculture system. As a result, an investigation (4) was undertaken (March 21-April 8, 1976) to determine the concentration of ammonia at which maximum growth rate occurs in *Neoagardhiella baileyi*. Each of the six tanks described in the density experiments was stocked with 1500 g of seaweed. Influent nutrient concentrations of the enriched seawater ranged from 4 to 70 μM NH_4^+ and from 1 to 14 μM PO_4^{-3}, respectively, in five of the experimental tanks, with an unenriched seawater control in the sixth tank. The continuous flow rates were equivalent to three tank volumes per day. Every three days the seaweeds were weighed and the biomass in each tank adjusted to the initial stocking density. Growth rate (Fig. 7) increased with increasing nitrogen concentration

up to a concentration of approximately 0.8 μM NH_4^+ but remained constant at higher concentrations.

At the conclusion of the experiment described above the carrageenan content of the plants was determined. Details of the methods and results have been previously reported (4). Carrageenan content (Fig. 7) was highest at a residual nitrogen concentration of 0.8 μM NH_4^+ and decreased at both higher and lower nitrogen levels. These results are similar to those of Neish and Shacklock (18) who found that *Chondrus* grown in unenriched seawater has a higher carrageenan content than when grown in nitrogen-enriched seawater. Our results show, however, that even higher levels of carrageenan can be produced at low (0.5-1.2 μM) NH_4^+ concentrations than in unenriched seawater.

In another study[2] it was determined that the half-saturation constants for growth in *Gracilaria* and *Neoagardhiella* are approximately 0.5 μM NH_4^+ or NO_3^-. These constants are very low, demonstrating that seaweeds can utilize very low concentrations of inorganic nitrogen.

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[2]DeBoer, J.A., H.J. Guigli, T. L. Israel, C. F. D'Elia, and F. G. Whoriskey. Studies on the cultivation of the macroscopic red algae, *Gracilaria* sp. and *Neoagardhiella baileyi* (Harvey ex Kutzing) Wynne and Taylor. I. Growth rates in cultures supplied with nitrate, ammonium, or sewage effluent. (In prep.)

Half saturation constants for growth in phytoplankton are usually in the 0.1-5 μM range for inorganic nitrogen. As far as we are aware, there have been no other studies describing the half saturation constants for growth or uptake of inorganic nitrogen by seaweeds. This study[2] also showed that ammonia-grown *Gracilaria* and *Neoagardhiella* exhibit higher growth rates than nitrgate-grown plants, that both species show a decided preference for ammonia over nitrate in mixtures of the two, and that growth of both species is essentially equivalent whether the nitrogen source is secondary sewage effluent or chemical fertilizers.

These results indicate that in order to maximize biomass and carrageenan production, the inorganic nitrogen concentration should be maintained at concentrations of 0.5-1.5 μM NH_4^+. Because of diel and other changes in the rate of nitrogen uptake by the seaweeds, it is difficult to constantly maintain an optimum nutrient concentration at all times. Some simple indicator of the nutrient condition or status of the plants would greatly facilitate large-scale culture operations. The nitrogen:carbon ratio (N/C) of the plants may serve as that indicator. Growth rate (Fig. 8) increases with increasing N/C atomic ratio up to approximately 0.85 with no increase in growth rate at higher N/C ratios. This and other studies[2]

(4) indicate that plants having an N/C ratio above 0.85 are probably not nitrogen limited while lower ratios suggest nitrogen limitation. Additional studies under a variety of environmental conditions are necessary to substantiate these findings, but these initial results indicate that large deviations in the N/C ratio can be used to predict a nitrogen deficiency or surplus. Such an indicator would be extremely useful in seaweed mariculture to know when and how much to fertilize to maximize biomass and phycocolloid production.

It may also be possible to use a simple color index as an indicator of nutrient status and phycocolloid content (4). Plant color in *Neoagardhiella* is due primarily to the relative proportions of chlorophyll and the red accessory pigment, phycoerythrin. Fig. 8 shows the phycoerythrin concentration as a function of N/C. Phycoerythrin concentration increased rather consistently with increasing N/C. Plants with high concentrations of the red pigment appear dark reddish-brown while those with very low levels of the pigment are yellow to straw colored. However, before such a simple color index of phycocolloid content and nitrogen status is used as a rule of thumb, it must be verified that other nutritional deficiencies that may not affect carrageenan levels do not influence the development of the accessory pigment in the alga.

At very high light intensities, for instance, photo-oxidation of the pigments occurs, so that in those circumstances pigmentation may not be a valid indicator of nutritional status and carrageenan content.

One common observation from all of the above experiments is that if the seaweed density varies greatly from the optimum or the nitrogen concentration is higher than is necessary to support the maximum rate of growth, algal contaminants often proliferate to the extent that both the growth and the economic value of the cultured species are significantly decreased. We have found that if the plants are grown near the optimum density and at optimum nutrient concentrations (1-2 μM NH_4^+ or NO_3^-) the algal contaminants do not become a serious problem. Additionally, as discussed above, we have shown that the carrageenan content is also higher in plants grown at low nitrogen concentrations. The agar content in *Gracilaria* sp. is also higher at low nitrogen concentrations[3].

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[3] DeBoer, J. A. and F. G. Whoriskey. Studies on the cultivation of the macroscopic red algae, *Gracilaria* sp. and *Neoagardhiella baileyi* (Harvey ex Kutzing) Wynne and Taylor III. Relationships between nutrient concentration and phycocolloid content. (In prep.)

SEAWEED MARICULTURE - PRODUCTIVITY

The polyculture experiment was conducted prior to the density and nutrient experiments. The densities in the raceways were, at times, far above those optimum for the maximum rate of production. The production rates were, therefore, probably lower than might have been obtained if optimum densities had been maintained throughout the year. Nevertheless, the mean annual dry weight production was 17 $g/m^2 \cdot day$ or 63 metric tons/hectare·year for *Neoagardhiella* and 9 $g/m^2 \cdot day$ or 33 t/ha·year for *Gracilaria*. These rates may be higher than would be realized in a commercial enterprise because the raceways were maintained at elevated temperatures for approximately six months of the year. Annual production rates based on a 5 1/2 month growing season when the raceways were not heated (May 8 - October 20, 1975) were 46 t/ha·year and 28 t/ha·year for *Neoagardhiella* and *Gracilaria*, respectively (Table II).

The yields obtained in this study were similar to those recently reported for *Gracilaria* sp. and *Hypnea musciformis* grown in essentially the same way but for shorter periods of time at the Harbor Branch Foundation in Florida. In other small scale, short duration experiments, production rates of cultured seaweeds exceed these values 2-3 fold (3, 10, 19, 31, 32) but these yields have not been substantiated by large scale, long term production studies. However, given favorable

growing conditions it does not seem unrealistic to expect production rates to exceed 50 t/ha/yr based on a five-six month growing season in temperate latitudes. The yields obtained in both the Woods Hole and Florida studies are considerably greater than literature values reported for other highly profitable seaweed crops (Table II) and are as high or higher than many agricultural crops (5, 11).

CONCLUSIONS

Most agarophytes and carrageenophytes currently cultivated are grown attached to ropes or nets (1, 8, 13, 16). These methods, although profitable in some areas, are very labor intensive and are, therefore, best suited to countries having low labor costs. The method used in the present study, growing unattached plants in raceways or ponds, is a more versatile means of culture and one that could easily be mechanized if desired.

We have used domestic sewage effluent as a nutrient source in the waste recycling-polyculture systems in Woods Hole and in Florida but other nutrient sources could also be used. Alternative nutrient sources include agricultural wastes from cattle feed lots or swine farms, wastes from seafood processing plants or other food processing wastes, and wastes from open animal mariculture systems such as

penaeid shrimp farms. In all of these applications seaweeds can be used to lower the nutrient and heavy metals content of these wastes to enable the discharge to meet state and federal regulations. Seaweeds may also be incorporated into a closed animal mariculture system, serving as a biological filter to remove toxic metabolic wastes of the animals. Upwelled, nutrient rich waters may also be used as a nutrient source in seaweed mariculture systems (10), and, of course, conventional commercial fertilizers will serve as adequate (though perhaps more costly) nutrient sources in seaweed monoculture operations.

In summary, it appears that cultivated seaweeds are among the most productive primary producers and that the prospects for seaweed cultivation look optimistic, however, there is still a need for considerable basic and applied research on the biology of the macroscopic marine algae. Both biomass and phycocolloid production are undoubtedly functions of many inter-related variables including: availability of chemical nutrients, light intensity, light quality, photoperiod, turbidity, CO_2 availability, mixing, rate of water exchange, temperature, population density, culture depth, water quality (toxins, growth enhancement factors, pH), and the seaweeds themselves (differences between species and

physiological races, seasonality of growth, etc.). Results of our studies on some of these variables suggest that, if properly managed, the yields of seaweed mariculture can be substantially increased.

ACKNOWLEDGEMENTS

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Table I. Culture conditions in the seaweed raceways during 1975-1976.

Dates	Nutrients (μM) ΣiN	PO_4^{-3}	Water flow l/min*	Density (kg wet wt/m^2) *Neoagardhiella*	*Gracilaria*
Mar 20-Apr 15	37	12	48	3.9	2.8
Apr 15-May 6	14	8	48	9.5	3.9
May 6-May 20	21	7	72	8.9	5.5
May 20-May 28	11	2	72	12.3	6.4
May 28-Jun 5	27	7	72	10.1	8.1
Jun 5-Jun 12	16	7	72	11.5	8.4
Jun 12-Jun 27	34	9	96	8.2	10.2
Jun 27-Jul 16	75	13	138	5.5	5.9
Jul 16-Aug 14	58	10	96	6.6	6.3
Aug 14-Aug 27	81	14	48-144	5.9	6.5
Aug 27-Sep 18	-	-	48-144	4.2	4.0
Sep 18-Oct 6	-	-	48-144	3.1	2.6
Oct 6-Nov 7	-	-	70	2.6	1.7
Nov 7-Nov 21	44	8	72	3.1	2.8
Nov 21-Dec 19	37	5	60-80	2.0	2.2
Dec 19-Feb 20	40	6	60-80	3.0	-
Feb 20-Mar 19	-	-	60-80	2.9	-

*48 l/min = 3 exchanges/day

Table II. Comparative dry weight productivity values for cultivated seaweed crops.

Crop	Location	Annual Production t/ha	Culture Method	Reference
Neoagardhiella	Massachusetts, USA	46	Raceway	This study
Gracilaria	Massachusetts, USA	28	Raceway	This study
Gracilaria	Florida, USA	46	Tank	11
Hypnea	Florida, USA	39	Tank	11
Iridaea	Washington, USA	20	Tank	31
Gracilaria	Taiwan	7-12	Pond	28
Gracilaria	Taiwan	2	Net	21
Gracilaria	Japan	0.4-1.3	Bay	12
Eucheuma	The Philippines	13	Net	21
Gelidium	Japan	1.5	Stones	21
Gelidium	Korea	1.4	Stones?	6
Porphyra	Japan	0.3-3.0	Net	21,33

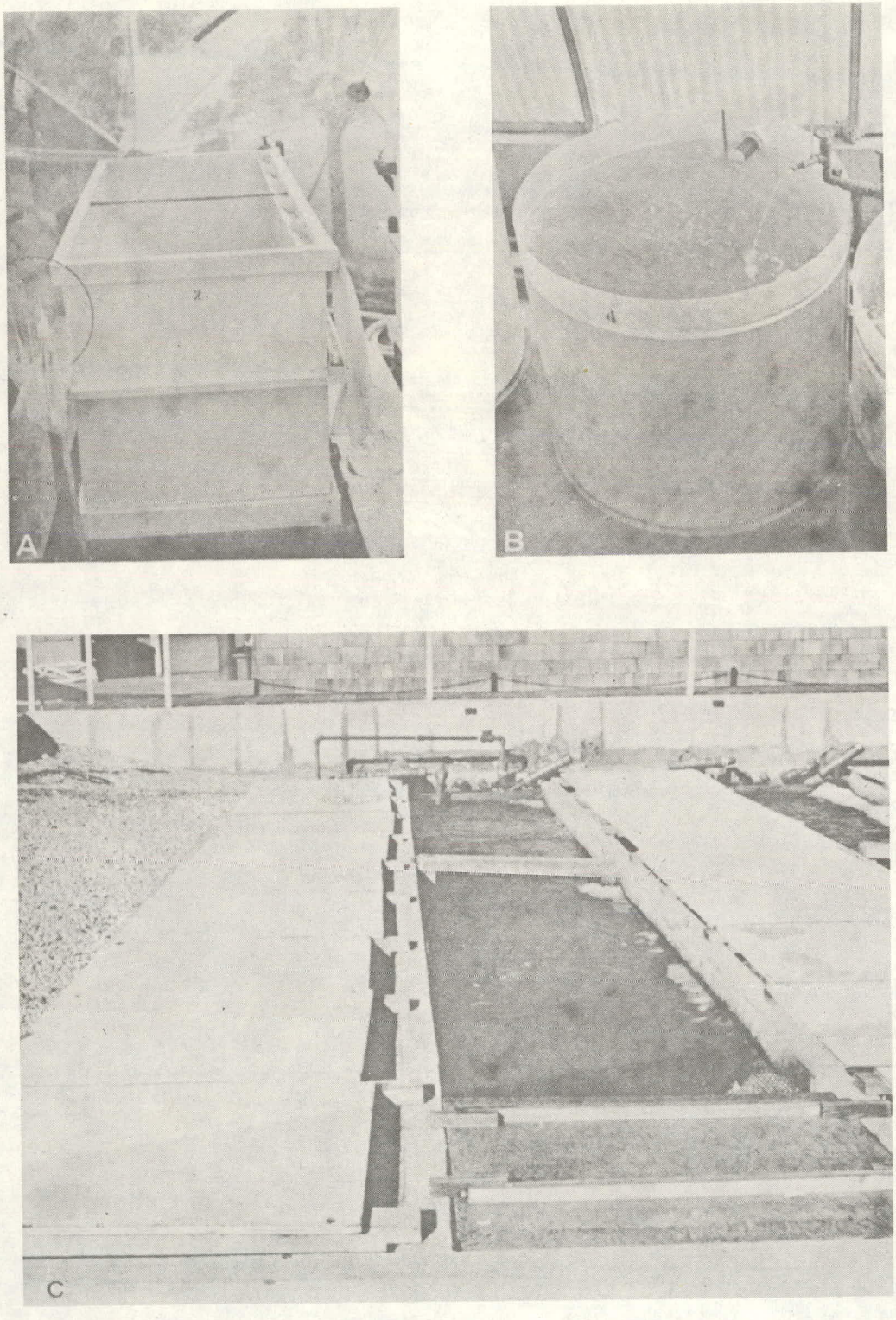

Figure 1. Enclosures used in the cultivation of seaweeds at the Woods Hole Oceanographic Institution. A - Rectangular plywood tanks. B - Circular fiberglass tanks. C - Concrete raceways.

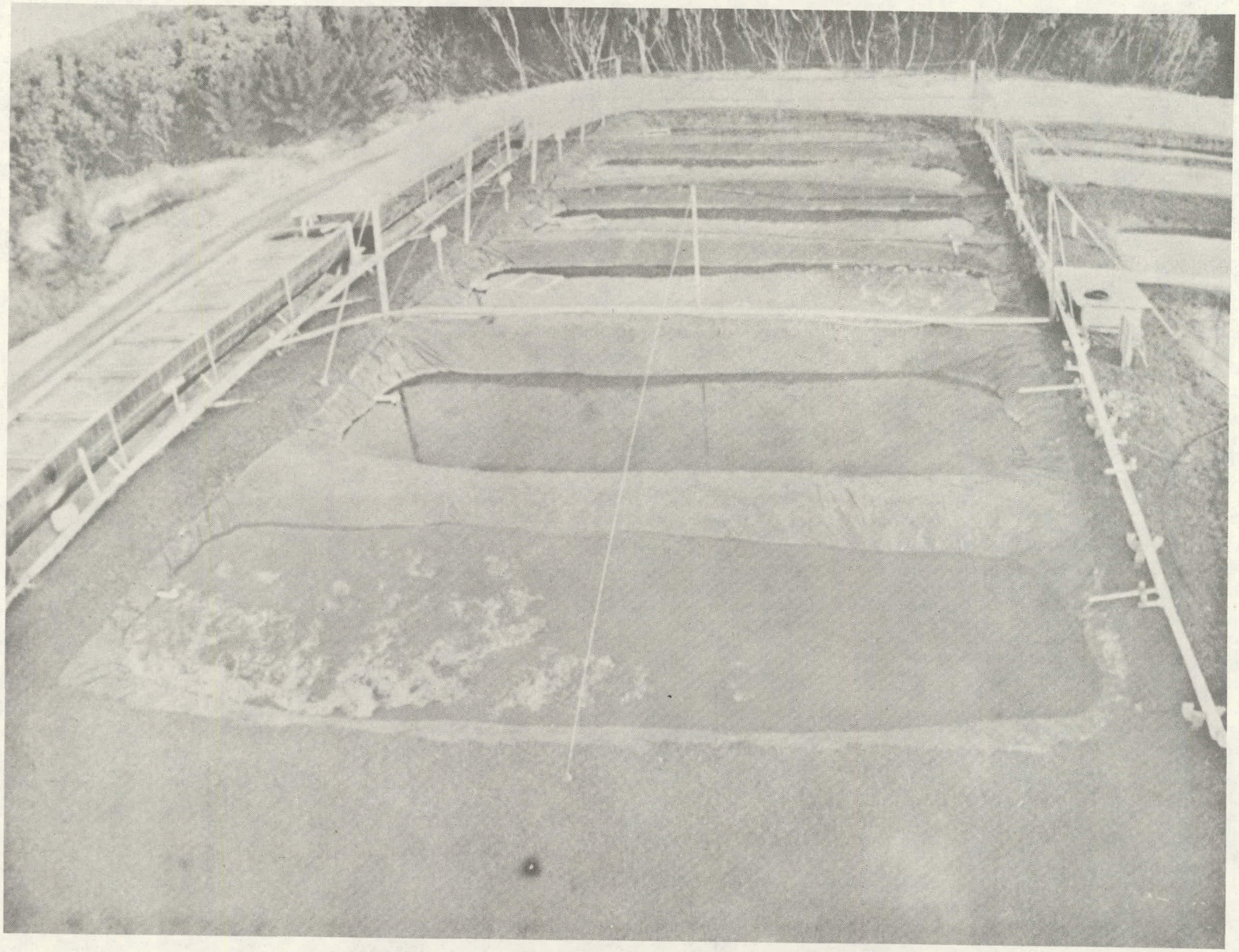

Figure 2. Aluminum raceways (upper left) and PVC-lined ponds (right) used to cultivate seaweeds at the Harbor Branch Foundation in Ft. Pierce, Florida.

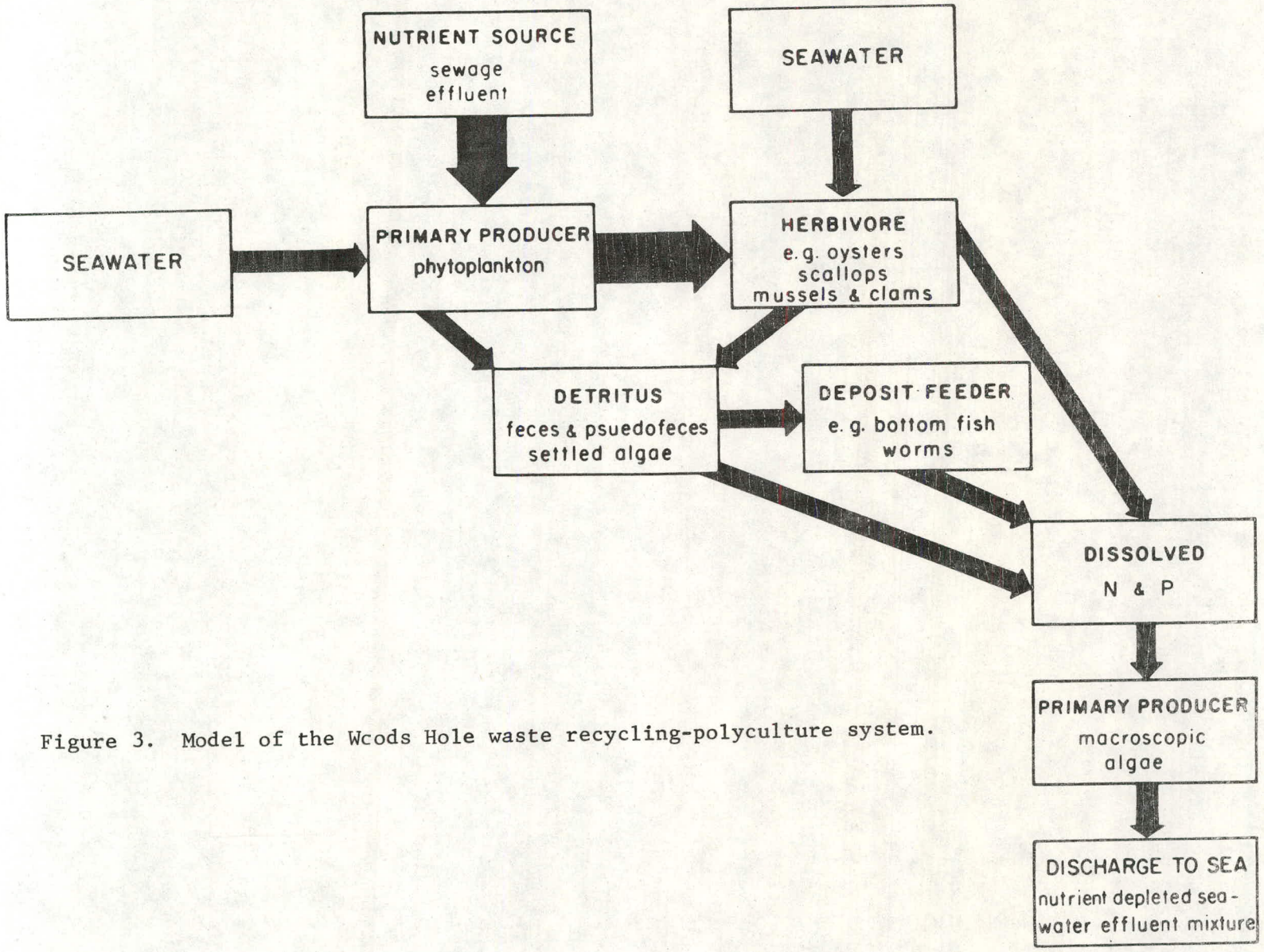

Figure 3. Model of the Wcods Hole waste recycling-polyculture system.

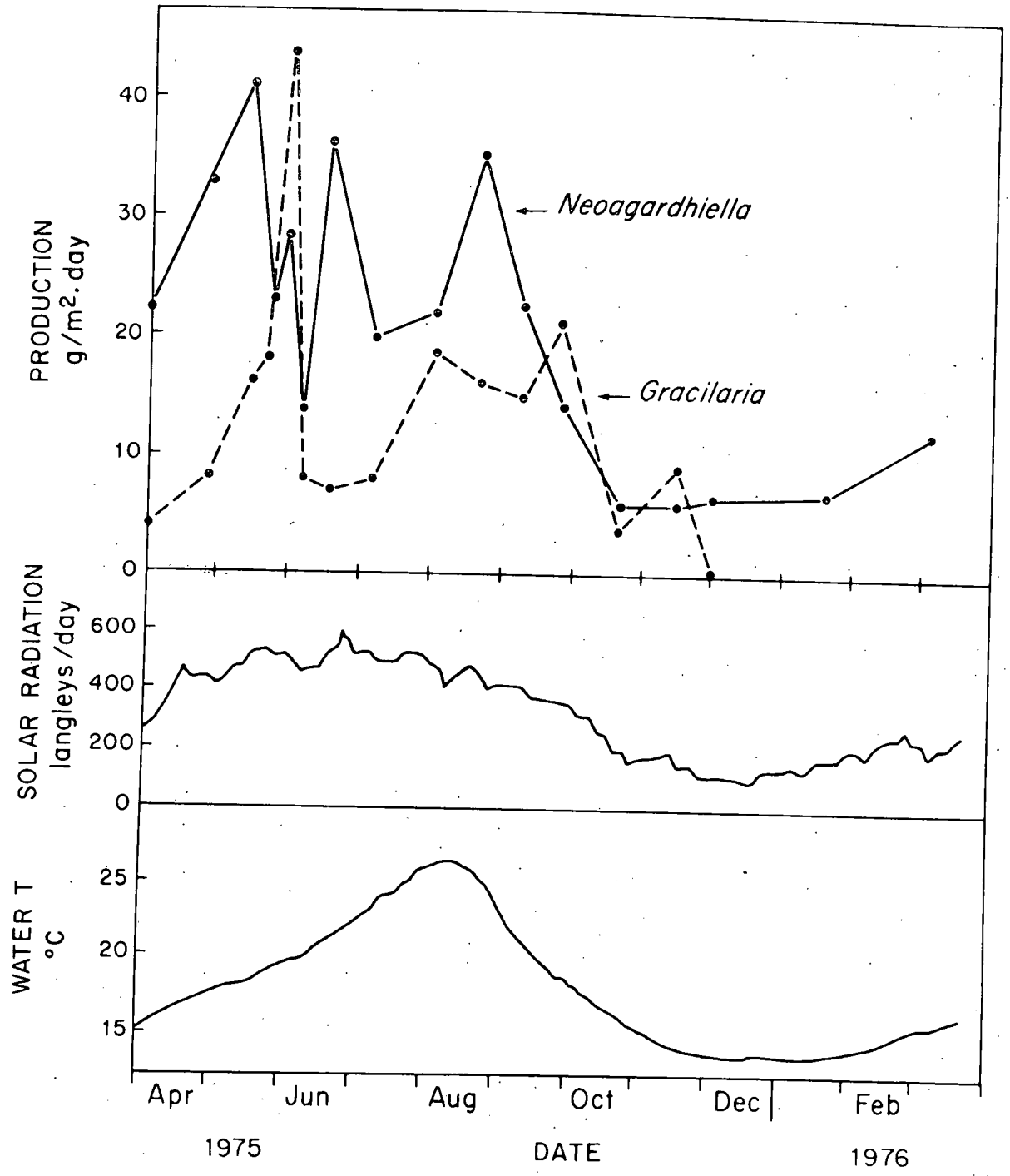

Figure 4. Production rates of seaweeds grown in raceways during 1975-76.

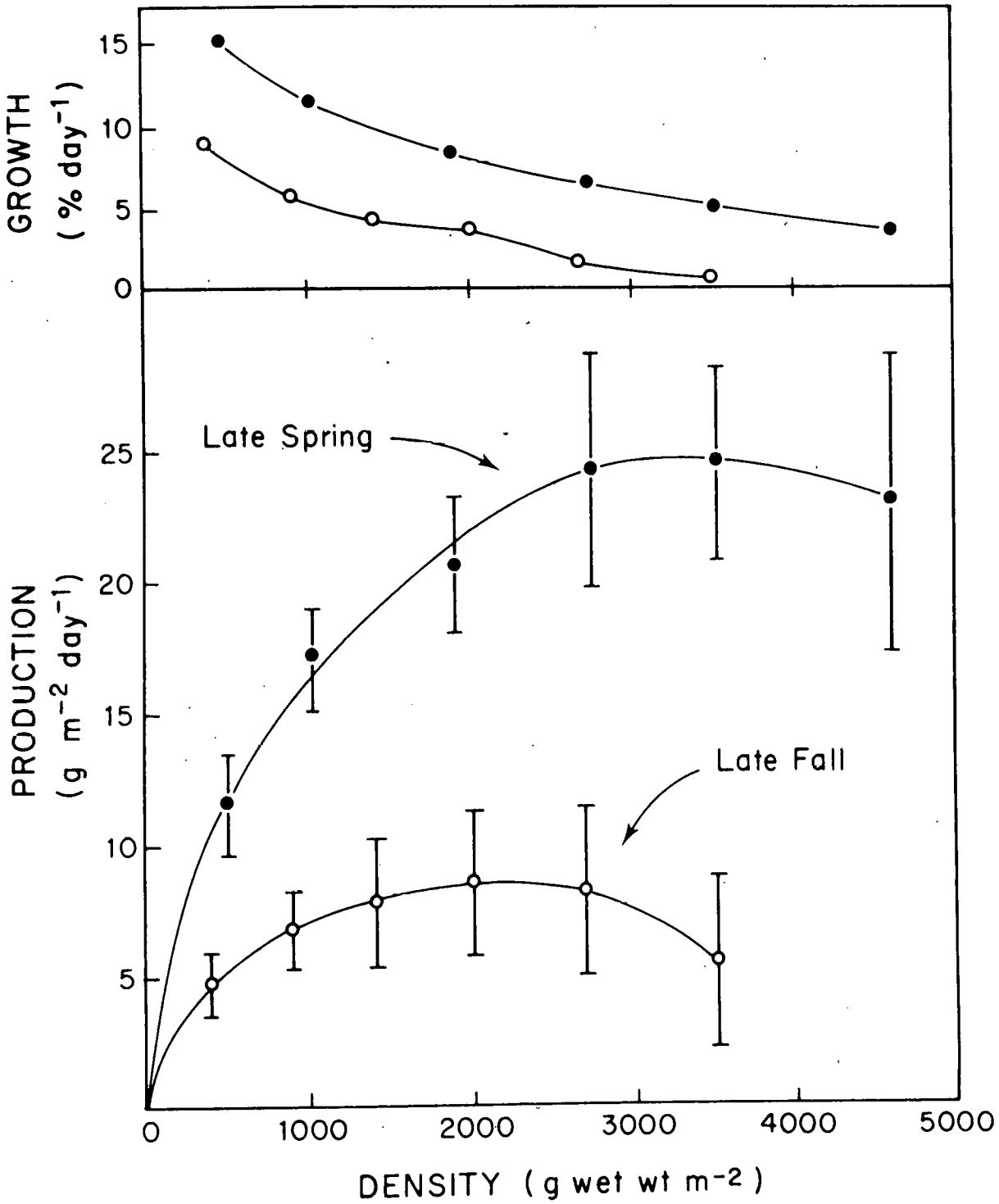

Figure 5 Growth and production rates of Gracilaria as a function of culture density.

