Woods Hole Oceanographic Institution

MARINE POLYCULTURE BASED ON NATURAL FOOD CHAINS AND RECYCLED WASTES

By

Dr. John H. Ryther

October 1976

TECHNICAL REPORT

Prepared for the Department of Commerce, NOAA Office of Sea Grant under Grant No. 04-6-158-44016, the United States Energy Research and Development Administration E(11-1)-2948 and the Jesse Smith Noyes Foundation, Inc.

WOODS HOLE, MASSACHUSETTS 02543

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Approved for Distribution: George Grice

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Table of Contents

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

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

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

SUMMARY

Summary

Research has continued during the past Sea Grant fiscal year (July 1, 1975-June 30, 1976) on the development, testing, and evaluation of a combined waste recycling-marine polyculture system. The concept of the system is to grow unicellular marine algae (phytoplankton) in mixtures of seawater and the effluent from a secondary sewage treatment plant. The algae grown in continuous flow-through cultures, are then fed to bivalve molluscs, such as oysters, clams, scallops, or mussels. The algae remove the nutrients from the wastewater and the molluscs remove the algae. Finfish (winter flounder) and crustacea (American lobster) are stocked as post-larval or juvenile animals together with the molluscs to feed upon the solid wastes (feces and pseudo feces) produced by the shellfish and upon the small invertebrate fauna (polychaete worms, amphipods, etc.) that are supported by these wastes. Seaweeds constitute a final polishing stage to the system, receiving the effluent from the animal culture system and removing from it nutrients regenerated through excretion and metabolism of the animals as well as any nutrients from the wastewater not initially removed by the phytoplankton.

The dual objectives of the system are to remove nutrients from treated wastewater prior to its discharge to the environment (i.e., a biological tertiary sewage treatment process) and, at the same time

grow commercially-valuable crops of shellfish with secondary crops of lobster, flounder, and seaweeds. With respect to the latter, the species grown are representatives of the red algae (Rhodophyceae) that have commercial value for their content of hydrocolloids (agar or carrageenan), products that are used as stabilizers and emulsifiers in the food, drug, and cosmetic industries.

As a practical approach to the problem of nutrient removal from the wastewater, main emphasis has been given to nitrogen. Because phosphorus is present in domestic sewage at higher concentrations relative to nitrogen than these two elements are assimilated by and exist in plants (including marine algae), a residue of phosphorus will inevitably remain after all the available nitrogen has been removed by the algae. However, it has been clearly demonstrated that wastewater-seawater mixtures from which all nitrogen has been removed are incapable of supporting further growth of algae. Thus nitrogen removal has been the objective of the nutrient removal (i.e., tertiary treatment) part of the system, and its efficiency and performance in that regard has been monitored and reported in terms of nitrogen.

The pilot-plant facility that has been in operation since October, 1973, consists of six 50' x 50' x 3' deep PVC-lined earth ponds for growing the phytoplankton and five 40' x 4' x 5' deep and three 40' x 8' x 5' deep cement raceways for growing the shellfish and other animals and the seaweeds. Approximately 500 gallons/minute

of seawater is pumped into the laboratory facility where it may be filtered, heated, or otherwise conditioned before being dispensed to the ponds or raceways. Approximately 4,000 gallons/day of effluent from the Town of Wareham activated sludge secondary sewage treatment plant is trucked daily to the facility and discharged into buried fibreglass tanks from which it is pumped into a headbox and then dispersed to the ponds.

The major problems that were encountered during the first year (1974-5) of operation of the "pilot-plant" facility are discussed below together with a brief summary of the accomplishments made during the past year and progress towards a better understanding and final resolution of these problems. A more complete discussion of these subjects is given in the individual reports.

I. Phytoplankton culture

Healthy cultures of phytoplankton have been maintained in the ponds for sustained periods of time, at high densities and yields and with good nutrient removal performance. However, it has not been possible to control the species of phytoplankton that appeared, become dominant, and persisted in the cultures. Seawater pumped to the laboratory and pre-filtered through sand filters was, at its point of delivery to the ponds, further filtered through 1 μm wound fiber filters. This

fine-filtered seawater was then inoculated with large (up to 200 liter) cultures of various desired species of phytoplankton (i.e., those known to be good food organisms for bivalve molluscs) such as Thalassiosira pseudonana, Skeletonema costatum, and Monochrysis lutheri. In several cases, these inocula were added to the ponds continuously from smaller (400-liter) flow-through cultures. The sewage effluent-seawater media was also further enriched with silica, vitamins, and various trace elements.

In most cases the species inoculated developed into healthy and reasonably-dense mass cultures in the ponds, but these persisted for no more than a few days to a week, following which they invariably "crashed" (i.e., the cells coagulated or clumped and sank to the bottom of the pond leaving an absolutely clear culture medium, usually over a period of one day or, most commonly, one night). The reason why these cultures "crash" is still not known, but it appears to happen in every case in which attempts have been made to grow mass outdoor cultures of desired species of marine phytoplankton, usually small centric diatoms, (e.g., Roels, Haines et al. in St. Croix, Pruter et al. at the University of Delaware, Petrovits at Cultured Clam Inc., Dennis, Mass.). In most cases, these workers have adopted the expedient of permanently maintaining inocula of the desired species from which new mass cultures may be restocked each time the cultures crash. We have concluded, however, that such a mode of operation would not be economically viable in a commercial application. Instead,

we have been attempting, through both the large outdoor mass cultures, and small-scale, controlled, laboratory chemostat cultures to understand the environmental factors that influence and control natural species succession and dominance in mass algal cultures.

If unfiltered or coarse, sand filtered seawater, enriched with sewage effluent or inorganic chemical nutrients, is added directly to the ponds, or if the "crashed" cultures of inoculated species of phytoplankton are allowed to remain untouched, cultures develop of some one of a few species of phytoplankton "weeds". Of these, the small pennate diatom Phaeodactylum tricornutum is the most common and persistent, dominating completely the mass cultures during most of the year. In mid-winter when temperatures range from 0° to about 8°C, Phaeodactylum may be replaced by Skeletonema costatum, one of the more desirable centric diatoms. In mid-summer, when pond temperatures exceed about 25°C, Phaeodactylum is succeeded first by other pennate diatoms such as Nitzschia closterium or Amphora sp. and, at still higher temperatures, by green flagellates or green coccoid algae such as Nannochloris sp. or Stichococcus sp.

Both the mass culture and laboratory studies have shown that temperature is the single most important controlling factor that determines species in the mass cultures. However, that control is limited to a seasonal selection of one of a very small number of species that apparantly are able to develop, dominate, and persist in the highly

eutrophic mass culture environment. With the exception of Skeletonema, none of those that have persisted in our cultures are normally common or dominant in moderately-eutrophic coastal or inshore marine waters.

It is therefore our tentative conclusion that artificial bivalve mollusc culture systems that depend on cultured microorganisms for food, assuming that these organisms will have to be grown out-of-doors in mass cultures as dense and productive as possible for economic reasons, will inevitably be restricted to the availability of a very few "weed" species of algae until or unless some breakthrough is achieved in our understanding and application of species control in mass algal cultures. In the latter respect, one intriguing factor that remains to be tested and evaluated is the possibility that organic mediators produced by the algae themselves may selectively inhibit the growth of the more normal algal species and/or enhance that of the weeds. Although often involved as an explanation for algal succession and dominance, such "ectocrines" remain to be demonstrated convincingly as controlling factors in the environment.

The yields of phytoplankton and the concurrent nutrient removal capacities of the mass algal cultures that have been achieved during the past year agree substantially with those projected from results of the first year's observations (i.e., mean ash-free dry weight yields ranging from about 3 g/m^2/day in winter to 9 g/m^2/day in summer, with short-term maxima of about 12 g/m^2/day). The earlier conclusion was also confirmed that yields are temperature independent and controlled primarily by solar radiation.

More and better information was obtained during the past year concerning the measured mass flow and balance of nitrogen through the phytoplankton pond system. Development of a new technique for measuring dissolved organic nitrogen in seawater has permitted our filling that gap in the mass balance determination. In that connection, it was gratifying to find that no significant net production of dissolved organic nitrogen occurs in the algal ponds. In other words, the inorganic nitrogen contained in the wastewater effluent is not simply being converted to dissolved organic form and discharged to the environment in a hitherto undetectable form.

For the first time also, measurements have been made of the rate of settling of phytoplankton from the algal ponds. Despite very weak circulation of the cultures, a very small fraction (less than 5%) of the algal production is apparently lost to the system by sedimentation in the ponds.

Despite the fact that losses of nitrogen to the system by the above two mechanisms were small, there was a rather large and persistent discrepancy noted in the nitrogen mass balance calculations between that removed from solution from the wastewater effluent and the significantly smaller amount harvested from the ponds as phytoplankton. In our earlier, small-scale experimental studies a similar discrepancy (i.e., up to 50% of the nitrogen removal) was observed. This was then attributed to loss as gaseous ammonia to the atmosphere at the high pH levels (9.5-10.0) attained in the cultures during daytime. Such appears to

be a logical explanation in our larger system and has the very significant implication that the nutrient-removal capacity of the system, projected last year only from the yields of algae and their nitrogen content, were conservative by as much as a factor of two.

II. Bivalve mollusc culture

During the first year of operation of the present facility, the shellfish culture raceways were heavily stocked with large numbers of juvenile or seed American oysters (Crassostrea virginica) and hard clams (Mercenaria mercenaria). These animals failed to grow significantly and, for the most part to survive during the following 18 months. Two possible explanations for this lack of success were suggested in the last report. 1) The seed shellfish in question were stunted or otherwise inferior stocks or they had suffered stress or injury during transport from their sources (commercial hatcheries on Long Island, New York). 2) The phytoplankton grown in the mass algal cultures, predominantly Phaeodactylum tricornutum during most of the year, was inferior and unsuitable as food for the shellfish.

The first of these two explanations has now been conclusively ruled out. During the past year, new stocks of American oysters were obtained from the same Long Island hatchery. These were newly-set, healthy, actively-growing seed obtained in two separate lots during the spring. In addition, small lots of both oysters and clams were obtained from other sources. In no case did either species grow or even survive in the culture system.

Since Phaeodactylum and various green algae were already known to be poor to indifferent foods for larval and very young juvenile clams and oysters, the second explanation therefore appeared to be the correct one, and success of the system appeared dependent upon the ability to control the species and to grow other, more desirable food organisms in the mass algal cultures.

At the same time these tentative conclusions were reached, however, small numbers of juvenile Manila clams (Venerupis semidecussata, also referred to as Tapes semidecussata) and European oysters Ostrea edulis) were obtained. Both species survived well and grew within the culture system and on the same food that failed to support C. virginica and M. mercenaria. Venerupis grew slowly, though apparently not unusually so for the species. Ostrea grew very rapidly, from 1.5 cm seed to 4.5 cm marketable adults in about five months.

As a result of this experience new, larger lots of O. edulis were obtained from several sources and stocks of Japanese oysters (Crassostrea gigas) were also obtained and introduced during the fall and winter of 1975-6. The results with O. edulis' have been somewhat equivocal, some growing well and others dying, but the reason for this is now believed to be damage or injury of some of the seed during shipment (i.e., from as far as the U.K.) The C. gigas stocks have all grown well.

Thus the earlier problem of the inability to control species in the algal ponds and to produce phytoplankton suitable as food for

the indigenous species of oysters and clams, if not solved, appears to have been circumvented by use of exotic shellfish species capable of utilizing the kinds of algae that can now be mass produced in our cultures. Very preliminary results also indicate that the local bay scallop (Argopecten irradians) may be included among the latter group, but evaluation of that species was hindered last year by scarcity of seed stocks. The interesting question of why some bivalve species but not others can subsist on our phytoplankton "weeds" remains to be answered.

Once the problem of how to grow shellfish had been resolved (i.e., by species selection of the animals rather than their food), the remainder of the year was devoted to studies aimed at the optimization of the shellfish culture system, not only for growth of the animals per se but more specifically for maximum production of a high-quality, marketable product. Such variables as food concentration, method of food presentation, stocking density, location of animals in the culture system, and water temperatures were all looked at simultaneously in a complex experimental design. These studies are still in progress and will require additional time for completion, as the animals must be grown from 5-10 mm seed to marketable adults to obtain conclusive results.

Of particular interest in the shellfish experiments is the effect of temperature, for it apparently has different effects upon

such physiological functions as shell growth, meat growth, gonad development, and general condition of the animal with respect to its food value and marketability. What may prove to be optimal temperature for overall growth or size increase of the animals (which is species specific but is 20°-25°C for several species) may be quite different from the optimum for meat production, condition index, and market value (15°C or less for the same species). Although rather basic, such information is surprisingly scarce and poorly documented.

III. Seaweed culture

Initially, the seaweed species used as the final "polishing" step of our multi-stage polyculture system was Chondrus crispus (Irish moss), the local red algae harvested in New England and the Canadian Maritimes as a source of carrageenan. There is a ready market for Chondrus, it has been well studied, and it has been the subject of intensive culture research by A. C Neish and his collaborators in Nova Scotia, who kindly supplied us with a fast-growing strain (T-4) isolated in their screening tests. We have found, however, that Chondrus at best grew slowly in our system (both the Neish T-4 strain and local clones), it became heavily epiphytized with other species of undesirable algae, and it could not tolerate our high (> 20°C) summer temperatures. Chondrus was therefore replaced with two more temperate species that occur in the Woods Hole region as summer annuals.

These are Neoagardhiella baileyi, a carrageenan producer like Chondrus, and Gracilaria foliifera, an agar-containing plant.

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

So successful has been the seaweed stage of our polyculture system that experiments have now been undertaken to investigate the use of these algae in a one-step nutrient removal-aquaculture system. In this approach, the seaweeds are grown directly on mixtures of sewage effluent and seawater, the plants performing the dual function of nutrient removal from the wastewater while producing a commercially valuable crop in the process. Not only is this a far more simple approach than the multi-stage polyculture system described above, but it avoids most of the public health problems and the associated legal and social constraints created by growing shellfish and other human foods in sewage effluent.

Towards the end of the past Sea Grant year, new support became available from ERDA to investigate the potential energy value of seaweeds grown in wastewater, harvested, and fermented to methane or other energy sources or petroleum - sparing industrial feed stocks.

Whether seaweeds are used in a polyculture or monoculture system, and for whatever ultimate application, the same problems apply to their cultivation. To date the approach has been largely empirical and the yields reported above, impressive as they are, have been obtained through trial and error. Surprisingly little, in fact, is known or documented concerning the nutrient uptake kinetics and other related aspects of the physiology of the macroscopic algae. Such information is, however, essential to any efforts at optimization of growth, yields, hydrocolloid production, and nutrient removal capacity of these plants. During the past year, therefore, research in this important area was initiated.

One of the most fundamental questions in seaweed culture is how dense a population should be maintained (i.e., what biomass per unit area and/or volume) to achieve maximum yields. This, however, cannot be simply answered, since it is dependent upon incident radiation and may differ from species to species. Our experiments have demonstrated that specific growth rate (growth per unit weight or percent increase per unit of time) is very high (20-30% per day) at very low densities of the plants, but that this value decreases rapidly with increasing densities to a value of zero at high biomass levels. Many people

mistakenly confuse specific growth rates with yields (i.e., production per unit of area and time), the latter being the product of specific growth rate and density. Maximum yields are obtained at intermediate densities that differ, depending upon season, solar radiation, species, and perhaps other factors, but appear from our preliminary results to fall between two and four kg (wet weight)/m^2, at which specific growth rates range from about 4-8%/day.

In a continuous flow system, an equilibrium becomes established, under steady-state conditions, between the concentration of nutrients in the incoming seawater and that in the volume of the culture itself (and the effluent from the culture). Nutrient uptake and, over long periods of time, growth of the algae are determined by the residual concentration in the culture. Preliminary experiments suggest that the residual nitrogen concentration necessary for the maximum rate of uptake (and perhaps maximum growth rate) of Neoagardhiella is as high as 10 μ moles/l, ten times or more that for most marine phytoplankton. In other words, rather high residual levels of nitrogen must be maintained in the culture medium and the effluent to achieve maximum rates of nutrient removal by the seaweeds.

There appear to be other areas in which the nutrient uptake kinetics of the seaweeds differ significantly from that of the macroscopic algae. We have, for example, obtained evidence that nitrogen assimilation in the seaweeds is closely coupled with photosynthesis and that little or no uptake occurs in the dark. This is in direct

contrast to the phytoplankton, in which there is no diel periodicity in nutrient removal. The seaweeds also show a strong preference for ammonium over nitrate as a nitrogen source. Although such is also the case with phytoplankton, most species of the latter can readily adapt to nitrate utilization, in the absence of ammonium, through induction of a nitrate reductase system. Such a shift appears to be much slower and more complex in the seaweeds, though there is evidence that it eventually does take place.

Some very preliminary studies have also been carried out on the production of carrageenan by Neoagardhiella baileyi and the relationship between the content of hydrocolloid, residual nitrogen content of the culture medium, nitrogen:carbon ratio of the plant tissue, and the red pigment (phycoerythrin) content of the alga. By studying these relationships, it is hoped that the carrageenan content and hence the commercial value of the seaweeds can be quickly and easily assessed by visual inspection or by simple chemical tests.

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

The seaweed cultures normally contain large numbers of herbivorus crustacea. Because all of the plants are never harvested at any one time, and because these small grazers reproduce rapidly, large numbers of the animals accumulate in the plant populations. Thus, there is the possibility that a significant fraction of the algal production may be consumed by these animals. A study was therefore undertaken to measure feeding rates and to determine algal food preferences by two of the herbivorous crustacea commonly found in the seaweed cultures, the large isopod, Idotea baltica and the smaller tube-building amphipod, Ampithoe valida. Although both of the cultured seaweeds, Neoagardhiella baileyi and Gracilaria foliifera are eaten by both animals, neither ranks high in preference among the several algal species tested. From measured feeding rates and the observed density of the animals in the seaweed cultures, estimates could be made of the impact of their grazing on algal productivity. This was generally low, of the order of 1% during most of the year, but was significant (ca. 8%) when productivity was very low. It could be concluded from these studies that herbivorus grazing is probably not normally a problem in seaweed culture, but that it should be guarded against in situations where non-growing stocks of the seaweeds are being held for extended periods of time.

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Temperature-Influenced Species Competition in Mass Cultures of Marine Phytoplankton[1]

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SUMMARY

Five marine phytoplankton species (*Phaeodactylum tricornutum, Thalassiosira pseudonana, Skeletonema costatum, Monochrysis lutheri,* and *Dunaliella tertiolecta)* were grown in enriched laboratory continuous cultures and natural populations were mass cultured outdoors for 16 months. Competition among the species was shown to be highly dependent on temperature, although the actual production of plant organic matter at the low growth rates used was relatively independent of this variable. Control of marine species in mass cultures does not appear economically feasible, but this drawback may be overcome by selecting for herbivorous shellfish that are capable of assimilating those temperature-dependent phytoplankton species dominating in a particular locale.

INTRODUCTION

The successful mass culture of algae has long been of interest to scientists. This interest has ranged from the early attempts at producing single cell protein by growing freshwater algae on enriched media (compiled in the Carnegie Report[1]), to Oswald's efforts in using wastewater as a growth media for mass culturing freshwater algae for simultaneous treatment of wastes and protein production[2]. More recently, Ryther has applied Oswald's approach in developing a marine counterpart in which the cultured phytoplankton comprise the first link in a controlled marine food chain[3].

Although considerable success has been realized in optimizing algal yields - over 20 g dry wt/m^2/day in some studies[4, 5], one of the most disappointing aspects of large-scale mass culturing to date has been a virtual inability to control species selection. This result is unfortunate because the "weed" species that often dominate through natural selection in both freshwater and marine enriched cultures are usually not a desired end product; either they have undigestible cell walls as *Chlorella* or *Scenedesmus* in freshwater cultures[6], or they are undesired food for many herbivorous shellfish in a marine aquaculture system, e.g., *Phaeodactylum tricornutum*[7].

In large-scale outdoor cultures, particularly those in which wastewater is used as a nutrient source, it is uneconomical to control species selection through elimination of indigenous algae by filtration or sterilization and then inoculation with a desired species. The cost of these techniques, together with the gross opportunities for subsequent contamination and take over by "weed" species, has been a major deterrent to commercial production of this

potentially important source of protein.

In an ecological sense, the outcome of competition among phytoplankton species in controlled outdoor cultures is not nearly as complicated as in natural environments. This is because factors strongly influencing species dominance in natural systems such as competition for nutrients, grazing by predatory herbivores, and sinking can be eliminated in mass cultures by adding nutrients in excess, by selecting dilution rates (growth rates) higher than the maximum reproductive rates of secondary organisms, and by providing vigorous mixing. Thus for the most part, the two main environmental factors influencing competition are temperature and sunlight (intensity and duration). Because these two factors change simultaneously with the seasons, particularly in temperate climates, isolation of either variable in outdoor mass cultures is virtually impossible. Laboratory experimentation in which one of these parameters can be studied independently is then an important way to estimate the relative importance of these effects.

For the past 4 years we have been growing marine phytoplankton in mass cultures, both indoors and outdoors, on wastewater-seawater mixtures in Woods Hole, Massachusetts and Ft. Pierce, Florida. The main objectives have been to optimize biomass yields, monitor the assimilation and removal of dissolved nutrients (i.e., nitrogen and phosphorus), and understand the ecology of phytoplankton in enriched cultures. These objectives, to varying degrees, have been satisfied[5, 8-10]. Still, a complete understanding of species dominance in outdoor cultures remains unanswered. However, enough data have been accumulated during long-term studies outdoors in both Massachusetts and Florida, and

in controlled laboratory experiments to provide a clearer picture of how one important environmental factor, temperature, influences species dominance in enriched outdoor cultures of marine phytoplankton.

Presented here are the results of both a laboratory and outdoor study dealing with temperature-controlled competition among marine phytoplankton. These results are compared with data on species-temperature effects from both field observations in natural waters and previous mass culture experiments.

MATERIALS AND METHODS

Algal Cultures

Laboratory Cultures

Five phytoplankton species were grown in continuous cultures. The physical apparatus (the culture units - each 2.75 liters volume, and the temperature control and lighting systems) is described elsewhere[8]. Mixing was accomplished by stirring with magnetic bars (a magnetic stirrer was placed under each unit) and aeration, with compressed air. Light intensity was about 0.03 langleys/min.

The experiments involved culturing each species in enriched media (half secondarily treated wastewater - half seawater) at a growth (or dilution) rate of about 0.6/day and at $5^{\circ}C$ intervals in the range 10° - $30^{\circ}C$. For each temperature steady state biomass levels were established for the individual species; a contaminant species was then introduced and competition between the two species observed over an extended period. The species tested included

Phaeodactylum tricornutum (TX-1), *Thalassiosira pseudonana* (3H), *Skeletonema costatum* (Skel), *Monochrysis lutheri* (Mono), and *Dunaliella tertiolecta* (Dun). All species were obtained from the collection of R. R. L. Guillard at Woods Hole Oceanographic Institution, (W.H.O.I.) and were routinely maintained in pure culture and in exponential growth on a twofold dilution of f-medium[11] until inoculation into the continuous cultures. These species were chosen because they were previously shown to have excellent growth characteristics when cultured on wastewater-seawater mixtures[8]. For most of the experiments *P. tricornutum* was the contaminant, although *T. pseudonana* and *D. tertiolecta* were each used in some experiments.

Secondarily treated domestic wastewater was obtained from the trickling filter treatment plant at Otis Air Force Base, Cape Cod, Massachusetts, and seawater was collected off the W.H.O.I. dock. Media were prepared by first mixing the wastewater with seawater (previously filtered through 1-μm cartridge filters) at the desired dilution in 60-liter batches. The mixture was then dispensed to each of the 12-liter carboys by vacuum-pumping the contents through a 1.2-μm membrane filter housed in a teflon-coated filtering unit. No attempt was made to eliminate bacteria from the media, but all indigenous plankton were removed.

The growth vessels and feed lines were initially acid-washed, distilled water-rinsed, and autoclaved. The system, including the media supply bottles, was then installed and approximately one liter of a desired culture was added to each system. Media were then pumped in at the desired dilution rate and the system operated in this fashion for 7-10 days. Daily measurements for pH, tem-

perature, particulate carbon (PC), particulate nitrogen (PN) and cell counts were made on 50 ml samples. When relatively steady state conditions were established (i.e., virtually constant culture pH, PC, PN, and cell counts) the contaminant consisting of 25-50 ml of one culture was added to another culture. The contaminant initially represented no more than 2% of the total biomass (cell count basis) of the mixed cultures. The contaminated cultures were maintained undisturbed for a long enough time to observe the outcome of the competition (when >98% of the culture biomass-cell count basis-consisted of one species). Cell counts of the individual species in each culture were made daily.

Outdoor Cultures

Continuous mass cultures of marine phytoplankton were started in two 135,000-liter ponds in January, 1974 at the Environmental Systems Laboratory (ESL) of W.H.O.I. The ponds, each 15-meters square (with rounded corners) and about one-meter deep, were constructed of earth and sand with a 20-mil thick polyvinyl chloride plastic liner (Figure 1). Gentle mixing was accomplished by recirculation with two 1/3 HP centrifigal pumps. The ponds could be heated to about 15°C above ambient by recirculating pond water through large capacity heat exchangers that also provided additional mixing. From January through April, 1974 the ponds were heated to maintain temperatures in the range 12° - 16°C. Enriched seawater consisting of NO_3^- and PO_4^{-3} additions to natural seawater (to simulate the N and P content of a 25% wastewater-75% seawater mixture) was added continuously at a dilution rate of about

0.3/day. In May, 1974 secondarily treated wastewater from the Wareham, Massachusetts activated sludge facility was substituted for the artificial nutrients; at this time two additional ponds were started on a similar growth scheme. All the ponds were maintained in this fashion until October, 1974 when the first two ponds were again heated. In January, 1975 the heated ponds were discontinued, but the remaining unheated ponds were kept in operation until May, 1975 when the experiment was terminated.

