WHOI-75-41

# PRELIMINARY RESULTS WITH A PILOT PLANT WASTE RECYCLING MARINE-AQUACULTURE SYSTEM

By

John H. Ryther

WOODS HOLE OCEANOGRAPHIC INSTITUTION
Woods Hole, Massachusetts 02543

September 1975

TECHNICAL REPORT

*Prepared for the Office of Sea Grant under Grant 04-4-158-5 and 04-5-158-8 and NSF (RANN) Grant GI-43884.*



Approved for Distribution George Grice
George D. Grice, Chairman
Department of Biology

# Abstract

A combined waste recycling-marine aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonally by 3-4 fold and are controlled by solar radiation but not by temperature.

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined.

An alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual production of 183 tons of oyster meat 38,000 bushels of whole oysters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with half the above yields.

## Introduction

A biological tertiary sewage treatment-marine aquaculture system has been developed, tested, and evaluated over a one-year period on a "pilot-plant" scale at the Woods Hole Oceanographic Institution's Environmental Systems Laboratory (ESL). (Fig. 1) The effluent from secondary sewage treatment, mixed with seawater, is used as a source of nutrient to grow single-celled marine algae (phytoplankton) in mass (35,000 gallon), continuous flow-through cultures. Harvest from the algal cultures (experimentally varied from 25% to 75% of the culture volume/day), diluted with seawater, is fed into 40' x 4' x 5' (deep) cement raceways containing stacked trays of shellfish. The latter, stocked at densities ranging from 75,000 to 150,000 animals/raceway (1,500-3,000 per tray) have consisted of the American oyster (Crassostrea virginia) and the hard clam (Mercenaria mercenaria), with smaller numbers of other shellfish species. The phytoplankton remove the nutrients from the sewage effluent, which has varied experimentally from 10% to 50% in the effluent-seawater mixture. The shellfish remove the phytoplankton from the water. Effluent from the shellfish cultures (i.e., the pond harvest and diluting seawater) prior to its discharge is passed through a culture of seaweeds, grown in suspended culture in raceways adjacent to the shellfish cultures, which serve as final polishing step, removing nutrients not initially assimilated by the phytoplankton and those regenerated by excretion of the shellfish and decomposition

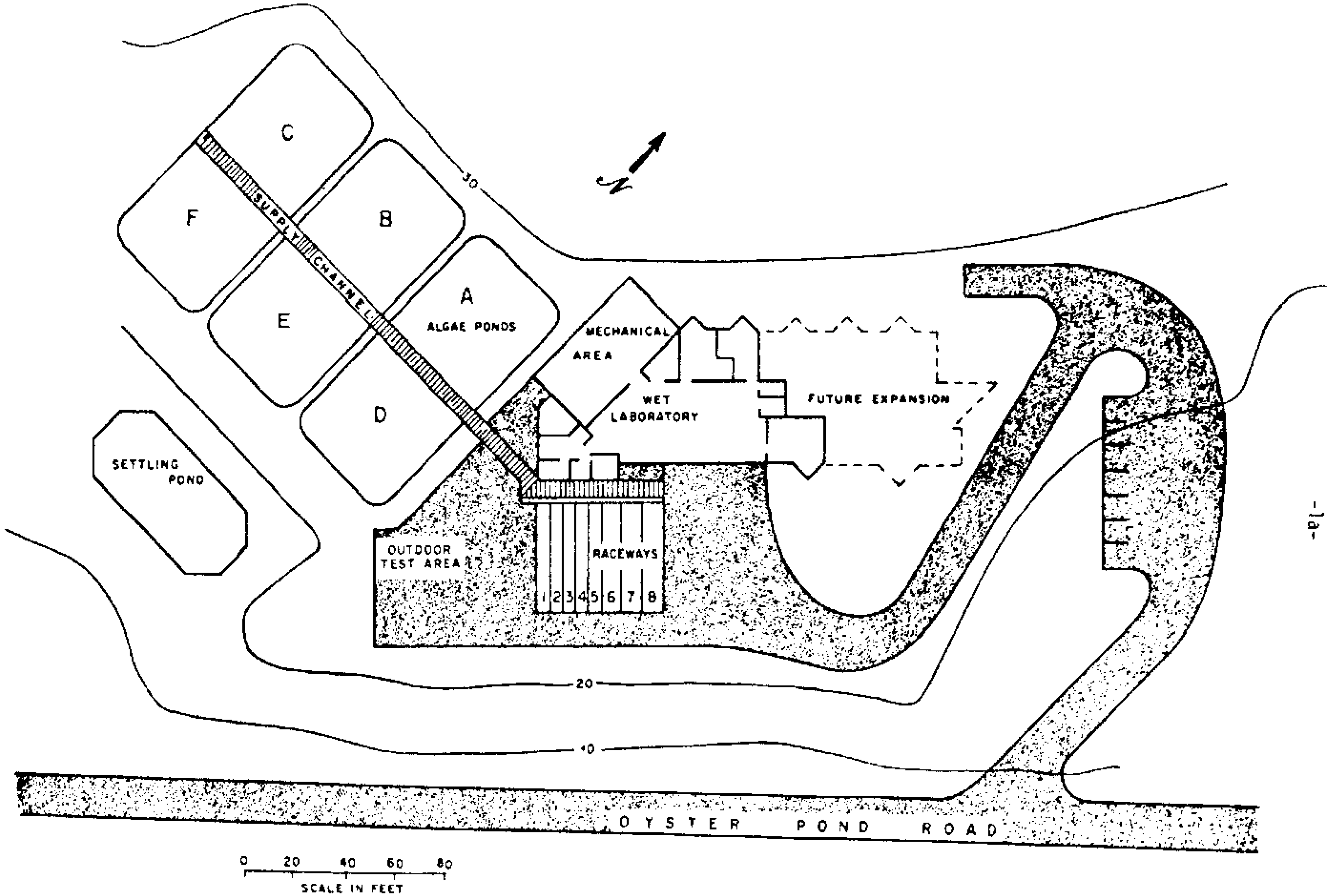



Figure 1 Environmental Systems Laboratory Woods Hole Oceanographic Institution

of their solid wastes. After initial experimentation with several seaweed species, research was concentrated on two red algae of potential commercial value, Gracilaria foliifera and Agardhiella tenera (which contain the polysaccharides, agar and carrageenan respectively).

Solid wastes produced by the shellfish and uneaten phytoplankton support dense populations of small invertebrates (amphipods, polychaete worms, etc.) These serve as food for secondary commercial crops of marine animals, the American lobster (Homarus americanus) and the winter or blackback flounder (Pseudopleuronectes americanus) which were stocked in respective raceways with the shellfish.

The primary objective of the research is to develop a biological tertiary sewage treatment process capable of removal of all inorganic nitrogen from secondary sewage effluent prior to its discharge into the environment. Earlier studies (1, 2) had established the fact that nitrogen is the nutrient limiting and controlling algal growth in and eutrophication of the coastal marine environment. Thus nitrogen removal may be considered as synonomous with tertiary sewage treatment of effluents to be discharged to the sea.

The second objective of the process is to develop a marine aquaculture system consisting of a primary crop of shellfish and secondary crops of other commercially-valuable marine organisms (seaweeds, lobsters, finfish), the value of which will pay for or help defray the cost of the tertiary sewage treatment process.

## Procedures and Results

A. Algae culture and nutrient removal.

Phytoplankton cultures have been maintained continuously in five of the six 35,000 gallon, 2,500 $ft^2$ algae ponds at ESL during the past year (the sixth pond was held out of production). Initially, the cultures were grown on inorganic chemical nutrients (commercial-grade ammonium chloride and monobasic sodium phosphate). Beginning in July, 1974, 8,000 gallons/day of effluent from the Town of Wareham secondary sewage treatment plant was trucked to ESL and discharged into one of the three buried 8,000 gallon fiberglass nutrient storage tanks. From there, the effluent was pumped to a head box in the ESL mechanical room and then distributed by gravity to the ponds.

For the remainder of the year, two to four of the pond cultures were grown on various mixtures of sewage effluent and seawater and the remainder on the inorganic nutrient medium, which was adjusted to the nitrogen and phosphorus levels of sewage effluent (typically 20-25 mg/1 N and 10-15 mg/1 P). The number of ponds operated on sewage effluent depended upon the sewage concentration and flow rate (% pond exchange/day) employed. For example, at 50% pond turnover with 25% sewage effluent and 75% seawater, over 4,000 gallons/day of effluent is required, over half the daily supply. Since it was desired to obtain maximum performance data of the algal cultures without any chance of their being nutrient limited, the usual procedure was to operate only two ponds with sewage effluent, at concentrations and turnover rates comparable to the above example, particularly during the high productivity period in summer, even although all nutrients were not removed.

Three times a week (M,W,F) the inorganic nitrogen and phosphorus input (sewage and seawater) and discharge (pond harvest) and the particulate (i.e., algal) carbon and nitrogen in the discharge were monitored. From these data, daily nutrient uptake and algal production could be calculated and expressed on a per volume and per area basis. This information is summarized in Table 1 on a seasonal basis, extrapolated to show areal requirements in acres per MGD of effluent (10,000 capita) for complete tertiary treatment (nitrogen removal). This ranges from 26 acres in summer to 77 acres in winter, with 19 acres for the best short-term performance in midsummer. Also shown in Table 1 are comparable data for production and nutrient removal by macroscopic algae (seaweeds), which will be discussed below.

In contrast to earlier experience with effluent from other treatment plants, in which the nitrogen is predominantly in the form of ammonia, the Wareham effluent is highly oxidized with 0-30% ammonia (depending upon time of year, performance of the plant, and perhaps other factors), the remaining nitrogen fraction being nitrate. This apparently does not affect algal production, though there is evidence that the ammonia is preferentially used first by the plants if a mixture of the two forms is present. To more nearly simulate sewage

**Table 1. Mean algal production** and nitrogen removal in effluent-enriched phytoplankton and seaweed cultures, on a seasonal basis. (Figures rounded) A = phytoplankton, B = seaweed*)

| | Winter | | Spring-Fall | | Summer | | Maximum | |
|---|---|---|---|---|---|---|---|---|
| | A | B | A | B | A | B | A | B |
| Mean algal production | | | | | | | | |
| (g dry weight/$m^2$/day)† | 3 | 3 | 6 | 5 | 9 | 13 | 12 | 16 |
| Nitrogen removal | | | | | | | | |
| g/$m^3$/day | 0.3 | 0.1 | 0.6 | 0.2 | 0.9 | .5 | 1.2 | .6 |
| lbs/acre/day‡ | 2.7 | 0.9 | 5.4 | 1.8 | 7.1 | 4.5 | 10.8 | 5.4 |
| Equivalent volume effluent treated§ | | | | | | | | |
| MGD/acre | .013 | .004 | .026 | .008 | .039 | .022 | .052 | .027 |
| Area requirement | | | | | | | | |
| Acres/MGD effluent ‖ | 77 | 223 | 37 | 112 | 26 | 45 | 19 | 37 |

*based on data for <u>Gracilaria foliifera</u>

†ash free

‡assuming cultures one meter deep

§assuming 24 mg N/l effluent or 200 lbs N/million gallons effluent

‖or acres/10,000 capita

effluent in the cultures that were fed inorganic chemical nutrient medium, the ammonium chloride was replaced by an equivalent amount of sodium nitrate. However, the latter proved unsuccessful, possibly due to toxic contaminants in the industrial-grade chemicals used, so practice reverted to the use of ammonium chloride. Generally speaking, the performance of the cultures with respect to algal growth and nutrient removal were the same whether sewage effluent or the chemical nutrient medium, adjusted to the same nitrogen concentration, were used.

During a period of approximately two months in the late winter-early spring of 1975, due to malfunction or poor operation of the treatment plant, the effluent was of poor quality, containing large quantities of undigested suspended solids. The resulting turbidity inhibited algal production and the dissolved and particulate organic matter made monitoring of nutrient utilization and algal production impossible during that period. That experience points out the necessity for high quality, completely oxidized, and clear secondary effluent for the successful operation and monitoring of the algal growth system.