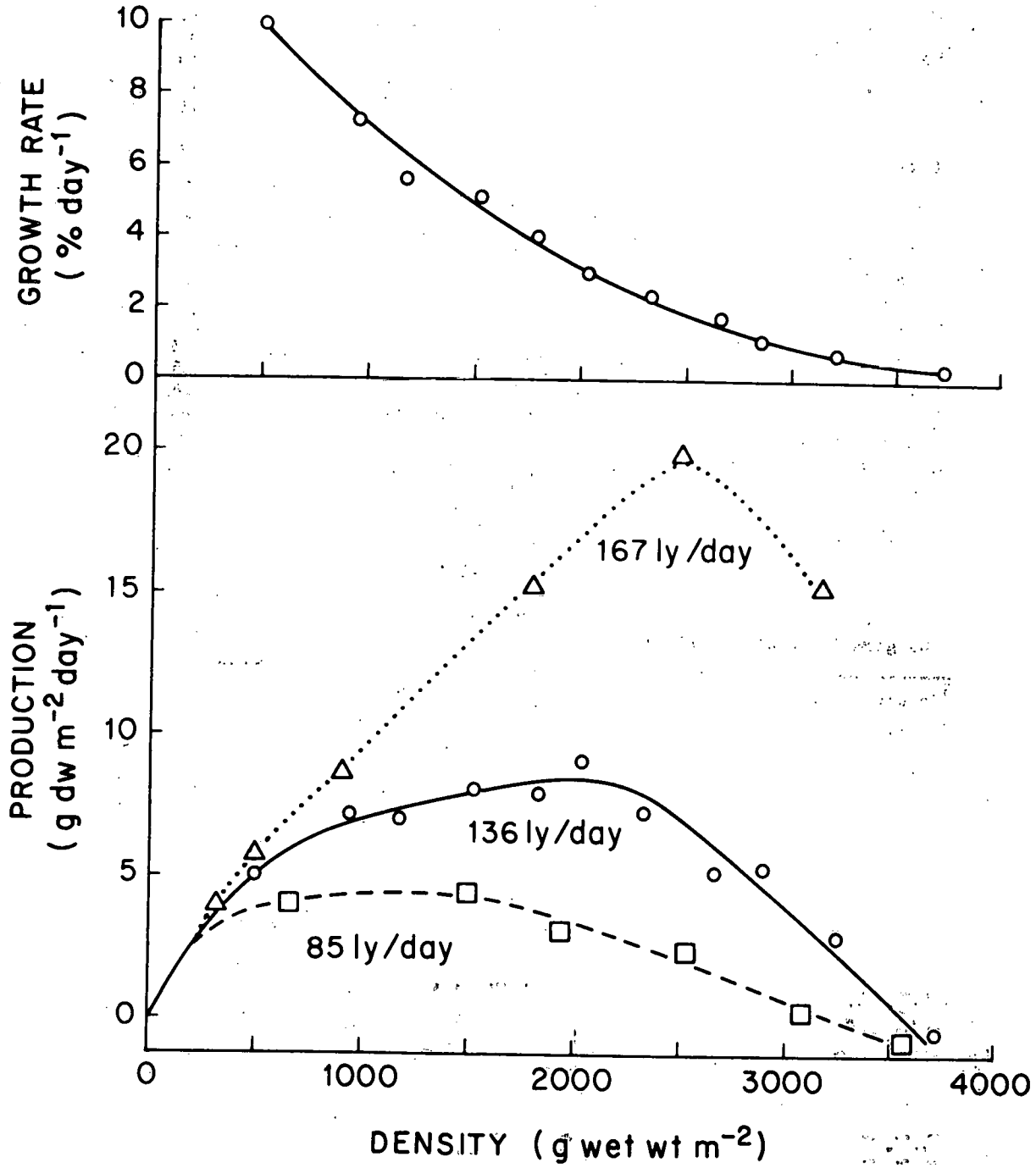

Figure 6. Growth and production rates of Neoagardhiella as a function of culture density.

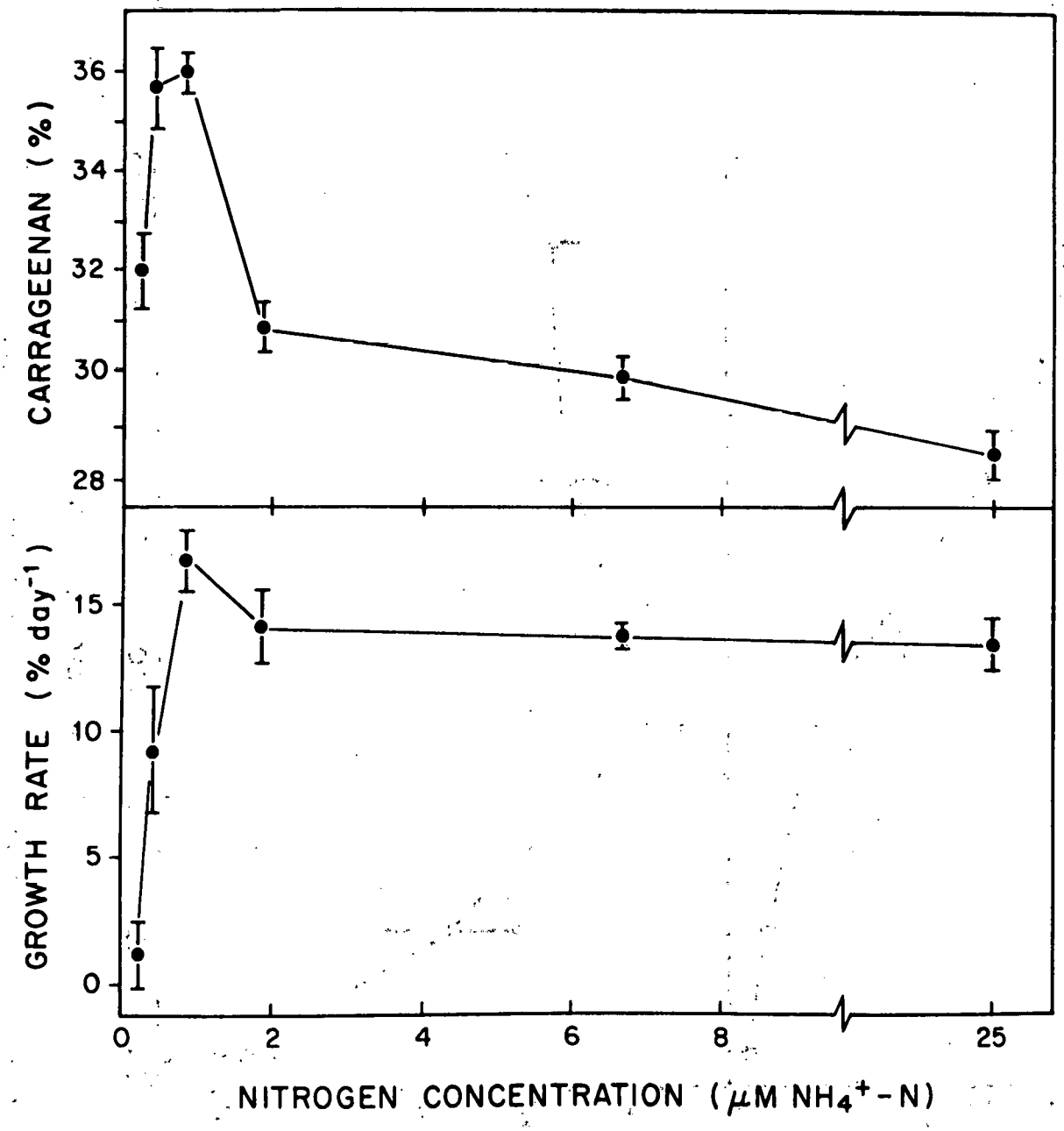

Figure 7. Effects of nitrogen enrichment on the growth rate and carrageenan content of Neoagardhiella.

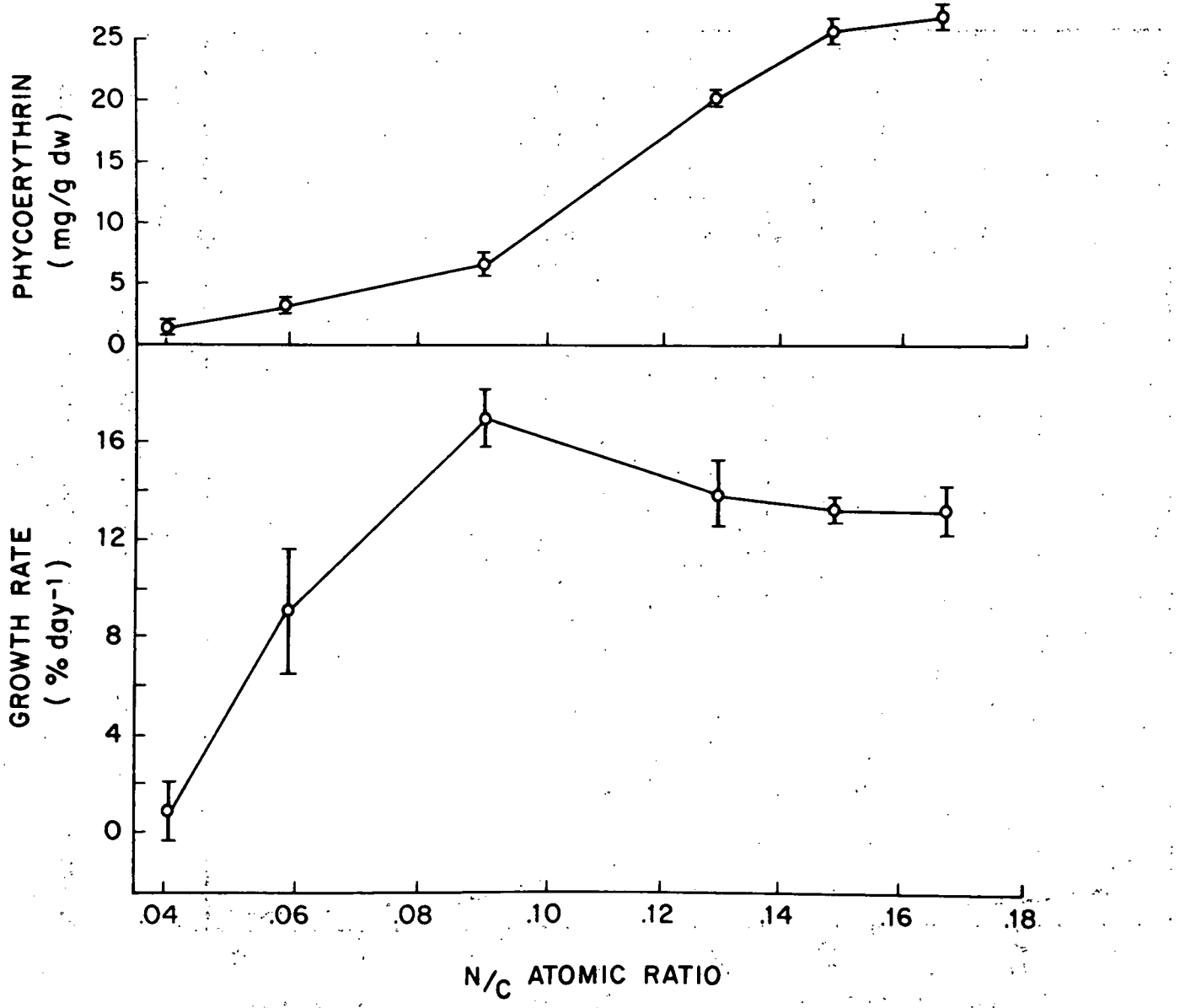

Figure 8. Phycoerythrin content and growth rate of Neoagardhiella as a function of the nitrogen:carbon ratio (by atoms) of the plants.

Cultivation of seaweeds as a biomass source for energy*

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Introduction:

The cultivation of photosynthetic crops for the specific purpose of converting their biomass to fuel is a new and, as yet, untried concept. The basic technology for such an undertaking is, of course, available in agriculture and the related fields involved in the production of food and fiber. However, the monetary value of plants grown for such purposes is an order of magnitude greater than their potential value as fuel (Greeley, 1976), even if all of their stored energy were recoverable. In view of the increasing world demand for food and fiber, it therefore seems unlikely that crops presently in production will find competitive use as an energy source in the foreseeable future, with the exception of those portions of cultivated plants that are not now utilized and are currently treated as waste.

It follows, then, not only that species not presently cultivated must be grown for this new purpose, but also that they must be grown in areas that are not suitable for the cultivation of food and fiber crops. Further, and most important, they must be produced within the framework of an entirely new budgetary concept, one that is based upon an energy rather than a financial balance sheet. The latter is, of course, always an important factor, particularly when alternative uses of the product (as discussed above) or the land used to grow it are considered. But costs and values of such commodities as food and energy change so rapidly that long-range

projections of the economic feasibility of energy plantations are at best tenuous.

What is incontestable, however, is the fact that crops grown as an energy source must, in their cultivation, harvest, processing, and conversion to fuel, consume less energy than they are capable of producing. Furthermore, the energy cost accounting must extend back to include the production of capital equipment, machinery, and fertilizer as well as the direct operational budget if the concept is to have any validity. Such an energy cost accounting has been done for U.S. corn production by Pimentel et al. (1973), who found an energy output:input ratio of 2.82 in 1970. If the entire plant were considered rather than the edible portion only, which is more relevant to the subject of energy conversion, a ratio between 5 and 9 is obtained (UK-ISES, 1975). However, of the energy contained in the plant biomass, only about half is recoverable through fermentation to methane with the technology of today, and the conversion itself is an energy-consuming process. Whatever the final net accounting, it is clear that an energy output:input ratio greater than one is necessary for a viable operation and the greater the ratio, the more attractive the concept becomes. In that connection, it would seem likely that a new plant cultivation technology will need to be developed for energy production, for the improved food yields of modern agricultural technology in recent

years have been accomplished only at the cost of even more rapidly decreasing energy output:input ratios (Pimentel et al., 1973; Pain and Phipps, 1975). In conclusion, then, it would appear that the new concept of energy plantations must involve new species of plants not now in cultivation on new areas unsuitable or marginal for food and fiber production and a new culture technology aimed at achieving the maximum productivity at the least possible expediture of energy.

It is proposed here that the macroscopic marine algae or "seaweeds" are particularly well suited to this purpose. These plants have been selected because (1) they are known or believed to be capable of exceptionally high levels of biological productivity and high organic yields per unit of time and space and (2) they grow naturally in existing coastal waters and wet lands and are capable of growing in simple impoundments on land areas that are incapable of supporting other crops used for food or fiber and for which there is little or no other existing or projected use.

Seaweed culture is a relatively new field with the exception of some rather primitive practices carried out in Asia. Few quantitative measurements of growth or yield have been made or documented in the literature. The present state of the art is reviewed briefly in the following section.

Current status of seaweed culture:

A rather simple method of cultivation of seaweeds has been practiced for many years in the Orient, where several species of these algae are used directly as food - among others, Porphyra (nori or laver), Undaria (wakami), Enteromorpha or Monostroma (aonori) in Japan and Laminaria (kelp) in China. Cultivation consists of growing the large, sporophyte plant from microscopic spores that are seeded onto twine nets on wooden frames, suspended ropes, or otherwise attached to other substrata in estuaries or sheltered coastal embayments (Bardach et al., 1972; Cheng, 1969). The seaweeds must be continually "weeded" by hand to remove epiphytes and the Chinese sometimes fertilize the surrounding water by spraying or allowing nutrients to seep from porous earthen jugs suspended on the culture ropes. A great deal of care and sophistication is also involved in the spore production phase of the operation, usually done in laboratories or "hatcheries" under controlled conditions.

It is difficult to determine yields from the scanty publications (in English) describing the above practices, but all indications are that they are not great - considerably less than one metric ton (dry wt) per hectare per year on the average. Furthermore, the processes are extremely labor intensive. Presumably these factors are not serious deterrents in the current social-economic setting of China, where agriculture is practiced in much the same way. In the more highly developed and industrialized society of Japan, such methods

of seaweed culture are possible only because of the high price of the dried product - $25 or more per kg.

In addition to their scattered, low-value use as cattle fodder, fertilizer, mulch, etc., seaweeds are utilized in the Western World primarily for their contained hydrocolloids. These substances are used as emulsifiers and stabilizers in a wide variety of foods, drugs, cosmetics, and other products. Different colloids with different properties and application are obtained from different species of seaweeds, the most commonly employed being algin (from the kelps) agar (from Gelidium and certain other red algae) and carrageenan (from Chondrus, Gigartina, and another group of the red algae). Traditionally, these seaweeds have been harvested from natural populations, dried, and shipped to factories in North America or Western Europe, where the hydrocolloids are extracted, refined, and sold to users. The principal source of agar, for example, has been Gelidium, exported from Japan to the rest of the world. Most of the algin has come from kelp beds harvested from the West Coast of the United States. Most of the world's supply of carrageenan comes from Chondrus populations in Eastern Canada and to a lesser extent New England and Northern Europe.

The above seaweed resources are limited in area and are now heavily exploited. At the same time, the demand for hydrocolloids has steadily increased. Furthermore, the industry has become considerably more sophisticated with the discovery that different chemical

species or blends of hydrocolloids from different seaweeds have different jelling or emulsifying properties that are appropriate for various different applications. These factors have together led to screening of different species and world-wide surveys of seaweed resources by the industry over the past decade or more, in an attempt to expand the base of its operation. One example of such expansion is the relatively new exploitation of the red alga, Eucheuma from the Philippines and other parts of Southeast Asia. From there it is shipped to the United States for extraction of its colloid, iota-carrageenan, for which special applications have been developed.

All of the above natural resources, old and new, have now become over-exploited to the extent that the industry in general is resource limited and attention has become focused on cultivation as the only viable long-term solution. A rather primitive form of seaweed farming for Eucheuma has already been developed in the Philippines (Doty, 1973; Parker, 1974), using a net-culture technique that is similar in many respects to the cultivation of edible seaweeds in Japan discussed above. Although highly labor intensive, the system appears very promising, with dry-weight yields from pilot farm operations reported at 13 MT/ha/year and projected yields of 30 MT/ha/year (Parker, loc.cit.). Another example of commercial seaweed farming is the culture of the agar-containing red alga, Gracilaria spp., recently developed in Taiwan by growing the seaweed unattached on

the bottoms of ponds originally designed for fish culture (Shang, 1976). Although the practice is reportedly labor intensive (i.e., 50% of operating costs), it appears to be profitable, particularly when done in conjunction with animal culture in a polyculture mode of operation. Yields of Gracilaria from these culture practices are reported as 9-10 tons/ha/year dry weight.

Other than the above few examples, there are no commercial seaweed culture operations presently in existence, but a large number of research projects on seaweed culture have evolved in the past decade, mostly in the past five years.

The giant kelp, Macrocystis pyrifera, is one of the most important resources of the California Coast, not only as the world's major source of algin, but also as the dominant species and habitat of the local ecosystem. Deterioration of the kelp beds over the past several decades from pollution, predation, or both, has been a matter of major concern. This has recently led J. Wheeler North and his associates (Cal. Inst. of Technology) to attempt rehabilitation of the kelp beds by cultivation techniques, basically the mass rearing of sporlings which are "seeded" in the environment to be repopulated (North, 1974). More recently, North and H. A. Wilcox (Navy Undersea Center, San Diego) have initiated a more ambitious kelp-farming operation which is planned to include an artificial upwelling system to provide the plants with cold, nutritent-rich waters (Jackson and North, 1973) and which has, as a major objective, the production

of organic matter as an energy (i.e., methane) source. It is too early to evaluate the results of this new project. Estimates of the productivity of natural kelp beds are of the order of 5 tons/ha/year (W. J. North, personal communication).

Starting at about the same time as North's kelp cultivation project (late 1960's), a group at the Canadian NRC Atlantic Regional Laboratory, Halifax, N.S. under A. C. Neish initiated research on the culture of Chondrus crispus. Here the approach was to grow the seaweeds unattached, without holdfasts, in suspension in tanks with flowing seawater in a greenhouse. Neish was highly successful with this approach, among other things isolating a strain of Chondrus (T-4) characterized by rapid growth and high carrageenan content, and he made significant progress over the following five-year period (Allen et al., 1971; Neish and Shacklock, 1971; Shacklock et al., 1972, 1974).

Following Neish's lead, two commercial seaweed companies, Marine Colloids, Inc. of Rockland, Maine and its Canadian subsidiary and Genu Products Canada Ltd. (a subsidiary of a Danish firm now owned by Hercules Corp., Wilmington, Del.) have started pilot Chondrus culture projects in Nova Scotia, with partial support from the Canadian Government, using different modifications of Neish's basic technique (unattached plants grown in suspended culture). Both of these pilot projects are in their very early stages (1-2 years) and only the most preliminary results are available. One of the major objectives

of the Marine Colloids project is to screen samples collected from a large number of natural Chondrus beds in an attempt to select improved strains with respect to growth, carrageenan content, and other favorable characteristics. Problems encountered by both projects include (1) control of algal contaminants (e.g., Ulva, Enteromorpha, Ectocarpus) in the culture system and (2) slow growth of the Chondrus.

During roughly the same period (i.e., 1970 to the present) a number of phycologists have conducted relatively small scale experiments in which the photosynthesis, growth, hydrocolloid content, and other characterists of Chondrus crispus (Prince and Kingsbury, 1973a, 1973b) and of other commercially-valuable red algae have been investigated (Dawes et al., 1974a, 1974b; Matthiesson and Dawes, 1974; Fralick and Matthieson, 1975; Waaland, 1973). The latter studies were partly funded by the seaweed industry with the objective of developing techniques for cultivation of carrageenan-containing species other than Chondrus. As an outgrowth of that research, Marine Colloids Inc. initiated a modest pilot experiment in the Florida Keys looking at the potential cultivation of Eucheuma spp., Hypnea musciformis, and other tropical and semitropical red algae, and has supported a similar effort in the cultivation of Iridaea and Gigartina, temperate Pacific species, in the Washington State area.

Beginning also about 1970, a project was started at the Woods Hole Oceanographic Institution under the direction of the senior author in which a waste recycling-marine aquaculture system was developed. Secondary sewage effluent, mixed with seawater, was used to grow unicellular algae and the algae were fed to oysters, clams, and other bivalve molluscs. The algae removed the nutrients (primarily nitrogen) from the wastewater and the shellfish removed the algae, the combined system providing a tertiary sewage treatment (nutrient removal) function as well as a crop of commercially-valuable marine organisms. However, metabolism of the bivalves and other animals in the aquaculture system resulted in remineralization of a portion of the nutrients contained in their food, and N and P were returned to the water through excretion of the animals and decomposition of their solid wastes. This phenomenon necessitated the addition of a final "polishing" step to the system to remove the regenerated nutrients, and this stage consisted of seaweeds grown in suspended culture. Initially Chondrus crispus was used in these experiments, including the T-4 strain obtained from Halifax. However, Chondrus grew slowly and became heavily overgrown with other species and it was subsequently replaced by other warm-water species that appear as summer annuals in the Woods Hole region. These have included Neoagardhiella baileyi, Gracilaria foliifera,

and Hypnea musciformis. Results of the Woods Hole waste recycling aquaculture experiment are summarized in Ryther (1976, 1977).

A similar experiment was initiated at St. Croix, Virgin Island by O. A. Roels and others involving the pumping of deep, nutrient-rich ocean water, in place of sewage effluent, as the basis for a unicellular algae-bivalve mollusc aquaculture system (Babb et al., 1972). Seaweeds have recently also been added to that system to remove wastes produced by the molluscs (Roels et al., 1975), but data are not yet available for that part of the program.

So successful was the growth of seaweeds, especially Neoagardhiella and Gracilaria, in the Woods Hole experiment that a separate project was initiated in which the seaweeds alone were grown in mixtures of sewage effluent and seawater as a one-step waste recycling-aquaculture system. In addition, seaweed culture experiments were started by the present authors in 1973 at the Harbor Branch Foundation, Ft. Pierce, Florida. Some of the results of the latter research are summarized in the following section.

Recent studies in the culture of Gracilaria foliifera[1]:

The specific growth rate of an alga is determined by its photosynthetic efficiency and may be considered as a biological constant if light, nutrients, CO_2, temperature, and other controlling factors are held at optimal levels. However, specific growth, expressed as increase per unit weight and time or percent increase of weight per unit of time, should not be confused with productivity or yield of an algal population, the production of organic matter per unit area and time, which is the derivative of the specific growth rate and the density or biomass of the seaweeds per unit area.

Because of self-shading and perhaps other factors, growth rate is highest at very low densities and decreases at a rate that is inversely proportional to density. As a result yield expressed as a function of density describes a bell-shaped curve with a maximum at some intermediate density that is both sufficient to insure maximum utilization of incident solar radiation and not too high to cause a loss of production from self-shading or other factors. The "other factors", such as CO_2, mineral nutrients (N, P), or inhibitory metabolites, may presumably be reduced or eliminated by increasing the flow of seawater and its contained nutrients through the seaweed populations, but nothing can be done practically in a commercial culture system to remove the limitations of natural solar radiation, the cost of artificial illumination being prohibitively expensive.

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[1] From Lapointe and Ryther (in preparation).

The objective of a seaweed culture system, therefore, is to maintain all other factors at optimal levels so that productivity is limited by incident solar radiation alone. It also follows that such other factors as water flow and nutrient concentrations should not exceed their optima, both because they may be inhibitory at higher levels but also because they represent cost factors that should be kept as low as possible without affecting yield.

The first step in designing a seaweed culture system, then, is to determine emperically the optimal density or biomass of algae and the optimal flow of seawater and concentration of nutrients to achieve maximum yields at a given input of incident solar radiation. To determine these operating parameters, a simple experimental system was developed at the Harbor Branch Foundation (Fort Pierce, Florida). This unit consisted of four 6-meter long, 0.4 m diameter PVC pipes that were longitudinally sectioned and divided into 0.75 m (50 liter) compartments by means of plywood sections. Each section was provided with a calibrated flow of enriched seawater by means of a manifold fed from a head box, and each section was also provided with a non-clogging over flow drain. Compressed air is fed into the bottom of each compartment through holes drilled along the bottom of the pipe connecting to an airline (a sectioned two-inch PVC pipe) cemented to the outside of the main pipe. Thirty-two individual growth assay chambers were produced in this way (Figures 1, 2).

The growth chambers were located out-of-doors in full sunlight.

Seawater was taken from the Harbor Branch Foundation ship channel which connects to the Indian River, a shallow lagoon of the Atlantic Ocean. No attempt was made to control water temperature, which ranged from 14°-30°C, or salinity, which ranged from 23-34°/oo, in the incoming seawater during 1976. Seawater was pumped into a reservoir tank holding several days supply for the experimental chambers. Prior to its use, the stored seawater was enriched with the desired concentrations of nitrogen and phosphorus, normally provided as sodium nitrate and monosodium (dibasic) phosphate at a ratio of 10:1 by atoms of N:P. A second reservoir contained unenriched seawater. Both enriched and unenriched seawater supplies were pumped to headboxes from which they were distributed to the experimental chambers at flow rates and mixtures to provide the desired rates of exchanges and nutrient concentrations.

Weighed amounts of seaweed were stocked in the experimental chambers to give the desired density (g/m^2). At intervals of 5-10 days, depending upon growth rate, the algae was removed from the chamber, shaken vigorously to remove water, and weighed. Establishment of the relationship between drained wet weight, dry weight, and ash-free dry weight (volatile solids), determined carefully on replicate samples of each species of seaweed (Table 1), permitted the expression of growth in any of these units from the wet weight measurements.

Growth and yield data have now been obtained for several species of seaweeds using the experimental system described above. The following discussions will be restricted to results with one species, the red

alga Gracilaria foliifera, which has proved to be the most productive and consistent form we have worked with to date.

The specific growth rates and yields of G. foliifera as functions of density or biomass of the seaweed are shown in Figure 3. These data are shown for both summer (August, 1976) and winter (January, 1977) conditions. At both times of year, growth rate decreased with increasing biomass, though both the absolute values and the range were much greater in summer than in winter. Yields (g dry wt /m^2·day) at both seasons peaked at densities of 2.0 - 3.0 kg wet wt /m^2 but with a rather broad plateau at both seasons and with some indication of a higher optimal density in winter than in summer. As would be expected, yields were higher, by a factor of about two-fold, in summer than in winter. The difference is believed to be due primarily to solar radiation but temperature may have had second order effects.

It is not possible to separate experimentally the effects of nutrient supply and flow rate of water or medium through a seaweed culture. If nutrient concentration is held constant, the rate of input of nutrients to the culture will vary proportionately with the flow rate. If, on the other hand, nutrients are added separately at a constant input rate while seawater flows are independently varied, the instantaneous concentration of nutrients entering the culture will be inversely proportional to seawater flow rate. To date, only the latter experiment has been conducted: i.e., flow rates were varied from 1 to 25 culture volume exchanges per day (35 to 870 ml/min through the 50-liter cultures) while holding the

input of nutrients separate and constant. An unenriched seawater "control" experiment was conducted at the highest flow rate (25 volumes/day). Gracilaria was stocked in the experimental chambers at a density of 2 kg/m^2 (wet wt) and the cultures were weighed and the incremental growth removed at intervals of 3-7 days over the one-month experimental period (October 6 - November 9, 1976).

The results of that experiment are shown in Figure 4. Algal yield ($g/m^2 \cdot day$) increased almost linearly with increasing flow rate up to and including the maximum employed (25 volumes/day). Although influent nutrient levels ranged from about 300 and 30 µmoles N and P respectively at the lowest flow rate to roughly 20 and 2 µmoles/ℓ respectively at the highest flow rate, the amount of nitrogen removed per day was remarkably constant at all flow rates and concentrations, as was the C:N ratio in the seaweeds themselves (i.e., as determined in dried material with a Perkin-Elmer Model 240 Elemental Analyzer). Nitrogen uptake thus appears to have been determined by total daily input and not by concentration or flow rate per se. However, the nutrient concentration of the unenriched seawater "control" was apparently insufficient, even at the high flow rate of 25 volumes/day. The seaweed could remove from the water only 20-25% as much nitrogen per day as in the enriched seawater, its C:N ratio increased significantly, and its growth fell sharply below that of the other, enriched cultures.

From the above experiment, it would appear that above some minimal nitrogen level, the growth of Gracilaria is independent of nitrogen con-

centration but strongly dependent upon flow rate. It should be emphasized that the minimal concentration is greater than the 4.4 μmoles/l present in the unenriched seawater at the time of the experiment, which in turn is greater than the total inorganic nitrogen concentration that is often present in surface coastal ocean waters. In other words unenriched seawater, no matter how rapid its rate of flow through the culture, may seldom have a high enough nutrient concentration to support even moderately high yields of seaweed.

The apparent dependence of growth and yield upon flow rate of seawater through the culture should not be interpreted as a cause-and-effect relationship. Flow rate affects many other factors that may influence growth. One example is pH, as shown in Figure 4. A more important factor is CO_2 tension and availability to the seaweed, which is inversely proportional to pH. Rate of removal of toxic or inhibitory metabolites of the seaweeds may be important. Finally, in the open and uncontrolled culture system used, the slower the water exchange, the greater the diurnal temperature fluctuation and, in rainy weather, the more variable the salinity of the cultures.

Clearly, the whole question of water exchange and nutrient concentration in flow-through seaweed cultures is an extremely complex but critically important subject that needs much more research.

Gracilaria foliifera was grown continuously from July 20, 1976 to February 11, 1977 in the culture chambers described above, maintaining a culture of 2 kg/m^2 (wet wt) by weighing the seaweed at approximately

one-week intervals and removing the incremental growth. The flow rate was 22 culture volume exchanges per day of seawater enriched with ca. 15 μmoles/l NO_3^--N and 4 μmoles/l $PO_4^{\equiv}$-P. Yield of the seaweed in g dry wt/ m^2·day is shown in Figure 5 together with incident solar radiation (as measured with an Epply pyroheliometer) and mean daily seawater temperature of the culture.

The close correlation between Gracilaria yield and solar radiation between July and December is obvious, with even minor perturbations of the latter reflected by irregularities in the growth of the seaweed. However, the seasonal increase in radiation after the first of the year was not immediately accompanied by an increase in algal yield, presumably due to the abnormally low (<12°C) water temperatures that occurred in January, 1977. As both light and temperature increased in February, the algae responded with renewed acceleration of growth.