No attempt was made to inoculate initially a particular phytoplankton species into the ponds. The seawater entering the ponds was sand-filtered to 20 μm so that natural populations of phytoplankton were added continuously; however, usually one species became dominant rapidly and changes occurred only on a seasonal basis.

Monitoring of the ponds consisted of tri-weekly analyses of dissolved nutrients (NH_4^+, NO_3^-, NO_2^-, PO_4^{-3}) along with cell counts and species identification. Temperature variations were recorded daily.

Analytical Techniques

Methods

Chemical analyses consisted of soluble NH_4^+,[12] NO_2^-,[13] NO_3^-,[14] and PO_4^{-3}.[15] Algal biomass measurements (PC and PN) were made on a carbon-hydrogen-nitrogen elemental analyzer (Perkin-Elmer Model 240). Cell counts were made with a Spencer Bright-line hemacytometer.

Media nutrient characteristics

Nitrogen and phosphorus concentrations in the wastewater-seawater mixtures

and the enriched seawater are shown in Table I. These nutrient levels, as shown in previous experiments, [8-10] are saturating for growth, and this fact was verified by repeated observations of N and P residuals in both the laboratory and outdoor cultures.

RESULTS

Laboratory Studies

Steady state biomass experiments

Steady state biomass levels were determined for the 5 species in the temperature range 10° - 30°C (Figure 2). Growth of *D. tertiolecta* increased progressively from zero at 10°C to over 30 mg/liter PC at 30°C. An opposite effect was demonstrated for *S. costatum:* over 35 mg/liter PC was produced at 10°C followed by a continuous decrease to cell washout at 30°C. *P. tricornutum* and *M. lutheri* grew best at 20°C and decreased in growth at higher and lower temperatures. Steady state biomass levels could not be maintained for either species at 30°C. *M. lutheri* displayed minimal growth at 10°C (3 mg/liter PC), whereas *P. tricornutum* at 20°C exhibited the highest growth of the 5 species - 46 mg/liter PC. Growth of *T. pseudonana* was generally unaffected by temperature changes: between 10° and 30°C PC levels were in the range 20-28 mg/liter.

Temperature effects on the carbon content per cell (PC:CC) were species specific (Table II). The PC:CC ratio of *S. costatum* decreased with temperature from a high value of 3.4 µg PC/10^5 cells at 10°C to 1.4 µg PC/10^5 cells at 25°C. This variation was the result of increases in the PC concentration as the cell count decreased. *P. tricornutum* and *M. lutheri* displayed minimal

PC:CC ratios of about 1.0-1.2 μg PC/10^5 cells between 15° and 20°C. The ratios at 10° and 25°C were only slightly higher (1.3-2.1). *D. tertiolecta* on the other hand, had a minimal PC:CC ratio between 20° and 25°C (1.7-1.9) but the ratio virtually doubled at both 15° and 30°C. The PC:CC ratio for *T. pseudonana* was relatively constant (0.8-1.1) between 10° and 30°C.

The cell carbon:nitrogen ratios (PC:PN) by weight were generally invarient with temperature for all species, varying between 5 and 6.5 (Table II). However, at 10°C the PC:PN ratio increased to 7.7 and 8.8 for *P. tricornutum* and *M. lutheri*, respectively.

Competition Experiments

The outcome of the competition experiments is summarized in Table III. In the temperature range 15°-20°C *P. tricornutum*, when introduced as a contaminant, was able to outgrow the 4 other species and virtually displace them from the continuous cultures (Figures 3 and 4). At 10° and 25°C, however, it failed to become established and steady state populations of the other species remained unperturbed. When *P. tricornutum* cultures were contaminated with *T. pseudonana* the same overall effect occurred. *T. pseudonana* outcompeted *P. tricornutum* at 10° and 25°C, whereas at 15° and 20°C the outcome was reversed (Figure 4). The temperature-control system failed during the 10°C experiment, but *T. pseudonana* had already comprised over 75% of the total cell count when this malfunction occurred.

At 30°C *D. tertiolecta* outcompeted *T. pseudonana*, the only other species that grew at this temperature; this effect resulted when either *D. tertiolecta*

was added as a contaminant, or when contaminated with *T. pseudonana* (Table III).

Outdoor Experiments

Variations in pond temperatures, PC, species numbers and composition are plotted in Figure 5 for one of the heated ponds (January-December, 1974) and one of the unheated ponds (December, 1974 - May, 1975). The other two ponds displayed similar characteristics and, for clarity, are not represented.

P. tricornutum was the dominant species between January and July, 1974. Pond temperatures increased during this time from 12° to 25°C (even though heat to the ponds was stopped in early May) with a corresponding increase in algal biomass (10 mg/liter PC to 20 mg/liter PC). In early August, when the temperature had risen to over 25°C, there was an infestation of a Monad-type flagellate that preyed upon *P. tricornutum*, resulting in the total collapse of the culture. The pond was cleaned and restarted by mid-August. Initially, a bloom of *Nitzschia closterium*, a pennate diatom, occurred. In early September *Stichococcus* sp., a small green nannoplankter, appeared and quickly dominated the culture, reaching a density of over 4.5×10^6/ml and 12 mg/liter PC by mid-September. Pond temperatures fell rapidly from 24°C in early September to 15°C by mid-October. This temperature decrease coincided with a rapid disappearance of *Stichococcus* sp., which was replaced by a culture of *P. tricornutum* that first appeared in the pond when the temperature fell to about 20°C. Heat was again applied to the pond in mid-October as the density of the *P. tricornutum* culture increased. When the temperature

rose to over 20°C in early November the biomass of *P. tricornutum* rapidly declined. However, as the pond temperature, even with the added heat, dipped to about 18°C in mid-November the culture rapidly recovered.

Experiments with the heated ponds were terminated in early December and full attention was focused on the unheated ponds that had originally been started in May, 1974, cleaned in November, and restarted. By early December the ambient pond temperature had fallen to about 5°C. A culture of *P. tricornutum* that had developed to a peak biomass at this time from startup in November began to decline. By mid-December the pond temperature was still hovering around 5°C when *Skeletonema costatum* appeared and rapidly increased in number as *P. tricornutum* disappeared. This species reached a peak biomass of about 0.5×10^6 cells/ml and 10 mg/liter PC when the temperature fell to a low of 0°C in mid-February. The culture persisted at this level until April when pond temperatures rose to 10°C and *P. tricornutum* reappeared and rapidly displaced *S. costatum*. By early May when the experiments were terminated *P. tricornutum* was again the dominant species.

DISCUSSION

Temperature Effect on Biomass Concentration

In the current laboratory and outdoor experiments dilution rates (specific growth rates) were fixed at fairly low levels of about 0.6/day and 0.3/day respectively for two reasons: 1) it was demonstrated in previous experiments that maximum yields were attained at dilution rates of about 0.5/day[5], and 2) logistic contraints in obtaining sufficient wastewater prevented testing

higher dilution rates in the outdoor ponds. Thus the results are restricted to temperature effects on phytoplankton biomass production at these fixed growth rates.

In the laboratory studies there was little effect of temperature on the production of organic matter: PC values between 30/mg/liter and 46 mg/liter were consistently attained for whichever species grew best at a particular temperature in the range 10^{o} - 30^{o}C. Light limitation undoubtedly resulted in the upper limit to biomass concentration at each temperature. A similar non-effect of temperature on plant production was duplicated in the outdoor experiments, although here the effect of varying light intensity on growth was evident. Biomass concentrations of *P. tricornutum* (10-12 mg/liter PC) during January - March, 1974 when the ponds were heated to about 15^{o}C were equal to concentrations in the *S. costatum* cultures during the same time of year in 1975 when ambient pond temperatures were between 0^{o} and 5^{o}C. However, during April of both years biomass levels increased significantly to 15-20 mg/liter PC, even though the rise in temperature in the heated ponds in 1974 (15^{o} to 18^{o}C) was much higher than in the unheated ponds in 1975 (5^{o} to 10^{o}C). Increased light intensity and duration during this period thus undoubtedly led to the increase in biomass.

Differences between the laboratory and pond biomass levels attained can be similarly attributed to differing light effects in the two culture techniques (e.g., continuous light and thin culture thickness in the laboratory versus diurnal light and relatively deep cultures outdoors).

From these results it is evident that temperature generally exerts as in-

significant influence on biomass concentration in mass culture, even down to temperatures of 0°C. Similar conclusions were reached on the basis of other mass culture experiments in Woods Hole, Massachusetts[9] and Ft. Pierce, Florida[5], and by Eppley[16], who examined the role of temperature in natural marine waters.

Temperature Effect on Species Dominance

The seasonal appearance of many species in natural marine waters and of key species such as *S. costatum* and *P. tricornutum* in mass cultures under defined temperature regimes, irrespective of seasons (Figure 5), is a prime example of how temperature, although having little effect on the production of organic matter, does indeed exert a tremendous influence on species competition.

Clearly, *S. costatum* and *P. tricornutum* were the dominant species outdoors at the cold (< 10°C) and intermediate (10° - 20°C) temperatures, respectively. It was not surprising that *S. costatum* dominated below 10°C; this species is often dominant in coastal waters during winter months even though its division rate decreases with decreasing temperature in the wide range 0° - 22°C[17]. *P. tricornutum*, on the other hand, although able to outcompete the other four species at 15° and 20°C in the laboratory and virtually always the dominant species during the 1½ year outdoor experiment when the temperature was between 10° and 20°C, is seldom found in natural waters in significant numbers at any time[10]. Interestingly, *P. tricornutum* was unable to compete with any of the other species in the laboratory at 10°C, but remained dominant

outdoors until the temperature fell to 5°C. It was then replaced by *S. costatum*, and did not appear again until the temperature rose to 10°C in the spring (Figure 5).

Two points are readily apparent from these results. First, these data represent an obvious case in which temperature optima found in the laboratory are obscured by the interactions of other environmental factors found outdoors: *P. tricornutum* was dominant at 10°C outdoors, but not in the laboratory. And second, competition outdoors may occur differently when temperatures are either falling or rising within a given range: *S. costatum* did not appear until the temperature fell to 5°C, but once dominant, was able to persist until the temperature rose back to 10°C. Relatively gentle mixing used in the outdoor ponds may have been the main factor responsible for this occurrence: only with complete mixing would the rate of washout be proportional to the reduced growth rate of a species that was adversely affected by a temperature change.

A similar situation occurred in the fall of 1974 when *Stichococcus* sp., which first appeared at 25°C, was still present at 15°C as *P. tricornutum* started to appear in large numbers. This delayed disappearance of *Stichococcus* sp. when the temperature became unfavorable is reminiscent of Ryther's[18] observation of a similar occurrence in Moriches Bay, Long Island, There, because of poor tidal exchange, *Stichococcus* and a related species *Nannocloris*, which both grew best between 20° and 25°C, still were present and viable in large numbers when the temperature fell to 15°C.

Real lag periods existed before species switch-over was observed in the

laboratory experiments (Figures 3 and 4). It is difficult to attribute any significance to the duration of the lags, which varied widely from one study to the other, because the number of contaminant cells inoculated into a particular monoculture was not strictly controlled. The delay in growth of the contaminant species is, however, similar to the lag period that initially occurs in batch cultures, and may have simply represented a period in which the contaminant cells were adjusting physiologically to a new environment.

Determination of the actual physiological and biochemical roles of temperature in influencing the outcome of competition was beyond the scope of this study. One hypothesis, suggested earlier as an explanation for the success of *P. tricornutum* is that this species may be excreting metabolites toxic to other phytoplankton[8]; the toxicity and/or rate of excretion of these substances may, in light of the current findings, be temperature-dependent.

Clearly though, the change in competitive advantage seen over the $5^{o}C$ intervals used in the laboratory experiments is suggestive that coexistence between two species should be possible at some intermediate temperature. However, outdoors the possibilities for coexistence at a given temperature are probably diminished because of the dynamic nature of the situation, together with the impact of other influencing factors.

S. costatum displayed two growth characteristics that may provide some insight into the ability of this species to compete well at low temperatures. First, based on an Arrenhius equation developed by Goldman and Carpenter[19], the maximum growth rate ($\hat{\mu}$) of phytoplankton at $10^{o}C$ should be 0.58/day. Indeed at $19^{o}C$ *S. costatum* was found to have a $\hat{\mu}$ of 1.27/day in continuous

culture[20], close to Goldman and Carpenter's predicted value of 1.21/day. Yet, at 10°C in the laboratory it was possible to maintain *S. costatum* at very high steady state biomass levels (35 mg/liter PC) when the growth rate was about 0.6/day. According to continuous culture theory, cell washout occurs when μ is reached[21]. Obviously then, *S. costatum* has a higher μ potential at low temperatures than predicted by the Arrenhius model of Goldman and Carpenter, an asset that *P. tricornutum* apparently does not have.

A second characteristic was that the cell weight of *S. costatum* increased as the temperature decreased: in the laboratory experiments the carbon and nitrogen content of this species virtually doubled from 1.9 µg PC/10^5 cells and 0.4 µg PN/10^5 cells at 20°C to 3.4 µg PC/10^5 cells and 0.6 µg PN/10^5 cells at 10°C (Table III). Jorgensen[22] found precisely the same temperature effect on the protein and carbon content per cell of *S. costatum* between 20° and 7°C. He also observed that photosynthetic rates of this species were independent of temperature and hypothesized that adaptation to low temperature was the result of an increased capacity to store enzymes required for photosynthesis as the temperature decreased. Morris and Glover[23] found a similar effect in other marine species, but contested Jorgensen's rationale by showing that photosynthetic rates were strongly temperature-dependent during log growth; however, because of a rapid decrease in photosynthesis as growth progressed at the higher temperatures (resulting in no apparent temperature effect on photosynthesis late in the log phase), they concluded that increased enzyme production could not be the adaptive mechanism by which cells maintain high

photosynthetic rates at low temperatures. However, none of these researchers explored the possibility that increased nutrient uptake rates at low temperature may be a mechanism by which certain species can compete successfully even though their growth rate is adversely affected.

This storage phenomenon was also present to varying degrees in *P. tricornutum* and *M. lutheri* at 10°C and *D. tertiolecta* at 15°C. *D. tertiolecta*, in fact, also displayed this characteristic at 30°C, which may have also contributed towards its ability to increase in organic matter while its cell count decreased at the higher temperature (Table II). However, the above argument, although satisfactory for explaining the dominance of *S. costatum* at low temperatures and *D. tertiolecta* at high temperatures, does not hold for explaining the competition results at the intermediate temperatures. As shown in Figure 4, although there was no variation in the PC:CC ratio of *T. pseudonana* with temperature, this species outcompeted *P. tricornutum* at 10° and 25°C, but not at 15° and 20°C. Similarly, even though the PC:CC ratio of *D. tertiolecta* was twice as large at 15°C than 20°C, it still could not outcompete *P. tricornutum* at either temperature.

The PC:PN ratios were similar and not temperature dependent in both the outdoor and laboratory cultures, whereas the PC:CC ratios of the dominant species outdoors were generally lower than their laboratory counterparts (Table IV). Packard et al.[24] similarly found no temperature effect on the PC:PN ratio of natural particulate samples from Pacific Ocean waters and concluded that enzyme storage was not a mechanism by which plankton cope with cold temperatures.

It is impossible to ascertain why algal cell weights were greater in the laboratory studies than outdoors without further experimentation. Possibly a combination of increased respiratory losses and decreased total photosynthesis caused by the diurnal light found outdoors led to this decreased cell weight. However, cell weights decreased by about the same amount (50%) for *S. costatum* and *P. tricornutum* from the laboratory to the outdoor cultures at 10°C; thus the ability of *S. costatum* to dominate at low temperatures outdoors may still have resulted from this nutrient storage phenomenon. Griffiths[25] indeed showed that the protein content of *P. tricornutum* varied in culture depending on the light-dark regime employed.

CONCLUSIONS

The results of this study, together with those from the previous mass culture experiments[5,9] (Table V) provide a fairly consistent picture of the temperature-related species changes that have occurred in enriched marine cultures. As summarized in Figure 6, *S. costatum* typically dominated below 10°C and *P. tricornutum* between 10° and 20°C. Between 20° and 25°C two species, *Nitzschia closterium*[5] and *Amphiphora* sp.,[9] have prevailed and above 25°C several species, including *Amphora* sp.,[9] *Stichococcus* sp., *Nannochloris* sp. and some unidentified small green flagellates, have at times dominated. The shaded areas in Figure 6 represent temperature ranges in which the cultures were unstable and species change over began to take place.

In general, most marine species that appear in mass cultures exhibit eurythermal qualities, that is, they can tolerate a broad band of temperature

variations. Ukeles[26] has indeed shown that many marine species tolerate temperatures between 15° and 25°C, and that a number have, to varying degrees, broader temperature-response spectra. Yet, many species that are highly adaptive and grow well over a wide temperature range, e.g., *T. pseudonana*, *M. lutheri*, and *D. tertiolecta* (Figure 2), are never found in outdoor mass cultures simply because in each temperature region they are out-performed by another species that has a more favorable, but limited response to temperature.

Based on these results, it appears that temperature exerts such a powerful influence on species dominance that species control in sustained mass cultures may not be practical. The energy requirements for artificial temperature control preclude any consideration of this technique for controlling species selection. Rather, for marine aquaculture systems designed to remove the grown phytoplankton by herbivorous feeding, it seems more desirable to select species of shellfish that are capable of filtering and assimilating phytoplankton that would normally dominate in a particular geographical locale. The fact that the production of phytoplankton biomass is independent of temperature and that marine diatoms (which in general are a preferred food source for shellfish) are the usual dominant species in mass cultures (see Figure 6), makes this an attractive approach for funneling nutrients through the marine food chain. In this regard, *P. tricornutum*, although a poor food for the American oyster *Crassostrea virginica*, is utilized more efficiently by the European oyster *Ostrea edulis*[3].

In natural waters the complex maze of interacting factors often obscures

the role of temperature, and it has been shown that temperature optima found for particular species in defined laboratory experiments do not always coincide with the temperatures at which these same species are often dominant in natural waters[27]. In outdoor mass cultures under defined conditions of dilution rate and nutrient enrichment the biological complexity is reduced and the role of temperature in affecting species distribution becomes clearer. Thus, although the production of plant organic matter is generally independent of temperature at low growth rates, and many species respond favorably over a wide temperature spectrum, competition is distinctly temperature dependent.

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Table I. Typical nutrient characteristics of media used in laboratory outdoor experiments. All concentrations are in mg/liter as N or P.

Source	NO_3^--N + NO_2-N	NH_4^+-N	ΣN^a	PO_4^{-3}-P
Laboratory				
50% wastewater-50% seawater	1.5	7	8.5	4
Outdoor				
25% wastewater-75% seawater	6.5	0	6.5	3
Enriched seawater	10	0	10	5

[a] $\Sigma N = NH_4^+-N + NO_2^- -N + NO_3^-N$

Table II. Biomass parameters for five species at steady state as a function of temperature.

Temperature °C	*Thalassiosira pseudonana*			*Dunaliella tertiolecta*			*Phaeodactylum tricornutum*			*Skeletonema costatum*			*Monochrysis lutheri*		
	CC[a]	PC:CC[b]	PC:PN[c]	CC	PC:CC	PC:PN	CC	PC:CC	PC:PN	CC	PC:CC	PC:PN	CC	PC:CC	PC:PN
10	2.6	1.0	6.1				0.7	1.6	7.7	1.1	3.4	5.8	0.2	2.1	8.8
15	2.6	1.1	6.6	0.5	3.4	5.7	3.0	1.0	5.2	1.2	2.7	5.6	1.5	1.2	6.1
20	3.1	0.8	6.1	1.5	1.7	6.4	4.2	1.1	6.4	1.6	1.9	5.0	2.8	1.2	6.1
25	1.8	1.1	6.4	1.5	1.9	5.3	3.0	1.2	5.3	1.6	1.4	5.0	2.0	1.3	7.3
30	2.4	1.0	4.7	1.0	3.2	6.2									

[a] 10^6 cells/ml

[b] µg particulate carbon/10^5 cells

[c] mg PC:mg PN

Table III. Outcome of competition experiments at different temperatures.

Initial Species	10°C		15°C		20°C		25°C		30°C	
	C[a]	F[b]	C	F	C	F	C	F	C	F
Phaeodactylum tricornutum (TX-1)	3H	3H	3H	TX-1	3H	TX-1	3H	3H		
Monochrysis lutheri (Mono)	TX-1	Mono	TX-1	TX-1	TX-1	TX-1	TX-1	Mono		
Skeletonema costatum (Skel)	TX-1	Skel	TX-1	TX-1	TX-1	TX-1				
Dunaliella tertiolecta (Dun)			TX-1	TX-1	TX-1	TX-1	TX-1	Dun	3H	Dun
Thalassiosira pseudonana (3H)	TX-1	3H	TX-1	TX-1	TX-1	TX-1	TX-1	3H	Dun	Dun

[a] C = Contaminant species.

[b] F = Dominant species at end of competition.

Table IV. Range of particulate carbon per cell values for natural phytoplankton populations in outdoor ponds.

Species	Temperature	PC:CC[a] µg PC/10^5 cells	PC:PN[a] (weight basis)
Phaeodactylum tricornutum	12-24	0.7-0.9	4.8-5.1
Stichococcus sp.	14-24	0.3-0.4	5.5-6.1
Skeletonema costatum	0-10	1.5-2.0	4.8-5.8

[a] Range of PC:CC and PC:PN ratios appeared to be random within stated temperature range.

Table V. Summary of phytoplankton species data from previous mass culture experiments in Woods Hole, Massachusetts and Ft. Pierce, Florida.

Location and Year	Month	Temperature Range-°C	Dominant Species	Reference
Woods Hole-1973				9
	May-mid June	13——22	*Phaeodactylum tricornutum*	
	mid June-mid August	22——27	*Amphiprora* sp.	
	mid August-mid September	27——20	*Amphora* sp.	
	mid September-November	20——13	*Amphiprora* sp.	
Ft. Pierce-1974				5
	January-November	16——31	*Nitzschia closterium*	

FIGURE LEGENDS

Figure 1. Photograph of large-scale outdoor phytoplankton ponds at ESL (Woods Hole Oceanographic Institution).

Figure 2. Steady state levels of particulate carbon as a function of temperature for five species grown in laboratory continuous cultures: open triangles - *Skeletonema costatum;* closed circles - *Thalassiosira pseudonana;* open circles - *Phaeodactylum tricornutum;* closed squares - *Monochrysis lutheri;* closed triangles - *Dunaliella tertiolecta.*

Figure 3. Competition between *Skeletonema costatum* (closed triangles) and *Phaeodactylum tricornutum* (open circles) at 10° and 15°C. In both studies monocultures of *S. costatum* were contaminated with *P. tricornutum.*

Figure 4. Competition between *Thalassiosira pseudonana* (closed circles) and *Phaeodactylum tricornutum* (open circles) at 10°, 15°, 20° and 25°C. *T. pseudonana* at 10° and 25°C and *P. tricornutum* at 15° and 20°C were the contaminants.

Figure 5. Summary of data collected in outdoor ponds from January, 1974 through April, 1975: A - Temperature data; B - Particulate carbon data; C - Species data (P = *Phaeodactylum tricornutum,* N = *Nitzschia closterium,* S = *Stichococcus* sp., SK = *Skeletonema costatum).*

Figure 6. Summary of phytoplankton species that have dominated in different temperature regions in current and previous enriched mass cultures. Shaded areas indicate regions of species instability.