Despite considerable effort and experimentation, including filling the algae ponds with 1μ-filtered seawater and inoculation with large (several hundred liter) cultures of several different species of unicellular algae, no success was obtained in controlling the species of algae that developed and persisted in the ponds. Cultures were always virtually unispecific, the species varying with the season. In winter, at temperatures between 0° and 9°C, the diatom _Skeletonema costatum_ occurred. During most of the remaining part of the year, at water temperatures of 10°-25°C, the diatom Phaeodactylum tricornutum, was the persistent alga. During a brief period of about one month in midsummer, when pond temperatures exceeded 25°C, unidentified green flagellates replaced the Phaeodactylum cultures. It is unlikely that the species of algae present affects rate of algal production or nutrient utilization, so this is not an important factor with respect to the tertiary treatment role of the system. However, some species are well recognized and documented as better food organisms for bivalve molluscs (3). Although Skeletonema is generally regarded as one of the better shellfish foods, Phaeodactylum is variously reported as poor to indifferent. The implications of this problem will be discussed further below, but species control remains a problem that will continue to receive high priority in future research.

Two of the algae ponds may be heated by circulating their contents through heat exchangers in the laboratory. These were operated at 15°-20°C throughout the winter when temperatures in the unheated ponds ranged from 0° to 5°C. Surprisingly, there was no difference in algal production between the heated and unheated ponds. Seasonal variations in algal production of three-fourfold and even species succession and dominance are apparently due to changes in incident solar radiation, with temperature a second order factor, at least in winter. This is an important finding, as it eliminates the need to consider heating an extensive area of shallow algal ponds in winter in any commercial application of the process in temperature latitudes. Unfortunately, however, the algal culture must still be heated to 15°-20°C before it can be utilized by the shellfish.

The culture ponds, which are approximately 50' x 50' x 3' deep were constructed from shaped sand and fine gravel lined with 20 mm black PVC. The exposed edges of the PVC liners are further covered with a 10 mm PVC "sacrificial" sheet that may be replaced when and if sun damage occurs. When filled to a depth of three feet, the pond volume is 35,000 gallons.

The cultures are kept in gentle circulation with two one-third HP (40 gal/min) cast iron pumps on opposite corners of the ponds. These recirculate the culture, the return jets entering above the surface to provide both momentum and aeration. This action is normally sufficient to keep the algal cells in suspension.

The continuous-flow cultures may be maintained for months at a time with little or no maintenance. Gradually, the accumulation of organic matter on the bottom and the development of a fringe of epiphytic green algae (usually Enteromorpha) at the water's edge around the periphery of the pond causes a reduction in algal production. This is exacerbated if the sewage effluent contains significant amounts of suspended solids When this occurs, normally at intervals of 3-6 months, the ponds are drained, cleaned, sprayed with dilute sodium hypochlorite, sun-dried, refilled, and reinoculated with an adiacent culture. This takes one to two man-days of effort per pond, and the new culture can be brought on line into production in about four days.

At pond temperatures exceeding 15°C when Phaeodactylum is the dominant alga in the cultures, an unidentified colorless protozoan flagellate, roughly the same size as the Phaeodactylum cells (i.e., 20-30μ in diameter) appears in the cultures and preys upon the diatoms. Unpredictably and very quickly, the flagellate at times proliferates throughout the culture and eliminates the algae. The cultures may be discarded and restarted, as described above, but if left alone, the flagellate population quickly subsides, presumably through lack of food, and the Phaeodactylum population reestablishes itself in about the same time (3-5 days) that it takes to start a new culture. This represents an undesirable interruption in algal production that should, if possible, be avoided. Studies have now been initiated and will be continued during the coming year on the identification, basic biology, and nutritional requirements of the flagellate predator so that, hopefully, conditions can be maintained that will inhibit its growth. Preliminary indications are that the organism favors high nutrient levels and perhaps the presence of dissolved organic compounds from the sewage effluent or excreted by the algae. It is hoped that the highest turnover rate and lowest nutrient level that are consistent with maximum algal production and nutrient removal (e.g., 10-20% effluent, 50% or more turnover per day) will also prevent growth of the flagellate population and thereby contribute to stability of algal production.

B. Bivalve mollusc culture

Harvest from the phytoplankton pond cultures (equivalent in volume to the daily turnover rate of the ponds) flows by gravity into respective cement raceways that are 40' x 4' x 5' deep. At its point of entry to the raceway, the algae culture is diluted with coarse-filtered seawater at ratios ranging from 1 to 5 parts seawater to 1 part culture, depending upon the season and other, related factors. Reasons for the dilution are: 1) to dilute the algal suspension to the degree necessary for the shellfish to filter and assimilate the food organisms most efficiently, a concentration believed to be of the order of $10^5$ cells/ml; 2) to provide a more rapid flow of water through the raceway to enhance shellfish feeding; 3) to prevent the accumulation of metabolites of the animals, particularly ammonia, to toxic levels; and 4) through use of heated seawater when and as needed, to bring the combined flow of algae and seawater to a temperature at which the shellfish will feed and grow throughout the year. Phytoplankton will grow equally well on heated and unheated pond cultures in winter, as discussed above but the unheated cultures must be heated to 15°-20°C or more (depending upon the shellfish species) before they are presented to the animals. One way of accomplishing this is to harvest the unheated cultures into the heated ponds where they can be raised to the desired temperature before being introduced to the shellfish. The other method, as mentioned above, is to dilute the culture with heated seawater to the extent necessary to bring the combined flow to the desired temperature. Both methods have been used successfully during the winter of 1974-75.

The relatively large ESL facility does not have the capacity to raise temperatures, by either of the above methods, of the combined algal culture-seawater mixture, at the desired flow rates, to levels above approximately 15°C in winter. Nor does it have the capability of providing a range of different temperatures to the raceway system while holding other factors (i.e., flow rates) constant. Finally, there is no capacity to cool water at our facility, and solar heating of the algal pond cultures together with the diluting seawater may result in peak summer raceway temperatures of 25°C. It has therefore not been possible to control temperatures in the animal culture system beyond a seasonal range of 15°-25°C This has led to some problems in attempting to assess shellfish growth over long periods of time as a function of other variables, such as food species, food concentration, flow rates, etc. Particularly, it has been difficult to assess the relative growth and potential value in our system of shellfish that have different optimal temperatures for feeding and growth. (e.g., The American oyster, Crassostrea virginia, grows more rapidly at 25°C or above while a strain of European oysters, Ostrea edulis, obtained from Maine grows best at about 15°C.)

The algae culture-seawater mixture enters one end of the 40-foot raceway and passes in a linear flow to the opposite end, where it enters the adjacent seaweed-stocked raceway, for final "polishing" of the effluent. Shellfish are stocked in wooden-frame, vexar-lined trays (mesh size depending upon size of the shellfish) at an initial density.

for the 1/2-1" seed, of 1,500 to 3,000 animals per tray, which is later thinned appropriately as the bivalves grow. The trays are stacked vertically, 7-8 trays per stack, the raceways accommodating 8 such stacks of trays, holding a total of some 150,000 seed shellfish. An airline extends along the side of the raceway on the bottom to provide aeration and vertical mixing of the water throughout its length. This has been found essential for mixing thoroughly the algae culture and diluting seawater and preventing a stratified flow down the length of the raceway, particularly in cold weather when heated seawater is used. In addition, aeration is important in maintaining high levels of oxygen and low levels of metabolites, particularly ammonia, everywhere in the raceway and especially near the bottom.

In spite of the large amount of work that has been done with oysters and, to a lesser extent, clams, mussels and other bivalves, no one has yet successfully grown these organisms in a large-scale, artificial rearing system employing cultured food organisms. Our initial attempt, involving the stocking of three shellfish raceways with 300,000 seed oysters (Crassostrea virginica) from Flower Brothers Hatchery, Bayville, Long Island (N.Y.) and 150,000 seed hard clams (Mercenaria mercenaria) from Long Island Oyster Farms, Northport, Long Island (N.Y.) during the the winter of 1973-74, was largely unsuccessful. Neither species grew significantly during the following 18 months and most of the oysters died during the summer of 1974. Initially the cause of this failure was believed to be an improper or inadequate food source, a diet predominantly of the diatom Phaeodactylum tricornutum. Recent experience, however, has required reevaluation of this explanation. A new batch of 150,000 seed oysters from Long Island Oyster Farms stocked on April 28, 1975, and smaller numbers of C. virginica, of the European oyster (Ostrea edulis), and of the Manila clam (Tapes semidecussata) which were introduced at various times later in 1974 and 1975 have all grown well, in some cases on a diet exclusively of Phaeodactylum. Table 2 is a summary of the experience to date with shellfish growth and mortality in the raceway system.

It is suspected, therefore, that the initial group of oysters and clams may have been hatchery "culls", or individuals whose growth had been irreversibly checked for some reason. Perhaps, also, culture conditions during the early part of the operation (temperature, dissoloved oxygen, ammonia concentration, flow rates, food concentration, etc.) were unfavorable. Fortuitously, either or both of these problems may have been corrected, but if so, it is not yet clear exactly what problem was corrected or how its reoccurrence may be avoided.

It is planned during the coming year, therefore, to obtain smaller numbers of a large variety of seed shellfish from as many different sources (commercial hatcheries, research institutions, etc.) as possible and to evaluate their comparative growth under the same culture conditions. These will include experiments with different species of bivalves and with the same species (C. virginica) obtained at different times, of different sizes and ages, from different hatcheries, and from different geographical regions. It is hoped thereby to select the best species

Table 2. Growth and cumulative survival of shellfish in algae-fed raceways (based on samples of 200 individuals).

| Species | *Crassostrea virginica* | | | *C. Virginica* | | | *C. virginica* | | | *C. virginica* | | | *Ostrea edulis* | | | *Mercenaria mercenaria* | | | *Iapes semidecussata* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Raceway No. | 1 | | | 5 | | | 1 | | | 1, 3 | | | 1 | | | 3 | | | 1 | | |
| No. stocked | 150,000 | | | 150,000 | | | 5,000 | | | 150,000 | | | 3,000 | | | 150,000 | | | 10,000 | | |
| Date stocked | 12/73 | | | 12/73 | | | 7/74 | | | 5/75 | | | 3/75 | | | 1/74 | | | 11/74 | | |
| Date | Length(cm) | | % survival | Length(cm) | | % surv. | Length(cm) | | % surv. | Length(cm) | | % surv. | Length(cm) | | % surv. | Length(cm) | | % surv. | Length(cm) | | % surv. |
| | $\bar{m}$ | $\sigma$ | | $\bar{m}$ | $\sigma$ | | $\bar{m}$ | $\sigma$ | | $\bar{m}$ | $\sigma$ | | $\bar{m}$ | $\sigma$ | | $\bar{m}$ | $\sigma$ | | $\bar{m}$ | $\sigma$ | |
| 12/73 | 2.7 | .8 | --- | 2.7 | .8 | --- | | | | | | | | | | | | | | | |
| 1/74 | | | | | | | | | | | | | | | | 1.5 | .1 | --- | | | |
| 2/74 | | | | | | | | | | | | | | | | 1.7 | .2 | 100 | | | |
| 3/74 | 2.8 | .7 | 96 | 2.8 | .8 | 100 | | | | | | | | | | | | | | | |
| 4/74 | 3.1 | 1.7 | 71 | 3.1 | .8 | 100 | | | | | | | | | | 1.7 | .2 | 100 | | | |
| 5/74 | 3.2 | .8 | 60 | 3.3 | .9 | 96 | | | | | | | | | | 1.6 | .4 | 99 | | | |
| 6/74 | 2.7 | .9 | 34 | 3.5 | .9 | 78 | | | | | | | | | | 2.0 | .3 | 99 | | | |
| 7/74 | | | | 3.4 | .5 | 78 | 2.3 | .3 | --- | | | | | | | | | | | | |
| 8/74 | | | | | | | | | | | | | | | | 2.0 | .2 | 97 | | | |
| 9/74 | | | | | | | 2.7 | .2 | 100 | | | | | | | | | | | | |
| 10/74 | 3.9 | .6 | 24 | | | | 3.5 | .3 | 100 | | | | | | | 2.1 | .2 | 97 | | | |
| 11/74 | | | | 3.9 | .7 | 2 | | | | | | | | | | | | | .5 | .1 | --- |
| 12/74 | 3.3 | .8 | 24 | | | | 3.6 | .5 | 100 | | | | | | | 2.1 | .3 | 97 | .7 | .1 | 100 |
| 1/75 | 3.8 | .6 | 24 | | | | 4.2 | .4 | 100 | | | | | | | | | | | | |
| 2/75 | | | | | | | | | | | | | | | | | | | .9 | .2 | 100 |
| 3/75 | 3.1 | 1.3 | 24 | | | | 3.8 | .8 | 93 | | | | 4.1 | .9 | --- | 2.1 | .3 | 90 | 1.1 | .2 | 100 |
| 4/75 | | | | 5.3 | .8 | 2 | 4.0 | .7 | 77 | | | | 5.8 | 1.1 | 100 | 2.0 | .2 | 87 | | | |
| 5/75 | | | | 5.2 | .8 | 2 | | | | 1.2 | .3 | --- | 7.5 | .6 | 100 | | | | 1.5 | .2 | 100 |
| 6/75 | | | | | | | | | | 1.9 | .3 | 100 | 7.4 | .8 | 100 | | | | 1.8 | .2 | 100 |

and define the best size and condition and the best stocking time for optimal growth under normal operating conditions of the system. Particularly, organisms capable of rapid growth on a unialgal diet consisting, for sustained periods of time, of *Phaeodactylum tricornutum* will be looked for.