The time-weighted mean production of Gracilaria was 30.7 g (dry wt)/m^2/ day. Since the time period involved was just under seven months and extended from very near the summer solstice through the winter solstice, it may be assumed that the mean yield for that period of time would be very close to an annual mean productivity value.

The above yield is approximately twice that previously reported for Gracilaria and several other species of seaweeds grown earlier in Florida in similar but somewhat larger culture chambers (volume 350-600 liters) and with a flow rate of 5-8 volume exchanges/day of enriched seawater at nutrient concentrations ranging from approximately 50 to 150 μmoles NO_3^--N

and 10-50 μmoles PO_4-P per liter (Lapointe et al., 1976). The difference appears to confirm the hypothesis presented above that higher yields are achieved with rapid water exchange and relatively low nutrient enrichment (eg. Figure 4).

A mean yield of 30.7 g/m^2/day is equivalent to an annual dry weight yield of 112 metric tons/ha/year. In Table 2 this figure is compared with annual yields of G. foliifera and Neoagardhiella baileyi at Woods Hole, Massachusetts (DeBoer et al., 1977) and with some of the highest sustained agricultural yields reported in the literature (Cooper, 1975). The Florida yield of Gracilaria is significantly higher than any of the other reported values. Total dry weight yields, however, are not equivalent to yields of organic matter or volatile solids, which are only about 50% of the dry weight of Gracilaria (Table 1) in contrast to 75-95% of terrestrial plants (Westlake, 1963). Yet half the reported yield of Gracilaria in Florida compares favorably with the total dry weight yields of most of the crops shown in Table 2.

Caution must also be used in extrapolating the yields of small, experimental systems to large agricultural or aquacultural systems. Much of the agricultural yield data appearing in Table 2 was also obtained from small experimental plots, but their physical nature was undoubtedly very similar to that of the large-scale agricultural systems they were simulating. Such is unfortunately not true of the small, highly-intensive seaweed culture systems reported upon here. Yields from the latter may be looked upon as representing something that is perhaps close to the biological potential of the marine algae, but it is

very doubtful that such systems could find large-scale application that would be cost effective either economically or from an energy accounting point of view.

The obvious next step in the assessment of seaweed culture as a biomass source for energy is the development of a simple, non-intensive culture system that is energy cost effective while still capable of producing yields that at least approach those reported above.

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Table 1. Relationship between wet weight, dry weight, and volatile solids in six species of algae.

Species	Dry weight* % wet weight	Volatile solids** % dry weight
Gracilaria foliifera	11	51
Neoagardhiella baileyi	8	50
Hypnea musciformis	12	60
Chaetomorpha linum	10	52
Enteromorpha clathrata	16	65
Ulva lactuca	12	77

*Dried @ 90°C for 48 hours.

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

Table 2. Productivity of agricultural crops[1] and seaweeds[2,3] on an annual basis. Yields of total dry matter in metric tons/hectare.year.

Crop	Country	Yield
Temperate		
Rye Grass	U.K.	23
Kale	U.K.	21
Sorghum	U.S., Illinois	16
Maize	U.K.	17
	U.K.	5 (grain)
	Canada, Ottawa	19
	Japan	26
	U.S., Iowa	16
	U.S., Kentucky	22
Potato	U.K.	11
	Netherlands	22
Sugar Beet	U.K.	23
	U.S., Washington	32
Wheat (spring)	U.K.	5 (grain)
	U.S., Washington	12 (grain)
	"	30 (total)
Barley	U.K.	7 (grain)
Rice	Japan	7 (grain)
Seaweeds[2]	U.S., Massachusetts	
Neoagardhiella baileyi		63
Gracilaria foliifera		33
Sub-Tropical		
Alfalfa	U.S., California	33
Sorghum	U.S., California	47
Bermuda Grass	U.S., Georgia	27
Sugar Beet	U.S., California	42
Potato	U.S., California	22
Wheat	Mexico	18
	U.S., California	7 (grain)
Rice	Australia, NSW	14 (grain)
	U.S., California	22
Maize	Egypt	29
	U.S., California	26
Seaweeds[3]	U.S., Florida	
Gracilaria foliifera		127
Tropical		
Napier Grass	El Salvador	85
	Puerto Rico	85
Sugar Cane	Hawaii	64
Oil Palm	Mayaysia	40
Sugar Beet	Hawaii (2 crops)	31
Cassava	Tanzania	31
	Malaysia	38
Sorghum	Philippines	7 (grain)
Maize	Thailand	16
	Peru	26
Rice	Australia, NT	11 (grain)
	Peru	22
Rice + Sorghum (multiple cropping)	Philippines	23 (grain)

[1] Cooper, J. P. (1975)

[2] Ryther et al. (1977)

[3] This study.

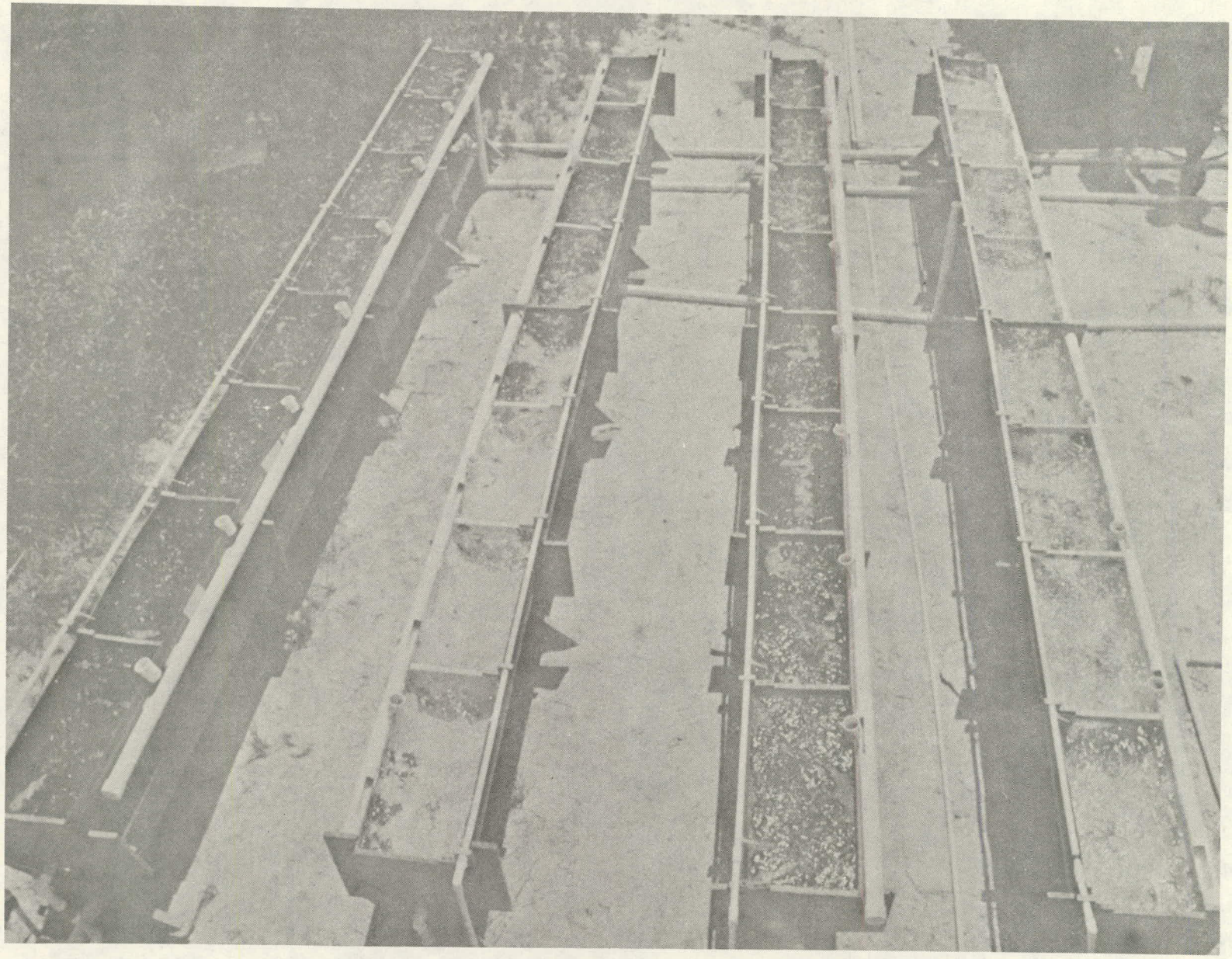

Figure 1. Experimental culture troughs for screening growth of seaweeds.

Figure 2. Close-up of seaweed screening troughs showing headbox for medium supply.

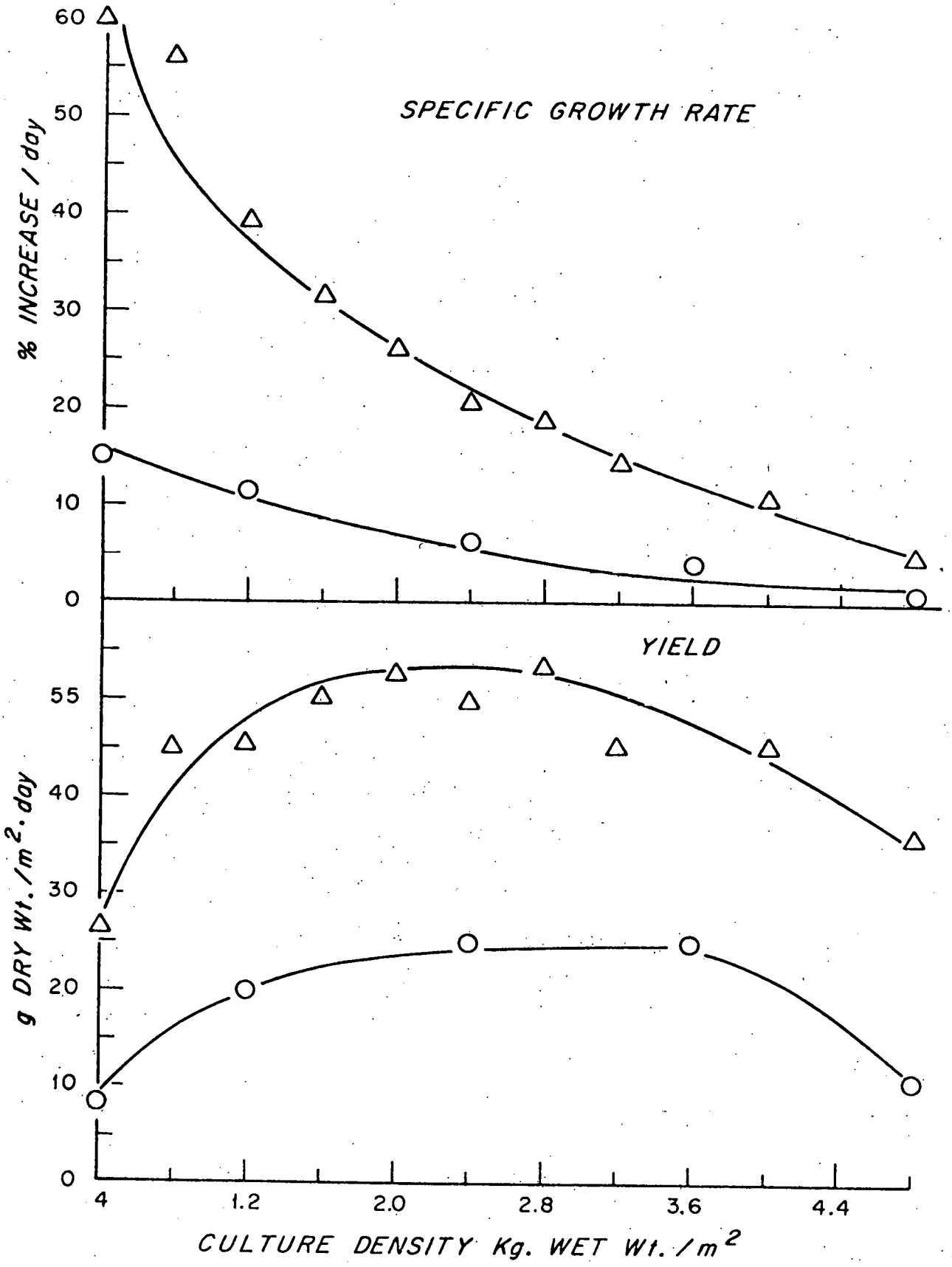

Figure 3 Effect of Culture Density on Production and Specific Growth of Gracilaria in Summer (△) and Winter (○).

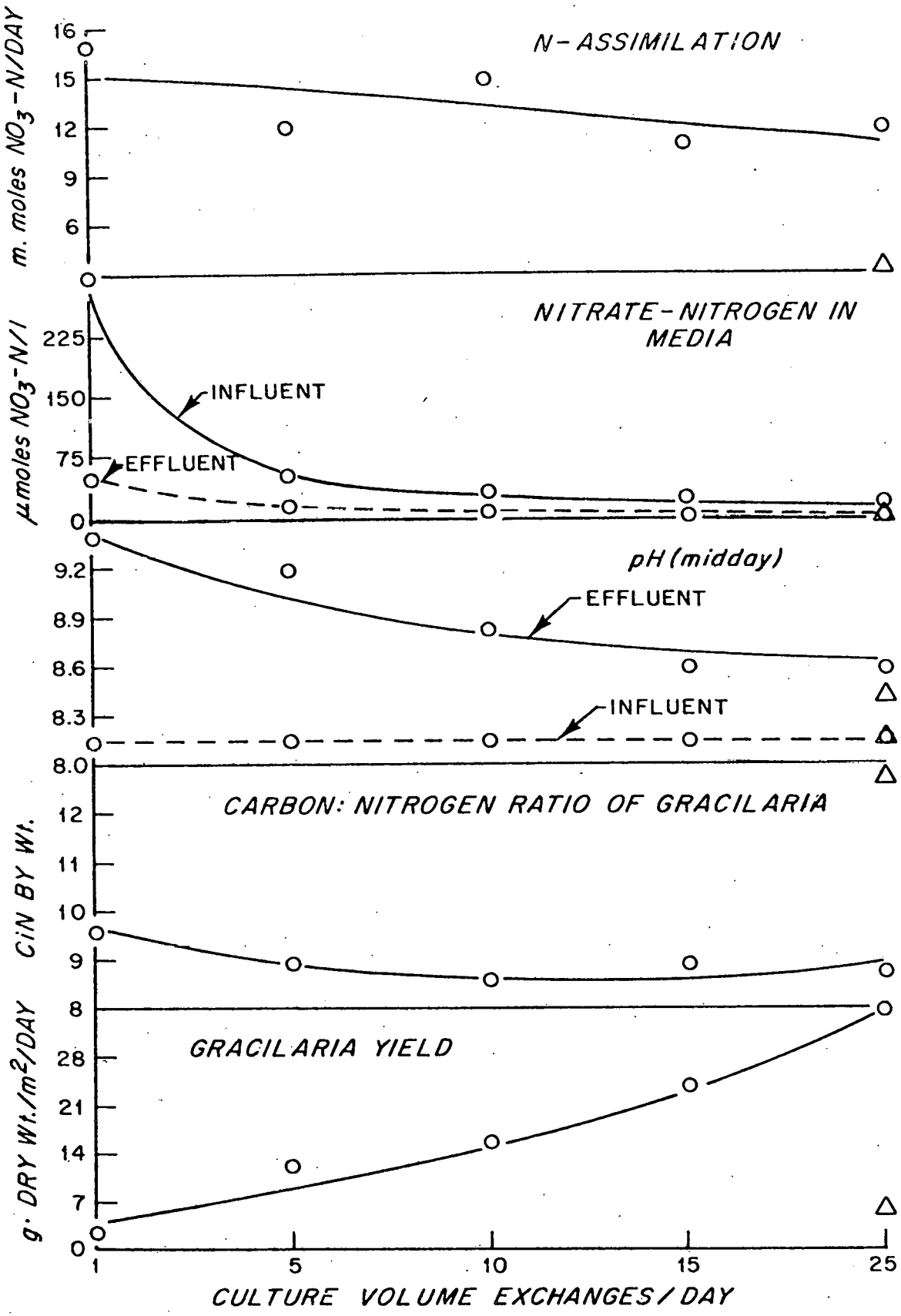

Figure 4 Effect of Culture Volume Exchange Rate on Growth and Nitrogen Uptake by Gracilaria in Enriched (O) and Unenriched (△) Media.

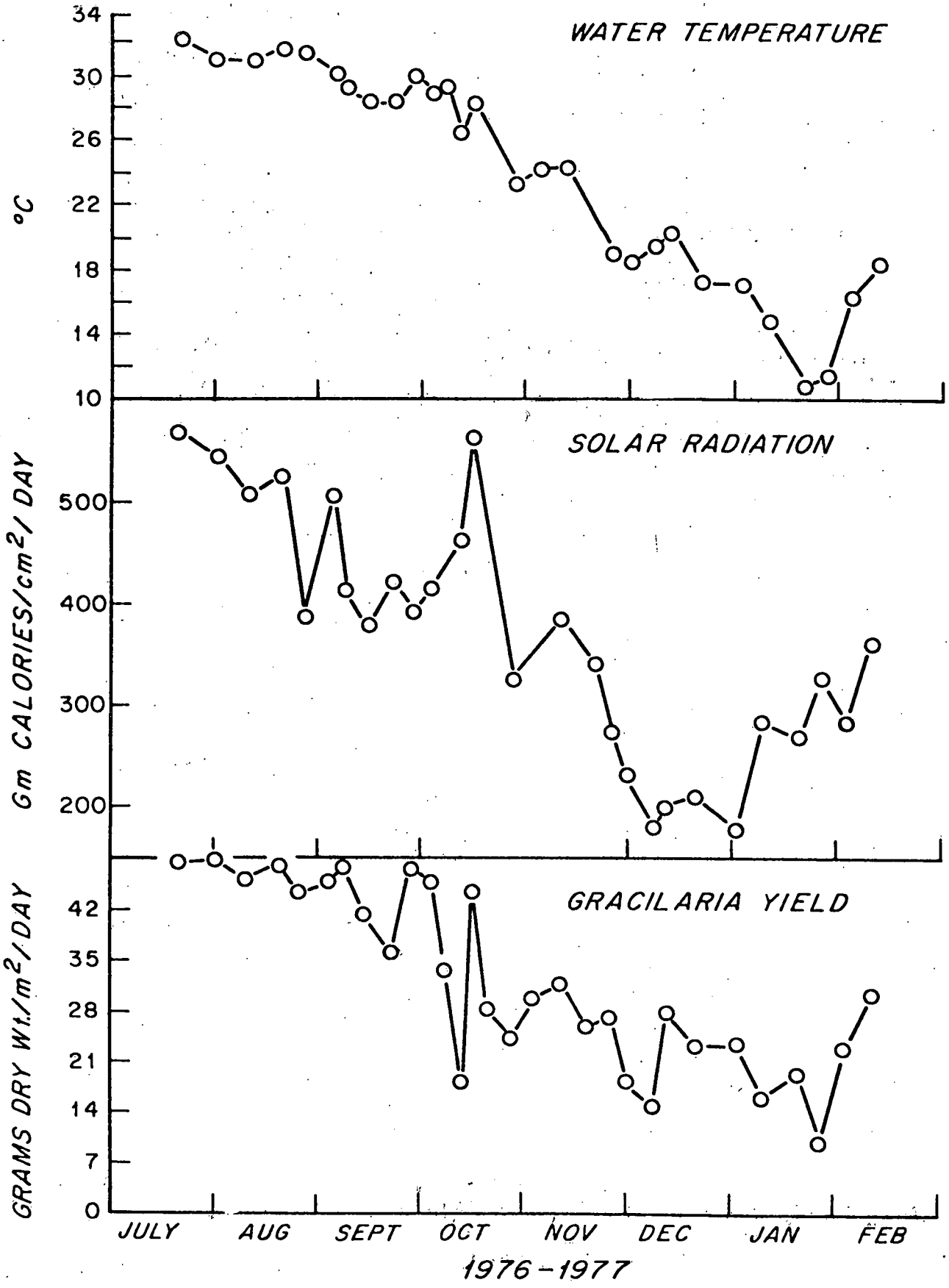

Figure 5 Yield of Gracilaria foliifera V. Angustissima, Solar Radiation, and Temperature: July - February, 1976-77.

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Some aspects of the growth and yield of *Gracilaria foliifera* in culture

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Abstract:

A series of outdoor, continuous-flow seawater cultures (50 liter; 0.23 m^2) were used to investigate the effects of culture density (kg/m^2, nutrient loading (total nitrogen input/day) with both NH_4^+-N and NO_3^--N, and turnover rate (flow rate/culture volume) on the growth and yield of Gracilaria foliifera v. angustissima (Harvey) Taylor (gigartinales). Although specific growth rates as high as 60% per day were recorded for Gracilaria at low densities (0.4 kg wet wt/m^2) in summer conditions, maximum year-round yields were obtained at densities of 2.0-3.0 kg wet wt/m^2. Above a minimal daioy nitrogen loading, yield of Gracilaria was independent of nutrient concentration, nitrogen loading or whether nitrogen was in the form of NH_4^+-N or NO_3^--N but was highly dependent upon flow rate. The time weighted mean annual production during 1976-77 was 34.8 dry wt/m^2/day or 127 metric tons/hectare/year based on 12 months continuous operation at near optimal densities and flow rates in the non-nutrient limited culture system.

The objective of a seaweed culture system is to maintain all other factors as nearly as possible at optimal levels so that productivity is limited by incident solar radiation alone. It also follows that such other factors as water flow and nutrient concentrations should not exceed their optima, both because they may be inhibitory at higher levels and because they represent cost factors that should be kept as low as possible without affecting yield.

Experimental culture system: Design and operation:

The first step in designing a seaweed culture system, then, is to determine emperically the optimal density or biomass of algae and the optimal flow of seawater and concentration of nutrients to achieve maximum yields at a given input of incident solar radiation. To determine these operating parameters, a simple experimental system was developed at the Harbor Branch Foundation in Fort Pierce, Florida. This unit consisted of four 6-meter long, 0.4 m diameter PVC pipes that were longitudinally sectioned and divided into 0.75 m (50 liter) compartments by means of plywood partitions. Each section was provided with a calibrated flow of enriched seawater by means of a manifold fed from a headbox, and also provided with a non-clogging overflow drain. Compressed air was fed into the bottom of each compartment through holes drilled along the bottom of the pipe connecting to an air line (a sectioned two-inch PVC pipe) cemented to the outside of the main

pipe. Thirty-two individual growth assay chambers were produced in this way.

The growth chambers were located out-of-doors in full sunlight. Seawater was taken from the Harbor Branch Foundation ship channel which connects to the Indian River, a shallow lagoon of the Atlantic Ocean. No attempt was made to control water temperature, which ranged from 12°-34°C, or salinity, which ranged from 20-34‰, in the incoming seawater during 1976-77. Seawater was pumped into a reservoir tank holding several days supply for the experimental chambers. Prior to its use, the stored seawater was enriched with the desired concentrations of nitrogen and phosphorus, normally provided as sodium nitrate and monosodium (dibasic) phosphate at a ratio of 10:1 by atoms of N:P. A second reservoir contained unenriched seawater. Both enriched and unenriched seawater supplies were pumped to headboxes from which they were distributed to the experimental chambers at flow rates and mixtures to provide the desired rates of exchanges and nutrient concentrations.

Weighed amounts of Gracilaria foliifera were stocked in the experimental chambers to give the desired density (g/m^2). At intervals of 5-10 days, depending upon growth rate, the algae was removed from the chamber, shaken vigorously to remove water, and weighed. Establishment of the relationship between drained wet weight and dry weight was determined carefully on replicate sam-

ples by oven drying at 90°C for 48 hours. Water samples were taken at midday for chemical analyses of PO_4^{3}-P (1), NH_4^{+}-N (2), and NO_3^{-}-N (3).

Effect of density on growth and yield:

The specific growth rate of an alga is determined by its photosynthetic efficiency and may be considered as a biological constant if light, nutrients, CO_2, temperature, and other controlling factors are held at optimal levels. However, specific growth, expressed as increase per unit weight and time or percent increase of weight per unit of time, should not be confused with productivity or yield of an algal population, the production of organic matter per unit area and time, which is the derivative of the specific growth rate and the density or biomass of the seaweeds per unit area.

Because of self-shading and perhaps other factors, growth rate is highest at very low densities and decreases at a rate that is inversely proportional to density. As a result yield expressed as a function of density describes a bell-shaped curve with a maximum utilization of incident solar radiation and not too high to cause a loss of production from self-shading or other factors.

The specific growth rates and yields of G. foliifera as functions of density or biomass of the seaweed are shown in Figure 1. These data are shown for both summer (August, 1976) and winter (January, 1977) conditions. At both times of year, growth rate

decreased with increasing biomass, though both the absolute values and the range were much greater in summer than in winter. Yields (g dry wt/m^2.day) at both seasons peaked at densities of 2.0-3.0 kg wet wt/m^2 but with a rather broad plateau at both seasons and with some indication of a higher optimal density in winter than in summer. As would be expected, yields were higher, by a factor of about twofold, in summer than in winter. The difference is believed to be due primarily to solar radiation but temperature may have had second order effects.

Effect of flow rate on yield:

It is not possible to separate experimentally the effects of nutrient supply and flow rate of water or medium through a seaweed culture. If nutrient concentration is held constant, the rate of input of nutrients to the culture will vary proportionately with the flow rate. If, on the other hand nutrients are added separately at a constant input rate while seawater flows are independently varied, the instantaneous concentration of nutrients entering the culture will be inversely proportional to seawater flow rate.

Both types of studies were conducted with G. foliifera in the experimental system described above and the results are shown in Figure 2. In both series of experiments, the seawater was passed through the cultures at flow rates of 1, 7.5, 15, and 30 culture volumes (50 liter) per day while simultaneously comparing growth

with both NH_4^+-N and NO_3^--N medium. In one experiment, nutrients were added separately at a constant rate to each culture so that daily nutrient loading remained constant but initial concentrations varied inversely with the flow rates of the diluting seawater. In the second experiment, the nutrients were mixed initially with the seawater so that all of the cultures received the same initial concentration but the daily loading was directly proportional to the flow rate. The nutrients consisted of phosphorus as monosodium dibasic phosphate ($NaH_2PO_4.H_2O$) and nitrogen as sodium nitrate ($NaNO_3$) or ammonium chloride (NH_4Cl). In the first experiment, the input concentrations of N and P ranged from 300 and 30 μmoles/l (at 1 exchange/day) to 10 and 1 μmoles/l (at 30 exchanges/day) respectively. In the second experiment, the constant concentrations of N and P were 50 and 5 μmoles/l respectively.

As seen in Figure 2, the important factor determining the yield of Gracilaria appears to be flow rate per se. At any given flow rate, the yields were essentially the same independent of the nutrient concentration, the daily nutrient loading, or whether the nitrogen was added as ammonia or nitrate. (There is, of course, a minimum nitrogen concentration and daily loading needed to achieve maximum yields at any flow rate, but those minima were exceeded in these experiments.)

Annual yield of Gracilaria:

Gracilaria foliifera was also grown continuously from July 20, 1976 to June 28, 1977 in the culture chambers described above, maintaining a culture of 2 kg/m^2 (wet wt) by weighing the seaweed at approximately one-week intervals and removing the incremental growth. The flow rate was 22 culture volume exchanges per day of seawater enriched with ca. 15 μmoles/l NO_3^--N and 4 μmoles/l $PO_4^=$-P. Yield of the seaweed in g dry wt/m^2.day is shown in Figure 3 together with incident solar radiation (as measured with an Epply pyroheliometer), mean daily seawater temperature and salinity of the culture.

The close correlation between Gracilaria yield and solar radiation between July and December is obvious, with even minor perturbations of the latter reflected by irregularities in the growth of the seaweed. However, the seasonal increase in radiation after the first of the year was not immediately accompanied by an increase in algal yield, presumably due to the abnormally low (<12°C) water temperatures that occurred in January, 1977. As both light and temperature increased in February, the algae responded with renewed acceleration of growth.

The time-weighted mean annual production of Gracilaria was 34.8 g (dry wt)/m^2/day or 127 metric tons/hectare.year. This figure approaches the theoretical limit to primary production (4) and probably represents the maximum attainable yield of Gracilaria in culture.

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Acknowledgements:

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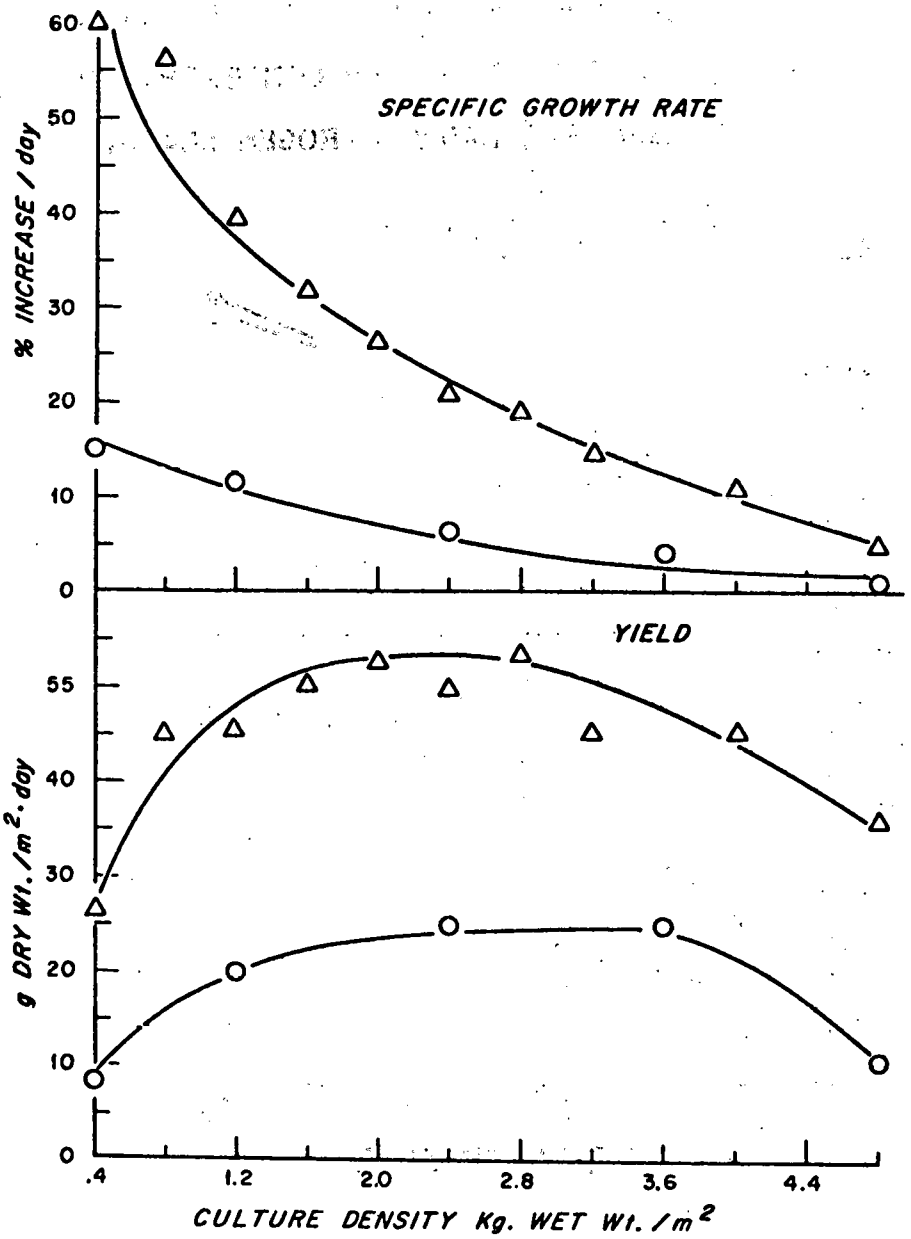

Figure 1. Effect of culture density on specific growth rate and yield of Gracilaria folifera v. angustissima in summer (Δ) and winter (o).