Fig. 1

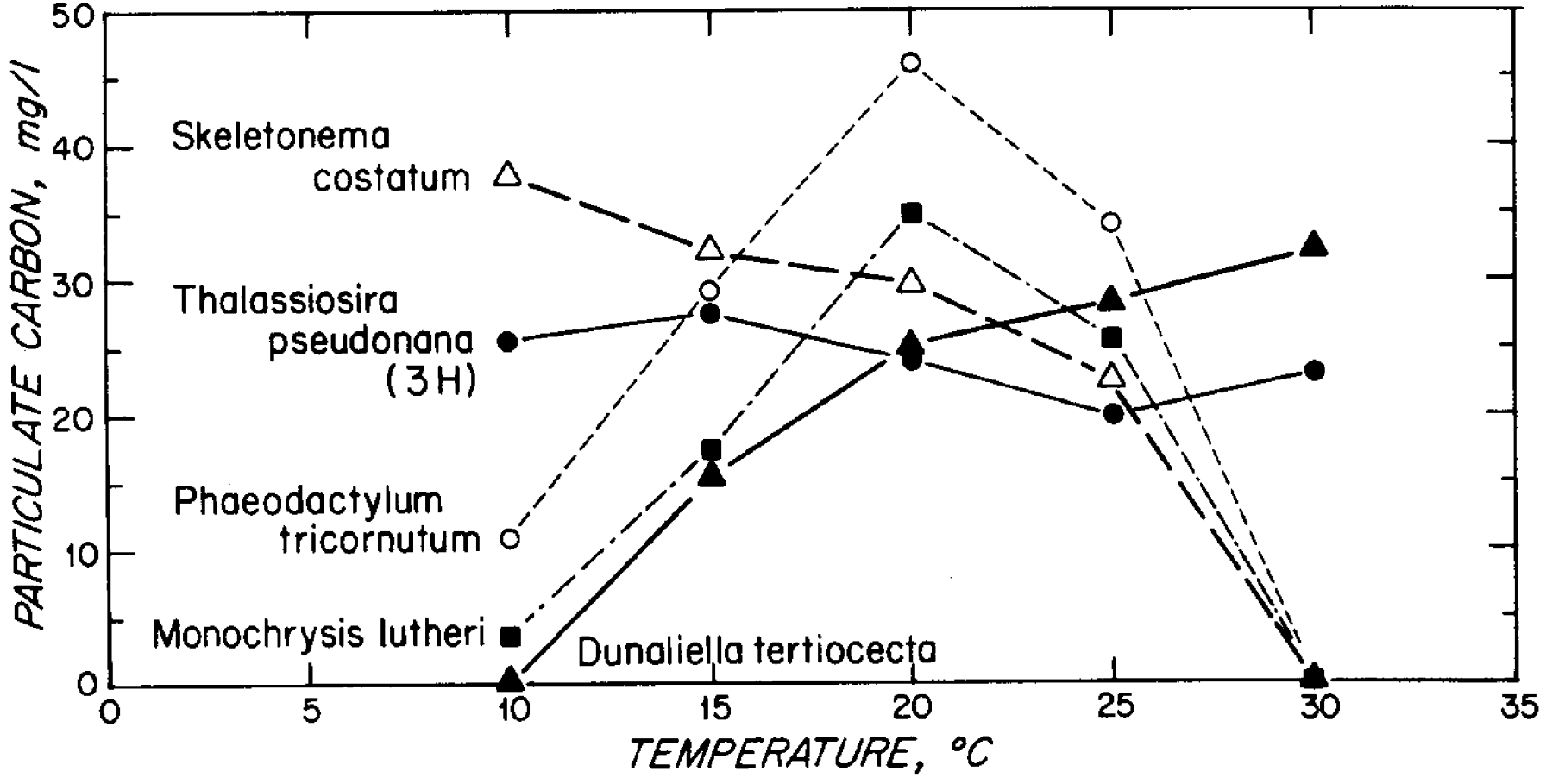
PARTICULATE CARBON, mg/l
TEMPERATURE, °C
Skeletonema costatum
Thalassiosira pseudonana (3H)
Phaeodactylum tricornutum
Monochrysis lutheri
Dunaliella tertiocecta
0
10
20
30
40
50
0
5
10
15
20
25
30
35

Fig. 2

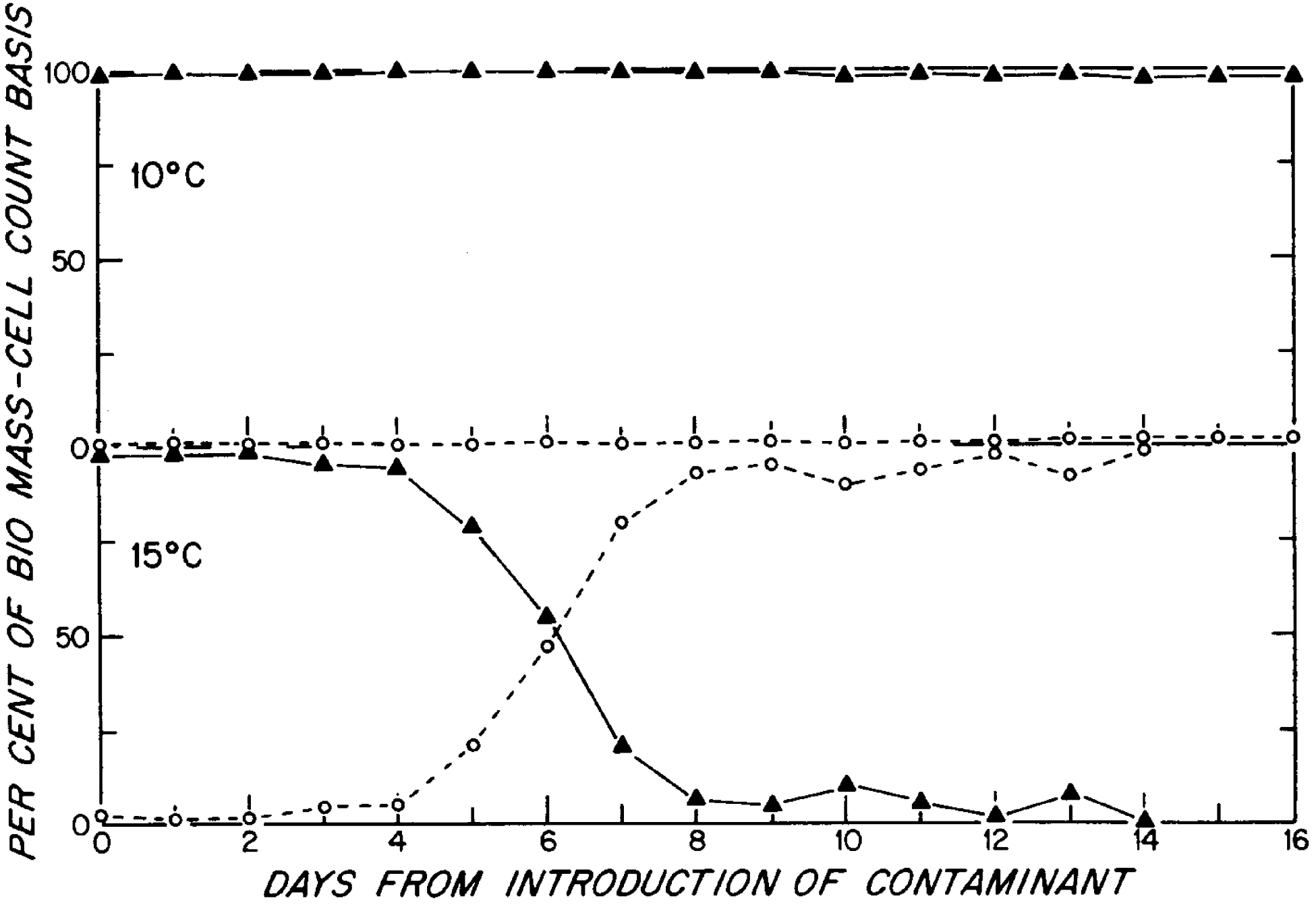
PER CENT OF BIO MASS-CELL COUNT BASIS
100
50
0
10°C
15°C
50
0
0
2
4
6
8
10
12
14
16
DAYS FROM INTRODUCTION OF CONTAMINANT

Fig. 3

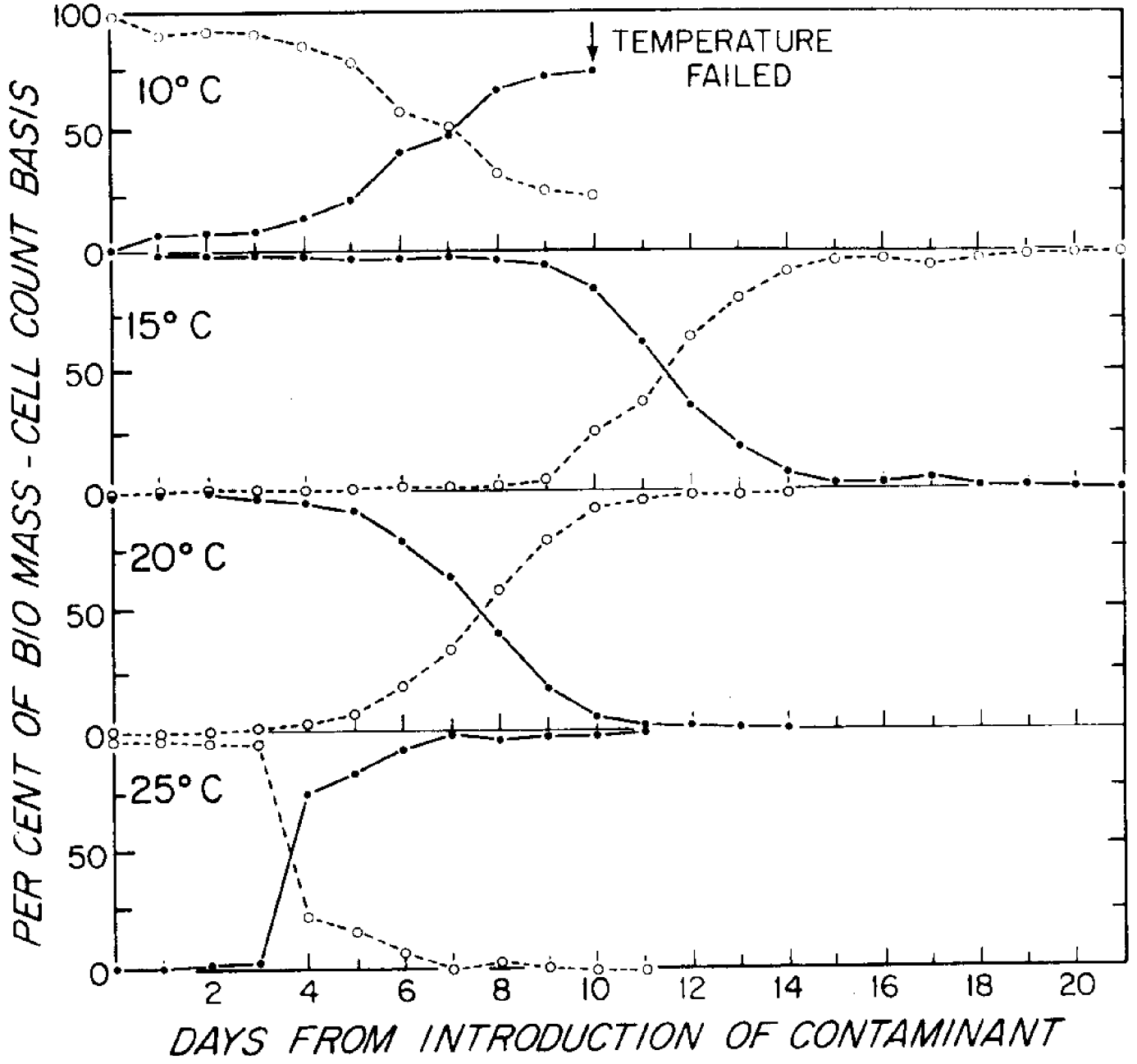
100
50
0
10° C
TEMPERATURE
FAILED
15° C
20° C
25° C
2
4
6
8
10
12
14
16
18
20
PER CENT OF BIO MASS - CELL COUNT BASIS
DAYS FROM INTRODUCTION OF CONTAMINANT

Fig. 4

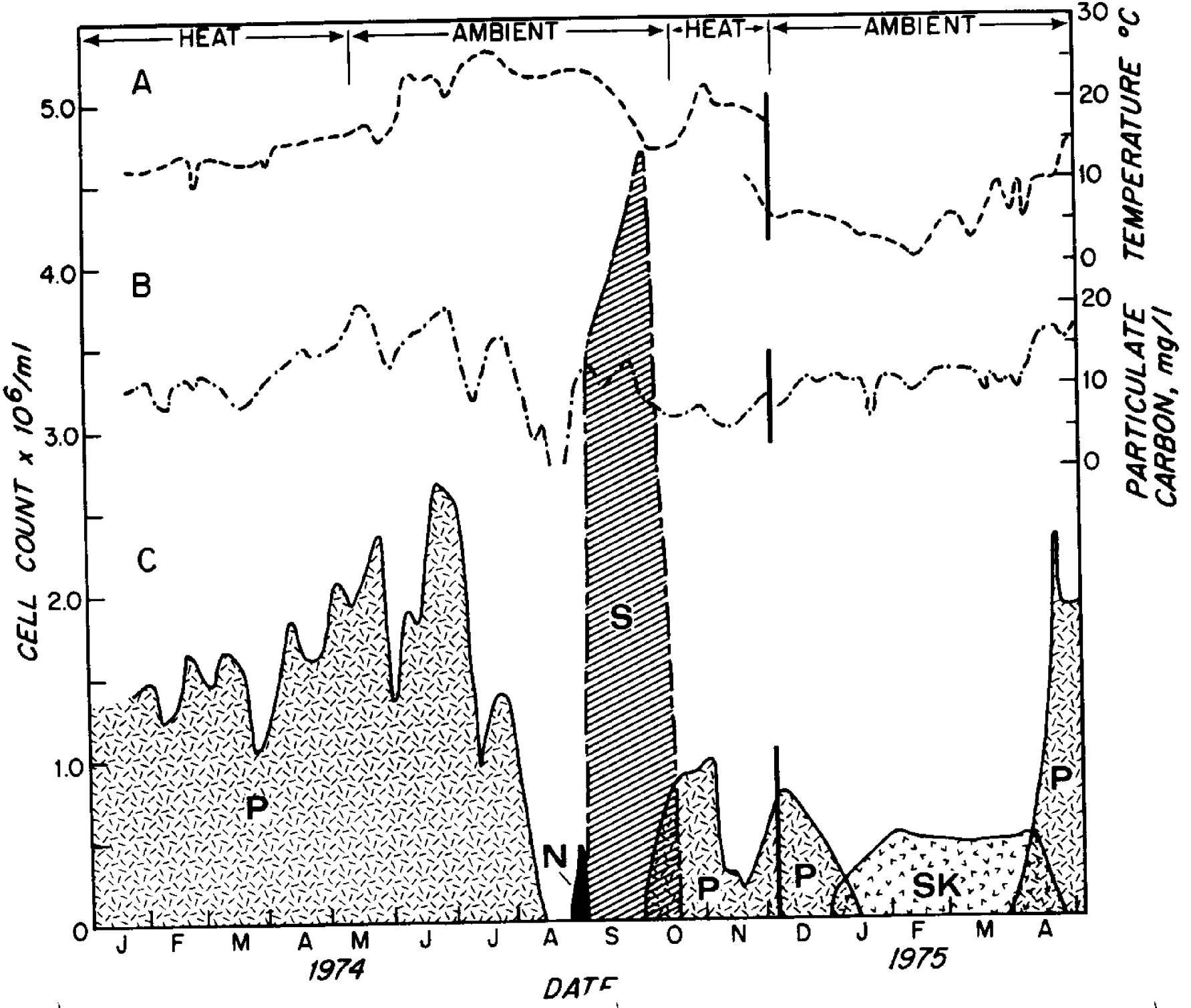
HEAT
AMBIENT
HEAT
AMBIENT
A
B
C
P
N
S
P
P
SK
P
CELL COUNT x 10⁶/ml
PARTICULATE CARBON, mg/l
TEMPERATURE °C
5.0
4.0
3.0
2.0
1.0
0
30
20
10
0
20
10
0
J F M A M J J A S O N D J F M A
1974
1975
DATE

Fig. 5

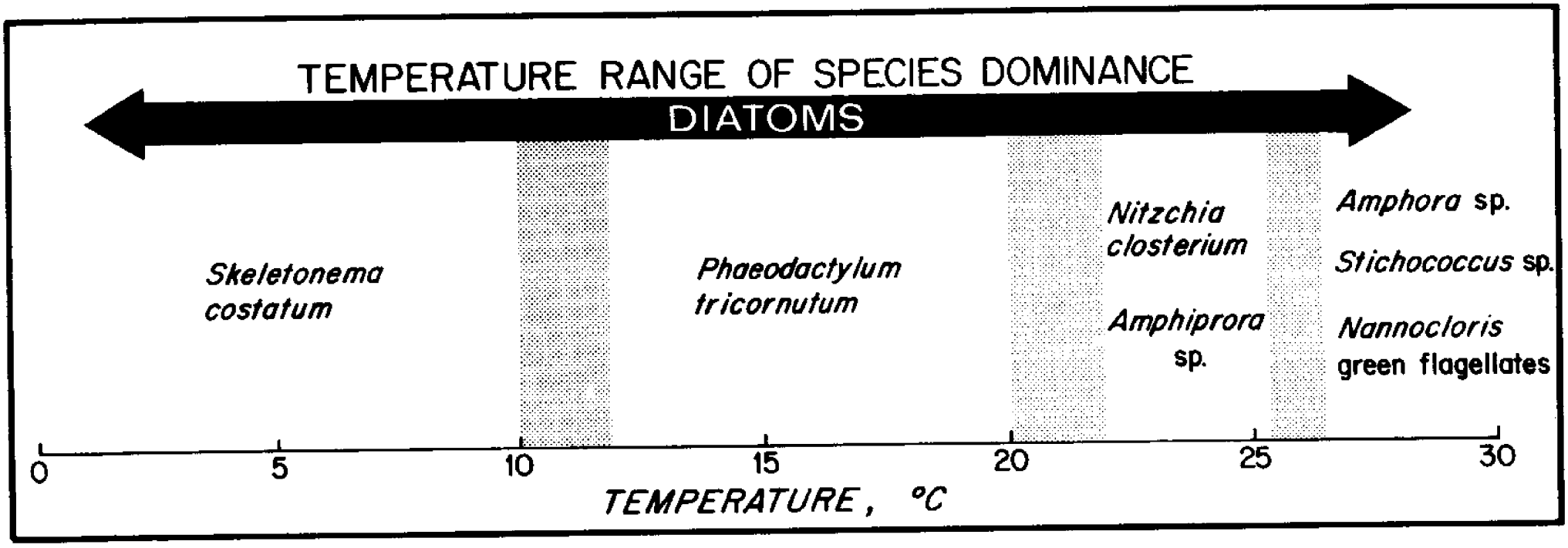
TEMPERATURE RANGE OF SPECIES DOMINANCE
DIATOMS
Skeletonema costatum
Phaeodactylum tricornutum
Nitzchia closterium
Amphiprora sp.
Amphora sp.
Stichococcus sp.
Nannocloris
green flagellates
0
5
10
15
20
25
30
TEMPERATURE, °C

Fig. 6

Temperature effects on phytoplankton growth in continuous culture[1]

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Running head: Temperature and phytoplankton growth.

[1]Contribution No. from the Woods Hole Oceanographic Institution.

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Abstract - Temperature has a powerful influence on the chemical composition of marine phytoplankton. A common characteristic of many phytoplankton is a minimum nutrient content (as nitrogen or carbon) per cell at the temperature optimum for cell division and increasing cellular biomass at lower and higher temperatures. Hence, cell division and nutrient uptake rates are uncoupled with respect to temperature. The role of temperature in phytoplankton ecology is unclear and doubts are raised concerning the predictive value of existing kinetic growth and uptake models.

It was recently demonstrated how temperature strongly influences the outcome of phytoplankton competition in mass cultures maintained on wastewater-seawater mixtures (Goldman and Ryther 1976). Although the objectives of that research were directed toward the development of waste recycling-aquaculture systems (Ryther et al. 1975), several aspects of those results appears to be of general importance to a more complete understanding of phytoplankton dynamics in natural, as well as controlled aquatic systems.

The direct influence of temperature on phytoplankton division rates is clearly established, that is, within defined temperature limits division rates increase with increasing temperature (Eppley 1972, Goldman and Carpenter 1974). Yet, as discussed in detail by Eppley (1972), a number of species display an increase in cellular biomass as the temperature is lowered. Jorgensen (1968) first observed this phenomenon in Skeletonema costatum; he showed that, whereas lowered temperatures led to lowered division rates, they conversely resulted in higher cellular carbon and nitrogen assimilation rates. Increased enzyme production as a result of higher cellular protein concentration present was hypothesized to be an adaptive mechanism for maintaining high photosynthetic rates at the lower temperatures. Morris and Glover (1974) contested this reasoning by showing that photosynthetic rates were independent of temperature only towards the end of batch growth, though they also demonstrated that the biomass per cell (measured as dry weight) of two other species, Phaeodactylum

tricornutum and Dunaliella tertiolecta, was inversely affected by temperature; this effect was not evident in Nitzschia closterium, however.

The results of previous mass culture experiments support the above observations. It was found that temperature strongly influences cellular chemical composition, not only at the lower, but at the higher temperatures as well In those experiments, described in detail elsewhere (Goldman and Ryther 1976), 5 phytoplankton species obtained from the culture collection of R.R.L. Guillard at Woods Hole Oceanographic Institution [Skeletonema costatum (Skel), Monochrysis lutheri (Mono), Phaeodactylum tricornutum (TX-1), Dunaliella tertiolecta (Dun), and Thalassiosira pseudonana (3H)] were grown in continuous cultures at a constant dilution rate of 0.6 day^{-1} with constant lighting (about 0.03 langleys min^{-1}, visible region) on enriched media consisting of 50% secondarily treated wastewater and 50% seawater. Steady state measurements were made in the temperature range 10°-30°C in 5°C intervals, for cell counts (CC) (Spencer Bright-line hemacytometer), and particulate carbon (PC) and nitrogen (PN) (Perkin-Elmer 240 Elemental Analyzer).

The data for steady state PN values are shown in Fig. 1 and cell numbers and PN CC^{-1} ratios in Fig. 2. The PN PC^{-1} ratios varied between about 5-7 (weight basis) for all species and temperatures so that the results are equally applicable for describing PC variations with temperature.

Each species responded to temperature variations in a somewhat different manner. Four species (T. pseudonana excepted) displayed a dramatic increase in the nitrogen (and carbon) content per cell (PN CC^{-1} ratio) at the lowest temperatures at which steady state biomass was measurable (Fig. 2). Dunaliella tertiolecta and P. tricornutum, in addition, had minimum PN CC^{-1} ratios at 20°C, and increasing ratios at higher as well as lower temperatures.

Based on the competition experiments from the mass culture study of Goldman and Ryther (1976) and the PN results of this analysis (Fig. 1), S. costatum at 10°C and D. tertiolecta at 30°C were the dominant and most efficient species in assimilating inorganic nitrogen and carbon. Yet, the anomalous feature of these results was that PN (and PC) values were highest when cell numbers were declining; hence the lower cell numbers were compensated for by higher nutrient uptake rates.

Nutrient uptake rates can be expressed either on a cell basis:

$$\rho_c = \mu Q = \mu \Delta S \text{ unit cell}^{-1} \qquad (1)$$

in which ρ_c is the cellular nutrient uptake rate, nutrient assimilated (ΔS) unit $cell^{-1}$ unit $time^{-1}$, μ is the specific growth rate, unit time, and Q is the cell quota, ΔS unit $cell^{-1}$, or on a total biomass basis:

$$\rho_B = \mu \Delta S \qquad (2)$$

in which ρ_B represents the amount of nutrient assimilated by the total cell population unit time^{-1}.

Because the dilution rate D is a continuous culture (flow rate culture volume^{-1}) is virtually equal to μ, and because D was kept constant at 0.6 day^{-1} in the experiments, the PN results from Fig. 1 and the PN CC^{-1} ratios from Fig. 2, when multiplied by 0.6 day^{-1}, represent the uptake rates ρ_B and ρ_c respectively.

Thus it is apparent that S. costatum at low temperatures and D. tertiolecta at high temperatures compensated for low cell production by an increase in cellular nutrient uptake rates, ρ_c. The net effect was that the ρ_B rates for S. costatum between 10°-20°C (3.5-3.8 mg PN liter^{-1} day^{-1}) were virtually independent of temperature.

It is tempting, but perhaps fruitless, at this point to speculate on the role this temperature-dependent uptake phenomenon plays in influencing species competition at the temperature extremes at which most natural marine populations grow (about 0° and 30+°C). It may or may not be coincidental that S. costatum, which strongly displays this uptake characteristic at lower temperatures, is often found in coastal marine waters during winter months (Curl and McLeod 1961; Braarud 1962; Smayda 1973). Malone (1975) in fact found that the assimilation ratio of natural populations in the New York Bight decreased with decreasing temperature down to 8°C, but at lower temperatures when S. costatum was dominant there was a dramatic rise in this ratio.

Three observations do appear noteworthy for further discussion, however. First, for a given growth rate actual biomass production measured as PN or PC appears to be fairly independent of temperature over a broad range. As seen in Fig. 1, the peak amount of PN produced was similar between 10°-30°C (5.3-7.2 mg liter^{-1}). The only major effect of temperature seemed to be on individual species growth: S. costatum grew well at the lowest temperatures (10°-15°C), P. tricornutum in the median range (15°-25°C) and D. tertiolecta at the highest temperature (30°C).

Second, we see here a classic example of the uncoupling of cell division and nutrient uptake. For a fixed growth rate, and depending on the species present, cellular uptake rates for both nitrogen and carbon varied widely with temperature. Williams (1971) in fact developed a model to demonstrate that the temperature optima for division and biomass production were not necessarily the same after he found a U-shaped response of biomass cell^{-1} ratio (as dry weight) versus temperature for the freshwater species Chlorella. Our results for D. tertiolecta and P. tricornutum are identical to those of Williams, and suggest that this type of a response to temperature may be characteristic of many algal species. In fact, it is conceivable that the PN CC^{-1} ratio of M. lutheri and S. costatum would have swung back up between 25° and 30°C had the temperature intervals been reduced to less than 5°C, since growth of these 2 species could not be maintained at 30°C (Fig. 2). On the other hand, there appears to be a

tight coupling between division and nutrient uptake, at least with respect to temperature, for species such as T. pseudanana (Fig. 2) and N. closterium (Morris and Glover 1974).

Currently, much emphasis is being placed on nutrient uptake and internal pool models (Droop 1973; Dugdale 1976). In light of the current findings, it appears that the usefulness of these models will be severely limited until the opposing temperature effects on cell division and nutrient uptake are accounted for. Because it is now evident that these are widely divergent and species-dependent responses to temperature, generalized use of such models, even with temperature functions included, may have limited predictive value.

The third observation concerns the reporting of growth rates. It is quickly apparent that growth rates measured as cell multiplication per unit time are not necessarily equivalent to growth rates measured as biomass increase per unit time. For example, Eppley (1972) describes 3 equations typically used to measure phytoplankton growth rates in batch culture and natural waters (Table 1). Equation 3 is based on increases in cell number, Equation 4 on PC changes, and Equation 5 on variations in PC chlorophyll^{-1} ratios. Because biomass per cell can change drastically with temperature, if one were to use only Equation 3 to compare growth rates at say 20° and 10°C for a species such as S. costatum, one could be easily misled into thinking that growth rate was directly a function of temperature. Yet, if Equations 4 or 5 were used the temperature dependency of growth rate would not be as evident.

Eppley (1972) emphasized this point by showing that significant increases in the PC chlorophyll^{-1} ratios of phytoplankton with decreasing temperature led to assimilation ratios not nearly as temperature dependent as might be expected. Smayda (1973) in his comprehensive study of Skeletonema blooms in Narragansett Bay, Rhode Island, showed a direct temperature dependency of division rate (measured as doublings day^{-1}) for S. costatum. The question arises as to whether this dependency would have been observed had growth rate been calculated according to Equations 4 or 5.

In conclusion, the role of temperature in pnytoplankton ecology is poorly understood. The opposing effects of temperature on division and nutrient uptake rates, together with the obvious dominance of certain species only in defined temperature regions, makes it extremely difficult to incorporate these effects into general phytoplankton growth models. The simple insertion of a temperature factor into growth rate equations such as done by Eppley (1972) and Goldman and Carpenter (1974) is obviously not sufficient.