It is also planned to conduct experiments with the same batch of seed oysters (i.e., obtained at the same time from the same hatchery) in which such factors will be varied as food concentration, flow rate, aeration, temperature, stocking density, size and configuration of trays, and any other conditions that are amenable to experimental manipulation in the raceway system. Many of these factors have been varied during the past year, but lack of significant growth of the shellfish under any conditions and infrequent monitoring of their size, condition, mortality, etc. made it impossible to reach any meaningful conclusions. During the coming year, the shellfish will be monitored much more frequently (at least once a week) and more intensively (from several different trays in each raceway) so that changes in growth and mortality may be attributed to both experimental and natural variations in the culture environment. It is also planned to carry out small scale experiments, using a few hundred to a few thousand animals in individual trays, water tables, or other containers where the effects of a wide range of experimental conditions (food concentration, flow rate, etc.) may be examined simultaneously. The object of these experiments, both large and small scale, will be to define, within the limits that can be achieved and controlled in the culture system, the optimal mode of operation for the best possible growth and survival of the shellfish.

C. Seaweed culture.

Seaweeds are used in the polyculture system as a "polishing step" to remove nutrients not initially assimilated by the phytoplankton and those put back into the culture system by excretion of the shellfish and other animals and the decomposition of their solid wastes. The objective is to achieve a nutrient-free final effluent that will meet standards of tertiary sewage treatment at the same time producing a crop of commercially valuable plants.

During the past year, seaweed research was restricted to red algae of several species that are of existing or potential commercial value for their content of agar or carrageenan. These have included *Chondrus crispus*, *Gracilaria foliifera*, *Agardhiella tenera*, and *Hypnea musciformis*. Of these, *Gracilaria* and *Agardhiella* have proved most successful. The following discussion concerns primarily the results obtained with *Gracilaria*.

As explained in the preceding section, water leaving the shellfish raceways passes through the adjacent raceway in the opposite direction where it is exposed to suspended cultures of seaweed before being discharged back to the ocean. The latter have the same dimensions as the shellfish raceway (40' x 4' x 5' deep) but have been modified with a sloping plywood bottom with a depth ranging from two feet, on the high side to the bottom (five feet) on the low side.

An air line on the bottom at the five-foot depth provides the vigorous circulation needed to keep the seaweed in suspension and to bring it continuously to the surface and to exposure to sunlight. The sloping bottom eliminates a dead area in the circulation cell in the corner opposite the air line, in which the seaweed would otherwise settle and collect.

Once a week, the seaweed population is harvested from the raceways with dip nets, drained, and weighed. Net production over the previous week is removed, returning a constant starting biomass of 50 kg/raceway. This routine has varied experimentially during the year, but the preceding figure was found empirically to be optimum for maximum daily production, which ranged from a mean of 3 grams dry weight (organic matter) $m^2$/day in winter to 10 grams/$m^2$/day in summer (dry weight is 10% of wet weight and contains an average of 40% ash in Gracilaria foliifera). The harvested seaweed is dried and packed in bales which have been sent to commercial seaweed firms for evaluation and assay for polysaccaride content. Information has not yet been received back from these organizations. Performance of the seaweed raceway as a polishing step in nutrient removal will be considered in the following section, in which nutrient mass balance for the whole system is discussed.

Occasionally fouling organisms, in particular the green alga Enteromorpha, invade seaweed cultures and grow epiphytically upon the cultured species. Under extreme conditions, the cultures must be discarded. Epiphytic growth is probably the single greatest problem in and constraint to commercial seaweed culture, particularly in the tropics and subtropics where conditions are otherwise ideal for such practices. For reasons not fully understood, this problem has not developed in the Woods Hole experiments. Occasionally, in smaller-scale experiments, the seaweeds have become overgrown with Enteromorpha, but this has never happened to any significant extent in the raceway cultures. Earlier, when Chondrus crispus was being grown, it became seriously epiphitized by filamentous red algae (Ceramium rubrum, Spermothamnion sp.). For that and other reasons, principally the relatively slow growth of Chondrus in our system, it was replaced by Gracilaria and Agardhiella. These two seaweeds have remained remarkably clean and free of epiphytes and epizoa and, in fact, when contaminated specimens from other, small-scale experiments or collected from nature are introduced to the raceways, they normally lose their epiphytes. Presumably, some fortuitious accident in the design or operation of the seaweed growth system results in the supression of epiphyte growth. It is hoped, in the near future, that the responsible factor or factors can be identified and defined.

During 1974, new experiments were initiated in which seaweeds have been grown alone, in a single-step waste recycling system, using mixtures of seawater and secondary sewage effluent in a continuous flow-through mode of operation. A series of plywood tanks 8' x 6' x 3' painted with white epoxy have been used in these experiments. As in the seaweed raceways, the bottom of the tank slopes from a depth of one foot to the three-foot bottom on the long (8') dimension. Because the tanks are wider and shallower than the raceways, the slope of the bottom is more gentle. Again, aeration is provided through an air line extending the length of the tank along the bottom, three-foot deep edge. Because of the more gentle slope, the seaweed is not carried over into the shallow side of the tanks, most of it sinking one to two feet before reaching the edge. As a result, some 15 $ft^2$, or almost one-third of the area of the tank, is not in production and yield per unit area is correspondingly lower than in the raceways. A bottom slope approaching 45° would appear to be an optimal design for such units.

Despite this design deficiency, high yields of as much as 16 grams ash-free dry weight/$m^2$/day have been achieved for short periods of time in summer, while average yields of 3 g/$m^2$/day in winter and 12 g/$m^2$/ in summer were sustained over long periods of time. Table 1 shows, in addition to the yields and nitrogen removal of the unicellular algae in the phytoplankton culture ponds, the corresponding data for the seaweeds grown on sewage effluent and seawater mixtures in the experimental tanks described above. Again the data have been extrapolated to show the potential and areal requirement of such a system in nutrient removal per MGD effluent. It may be seen that seaweed production is comparable to and, in summer, slightly better than unicellular algae production. However, because the seaweeds contain on the average less nitrogen per unit of ash-free dry weight (4% for seaweeds and about 10% for unicellular algae), the equal or higher rate of growth of seaweed is more than offset by its lower capacity for nitrogen removal per unit growth.

In one experiment, three of the above seaweed tanks were operated in series, with an input of 25% sewage effluent - 75% seawater mixture introduced into the first tank and then passing through the second and third tanks at flow rates equivalent to 50% of the individual tank volume turnover per day. The three tanks were initially stocked with 5,000, 3,000, and 1,000 grams respectively of Gracilaria, and the growth increment allowed to accumulate during the one-month period of the experiment. Inorganic nitrogen and phosphorus were monitored in the water entering and leaving each of the tanks. The data from this experiment is summarized in Table 3, where it may be seen that the three tank cultures progressively removed 99% of the incoming nitrogen. Nitrogen deficiency of the Gracilaria in the third tank was evident both in its pale yellow coloration, in contrast to the deep reddish-brown color of the plants in the first tank, and in its carbon:nitrogen ratio, which was 28 in contrast to 10 in the first tank. This has some practical significance, as the commercial product of the seaweeds (agar in Gracilaria) is reportedly elaborated more

Table 3. Nitrogen removal in experimental seaweed (Gracilaria foliifera) tanks operated in series, April 15 - May 15, 1975, under steady-state, continuous-flow conditions*

| Tank No. | Effluent N concentration† (ppm) | %N removal† | Seaweed production ($g/m^2/day$)** | C:N in seaweed |
|---|---|---|---|---|
| 1 | 0.96 | 60 | 3.4 | 10 |
| 2 | 0.07 | 77 | 2.5 | 12 |
| 3 | 0.02 | 99 | 1.2 | 28 |

*See text for description of experimental conditions

†Initial N concentration (input to Tank 1) = 2.41 ppm.

** Ash free dry weight

rapidly and to a greater degree in nitrogen-deficient plants. In a commercial seaweed culture application, using a raceway or channel-type culture configuration with a linear flow of water and nutrients, the seaweed should presumably be moved downstream in the system, away from the source of nutrients, and harvested from the far end following a period of exposure to nitrogen-free conditions.

The same experiment as described above is now being repeated, at the time of this writing. Conditions are identical except that the input of both sewage effluent and seawater has been doubled (one tank volume turnover per day and twice the daily flux of nutrients). Complete nitrogen removal is still being observed in this experiment, the results of which are not yet ready for publication. Ultimate capacity of the system will eventually be evaluated by this means.

D. Nutrient removal efficiency of the system as a whole.

As pointed out earlier, algal pond cultures were operated during the first year deliberately at nutrient (sewage effluent) concentrations higher than could be completely utilized by the phytoplankton. This was done to develop information on the maximum potential growth and nutrient assimilative capacity of the algae under non-nutrient limited conditions. The amount of nitrogen taken up by the algae from solution or the amount contained in the algal harvest, by direct measurement, could then be used to calculate the daily assimilative capacity of the system and this, in turn, to calculate the daily input of sewage effluent per unit area of algal pond for complete nitrogen removal. That information, based on a year's observation, is presented in Table I, also including the comparable data for a seaweed-based tertiary treatment system.

The above data, interpreted in terms of the ESL pond culture system, means that complete nitrogen removal could be expected in winter operating at a 25% pond volume turnover per day with an input of 10% sewage effluent and 90% seawater. In spring and fall, the effluent strength can be increased to 20% or the turnover rate doubled (50%), resulting in either case in doubling the nutrient input rate. In summer, the system should be able to assimilate completely the nitrogen from 30% effluent - 70% seawater mix at 25% turnover, or a 10% effluent - 90% seawater at 75% turnover rate per day.

Since it is costly to pump seawater, the higher effluent concentration at the lower exchange rate is the more economical mode of operation. There is some evidence, however, that stability of the cultures may be enhanced by low nutrient levels at high turnover rates, so the costs of labor (for cleaning and restarting cultures) and of building and operating stand-by cultures to provide for down-time may exceed the cost of pumping additional seawater.

Beginning in April, 1975, a series of pond experiments was initiated to test and evaluate the above conclusions. Two of the ponds have been operated continuously on a 10% sewage - 90% seawater mixture at 50% pond volume turnover per day and 20% sewage effluent - 80% seawater at 25% turnover per day respectively (i.e., the projected "spring" operating conditions discussed above), with the objective of achieving complete nitrogen removal.

At the same time, a new supply of 5-10 mm seed oysters (Crassostrea virginica) was obtained from Long Island Oyster Farms. These oysters had set in the hatchery in February, 1975, and were new, vigorously-growing animals. Growth has continued in the ESL raceways up to the present time with no detectable mortality. In addition, by the spring of 1975 the seaweed raceway culture system had been improved through experience to the extent that a dense, clean, vigorously-growing culture of Gracilaria was well established with stable, uninterrupted production of the seaweed.