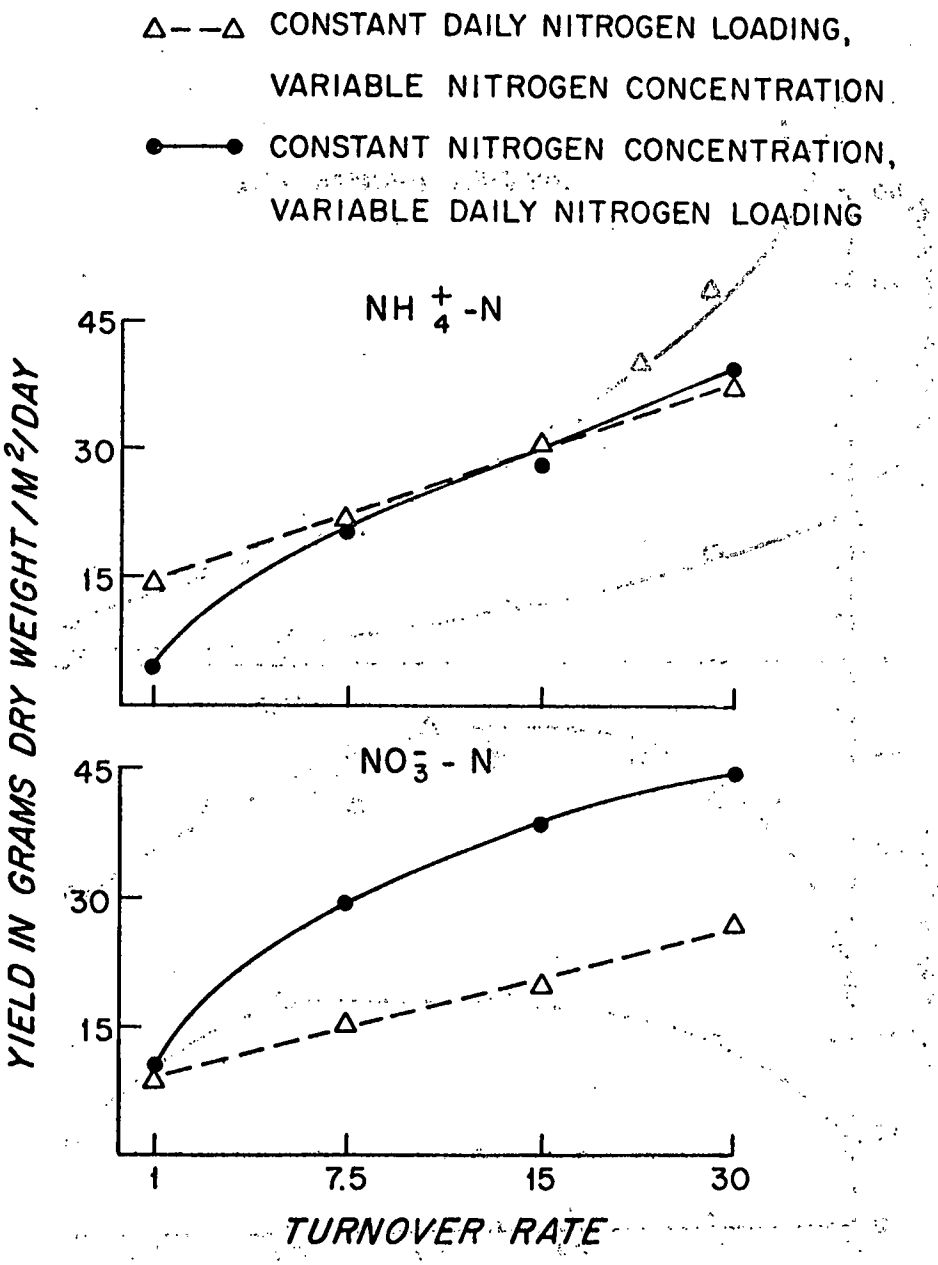

Figure 2. Effect of turnover rate (culture volume exchanges/day) on yield of Gracilaria folifera v. angustissima with both NH_4^--N and NO_3^--N medium.

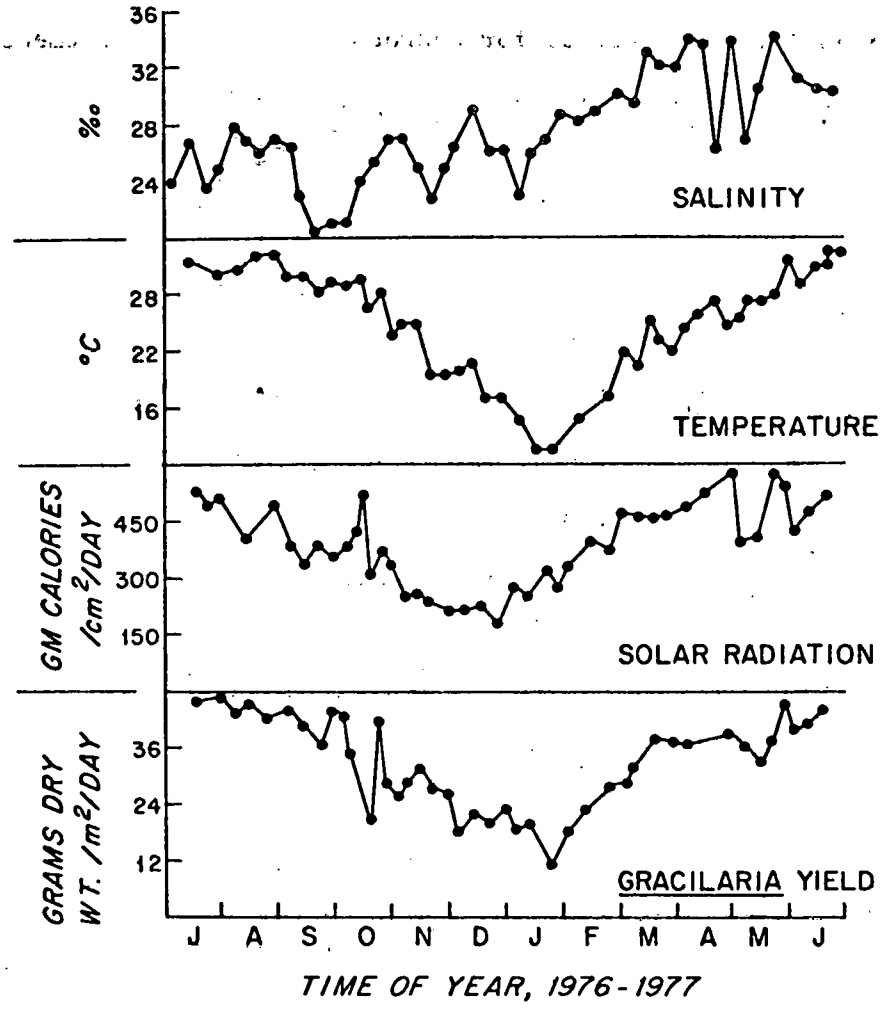

Figure 3. Yield of Gracilaria foliifera v. angustissima, solar radiation, temperature, and salinity: July-June, 1976-77.

Cultivation of seaweeds for hydrocollids, waste treatment, and biomass for energy conversion

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Abstract:

Development is described of seaweed culture systems in Woods Hole, Mass. and Ft. Pierce, Florida for nutrient removal from domestic and animal culture wastewater, for commercial value for their hydrocolloids, and for a biomass source for conversion to methane or other fuels. Of various species evaluated, the red alga Gracilaria foliifera appears the best suited for all three purposes because of its ability to be grown vegetatively and essentially trouble-free over long periods of time and because of its high dry-weight yields of 26 metric tons/hectare over a 165-day growing season in Woods Hole and 112 metric tons/hectare on a year-round basis in Florida.

Description of Culture Systems:

In the early 1970's, a project was initiated at the Woods Hole Oceanographic Institution with the objective of developing a combined advanced wastewater treatment-marine aquaculture system. Secondary sewage effluent, mixed with seawater, was used as a culture medium to grow unicellular algae on a continuous, flow-through mode. Overflow from the algae cultures, a series of 15 m-diameter, 1 m-deep ponds, was fed to oysters, clams, and other bivalve molluscs held in trays in 12 x 1.2 x 1.5 m deep concrete channels or raceways. Conceptually, the algae would remove the nutrients, primarily nitrogen and phosphorus, from the sewage effluent, thereby providing a biological tertiary sewage treatment, and the bivalves would remove the algae from the water, leaving a clean, nutrient-free final effluent. In fact, however, the molluscs can assimilate only a fraction of the food they ingest, and a significant portion of the nutrients initially present in the wastewater was regenerated by excretion of the animals and decomposition of their solid wastes and was released into the final effluent from the animal cultures, thereby defeating the objective of complete nutrient removal by the system. To correct that deficiency, seaweeds were added as a polishing step to remove nutrients produced by the animals prior to final discharge of the aquaculture effluent to the environment.

Seaweeds were grown in concrete raceways identical and immediately adjacent to those used for growing the shellfish except

that the bottoms were modified with plywood sheets to provide a sloping V-bottom, at the apex of which was located a perforated plastic pipe to provide vigorous aeration (Figure 1a). The seaweeds were thus grown in suspension using basically the techniques developed by Neish and his colleagues in Halifax, N.S. (1) and Harold Humm, University of South Florida (personal communication). Water was exchanged in the 23,000-liter raceway culture from three to ten times daily, depending upon the feeding regime used in the shellfish cultures.

The unicellular algae cultures (10-25% sewage effluent in seawater) were further diluted with one to ten times their volume of seawater prior to feeding to the shellfish, so the salinity of the effluent from the animal cultures (= input to the seaweed cultures), was nearly that of the ambient seawater and ranged from 26 to 29‰. Both the algal cultures, at times, and the diluting seawater entering the shellfish raceways were heated in winter to allow the bivalves to feed. Although cooling occurred before the water entered the seaweed cultures, temperatures of the latter never fell below 13°C in winter and reached a maximum of 26°C in mid-summer.

While the seaweed cultures were instituted primarily as an expedient to remove nutrients from the animal culture units, an attempt was made to grow commercially-valuable species that would add value to the system. Initially, Chondrus crispus (Irish moss), the carrageenan-bearing red alga that is commercially har-

vested in New England and the Canadian maritimes, was used, employing both strains collected locally in the Woods Hole area and the fast-growing T-4 strain obtained from the laboratory of A. C. Neish at Halifax, N. S. However, Chondrus grew slowly in the aquaculture system described above, the plants became heavily epiphytized by other red algae, and summer temperatures were clearly too high for the species; (2). Therefore other, more tropical species of the Rhodophyta that are summer annuals in the Woods Hole area were collected locally and introduced into the system. Of the species tried, Gracilaria foliifera, an agarophyte, and Neoagardhiella baileyi, a carrageenan-producing alga, proved most successful, growing well within the temperature regime of the aquaculture system throughout the year, remaining relatively free from epiphytes, and maintaining themselves indefinitely in a non-reproductive, non-fruiting, vegetative condition.

So successful was the seaweed culture portion of the aquaculture system described above that separate experiments were subsequently initiated in Woods Hole in which the seaweeds were grown directly on mixtures of secondary sewage effluent and seawater in a simplified, one-stage advanced wastewater treatment-aquaculture system. In these studies, the seaweeds were grown in plywood tanks 2.5 x 1.8 x 1.0 m deep also fitted with sloping bottoms with an airline at the base. Mixtures of 10-25% secondary sewage effluent in seawater or enrichment of the seawater with inorganic chemicals (NH_4Cl and $Na_2HPO_4 \cdot H_2O$) to give concentrations of nitrogen and

phosphorus comparable to sewage were passed through the seaweed cultures in separate experiments at exchange rates of two to four volumes per day.

At the same time that these studies were going on in Woods Hole, a smaller project with similar objectives was initiated at the Harbor Branch Laboratory in Fort Pierce, Florida. A part of the studies in Florida also involved seaweed culture in 350-liter and 600-liter plywood tanks of similar design to those used in Woods Hole (Figure 1b). Both sewage effluent and inorganic chemical media were used as sources of enrichment to grow the seaweeds, which consisted of Gracilaria foliifera and Hypnea musciformis (another carrageenan-containing alga) collected locally from the Indian River (3).

In 1976, research supported by the U. S. Energy Research and Development Agency (ERDA) on the mass culture of seaweeds as a biomass source for conversion to energy was initiated both at the Woods Hole, Mass. and Harbor Branch Foundation, Florida facilities. Since the objective in this research was to produce organic matter in toto, and not simply the commercially-valuable hydrocolloids, carrageenan and agar, of the Rhodophyta, the species of seaweed produced in culture was no longer of importance. Rather, the immediate goal was to select the species and develop a culture technique that would provide the highest possible yield of organic matter per unit of time and space and with the smallest possible input of energy.

For screening different species of algae for their growth potential in artificial culture, a standard assay system was developed consisting of four 6-m long, longitudinally-sectioned, 0.4 m diameter PVC pipes divided into 0.75-m long (50-liter capacity) compartments by means of plywood sections. Each section was provided with a calibrated flow of enriched seawater and a screened overflow drain. Thirty-two individual assay chambers were provided in this way (Figure 2).

Initial experiments were conducted with the assay system in Florida and with small experimental systems in Woods Hole to determine emperically the optimum culture conditions for maximum sustained growth of selected species of the seaweeds. The varibles examined included culture density of the seaweeds, rate of water exchange through the culture, rate of mixing and aeration, nutrient concentration, and species of nitrogen (ammonia vs. nitrate) in the enrichment. The results of those experiments will be reported in detail elsewhere. The resulting operating criteria are, of course, biased in favor of the species tested (i.e., other species may have different optima for best growth). Also, since those experiments were carried out simultaneously with or after some of the long-term growth studies, the latter were not in some cases designed for the best possible results.

In brief, the best growth of the seaweeds tested, principally Gracilaria foliifera and Neoagardhiella baileyi, were obtained in full sunlight, at relatively low nutrient concentrations (e.g., 10-

50 μmoles/l nitrogen and 1-5 μmoles/l phosphorus) supplied as ammonia and phosphate continuously at high turnover rates of at least 20 volumes per day (the highest rate tested) and with the culture maintained by periodic harvesting at a density of 2.0-4.0 kg (wet weight)/m^2 of culture surface.

A working definition of growth and yield:

Before discussing the results of the different culture studies, a word is needed concerning the distinction between growth and yield and the units used in expressing these parameters. The specific growth rate of an organism is the rate of increase per unit weight (grams/gram) or percent increase per unit of time. Sometimes this is expressed as doubling time (i.e., a doubling time of one week is roughly equivalent to a growth rate of 0.1 g/g.day or 10%/day).

Figure 3 illustrates the fact that specific growth rate decreases exponentially with increasing density of the culture. Both the magnitude of this quantity and its relationship to culture density are also influenced by other factors, of which incident solar radiation is probably the most important. Thus, values of specific growth rate of an alga, by themselves, are not meaningful.

The productivity or yield of an alga is the product of specific growth rate and density, and is expressed as growth per unit of time and area. Conventionally short-term yields are given as grams/m^2.day and sustained, large-scale yields as metric tons/hectare.year, both in units of total dry weight. It is these

figures that are of immediate concern to those interested in the utilization of seaweeds as a resource, for whatever purpose.

As the derivative of specific growth rate and culture density, yield describes a bell-shaped curve as a function of density (Figure 3). At low densities, the seaweeds are unable to utilize all of the incident radiation, while at high densities, self-shading by the plants decreases growth. As mentioned above, maximum yields were usually found at densities of 2-4 kg wet weight/m^2. In practice, that observation has led to the operating procedure of harvesting back the cultures from 4.0 to 2.0 kg/m^2 at whatever time interval is needed for such a doubling, depending upon time of year, etc.

Yields:

The yields of Gracilaria foliifera and Neoagardhiella baileyi obtained from the large concrete raceways as part of the waste recycling polyculture system are described in some detail by DeBoer et al. (4). Neoagardhiella had surprisingly high rates of production in the spring (22-41 g dry weight/m^2.day) and summer (20-36 g/m^2.day). Gracilaria production rates were quite variable, increasing from 4 g/m^2.day in April to 43 g/m^2.day in early June and decreasing again during the latter part of the summer to 7-18 g/m^2.day. Yields of both species declined during the fall and early winter. By mid-December the Gracilaria yield had fallen to zero and the plants had deteriorated, while the Neoagardhiella remained viable (although stunted) with a yield of 6 g/m.day.

The gross seasonal changes in production appear to be correlated with both water temperature and incident solar radiation. From June 1 to October 1, however, when the water temperatures and solar radition were presumably optimal for the seaweeds, production showed little correlation with those variables. Low production rates during that period were probably due to the high densities of seaweeds maintained in the raceways (as high as 12.3 and 10.2 kg fresh weight/m^2 for Neoagardhiella and Gracilaria, respectively). The effect of density on yield and the optimum density for best yield had not yet been determined at the time of these studies.

The mean annual dry weight production rate was 17.3 g/m^2.day or 63.2 metric tons/hectare.year for Neoagardhiella and 8.9 g/m^2.day or 32.9 t/ha.year for Gracilaria. These rates may be higher than would be realized in a commercial enterprise because the raceways were maintained at elevated temperatures for approximately six months of the year, a practice that would be economically prohibitive on a commercial basis. Annual production rates based on a 5 1/2 month growing season when the raceways were not heated (May 8, 1975-October 20, 1975) were 41.6 t/ha.year and 25.8 t/ha.year for N. baileyi and G. foliifera respectively, yields that are still not impressive despite the short season.

The production of G. foliifera and H. musciformis in experiments carreid out in Florida in 1974-75 in the 350- and 600-liter plywood box tanks (Figure 1b) are discussed by Lapointe et al. (4). Yields ranged from 4.5 to 17.6 g/m^2.day for Hypnea between February

and June and from 7.7 to 15.7 g/m^2.day for Gracilaria between April and September in several separate experiments that lasted from two to eight weeks each.

Average yields in Florida were higher than those observed in Massachusetts for G. foliifera and N. baileyi (see above). However, the same plants could not be maintained in continuous culture in Florida, as in Woods Hole, but periodically became heavily epiphytized and/or fragmented into small pieces and had to be discarded and replaced with new clones collected from the field.

With the introduction of the small (50-liter) screening tanks to the Florida site in 1976 (Figure 3) and the subsequent definition of more opimal culture conditions as described above, some of the difficulties that had been earlier encountered in growing seaweeds and particularly in maintaining cultures of the same clone over long periods of time have been resolved.

As mentioned above, the smaller culture units were designed to screen as large a number of seaweed species as possible for their growth potential in culture systems as part of the ERDA Fuels from Biomass Program. To date, 24 species including representatives of the red, green, and brown algae, have been tested. Of these, only six have shown significant growth potential in short-term (1-2 week) culture experiments. These include the greens Enteromorpha clathrata, Chaetomorpha linum, and Ulva lactuca and the reds Gracilaria foliifera, Gracilariopsis sjoestedtii, and Hypnea musciformis. Of the six, the three species of greens and H. musci-

formis could not be maintained over long periods of time in culture, but fragmented or disintegrated into spores after one to two weeks. Gracilariopis appears promising, but had not been cultured for more than three months at the time of this writing. However, the same clone of G. foliifera has now been maintained for a full year in the smaller (50-liter) culture mode. Yields of that culture from July, 1976 to February, 1977 are shown in Figure 4 together with data for water temperature and solar radiation. Generally there is a close correlation between yield and sunlight, but the abnormally low temperatures that occurred in the winter of 1976-77, which reached levels that were marginal for G. foliifera, depressed yields of the seaweed after solar radiation began to increase in January.

The time-weighted mean production of Gracilaria was 30.7 g (dry weight)/m^2/day. Since the time period involved was just under seven months and extended from very near the summer solstice through the winter solstice, it may be assumed that the mean yield for that period of time would be very close to an annual mean productivity value, a figure that is approximately twice that previously reported for Gracilaria and Hypnea grown earlier in Florida in the larger (350-600 liter) culture units.

A mean yield of 30.7 g/m^2/day is equivalent to an annual dry weight of 112 metric tons/ha/year, a figure that is more than twice the annual yields of G. foliifera and Neoagardhiella baileyi at Woods Hole, Massachusetts (4) and greater than the highest sus-

tained agricultural yields reported in the literature (5). With respect to the objective of biomass production as an energy source, however, it must be kept in mind that total dry weight yields are not equivalent to yields of organic matter or volatile solids, which are only about 50% of the dry weight of Gracilaria in contrast to 75-95% of terrestrial plants (6). On the other hand, half the yield of Gracilaria in Florida compares favorably with the total dry weight yields of most of the best agricultural yields referred to above (5).

Caution must also be used in extrapolating the yields of small, experimental systems to large agricultural or aquacultural systems. Much of the agricultural yield data was also obtained from small experimental plots, but their physical nature was undoubtedly very similar to that of the large-scale agricultural systems they were simulating. Such is unfortunately not true of the small, highly-intensive seaweed culture system reported upon here. Yields from the latter may be looked upon as representing something that is perhaps close to the biological potential of the marine algae, but it is very doubtful that such systems could find large-scale application that would be cost effective either economically or from an energy accounting point of view.

The obvious next step in the assessment of seaweed culture as a biomass source for energy is the development of a simple, non-intensive culture system that is energy cost effective while still capable of producing yields that at least approach those reported

above.

Waste treatment:

The original objective of growing seaweeds in the aquaculture systems designed in Woods Hole and tested there and in Florida was to remove nutrients from wastewater and/or animal culture effluents. Assessment of the performance of seaweeds in that role in a small, experimental scale (8) and at the larger, pilot-scale facility at Woods Hole (9) have demonstrated that the biological system, including the seaweeds, is capable of removing virtually 100% of inorganic nitrogen from solution. Because of the use of phosphate-detergents, the ratio of P:N is abnormally high in wastewater relative to that in organisms, so that only about half the phosphate can be removed biologically, through assimilation by the seaweeds, at the same time that all of the nitrogen is removed (10). However, it has also been convincingly demonstrated that further algal growth in the final effluent from the aquaculture system is not possible without further nitrogen supplementation (11), so the objective of tertiary wastewater treatment to prevent eutrophication of receiving waters is met with the system despite the residue of phosphorus.

Seaweed culture alone may function effectively as a advanced wastewater treatment system. Taking the dry weight annual yields of Gracilaria reported above of 26 tons/hectare for 165 days in Woods Hole and of 112 tons/ha for 365 days in Florida and assuming that the seaweed contains 5% of its dry weight as nitrogen, the

seaweeds are capable of removing 7.9 and 15.4 kg of nitrogen per hectare of culture per day in Woods Hole and Florida respectively. Since the per capita production of nitrogen in wastewater is approximately 10 grams per day, the nitrogen from a town of 10,000 could be removed completely for half the year in New England in a culture area of 13 hectares (32 acres), throughout the year in Florida in an area of 6 hectares (16 acres) using yield figures given in this report for the two regions. As mentioned above, extrapolation of the performance of the small experimental systems to large commercial scale is perhaps questionable, but the potential for an effective waste treatment system for nutrient removal based on seaweed culture does appear to exist. The value of a crop of some hundreds of dry tons of commercially valuable algae as the by-product of an advanced waste treatment plant of the size discussed above would also go a long way towards paying the cost of of the additional waste treatment.

Acknowledgements:

Contribution No. 4016 of the Woods Hole Oceanographic Institution and Contribution No. 71 of the Harbor Branch Foundation, Inc. This research was supported by Contract E(11-1)-2948 from the U.S. Energy Research and Development Agency, the NOAA Office of Sea Grant, Department of Commerce Grant No. 04-6-158-44016, and the Jessie Smith Noyes Foundation, Inc.

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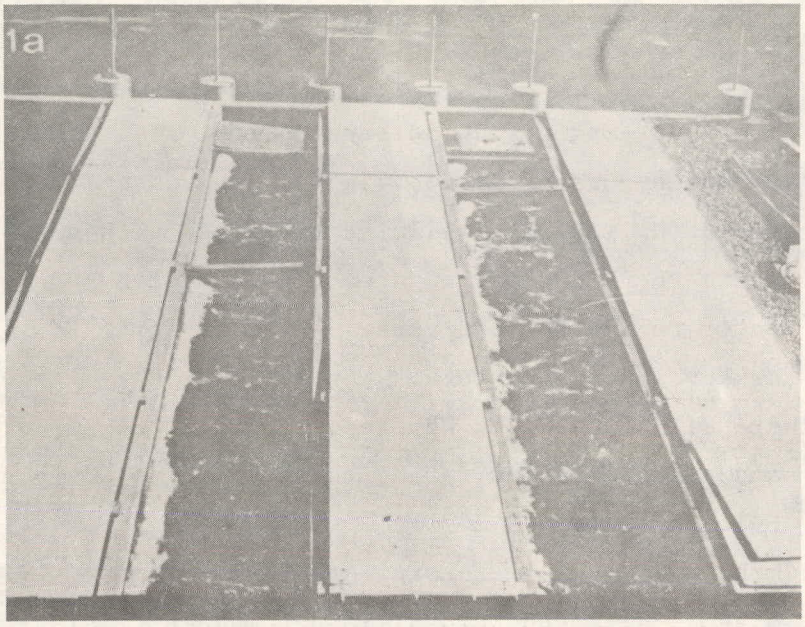

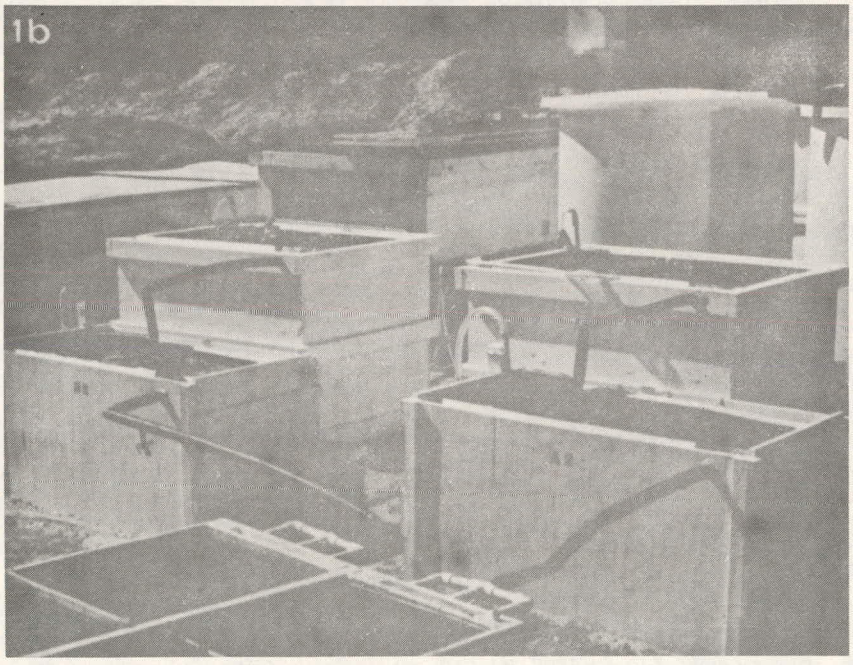

Figure 1a. Concrete raceway system of the Woods Hole waste recycling-marine aquaculture project. Covered raceways contain stacked trays of shellfish; open raceways contain seaweeds. Note aeration to maintain plants in suspension. 1b. Plywood "box" tanks (350 and 500-liter capacity) for growing seaweed at Harbor Branch Foundation, Ft. Pierce, FL.

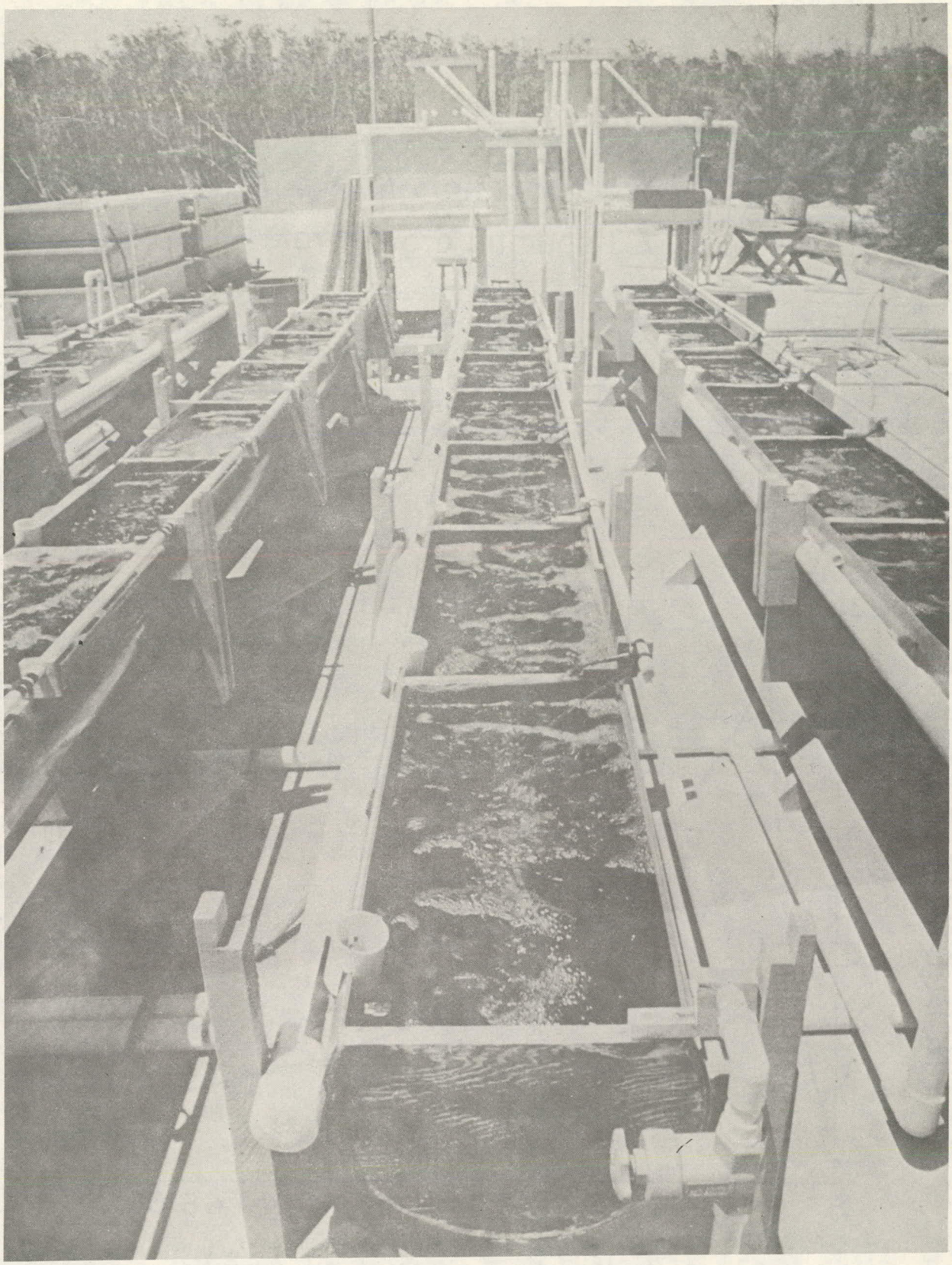

Figure 2. 50-liter capacity "screening" tanks for assaying growth potential of seaweeds at Harbor Branch Foundation, Ft. Pierce, FL. Headboxes for enriched seawater supply in background.