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Table 1. Equations used to calculate phytoplankton growth rates (from Eppley 1972).

Growth parameter	Equation	Number
Cell count	$\mu = t^{-1} \log_2 \left[\frac{CC + \Delta CC}{CC}\right]$	(3)
Particulate carbon	$\mu = t^{-1} \log_2 \left[\frac{PC + \Delta PC}{PC}\right]$	(4)
Carbon and chlorophyll a	$\mu = t^{-1} \log_2 \left[\frac{PC\ chl\ a^{-1} + \Delta PC\ chl\ a^{-1}}{PC\ chl\ a^{-1}}\right]$	(5)

Figure Legends

Fig. 1. Effect of temperature on steady state PN concentration for 5 phytoplankton species grown in continuous culture at a dilution rate of 0.6 day^{-1}.

Fig. 2 Effect of temperature on steady state cell numbers (solid lines) and PN CC^{-1} ratios (dashed lines) for 5 phytoplankton species grown in continuous culture at a dilution rate of 0.6 day^{-1}.

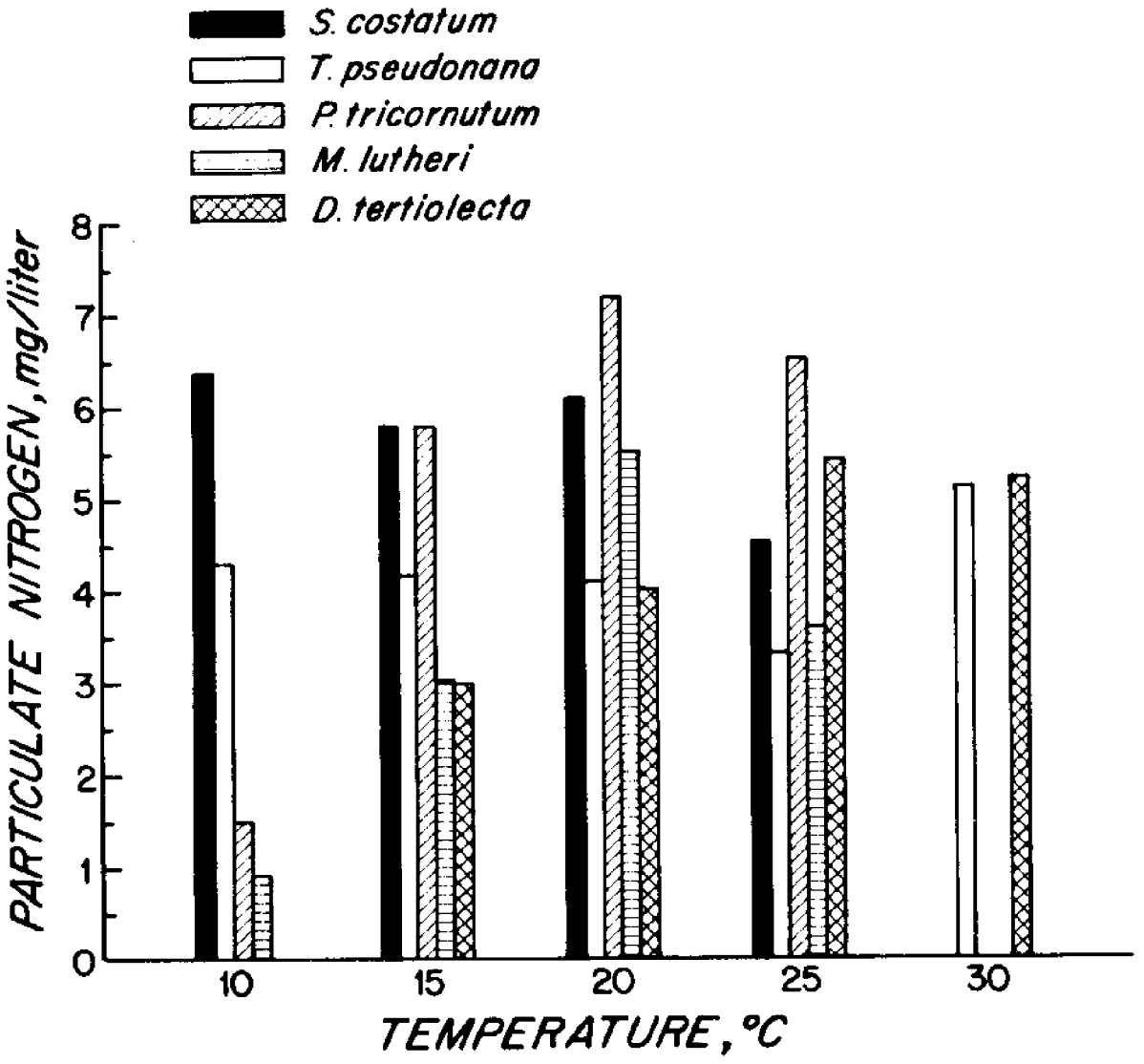
S. costatum
T. pseudonana
P. tricornutum
M. lutheri
D. tertiolecta
PARTICULATE NITROGEN, mg/liter
0
1
2
3
4
5
6
7
8
10
15
20
25
30
TEMPERATURE, °C

Fig.

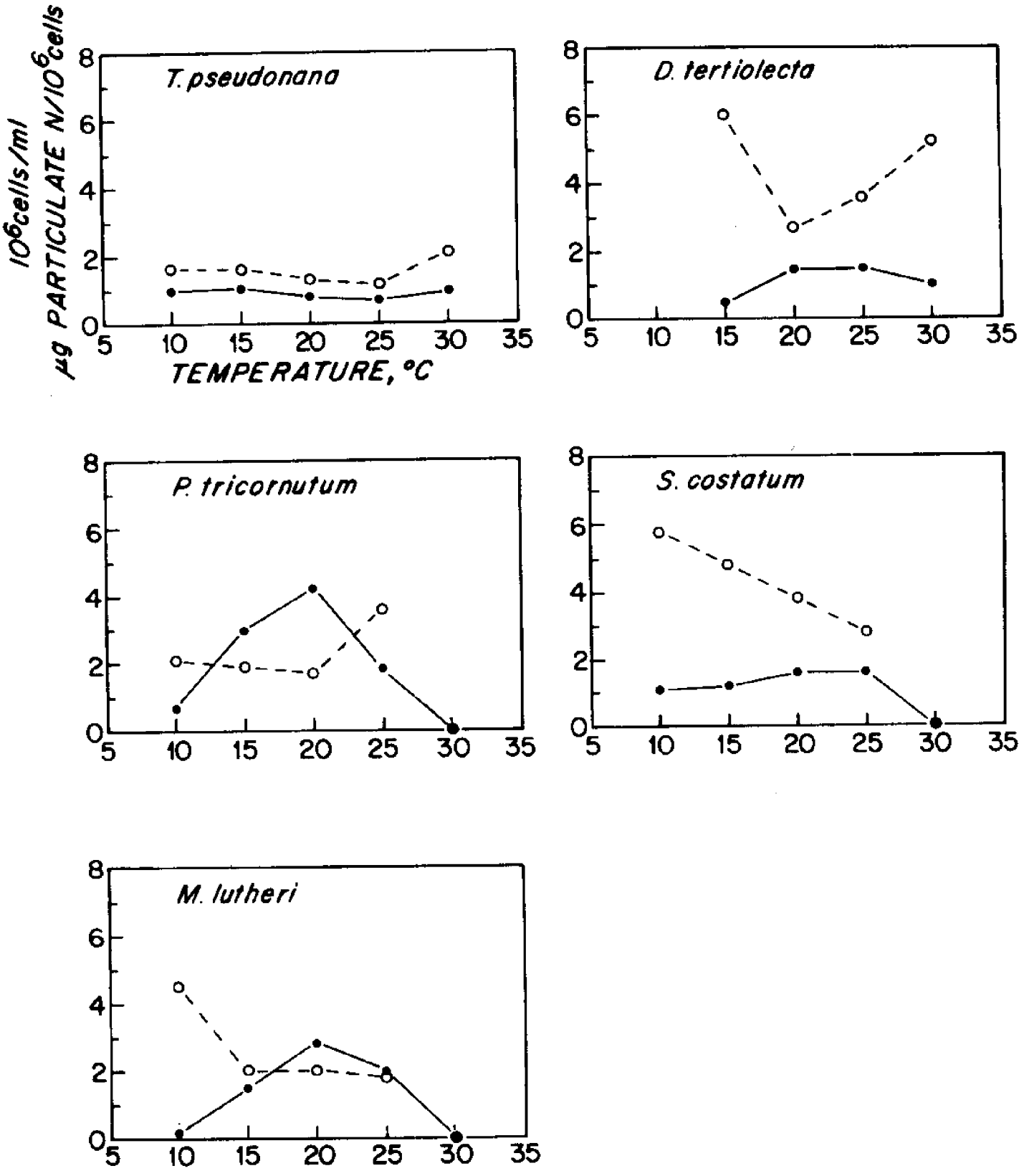
T. pseudonana
D. tertiolecta
P. tricornutum
S. costatum
M. lutheri
10^6cells/ml
µg PARTICULATE N/10^6cells
TEMPERATURE, °C
0
2
4
6
8
5
10
15
20
25
30
35

Fig. 2

Biomass Production in Mass Cultures of Marine Phytoplankton at Varying Temperatures*

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*Contribution No. from the Woods Hole Oceanographic Institution. This investigation was supported by NOAA Office of Sea Grant, Department of Commerce, under Grant No. 04-6-158-44016.

Abstract

Natural populations of marine phytoplankton obtained from a large outdoor pond were grown on wastewater-seawater mixtures in laboratory continuous cultures in the temperature range 5°-33°C. Virtually all of the influent inorganic nitrogen (14.0 mg ℓ^{-1}) was assimilated at every temperature tested. However, there was a distinct change in dominant species with temperature: below 19.8°C *Phaeodactylum tricornutum* was dominant, at 27°C *Nitzschia* sp. was the main species, and as the temperature increased above 27°C a blue-green alga, *Oscillatoria* sp., become increasingly dominant. These results are identical to those of Goldman and Ryther (1976), who examined temperature effects in both laboratory and outdoor mass cultures. There is some indication that the excellent growth of *P. tricornutum* below 10°C was related to a dramatic increase in the nutrient content per cell as the temperature decreases. Thus at low temperatures reduced division rates are compensated for by increased nutrient uptake rates. It follows that there is a transfer of phytoplankton protein from numerous small cells at intermediate temperatures to large cells that are reduced in numbers at lower temperatures, but which represent the same total organic matter. The effect of this phenomenon on year-round food chain efficiences in both controlled and natural marine ecosystems is unknown.

Introduction

There is increasing evidence to support the conclusion of Eppley (1972) that temperature plays a minor role in affecting primary production in marine waters (Mann, 1973; Goldman and Ryther, 1976). Eppley reasoned that, whereas temperature set an upper limit to phytoplankton growth rates, this growth potential is seldom realized in the oceans because other factors, most notably nutrient limitation act to severely restrict growth. The main role of temperature appears then to be its affect on species composition, as evidenced by repeated observations of distinct seasonal and geographical distributions of both micro and macro algae (Smayda, 1958; Braarud, 1961; Mann, 1973).

The same seasonal effects of temperature have been observed in enriched outdoor mass cultures of marine phytoplankton: significant species changes, but invarient total production of organic matter (Ryther, 1975; Goldman and Ryther, 1976). As might be expected, the seasonal effects of sunlight intensity on phytoplankton production were significant in those studies, but when cultures were artificially heated during winter months to 15^{o}-20^{o}C, yields were no better than at ambient temperatures (0^{o}-5^{o}C), although there were distinct changes in species composition (Goldman and Ryther, 1976).

Because it was impossible to completely isolate temperature as the only variable in the outdoor studies, laboratory continuous cultures were used to grow natural populations of phytoplankton under constant light intensity. The phytoplankton originated from an outdoor mass culture and were grown at relatively low dilution rates and on mixtures of wastewater and seawater, conditions similar to those typically used outdoors in the aquaculture re-

search program at the Environmental Systems Laboratory (ESL) of Woods Hole Oceanographic Institution (WHOI) (see Ryther *et al.*, 1975). Observations of biomass production and species distribution were made at temperatures between 5^{o} and 33^{o}C. Of major interest in these experiments was the effect of temperature on nitrogen assimilation since the flow of nitrogen in natural and controlled food chains is a prime indicator of ecological efficiency.

Materials and Methods

Experimental Apparatus

A bank of 4-one liter continuous cultures described in detail previously (Brewer and Goldman, 1976) was used. Experiments were performed at 8 temperatures: 10.3^{o}, 15.0^{o}, 19.8^{o} and 27.0^{o}C in the first two runs and then switched to 5^{o}, 8.5^{o}, 30.2^{o} and 33.0^{o}C respectively in the 3rd experiment. Temperature control was maintained by circulating cooling water from constant-temperature baths (Forma 2095) through glass water jackets on the outer surface of each unit. Visible light intensity of about 0.025 langleys min^{-1} was established with a bank of 4-40W Luxor ® vite-lite fluorescent lamps.

Nutrient media were peristaltically pumped (Harvard 1212) to each unit from individual 4-liter glass storage bottles. All tubing was glass except for silicone tubing joints and similar tubing inserted through the pump.

Nutrient Media

Nutrient media consisted of mixtures of secondarily treated wastewater from the Wareham, Massachusetts activated sludge treatment plant with seawater from Vineyard Sound, Massachusetts in ratios of 1:3 (wastewater-sea-

water) in the 1st experiment and 1:1 in the remaining 2 runs. To simulate as closely as possible the experimental design followed in concurrent large-scale outdoor experiments (Ryther *et al.*, 1975; Goldman and Ryther, 1976), wastewater was obtained directly from the storage and supply system of ESL where the experiments were performed, and 20 μ sand-filtered seawater was obtained from the laboratory's flowing seawater system. Fresh media were prepared daily. Nutrient characteristics of the mixtures are shown in Fig. 1. Ammonia accounted for about 50% of the dissolved inorganic nitrogen concentration ($\Sigma N = NH_4^+ + NO_2^- - NO_3^-$) in the 1:3 mixtures and about 75% in the 1:1 mixtures, the differences being attributed to changing degrees of oxidation at the treatment plant during the month long study (November - December, 1975). The N:P ratios (by atoms) were always 10 or less, indicating that nitrogen was more limiting for growth than phosphorus (Goldman, 1976a).

Analytical Techniques

Soluble NH_4^+ (Solorzano, 1969), NO_2^- (Bendschneider, 1952), NO_3^- (Wood *et al.*, 1967), ortho PO_4^{-3} (Murphy and Riley, 1962), and total dissolved nitrogen-TDS (D'Elia *et al.* in prep.) were determined on the nutrient media. Particulate carbon (PC) and nitrogen (PN) (Perkin Elmer 240 elemental analyzer) and cell counts of individual species (Spencer Bright-line hemacytometer) were measured on the cultures.

Test Algae and Culture Operation

The initial inoculum consisted of a heterogeneous mixture of phytoplankton

taken from one of the 120,000 liter outdoor ponds at ESL that was in operation at the time the laboratory study was performed. (See Ryther *et al.*, 1975; Goldman and Ryther, 1976). The four units were first completely filled with the pond culture and then pumping of media (1:3 wastewater-seawater mixture) was begun (Experiment 1). As noted, temperatures were in the range 10.3° to 27.0°C. After 14 days of operation, the cultures were drained, cleaned, and reinoculated with the pooled cultures saved from the first experiment. The wastewater fraction was increased to 50% and continuous flow restarted (Experiment 2). After eight days culture temperatures were changed as described and media flow continued for an additional seven days (Experiment 3), when the study was terminated. The dilution rate (flow rate culture volume^{-1}) in the 4 units was maintained at about 0.33 day^{-1} for the duration of the study.

Cell counts were determined daily and culture PC and PN were analyzed at the end of each experiment.

Results

It was clear that temperature had virtually no influence on the production of plant organic matter, measured as PN (Fig. 1). At both levels of influent inorganic nitrogen (14.0 mg ℓ^{-1} for the 1:1 mixtures and 6.0 mg ℓ^{-1} for the 1:3 mixtures) there was almost complete assimilation of available N regardless of the temperature. However, at both nitrogen levels there was a distinct pattern of changes in species composition as the temperature was varied.

Three species, *Phaeodactylum tricornutum*, *Nitzschia* sp. and *Oscillatoria* sp., comprised the culture populations in varying relative amounts depending on the temperature (Table 2). *P. tricornutum* virtually dominated below 10.3°C, with only a trace of *Nitzschia* sp. present. In the intermediate temperatures (10.3°-19.8°C) both *P. tricornutum* and *Nitzschia* sp. were present, although the relative proportions of the two species were dependent on both nutrient level and temperature. In the 1:3 mixtures *P. tricornutum* comprised 38% of the population (cell count basis) at 10.3°C, increasing to 44% at 15°C and 70% at 19.8°C. A similar trend of increasing proportions of *P. tricornutum* with increasing temperature was noted with the 1:1 mixtures, but at higher values (77% at 10.3°C, 83% at 15°C and 87% at 19.8°C).

At 27.0°C *Phaeodactylum tricornutum* did not survive and the appearance of *Oscillatoria* sp., a blue green alga, was observed. It was impossible to accurately count the number of individual *Oscillatoria* cells because they were present in long overlapping strands; but based on a rough visual estimate, this species increased from about 25% of the algal population at 27.0°C for both mixtures to about 75% of the population at 33.0°C with the 1:1 mixtures (Table 2). *Nitzschia* sp. made up the balance of the cultures at these high temperatures.

Discussion

The basic features of these results are identical to those of the previous study (Goldman and Ryther, 1976): virtually no effects of temperature on the assimilation of nitrogen and the production of organic matter (Fig. 1),

but significant effects on the composition of species (Table 2). These trends were evident regardless of the wastewater-seawater mixture used.

The range of temperatures at which the three observed species dominated is likewise consistant with the results from previous outdoor studies. *Phaeodactylum*, which dominated between 5° and 20°C but did not grow at 27°C in the current experiments, has been the dominant alga on numerous occasions in mass cultures maintained in Woods Hole when the temperature was between 5°-25°C (Goldman and Ryther, 1975; 1976). Similarly, *Nitzschia* sp. has dominated in outdoor mass cultures in Woods Hole for short periods, but only at > 25°C (Goldman and Ryther, 1976), and was the dominant alga during a six month long mass culture study in Fort Pierce, Florida, when water temperatures were between 20°-30°C (Goldman *et al.*, 1975). Although present in varying numbers at all temperatures used in this study, it was most prevalent at 27°C.

The *Oscillatoria* sp., seen in increasing numbers above 27°C in the current work, has never been dominant in the outdoor experiments at Woods Hole, most probably because water temperatures there rarely rise above 30°C. Yet, this species has been observed as a contaminant in many of these experiments at all temperatures. In fact, it was previously isolated and was one of a number of marine algae (along with *P. tricornutum* and *Nitzschia* sp.) that grew well in continuous monoculture on wastewater-seawater mixtures at 21°C (Goldman and Stanley, 1975). Its increasing appearance above 27°C is interesting because there is virtually no previous documentation of a filimentous blue green alga other than *Trichodesmium* (Carpenter and McCarthy

1976), dominating in an enriched marine system at high temperatures. The dominance of this species may thus be unique to laboratory cultures. Clearly though it has a temperature response that is generally characteristic of thermophilic blue green algae.

One seemingly anomalous result of the species composition data is that in the temperature range 10.3°-19.8°C there were relatively higher proportions of *Nitzschia* sp. at each temperature in the 1:3 wastewater-seawater mixture than in the 1:1 mixture, although the trend of decreasing *Nitzschia* and increasing *Phaeodactylum* numbers with increasing temperature in this range was the same in both mixtures (Table 2). Precisely what caused this result remains unclear, although a biomass-dependent interaction between the two species may have been responsible. For example, if *P. tricornutum* were excreting a toxic metabolite, as suggested earlier (Goldman, 1976b), the effect could have been biomass dependent, thus accounting for the relatively better success of *P. tricornutum* at the higher nutrient, and resulting greater biomass levels. An equally plausable hypothesis is that during Experiment 1 with the lower wastewater fractions *P. tricornutum* (but not *Nitzschia* sp.) was adversely affected by a nutrient limitation or by the presence of toxicity, but, because of the heterogeneous and constantly changing nature of the wastewater, this affect disappeared during the latter experiments.

Nitzschia sp. appears to be a highly eurythermal species as it was present at every temperature in varying numbers. It's relative success at two widely divergent temperatures (10.3° and 27°C) may have resulted simply be-

cause in the culture system imposed, these were the temperature regions in which, for one reason or another, the other species did not grow particularly well; hence the competitive pressure on *Nitzschia* sp. was eased and it appeared in relatively higher numbers. Perhaps this is a common characteristic of species that display a wide tolerance to environmental factors such as temperature: they are everpresent, but in each temperature region are outcompeted by another species that has a limited but more positive response to temperature.

The dominance of *Phaeodactylum* at temperatures below $10^{o}C$ is not completely consistant with the results from the study of Goldman and Ryther (1976); there it was shown that *P. tricornutum* in the laboratory cultures did not grow well at $10^{o}C$, although it was dominant outdoors during December 1974 - January 1975 down to $5^{o}C$. It may be that the clone TX-1 from the WHOI culture collection used in the earlier study, although isolated several years previously from a similar outdoor culture at Woods Hole (Goldman and Stanley, 1974), has lost its ability to withstand very low temperatures, whereas the *P. tricornutum* that dominated in the current experiments came directly from the ponds and still was adaptative to low temperatures.

As pointed out by Goldman (in prep.), a common characteristic of phytoplankton that grow at low temperatures ($< 10^{o}C$) is a large increase in the organic content per cell; thus cold adapted cells seem to compensate for lower division rates by increasing their nutrient uptake rates.

It was impossible to precisely determine whether this characteristic was evident in *P. tricornutum* in the current study because *Nitzschia* cells

were present at all temperatures. However, there is some evidence that *Nitzschia closterium* does not display a biomass cell^{-1} change with temperature (Morris and Glover, 1974). If the *Nitzschia* sp. that appeared in this study has a similar temperature response, then a rough estimate of the PN cell^{-1} ratio of *P. tricornutum* can be obtained by subtracting the fraction of PN produced by the *Nitzschia* cells at each temperature, assuming no change in this species' PN cell^{-1} ratio. The PN cell^{-1} ratios of *P. tricornutum* were thus calculated and plotted in Fig. 2. Also included in this plot were similar data for *P. tricornutum* from previous studies (Goldman and Stanley, 1974; Goldman and Ryther, 1976) and unpublished work. The shaded portion in Fig. 2 encompasses all the data, except one point from the current study, and seems to characterize a U-shaped curve of PN cell^{-1} versus temperature.

Although the crudeness of the above presentation is recognized (aside from the above assumptions, data from various experiments that represented different growth conditions were pooled), the trend of increasing cell biomass of *Phaeodactylum* at both high (>20°C) and low (<10°C) temperatures is similar to results with other algal species (Williams, 1971; Eppley, 1972; Goldman and Ryther, 1976). It appears then that a common characteristic of many species is to compensate for reduced cell division at temperature extremes by an increase in cellular nutrient uptake rates. The net result is that the nutrient assimilation rate for the whole cell population appears independent of temperature. For example, in the current study the dilution rate (D), which is virtually equal to the growth rate in continuous culture, was held constant at about 0.33 day^{-1}. Because the total nitrogen uptake rate ρ_N

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Table 1. Nitrogen and phosphorus concentrations in wastewater-seawater mixtures in mg ℓ^{-1} as N or P.

Ratio of wastewater-seawater mixture	ΣN[a]	TDN[b]	Average $NH_4^+/\Sigma N$ Ratio	PO_4^{-3}	N:P (atoms)
1:1	6.0	7.5	0.46	1.7	7.8
1:3	14.0	15.2	0.73	3.1	10.0

[a] $\Sigma N = NH_4^+ + NO_2^- + NO_3^-$.

[b] TDN = total dissolved nitrogen.

Table 2. Fractional distribution of phytoplankton species grown in wastewater-seawater mixtures as a function of temperature.

Temperature °C	*Phaeodactylum tricornutum* 1:3[a]	1:1	*Nitzschia closterium* 1:3	1:1	*Oscillatoria* sp. 1:3	1:1
5.0		0.95		0.05		0
8.5		0.99		0.01		0
10.3	0.38	0.77	0.62	0.23	0	0
15.0	0.44	0.83	0.56	0.17	0	0
19.8	0.70	0.87	0.30	0.13	0	0
27.0	0	0	0.75[b]	0.75	0.25	0.25
30.2		0		0.50		0.50
33.0		0		0.25		0.75

[a]Wastewater-seawater ratio.

[b]Underlined values are gross estimates made from visual observations of organisms in microscopic field.

Figure Legends

Figure 1. Concentrations of particulate nitrogen produced between 5^{o}-33^{o}C for mixed phytoplankton populations in continuous culture. Solid bars - 1:1 wastewater-seawater mixtures; shaded bars - 1:3 mixtures. Dashed lines represent influent ΣN concentrations.

Figure 2. Relationship between PN $cell^{-1}$ ratio and temperature for *Phaeodactylum tricornutum*. Solid symbols are for experiments with current continuous culture apparatus, open symbols for system described in Goldman and Stanley (1974). Solid circles - data from this study; solid squares - average values (with indicated replicates and range bars) from unpublished data; open circles - data from Goldman and Ryther (1976); open triangle - data from Goldman and Stanley (1974).