Conditions were appropriate, therefore, for evaluation of the nitrogen balance and mass flow through the entire system. In so doing, half the algal harvest from one pond was fed into one shellfish raceway and its discharge into one seaweed raceway, the three units serving as one module of a prototype tertiary sewage treatment-aquaculture system.

Table 4, shows the daily mass flow of nitrogen through the three-step system under the operating conditions defined above, considering only the half of the pond culture that was fed to the shellfish. Of the nitrogen (nitrate, nitrite, and ammonia) daily entering the pond as sewage effluent (84 grams) and seawater (1 g), over 98% (83.5 g) was removed by the phytoplankton. The remaining 1.5 g, together with the algae, was fed to the shellfish raceway, where it was mixed with twice

Table 4. Mass flow of inorganic nitrogen (ammonia, nitrite, and nitrate) through the phytoplankton-oyster-seaweed system; May, 1975.

| | | grams N/day | |
|---|---|---|---|
| 1. | Phytoplankton pond input | | |
| | sewage effluent | 84 | |
| | seawater | 1 | 85 |
| 2. | Phytoplankton pond output | | 1.5 |
| 3. | Shellfish raceway input | | |
| | phytoplankton pond harvest | 1.5 | |
| | seawater | 3.0 | 4.5 |
| 4. | Shellfish raceway output | | 27 |
| | (= seaweed raceway input) | | |
| 5. | Seaweed raceway output | | |
| | (final effluent from system) | | 9.4 |
| | Total N removal efficiency (including seawater) | | 89.3% |
| | Effluent N removal efficiency | | 93.6% |

its volume of seawater. Since the latter contained the same concentration of inorganic nitrogen as the pond effluent (0.04 ppm), the seawater contributed twice as much nitrogen as the effluent (total 4.5 g). To this, the shellfish raceway added 22.5 g of dissolved inorganic nitrogen through excretion, decomposition, or other sources, roughly 25% of the amount that entered the raceway as phytoplankton. Of the total output of 27 g nitrogen from the shellfish raceway, 18 g were removed by the seaweeds, leaving a final residual of 9 grams, 10% of the initial input of the sewage effluent and seawater, for a total removal efficiency of the system as a whole of 90%.

The complete system, as described, was not "balanced" with respect to the size and biomass of the seaweed culture relative to the other components, since the experiments were designed to determine, among other things, what that balance should be. (i.e., It was not known at the outset what fraction of the unicellular algal nitrogen would be regenerated by the shellfish.) Since the seaweed removed two-thirds of the regenerated nitrogen, it can be assumed that expansion of the seaweed culture by one-third (from 160 $ft^2$ to 240 $ft^2$ in the pilot facility) would result in complete nitrogen removal of the final effluent.

The above results are typical of those that were obtained in over one month of continuous operation, with extremely little variability with the exception of one five-day period, when predation by colorless flagellates (discussed in an earlier section) temporarily reduced phytoplankton production. It should be pointed out, however, that this monitoring period, in April-June, 1975, coincided with the and took place under conditions of the best possible performance of the system, and is not representative of average performance throughout the year.

In both modes of operation of the pond cultures (i e., 10% effluent-50% turnover and 20% sewage-25% turnover), 95-99% nitrogen removal by the unicellular algae has consistently been achieved except for brief periods mentioned above when algal production is depressed by the protozoan predator. The latter has been more frequent and pronounced in the more heavily enriched (20% effluent) culture. More recently, at the time of this writing, the nutrient concentration in the sewage effluent has unaccountably increased by about 25%, giving a total daily input of nitrogen to the ponds of about 210 grams, in contrast to the 170 g reported in Table 4. This is equivalent to roughly 25% effluent of the strength used up to this time. Nitrogen removal is still virtually complete, ranging from about 90% on cloudy days to as much as 99.9% on clear sunny days, at which times the effluents from the ponds contain less nitrogen than the incoming seawater.

E. Culture of secondary animal crops.

Solid wastes (feces and pseudofeces) produced by the shellfish and/or uneaten phytoplankton cells which settle out from suspension in the shellfish raceways provide sources of food for large quantities of several species of small, invertebrate detritovores that presumably enter the system as larvae in the coarse-filtered seawater used to dilute the phytoplankton pond harvest. Prominent among such invertebrates are amphipods (Corophium, Jassa, and Gammarus), polychaetes (Capitella capitata), bryozoans, tunicates, and mussels. This small invertebrate fauna serves the dual purpose of preventing the accumulation of solid organic wastes in the raceways and providing a source of food for secondary crops of carnivores or omnivores of potential commercial value. The latter has included the American lobster (Homarus americanus) and the winter or blackback flounder (Pseudopleuronectes americanus) (Table 5).

In July, 1974, 474 juvenile (0 and 1 year class) flounder were collected locally and stocked in one of the oyster raceways. Their size distribution was, of course, bimodal for the two-year classes, but averaged 7.0 cm. In October, 1974, the raceway was drained and 124 fish recovered, averaging 11.0 cm in length. In April, 1975, 69 fish were recovered, averaging 16.75 cm in length. The surviving fish thus more than doubled in size in 9 months. If the observed linear growth rate were to continue, the fish would reach a marketable size of 25 cm (1/2-1 lb) in another 9 months, or 18 months from the time of stocking as juveniles.

The fish were obtained by beach seine some distance from the laboratory, measured immediately, and stocked. The smaller (0-year

Table 5. Growth and survival of winter flounder and American lobsters in oyster raceways.

| Winter flounder (Pseudopleuronectes americanus) | | | |
|---|---|---|---|
| Date | Number | % survival | Size (mm) |
| 7/74 | 474 | ---- | 70 |
| 10/74 | 99 | 21 | 110 |
| 4/75 | 69 | 14.5 | 167 |

| American lobster (Homarus americanus) | | | |
|---|---|---|---|
| Date | Number | % survival | Size (mm)* |
| 9/74 | 390 | ---- | 9.0 |
| 11/74 | 256 | 66 | 13.4 |
| 4/75 | 124 | 32 | 25.0 |

*carapace length

class) fish are very delicate and subject to injury during collection and transport, and they very likely suffered a large initial mortality. Some fish may also have escaped, since flounder were found in adjacent raceways during the summer of 1974. Thus, the small survival (15%) of flounder is not necessarily representative of the carrying capacity of the raceway. The fish recovered in April, 1975, were extremely fat, healthy in appearance, with good natural coloration, and with no sign of disease. There was still an abundance of the small, invertebrate food organisms in the raceway, so it is unlikely that food was limiting.

The surviving 69 fish represented a density in the raceway of 0.4 fish/$ft^2$, which is a dense population if the bottom of the raceway alone were considered. However, many flounder were observed in the shellfish trays resting on the oysters and presumably feeding on the invertebrates associated with the shellfish. The area of the trays (483 $ft^2$ for 64 trays) increases the total habitat of the raceway by fourfold and should thereby increase the habitat of the system for bottom-dwelling fishes such as flounder proportionately, if sufficient food is available. A more careful and heavy stocking of the larger size juvenile fish (1-year class) that have been held long enough to eliminate mortality due to initial injury should provide the needed information on carrying capacity and potential rate of production of flounder in the system.

Egg-bearing lobsters were obtained from commercial fishermen, by special permit, and were held in the laboratory until the eggs hatched (i.e., in spring, when water temperatures reach 15°-20°C). The larvae were transfered to specially-constructed larval rearing tanks where they were fed live or frozen brine shrimp (Artemia salina). After metamorphosis to juvenile lobsters (10-14 days), they were segregated into small containers, to prevent cannibalism, and fed the same food until they had molted an additional 3-4 times and attained a mean size of 9 mm carapace length and 0.18 grams. A total of 390 of these lobsters were then stocked in September, 1974 in segregated (screened-off) portions of two oyster raceways, each group together with two stacks (16 trays) of oysters. In April, 1975, a total of 124 lobsters were recovered which had a mean size of 25 mm carapace length and a mean weight of 18 grams. These ranged widely, however, in their size distribution, from 10 to 52 mm carapace length. The larger individuals, some 150 mm total length, attained a size in eight months that is not reached by wild lobsters in New England in less than three years, and is comparable to the best growth obtained with segregated lobsters held in captivity at elevated temperatures and fed artifically.

A survival rate of 32% in an animal as cannibalistic as the lobster is remarkedly good. Post-larval lobsters can be produced in vast numbers quite inexpensively, so even quite a small percentage survival to adult animals could be economically attractive. The important factor, however, is not survival or mortality of the original stock, but the carrying capacity of the system. Lobsters were found living in the oyster trays and on the bottom of the segregated portions of the raceways, a total area of 365 $ft^2$ or about 0.3 lobsters/$ft^2$. On that basis, a complete 40' x 4' raceway with 64 trays of oysters can support some 200 lobsters for their first eight months. How long it will take for them to reach legal marketable size (81 mm carapace length in Massachusetts), and how many will survive to that size must await further observations.

Other lobster experiments were conducted in which post-larval animals were stocked at various densities in individual trays or screened-off portions of trays of oysters. Growth, again on the natural food that developed in the oyster trays, was comparable to that reported above, but after reaching a size of 13-17 mm carapace length (10th-12th molt), cannibalism reduced the experimental populations essentially to one lobster per tray or per compartment.

Although there is much current interest in commercial lobster culture, the logistics and economic problems of maintaining lobsters individually in separate containers (because of cannibalism) and feedint each lobster individually a prepared, artificial food has discouraged any serious undertaking. If, however, post-larval lobsters could be segreated in compartmentalized trays of oysters and allowed to feed on natural food, so that they would not have to be handled or inspected except at relatively long intervals (e.g., 3-6 months) when the oysters would need to be thinned and culled in any case, the economic prospects would be considerably more attractive.

New experiments are now being initiated in oyster culture using plastic, commercial grow-out trays (Nestier Division, Vanguard Industries, Cincinnati, Ohio) that measure 23" x 23" x 2 7/8". Six hundred and forty such trays will fit into one shellfish raceway. Even at a stocking density of one lobster per tray, this would be equivalent to 175,000 lobsters per acre of shellfish raceways five feet deep. Conceivably, two or even four lobsters per tray could be stocked by compartmentalizing the trays. The value of the secondary crop of lobsters could thus rival if not exceed that of the primary crop of shellfish. Such speculation, however, is premature until more is known about the space requirements of the lobsters and most important, the amount and rate of production of food in such a system, for it is the latter that will ultimately determine the potential yield of lobsters. These subjects will be investigated in the coming year.

## Summary and Conclusions

1. Tertiary treatment requirements.

Nitrogen removal is equivalent to tertiary sewage treatment of domestic wastewaters discharged into coastal marine environments, if the objective is to prevent algal growth in and eutrophication of the receiving waters. Repeated bioassays have demonstrated that effluent-seawater mixtures from which nitrogen has been removed are incapable of supporting the growth of algae, despite residues of phosphate and perhaps other nutrients.

2. Nitrogen removal by unicellular marine algae.

Complete nitrogen removal may be accomplished by growing unicellular marine algae in continuous, flow-through cultures in effluent-seawater mixtures. Shallow (1 meter), 2,500 $ft^2$, PVC-lined ponds, gently circulated to maintain the algae in suspension, have been used for this purpose. Optimal and maximum possible size of ponds to achieve the same or an improved performance have not been determined.

3. Factors controlling algal growth and nutrient removal.

Algal growth and nitrogen removal are independent of temperature (0°-25°C) but controlled by incident solar radiation, ranging in magnitude seasonally by three-fourfold in temperature latitudes. For the same reason, nitrogen removal is a function of surface area of the algae ponds and is largely independent of depth.

4. Algal production and nitrogen removal.

Sustained algal production in pond cultures has ranged from 3 grams ash-free dry weight per square meter of pond surface per day in winter to 9 $g/m^2$/day in summer with intermediate levels in spring and fall. Maximum production for short periods of time in summer has reached 12 $g/m^2$/day. A production of 10 $g/m^2$/day is equivalent to nitrogen removal (assimilation) of 1 $g/m^2$/day or 9 lbs/acre/day.

5. Area requirement for nitrogen removal.

At the latitude of Woods Hole, Massachusetts, the pond area required for complete nitrogen removal from one million gallons per day (1 MGD) of secondary sewage effluent (10,000 capita) ranges from 26 acres in summer to 77 acres in winter. At lower latitudes, as in Southern United States, a seasonal range of 26-37 acres per MGD effluent would be required.