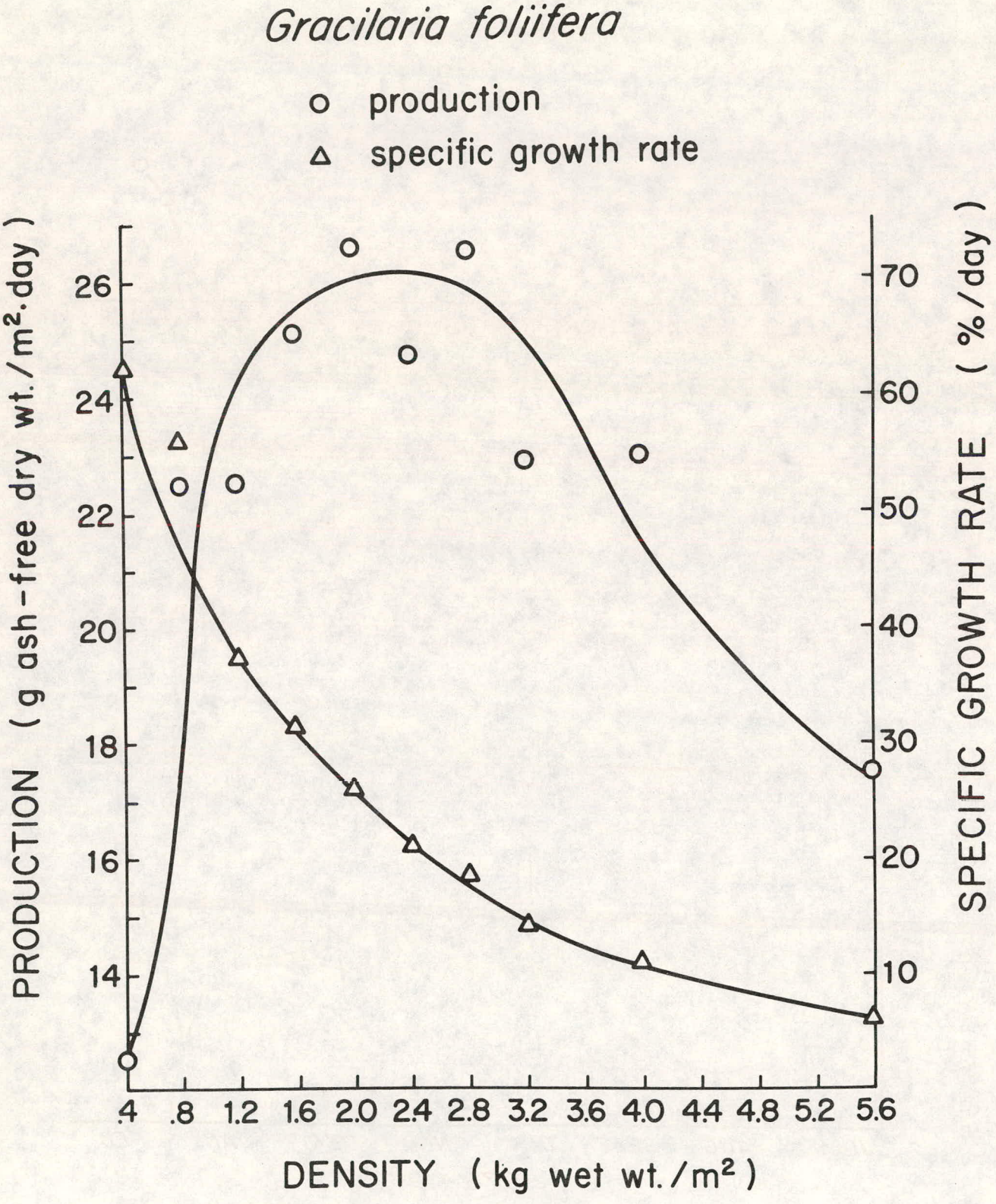

Figure 3. Effect of culture density on specific growth rate and yield of Gracilaria foliifera.

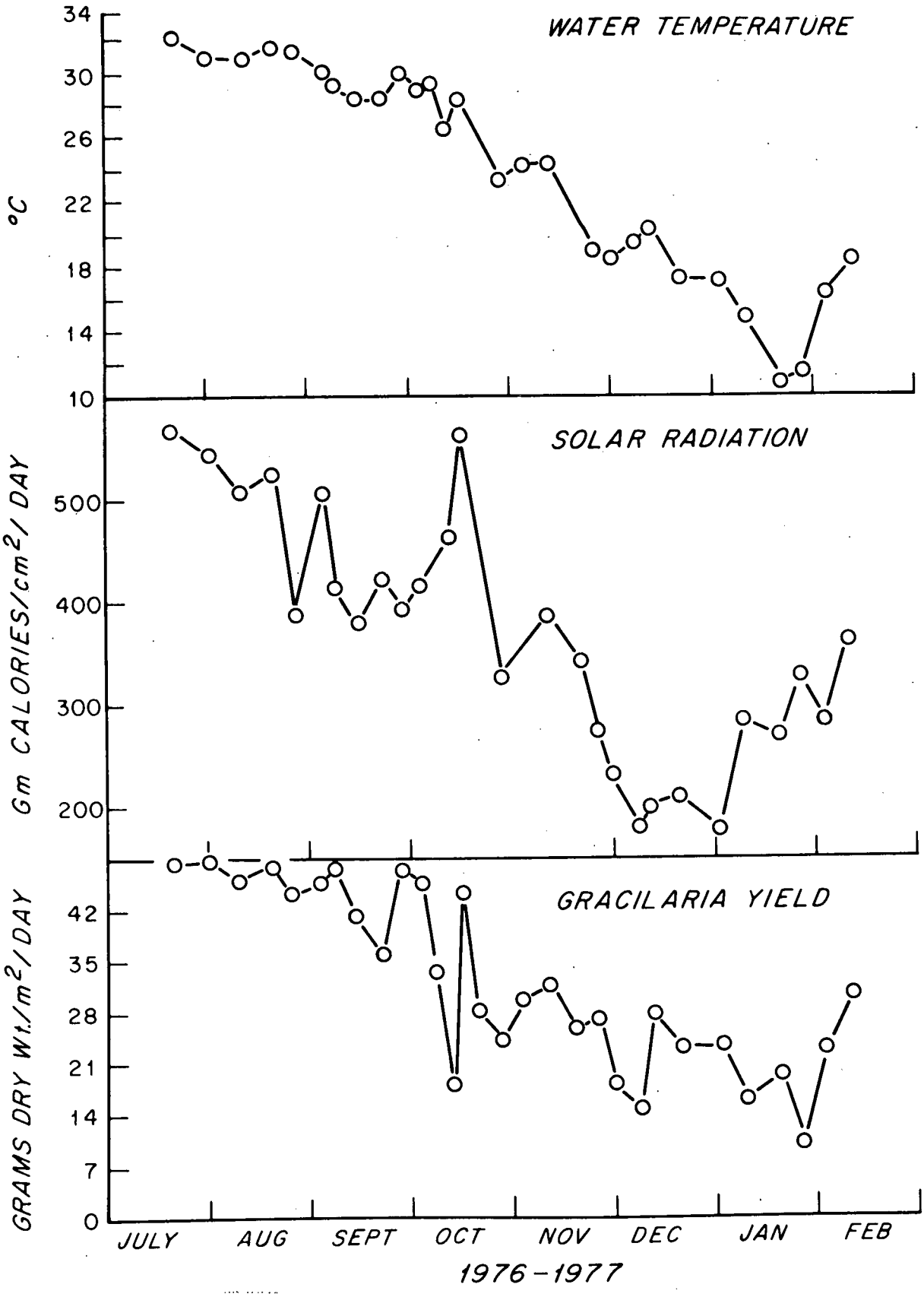

Figure 4. Yield of Gracilaria foliifera, solar radiation, and water temperature in screening tanks at Harbor Branch Foundation Ft. Pierce, FL; July-February, 1976-77.

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Growth and yields of different species of seaweeds in an intensive, outdoor culture system

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Most of the effort and interest in seaweed cultivation to date has been directed towards those species that are used or have potential value as food or as a source of the commercially-valuable hydrocolloids agar, alginic acid, or carrageenan (e.g., see reviews in Ryther et al., 1977; Mathieson, 1975). These are primarily representatives of the red algae (Rhodophyta) and a few species of the brown algae (Phaeophyta).

In considering seaweeds as a biomass source for conversion to methane or other fuels, however, the only relevant criterion of value is the total amount of organic matter that is produced per unit of area and time, usually expressed as g dry weight/m^2 day or m tons dry weight/hectare-year. This is a characterictic of all phytosynthetic plants and therefore of all kinds of seaweeds, regardless of their taxonomic position or specific chemical composition or nutritional value.

Selection of the best one or more seaweed species for biomass production therefore requires a rather extensive screening program to evaluate comparative long-term or sustained organic yields of as many species as possible when grown under the same standard culture conditions, preferably conditions that at least approximate those under which large-scale seaweed cultivation might be carried out within the context of an energy plantation. Unfortunately, such large-scale culture systems have not yet been developed or defined.

An alternative procedure is to screen seaweed species for their growth under highly intensive culture conditions that were developed to demonstrate the maximum growth potential of certain of the commercially-valuable species of red algae. While these methods are recognized to be economically impractical even for hydrocolloid production (which in itself is orders of magnitude more valuable than biomass for conversion to an energy source), they at least provide data on the biological potential of growth under the best of conditions against which that obtained by more economically attractive methods may be evaluated. For several species of the red algae, these optimal growth conditions were found to be: (1) maintenance of the seaweed thalli in relatively small fragments in suspended culture by means of vigorous aeration, (2) maintenance of a biomass of 2-4 kg wet wt/m^2 culture surface area, (3) rapid exchange of culture volume of at least 25 times per day, (4) relatively low concentration of major nutrients of the order or 10 μmoles/l nitrogen and 1 μmole/l phosphorus.

It was judged to be impractical to control such factors as temperature, salinity, and incident solar radiation in any large-scale culture system, so no attempt was made to determine optima for those environmental variables. Rather, they were monitored along with growth of the seaweeds throughout the year so that their influence on growth could at least be estimated by correlation.

To evaluate the growth potential of a large number of species of seaweeds under the idealized culture conditions described above, it was necessary to construct a large number of culture chambers so that many species could be screened at the same time, thereby eliminating, at least for that group of species, the differential effects of the uncontrolled variables of temperature, salinity, and light intensity.

A relatively simple and inexpensive experimental system was developed for this purpose at the Harbor Branch Foundation. This unit consisted of four 6-meter long, 0.4 m diameter PVC pipes that were longitudinally sectioned and divided into 0.75 m (50 liter) compartments by means of plywood sections. Each section was provided with a calibrated flow of enriched seawater by means of a manifold fed from a headbox, and each section was also provided with a non-clogging overflow drain. Compressed air is fed into the bottom of each compartment through holes drilled along the bottom of the pipe connecting to an airline (a sectioned two-inch PVC pipe) cemented to the outside of the main pipe. Thirty-two individual growth assay chambers were produced in this way (Fig. 1).

The growth chambers were located out-of-doors in full sunlight. Seawater was taken from the Harbor Branch Foundation ship channel which connects to the Indian River, a shallow lagoon of the Atlantic Ocean. No attempt was made to control water temperature, which ranged from 14°-30°C, or salinity, which ranged from 23-34‰, in the incoming seawater.

Seawater was pumped into a reservoir tank holding several days supply for the experimental chambers. Prior to its use, the stored seawater was enriched with the desired concentrations of nitrogen and phosphorus, normally provided as sodium nitrate and monosodium (dibasic) phosphate at a ratio of 10:1 by atoms of N:P. A second reservoir contained unenriched seawater. Both enriched and unenriched seawater supplies were pumped to headboxes from which they were distributed to the experimental chambers at flowrates and mixtures to provide the desired rates of exchanges and nutrient concentrations.

Weighed amounts of seaweed were stocked in the experimental chambers to give the desired density (g/m^2). At intervals of 5-10 days, depending upon growth rate, the algae was removed from the chamber, shaken vigorously to remove water, and weighed. Establishment of the relationship between drained wet weight, dry weight and ash-free dry weight (volatile solids), determined carefully on replicate samples of each species of seaweed, permitted the expression of growth in any of these units from the wet weight measurements.

A total of 42 species of seaweeds indigenous to the coastal waters of Central Florida have been evaluated to date. That number includes six green algae (Chlorophyta), two brown algae (Phaeophyta) and the remainder, representatives of the red algae (Rhodophyta). The latter group included 11 species or varieties

of the large genus Gracilaria, of which over 100 species have been described, as well as the closely-related Gracilariopsis skoestedtii.

About half of the seaweeds tested failed to grow at all or to survive in the artificial culture system. Of the rest, various species of Gracilaria and the related Gracilariopsis grew best along with two other species of red algae (Neoagardhiella baileyi and Hypnea musciformis) and, for very brief periods of time, three species of the green algae (Ulva lactuca, Enteromorpha intestinalis, and Chaetomorpha linum).

Of all those tested, however, only one, Gracilaria foliifera V. angustissima, grew well, at relatively high levels of organic productivity, in the culture system throughout the entire year.

The results of the screening tests are shown qualitiatively in Table 1 and quantitatively, for those species that showed good growth for some significant period of time during the year, in Figure 2.

The usual fate of the seaweeds in the experimental chambers was to (1) become overgrown with epiphytes including both macroscopic algae and pennate diatoms which eventually killed the culture, (2) fragment into very small pieces that washed out of the culture system, or (3) disintegrate into reproductive spores that washed out of the culture system. The latter occurred routinely with all of the green algae tested.

Figure 2 is misleading in that the growth shown for restricted periods of the year for most species is a function of the availability to replacement of the particular alga by collection of new specimens from the local environment after a given culture had become overgrown with a epiphytes or had otherwise deceased. Similarly, the continuous growth of G. foliifera V. angustissima throughout the year is misleading in that the same culture was not maintained for that period, but new material was periodically collected and used to replace the old material as it became heavily epiphytized and necrotic, in this case the new material being available throughout the entire year from the wild or from other cultures maintained at the laboratory.

Epiphytization is, by any criteria, the most serious problem and constraint to seaweed culture. While a given species, such as G. foliifera, may be capable of growing throughout the year at a high level of organic productivity, its periodic overgrowth by epiphytes, necessitating its complete replacement in the culture system, makes its large scale cultivation impractical at the present time. Nor is it feasible to consider growing the epiphytes themselves as a biomass source. After over-growing the host plant to the point where the latter ceases to grow and dies, the epiphyte itself declines and usually stops growing entirely if it survives at all. Often, the epiphyte is one of the green algae (e.g., Enteromorpha, Chaetomorpha) that periodically becomes reproductive and disappears from the culture.

One final reservation should be pointed out concerning the screening program described above. The intensive culture system employed was developed empirically, by trial and error and by modification from other experimental culture systems, specifically for the growth of various species of red seaweeds of commercial value. It is therefore perhaps not coincidental that red algae were found to grow most successfully by the methods employed. Other, quite different techniques and conditions may be needed for comparable growth of some of the other groups of algae indigenous to the area.

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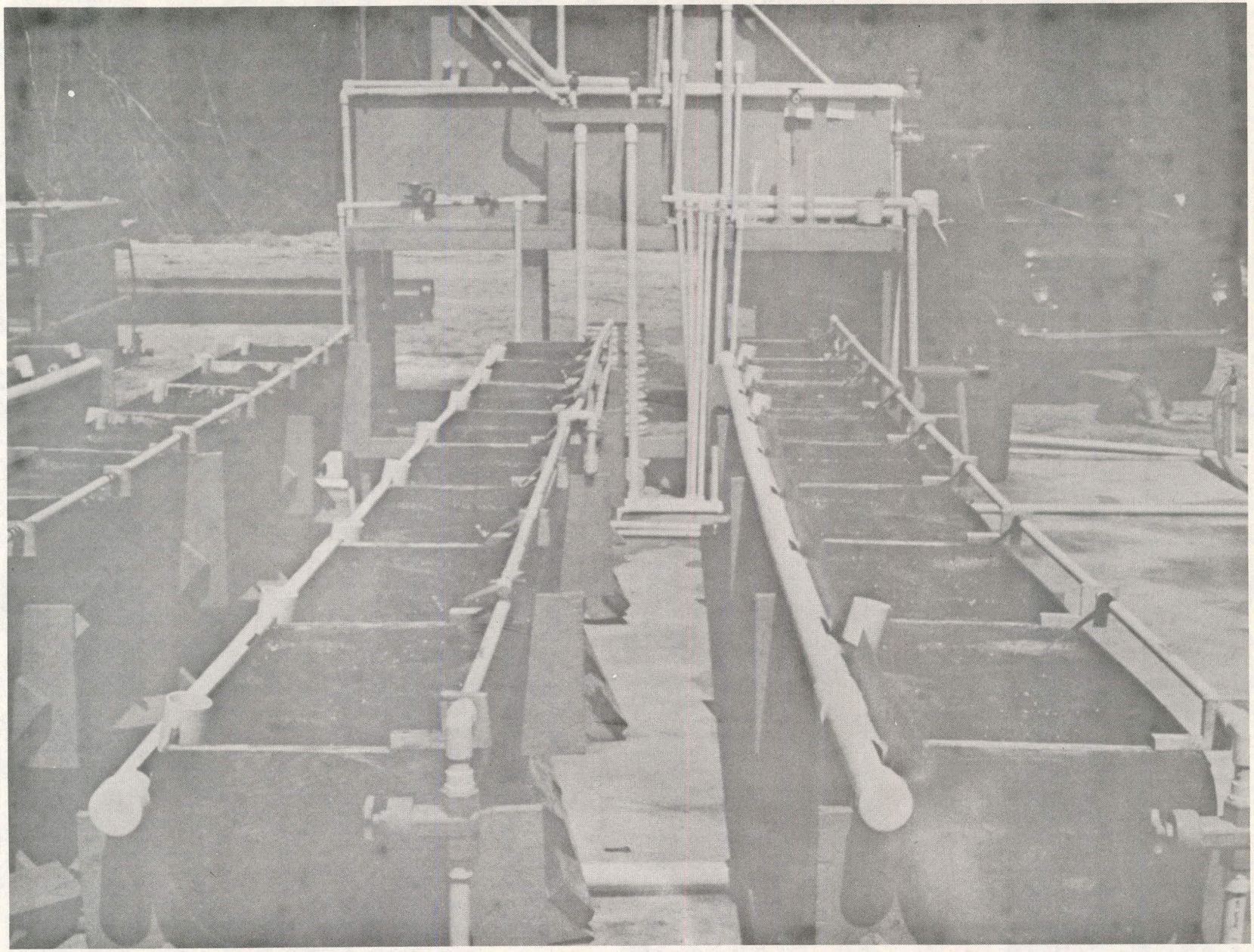

Figure 1. Growth assay chambers constructed from sectioned PVC pipe.

Table 1. Marine algae screened at the Harbor Branch Foundation, Ft. Pierce, Florida. + = growth; - = no growth.

	Summer	Fall	Winter	Spring
Chlorophyta				
Enteromorpha intestinalis			+	
Enteromorpha clathrata	+			
Chaetomorpha linum	+			+
Ulva lactuca			+	+
Codium decorticatum			+	+
Cladophora flexuosa			+	
Rhodophyta				
Gracilaria foliifera V. *angustissima*	+	+	+	+
Gracilaria foliifera		+	+	+
Gracilaria blodgettii	-			
Gracilaria debilis		+	+	
Gracilaria verrucosa		+	+	+
Gracilaria armata	+			+
Gracilaria compressa		+	+	
Gracilaria mammillaris		-	-	
Gracilaria ferox		-	-	+
Gracilaria cervicornis				+
Gracilaria sp.			-	
Gracilariopsis sjoestedtii				+
Neoagardhiella baileyi		+	+	
Solieria tenera		+		
Ceramium rubrum			+	
Spyridia filamentosa			+	+
Polysiphonia subtilissima			+	
Eucheuma isiforme		-	-	
Eucheuma sp.	-	-		
Hypnea musciformis		+	+	+
Hypnea cervicornis			+	+
Chondria tenuissima	-	-	-	
Acanthophora spicifera	-	-		
Acanthophora muscoides	-			
Dasya sp.	-	-	-	
Halymenia agardhii	-			
Halymenia floridana	-			
Chrysymenia halymenioides	-			
Botryocladia sp.	-	-		
Bryothamnion triquetrum	-	-		
Bryothamnion sp.		-		
Gigartina acicularis		-		
Gelidiopsis intricata		-		
Laurencia papillosa		-		
Phaeophyta				
Dictyota dichotoma	-	-		
Padina vickersiae	-	-	-	

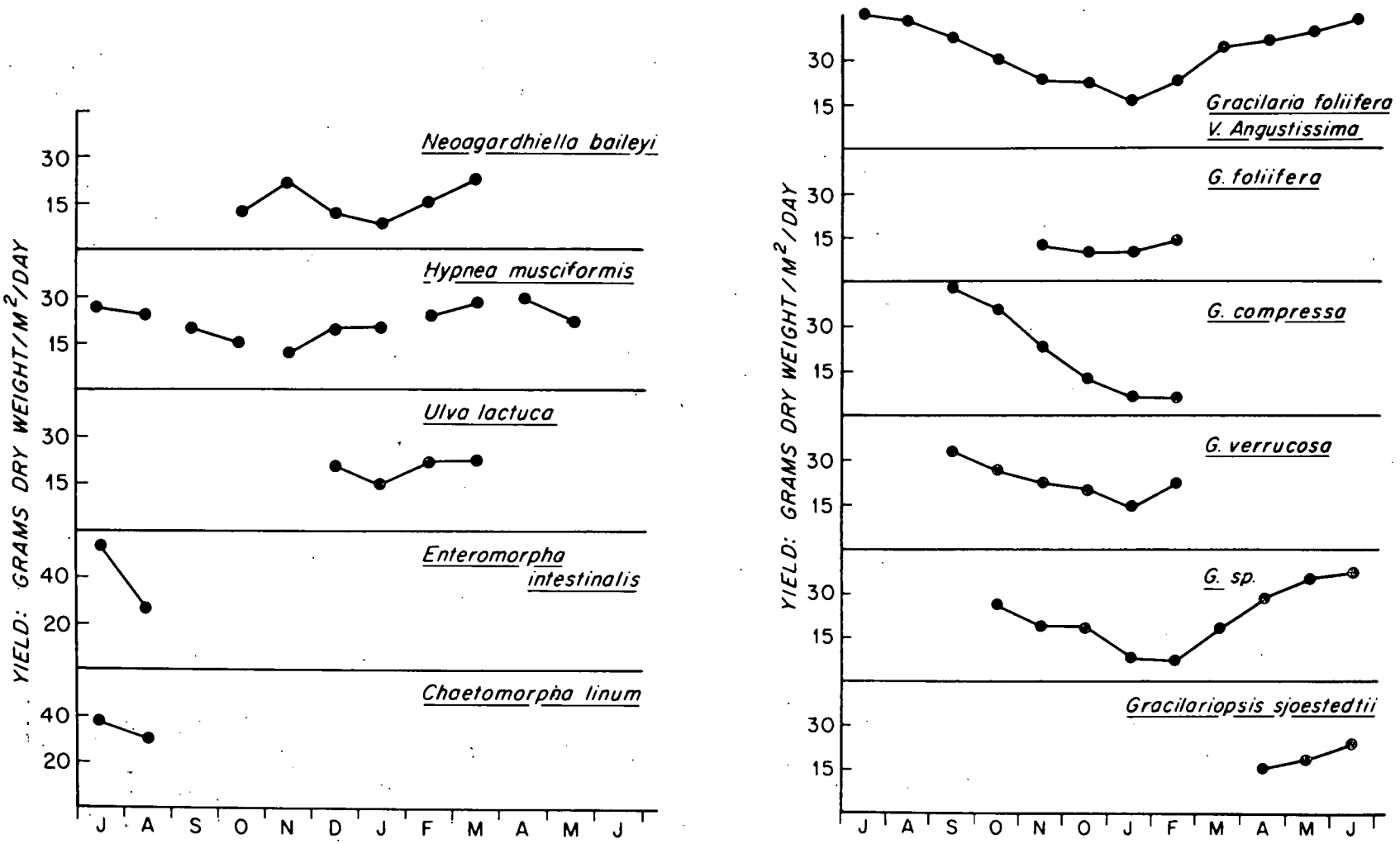

Figure 2. Yields of those species of seaweeds that grew successfully for significant periods of time during the screening tests.

Growth of *Gracilaria foliifera* in non-intensive culture systems

by

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One of the problems with growing algae in suspended culture (e.g., Lapointe et al., 1976; Ryther et al., 1978) is that large volumes of water and compressed air must be supplied to the cultures to achieve the best results. Such practices are highly energy intensive and, on a large commercial scale, would almost certainly prove not to be cost-effective, either economically or in terms of the net energy output:input ratios. For that reason, efforts have also been made simultaneously to develop other, non energy-intensive culture methods and to determine long-term yields of seaweeds grown under those conditions, in each case using the same strain of G. foliifera V. angustissima for the assessment.

Other species of Gracilaria are currently farmed in Taiwan in a non-intensive culture method, primarily in shallow ponds that were formerly used for fish culture (primarily for milkfish, Chanos chanos) (Shang, 1976). Gracilaria farms are started by seeding the ponds with vegetative fragments of the alga. The Gracilaria plants are not tied to netting or otherwise attached, but they are usually held down by bamboo stakes or covered with netting to prevent their being washed to one end of the pond by wind action. The alga grows at depths ranging from 20 to 80 cm in water ranging in salinity from 8 to 30‰ and at temperatures of 10 to 30°C, with one exchange of water every two to three days.

Gracilaria farming is highly labor intensive, with epiphytes (other undesirable species of algae overgrowing the plants)

representing the major problem. This is sometimes partially controlled by introducing herbivorous fishes (milkfish, Tilapia) into the ponds, but the latter may also consume the Gracilaria if the epiphytes are not adequate fodder for the fishes. Yields are reported to be 10 metric tons (dry wt)/ha.year.

At the Harbor Branch Foundation, four non-intensive methods of growing Gracilaria have been assessed. These are described below.

1) Plastic mesh trays made from ca. 2 cm Vexar on 2.5 cm diameter PVC pipe frames measuring 1 m x 1 m x 25 cm deep (Fig. 1) were either floated at the surface or anchored to the bottom in about 1 m of water in the Indian River (a large, shallow embayment behind the barrier beach along Florida's central East Coast). Gracilaria was stocked in the trays at a density of approximately 2.0 kg wet wt/m^2. The seaweed was weighed and its incremental growth since the previous weighing removed at one-week intervals. Trays anchored to the bottom, by tying to aluminum stakes, were covered with a layer of Vexar mesh to prevent the algae from washing out.

Trays were placed in several locations in the Indian River, at distances ranging from 0.5 to about 10 miles from the nearest inlet to the Atlantic Ocean and in areas receiving various amounts of tidal current flow.

Growth of Gracilaria was variable but unpredictable and appeared to be independent of depth (surface or bottom) or location in the Indian River. Yields ranged from 0 to 6 g dry wt/m^2.day and averaged about 2.5 g/m^2.day. Cultures gradually become covered with silt and epiphytes and died after 4-8 weeks.

2) The same plastic mesh trays were floated or sunk to the bottoms of 13 x 5 x 1 m deep PVC-lined ponds (Fig. 2), through which seawater was passed at rates of either 0.5 or 5.0 pond volumes (ca. 20,000 liters) per day. The seawater was enriched with treated sewage effluent or with sodium phosphate and sodium nitrate so as to give N and P concentrations of 1000 and 100 μmoles/l respectively at the slow (0.5/day) turnover rate and 100 and 10 μmoles/l respectively and the fast (5/day) exchange rate. Trays were floated at the surface or covered and sunk to the bottoms of the ponds, as in (1). When the trays were floated, water was pumped from the bottom of the pond and sprayed down on the algae, which was barely submerged beneath the water surface. Otherwise, water circulated through the ponds passed beneath the trays and not through the algae. Gracilaria was stocked in the trays at the same density and harvested in the same routine as in (1)

Growth was highly variable and unpredictable and was apparently independent of culture depth, flow rate of enriched seawater, or nutrient concentration. Yields ranged from 0 to 12 g dry wt/m^2. day and averaged about 5.0 g/m^2/day. Culture become heavily epiphytized and growth ceased after about 8 weeks.

3) Concrete burial vaults measuring 2.2 x 0.8 x 0.6 m deep (ID) were made into culture chambers with PVC piping for input and overflow drains (Fig. 3). G. foliifera was stocked in the vault cultures at the same density and harvested in the same way (1).

Seawater was enriched with treated sewage effluent adjusted with sodium phosphate and sodium nitrate so as to give final concentrations of 50 μmoles/1 N and 5 μmoles/1 P. The enriched seawater was passed through the vaults at an exchange rate of 21 volumes per day.

Growth in the vault cultures of G. foliifera newly collected from the wild or taken from stock cultures was initially high, reaching maximum values of 25 g dry wt/m^2.day. However, yields gradually decreased with the age of the culture to levels of 5-10 g/m^2.day and eventually ceased growing entirely. Mean yield during the time the cultures remained viable was approximately 10 g/m^2.day.

4) The technique for growing Gracilaria that was most recently developed at the Florida research facility involved growing the seaweed in trays completely out of water but continuously irrigated by enriched seawater sprayed down on the algae from above (Fig. 4). This method is based upon a technique described by A.R.O. Chapman and attributed by him to

Seawater enriched with 100 μmoles N and 15 μmoles/1 P as sodium nitrate and sodium phosphate respectively was pumped through

a series of household shower heads upon plastic 0.6 x 0.6 x 0.08 m trays (constructed as shellfish grow-out trays by Nestier Corp., Cincinnati, Ohio), using four shower heads per tray, at a rate of irrigation that was equivalent to approximately 50 liters per min. per m^2 of tray surface. The enriched seawater used for the spray was held in a 400-liter reservoir which was continuously recirculated (i.e., every eight minutes). In addition the reservoir was continuously replaced by new enriched seawater at a rate of 20 volume exchanges per day, or a little more than five liters per minute. The choice of the exchange rate of the reservoir, the nutrient content of the enriched seawater, and the rate of spraying the seaweed were all arbitrary and will be the subjects of designed experimental variation in future studies.