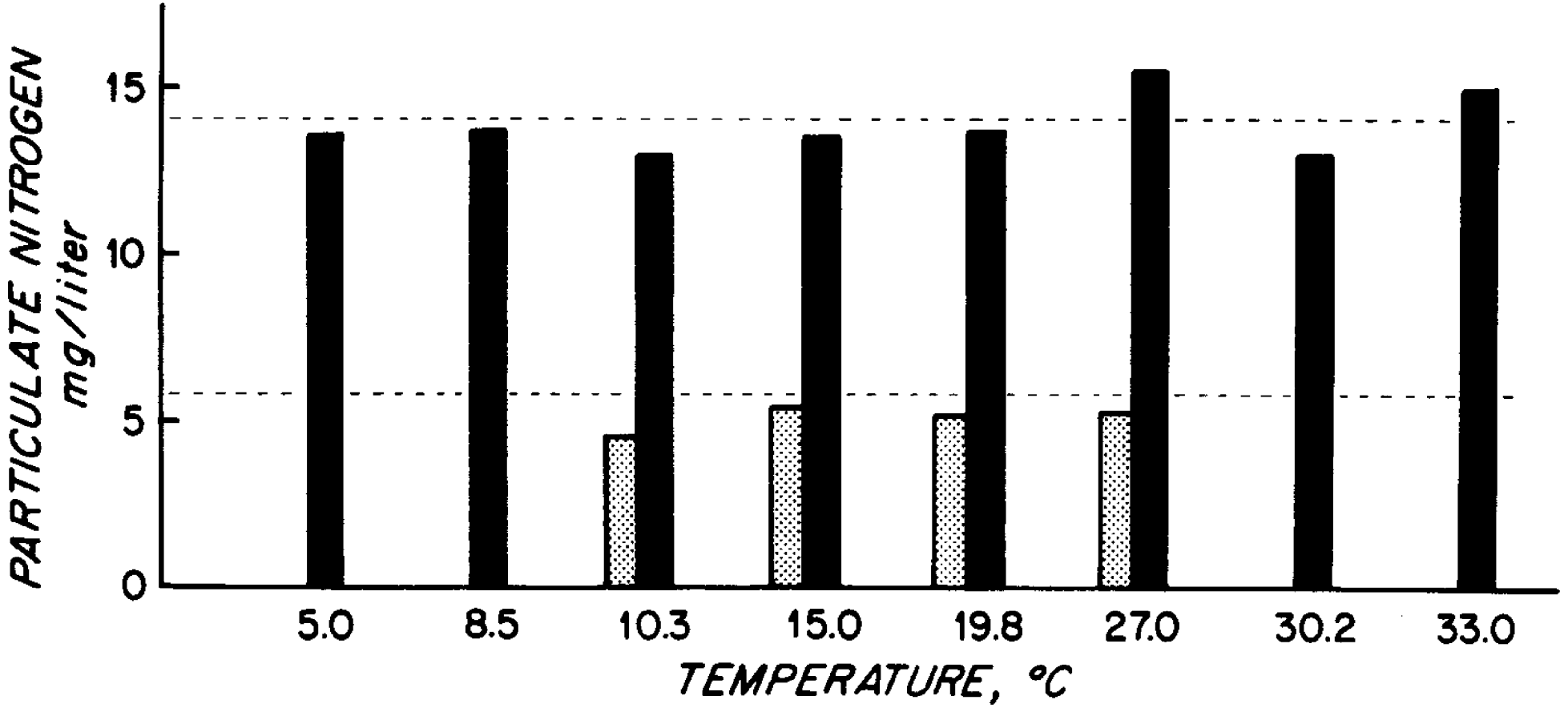
PARTICULATE NITROGEN
mg/liter
15
10
5
0
5.0
8.5
10.3
15.0
19.8
27.0
30.2
33.0
TEMPERATURE, °C

Fig. 1

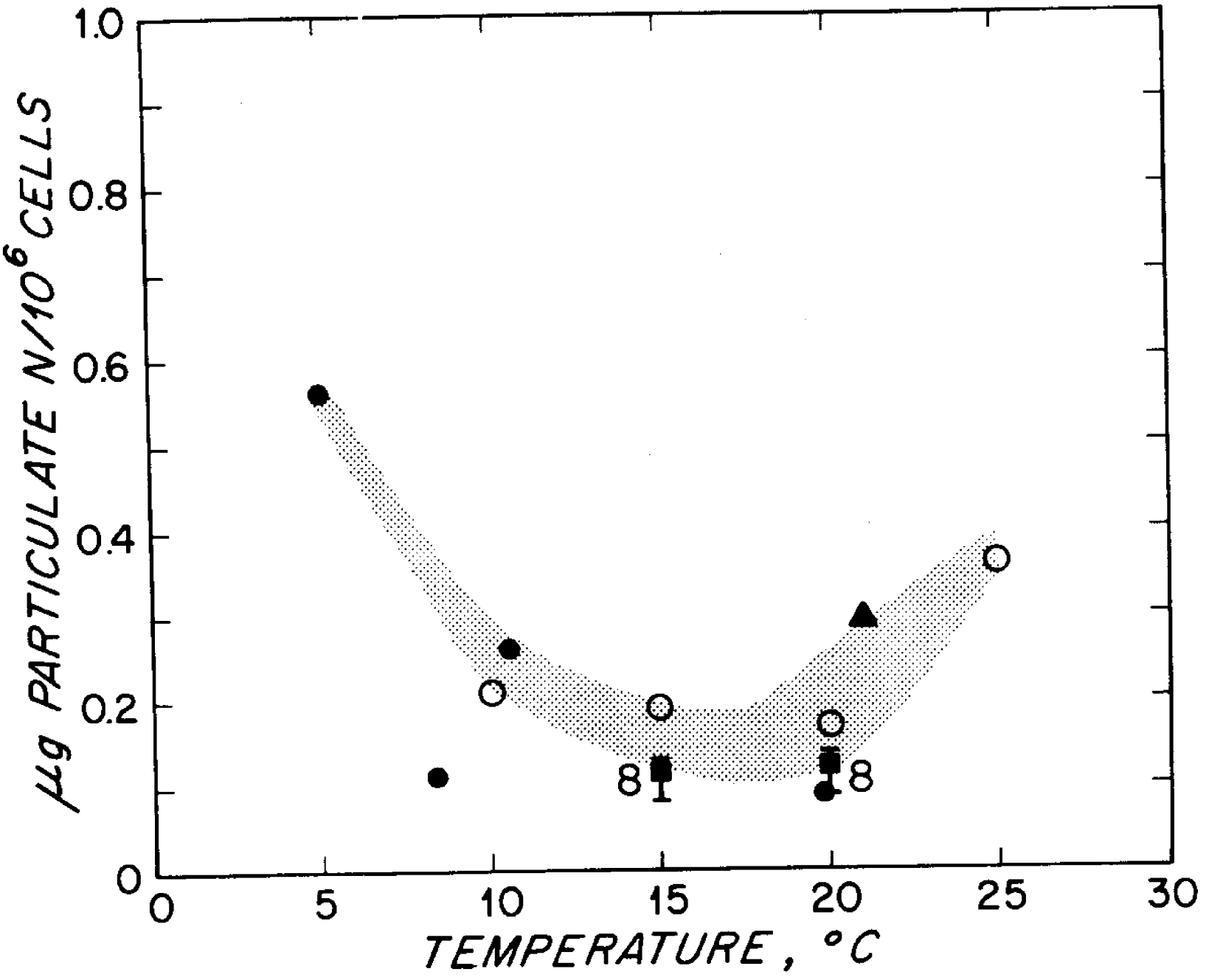
1.0
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
30
μg PARTICULATE N/10^6 CELLS
TEMPERATURE, °C

Fig.

PRODUCTIVITY AND NITROGEN BALANCE IN LARGE SCALE PHYTOPLANKTON CULTURES[1]

by

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INTRODUCTION

Recently, Goldman and Ryther (1975) reported on the nutrient transformations and productivity of 539 gal (2020 liter) marine phytoplankton cultures grown in a secondarily treated sewage effluent-seawater medium. Such cultures comprise the first of a three stage combination tertiary sewage treatment-aquaculture system which is described more fully elsewhere (Ryther, *et al.*, 1975). In this system, nitrogen, typically the growth-limiting nutrient in coastal receiving water (Ryther and Dunstan, 1971), is removed from secondary sewage effluent not only by its incorporation into the phytoplankton, but also by the volatilization of NH_3 dissociated from NH_4^+ at high midday pond pH's. Thus, in addition to an impressive algal yield of up to 6 g of particulate carbon (PC) and 1 g of particulate nitrogen (PN) per square meter per day, substantially more nitrogen can potentially be removed from the wastewater by means of NH_3 evolution.

A scaled up "pilot plant" version of the system was constructed at Woods Hole in 1973 and, beginning in July, 1974, effluent from the nearby Town of Wareham, Mass., mixed with seawater, has been used to grow marine phytoplankton in 35,000 gal (133,000 liter) ponds. These ponds (described below), although operated in a similar, continuous-culture fashion to those described by Goldman and Ryther (1975),are less sophisticated in design. Particularly noteworthy in that respect is that recirculation and agitation in

our large ponds, each with two 1/2 HP pumps, is considerably less intensive on an areal basis than in the Goldman and Ryther ponds, each of which were supplied with a 1/2 HP pump and a motor driven rotating arm. Thus, the present system is significantly less costly to build and energy intensive to operate.

This report discusses the performance of the "pilot plant" phytoplankton culture system much as was done previously for the smaller unit. In addition, we report more fully on the mass balance of nitrogen in the algal cultures.

Pond design and operation

The six ponds approximately 50'x50'x3' (15x15x1 m) are 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 "sacrificial" PVC sheet that may be replaced when and if sun damage occurs. Each culture is kept in gentle circulation with two 1/2 HP (40 gal/min) cast iron pumps on opposite corners of the ponds. These circulate the cultures, 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. Two of the ponds can be heated to about 15°C above ambient by recirculating pond water through large capacity impervious-carbon heat exchanges. Additional mixing, with or without heating, can be provided to the same two ponds by the pumps incorporated in the heating system.

Varying percentages of sand-filtered seawater and secondary effluent or an inorganic nutrient medium can be supplied as desired through separate piping systems, and dilution rates in excess of 2.0 pond volumes per day can, if required, be simultaneously attained in all six ponds.

Normally, ponds are filled with the desired mixture of seawater and sewage effluent (or inorganic nutrients) and circulated until a culture develops from the phytoplankton naturally present in the sand-filtered seawater. Occasionally, the new cultures are inoculated from an existing pond culture or from a smaller culture of some desired species grown for that purpose. Once the standing culture has developed, seawater and nutrients are delivered to the pond on a continuous-flow regime.

Sampling

Samples for chemical nutrient analyses were taken usually at 0830 AM, once a week. Since no distinct diel variation in nutrient assimilation was previously observed (Goldman and Ryther, 1975) the time of sampling was not considered critical and a single daily sample adequate for nitrogen mass balance calculations was taken. Cumulative and differential algal cell counts were taken biweekly, usually on the day of the weekly sampling and 3 days later,using a Spencer Brightline haemocytometer. Maximum and minimum temperature, flow rate, midday pH, and *in vivo* chlorophyll fluorescence were recorded each work-day.

A Turner fluorometer (model 111) with a Corning CS. 5-60 primary and a Corning CS. 2-64 secondary filter was employed to obtain fluorescence readings (Lorenzen, 1966). Continuous monitoring of solar irradiance was provided by an Eppley Model 790 pyranometer.

Chemical analyses

Analysis for PO_4^{3-}, NO_3^-, and NO_2^-, were as given in Strickland and Parsons (1972). NH_4^+ analysis was according to Solórzano (1969). Dissolved inorganic nitrogen (DIN) was calculated as the sum of values obtained for NO_2^-, NO_3^-, and NH_4^+. Dissolved total nitrogen (DTN) was by the method of D'Elia, *et al.* (unpublished ms.) Dissolved organic nitrogen (DON) was determined by difference between DTN and DIN. Particulate nitrogen (PN) and particulate carbon (PC) were determined using a Perkin-Elmer model 240 CHN elemental analyzer. Samples for the latter were filtered onto 0.45 µm glass fiber filters which had been precombusted at 550°C.

Sediment deposition measurements

The sediment trap used was a weighted cylindrical polycarbonate sample bottle cut so that it was twice as tall (11.8 cm) as it was wide (5.9 cm); such a trap design is 100% efficient in accumulating sedimenting material at current speeds of less than 10 cm x s^{-1} (W. Gardner, personal communication). Upon recovery of the trap, pond water above the accumulated sediment was aspirated off and the

sediment suspended in 0.45 μm pore size filtered seawater for C and N analysis as above.

RESULTS AND DISCUSSION

Wastewater, inorganic nutrient medium, and seawater

During the current study, 4,000 gal (15,200 liters) per day of secondary, unchlorinated effluent from the Town of Wareham activated-sludge waste treatment plant was trucked to the laboratory and discharged into fiberglass storage tanks. From there the effluent was pumped to a headbox for gravity distribution to the ponds. Fig. 1 shows the N and P characteristics of this effluent. DIN:PO_4^{3-} ratios were typically less than 8:1, indicating an excess of phosphorus to nitrogen relative to the proportion of the two elements required by phytoplankton (Ryther and Dunstan, 1971). We had no control over the characteristics of the dissolved nitrogen in the effluent, which was normally highly oxidized, typically containing about 75% NO_3^--N, with the remainder being DON and NH_4^+-N in approximately equal amounts.

Some pond cultures were maintained on an inorganic chemical nutrient medium containing N as NH_4Cl and P as $NaH_2PO_4 \cdot H_2O$ in a 10:1 molar ratio at nitrogen levels designed to simulate those achieved by sewage addition. Generally speaking, the performance of the cultures with respect to algal growth was the same whether sewage effluent or chemical nutrients were added.

Nitrogen and phosphorus contributions by the seawater were always neglible, however it did make available a substantial amount of dissolved inorganic carbon (as bicarbonate). As at most we diluted the seawater to 80% of full strength, adequate dissolved inorganic carbon could be provided by the seawater alone to support a biomass level of at least 18 mg C . l^{-1}. Additional carbon (as CO_2) was of course available via surface exchange with the atmosphere and aeration.

Algal species control

Despite considerable effort and experimentation, including filling the algae ponds with 1 µm filtered seawater and inoculation with large (several hundred liter) cultures of *Monochrysis*, *Isochrysis*, *Skeletonema*, or *Thalassiosira*, no success has yet been obtained in controlling the species of algae that have developed and persisted in the ponds. Cultures were always virtually unispecific, the species varying with the season. It has been suggested that temperature plays a major role in determining which species predominates (Goldman and Ryther, 1976). In general, the diatom, *Phaeodactylum tricornutum* persisted during the current study. In past years this was true with the exception of winter temperatures 10°C, when *Skeletonema costatum* became dominant, and summer temperatures when unidentified green flagellates replaced *Phaeodactylum*. As it is unlikely that the species of alga present greatly affects the rate of algal production or nutrient utilization, species composition is not believed to be a particularly important factor with respect to the tertiary treatment role of this part of the system.

Pond stability

Fig. 2 shows *in vivo* fluorescence and cell counts in a typical pond during the spring of 1976. *Phaeodactylum tricornutum* predominated, normally comprising more than 90% of the cell count. Next most common, in terms of numbers of cells, was *Nannochloris atomis*, a small green coccoid algae, but the latter did not appear to contribute much biomass, as relative fluorescence was negligible in mid-March when *Nannochloris* counts were highest. The culture, like others (not shown), did display considerable variation in both cell numbers and relative fluorescence, being affected considerably by weather-related changes in insolation and altered experimental conditions; other as yet unresolved factors undoubtedly account for much of this variability. The culture persisted from its inception in late March for nearly three months, until when in early June a colorless, flagellated protozoan, tentatively identified as a monad, appeared in the cultures and eliminated the algae within a few days' time. Other cultures were similarly destroyed by this flagellate. Such cultures may be discarded and restarted, but , if left alone, the flagellate population quickly subsides, presumably through lack of food, and the *Phaeodactylum* population usually reestablishes itself within several days. Analogous observations were reported for mass cultures of *Phaeodactylum* (Raymont and Adams, 1958; Ansell *et al.*, 1963). Others have also reported rapid consumption of mass algal cultures by protozoans (*see* Tamiya, 1957).

N/C ratio vs residual DIN concentration

Fig. 3 shows the N/C ratios in the particulate material vs residual (i.e. effluent) DIN concentrations in the ponds over the study period. At residual DIN concentrations over approximately 5 µM, the N/C ratio averaged approximately 0.17. Caperon and Meyer (1972) suggest: "In rare instances where the particulate organic material is heavily dominated by phytoplankton [such as in the present case], the N/C ratio could be a meaningful indicator of steady-state growth rate." From their data obtained for several algal species, and N/C ratio of 0.17 corresponded with growth rates greater than 1.0 d^{-1}. It would seem reasonable to assume that a similar correlation exists in our phytoplankton ponds. Thus by keeping residual DIN concentrations above approximately 5 µM, growth rates of at least 1.0 d^{-1} can be sustained along with maximal incorporation into phytoplankton biomass.

In vivo fluorescence vs biomass

In vivo fluorescence recorded on the day that particulate carbon (PC) and particulate nitrogen (PN) determinations were made was graphed against PC and PN to determine whether there is a relationship between PC and PN biomass concentrations and relative fluorescence (Fig. 4). It is evident that in both cases, a hyperbolic relationship exists, with the ratios of PC or PN to relative

fluorescence declining at higher values. This perhaps reflects "shade-adaptation" as the chlorophyll content (and hence *in vivo* fluorescence) of many algae has been shown to be inversely proportional to light intensity during growth (e.g. Brown and Richardson, 1968). As the highest ratios of fluorescences to PC and PN were obtained in cultures with the lowest dilution rates (and thus, growth rates - see below), such cultures may be more strongly light-limited than are cultures at higher dilution rates. According to Meeks (1974) "when light is limiting for growth the chlorophyll content increases and it decreases when light is not limiting."

It is important to note here that in the vast majority of samples represented in Fig. 4, the N/C ratio was greater than 0.15, and thus growth could be considered not to be nitrogen-limited (see above). One would expect poorer correlation between relative fluorescence and biomass levels if nitrogen limitation existed, as chlorophyll synthesis and content of algae are markedly reduced under such conditions (cf. Meeks, 1974; Caperon and Meyer, 1972).

Since the ordinal intercepts in Fig. 4A and B are at positive PC and PN values, it is apparent that a certain portion of the particulate material is not algae, but rather detrital or non-algal living material. Similarly, Menzel and Ryther (1964) found nitrogen and carbon in the absence of chlorophyll when they regressed

nitrogen and carbon against chlorophyll in oceanic samples; they interpreted this as "residual detritus." While in our ponds a certain amount of this residual PC and PN was certainly allochthonous (coming in with sewage and seawater), analysis of seawater and sewage typically did not fully account for this much residual material. Consequently airborne inputs (leaves, pollen, etc.) or autochthonous origins of some of this material are likely.

Standing biomass vs time of year

Fig. 5 shows standing biomass levels expressed as particulate organic carbon in ponds at a 0.25 d^{-1} dilution rate from mid-November, 1975 through the end of June, 1976. All points shown here represent cultures in which the N:C ratio was 0.14 or more, adequate to maintain a growth rate much greater than 0.25 d^{-1} (see above) and the cultures are therefore assumed not to be nitrogen-limited. The two ponds which could be heated were at times during January and February operated 5° to 10°C above ambient temperatures of the other ponds (0°-5°C). Except for these times when the unheated ponds were frozen over (on several occasions in January and Feburary), and therefore out of production, surprisingly there was no difference noted in biomass sustained in heated vs unheated ponds: consequently no distinction has been made for data taken from heated and unheated ponds. Similarly, this was observed the previous winter, when the heated ponds were held at approximately 15°C above ambient (Ryther, 1975).

Superimposed on carbon biomass levels in Fig. 5 is a curve showing mean daily solar irradiance accumulated and averaged on a monthly basis. While this curve is supplied for comparison and not to indicate a strict mathematical relationship, a close correlation between carbon concentrations and irradiance is evident. This indicates that biomass levels are controlled by available light, and that production is therefore, light-limited. As nutrients were all supplied in excess, it is suggested that light behaves much the same as a limiting nutrient in a chemostat (*see* Herbert, *et al.* 1956 for details about continuous culture theory). In the latter case, the biomass of the organisms in culture increases until the residual concentration of the limiting substrate in the reactor declines and reduces growth to the point that it becomes equal to the dilution rate. Thereafter a steady-state biomass level is maintained in the reactor. Similarly with light in the present case, at low biomass concentrations, enough light penetrates the culture that growth exceeds dilution and the level of biomass increases faster than it is flushed out of the ponds. Once biomass levels are high enough that light becomes limiting through self-shading, growth equals dilution and a steady-state biomass level is maintained for the given level of solar radiation. In practice, of course, true steady-state conditions are not possible in cultures grown out-of-doors in the variable conditions of natural incident radiation (which may differ from day to day by an order

of magnitude), but the long-term trend is nevertheless obvious in the data shown in Fig. 5.

Effect of dilution rate on biomass levels

Between May and July, 1976 (the period of optimal irradiance), five dilution rates, i.e. 0.25, 0.35, 0.45, 0.50, and 0.75 d^{-1} were tested in light-limited ponds. Under reasonably steady-state conditions [i.e. when less than a ±15% day to day change in relative fluorescence was observed], there was an inverse relationship between algal biomass concentration and dilution rate (Fig. 6A). A similar observation was made by Shelef, *et al.* (1973) and Goldman and Ryther (1975). We suggest, as did Shelef *et al.* (1973), that biomass is established at levels at which self-shading produces a specific growth rate equal to the dilution rate.

The linear regression of the data in Fig. 6 was calculated for empirical use in estimation of pond output, O, on an areal basis by the formula

$$O = \frac{VD\tilde{x}}{A} \qquad (1)$$

where V = pond volume, D = dilution rate, and $\tilde{x}$ = steady-state biomass concentration, and A = pond area. Fig. 6B shows a curve relating O to D; note that the highest outputs were found at dilution rates between 0.55 and 0.65 d^{-1}. Similarly, Shelef *et al.* (1973) and Goldman and Ryther (1975), respectively, found maximal summer yields to occur at dilution rates of 0.5 and 0.75 d^{-1}. At a

dilution rate of 0.6 d^{-1} one can expect a summertime net productivity in our ponds to average almost 5 g of C· m^{-2} · d^{-1} (and assuming an N/C molar ratio of 0.17, 1.0 g of N . m^{-2} . d^{-1}). This yield is slightly less (~15%) than has been reported before at this location and less than 50% of that found at a more favorable geographic location, i.e. Fort Pierce, Florida (Goldman, *et al.*, 1975).

It is interesting to note that higher biomass concentrations were achieved in the Goldman and Ryther (1975) study. For instance, at a dilution rate of 0.25 d^{-1}, biomass was approximately 28 mg C · $liter^{-1}$ vs 16 mg C · $liter^{-1}$ in the present study. Considering that the pond depth in the present study was slightly less than twice that of the previous study, on an areal basis approximately the same biomass levels were obtained in the two studies. Thus, it would appear that pond depth could have been reduced in the present study without affecting yields. The advantage to reducing depth would be that, for the same algal yield, only half as much seawater pumping would be required though the same amount of nutrient (effluent) would be utilized. In addition, a smaller volume of water would need to be heated, filtered, or otherwise conditioned, whatever the requirements might be.

Sedimentation

As the ponds were not provided with mechanical scraping arms to prevent sedimentary buildup and the establishment of benthic diatom populations, there was some accumulation of material on the bottoms even in the healthiest cultures. To obtain a rough estimate of the amount of this accumulation, a sediment trap was placed at the bottom of one pond in mid-April and recovered two weeks later. The

accumulated "sludge" was then analyzed. 30.1 mg N and 208 mg C $\cdot$ m^{-2} $\cdot$ d^{-1}, respectively, were deposited. The productivity of the pond during that time averaged 784 mg N and 4100 mg C $\cdot$ m^{-2} $\cdot$ d^{-1}. Thus, sedimentation accounted for roughly 4% of the nitrogen and 5% of the carbon fixed in the photosynthetic production (output) of that pond. While the percentage of the productivity lost to the bottom does not appear to be large, nonetheless, after several months, a considerable quantity of accumulated material will, of course, build up. This material could conceivably be mechanically removed from time to time and combined with sludges obtained in primary and secondary treatments.

At times, for unknown reasons, within hours an entire culture would appear to flocculate and sediment out of suspension. As that did not happen often, and since cultures could be successfully restarted quickly thereafter, it was not considered to be a critical problem in practice.

Dissolved organic nitrogen flux

Fig. 7 shows daily fluxes of dissolved organic nitrogen (DON) as a function of particulate nitrogen (PN) production. The DON fluxes were calculated as the difference between incoming levels in the sewage effluent and seawater and those in the pond effluent. Note that the data points cluster closely around the abscissa regardless of the PN production. A frequency histogram of DON values alone (Fig. 7) shows a normal Gaussian distribution with a

mean of 0.0822 g atoms · $pond^{-1}$ · day^{-1} which when tested at the 5% level was not significantly different from zero; this indicates that on the average there is little or no net DON production in the ponds. Conceivably, the spread in the data is the result of analytical error in measuring DON fluxes, rather than actual DON variability.

While this study shows that net production of DON in the ponds is zero or close to zero, it does not indicate that the phytoplankton are not excreting DON. The observed negligible net production may be the result of heterotrophic uptake into PN at a rate equal to that of DON excretion by the phytoplankton.

DTN removal vs PN output

To assess the mass balance for N in the ponds, daily output of particulate nitrogen (calculated using equation 1), and corresponding dissolved total nitrogen (DTN) removals (calculated analogously) were plotted (Fig. 8). A 1:1 relationship between them would indicate thatall DTN removed could be accounted for in the suspended PN output of the ponds. A line indicating this relationship has been drawn in Fig. 8. It is evident that on the average, PN output was less than DTN removal, as all but five points

fall below the line. Three explanations for this seem reasonable: 1) that NH_4^+ was evolved as NH_3 to the atmosphere at high pH's and did not therefore enter the PN pool, 2) that PN sedimentation had occurred, indicating that PN *production* was greater than PN *output* and 3) that denitrification occurred.