6. Seawater pumping requirement.

A total of 7-9 MGD of seawater must be pumped per 1 MGD sewage effluent treated to dilute the effluent and provide a suitable medium for growth of the marine algae.

7. Algal species.

Pond cultures consist of uni-specific populations of single-celled algae, usually diatoms. At Woods Hole, Mass. cultures have consisted of the diatoms Skeletonema costatum in winter and Phaeodactylum tricornutum the rest of the year except for a brief period (ca. 1 month) in midsummer, at pond temperatures > 25°C, when green flagellates appeared. Efforts to control the species of algae in the ponds have been unsuccessful.

8. Culture stability.

Cultures are stable with respect to cell density (biomass), growth rate, and nutrient removal for long periods of time (months). Gradual accumulation of sediment requires periodic draining and cleaning of ponds every 3-6 months, requiring 1-2 man-days effort per 2,500 $ft^2$ pond with down-time of 3-5 days.

Occasionally and unpredictably, predation of the algae by an unidentified protozoan depletes the algal populations and temporarily reduces or stops cell production. This situation normally corrects itself within 3-5 days. Operating conditions that will inhibit development of the protozoan predator without depressing algal growth (i.e., low effluent concentrations at high turnover rates) are currently being investigated.

9. Algal removal and shellfish culture.

To complete the tertiary sewage treatment process, the algal cells that have assimilated nutrients from the sewage effluent must themselves be removed from the water. Filter-feeding bivalve molluscs are used for this purpose. The continuous-flow algal pond harvest is fed by gravity to cement channels or raceways containing stacked trays of oysters, clams, or other bivalves. Algal cultures must be diluted with coarse-filtered seawater to reduce cell densities to the degree that they can be used efficiently by the shellfish and to prevent accumulation of toxic metabolites of the shellfish (e.g., ammonia). Shellfish must also be supplied with vigorous aeration to mix cultures with diluting seawater, to prevent stratified flow through raceways, and to insure an adequate oxygen supply.

10. Seawater pumping requirement for shellfish culture and for entire system.

Algal cultures are diluted with an equal volume of seawater in winter, as much as five times the culture volume in summer. Total seawater requirements for the algal and shellfish cultures, per MGD effluent treated, range from 17 MGD in winter to 50 MGD in summer.

11. Shellfish production.

Shellfish culture during the first year of operation of the pilot plant facility was largely unsuccessful, with poor growth and high mortality of seed clams and oysters. This was due to unresolved problems believed to be: 1) unfavorable culture conditions in the raceway system; 2) unfavorable algal food (a diet for most of the year of the diatom, *Phaeodactylum tricornutum*, variously reported in the literature as a poor to indifferent food for bivalves); or 3) an inferior stock of shellfish that were stunted or whose growth was irreversibly checked prior to acquisition.

12. Projected shellfish prduction.

Based on data from small-scale experiments and from the literature (4), the algae produced from 1 MGD sewage effluent is sufficient to produce eleven million market-sized (3-4") oysters per year, which is equivalent to 183 tons of oyster meat or 38,000 bushels of whole oysters, assuming continuous feeding and growth throughout the year. Held in raceways or channels five-feet deep in stacked trays, the annual crop would require an area of approximately six acres assuming maintenance of a mixed population of juvenile and adult animals with periodic stocking and harvesting. These data are speculative until a successful method of shellfish production is demonstrated and evaluated in the pilot plant project.

13 Temperature requirements for shellfish production.

Indigenous bivalve molluscs require a minimum temperature of about 15°C and an optimum temperature of about 20°C or more (depending upon species) for feeding and growth. Algal cultures may be grown at ambient seawater temperatures throughout the year, with no advantage from heating (i.e., growth is controlled by solar radiation), but unheated algal cultures cannot be fed upon by the shellfish if temperatures of the culture and diluting seawater are together less than about 15°C. Diluting seawater could be heated more practically than could the shallow, extensive algal cultures, but neither could be done economically. Use of cooling water effluent from power generating stations ($\Delta t$ normally 10°-15°C above ambient) is not sufficient to raise temperatures of the algal food to the degree necessary in winter in regions of temperate climate, but at best could only extend the shellfish growing season.

14. Implications of thermal constraints to shellfish culture in operation of the system.

Because of the temperature requirements for shellfish growth (see 13), year-around operation of the system is restricted to tropical to semi-tropical regions. In temperate latitudes, operation is restricted to 4-6 months per year. This may be an acceptable alternative in coastal resort areas, where both the need for tertiary treatment and population pressure are greatest in summer. Over two-thirds of annual algal production occurs during the six warmer months (i.e., in Woods Hole, Mass.), so losses in nutrient-removal capacity and shellfish growth during the remaining six months would be minimized. Winter storage of effluent in lagoons, as is now practiced in some terrestrial waste recycling systems, is a possible alternative to discharge of such wastes when the aquaculture system is inoperative.

15. Regeneration of nutrients by shellfish and final nutrient removal by seaweeds.

In a balanced system in which there are enough shellfish to consume all of the unicellular algae provided as food ($10^8$-$10^9$ cells/animal/day depending upon size) approximately 25% of the nitrogen contained in the algae cells is regenerated as inorganic nitrogen, principally ammonia, by excretion of the shellfish and other animals in the shellfish culture system and decomposition of their solid wastes. These may be removed by a final "nutrient polishing" stage consisting of cultures of one or more species of seaweeds. Red algae of existing or potential commercial value for their hydrocolloid (carrageenan or agar) content are used for this purpose.

Seaweeds are grown in cement channels or raceways similar to those used for shellfish culture but with a sloping (ca. 45°) bottom and with vigorous aeration to maintain the plants in suspension and exposure to sunlight.

16. Seaweed production and nutrient removal capacity.

On the basis of inorganic nitrogen mass flow data in the complete pilot system, an area 8.5% that of the unicellular algal pond requirement is needed for total nitrogen removal of regenerated wastes by the animals. This is equivalent to 2.2 acres per MGD effluent treated. However, the mass flow data are based on short term measurements in May, when the system was operating near maximum efficiency and therefore represent "best performance" conditions.

Continuous, long-term seaweed production during late spring, summer, and early fall (i.e., when shellfish production is possible) averages 10 grams (ash-free dry weight)/$m^2$/day or 89 lbs/acre/day. This is equivalent to a nitrogen removal capacity (assuming N = 4% ash-free dry weight) of 3.5 lbs/acre/day.

The residual nitrogen produced by the animal system may therefore be removed by seaweed culture in an area of 14 acres per MGD effluent treated during a six-months growing season at temperatue latitudes, presumably throughout the year in tropical to semi-tropical environments.

Seaweed production from the "polishing stage" is 3,350 tons wet weight per year per MGD effluent treated, half of that figure for a six-months growing season.

17. Total areal requirement for complete system.

Considering only a six-months operating cycle in Northern climates and a year-around operation in the South, the areal requirements per MGD effluent treated for the complete nitrogen removal-aquaculture system consists of approximately 28 acres of algae ponds, 6 acres of shellfish cultures, and 14 acres of seaweed culture, for a total of 48 acres/MGD effluent. Mean depth of algae and seaweed cultures is three feet, that of the shellfish cultures, five feet.

18. A nitrogen-removal system based on seaweed culture only.

A tertiary sewage treatment (N-removal) system consisting only of seaweed culture may be used as an alternative to the combined unicellular algae-shellfish-seaweed culture. Production of seaweeds, as ash-free organic matter per unit of culture area per day, is comparable or perhaps slightly higher than that of unicellular algae. However, seaweeds contain less nitrogen per unit weight and therefore assimilate less nitrogen per unit growth than unicellular algae. Consequently a larger culture area is required for seaweeds than for unicellular algae to accomplish the same nitrogen removal.

19. Seasonal aspects of seaweed culture.

Experience with the pilot plant facility to date has been restricted to the culture of semi-tropical seaweed species (forms that are also summer annuals in New England), such as Gracilaria foliifera, Agardhiella tenera, and Hypnea musciformis. In winter, this was done in heated (ca. 15°C) water. Sustained production of *Gracilaria* has ranged approximately fourfold, from 3 grams (ash-free dry weight)/$m^2$/day in winter to 13 g/$m^2$/day in summer, presumably due to changes in solar radiation. Neither the above species nor the colder-water species *Chondrus crispus*, grows in nature or in unheated culture in winter. It is therefore assumed that seaweed culture is restricted to seasonal operation in the North but could be carried out throughout the year in tropical to semi-tropical climates.

20. Areal requirements and yields of seaweed culture.

Areal requirement for a seaweed culture system for nitrogen removal is approximately 60 acres per MGD effluent with an annual production of 16,300 tons (wet weight) of seaweed, half of that for a six-month seasonal operation.

21. Seawater pumping requirement for a seaweed-based waste recycling system.

Although not yet conclusively determined, tentative evidence is that best growth of and nitrogen removal by seaweeds occurs at nitrogen concentration of 2.5-3.0 mg/l, equivalent to approximately 10% wastewater effluent, at a flow rate of one culture volume exchange per day. Seawater required per MGD effluent treated is therefore 9 MGD.

22. Production of other animals grown in the shellfish cultures.

Shellfish cultures produce large quantities of solid, organic wastes (feces and pseudofeces). Small invertebrates (worms, crustacea, etc.) entering the shellfish culture as larvae in the diluting, coarse-filtered seawater, establish themselves and multiply in the shellfish trays and on the bottoms and sides of the raceway system. This small invertebrate fauna keeps organic sediment from accumulating in the shellfish culture and may also serve as a source of food for larger animals. Winter (blackback) flounder (Pseudopleuronectes americanus) and American lobsters (Homarus americanus), stocked as juveniles with the shellfish, have shown good growth and reasonable survival with no supplemental feeding. Carrying capacity of the system and potential yields of such animals with respect to food supply and its rate of production and habitat have not yet been determined.

23. Trace contaminate and pathogen uptake in organisms cultured in sewage effluent and related public health problems.

Problems associated with the uptake of trace contaminates (heavy metals, organic compounds) and pathogens (principally viruses) from the treated sewage effluent by the shellfish and other cultured organisms, and depuration of these substances by the organisms are subjects of an independent study now in progress. Results, when available, will be presented separately.

24. Preliminary results of research in Florida.

Smaller scale studies are currently being undertaken in Fort Pierce, Florida under support of the Harbor Branch Foundation, Inc. Growth yields, and nutrient removal by both unicellular algae and seaweeds are being studied on a seasonal basis. While it is unrealistic to compare the performance of the pilot-scale Woods Hole facility with that of the smaller Florida project, and results from the latter are not yet available for a complete year, preliminary indications are that growth and nutrient removal of both unicellular and macroscopic algae are, on the average considerably greater throughout the year in Florida than for the Spring-Fall period in Woods Hole (5, 6). On that basis, extrapolation of the seasonal performance of the Woods Hole system to a year-around operation in a climate such as Florida is not unreasonable and may be conservative.

References

1. Ryther, J. H. and W. M. Dunstan. 1971. Nitrogen, phosphorus, and eutrophication of the coastal marine environment. Science 171: 1008-1013.

2. Goldman, J. C., K. R. Tenore, and H. I. Stanley. 1974. Inorganic nitrogen removal in a combined tertiary treatment-marine aquaculture system: Part II. Algal bioassays. Water Research 8: 55-59.

3. Ryther, J. H. and J. C. Goldman. (In Press) Microorganisms as food in mariculture. Ann. Rev. Microbiology 1975.

4. Tenore, K. R., J. C. Goldman, and J. P. Clarner. 1973. The food chain dynamics of the oyster, clam, and mussel in an aquaculture food chain. J. Exp. Mar. Biol. Ecol. 12: 157-165.

5. Goldman, J. C., J. H. Ryther, and L. D. Williams. 1975. Mass production of marine algae in outdoor cultures. Nature 254: 594-595.

6. Lapointe, B. L., L. D. Williams, J. C. Goldman, and J. H. Ryther. The mass culture of macroscopic marine algae. Presented at the 38th Annual Meeting of ASLO, Halifax, Nova Scotia, June 23-26, 1975.