Gracilaria was stocked at different densities ranging from less than one to 12 kg wet wt/m^2 of tray surface, to determine optimal density for best growth. In contrast to suspended cultures, where yields fall off sharply above or below a density of 2-4 kg/m^2 (e.g., Ryther et al., 1978), growth was relatively independent of density in the spray-cultures over the entire range tested above approximately 2 kg/m^2. Yields averaged about 20 g/m^2.day over an eight-week period during the fall of 1977. One reason for the good growth at high algal densities was the fact that the seaweed grew vertically upward in the trays. Eventually, the Gracilaria become heavily epiphytized in this, as in all other

culture methods tested, and the cultures had to be discarded and started with newly-collected material.

The yields and other characteristics of Gracilaria obtained by the four non-intensive culture methods described above are shown in Table 1, which also includes that from the commercial pond culture in Taiwan, as described above and by Shang (1976), and the yield from the highly-intensive culture method described elsewhere in this report (e.g., Ryther et al., 1978)

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Table 1. Yields and other characteristics of Gracilaria grown by different culture methods.

Method	$g/m^2.day$* (mean)	$g/m^2.day$* (range)	t/ha.yr*	Exchange rate (volumes/day)
Ponds, Taiwan (Shang, 1976)	2.7		10	0.3-0.5
Indian River cages	2.5	0-10	9	not known
Pond cages	5.0	0-10	18	0.5-5.0
Cement vaults	10.0	0-25	37	21
Spray cultures	20	0-35	73	20
50-l PVC tanks, aerated (suspended culture) (Ryther et al., 1978)	35	0-55	127	22

*Total dry weight (ash included).

Figure legends

Figure 1. Vexar trays used for culture methods (1) and (2).

Figure 2. PVC-lined ponds used for try culture (method 2).

Figure 3. Concrete burial vaults used for seaweed culture (method 3).

Figure 4. Spray method (4) for growing Gracilaria.

Fig. 1

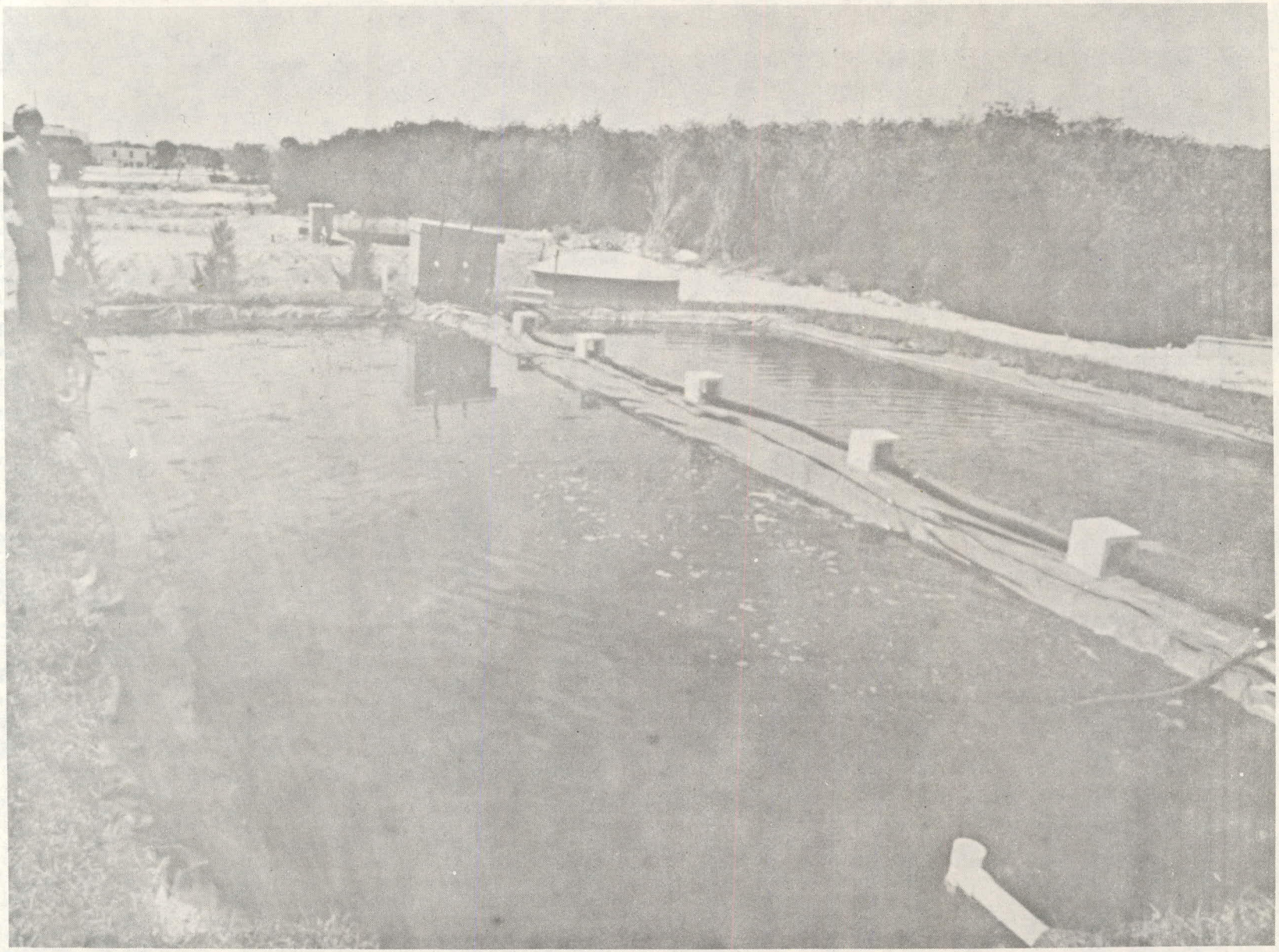

Fig. 2

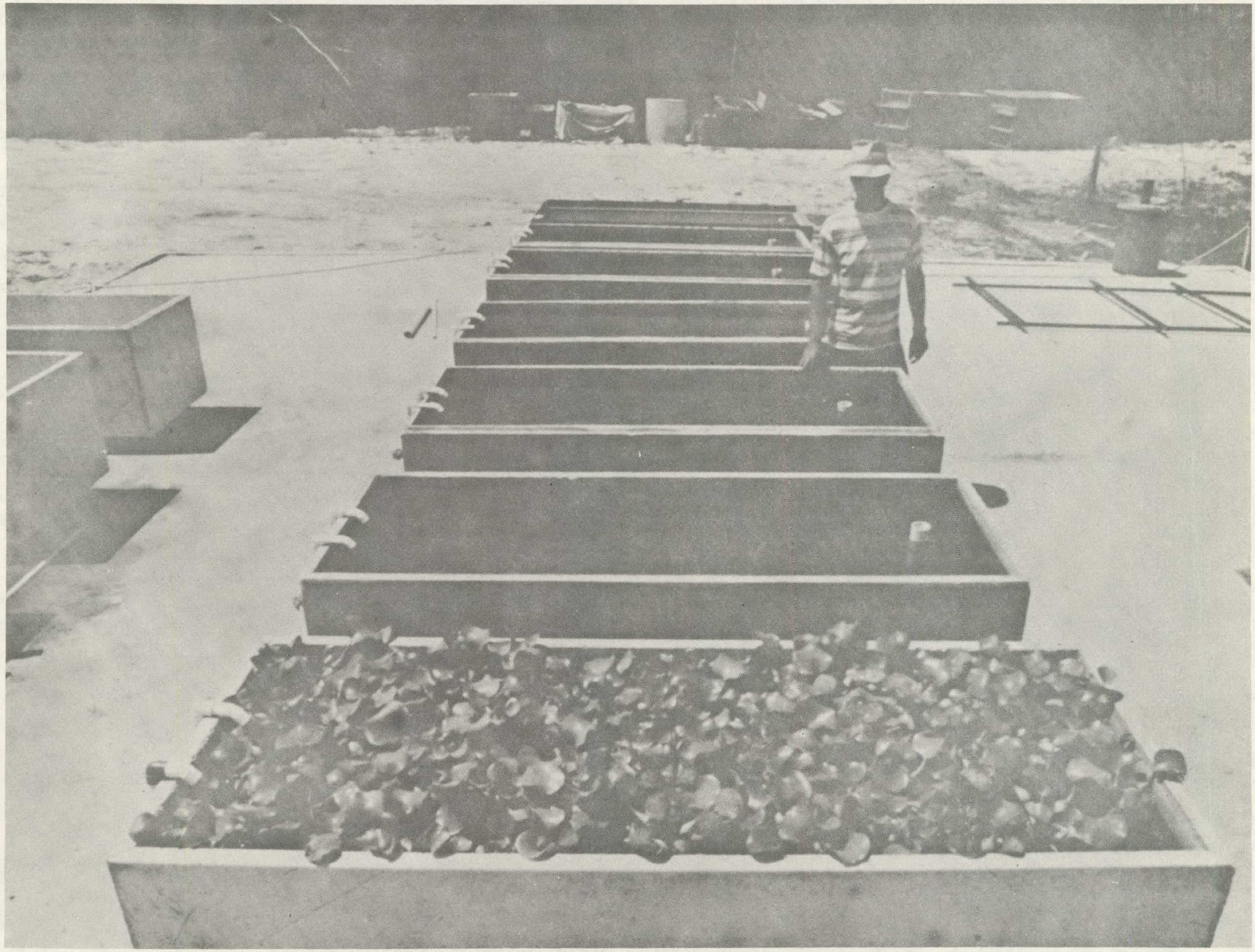

Fig. 3

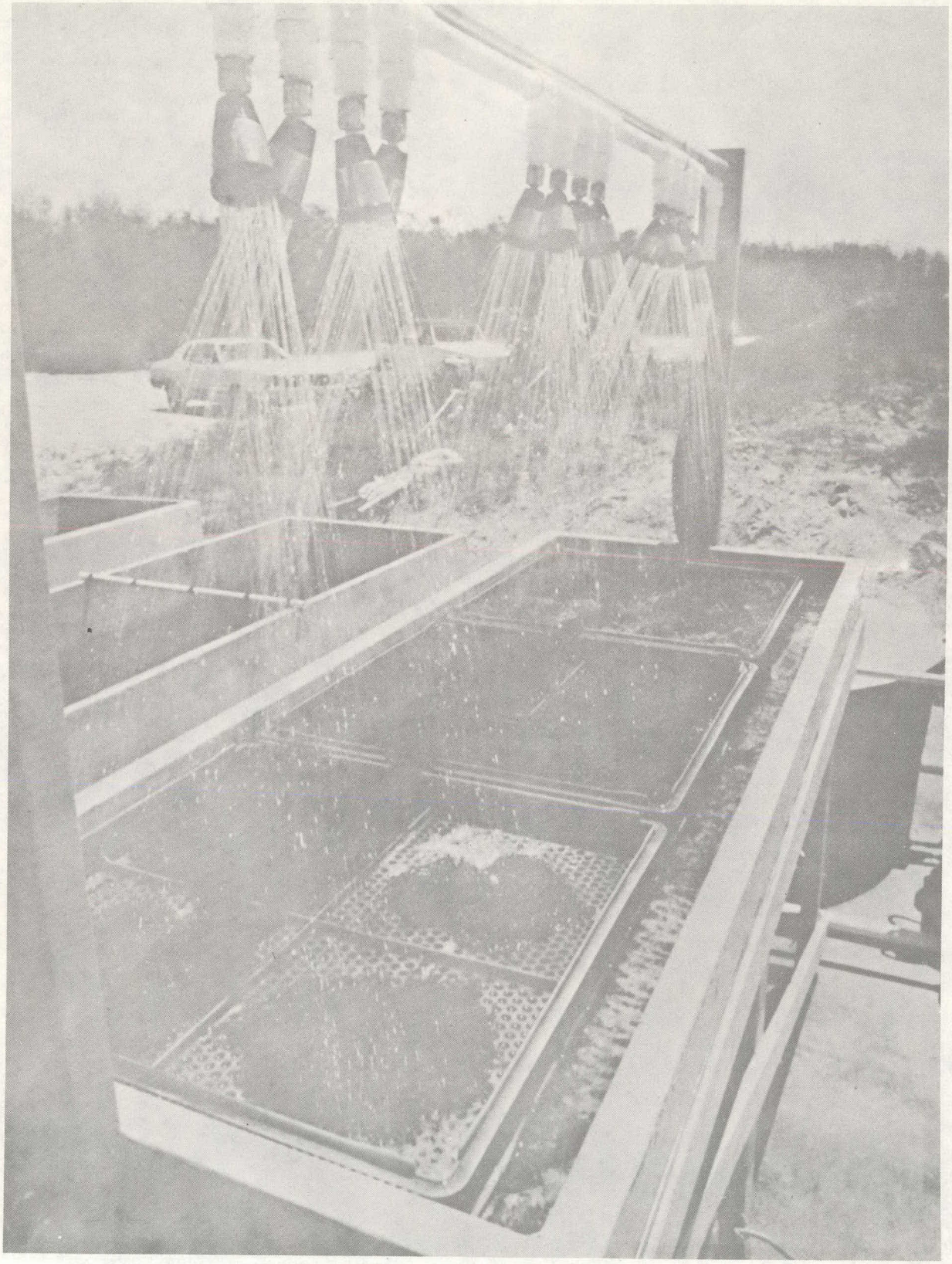

Fig. 4

Growth and yields of the freshwater weeds *Eichhornia crassipes* (water hyacinth), *Lemna minor* (duckweed), and *Hydrilla verticillata*

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Freshwater aquatic weeds grow throughout the world in lakes, ponds, rivers, and canals Where nutrient inputs from wastewater, agricultural runoff, and other source are sufficient, these plants spread rapidly, choking the waterways and rendering them impassible, and generally assuming nuisance proportions. Over $3 million is spend annually in Florida alone for aquatic weed control.

Of the many species of common freshwater aquatic plants, several are particularly notorious for their abundance and presumed rapid growth rates. These include emergent species such as water hyacinth (Eichhornia crassipes) and the duckweeds (Lemna, Spirodela, Wolffia), only the roots of which are in the water, and completely submerged species of higher plants such as Hydrilla, Egeria, Ceratophyllum (coontail), and Najas (Southern naiad).

Although high levels of productivity are attributed to all of the above aquatic "weed" species, in fact their yields have seldom if ever been actually measured and reliable data are almost entirely lacking in the scientific literature.

Detailed economic analyses have been carried out of hypothetical water hyacinth farms for municipal wastewater treatment (Robinson et al., 1976) and for energy conversion (Lecuyer et al., 1976), but the authors of the first of these reports admit to an uncertainty in the potential yield of these plants between 8 and 151 tons/hectare/year. The latter figure (equivalent to about 60 tons/acre), which

is most frequently quoted in projections concerning the potential productivity of water hyacinths, has its origin in Westlake (1963). However that author actually states "It seems possible that about 150 m tons/ha/year might be obtained in a good climate if Eichhornia crassipes could be grown so that young plants always predominated and the water surface was always completely covered... etc." Earlier in the same article, Westlake states "However, reliable data on its (hyacinth's) annual productivity has not been found." The latter fact, unfortunately, hold true up to the present.

Experiments were initiated in the Spring of 1977 at the Harbor Branch Foundation (Ft. Pierce, Florida) for measurement of the productivity of three species of freshwater needs, the floating plants water hyacinth (Eichhornia crassipes) and duckweed (Lemna sp.) and the submerged, rooted plant Hydrilla verticillata. These studies were carried out in 12 x 2.4 x 1.2 m deep PVC-lined earthen ponds (15,000-20,000 liter volume, ca. 30 m^2 area) and in concrete burial vaults measuring 2.2 x 0.8 x 0.6 m through which well water enriched with sodium nitrate and sodium phosphate was passed at varying residence times (0.5 to 16.5 days) and varying concentrations of N (50-1500 μmoles/l) and P (5-150 μmoles/l).

Experiments with Hydrilla were delayed because of the problem of how to measure productivity (i.e., weight increase per unit time and area) in a plant that grows only when rooted to the bottom, the culture of which must be virtually destroyed to obtain the measurement.

This problem was resolved when it was found that Hydrilla would grow if the root ends were tied to a piece of Vexar screening suspended a short distance off the bottom of the pond. In this way, the entire section of Vexar mesh with plants attached could be removed and weighed without harm to the plants or their root systems.

Some very preliminary experiments were also carried out with the giant duckweed (Spirodela polyrhiza), water lettuce (Pistia stratioties) and the filimentous algae Hydrodictyon and Rhizoclonium but these will not be reported upon here except for one experiment with Spirodela.

The general procedure used in measuring the yields of these freshwater plants was the same as that employed for measuring the productivity of seaweeds (e.g., Lapointe et al., 1976). The entire culture was removed from the pond or vault at intervals of approximately one week and the excess water drained from the plants by shaking them gently in mesh bags or in Vexar screen baskets. The culture was then weighed and returned to the water as quickly as possible to avoid damage by desiccation. In the case of water hyacinth and duckweed, the incremental growth since the previous weighing was routinely removed and a constant starting biomass and culture density thereby returned to the culture unit.

In the case of Hydrilla, harvesting the incremental growth was attempted by cutting the tops of the plants off approximately six inches below the water surface. However, it was found that cutting

arrested the growth of the plants for a period of one to two weeks until a new growing tip was developed. That practice was therefore discontinued and thereafter the cultures of that species were not harvested back to a constant starting biomass each time they were weighed, but rather incremental growth was allowed to accumulate in the culture.

In the cultures of water hyacinth and duckweed, where a constant starting biomass was maintained, it was necessary to determine the density at which maximum growth and yield occur. At densities that are too low, all of the incident sunlight is not absorbed by the plants. At excessively high densities, on the other hand, self-shading of the plants and other adverse effects of over-crowding occur.

Table 1 shows the results of a series of experiments with water hyacinth and two species of duckweed in which yield was measured as a function of starting density of the culture. Each data point in Table 1 represents the mean daily rate of production, expressed in grams dry weight per square meter of culture surface, obtained from five one-week growth periods. The experiments were all carried out in the Fall of 1977, when growth of all three species was significantly lower than that observed in late spring and summer. It is possible that maximum yields would have occurred at somewhat higher densities in mid-summer.

The results summarized in Table 1 show that the best yields of the duckweeds occurred at the relatively low density of 0.25

kg/m^2 while that of water hyacinth occurred at a density of 8 kg/m^2, but with an almost constant yield in the entire range of 5-14 kg/m^2. Natural stands of water hyacinth reportedly attain densities as high as 40 kg/m^2 (Penfound and Earle, 1948) but it must be assumed from the present results that little growth can occur under such conditions.

The relatively poor growth of the duckweeds is somewhat surprising but appears to result from the fact that these plants cannot attain a high density per unit area because, once they cover the water surface completely, there is no direction in which their biomass can expand. Hyacinths, on the other hand, can grow upward vertically and may reach a height of as much as one meter above the water surface. Thus, while the specific growth rate (% increase per day) for duckweed and hyacinths may be comparable under optimal growth conditions for each species, the fact that this optimum may persist at a much higher density for hyacinths than for the duckweeds means that yield, the product of specific growth rate and density, will be quite different for the two species. It is possible that higher yields of duckweed could be achieved if the plants were maintained at a higher starting density and then harvested back more frequently than the weekly periodicity that has been used routinely in all of these studies. This possibility will be investiaged in future studies.

Experiments on the growth of seaweeds in suspended culture showed that yields were strongly dependent upon the rate of exchange of water (i.e , residence time) in the cultures, even if nutrient concentrations were varied inversely with flow rate so as to deliver the same quantity of nutrients to the plants per unit of time (Ryther et al , in press). Similar experiments were therefore conducted with water hyacinths, duckweed, and Hydrilla varying the water exchange rate by 0.06, 0.20, and 2.0 volumes per day (16.5, 5.0, and 0.5 days residence time, respectively). In each case, the concentration of nutrients was also varied, as in the seaweed experiments, to provide roughly the same daily inputs of nitrogen and phosphorus. The results, shown in Tables 2-4, indicate that the freshwater plants grow slightly better at the most rapid exchange of water but that the differences are relatively small, in sharp contrast to the situation found with the seaweeds.

However, it was found that when the more rapidly growing water hyacinths were maintained at the lowest rate of water exchange (16.5 days residence time) for long periods of time (months), the plants gradually became chloratic and somewhat flaccid in appearance and they became heavily infested with mites, and their yields declined sharply. This situation was reversed by spraying a commercial mixture of minor nutrients directly on the foliage at one-week intervals. It was clear from that experience that the local well water enriched only with nitrogen and phosphorus was an inadequate

culture medium for long-term growth of the plants, some one or more essential element or compound eventually becoming growth limiting. It is therefore planned to formulate a complete enrichment medium for future studies of these freshwater plants. With respect to the experiments summarized in Tables 2-4, it is therefore believed that such differences as were observed in yields obtained at the different residence times were probably due to limitation of some nutrient other than nitrogen and phosphorus in the freshwater supply.

Continuous cultures of water hyacinth and duckweed have been maintained since May, 1977 and of Hydrilla since August, 1977 until the time this report was written, 8 and 5 months respectively. However, the same clone of each species has not been grown over that entire period. The major pond culture of hyacinths died back and became heavily infested by insects, as discussed above, and, though it did eventually recover, no growth data from it were obtained from 9/2 to 11/1. To obtain a continuous record of water hyacinth yields, it was necessary to use data for that period from the concrete vaults where other experiments with that species were in progress, using only those experiments in which the operating conditions were comparable to those in the pond culture. The mean weekly yields of water hyacinths from May to November are given in Table 5 and show a range of 6.2 to 26.2 grams (dry weight)/m^2.day with a mean for the entire period of 16.2 g/m^2.day. A clear

seasonal periodicity was observed, with yields in the late fall somewhat less than half those observed in mid-summer. It must be assumed that yields for the remaining four months of the year will be still lower and that the mean annual yield will be significantly less than those obtained for the Spring-Fall period.

There is no indication that the seasonal periodicity in water hyacinth growth is caused by anything more than the normal seasonal changes in solar radiation and temperature. Some of the plants were in flower during the entire period, but this did not appear to have affected the productivity of the population as a whole.

The mean weekly yields of duckweed (Lemna minor) for roughly the same period are shown in Table 6. Once at the end of July and again in mid-September, the duckweed pond became completely overgrown with freshwater filimentous algae of the genera Hydrodictyon and Rizoclonium and had to be discarded and replaced with new plants collected nearby. Again, in mid-August, a severe wind storm literally blew the plants out of the pond and dispersed them over the country side, also requiring replacement of the culture. With the exception of those brief hiatuses, a continuous record of yields has been kept showing a range of 2.1 to 7.0 grams dry weight/m^2.day and a mean for the May-November period of 4.6 g/m^2.day, only 28% of that for water hyacinth. Although the highest yields of duckweed occurred in July, as was true with water hyacinths, the seasonal variability in duckweed growth is not as great as that of

hyacinths. Duckweed is apparently more cold resistant than hyacinths, having persisted in the wild throughout the cold winter of 1976-77 in central Florida, when the water hyacinths were uniformly killed back. Duckweed also extends much further north in its natural range than does the water hyacinth. It is likely, therefore, that the yield of duckweed in winter and its mean annual production will not decline as much as that of the water hyacinth from the values shown in Tables 5 and 6.

Hydrilla was grown in ponds and vaults with its roots attached to square panels of Vexar mesh, as described above. Growth in the two kinds of culture units was essentially the same. Yields from August 24 to November 17 ranged from zero to 14 grams dry weight/m^2.day and averaged 5.1 g/m^2.day (Table 7). However, there was observed a distinct seasonality in the growth of this species that was not directly the result of sunlight and temperature changes. In October, reportedly as a result of a photoperiodic response to the shortening days, the plants all developed flowers and virtually stopped growing. While the blossoms of Hydrilla are extremely small and their production can utilize no more than a negligible fraction of the photosynthetic organic production of the plant, florescence is apparently accompanied by other physiological changes, including tuber formation, and vegitative growth slows or stops and does not recommence until the following spring (Dr. William Haller, U. Florida, Gainesville, FL, personal communication).

It follows, then, that the mean annual daily yield of Hydrilla will be considerably less than the 5.1 g/m^2.day observed during the August-November period. However, the plant has not yet been worked with long enough to have any feeling for whether or not the yields in culture to date are representative of those in nature or represent the best that the plant can do.

There was some question of whether or not attachment of the plants to Vexar mesh would result in growth as good as that of plants rooted to bottom. An experiment was therefore conducted to compare the growth of plants attached to the screening with those rooted in sand and in mud (Table 8). Growth was roughly comparable in all three cases, though the experiment was done late in the year when growth was greatly reduced in any case, so the results are somewhat questionable and the experiment needs to be repeated under more favorable conditions.

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Table 1. Effect of culture density on the yield of three species of fresh-water plants harvested weekly and maintained at the indicated starting density.

Culture density	Yield (g dry wt/m^2/day)		
kg wet wt/m^2	Lemna minor	Spirodela polyrhiza	Eichhornia crassipes
0.05	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		6.0
3.00	0.6		
5.00			9.0
8.00			9.7
11.00			9.0
14.00			4.1
17.00			4.0

Table 2. Effect of water exchange rate (residence time) on the yield of water hyacinth grown in ponds and harvested at weekly intervals and maintained at a constant starting density of 10 kg wet wt/m^2.

Dates (1977)	Residence time (days)	Influent N (μmoles/l)	Influent P (μmoles/l)	Mean yield g dry wt/m^2.day
6/3-9/7	16.5	1500	150	11.1
5/16-9/7	5.0	1000	100	15.0
5/16-6/3	0.5	100	10	16.7

Table 3. Effect of water exchange rate (residence time) on the yield of duckweed (Lemna minor) grown in ponds, harvested weekly, and maintained at a constant starting density of 0.5 kg wet wt/m^2.

Dates (1977)	Residence time (days)	Influent N (μmoles/l)	Influent P (μmoles/l)	Mean yield (g dry wt/m^2.day
6/6-11/16	16.5	1500	150	3.8
6/6-11/30	5.0	500	50	4.6
5/16-11/30	0.5	50	5	4.5

Table 4. Effect of water exchange rate (residence time) on the yield of Hydrilla verticillata grown in ponds and vaults with roots attached to Vexar screening. Plants weighed weekly but not harvested during growth period.

Dates (1977)	Residence time (days)	Influent N (μmoles/l)	Influent P (μmoles/l)	Mean yield (g dry wt/m^2/day
8/24-11/17	5.0	500	50	5.1
7/7-11/8	0.5	50	5	8.5

Table 5. Yields of water hyacinth grown in ponds or vaults at a water exchange rate (residence time) of 0.5 days in well water enriched with N (100 μmole/l) and P (10 μmoles/l), harvested weekly and maintained at a constant starting density of 10 kg (wet wt)/m^2.

Time interval (1977)	Mean yield (g dry wt/m^2/day)
5/16-5/24	14.9
5/24-6/3	18.5
6/27-7/5	7.9
7/5-7/12	18.5
7/12-7/19	21.6
7/19-7/26	23.5
7/26-8/2	25.4
8/2-8/9	26.2
11/1-11/8	6.2
11/8-11/15	9.9
11/15-11/22	15.3
11/22-11/30	11.5
11/30-12/9	10.6
Mean	16.2

Table 6. Yields of duckweed (Lemna minor) grown in ponds at a water exchange rate (residence time) of 5 days in well water enriched with N (500 μmoles/l) and P (50 μmoles/l), harvested weekly and maintained at a constant starting density of 0.5 kg wt wt/m^2.

Dates (1977)	Mean yield (g dry wt/m^2/day
5/18-6/6	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
7/15-722	6.1
7/22-7/29	6.0
7/29-8/3	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 (culture overgrown, restocked)
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
Mean	4.6

Table 7. Yields of Hydrilla verticillata grown in ponds or vaults with roots attached to Vexar screening at a water exchange rate (residence time) of 5 days in well water enriched with N (500 μmoles/l) and P (50 μmoles/l). Plants weighed weekly but not harvested during growth period.

Dates (1977)	Mean yield (g dry wt/m^2/day)
8/24-9/6	7.0
9/6-9/13	9.8
9/13-9/22	14.0
9/22-9/30	9.2
9/22-10/5	7.6
10/5-10/12	13.2
10/7-10/19	6.5
10/19-10/26	3.2
10/26-11/2	0.0
11/2-11/9	1.7
11/9-11/17	1.5
Mean	5.1

Table 8. Comparative yields of Hydrilla verticillata in vaults with roots attached to Vexar screening and rooted in sand and mud, with water exchange rate (residence time) of 5 days in well water enriched with N (500 μmoles/l) and P (50 μmoles/l). Cultures all started at a density of 2.0 kg wet wt/m^2. Yields measured during the period 10/14-11/7/77.

Substrate	Mean yield (g dry wt/m^2/day)
Vexar screening	2.9
Mud	3.8
Sand	2.5

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Availability of digester residues from anaerobic fermentation of seaweeds and freshwater plants as nutrient sources for growth of the same plants.

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Dynatech Report No. 1686
Dynatech Project No. WOD-1

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Section 1
SUMMARY

Experiments were carried out to provide Woods Hole Oceanographic Institute (WHOI) with nutrient rich effluent from the anaerobic fermentation of aquatic biomass. This effluent is to be used by WHOI to evaluate nutrient recycle in the cultivation of aquatic biomass. Two freshwater aquatic plants and two marine algal species were used; these are potential candidates for mass cultivation and ultimate conversion to synthetic fuels. Plants tested were the freshwater species Duckweed (Lemna sp.) and Hydrilla. Marine (saltwater) algae tested were the red alga Gracilaria sp. and the green alga Ulva lactuca. As part of the experiments, preliminary base-line information was obtained on the conversion to fuel gas under selected fermentation conditions for each type of aquatic biomass.

Under thermophilic conditions (58°C), Duckweed exhibited a methane yield of 3.91 ft^3/lb v.s. relative to a yield of 2.15 ft^3/lb v.s. under mesophilic conditions (37°C). Other tests were conducted at mesophilic temperatures and CSTR digester retention times of 26 days using sewage sludge as a source of supplemental nutrients. All experiments were carried out in 50 liter (15 gallon) continuously stirred fermenters. Net methane yield, expressed in ft^3 CH_4 @ STP per pound of volatile solids fed, was 2.15 for Duckweed, 2.91 for Hydrilla, near zero for Gracilaria and 2.45 for Ulva lactuca at the mesophilic conditions. These results are expressed as averages over a 77 day experimental interval. However, improved methane yields from the marine algae were observed during the later stages of the experiment, possibly indicative of culture adaption. Plans for continued work are discussed.

Section II

INTRODUCTION

A number of investigators have proposed the growth of biomass crops as a source of renewable fuel. After harvesting, biomass crops may be converted by any of a variety of methods to readily usable liquid or gaseous fuels, compatible with existing distribution and use patterns. A specific process under consideration is growth of freshwater aquatic or marine (saltwater) biomass, followed by anaerobic fermentation (digestion) of the biomass to methane. Among attractive aspects of this concept are (1) aquatic biomass growth systems would not compete with land-based food and fiber production, and (2) the anaerobic fermentation (digestion) process promises a relatively economic method for conversion for which much of the technology is developed. However, both processing sequences and economics for these processing sequences must be established.