We were unable to establish how much of the difference between DTN removal and PN output was accounted for by NH_3 evolution as no clear pattern existed between NH_4^+:DIN input ratios and the PN output: DTN removal ratios. Undoubtedly, NH_3 evolution occurs to a considerable extent, for pH values of greater than 10 were commonly observed when the ponds were warmest (Fig. 9). At these pH's and temperatures, the equilibrium between NH_4^+ and NH_3 is strongly in favor of the latter (Cohen, 1972). As Goldman and Ryther (1975) found strong evidence for NH_3 evolution in the ponds, we suspect that this too is an important factor here as well. Thus, at high pH and temperature, increased nitrogen stripping can be provided by increasing influent NH_4^+ loading and increasing aeration to take advantage of NH_3 evolution.

Some sedimentation of PN produced does of course occur (see above), but from our estimates of the percentage of PN output that this represents, it probably only accounts for a small fraction of the difference between PN output and DTN removal.

Denitrification rates have not been assessed in the ponds, but probably should be. We would not expect this to occur in the water, as the process is an anaerobic one, though some denitrification may

occur in the presence of detrital particles due to the formation of anaerobic microzones of bacterial activity within the particles (Jannasch, 1960). However, the pond sediments, which when disturbed often smell strongly of H_2S, are assumed to be anaerobic and could thus support a population of denitrifying bacteria.

While we suspect that one or more of the above reasons accounts for the difference between PN output and DTN removal, we are aware of the possibility that no difference may in fact exist and that experimental artifacts are responsible for the apparent difference. For instance, as we always sampled at the same time of day, we may have obtained an erroneous picture of the actual 24-hour balance in that PN output was underestimated, DTN removal overestimated, or both. However, we are inclined to discount those possibilities as both PN standing stock and residual DIN concentrations did not show significant diel variation in a previous study (Goldman and Ryther, 1975).

Pond productivity, Nov. 1975 - June, 1976

As we designed our experiments to evaluate pond nutrient removal and production under varying dilution rates and nutrient loadings, a summary of "optimal" pond productivity over the study period would not be provided by merely averaging weekly data we collected. Consequently, we tabulated only the data from the ponds with the greatest algal yields

at weekly sampling time (Table 1) to provide an idea of productivities one might expect on a yearly basis.

Yields ranged from 0.99 gC . m^{-2} . d^{-1} to 5.04 gC . m^{-2} . d^{-1} with a mean of 3.34 gC . m^{-2} . d^{-1}. As our sampling times are spread out fairly well over the period from maximal to minimal irradiance, the mean is probably representative of daily net production averaged on a yearly basis. PN yield ranged from a low of 0.185 g · m^{-2} d^{-1} to a high of 1.04 g · m^{-2} ·d^{-1} with a mean of about 0.66 g · $m^{-1}d^{-1}$. Thus, the average N/C ratio in the harvested algae as might have been expected from previous discussion, was about 0.17, equivalent to a nitrogen content of about 8% of ash-free dry weight, algae that are roughly 50% protein.

As DTN removal can be expected to depend on N loading, NH_4^+/DIN ratios, pH, temperature, and amount of aeration (to purge NH_3 when present) it makes little sense to generalize about anticipated average daily DTN removal except to say that it should be greater than PN production by the phytoplankton. The strategy for DTN removal will depend on the goals desired. As maximal particulate yields can be obtained with residual DIN concentrations held at least as low as 5 μM, high yield and better than 90% N stripping can be achieved simultaneously. Conversely, by increasing DIN loading (as NH_4^+) in the pond influent and hence residual DIN concentration, similar algal yields but higher N removals can be obtained, though at the cost of a lower percentage of removal of the available DIN.

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Table 1. Productivity and N removal in algal ponds.

Date	Dilution rate (d^{-1})	Dissolved total nitrogen removed*	Particulate nitrogen produced*	Particulate carbon produced*
11/18/75	0.25	188	217	1104
11/25/75	0.25	239	456	2244
12/2/75	0.25	312	237	1296
12/9/75	0.25	295	185	990
3/9/76	0.25	504	391	2136
3/23/76	0.25	526	400	2400
3/30/76	0.35	722	668	3516
4/6/76	0.35	712	636	3408
4/13/76	0.35	951	644	3276
4/21/76	0.35	1218	917	4932
4/27/76	0.45	1428	1039	4020
5/4/76	0.35	1123	734	3996
5/11/76	0.35	1228	965	4512
5/18/76	0.50	1392	745	3804
5/25/76	0.50	1616	1035	5040
6/1/76	0.75	2324	970	4644
6/8/76	0.75	1610	707	3528
6/15/76	0.50	1018	693	4536
6/22/76	0.50	1056	755	3876
6/29/76	0.50	1331	750	3648

*mg m^{-2} d^{-1}.

FIGURE LEGENDS

Fig. 1. Nutrient characteristics of Wareham secondary effluent, March-July, 1976.

Fig. 2. Relative fluorescence and algal cell counts in a typical pond, March-June, 1976.

Fig. 3. N:C ratios (molar) in particulate samples vs residual dissolved inorganic nitrogen (DIN) concentrations in ponds.

Fig. 4. A. Particulate carbon (PC) concentration and B. Particulate nitrogen (PN) concentration (PN) vs relative fluorescence (RFL). Fitted curves are empirical relationships for predictive purposes only. They are least squares fits of second-order polynomial equations.

Fig. 5. Mean daily irradiance (based on monthly averages) and standing stock biomass in poinds at a dilution rate of 0.25 d^{-1}, Nov. 1975-July, 1976.

Fig. 6. Particulate carbon (PC) levels during May-June, 1976, vs dilution rate (DR). PC levels included are only from ponds which maintained less than ±10% variation over a three day period including the sampling date. B. Pond output, per square meter, calculated using the regression obtained in A, vs dilution rate.

Fig. 7. Dissolved inorganic nitrogen (DON) flux vs particulate nitrogen (PN) production in ponds. The frequency histogram shows the distribution of the ordinal values.

Fig. 8. Particulate nitrogen (PN) output vs dissolved total nitrogen (DTN) removal in the ponds.

Fig. 9. Pond pH (A) and temperature (B) in 0.25 d^{-1} dilution rate ponds, January-July, 1976.

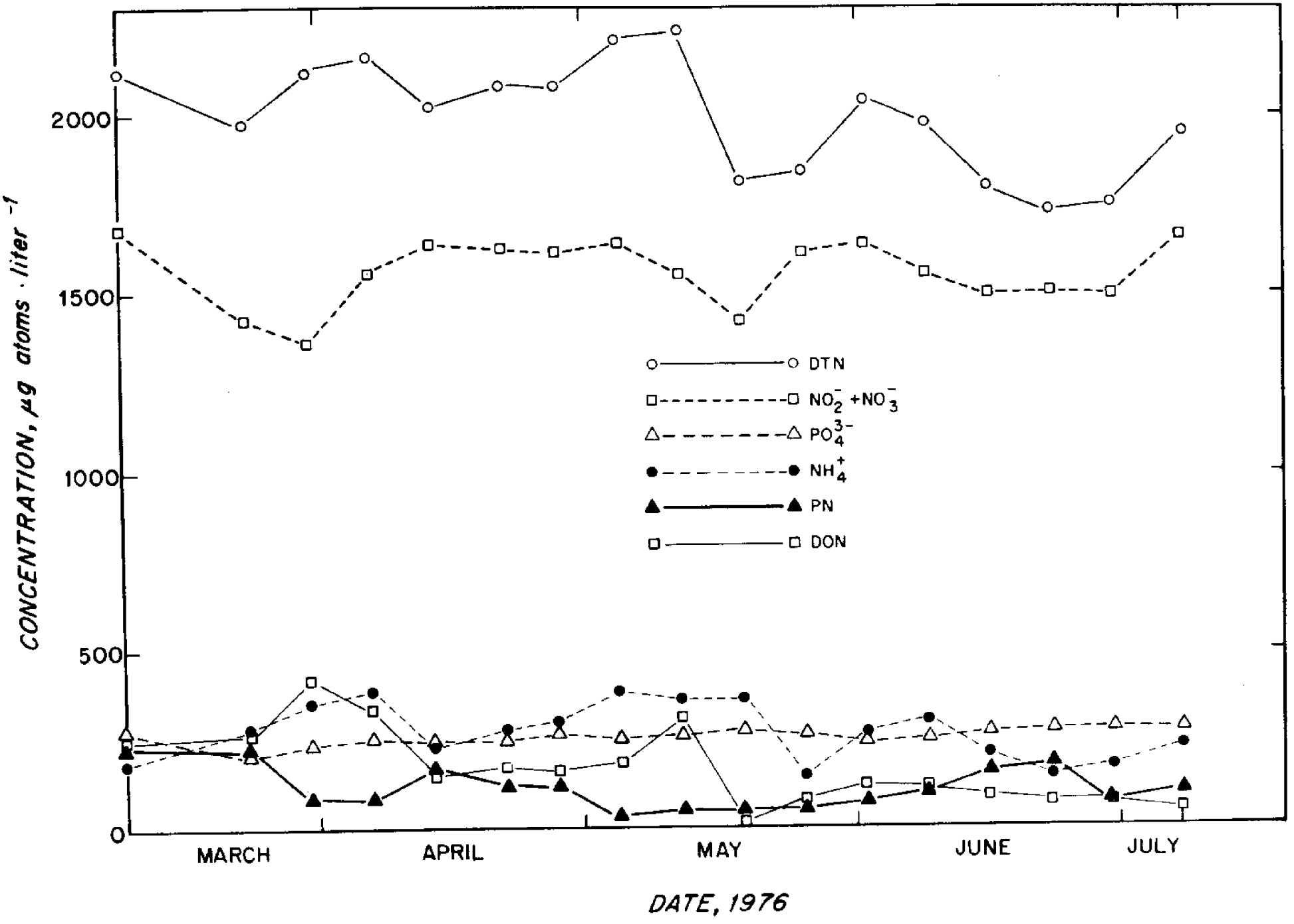
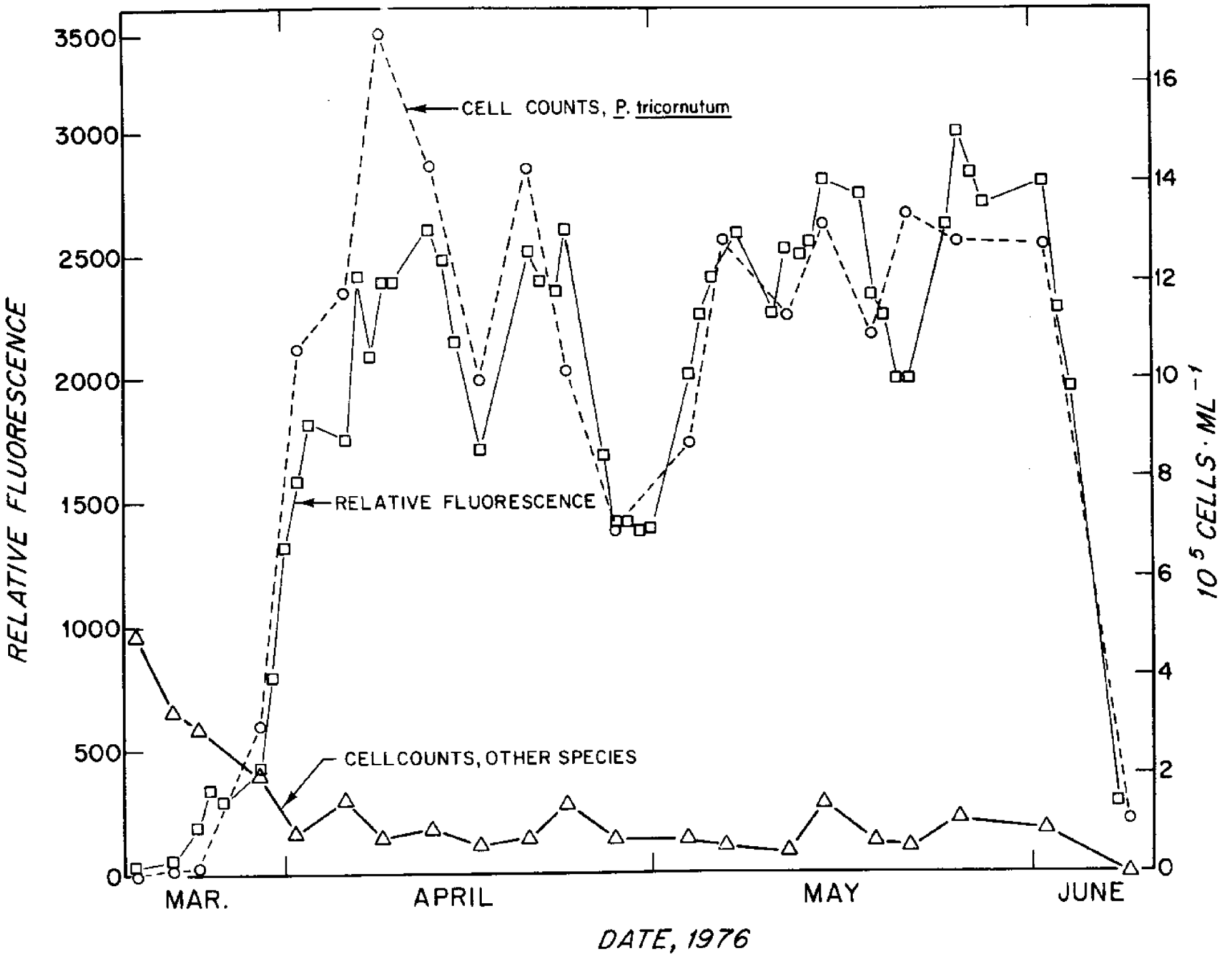

2000
1500
1000
500
0
CONCENTRATION, μg atoms · liter -1
DTN
NO2- +NO3-
PO43-
NH4+
PN
DON
MARCH
APRIL
MAY
JUNE
JULY
DATE, 1976

Fig. 1


CELL COUNTS, P. tricornutum
RELATIVE FLUORESCENCE
CELLCOUNTS, OTHER SPECIES
RELATIVE FLUORESCENCE
3500
3000
2500
2000
1500
1000
500
0
16
14
12
10
8
6
4
2
0
MAR.
APRIL
MAY
JUNE
DATE, 1976
10^5 CELLS · ML^{-1}

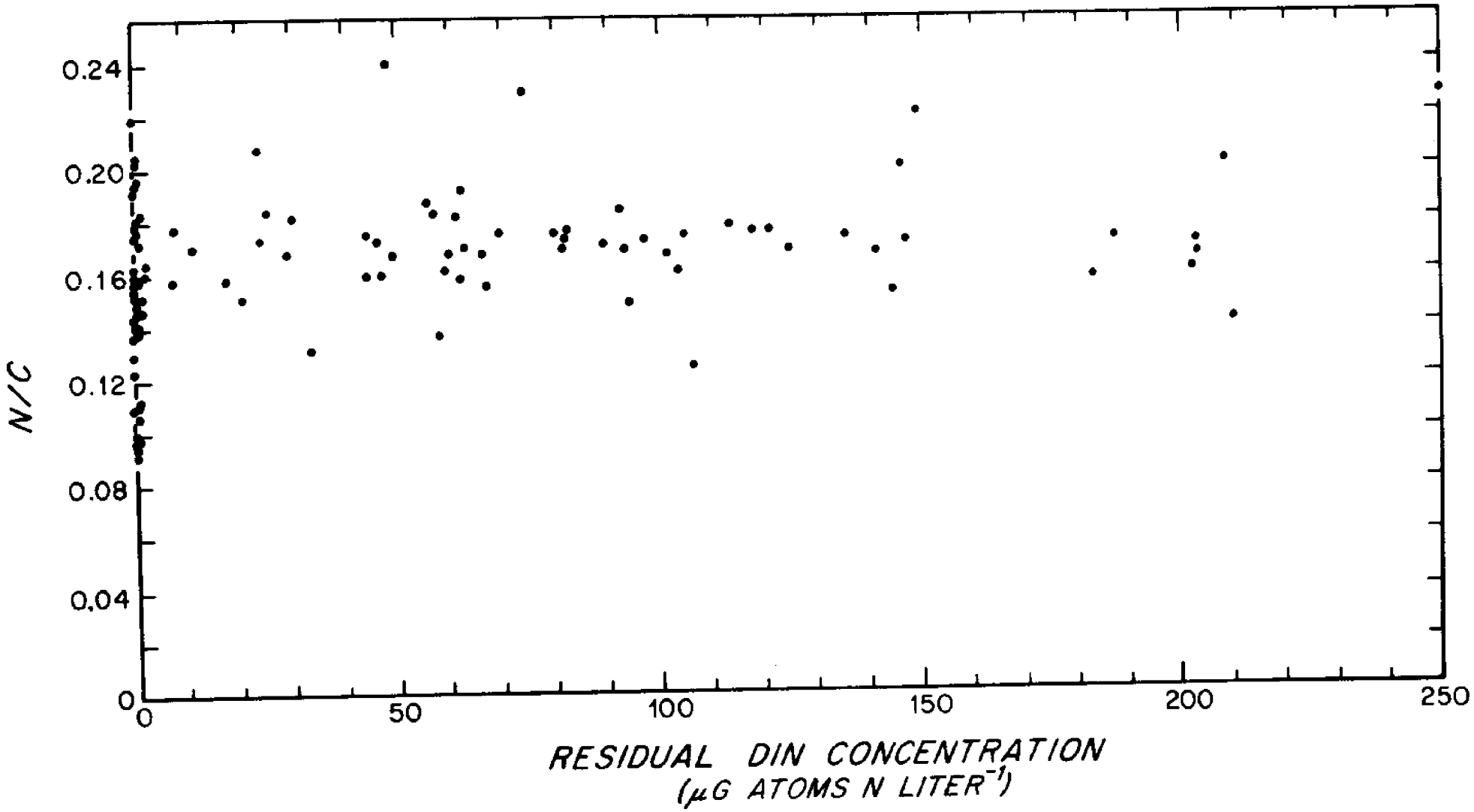
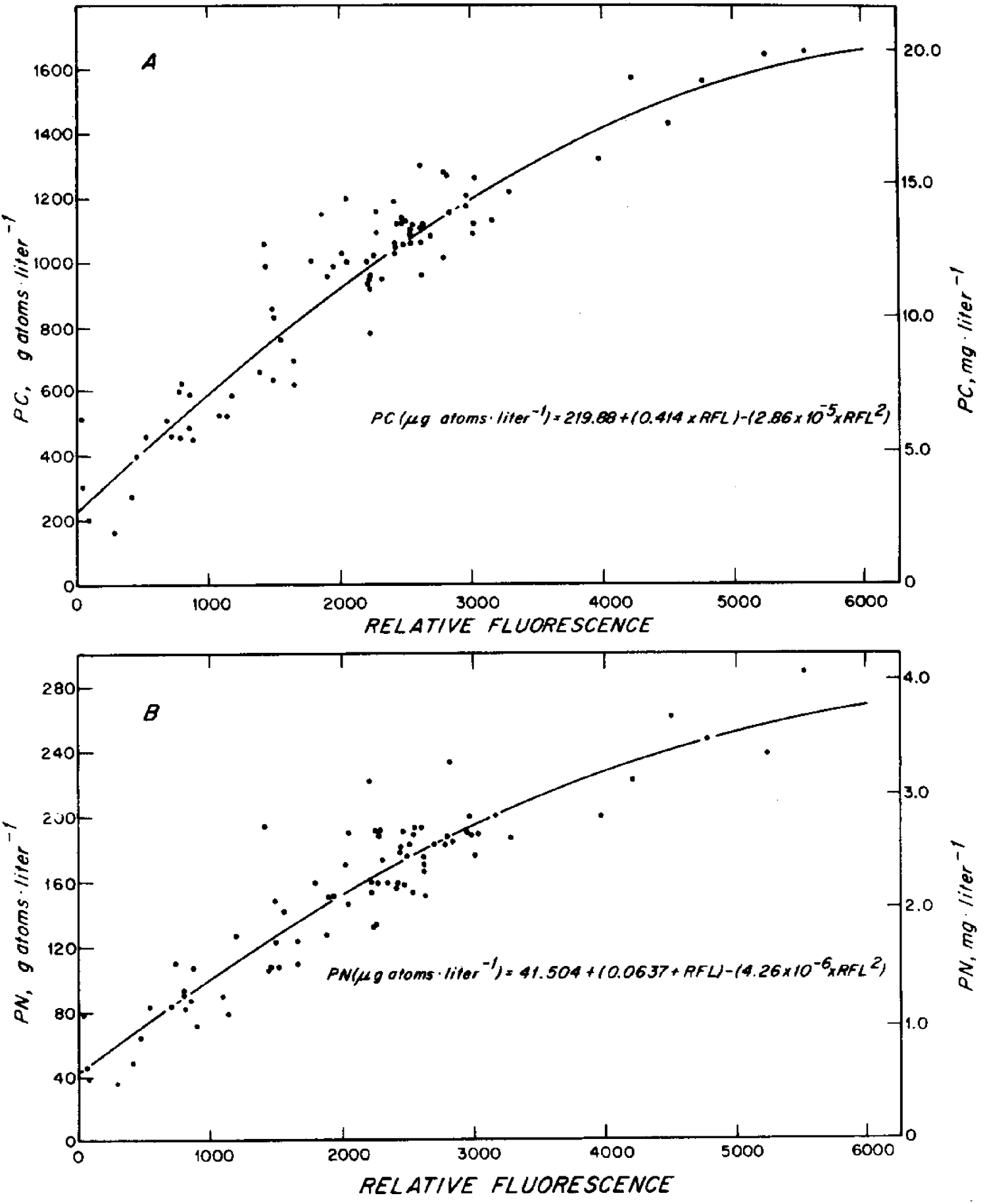

0.24
0.20
0.16
0.12
0.08
0.04
0
N/C
0
50
100
150
200
250
RESIDUAL DIN CONCENTRATION
(μG ATOMS N LITER⁻¹)

Fig. 3


A
PC, g atoms · liter⁻¹
PC, mg · liter⁻¹
PC (μg atoms · liter⁻¹) = 219.88 + (0.414 x RFL) - (2.86 x 10⁻⁵ x RFL²)
RELATIVE FLUORESCENCE
B
PN, g atoms · liter⁻¹
PN, mg · liter⁻¹
PN(μg atoms · liter⁻¹) = 41.504 + (0.0637 + RFL) - (4.26 x 10⁻⁶ x RFL²)
RELATIVE FLUORESCENCE

Fig

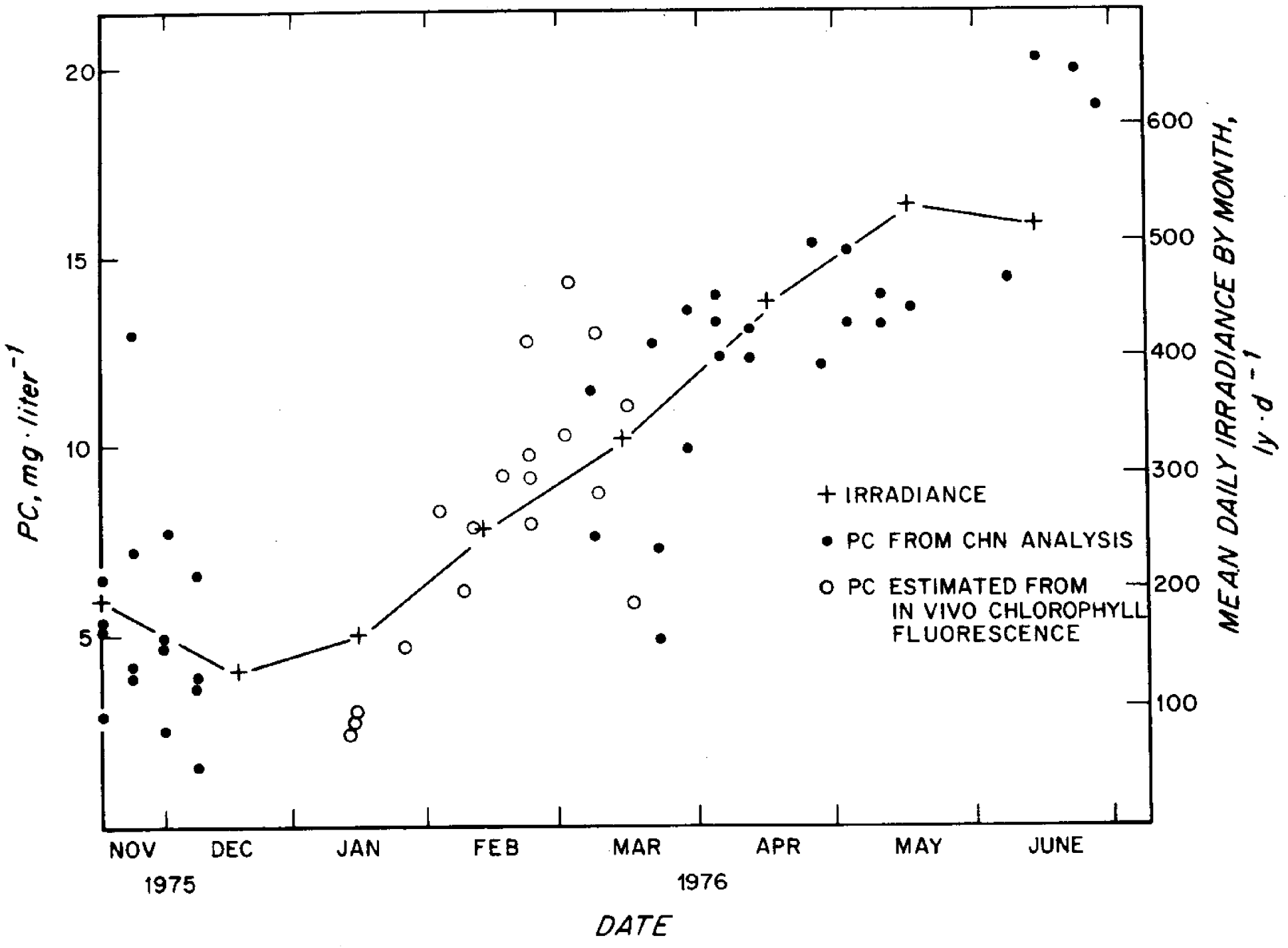

PC, mg · liter⁻¹
MEAN DAILY IRRADIANCE BY MONTH, ly · d⁻¹
20
15
10
5
600
500
400
300
200
100
+ IRRADIANCE
• PC FROM CHN ANALYSIS
o PC ESTIMATED FROM IN VIVO CHLOROPHYLL FLUORESCENCE
NOV
DEC
JAN
FEB
MAR
APR
MAY
JUNE
1975
1976
DATE