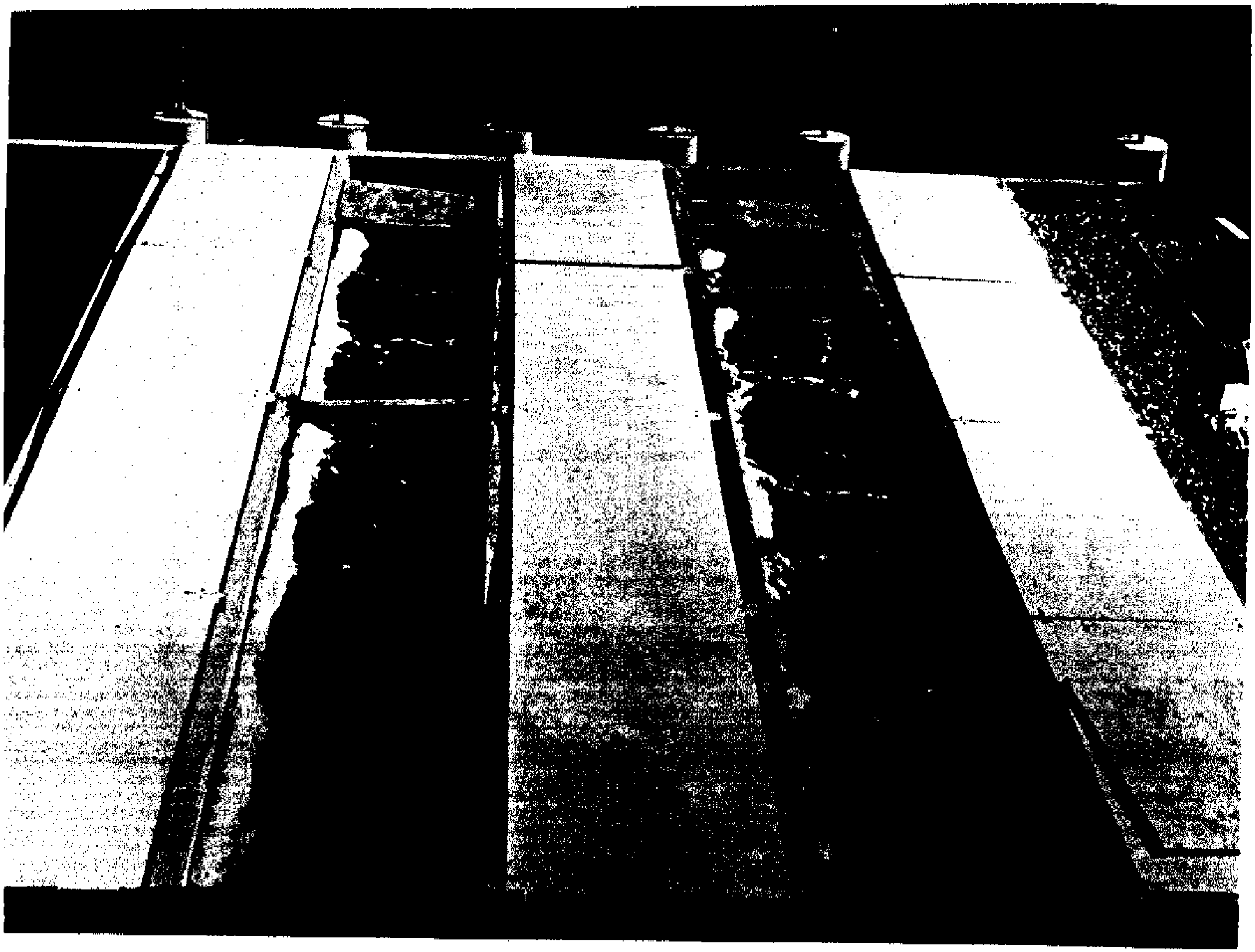


Figure 2. Environmental Systems Laboratory raceway system. Plywood covered raceways contain shellfish, flounder, and lobsters. Open raceways contain seaweeds Gracilaria, Agardhiella, and Hypnea grown in air-suspended culture.

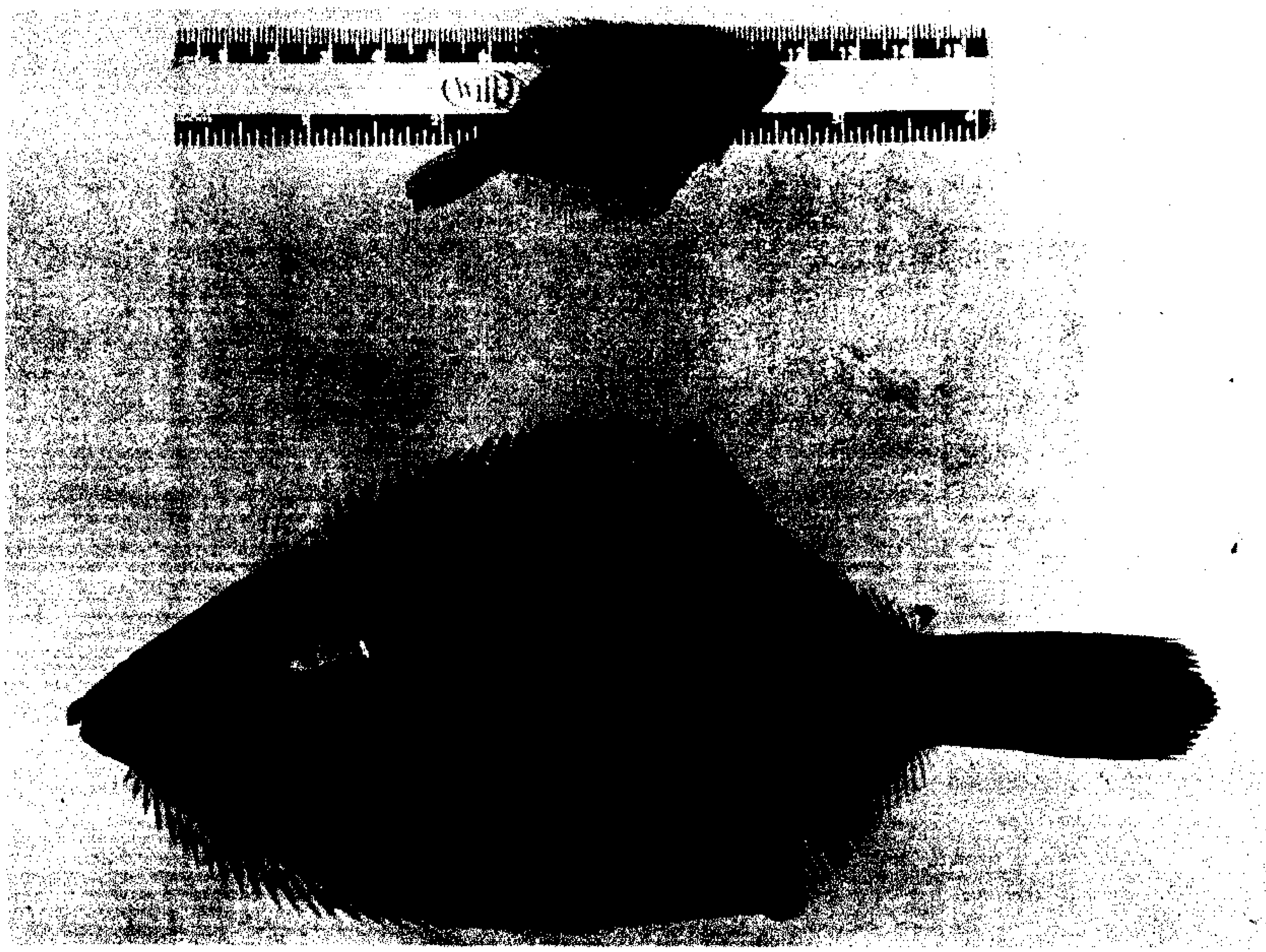


Figure 3. Growth of winter flounder (Pseudopleuronectes americanus) from July, 1974 (7.0 cm) to April, 1975 (16.7 cm).

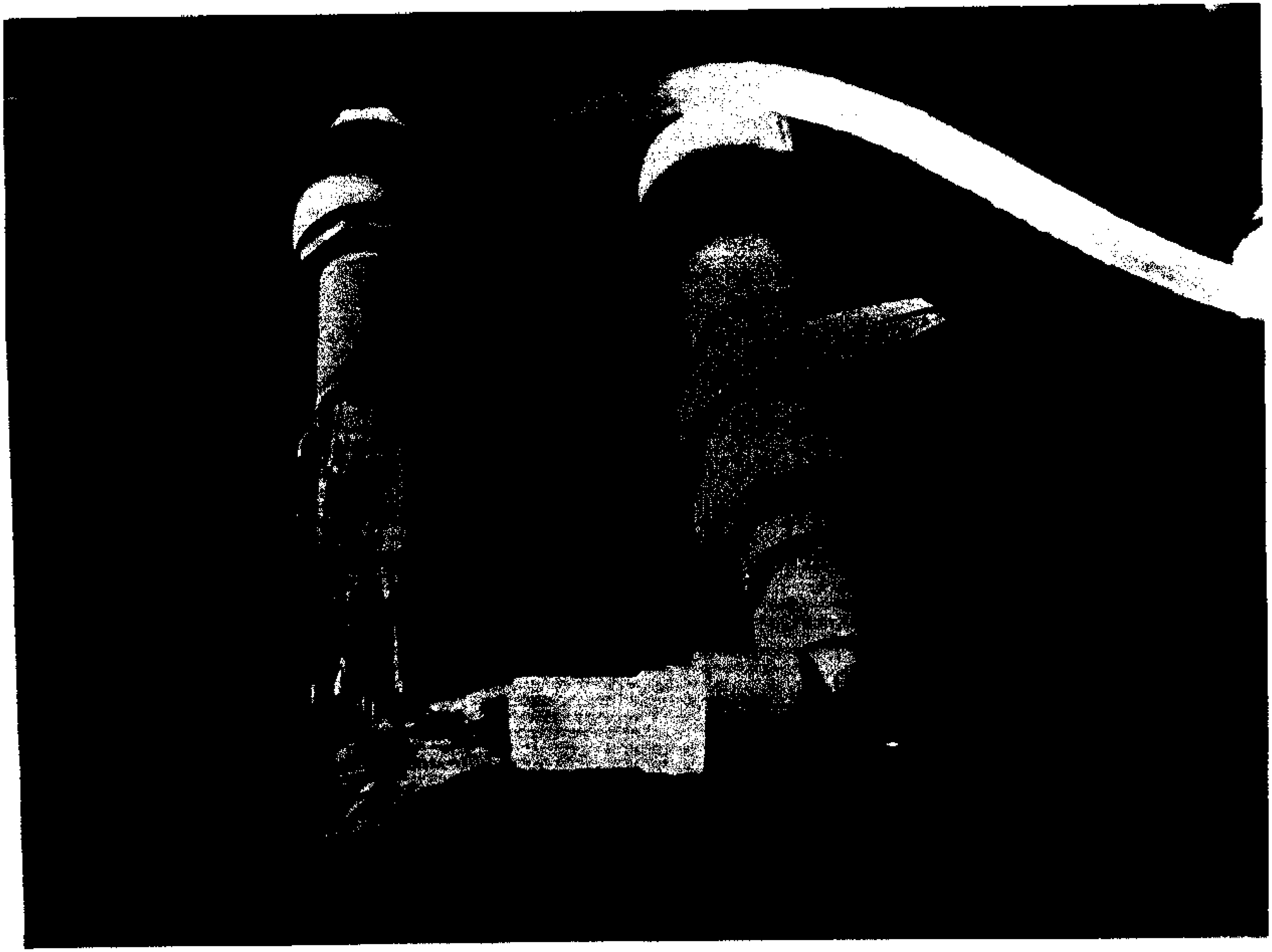


Figure 4. Sewage effluent (left) and filtered seawater (right) entering algae pond in the continuous-flow culture system at 0.5 pond-volume turnovers/day.