As part of the requisite examination to determine the feasibility of water-based growth systems to produce fuel and other products from biomass, a program is being conducted by the Woods Hole Oceanographic Institute (WHOI), Woods Hole, Mass., for the Fuels from Biomass Branch of the Department of Energy, Washington, D.C. In this program, Woods Hole is establishing culture requirements, optimum growth conditions and yields for several candidate marine algae and freshwater plants at the Harbor Branch Foundation, Ft. Pierce, Florida. Biomass produced in these experiments is then utilized in digestion experiments at Dynatech R/D and the effluent returned to WHOI for use in nutrient recycle experiments. This report describes initial results of that portion of the program being conducted at Dynatech R/D.

The objective of the experiments being conducted by Dynatech is to supply to WHOI the nutrient rich effluent from the digestion of the

freshwater weeds Duckweed (Lemna sp.) and Hydrilla, the red marine alga Gracilaria ceae and the green alga Ulva lactuca. The scope of work is limited to the evaluation of the digestion characteristics of each of these species under representative conditions. It is anticipated that results from this work will be helpful in establishing likely economics of the combined growth/digestion process. Because of the necessity to compare results with other workers, the initial work is being carried out under "typical" conditions employing mesophilic stirred task reactors. Limited additional work is currently being conducted under thermophilic conditions and also on pretreatment.

Details of experiments, results, and plans for future work are presented in the sections which follow.

Section III

EXPERIMENTAL

3.1 Biomass Handling

The biomass grown at the Harbor Branch Foundation was dried to 10-20% moisture and shipped in drums to Dynatech, where it was tested for moisture content, total solids and volatile solids and stored until use. To prevent hydration and/or dehydration each test lot was sealed in polyethylene garbage bags except at times when samples were withdrawn. Testing was as described in Standard Methods, 13th edition.

3.2 Digesters

Digesters consisted of modified 50 liter carboys equipped with three horizontal one-foot stirrer bars mounted on a central vertical shaft. All units were stirred at 100 rpm using a shaft/gearbox arrangement allowing either 3 or 6 digesters to be stirred with a single synchronous constant-speed drive motor. Thus stirring was identical for all units used in this work. Gas-tight oil-sealed bearings were used on all units which also served as shaft seals. Medium withdrawal and feed were accomplished by means of a stoppered port above the liquid level in the digester. To insure gas tightness this stopper was greased and held in by a tensioning spring. Temperature was controlled either by placing the digesters in a constant temperature incubator room or constant temperature ethylene glycol baths. Feeding of digesters was accomplished by "draw and fill" daily, withdrawing effluent and adding the required amount of medium to give the desired retention time.

Since digesters were generally operated with supplemental sewage sludge, which was the same for all digesters daily, a control digester was operated which was fed the same amount of sewage sludge alone. Methane output

from this digester was measured and used to compensate for the production due to the sewage sludge component, in order to compute net yield from each biomass.

3.3 Computation of Methane Production

Exit gas composition was analyzed daily by means of peak height analysis with a Fisher model 25 v gas partitioner, calibrating against a CH_4/CO_2 standard of known composition. Previous tests on other programs have established that this method of analysis is linear and reproducible to $\pm$ 1% for methane and carbon dioxide components. Exit gas output was measured, also daily, with precision gas wet test meters (Model 63126, Chicago, Ill.). Methane output was computed from the composition and output of the exit gas stream, correcting for temperature and water vapor content to obtain methane output at STP (0°C, 1 atm,).

3.4 Other Routine Analyses

Each biomass, on receipt from the Harbor Branch Foundation, was analyzed for total and volatile solids as noted. Sewage sludge was likewise analyzed. Although total and volatile solids were also analyzed in the digester effluent, it proved difficult to obtain representative samples from the heterogeneous digester contents, and results showed considerable scatter.

Periodically, effluent from digesters was analyzed for free ammonia nitrogen and dissolved orthophosphate to insure that levels of these nutrients were adequate. Ammonia nitrogen was analyzed with an ion-selective ammonia electrode (Model 95-10, Orion Instruments, Cambridge, Mass.) and dissolved orthophosphate by a modification of the method of Fiske and Subbarow using test kits from Fisher Scientific.

pH was measured daily. Spot checks of volatile organic acid levels were conducted using a Gow-Mac Model 69-750 gas chromatograph with FID, using a 2 foot porapak QS column at 180°C.

Section IV

RESULTS

4.1 Total and Volatile Solids of Biomass

Upon receipt, duplicate assays were performed on each of the four species for total solids and volatile solids. Where two containers of one species were received, lots were taken from the separate containers. Results of these assays are presented in Table 4.1; this feedstock with characteristics shown has been used to date in the study.

4.2 Initial Mesophilic Digestion Trials

Initial trials were conducted according to the plan outlined in the original proposal of December 1976, e.g., at 15 day retention time, 37°C, using a 5% solids feed with a 90/10 ratio of biomass solids to sewage sludge solids. Also, in this initial trial an objective was to determine whether "as received" materials could be readily digested, avoiding both the extra costs and energy requirement for comminution. Digesters were started with effluent from the Nut Island Sewage Treatment Plant, Quincy, Mass. Upon observing satisfactory digester operation on pure sewage sludge for two weeks, test biomass feed was begun as outlined. Two problems were observed on this feed regimen. First, the biomass (particularly duckweed) tended to either float on the surface of the digester, or clump. Secondly, volatile acid buildups began after about one week, with concomitant decreases in methane production. The souring problem was not controlled with base addition (these data are not shown), and supplemental ammonia addition to 200 mg/l was of no benefit.

Because of the limited scope of the program, and the necessity to determine the basic yield data, less stringent operating conditions were chosen to assure satisfactory digestion as outlined in 4.3 below.

Table 4.1

TOTAL AND VOLATILE SOLIDS OF BIOMASS AS RECEIVED FROM HARBOR BRANCH FOUNDATION

Total Solids (percent by weight)

Biomass Species	Determination		Average
	#1	#2	
Gracilaria ceae	93.76	93.51	93.6
Ulva lactuca	86.61	96.96	86.8
Duckweed	90.63	90.11	90.4
Hydrilla	89.44	89.58	89.5

Volatile Solids (As percentage of total solids)

Biomass Species	Determination		Average
	#1	#2	
Gracilaria ceae	65.37	72.96	69.2
Ulva lactuca	57.95	52.94	55.6
Duckweed	78.47	69.72	74.1
Hydrilla	80.29	71.55	69.2

4.3 Mesophilic Digestion at Long Retention Time

In view of these initial results, several modifications were made in mesophilic digester operating procedures. Digester head space was reduced to the minimum volume possible, to minimize potential problems due to oxygen entry during feeding, by increasing fermenter liquid contents from 50 to 52 liters. Feed composition was changed to 50 grams of the test biomass in one liter of water daily, with one liter of supplemental sewage sludge (40-50 gm/l solids) to give a total of two liters of feed daily and a 26-day retention time. Size reduction of each test biomass was carried out by blending each 50 gram lot of the test biomass for 30 seconds in one liter of water in a Waring blender. Activated carbon (Hydrodarco H, ICI America, which was shown in previous work at Dynatech to facilitate digestion of various substrates) was added in 50 gram lots approximately once weekly. Finally, supplemental ammonia was added at intervals of approximately once a week, to bring the free ammonia level up to 170-200 ppm (10-12 meq/l) when necessary.

Following these procedures, digester operation was satisfactory from the standpoint that "souring" did not occur (as evidenced by pH and spot acid checks by GLC) and methane output was continuous.

Methane output due to the sludge component was measured in one or two control digesters, maintained identically to the biomass digesters except that biomass was not added. Figure 4.3.1 compares initial results for two control sewage sludge digesters, operated identically, over the same eight day interval. Agreement between output of these two digesters was considered sufficiently close so that only one control digester was used in subsequent work.

Figures 4.3.2 through 4.3.5 show methane production (S.T.P., exclusive of water vapor and associated carbon dioxide), for digesters operated mesophilically as described on Duckweed, Hydrilla, Gracilaria sp. and Ulva lactuca. Also shown for reference in each figure is the methane output for the control digester operated concurrently on sewage sludge alone.

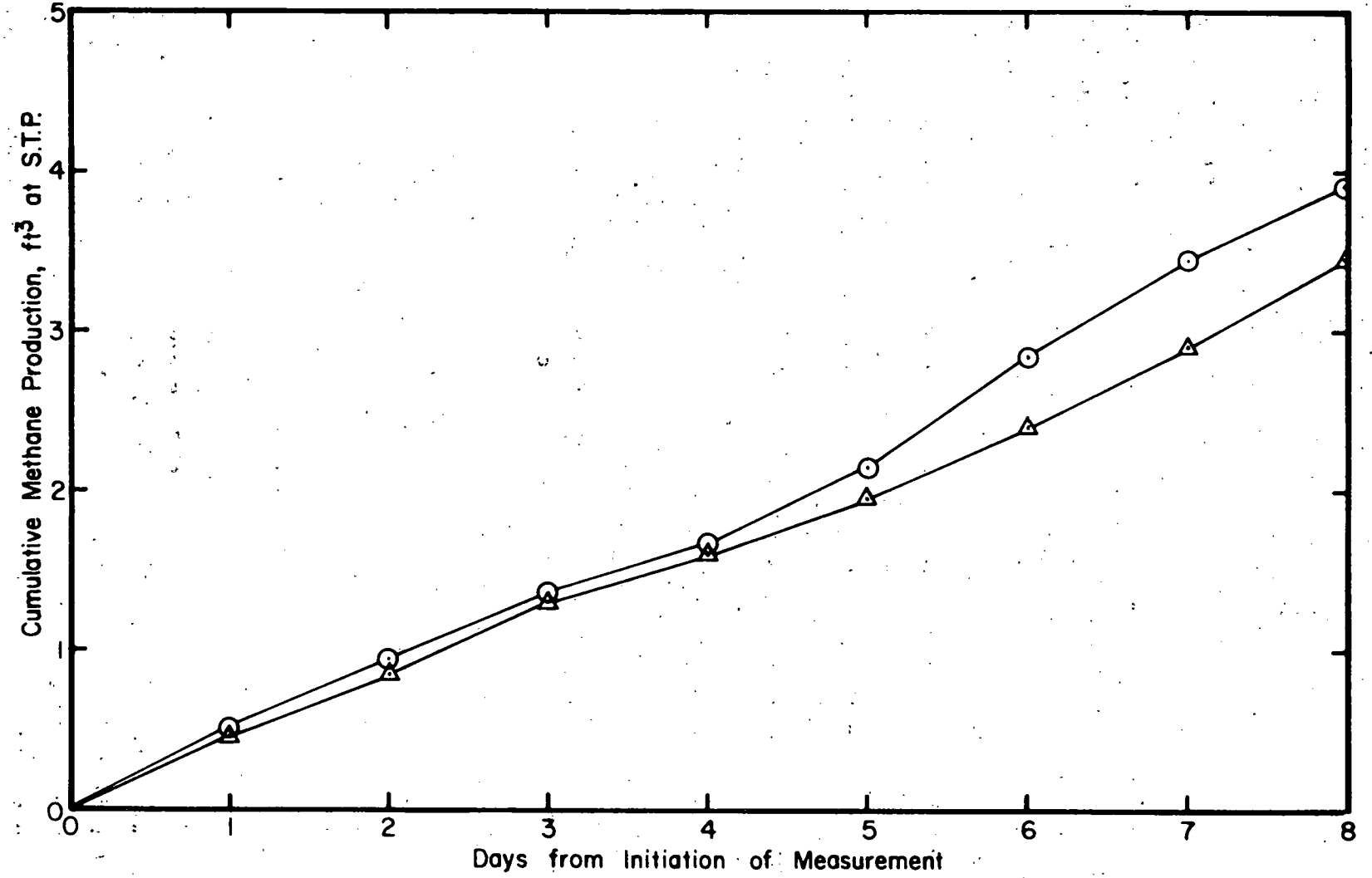

Figure 4.3.1

Performance Comparison of Two Digesters
On Identical Sewage Sludge Feeds
(1ℓ sludge + 1ℓ H_2O daily)

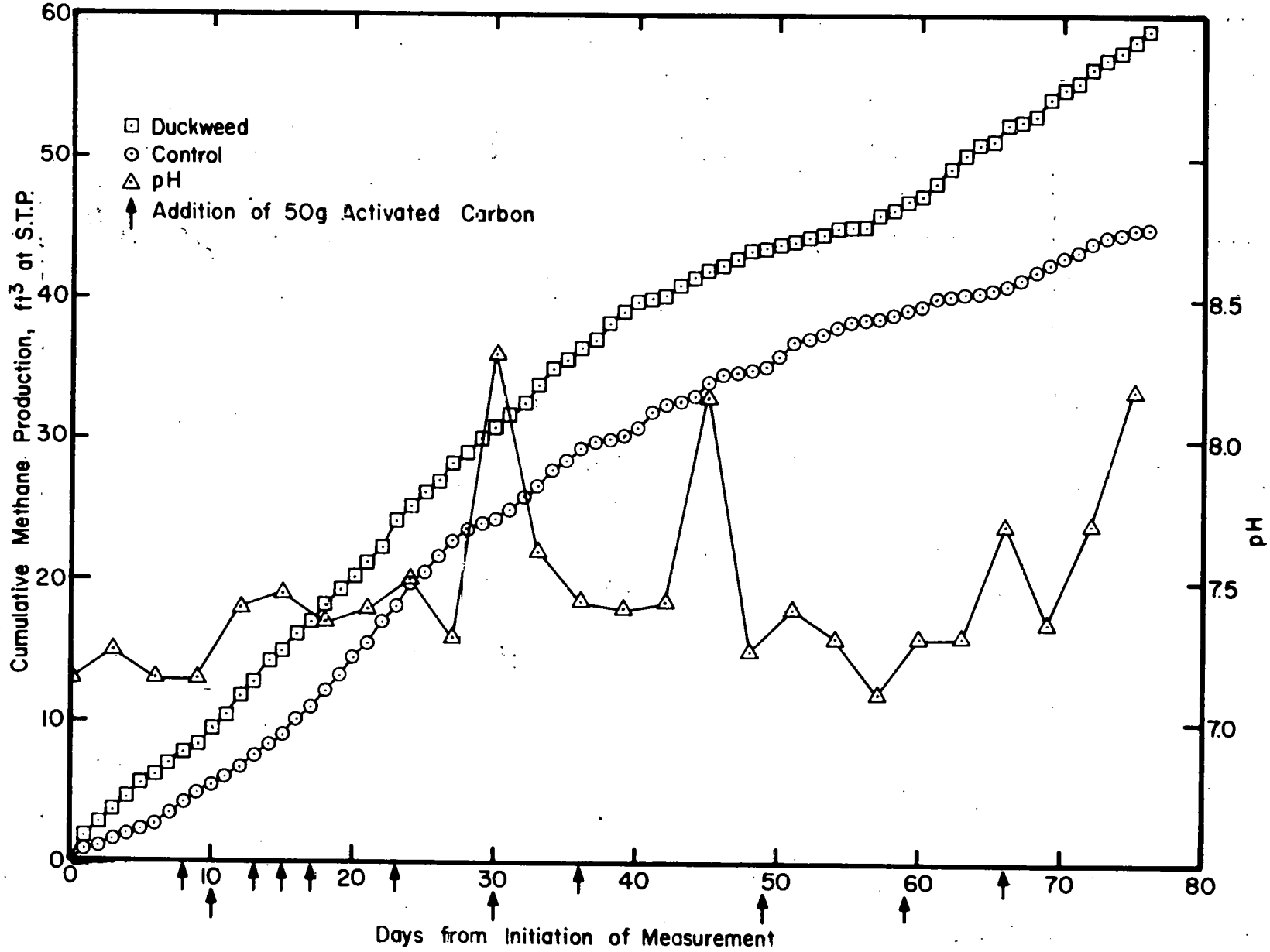

Figure 4.3.2

Methane Production by Duckweed
Digester and Control

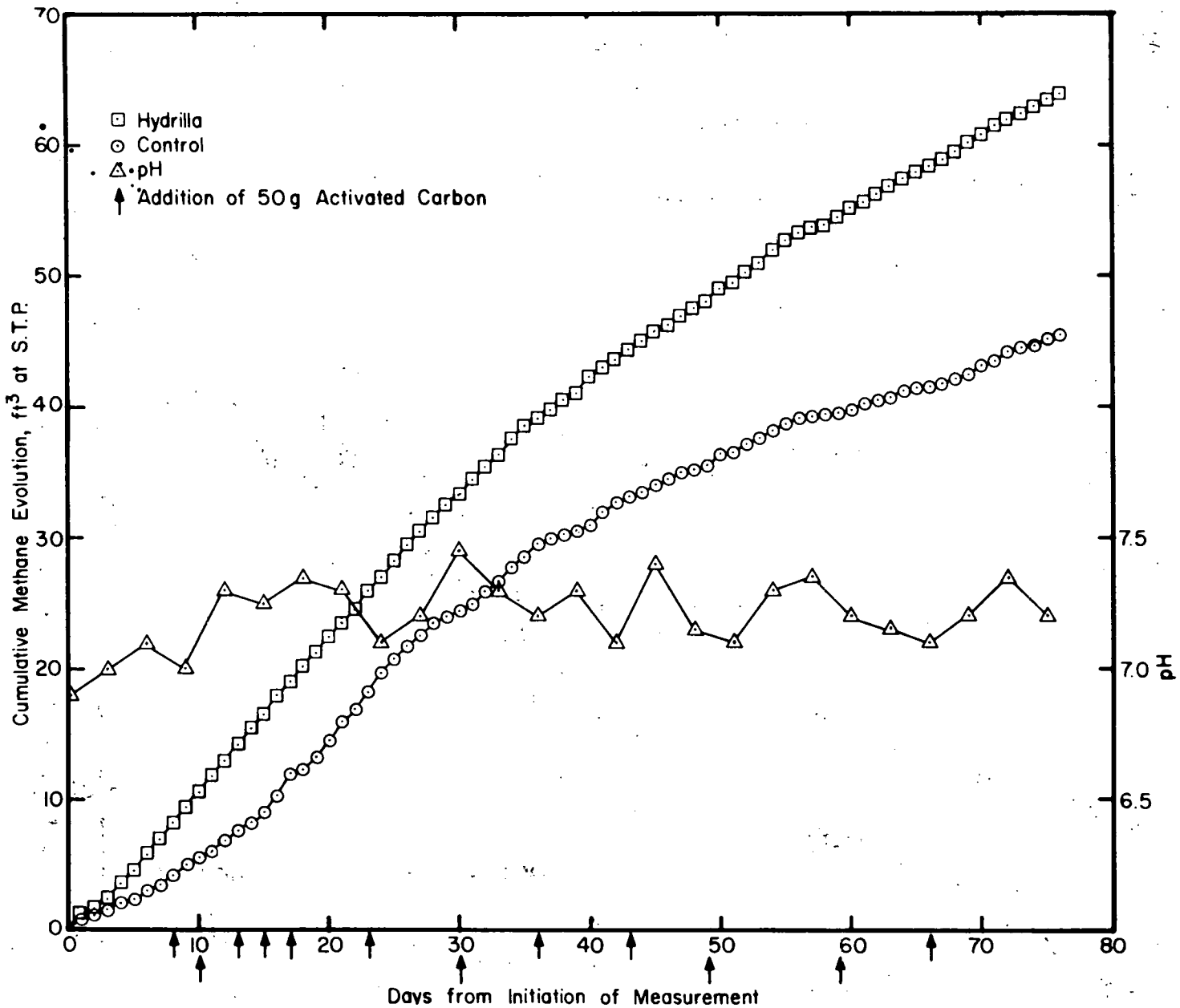

Figure 4.3.3

Methane Production by Hydrilla Digester and Control

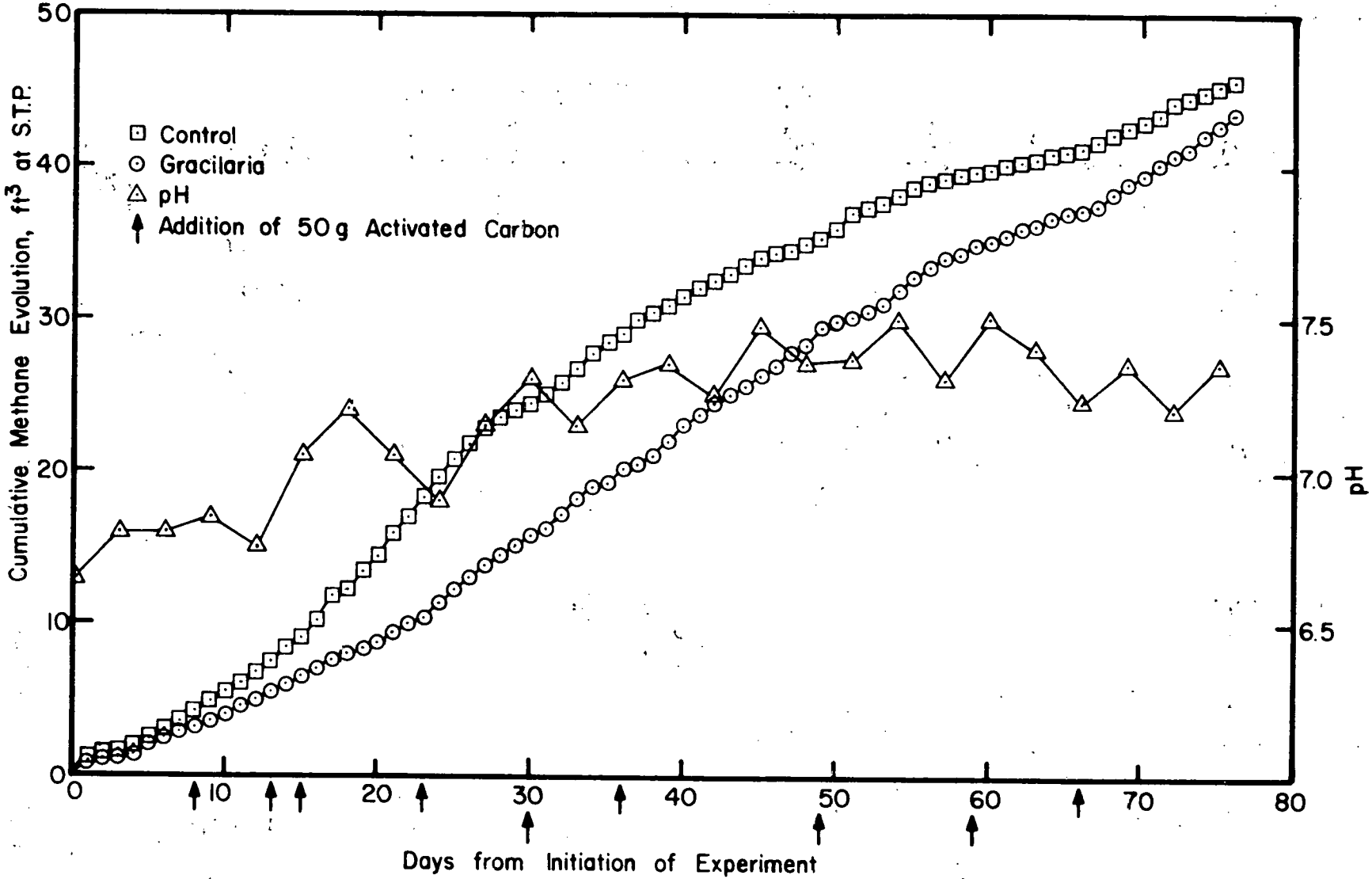

Figure 4.3.4

Methane Production by Gracilaria (Red Algae)
Digester and Control

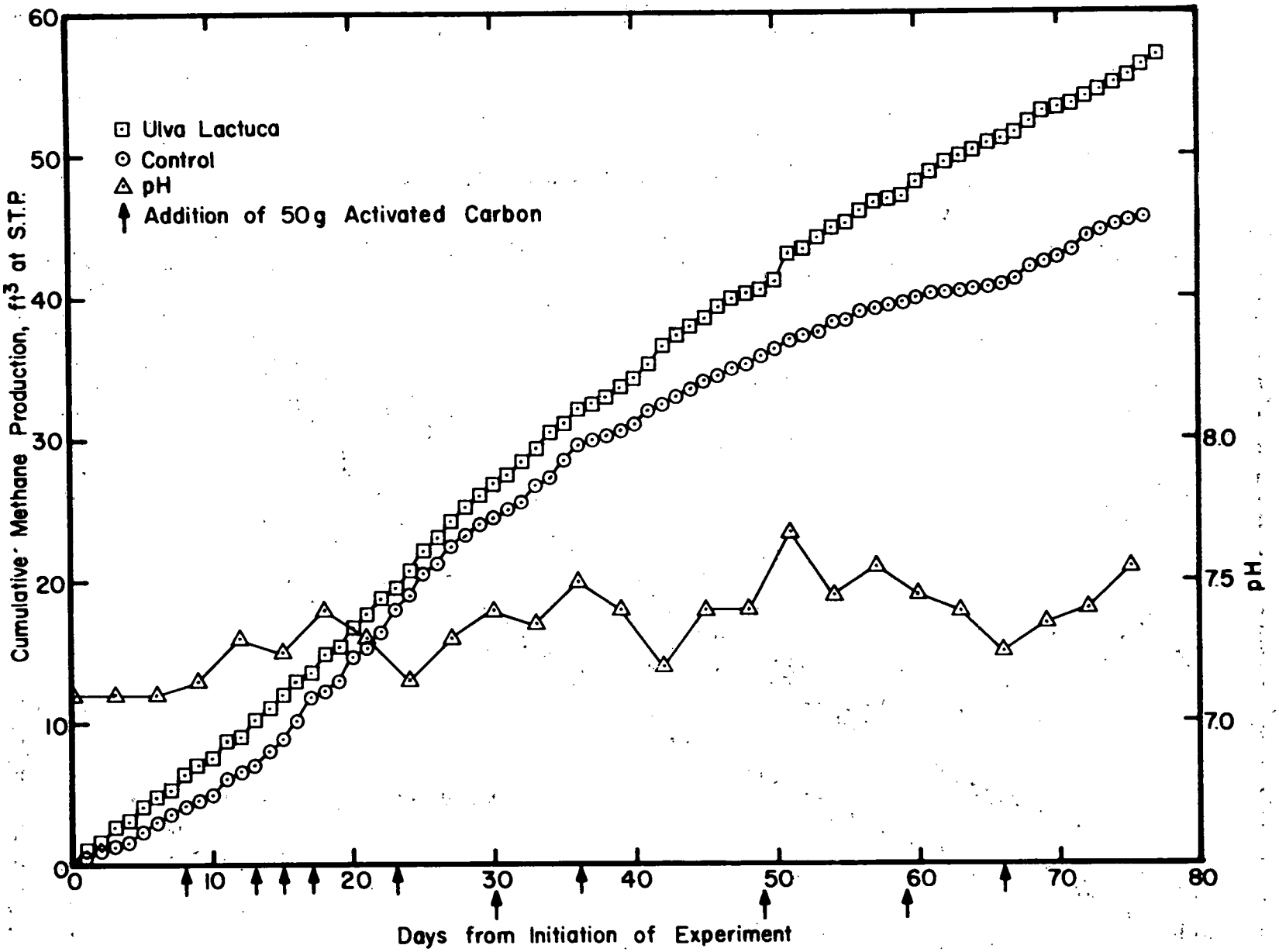

Figure 4.3.5

Methane Production by Ulva Lactuca (Green Algae)
Digester and Control

Net methane from each test biomass is represented by the difference between the test and control digester. The 77 day period of operation represents a balance between time constraints in the program and the necessity to operate for a multiple of the retention time to achieve a reasonable steady state as well as to eliminate effects due to day-to-day variation in digester methane output.

Methane yields computed from outputs over the entire 77-day interval are summarized in Table 4.3.1. The methane yield corresponding to stoichiometric conversion of cellulose is 6.64 ft^3/lb. Based on this "ideal" value for cellulose conversion, percentage conversions for Duckweed, Hydrilla, Gracilaria sp. and Ulva lactuca were 32%, 44%, near zero, and 37% on a volatile solids basis.

Conversion of the aquatic plant species was within normal ranges, and the behavior of these species during digestion also appeared normal. However, the marine algae present a more complex picture. Low digestion rates and conversions during the beginning weeks of the experiment with marine algae are compatible with the hypothesis that digestion was inhibited (this possibility is discussed in more detail later) or else that the culture was not well-adapted to the substrate. Conversion clearly improved during later stages of the experiment. It is encouraging to note that if net yields are recomputed from day 40 onward from data shown in Figures 4.3.4 and 4.3.5, Ulva lactuca exhibited a net yield of 3.02 ft^3CH_4/lb. v.s. and Gracilaria ceae a net yield of 1.82 ft^3CH_4/lb. v.s., much improved over the average for the entire interval shown in Table 4.3.1. This improvement could either indicate adaption to the substrate, or the development of tolerance to an inhibitory factor or factors, as discussed in 4.4 below. In any event, later data from this experiment are encouraging for digestion of the marine algal species.

Table 4.3.1

MESOPHILIC CONVERSION EFFICIENCY OF TEST BIOMASS SPECIES

(See text for conditions)

Substrate	Ft^3CH_4 (STP) / lb Volatile Solids	Ft^3CH_4 (STP) / lb Total Solids
Duckweed	2.15	1.59
Hydrilla	2.91	2.21
Gracilaria	~0 (1)	~0
Ulva Lactuca	2.45 (1)	1.36

Note (1) Conversion efficiency of the two marine algae improved in later stages of the experiment - see text.

4.4 Investigation of Inhibition: H_2S Levels in Output Gas

The low initial net methane yield from Ulva lactuca and the "negative" yield from Gracilaria presented an obvious puzzle and problem. One possible explanation is that high ionic strength due to high ash content of the marine algae is inhibitory to digestion. (An experiment designed to get around difficulties caused by inhibition is discussed in 5.3).

Another possible explanation which was entertained relates to possible consequences of the high degree of sulfonation of polysaccharides in the two marine algae. Table 4.4.1 shows a published analysis for the sulfonated glucuronic acid moiety for Ulva lactuca (Reference 1). According to this particular published analysis, there are approximately 2.5 sulfonic acid groups per glucuronic acid subunit. It is well-known that sulfate-reducing bacteria will utilize the oxygen in sulfate to oxidize organic substrates while producing H_2S. If, for example, the reducing species is glucose, the reaction may be written

$$12\ SO_4^{=} + 5\ C_6H_{12}O_6$$

$$12\ H_2S + 30\ CO_2 + 18\ H_2O$$

and with a mole ratio of 2.4 sulfates to one glucose, all of the glucose BOD may be utilized to reduce sulfate leaving none for the competing reaction of methane formation. In practice, some BOD, amounting to 10-25% of that metabolized in sulfate reduction, will also be utilized for biomass formation, and this is another "sink" for BOD which will decrease the amount available to form methane. Thus the "negative" methane yield on Gracilaria could (at least in principle) be explained by sulfate reduction (H_2S toxicity, discussed later, was also a possibility which was considered).

By the stoichiometry presented, reduction of sufficient BOD to produce one mole of methane should lead to the appearance of 0.8 moles of H_2S. The appearance of a sufficient quantity of H_2S in the exit gas, over about 10%, would indicate that this sulfate reduction hypothesis is correct.