Fig. 5

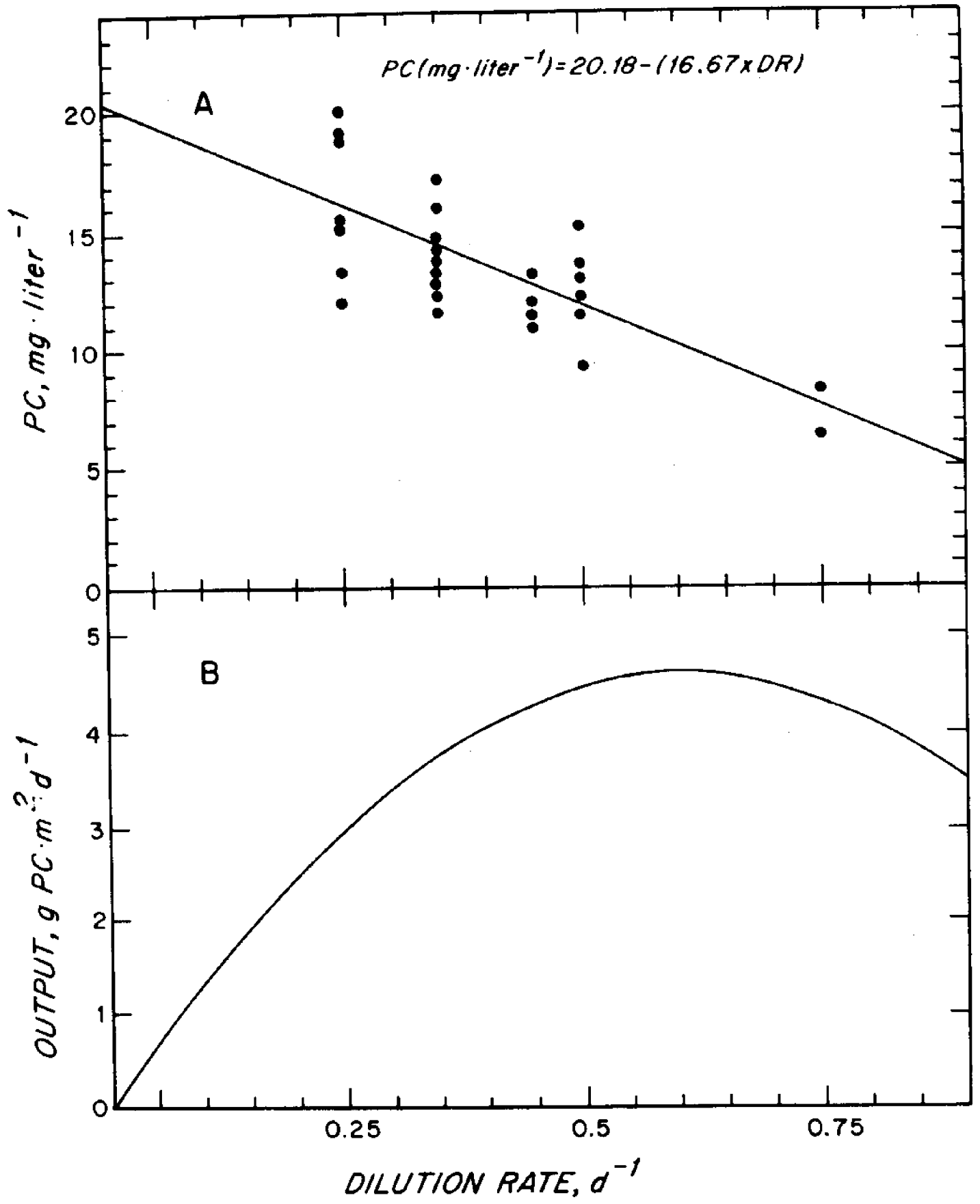

PC(mg·liter⁻¹)=20.18-(16.67xDR)
A
B
PC, mg·liter⁻¹
OUTPUT, g PC·m⁻²·d⁻¹
DILUTION RATE, d⁻¹
0
5
10
15
20
1
2
3
4
0.25
0.5
0.75

Fig.

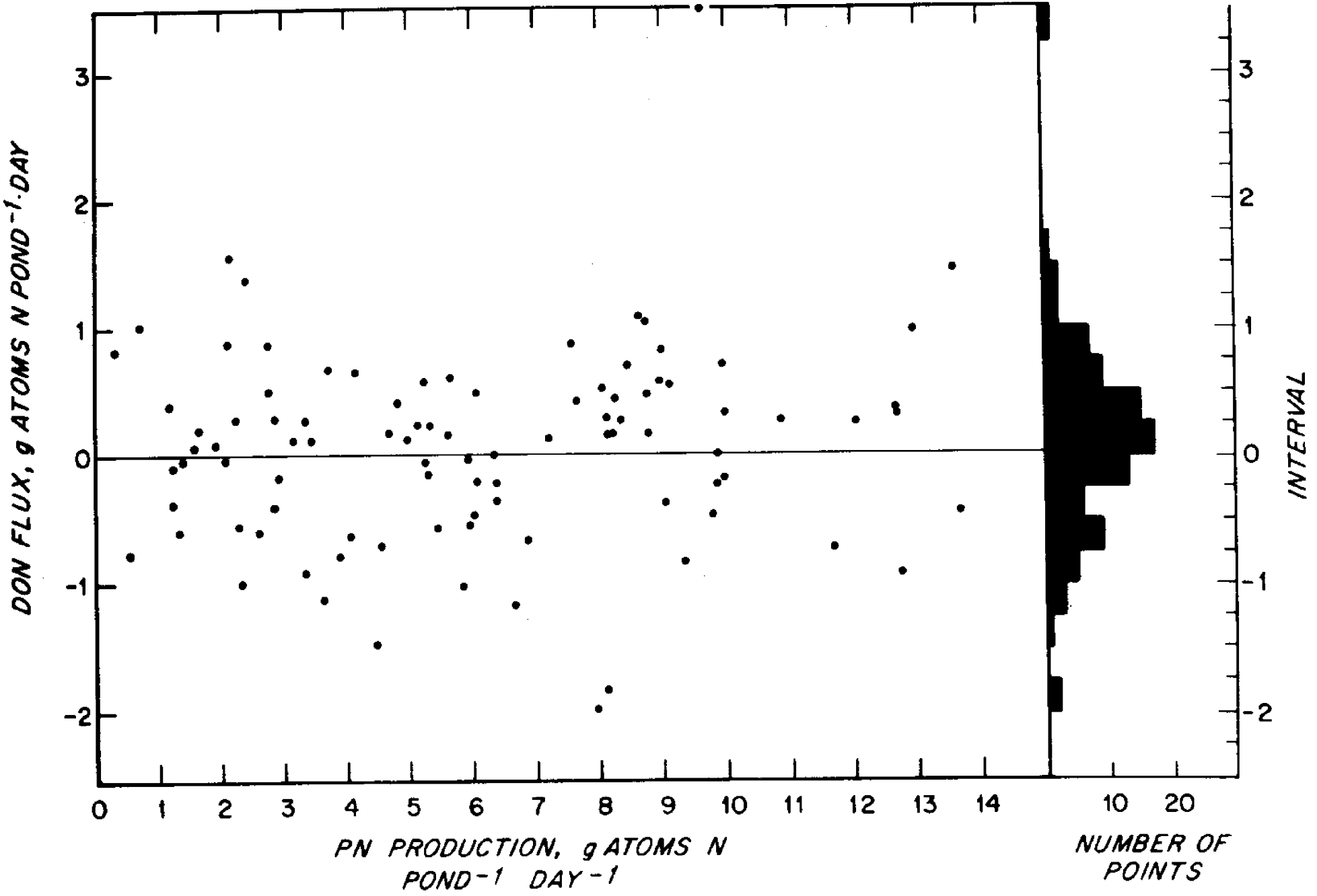

DON FLUX, g ATOMS N POND⁻¹·DAY
3
2
1
0
-1
-2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14
PN PRODUCTION, g ATOMS N POND⁻¹ DAY⁻¹
10 20
NUMBER OF POINTS
INTERVAL

Fig. 7

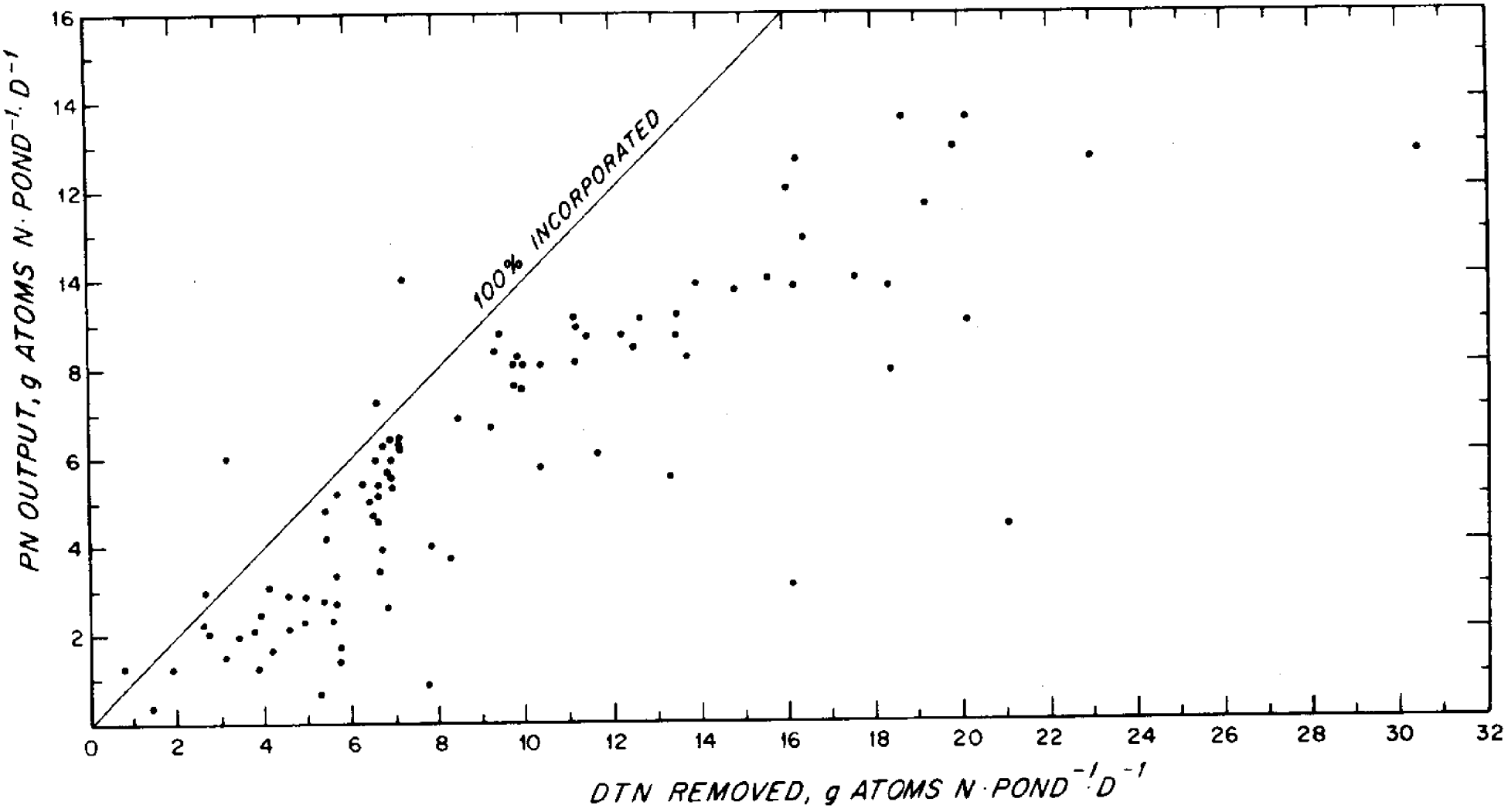
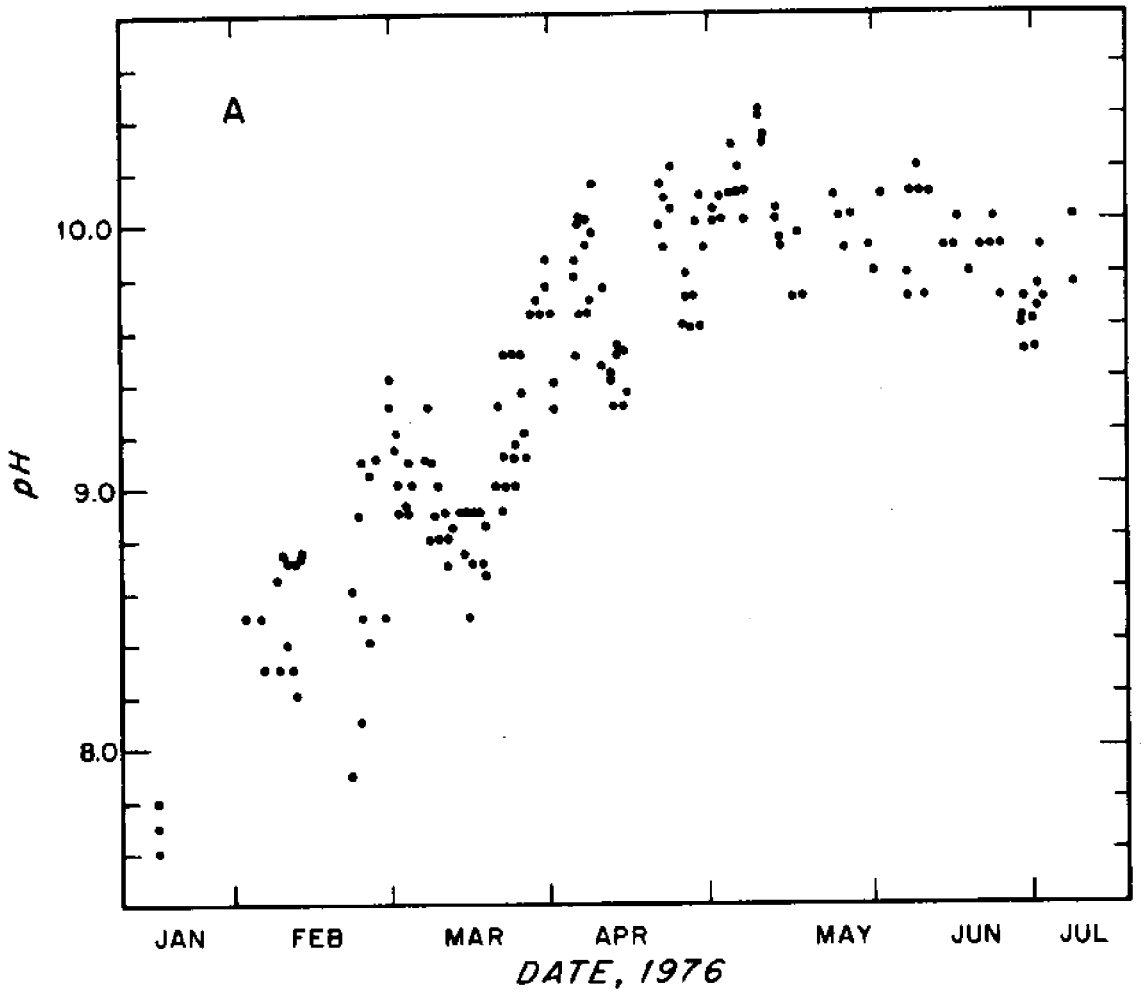

PN OUTPUT, g ATOMS N·POND⁻¹·D⁻¹
DTN REMOVED, g ATOMS N·POND⁻¹D⁻¹
100% INCORPORATED
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32

Fig. 8


A
10.0
9.0
8.0
pH
JAN
FEB
MAR
APR
MAY
JUN
JUL
DATE, 1976

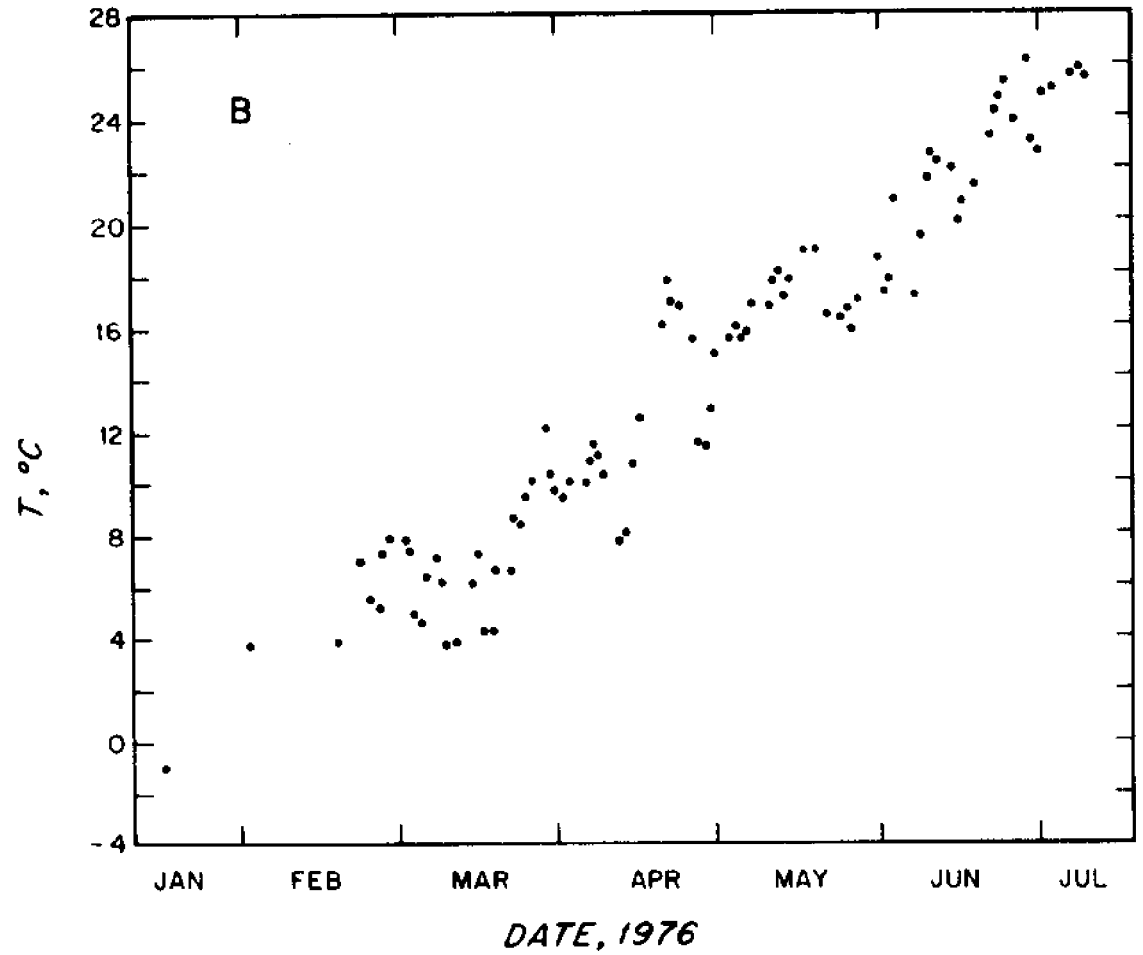

B
28
24
20
16
12
8
4
0
-4
T, °C
JAN
FEB
MAR
APR
MAY
JUN
JUL
DATE, 1976

Fig. 9

Determination of total nitrogen in aqueous samples using persulfate digestion[1]

by

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Abstract

Determination of total nitrogen in aqueous samples after potassium persulfate digestion compared favorably in both precision and nitrogen recovery with determinations obtained using Kjeldahl digestion.

The determination of total nitrogen ("TN" = inorganic and organic fixed nitrogen) has, in the past, largely depended either on acid Kjeldahl digestion (cf. Strickland and Parsons 1972) or on photo-oxidation (Armstrong, Williams, and Strickland 1966) of nitrogenous organics. The former procedure is quite tedious to perform and yields a total Kjeldahl nitrogen (TKN) value which includes only organic N and NH_4^+-N (not NO_2^- and NO_3^--N). The latter procedure is less tedious and provides a value which includes NO_2^- and NO_3^--N, but some compounds (e.g., urea and ethylenediaminetetraacetic acid--EDTA) have been reported to be refractory to this type of oxidation (Henriksen 1970; Afghan, Goulden, and Ryan 1971). In addition, a substantial investment in photo-oxidation equipment is necessary if a large number of samples are processed per day.

A persulfate oxidation technique for TN determination has also been developed Koroleff (1969, 1970). Koroleff found that while there is no single N product of non-alkaline persulfate oxidation (presumably NO_3^-, NOCl, and other compounds are produced), under alkaline conditions, NO_3^-, readily reduced to NO_2^- for analysis, was the sole product. Thus, total persulfate nitrogen (TPN) should be equivalent to TKN plus NO_2^- and NO_3^--N. Koroleff's procedure, although apparently in wide use in Scandinavia (Ekedahl, Junker, and Röndell 1975) to our knowledge has received little attention elsewhere. Here we report some minor modifications to his procedure and evaluate its efficacy relative to a modified Kjeldahl procedure.

All reagents used should be of analytical reagent grade. N-free distilled water (NFDW) is prepared by UV oxidation and deionization or by double distillation from acid persulfate and alkaline permanganate respectively. Glassware is pre-rinsed in dilute HCl and NFDW.

TPN reagents. 1. Oxidizing reagent. 3.0 g of NaOH and 6.7 g of low N (< 0.001%) potassium persulfate (peroxydisulfate), $K_2S_2O_8$, are dissolved in one liter NFDW just before use.

2. 0.3 N HCl. Stable for months.

3. Buffer solution. 30.9 g H_3BO_3 are dissolved in deionized water, 101 ml of 1 M NaOH are added, and the solution made to one liter. Stable for months.

4. NO_3^- reduction columns and NO_2^- reagents are as given by Strickland and Parsons (1972).

TPN procedure. 15.0 ml of oxidizing reagent are added to 10.0 ml of sample in 25 x 150 mm (50 ml capacity) borosilicate screw cap culture tubes. A $Mg(OH)_2$ precipitate forms in seawater samples. Blanks for undiluted samples consists of 15.0 ml of oxidizing reagent only. The tubes are capped immediately with size 24 polypropylene screw closures (e.g. Nalgene No. 0240 - for reasons not clear to us, some caps cannot withstand autoclaving, hence test them before use). Samples are autoclaved at 100° to 110° (the optimal temperature range for persulfate digestion - Williams 1969) for at least one-half hour and brought back to atmospheric pressure slowly to avoid breaking

the tubes. The tubes can then be removed and cooled to room temperature in running tap water. 1.5 ml of 0.3 N HCl are added to each sample. The samples are mixed with a Vortex mixer until the precipitate dissolves, 2.0 ml of buffer solution is added, and then deionized water to a mark on the tube indicating 50 ml (alternatively, 23.5 ml of a stock solution of 2 parts buffer solution to 21.5 parts of deionized water can be added). 20 ml of each sample are washed through the nitrate reduction column in small aliquots which are discarded. The final 30 ml is passed through the column and analyzed for NO_2^- (cf. Strickland and Parsons 1972). As the borate buffer does not appear to complex Cd^{++} well, the columns will tend to clog unless rinsed every few samples with about 10 ml of dilute NH_4Cl solution (cf. Strickland and Parsons 1972).

TKN reagents. 1. Digestion mixture. 50 parts of concentrated H_2SO_4 to 50 parts NFDW to 5 parts 5% $CuSO_4$.

2. Ammonium reagents. Phenol and alkaline solutions as given by Solórzano (1969). Sodium nitroprusside solution--0.25% of sodium nitroprusside. Oxidizing solution--0.2 g of sodium dichloroisocyanurate (Liddicoat, Tibbits, and Butler 1975) per 100 ml of alkaline solution.

TKN procedure. 2.0 ml of digestion mixture and two glass beads are added to 25.0 ml of sample in a Kjeldahl flask. Place the flask on a heating rack, and after first volatilizing the water in the sample, digest until the remaining solution turns clear. Cool and, rinsing with a minimum amount of NFDW, transfer to a 60 ml beaker.

Cool the beaker to room temperature in an ice bath and adjust the pH of the solution to 5.0-5.2 with NaOH. Quantitatively pour a portion or all (depending on the amount of N expected) of the solution into a 50 ml volumetric flask and dilute to volume. Samples can be stored overnight at this point. The reagents for NH_4^+ determination are added as prescribed by Solórzano (1969), and the color developed in the dark (Gravitz and Gleye 1975) for a consistent period of time (not less than 90 minutes) at room temperature. The factor (F) relating absorbance to NH_4^+ concentration should be determined by difference in absorbance from NH_4Cl-spiked and unspiked sea water. TKN blanks are obtained using NFSW. Absorbances are recorded at 640 nm in a 1 cm cuvette.

We have found that although the $CuSO_4$ catalyst in the digestion mixture gives a slight blue color after addition of the NH_4^+ reagents (Nicholls 1975), it does not interfere with indolphenol blue color formation. We have chosen to retain the Cu catalyst for samples in the higher TKN ranges (in lower ranges it probably can be omitted; Nicholls 1975). The present procedure affords a range of detection from approximately 2-50 µg at $N.L^{-1}$.

Results and Discussion. During the past year we have randomly used NH_4^+, NO_3^-, glycine, EDTA, and urea as standards to obtain the factor (F) relating 1 cm cuvette absorbance to TPN concentration. Table 1 shows a comparison of mean percentage recoveries the various standards yielded when NO_3^- recovery is considered equivalent to 100%.