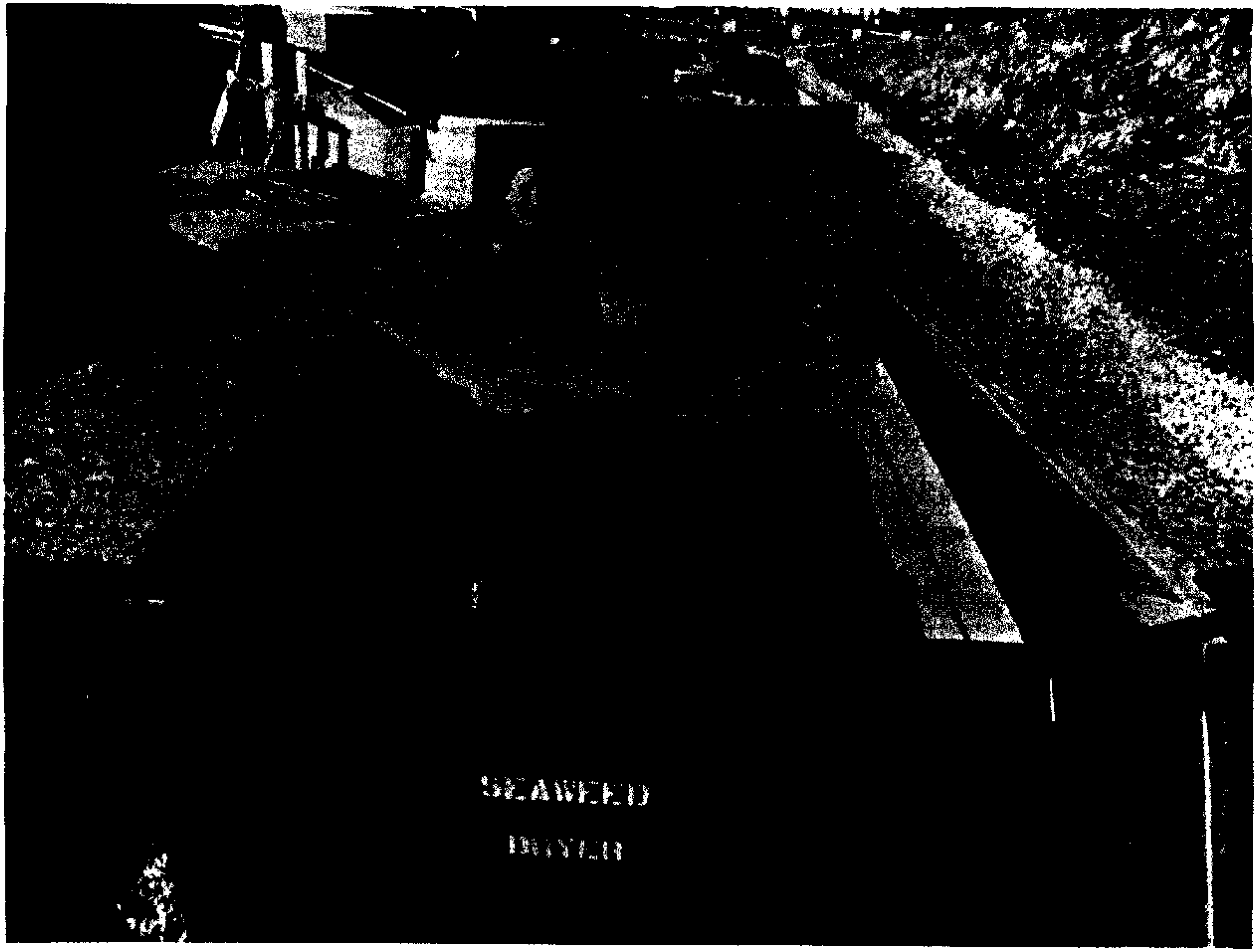



Figure 5. Weekly yield of seaweed (*Gracilaria*) from one raceway being dried prior to shipment to commercial seaweed firm for hydrocolloid assay.

Figure 6. Growth of lobsters (Homarus americanus) in two months from hatching (0.75 g) and one year from hatching (310 g) in oyster raceway with no supplemental feeding at an annual temperature range of 15-25°C.

Figure 7. Lobsters produced in heated shellfish raceway in 8 months with no supplemental feeding.

Figure 8. The red seaweed <u>Gracilaria foliifera</u> grown in suspended culture in the raceway system.

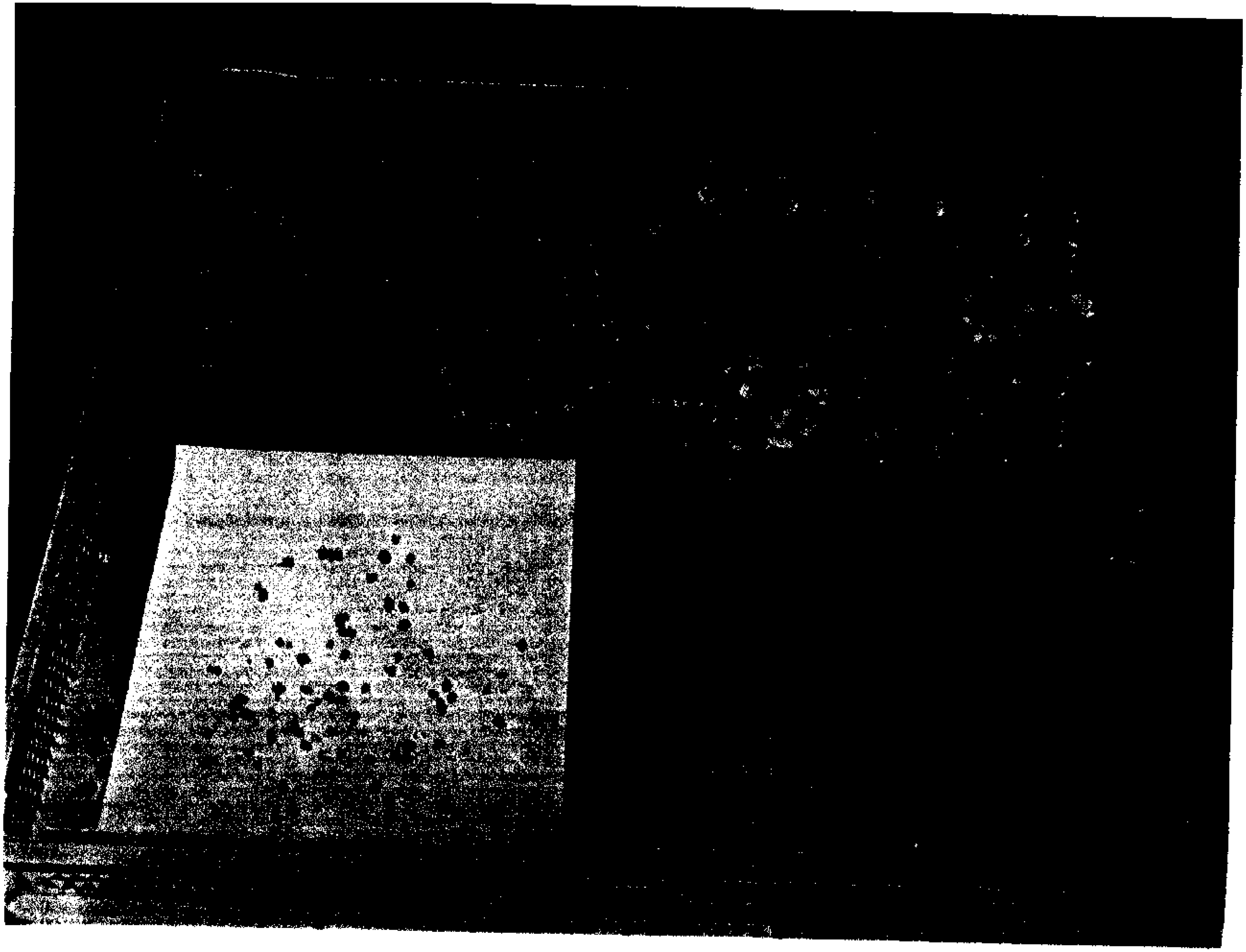
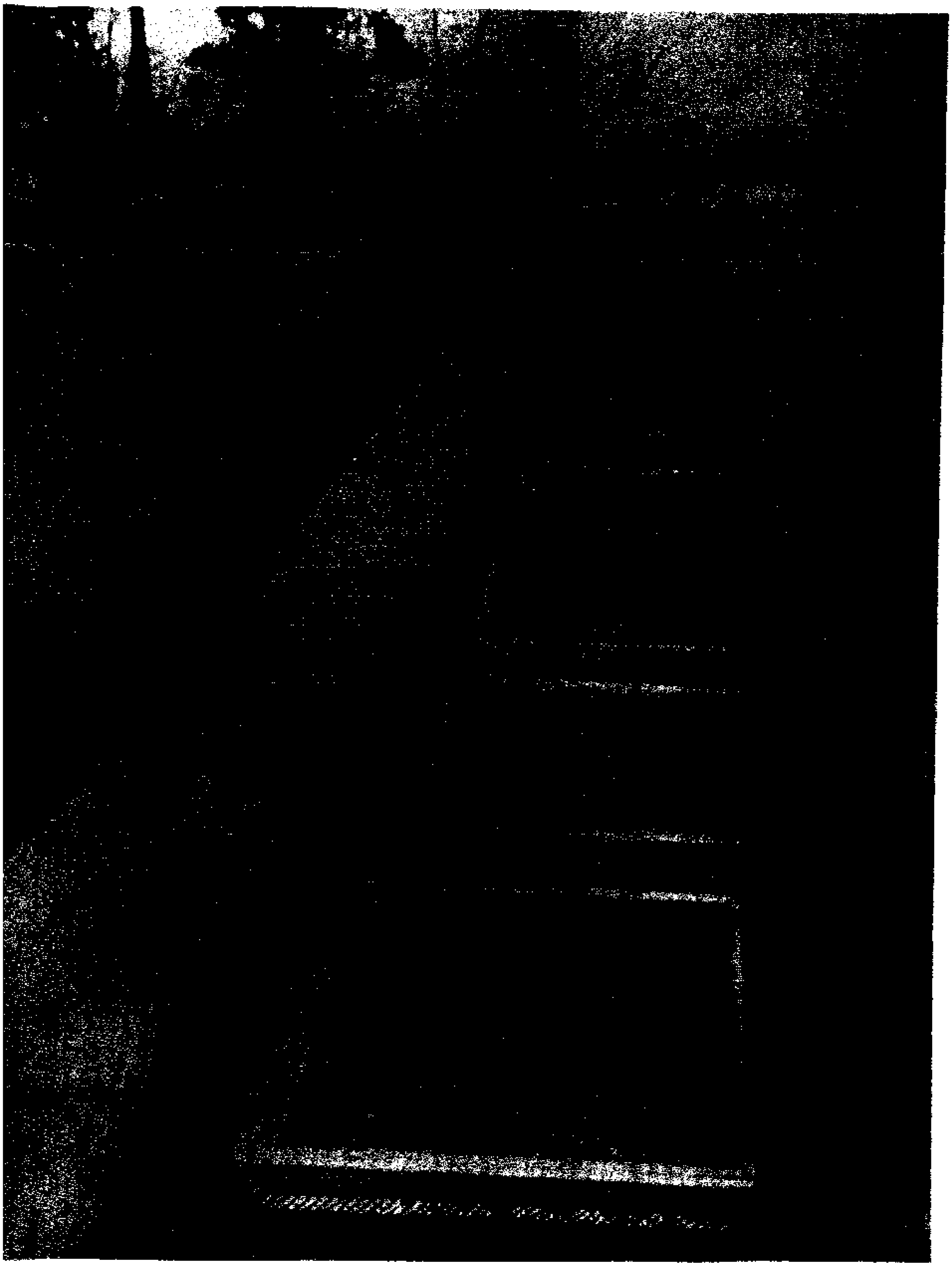
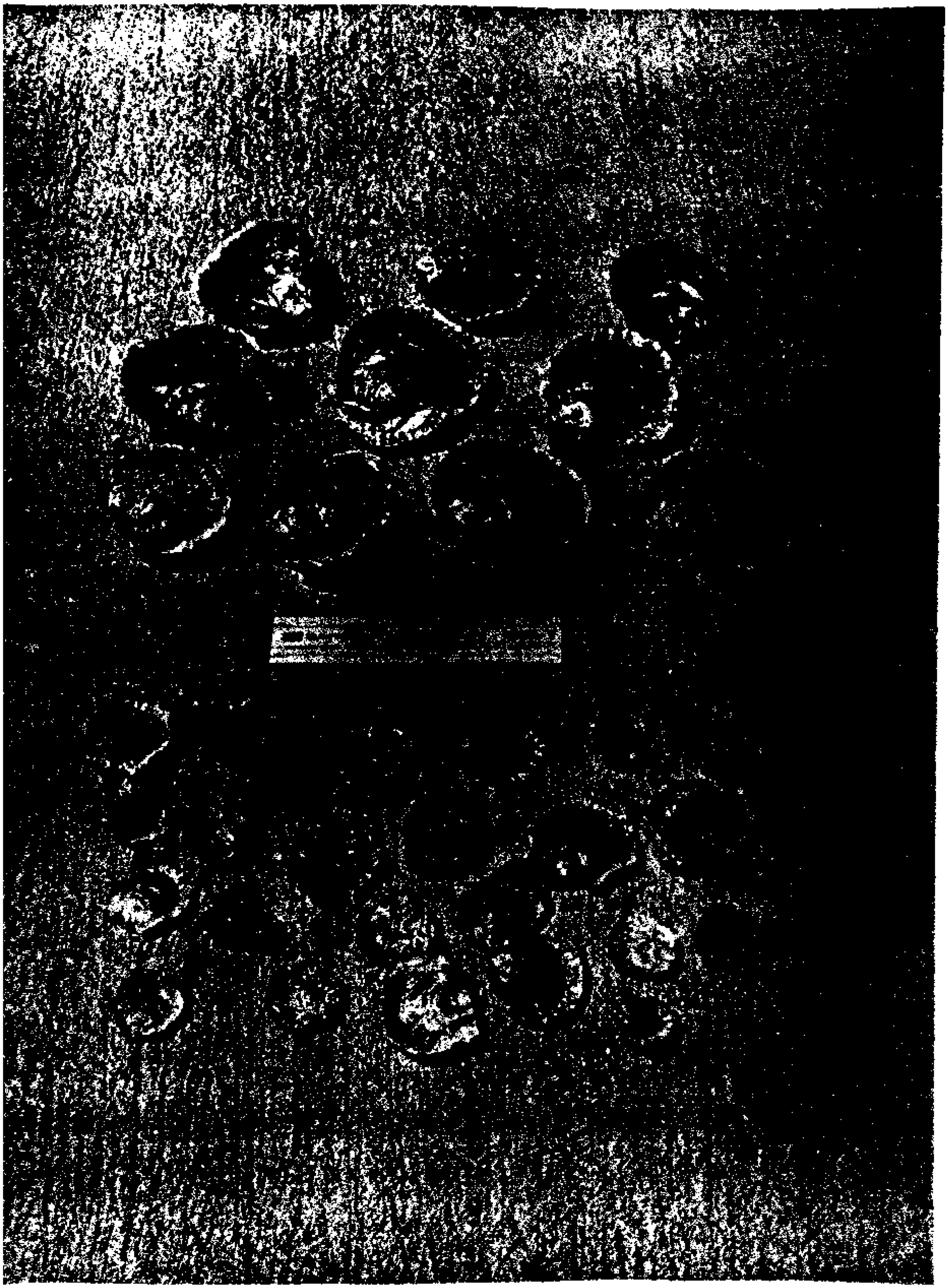