To test the hypothesis, H_2S was analyzed in the exit gas from the two marine algae digesters on 9/15/77. This was accomplished by adding 25 ml of a 2% zinc acetate solution (with 2% HCl added to prevent $ZnCO_3$ precipitation) to 250 ml gas sample bottles containing the exit gases. Bottles were shaken vigorously for several minutes and the resulting ZnS precipitate was collected on a millipore filter, washed with distilled water, dried and weighed. This method of gravimetric analysis gave H_2S levels for the Ulva Lactuca and Gracilaria digesters of 0.95% and 1.22%. To verify the analysis, the Fisher gas partitioner column was modified for H_2S detection by removing the drierite tube on the inlet line. It was then calibrated with H_2S standards, made by aspiring appropriate volumes of air/H_2S into a gas sample syringe. Another sample of exit gas from the Gracilaria digester assayed 1.24% H_2S, confirming the gravimetric test results. Thus the observed hydrogen sulfide levels in the exit gas appear to be insufficient to support the hypothesis that BOD utilization through sulfate reduction was responsible for low methane yields observed on marine algae.

Another possibility which was considered was that the observed H_2S levels were toxic of themselves. Lawrence and McCarty (Reference 2) report toxicity at H_2S levels above 200-300 mg/l. However, it may easily be computed from available ionization relations and solubility data that dissolved sulfide levels in equilibrium with 1% H_2S in the gas phase are only 10-20 mg/l. From these results it would appear that sulfate/H_2S problems were not responsible for poor conversion of the marine algae during early portions of the experiment. Further, it should be noted that the sulfide toxicity question has been rendered moot by improved results later in the experiment.

4.5 Alkaline Pretreatment Experiment with Duckweed

Mild alkaline pretreatment has been shown in a number of cases (for example in References 3 and 4) to improve digestibility of various biomass substrates. The effect of alkaline pretreatment on Duckweed was tested by soaking 50 g lots of Duckweed in saturated lime (pH = 11.2) for 5 days at room temperature. It was found that 1.2 grams of lime were required, in addition to that needed to bring the slurry initially to pH 11.2, to titrate acid and/or buffering components in the 50 gram lots of Duckweed and to keep pH at 11.2 for 5 days. Following the pretreatment step, the lime/duckweed slurry was neutralized to pH $\sim$7.3 by sparging CO_2 gas through it, and then fed to the digester.

Results for digestion of the pretreated Duckweed are shown in Figure 4.5.1. This pretreatment step appeared to reduce, rather than increase digestibility. A possible explanation (quite speculative) is that the alkali reacted in this case to form a substance inhibitory to the digestion process. The best that can be said is that the methanogenic culture got used to the situation and appeared to be able to convert the alkaline pretreated material almost as well as the Duckweed which did not receive pretreatment. Net conversion for days 30-68 in Figure 4.5 may be computed to be 1.96 ft^3/lb. v.s., to be compared with 2.15 ft^3/lb. v.s. for Duckweed which was not pretreated.

4.6 Thermophilic Operation: Digestion of Duckweed

Thermophilic (55-60°C) digestion is known, from results of numerous investigators including Cooney and Wise (Reference 5) and the work of J. T. Pfeffer over the last several years at the University of Illinois, to result in more rapid and complete conversion of substrates than mesophilic (35°-45°C) operation. From kinetic information compiled

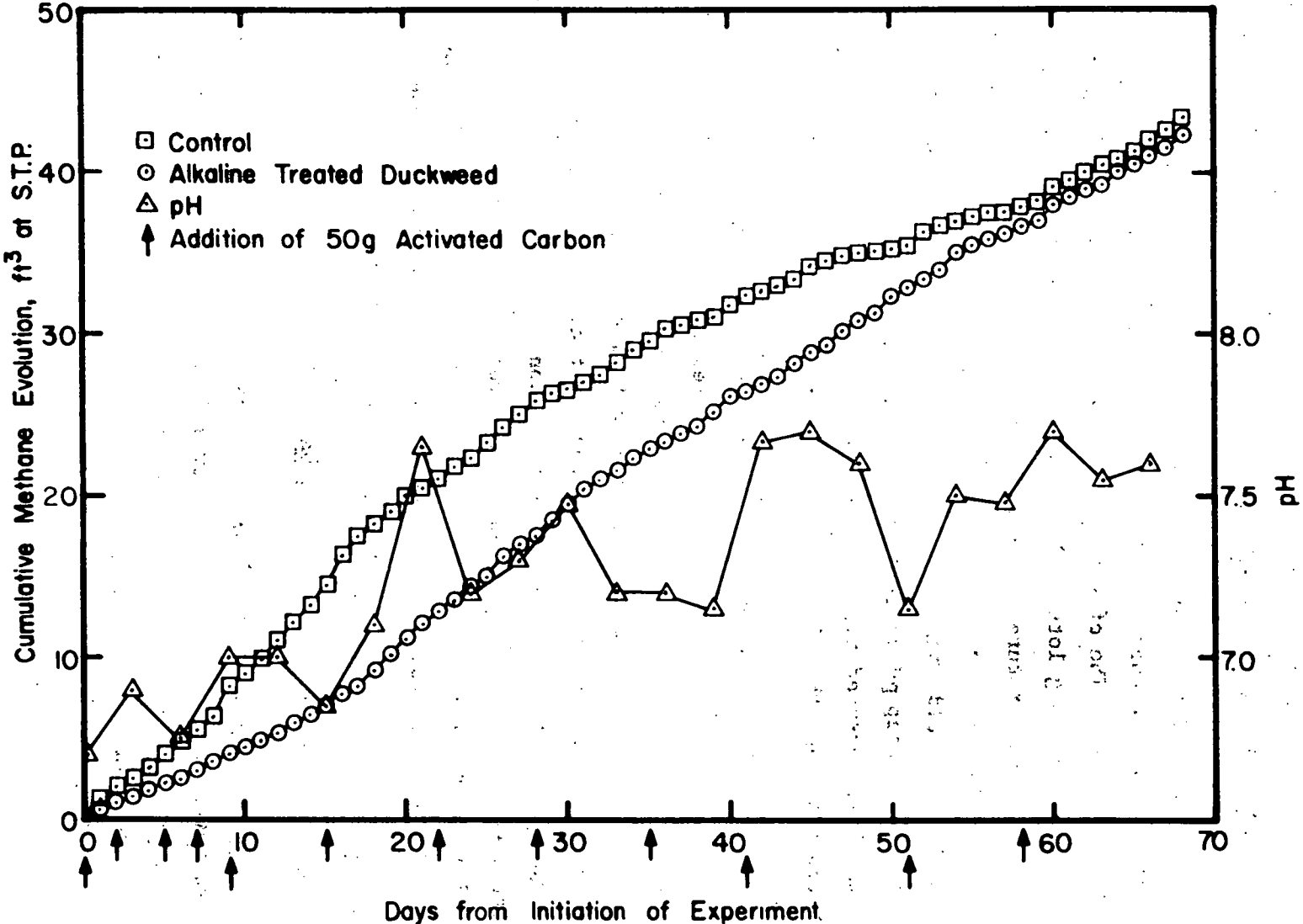

Figure 4.5.1

Methane Production by Alkaline Treated
Duckweed Digester and Control

by Ashare et. al. (Reference 6) it would be expected that combined rate coefficients of the thermophilic process, assuming Ea ≈ 15 Kcal, are about four times those of the mesophilic process. To place this statement in another perspective, thermophilic operation at a given retention time should give fractional conversions effectively equivalent to mesophilic operatation for a retention time four times as long.

The thermophilic culture was developed following a procedure similar to that of Varel et. al., (Reference 7), that is, by taking mesophilic sewage digester effluent (37°C) along with some supplemental sludge substrate and simply placing it in two CSTR digesters maintained at 58°C. An effluent/sludge ratio of 3:1 was used, and pH remained near 7.0 at all times. Some aspects of this culture development procedure are worth noting. In the procedure the thermophilic digesters were opened daily both to introduce substrate and to withdraw samples for pH. Additionally, they were continuously agitated. Negligible methanogenesis was observed over the first 33 days, which is in contrast to the finding of Varel, et. al. in Reference 7 who observed thermophilic digestion of manure within 10 days. At this point, consideration was given to the possibility that oxygen introduced during the daily manipulations (with little or no gas evolution, air introduced was not being flushed from the head space) was inhibiting the culture. In the presence of agitation, contacting of culture medium with air (although it can only be rather imprecisely estimated in the absence of measurement) was probably such that methanogens were exposed to significant activites of oxygen. (This author is not aware of any quantitative information regarding such inhibition, except that it is reported to exist.) In static systems, it is easily demonstrated utilizing extant mass transfer correlations and assuming that facultative aerobes are present, that oxygen activity in the bulk of the liquid (even if a gas-liquid interface is exposed to oxygen) is identically zero. Accordingly, stirring was turned off in one of the two thermophilic reactors, and in this reactor rapid methanogenesis was observed within 5 days after cessation of stirring, and steady-state thermophilic digestion was developed within

10 days. At that time stirring was resumed. Effluent from this reactor was used to seed the other reactor, satisfactory steady-state operation was observed for both reactors, and biomass feed was initiated. Although in most cases no deleterious effects of oxygen on digestion have been observed in this or other laboratories, it would appear that oxygen effects could have caused problems in this instance. It also seems from these results that subjecting a mesophilic culture to a single step change to thermophilic conditions is as good a way as any (e.g., as opposed to incremental temperature changes) to establish a thermophilic culture. Total elapsed time between initiation of culture development and steady-state thermophilic operation was 43 days, which is less time than has been required to develop thermophilic cultures through stepwise increases in other work, for example in Reference 5.

The same feeding procedures and the same 26-day retention time were used in thermophilic as in mesophilic work. Thus the only variable in the experiment was temperature. As in mesophilic work, a control was maintained which was fed sewage sludge alone. Figure 4.6.1 shows cumulative output for the Duckweed digester and the control. Over a 42 day interval, Duckweed showed a net yield of 3.91 ft^3/lb v.s., as opposed to 2.15 ft^3/lb v.s. under mesophilic conditions. While the thermophilic Duckweed digester was operated for only 1.6 retention times, it is clear that thermophilic operation gave substantial improvement.

Available data also enable a comparison of the relative effectiveness of thermophilic and mesophilic conversion of sewage sludge. For the 42-day experimental interval, the thermophilic and mesophilic digesters receiving sewage sludge produced 17.1 and 15.6 ft^3 of net methane, respectively, so that the advantage of thermophilic digestion for sewage sludge is much less pronounced. While a detailed discussion of the reasons for this behavior is beyond the scope of this report, some speculation may be offered. In circumstances where hydrolysis of cellulose is the rate-limiting

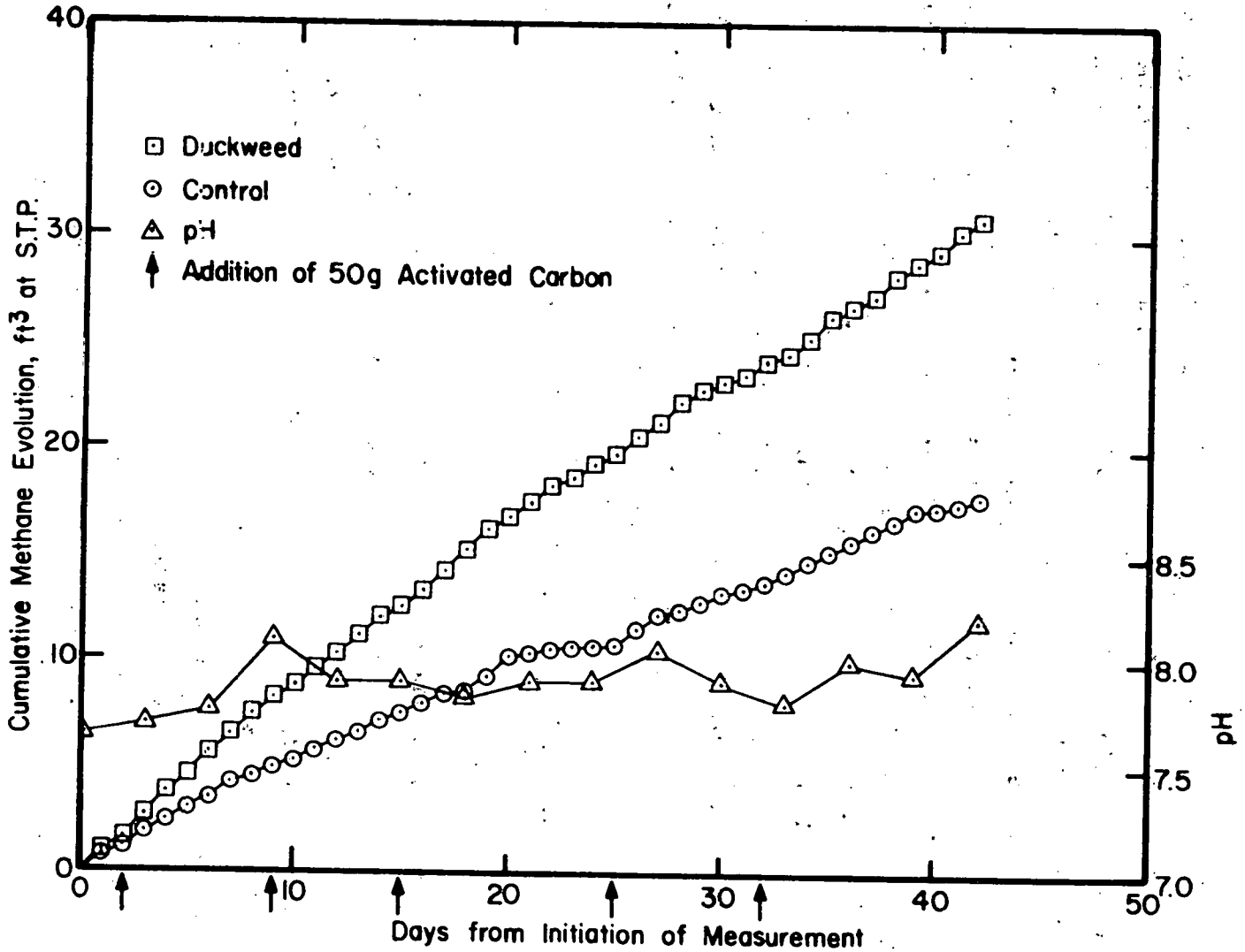

Figure 4.6.1

Methane Production by Thermophilic
Duckweed Digester and Control

step, solid substrate particles are consumed from the outside in, and the time requirement for conversion, or conversely, fractional conversion at a given retention time, will reflect the kinetics of utilization of a heterogeneous distribution of particle sizes by surface attack. Although the size of Duckweed particles after blending was not measured, it was observed that the suspension after blending still contained pieces of plant, in the order of 1 mm. (This is a much larger particle size than the 20-100 μ typical of paper fiber in municipal solid waste or of particles in sewage in Reference 10.) In the case of "large" particle size substrates such as this, it would be expected that maximum fractional conversions would occur at long retention times, or the equivalent attained through shorter-term thermophilic operation. (Along with this speculation, however, should be noted that lower mesophilic yields may have been due in part to loss of organic acids in the effluent as discussed in 4.7.1).

4.7 Results of Chemical Analyses on Digester Effluent

4.7.1 Volatile Acids

Because of the satisfactory pH behavior shown by all digesters during the course of the program, it was not considered necessary to conduct frequent volatile acid analyses. Three analyses were conducted during the 77-day interval of digester operations. Results of these are shown in Table 4.7.1.1. Athough pH was within normal ranges, it is seen that measurable levels of acids were detectable in most digesters, which with operating procedures described was necessarily withdrawn from the units in the aqueous phase at the rate of 2ℓ/day. For interest, the potential methane which could have been formed if the acids withdrawn were instead completely converted is shown to the right of the acid levels measured on 9/4/77. Also shown for comparison is the average yield of each digester in ft^3/day.

It may be seen that the potential methane yield which was lost to acids leaving in the aqueous effluent was significant in the case of the

Table 4.7.1.1

RESULTS OF VOLATILE ACID ANALYSES ON ANAEROBIC DIGESTERS

(1) Assay of 9/5/77

	Digester	Acid Levels, meq/l				Average Gas Over Interval Ft^3/day	Potential Gas Lost With Acids Leaving At Concentraion Shown, Ft^3/day	Potential Gas Lost As Percent of Observed Production (Percent)
		Acetic	Propionic	Butyric	Valeric			
Mesophilic	Hydrilla	4	2	2	0.9	0.839	0.024	2.9
	Control	2.5	1	1	0.3	0.599	0.013	2.2
	Gracilaria	9.5	18.5	0	0	0.572	0.066	11.5
	Alk. P.T.	10.5	23	0	0	0.559	0.080	14.3
	Duckweed	2.5	1.0	0.3	0	0.740	0.008	1.1
	Ulva Lactuca	58	7.5	0.5	0	0.776	0.115	14.8
Thermo	Duckweed	6	4	0.5	0	0.733	0.023	3.1
	Control	3	3	0.3	0	0.424	0.014	3.3

Gracilaria, alkaline pretreated Duckweed, and also the mesophilic Duckweed digester. The fact that acids were incompletely converted in these cases indicates that CSTR digestion is probably not the best method for assessing convertibility of these substrates, in terms of determining their ultimate methane potential. Although the yield data are certainly valid for operating conditions chosen, the net methane production is also certainly less than the maximum possible. An alternative method for assessing methane potential which would eliminate various sources of error is to operate batch reactors to completion, and plans to do this are outlined in Section 5.3.

4.7.2 Phosphate Levels

Because of the high level of phosphate which enters municipal wastewaters with detergents, it was anticipated that phosphate levels sufficient to support digestion would be introduced with the sewage sludge component. Assays for free dissolved orthophosphate in the supernatant liquid after centrifugation were carried out on 9/23/77, shown in Table 4.7.2.1, which showed phosphate levels to be ample.

4.7.3 Ammonia Nitrogen Levels and Ammonia Nitrogen Supplementation

Initially, free ammonia nitrogen measurements were conducted (as discussed in Section 3) with the expectation that these would confirm the presence of adequate ammonia nitrogen levels. However, these showed, instead, that free ammonia levels were dropping to dangerously low levels, in the order of those reported by Thiel, et. al. (Reference 8) and Sanders, et. al. (Reference 9) to cause digester failure, e.g., < 50 PPM NH_3, or $\leq$ 3 meq/1. Accordingly, ammonia nitrogen was measured once weekly and sufficient ammonia nitrogen was added after each measurement to bring ammonia nitrogen levels back up to 170-200 PPM (10-12 meq/1). Table 4.7.3.1 shows results of measurements and ammonia nitrogen supplementation carried out.

Table 4.7.3.1

MEASURED FREE AMMONIA NITROGEN LEVELS, AND HISTORY OF AMMONIA NITROGEN SUPPLEMENTATION TO DIGESTERS

KEY: M = Measured Ammonia Level, mmol/l
A = Total Ammonia Added, mmols/digester

	Mesophilic Digesters												Thermophilic Digesters			
Date	Hydrilla		Control		Gracilaria		Alk. P.T. Duckweed		Ulva Lactuca		Duckweed		Duckweed		Control	
	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
8/10/77	2.3	460	4.4	406	4.2	411	5.8	369	4.5	403	7.7	320	N.A.	N.A.	N.A.	N.A.
8/16/77	3.9	408	4.5	376	6.8	258	7.1	244	4.7	366	6.6	269	12.3	---	14.1	---
8/23/77	2.6	476	2.8	463	4.4	384	5.4	333	2.7	470	3.9	409	7.6	214	7.6	214
8/26/77	4.2	391	5.5	323	7.9	201	7.9	201	5.5	323	7.6	214	11.2	---	10.0	---
9/3/77	N.M.	330	N.M.	330	N.M.	330	N.M.	330	N.M.	330	N.M.	330	N.M.	330	N.M.	330
9/6/77	3.3	440	3.3	440	5.5	326	5.5	326	3.0	455	5.0	351	6.0	298	7.1	241
9/12/77	4.8	361	4.8	361	4.8	361	7.0	247	4.8	361	5.6	320	7.0	247	7.0	247
9/15/77	3.7	412	3.7	412	5.0	351	4.0	402	3.7	419	4.0	402	7.4	226	7.4	226
9/20/77	5.0	550	5.3	550	5.3	550	5.1	550	4.9	550	5.1	550	5.0	550	5.1	550
9/26/77	9.7	107	5.0	351	2.5	483	3.5	427	3.7	419	3.5	427	5.4	330	3.7	419
9/29/77	4.7	366	5.6	320	5.0	351	2.6	473	3.7	419	3.5	430	5.3	336	2.6	473
10/3/77	---	---	---	---	3.5	---	4.7	---	3.5	---	4.7	---	5.0	---	7.0	---
10/5/77	4.8	363	2.0	305	2.2	494	5.2	339	1.5	534	7.0	247	7.0	247	4.8	363
10/10/77	4.0	---	4.0	---	3.8	---	7.0	---	4.4	---	4.2	---	4.2	---	7.2	---
10/19/77	29.4	---	10.0	---	24.1	---	23.5	---	10.9	---	28.8	---	11.8	---	9.9	---

Table 4.7.1.1 (continued)

RESULTS OF VOLATILE ACID ANALYSES ON ANAEROBIC DIGESTERS

Assay of 10/5/77

	Digester	Acid Levels, meq/l			
		Acetic	Propionic	Butyric	Valeric
Mesophilic	Hydrilla	21	0	0	0
	Control	0	0	0	0
	Gracilaria	6	8.5	0	0
	Alk. P.T. Duckweed	2	12.5	0	0
	Ulva Lactuca	2	0	0	0
	Duckweed	76	8	0	0
Thermo	Duckweed	0	0	0	0
	Control	0	0	0	0

Assay of 11/1/71

	Digester	Acid Levels, meq/l			
		Acetic	Propionic	Butyric	Valeric
Mesophilic	Hydrilla	0	0	0	0
	Control	0	0	0	0
	Gracilaria	3.5	0.75	0	0
	Alk. P.T. Duckweed	7.5	5	0.5	0
	Ulva Lactuca	1.5	0.5	0	0
	Duckweed	7.5	0	0	0
Thermo	Hydrilla	1.3	15.5	1.5	1.0
	Control	1.5	1	0	0

Table 4.7.2.1

RESULTS OF ASSAYS FOR FREE PHOSPHATE IN AQUEOUS DIGESTER SUPERNATANT (9/23/77)

	Digester	Phosphate Level, meq/l
Mesophilic	Hydrilla	4.5
	Control	2.6
	Gracilaria	7.7
	Alk. P.T. Duckweed	3.3
	Ulva Lactuca	3.0
	Duckweed	5.2
Thermo	Duckweed	3.75
	Control	2.3

4.8 Initial Effluent Shipment to Harbor Branch Foundation

Effluent generated as described was collected daily from digesters and stored in individual drums, one for each digester. An initial shipment of effluent drums was sent out on 9/5/77 to Harbor Branch, but was returned on 9/9 by the shipper because of leaks. These drums were provided with foamed plastic inserts to prevent further leaks, and effluent continued to be collected in the drums between 9/13 and 9/28, at which time, they were again shipped. This is the lot which was ultimately received by Harbor Branch in Ft. Pierce, Fla. for analysis and recycle studies.

Of interest to Harbor Branch, in addition to feed components to each digester, which have been described, is the degree of nutrient supplementation. The only supplement added (other than activated carbon, which may be regarded as inert) to the initial shipment was ammonia nitrogen. Effluent received by Harbor Branch is that collected between 8/9/77 and 9/28/77. Since some material was lost in the leak episode, and effluent was not collected between 9/5 and 9/12, the level of cumulative ammonia supplementation is somewhat uncertain, but may be computed within reasonable limits as follows.

The fraction of ammonia nitrogen which was added to any given digester which remains in the digester at any given time, θ, after addition is

$$\frac{A}{Ao} = e^{-\frac{26}{\theta}} \qquad (4.8.1)$$

where A = ammonia nitrogen remaining in digester
Ao = ammonia nitrogen added
θ = time, days, after the supplementation.

The supplemental ammonia nitrogen delivered to the drum with the effluent, therefore, is Ao -A, with the reservation that some material was lost through leaks. (The effect of material not collected between 9/5 and 9/12 may be computed.) The effect of material loss in the leak episode is

that later addition after 9/12 should be weighted more heavily than they actually are by equation 4.8.1 above. However, it is estimated that the amount of supplemental ammonia computed by 4.8.1 is accurate to within 20%. Computed supplemental ammonia levels are presented in Table 4.8.1, assuming complete collection of material between 8/9 and 9/5, and 9/12 and 9/28 for each drum shipped.

Section 5

FUTURE WORK

5.1 Thermophilic Operation

Immediate plans for thermophilic work are to discontinue Duckweed and to begin digesting Hydrilla which has shown the best mesophilic yields to date. Although it would be desirable to thermophilically digest Ulva Lactuca and Gracilaria as well, constraints on the program are such that it does not appear that it will be possible to do this. An alternate method for determining methane potential of the two marine algae is discussed later (Section 5.3).

5.2 Hydrogen Sulfide Removal Experiments with Gracilaria and Ulva Lactuca Using FeO

To examine further whether problems with marine algae are in any way related to high H_2S levels, due to reduction of sulfonic acid groups, it is planned to conduct a short-term experiment (about two weeks) in which ferrous oxide is added to these two digesters. The relevant reaction is

$$FeO + H_2S \rightarrow FeS + H_2O \qquad (5.2.1)$$

Ferrous oxide will be added in an excess sufficient to take care of any likely levels of H_2S evolution. It has been calculated as part of this work that Fe^{++} level is fixed by carbonate ions in solution at about 1.4×10^{-2}M (pH = 7, 0.5 atm. CO_2 activity) a level which from solubility product considerations dictates an equilibrium H_2S activity of about 20×10^{-6} atm, or 20 PPM in the gas phase. This experiment will be continued for about two weeks.

5.3 Small Stationary Flask Digestion Experiments

Previous experimental results have indicated that there can be drawbacks with CSTR digestion, in that under many operating conditions unconverted acids may leave the digester, effectively reducing methane yield, and also in that, even if acids are completely converted to methane, fractional conversions will vary significantly with retention time because of the nature of the hydrolytic process involving solid substrates. There is also, in CSTR work, the consideration that kinetic coefficients may be altered by such factors as high ionic strength as outlined by McCarty et. al. Reference 12, (or high H_2S) and such effects could well have been operative in the marine algae digestion work.

For these reasons, it would be desirable to have a measure of the maximum methane potential of each of the four biomass species to serve as a check on CSTR results. This will be done by carrying the batch digestion of test lots of biomass to completion. As background, Augenstein, et. al. (Reference 3 and also unpublished work) have shown that methane yields 30-60% higher than in 25 day mesophilic CSTR digestion were attainable by carrying appropriately seeded batch and continuous reactors to completion with municipal solid waste and newsprint feedstocks. (It is to be noted that in the cited work, low CSTR conversions relative to batch results were not due to acid loss in the effluent. Rather, the yield difference appeared to be due to the fact that the allowance of a sufficiently long batch reaction time enabled more complete substrate consumption relative to the CSTR work.)

It is planned to carry out batch digestion tests with stationary reactors of 2 l. capacity, which will be charged with feed identical to that introduced to the CSTR digesters, e.g., 50 grams (dry basis) of the test biomass and one liter of sewage sludge in a total of 2 liters. Reactors will be appropriately buffered and seeded. A control will also be operated with sewage sludge alone to determine the methane due to the sludge component. Gas collection will be by water displacement in collectors (which

are being constructed for this purpose). It is anticipated that this experiment will require several months. However, the "completion" of substrate digestion, by this procedure should give an accurate measure of the total methane potential of each biomass substrate; this will be true even though inhibition or kinetic effects may cause digestion to proceed at differing rates for each test substrate. This information will be useful since there exists the possibility that digestion processes may be developed which will give near-maximal conversion, as outlined below.

5.4 Large Stationary Reactor Experiments with Duckweed and Hydrilla

One possible processing sequence has been considered as an alternative to conventional CSTR digestion is to make use of large, low capital-cost lined, covered pits to carry out digestion for long retention times, and thereby attain high fractional conversions inexpensively. Estimated performance of such digesters is presented in References 3 and 11. It is notable that such reactors are estimated to be 10-20 times less expensive on a unit volume basis than typical concrete tanks (Reference 11) and the processing could consist, in the limit, of simply wetting large lots of substrate with an appropriate buffer/nutrient/inoculum mixture, covering large piles of it (if solid) or pits (if liquid) and piping off the product gas. It has been established that digestion proceeds at quite reasonable rates without stirring (Reference 3 and 13), and this particular processing technology would be expected to have minimal energy consumption and a very favorable energy balance. Whether or not such systems are as workable as they appear (and problems with this technology certainly remain to be addressed) it would be desirable to have a measure of the behavior of the test biomass species under conditions likely to be typical of such low capital cost processing. Woods Hole has, additionally, indicated that they would like as much effluent as is possible.

It is planned to carry out two larger stationary digestion experiments, of 50 l capacity, with Hydrilla and Duckweed. Since previous

work (Reference 3) was established that digestion will proceed at high total solids of as much as 45% by weight with municipal waste, solids loading will be as high as possible in these reactors. It is presently planned to add the biomass without comminution, since this would allow cost and energy savings in operation of a full-scale digestion process. Additionally, since sewage sludge is unlikely to be available in quantity for most biomass digestion processes, the amount of sewage sludge added in this particular experiment will be kept to an absolute minimum, currently planned at less than 1/2% by weight of total solids.

This experiment should show whether there is the potential for the low-capital cost digestion of Duckweed and Hydrilla. Additionally, it will make available the residue after digestion of several kilograms of each plant to Harbor Branch. As with the stationary flask experiments, it is anticipated that this experiment will require several months.

If resources permit, two similar experiments will be conducted with the marine algae.

5.5 Analysis for Elemental Composition of Biomass

Samples of each biomass are being sent to an outside laboratory for elemental composition, for C, H, N, and S as a fraction of total weights. This information will allow an estimate of nutrient nitrogen which is freed (if any) during digestion, and which therefore would be readily available in the recycled effluent. The determination of this ratio of freed to cell and residue-bound nitrogen will be important to establishing the likely practicality of recycle of effluent to the biomass growth system.

5.6 Future Deliveries of Effluent Samples to Harbor Branch

Current plans are to send to Harbor Branch all remaining effluent from digestion of Duckweed, Gracilaria, Ulva Lactuca, and Hydrilla, as well as the sewage sludge control, as soon as the iron oxide addition experiment is completed with the marine algae. It is anticipated that this

shipment will be made in December, 1977. Information will be furnished with this shipment on additions which were made to each digester, e.g., NH_4OH and in the case of the marine algae, the amount of FeO added. A later shipment will be made of the residue from the 50 l stationary reactor experiments with Hydrilla and Duckweed when this experiment is complete. It is presently anticipated that this shipment will be made in the early spring.

Table 4.8.3.1

SUPPLEMENTAL AMMONIA CONTENT OF DIGESTER EFFLUENT DRUMS[1] SENT TO HARBOR BRANCH FOUNDATION

Drum Contents	Computed Supplemental Ammonia, Gm. Mol/Drum[2]
Hydrilla Effluent	1.92
Gracilaria Effluent	1.57
Alkaline Pretreated Duckweed Effluent	1.47
Ulva Lactuca Effluent	1.83
Duckweed Effluent	1.54

1. Mesophilic Digesters
2. Total Supplemental Ammonia in Drum (In addition to ammonia entering with biomass and sewage sludge). Computed as stated in text.

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