Figure 9. Growth of the Manila clam Venerupis (Tapes) semidecussata from November, 1974 (5.5 mm) to August, 1975 (20.5 mm).



'igure 10. Stacked trays of oysters in raceway system. Each 40' x 4' x 5' raceway holds approximately 48 trays each with 3,000 seed oysters.



ure 11. Growth of European osyters (Ostrea edulis) from March, 1975 (44 mm) to August, 1975 (83 mm).

October 1973

DISTRIBUTION FOR SEA GRANT REPORTS

| No. of Copies | Address |
| --- | --- |
| 3 | National Sea Grant Depository<br>Pell Marine Science Laboratory<br>University of Rhode Island<br>Narragansett Bay Campus<br>Narragansett, RI 02882 |
| 1 | Sea Grant 70's<br>Center for Marine Resources<br>Texas A&M University<br>College Station, TX 77843 |
| 5 | Office of Sea Grant<br>NOAA, U.S. Dept. of Commerce<br>Rockville, MD 20852 |
| 50 | Chief, Technical Information Div. D83<br>National Oceanic & Atmospheric Admin.<br>Rockville, MD 20852 |
| 1 | Charles W. Mason, Documents Librarian<br>Morris Library<br>University of Delaware<br>Newark, DE 19711 |

Woods Hole Oceanographic Institution
WHOI-75-41

PRELIMINARY RESULTS WITH A PILOT PLANT WASTE RECYCLING MARINE-AQUACULTURE SYSTEM by John H. Ryther. 51 pages. September 1975. Sea Grants 04-4-158-5 and 04-5-158-8 and NSF (RANN) Grant GI-43884.

A combined waste recycling-marine aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonally by 3-4 fold and are controlled by solar radiation but not by temperature.

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined. (con't on back)

1. Aquaculture
2. Secondary sewage
3. Unicellular marine algae

I. Ryther, John H.
II. 04-4-158-5
III. 04-5-158-8
IV. NSF (RANN) GI-43884

This card is UNCLASSIFIED

Woods Hole Oceanographic Institution
WHOI-75-41

PRELIMINARY RESULTS WITH A PILOT PLANT WASTE RECYCLING MARINE-AQUACULTURE SYSTEM by John H. Ryther. 51 pages. September 1975. Sea Grants 04-4-158-5 and 04-5-158-8 and NSF (RANN) Grant GI-43884.

A combined waste recycling-marine aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonally by 3-4 fold and are controlled by solar radiation but not by temperature.

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined. (con't on back)

1. Aquaculture
2. Secondary sewage
3. Unicellular marine algae

I. Ryther, John H.
II. 04-4-158-5
III. 04-5-158-8
IV. NSF (RANN) GI-43884

This card is UNCLASSIFIED

Woods Hole Oceanographic Institution
WHOI-75-41

PRELIMINARY RESULTS WITH A PILOT PLANT WASTE RECYCLING MARINE-AQUACULTURE SYSTEM by John H. Ryther. 51 pages. September 1975. Sea Grants 04-4-158-5 and 04-5-158-8 and NSF (RANN) Grant GI-43884.

A combined waste recycling-marine aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonally by 3-4 fold and are controlled by solar radiation but not by temperature.

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined. (con't on back)

1. Aquaculture
2. Secondary sewage
3. Unicellular marine algae

I. Ryther, John H.
II. 04-4-158-5
III. 04-5-158-8
IV. NSF (RANN) GI-43884

This card is UNCLASSIFIED

Woods Hole Oceanographic Institution
WHOI-75-41

PRELIMINARY RESULTS WITH A PILOT PLANT WASTE RECYCLING MARINE-AQUACULTURE SYSTEM by John H. Ryther. 51 pages. September 1975. Sea Grants 04-4-158-5 and 04-5-158-8 and NSF (RANN) Grant GI-43884.

A combined waste recycling-marine aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonally by 3-4 fold and are controlled by solar radiation but not by temperature.

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined. (con't on back)

1. Aquaculture
2. Secondary sewage
3. Unicellular marine algae

I. Ryther, John H.
II. 04-4-158-5
III. 04-5-158-8
IV. NSF (RANN) GI-43884

This card is UNCLASSIFIED

(Cont.)

An alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual production of 183 tons of oyster meat (38,000 bushels of whole oysters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with half the above yields.

(Cont.)

An alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual production of 183 tons of oyster meat (38,000 bushels of whole oysters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with half the above yields.

(Cont.)

An alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual production of 183 tons of oyster meat (38,000 bushels of whole oysters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with half the above yields.

(Cont.)

An alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual production of 183 tons of oyster meat (38,000 bushels of whole oysters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with half the above yields.

**BIBLIOGRAPHIC DATA SHEET**

1. Report No.

2.

3. Recipient's Accession No.

4. Title and Subtitle

PRELIMINARY RESULTS WITH A PILOT PLANT WASTE RECYCLING MARINE-AQUACULTURE SYSTEM

5. Report Date

September 1975

6.

7. Author(s)

John H. Ryther

8. Performing Organization Rept. No. WHOI-75-41

9. Performing Organization Name and Address

Woods Hole Oceanographic Institution
Woods Hole, MA 02543

10. Project/Task/Work Unit No.

11. Contract/Grant No.

04-4-158-5, 04-5-158-8
NSF (RANN) GI-43884

12. Sponsoring Organization Name and Address

Sea Grant
National Science Foundation

13. Type of Report & Period Covered

Technical

14.

15. Supplementary Notes

16. Abstracts A combined waste recycling marine-aquaculture system capable of complete nitrogen removal from treated domestic wastewater has been developed and tested on a pilot-plant scale over a one-year period. Effluent from secondary sewage treatment, mixed with seawater, is used to grow unicellular marine algae in large, continuous-flow, outdoor mass cultures. Harvest from the algae cultures is fed to oysters and other filter-feeding bivalve molluscs and to secondary crops of flounder or lobsters. Dissolved wastes produced by the animals are assimilated by cultures of commercially-valuable seaweeds.

Successful cultures of unicellular algae, mostly diatoms, and seaweeds have been sustained over long periods of time (months) with only minor problems. Algal yields and nitrogen removal capacity vary seasonably by 3-4 fold and are controlled by solar radiation but not by temperature. (cont. on next page)

17. Key Words and Document Analysis. 17a. Descriptors

1. Aquaculture
2. Secondary sewage
3. Unicellular marine algae

17b. Identifiers/Open-Ended Terms

17c. COSATI Field/Group

18. Availability Statement

19. Security Class (This Report) UNCLASSIFIED

20. Security Class (This Page) UNCLASSIFIED

21. No. of Pages 51

22. Price

FORM NTIS-35 (REV. 3-72) USCOMM-DC 14952-P72

| BIBLIOGRAPHIC DATA SHEET | 1. Report No. | 2. | 3. Recipient's Accession No. |
|---|---|---|---|
| 4. Title and Subtitle | | | 5. Report Date |
| | | | 6. |
| 7. Author(s) | | | 8. Performing Organization Rept. No. |
| 9. Performing Organization Name and Address | | | 10. Project/Task/Work Unit No. |
| | | | 11. Contract/Grant No. |
| 12. Sponsoring Organization Name and Address | | | 13. Type of Report & Period Covered |
| | | | 14. |

15. Supplementary Notes

16. Abstracts (cont.)

Bivalve mollusc culture was unsuccessful during the first year of operation. Inability to control the species of unicellular algae in the mass cultures and other possible reasons are suspected and are now being investigated. Good growth of flounders and lobsters was obtained, but the carrying capacity of the system and potential yields of these secondary crops has not yet been determined.

As an alternative nitrogen-removal system consisting only of seaweeds fed a continuous flow of secondary sewage effluent mixed with seawater has also been evaluated.

Assuming that problems with shellfish culture can be resolved, the combined unicellular algae-shellfish-seaweed system is capable of complete nitrogen removal from wastewater effluent within an area of 48 acres and with an estimated annual

~~17. Key Words and Document Analysis. 17a. Descriptors~~

production of 183 tons of oyster meat (38,000 bushels of whole oysters), 3,350 tons (wet weight) of seaweeds and undetermined quantities of flounder and/or lobsters per MGD effluent treated (wastes per 10,000 capita). A seaweed system alone is capable of nitrogen removal in an area of 60 acres with annual production of 16,300 tons wet weight of seaweeds.

The above figures are for year-around operation as would be possible in tropical to semi-tropical climates. Operation of the system in temperate climates is possible within the same areas but only on a seasonal basis (approximately six months) with

~~17b. Identifiers/Open-Ended Terms~~

half the above yields.

17c. COSATI Field/Group

| 18. Availability Statement | 19. Security Class (This Report) UNCLASSIFIED | 21. No. of Pages |
|---|---|---|
| | 20. Security Class (This Page) UNCLASSIFIED | 22. Price |