Except for NH_4^+ ($0.05 < p < 0.1$; t-test), there was no significant difference (i.e. $p < 0.1$) in N recovery between NO_3^- and the other standards. Presumably, the significant difference in NH_4^+-N recovery was due to its dissociation and partial volatilization upon addition of alkaline persulfate reagent as this raises the pH to about 10.8. However, recovery of NH_4^+-N was excellent (96%) and should amount to an inconsequential error except in samples containing mostly NH_4^+-N. Note that the quantitative recovery of EDTA-N and urea-N by the TPN procedure is in contrast to the poor recovery by UV oxidation (Afghan, et al. 1971).

To test the effect of N concentration on its recovery as TPN, we prepared various dilutions of an algal culture (containing mostly particulate organic N) and subjected them to persulfate digestion on two successive occasions. An equivalent recovery was observed at all dilutions (Fig. 1).

The precision of total N determinations by persulfate and Kjeldahl methods was compared by randomly selecting data from routine sea water samples we have processed (Table 2). The overall coefficient of variation (CV%) for both was about 5%. The standard errors we report for TKN-TN are better than might be expected using the Strickland and Parsons (1972) procedure for TKN alone. We do not feel that the Kjeldahl precision can be improved substantially. The precision of the persulfate method can probably be increased as additional refinements are incorporated.

Given the relative convenience of the persulfate procedure, standard errors can also be reduced by increasing replication. Note, however, that since TKN-TN is the sum of TKN and NO_2^- and NO_3^--N, some error is accounted for by the latter analysis. Thus, the determination of TKN alone (i.e. NH_4^+ + organic N) in samples containing a high percentage of NO_2^- and NO_3^--N, is not as precisely determined by difference of TPN and NO_2^- and NO_3^--N.

TPN recovery was compared to TKN-TN recovery on 21 paired samples containing 20 to 35 μg at $N.L^{-1}$ and varying ratios of inorganic to organic N (Table 3). The mean values of 27.83 for TKN-TN and 26.34 for TPN compared favorably. However, treating the TKN-TN and TPN values as duplicates, we found a higher CV% (ca. 13%) than for duplicates of either method alone (ca. 5%). This suggests that there is perhaps some slight difference in what is measured by the two procedures.

Also shown in Table 3 is a comparison of particulate nitrogen (PN) determinations using either the TPN technique (found by difference in TPN in filtered and unfiltered sample) or the Perkin-Elmer model 240 CHN analyzer (on precombusted glass filters). There is again fairly close agreement between TPN values and those obtained by another procedure. We do not, however, recommend PN determination using persulfate analysis "by difference" as the precision is poor.

We have encountered difficulties in obtaining low N persulfate. Generally, B&A and Baker products have produced the lowest blanks (0.020 to 0.050 absorbance in 1 cm cells). NFDW accounted for only

about 0.01 absorbance units. Recrystallization of persulfate may improve its quality. One can expect a factor of about 110 for TPN when using a 1 cm cell. Since day to day variation in F with four pairs of blanks and standards has been as great a ±10%, probably due to poor replication rather than a true change in F, it is probably best to establish an overall F value recalculated on a continuous basis, provided that regular checks are made to ensure that the NO_3^--reducing ability of the columns remains constant.

A distinct advantage to the TPN procedures is that it can be used at sea. Samples can either be digested in precombusted ampoules or sealed pyrex tubes while at sea for analysis later on shore, or the whole operation completed on board ship. Considering the other advantages of good recovery, convenience, and equipment requirements, the persulfate method should find wide use in laboratories analyzing aqueous samples for TN.

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Table 1. Mean percentage N recoveries, relative to NO_3^-, of various N standards.

N Standard	Number of blank-standard pairs	Percentage recovery
NO_3^-	17	100
NH_4^+	19	96.2
Glycine	19	100.1
Urea	14	98.9
EDTA	18	97.8

Table 2. Precision of total N determinations by persulfate and Kjeldahl methods.

Range*	TPN				TKN + NO_2^- & NO_3^-§			
(µg at $N.L^{-1}$)	$\bar{X}$ (µg at $N.L^{-1}$)	N (pairs)	2SE+	CV%‡	$\bar{X}$ (µg at $N.L^{-1}$)	N (pairs)	2SE	CV%
Below 20	14.2	23	1.76	8.7	14.3	12	1.06	5.3
20-40	26.9	14	2.26	5.9	27.1	12	2.62	6.9
40-60	50.7	11	3.14	8.6	47.3	3	2.88	7.3
60-80	70.9	20	5.24	5.2	70.1	3	2.24	2.2
80-100	88.2	12	3.94	3.2				
100-120	110.9	16	5.72	3.7				

*Data produced in our laboratories over the past year were collected at random and for comparison divided arbitrarily into the concentration ranges shown.

+Two standard errors of the mean of two determinations (95% confidence interval), i.e. $2s/\sqrt{2}$, where s is the standard deviation; s is calculated by the formula, $s = (\Sigma d^2/2N)^{1/2}$, where d is the difference between duplicates (Youden, 1959).

‡CV% = coefficient of variation = $100s/\bar{X}$, where $\bar{X}$ is the mean.

§TKN (total Kjeldahl nitrogen) does not include NO_3^- & NO_2^--N, hence for comparison with TPN (total persulfate nitrogen) their values must be added to TKN.

Table 3. N recovery by the persulfate technique relative to Kjeldahl and CHN analysis.

	TPN vs. TKN + NO_2^- & NO_3^-	TPN vs. CHN
Mean	26.34:27.83	131.5:120.0
Number of pairs	21	34
% difference	5	10

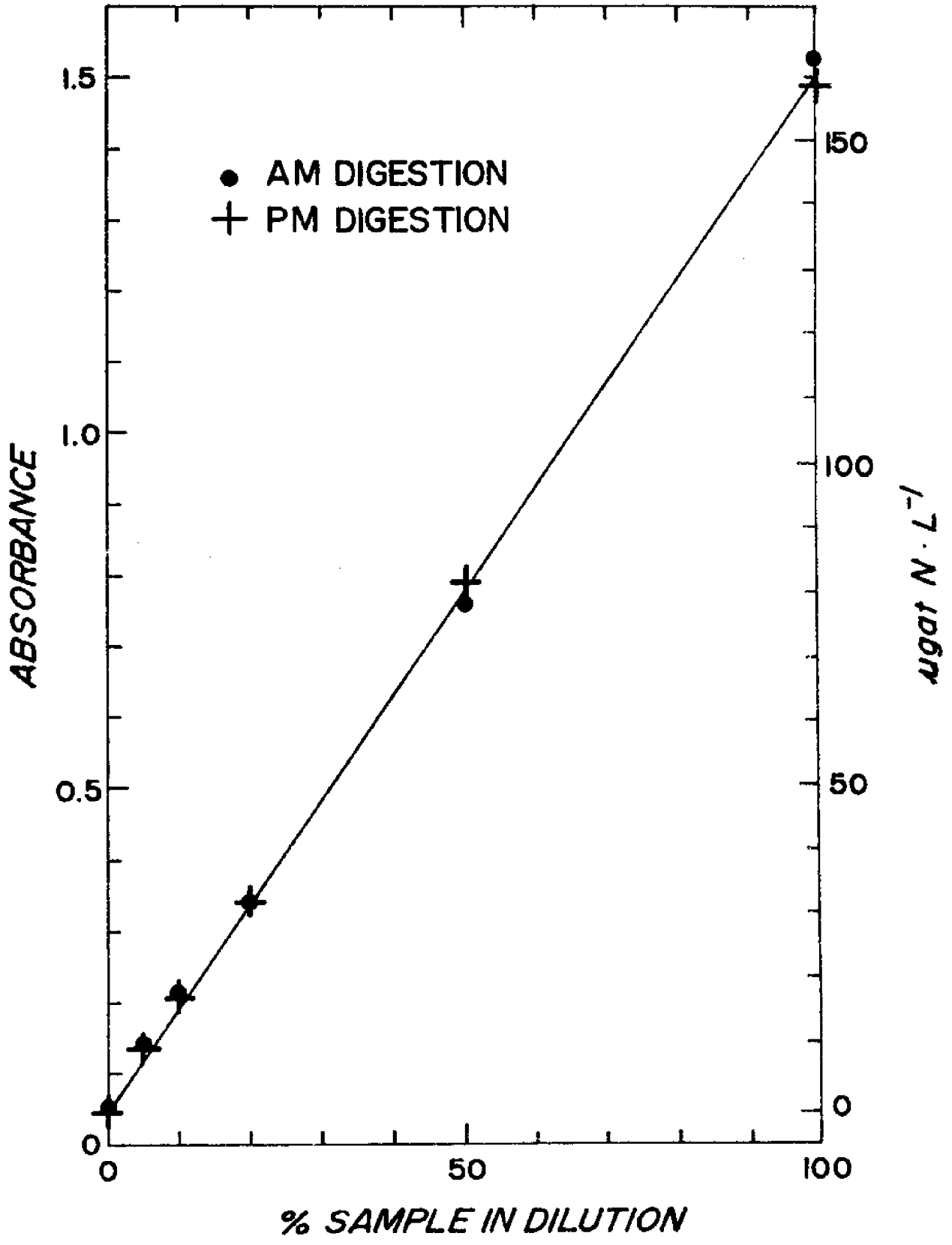

Fig. 1. Recovery of N in various dilutions of an algal culture. Dilutions were subjected to persulfate digestion on two successive occasions (a.m. + p.m.).

The growth of six species of bivalve molluscs in a waste-recycling aquaculture system*

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INTRODUCTION

The feasibility of the mass culture of marine phytoplankton in sewage-seawater mixtures as a method of tertiary wastewater treatment has been illustrated in recent studies at Woods Hole Oceanographic Institution (Goldman and Ryther, 1975). Initial attempts to utilize these cultures to grow the bivalves Crassostrea virginica and Mercenaria mercenaria met with little success (Ryther, 1975), a fact that was considered to be related to the inability to control the predominant species of phytoplankton. The diatom Phaeodactylum tricornutum was found to predominate over a wide temperature range in light limited, highly eutrophic culture regimes (Goldman and Ryther, 1976). Although P. tricornutum has proved to be of little use in the laboratory culture of juvenile bivalves (Walne, 1970), its ease of maintenance in mass cultures warrants investigation into its use in the growth of "seed" oysters or clams as an integral part of a waste recycling-aquaculture system of the type proposed by Ryther et al. (1972). The present work reports the results of initial studies during the period November 1975-May 1976 investigating the comparative growth of six species of commercially important bivalve molluscs in the waste recycling-aquaculture system at Woods Hole Oceanographic Institution, and attempts to optimize growth within the system through the manipulation of temperature and food concentration.

METHODS

Algal culture

Algae were cultured outdoors in six 120,000 l ponds of 15 m diameter. Sand filtered seawater was supplied to the ponds at a rate of 24-72 l/min (corresponding to 0.25-0.75 turnovers of pond volume per day depending upon incident sunlight levels) and enriched with either secondary treated sewage effluent to 100-200 µg at/l N and 40-80 µg at/l P, or inorganic nutrients (NH_4Cl and $NaH_2PO_4 \cdot H_2O$ to 200 µg at/l N and 40 µg at/l P respectively). The harvested algae overflowed through a centrally placed standpipe into concrete raceways containing the shellfish.

Shellfish raceways

Shellfish were held in green plastic "Nestier" trays (60 x 60 x 6 cm) in concrete raceways (12 x 1.2 x 1.5 m). The Nestier trays were stacked twenty deep, twenty-four stacks of trays being arranged in pairs along the length of the raceway. Stack positions were numbered 1-12 along the length of the raceway. Raceways were supplied with both heated and ambient filtered sea water and the algal food harvested from the culture ponds. Mixing was effected by means of an air line along the bottom corner of the raceway.

As algal culture densities varied with incident light in the range 0.5-1.5 x 10^6 cells/ml, a regime was chosen whereby algal cultures were diluted fivefold (1 part algae plus 4 parts sea water)

on entry to the raceway giving an initial cell concentration in the range 1-3 x 10^5/ml. Two raceways were used, these being held at 20°C and 15°C respectively. During the period November 1975-February 1976 both algal food and sea water were supplied at one end of the raceway and flowed to waste, via a culture of macroscopic algae, at the other. Although this mode of operation ensures efficient removal of algal food, only a small number of bivalves are exposed to an optimum concentration of food (10^4-10^5 cells/ml, see Walne, 1970). Excessive dilution with filtered sea water for the maintenance of such optima is unfeasible due to the volumes of the latter required. During the period November-December 1975 food concentration was regularly assayed fluorometrically throughout the length and depth of the raceway. At the stocking density of animals used, which are described in more detail in the following text and tables, and the flow rate regime described previously, a decrease in food concentration from 10^5-10^4 cells/ml was evident over a distance corresponding to one quarter of the length of the raceway (three stacks of shellfish). Therefore, during the period March-May 1976 the mode of raceway operation was changed such that sea water was supplied at one end of the raceway whereas algal food was supplied at four points along it. This latter mode proved effective in supplying the majority of the raceway with an optimal food concentration (2.5-7.5 x 10^3 cells/ml) whilst ensuring adequate removal of algae.

Throughout the course of the study attempts were made to feed both raceways with equal amounts of algal food from the same pond(s), however, the mechanical limitations of the experimental system combined with the occasional failure of an algal culture sometimes resulted in raceways being fed from different ponds. Raceways were covered by wooden boards during the experiment to discourage the growth of epiphytic filamentous algae.

Experimental animals

Crassostrea gigas seed oysters were obtained in two lots from International Shellfish Enterprises, Moss Landing, California in September 1975 (stock A), and from Sea Salter Shellfish Ltd., Whitstable, U.K. in November 1975 (stock B). Venerupis semidecussata were obtained from International Shellfish Enterprises, Moss Landing as 3 mm seed in November 1974. Mercenaria mercenaria and Crassostrea virginica (3 lots) were obtained from Long Island Oyster Farms during January 1974 and May 1975 respectively. Mytilus edulis were collected locally in September 1975. Ostrea edulis were obtained in two lots from Mr. Walter Foster, Bar Harbor, Maine in March 1975 (stock A), and from the Fisheries Experiment Station, Conwy, U.K. in December 1975 (stock B). A further stock of O. edulis was obtained from Seed Oysters U.K. Ltd. in October 1975. However a considerable percentage of these animals was dead on arrival. Further mortalities occurred within a period of two to three months. This suggests either poor condition

of the original stock or mishandling during transit. Consequently growth data for this stock has not been reported.

All stocks were maintained in the aquaculture facility at Woods Hole from collection to the commencing of the grow trial. During this period both V. semidecussata and O. edulis (A) exhibited considerable growth as is witnessed by the initial experimental values given in the results tables.

Sampling of bivalves

Standard populations of each stock, the size of which was dependent upon the initial size of the experimental animals, (see Tables I-VIII) were selected and placed on both the top and the bottom of stacks of animals in positions 1, 7 and 12 in each of the two raceways. In this manner it was hoped to gain data on growth throughout both the length and depth of the raceway for all species at two temperatures. Concurrently animals from stock C. gigas (B) were placed in experimental trays at the same raceway positions but at a variety of densities (250, 500, 750, 1 000, 1 250 and 1 500 animals per tray, mean live weight 1.2 g/animal) to investigate the effect of density of shellfish on growth and to assess the maximum biomass that can be sustained in an experimental "Nestier" tray. The remaining twenty-one stacks of shellfish per raceway were filled with excess populations of C. gigas (B), M. mercenaria, C. virginica and O. edulis (A) at approximately the same densities as experimental populations.

The comparative growth trial commenced on November 5, 1975. At intervals of four weeks stacks containing experimental animals were removed from the raceway and cleaned of adherent sediment, fecal material and, where possible, epifauna. Populations were removed from their Nestier tray, the dead individuals enumerated and removed, and weighed on a beam balance to the nearest gram. A mean live weight was subsequently calculated. A subsample of 10-20 animals was removed from each population for the estimation of dry meat and dry shell weights following drying at 100°C for 24 hr. The remainder of the population was subsequently returned to the raceway. Sampling was effected during the months of December 1975, and January, March, April and May 1976. It was not considered wise to attempt sampling during February 1976 due to the excessively cold weather and the potential harm to the experimental populations.

RESULTS

Comparative growth of different stocks

Data are summarized in Tables I-VIII inclusive. Each Table gives data for one stock of animals at all raceway positions during the trial period. Stocks C. gigas (A) and M. edulis were tested at 20°C only as insufficient animals were available for a two-temperature comparison. In only one case, position 1 at 20°C, was any consistent difference evident between the growth of populations at the

top and bottom of a stack (see Table II). Thus, for simplicity, reported values are the mean values of the two populations of the same stock in one stack. Values for live weight (W g), dry meat weight (M mg), dry meat condition index (Ci, dry meat x 1 000/dry shell), and the number of animals per tray (N) are given. Relevant values for dry shell weight, total biomass per tray and percentage mortality can thus be calculated from the present data.

The initial values of November 1975 are reported together with sampling dates in January 1976, March 1976 and May 1976. The intervening periods may be summarized as follows:

1) November 1975-January 1976: Algal food introduced only at the end of the raceway. Algal cultures grew well and required little maintenance.

2) January-March 1976: Very cold weather. Algal cultures were often iced over or cell densities were low due to low incident light levels. Raceways were often fed seperate ponds due to management problems.

3) March-May 1976: Good algal growth with the harvest being introduced at four points in the raceway.

Crassostrea gigas (Tables I and II)

Stock A exhibited good growth only at position 1 during the period November 1975-March 1976, growth at positions 7 and 12 being considerably enhanced during the period March-May 1976. Despite

considerable increases in dry meat weight at position 1 only a marginal increase in condition index was noted indicating a proportionate increase in dry shell weight. Mortality for the period November 1975- May 1976 was only 24% at position 1 and total biomass increased by 302.6%. Mortality was considerably higher at positions 7 and 12 (51.6% and 48.4% respectively), however this may have been due to an excessively prolonged period of exposure to lower food levels coincident with the high metabolic demands associated with high temperatures.

Stock B exhibited a similar pattern of growth to stock A at 20°C. Some enhancement of growth was apparent at the top of the stack in position 1 at 20°C as mentioned earlier. This may have been due to ineffective mixing of algal food in the region directly adjacent to the inflow. Despite considerable growth a reduction in condition index was evident in all populations at 20°C, however, accompanying mortality was less than recorded for stock A. Values for both live weight and dry meat weight recorded in all positions at 15°C were either comparable to, or higher than at 20°C. Values for the condition index were consistently higher at 15°C than at 20°C although values for both temperatures for May 1976 were lower than initial values. This latter point is probably related to the higher mortality recorded at 15°C, the cause of which is discussed at some length later in the present text. During the period November 1975- May 1976, a 134.8% mean increase in biomass was recorded for all C. gigas (B) standard populations at 20°C compared to a value of 128.4% at 15°C. However, mean dry meat weight for the six populations at

15°C increased by 106.5%, compared to 59.6% for populations at 20°C, for the corresponding period.

Venerupis semidecussata (Table III)

Growth patterns in the 20°C raceway resembled those of population C. gigas (A). Consistently higher values of live weight, dry meat weight and condition index were recorded throughout the study at all position at 15°C. Mortalities were comparable at positions 1 and 12 for both temperatures (4.3-5.2%) although somewhat higher at position 7 at 15°C (17.1% vs. 6.7%). Meat production at 15°C was exceptionally good, a 656% increase in dry meat weight being recorded at position 1 over the period November 1975-May 1976.

Mercenaria mercenaria (Table IV)

The previously poor records of growth of this species in the present system were confirmed in the present study with only small increments in live weight being recorded. Meat growth was evident at position 1 at 20°C, and at all positions at 15°C. A consistent increase in condition index over the period November 1975-May 1976 indicates that the stock was in relatively poor condition at the beginning of the study, a result consistent with the lack of growth evidenced since the stocks introduction to the present system in December 1974. Mortality varied between 15% and 42% for the period November 1975-May 1976.

Crassostrea virginica (Table V)

As all three stocks of the above species that were tested gave similar results, recorded values are given for only one of them. Growth increments during the period November 1975-January 1976 were consistently small except in position 1 at 20°C. A drop in condition index was noted in all populations. Sampling was discontinued in January 1976.

Mytilus edulis (Table VI)

Good growth was noted at position 1 only, however mortalities were high (45%) and a 28% decrease in total biomass of this population was recorded during the period November 1975-January 1976. Sampling was discontinued in January 1976. Recent work by Bayne, Widdows and Worrall (1975) suggests that the cause of this high mortality may be the experimental temperature used as Mytilus exhibits increasing difficulty in acclimating physiologically as water temperatures rise in excess of 20°C. The potential of this species for growth at a lower temperature remains to be evaluated at a later date.

Ostrea edulis (Tables VII and VIII)

Only live weight and mortality data were collected for population O. edulis (A) as individuals were too large to allow reasonable matching between populations. Similarly insufficient numbers were present in a population to allow sacrificial sampling. Good growth was noted

only in position 1 at either temperature. Some mortality was evident towards the end of the study, this usually occurring in the larger individuals resulting in a decrease in mean live weight. It should be noted that these animals had grown to well in excess of market size (7.65 cm length) from individuals of approximately 4.1 cm length during a period of some five months (March-August 1975) prior to commencing the present study (Ryther 1975).

Ostrea edulis (B) were included into the trials for the period March-May 1976. Unfortunately no dry meat and dry shell weight data was taken for initial samples. Table VIII reports data for March, April and May 1976, a considerable increase in live weight was noted only at position 1 at 20°C. A high dry meat weight increment was noted in position 1 at 15°C between March and April 1976. Mortalities were considerable in all populations (24-29%), negating any increase in total biomass of the population.

The effect of density of shellfish on growth: population C. gigas (B)

A marked increase (125-422%) in biomass per tray (Table IX) was noted in position 1 at 20°C. At positions 7 and 12 biomass increments were consistently in the range 48-63% for all densities. No consistent patterns were evident in % mortality (Table X) that could be related to either density or position of an experimental population at 20°C.

At 15°C maximum increments in biomass were recorded at either January or March 1976 (156-212% at position 1, 132-185% at position 7,

and 128-167% at position 12). Subsequent mortalities resulted in a drop in biomass at all densities and positions. In all but one case mortalities were greater for populations at 15°C than at 20°C, a topic that is discussed in some length in the following section of the present text.

DISCUSSION

The present study deals with a food chain analogous to that occurring in nature but maintaining certain unique characteristics of its own. The most notable is the predominance in the algal cultures of the diatom P. tricornutum, a species that is present but never predominant in natural, less eutrophic, situations. Until such times that the causes of this predominance are clarified and the subsequent steps taken to maintain mass cultures of alternative, more desirable, marine phytoplankton species the onus is upon researchers to investigate methods of utilizing this potential food source in the production of animal protein. Despite dilution of the cultured algae with sea water, and a small (5-10%) proportion of subordinate species that survive in mass cultures, the major proportion of the food presented to the bivalve species in the present study was P. tricornutum. A considerable variation in response to such a dietary regime was evident in the six species tested. Crassostrea gigas and V. semidecussata exhibited good potential as prospects for culture in a waste recycling facility. The terminal size of specimens of population O. edulis (A)

suggests good potential for this species also, although confirmatory data from population O. edulis (B) has not yet been forthcoming. As these two stocks came from different sources it is possible that these growth differences may be explained by the experimental populations being of different geographic strains - a possibility that remains to be explored. Results obtained from M. mercenaria and C. virginica are discouraging of further effort. The potential of M. edulis has yet to be fully evaluated.

Similar large scale growth experiments in pilot upwelling aquaculture systems have been reported by Roels et al. (1975). Roels' outdoor cultures used deep oceanic water inoculated with large volumes of the diatoms Thalassiosira pseudonana (3H), Chaetoceros sp. or Bellerochia sp. These monospecific cultures rapidly became infected with endemic species (Malone et al. 1975) and were thus maintained for only one to four weeks before being discarded and restarted with a different diatom. Thus shellfish were fed alternating supplies of cultures with one predominant species. In this regime the bivalves C. gigas, V. semidecussata, O. edulis, Argopecten irradians, and a hybrid clam (M. mercenaria x M. campechiensis) all grew well, but both C. virginica and M. mercenaria failed to grow in the system. The similarities between these results and those of the present study are notable and suggests that the inability of C. virginica and M. mercenaria to grow well may not be related to the food species per se, but may be due to the inability of any one food species to form a

balanced diet for either of these bivalve species. Such a hypothesis does however contrast markedly with the results of Epifanio et al. (1975) who obtained good growth of C. virginica over a period of thirty weeks post settlement on a monospecific diet of T. pseudonana. In the same study, and using the same six bivalve species as the present study, Epifanio et al. compared growth over a ten-week period in a closed culture system on monospecific diets of either P. tricornutum or T. pseudonana. In no case was growth recorded when P. tricornutum was used as food. The ability of C. gigas, V. semidecussata and O. edulis to grow in the present study would thus appear to be due to the provision of some dietary necessity, absent in pure cultures of P. tricornutum, from either the subordinate phytoplankton species present in the mass cultures, or from dissolved compounds present in the sewage itself.

The present study also raises the question of optimal temperature regimes in bivalve aquaculture systems. Seasonal, temperature related cycles of growth, gametogenesis, spawning and relative inactivity have been described for may bivalve species (Ansell 1972, Ansell et al. 1964, Ansell and Trevallian 1967, Masumoto et al. 1934, Walne and Mann 1975). The use of sustained elevated culture temperatures will obviously disrupt such cycles. An optimal temperature regime must draw a compromise between the stimulation of feeding and meat production whilst attempting to minimize excessive shell growth, the production of gonadal material and the potential physiological stresses

associated with high temperatures. In the present study both C. gigas and V. semidecussata exhibit higher condition indices at 15°C than 20°C, and appear better able to grow under conditions of reduced food concentrations, i.e., at positions 7 and 12 in the raceway, at this lower temperature. To date relevant information on sustained elevated culture temperatures is minimal and further physiologically orientated growth studies are urgently needed.

In attempting to build a market predictive model of bivalve growth in such an aquaculture system temperature is only one factor of many that needs to be considered. Growth rate, mortality, maximum sustainable biomass of shellfish per culture unit, optimal food concentration for conversion and removal efficiency, depuration, water circulation and epifaunal fouling are amongst the biological problems encountered. These must, of course, be superimposed on the accompanying engineering and economic problems to formulate a total evaluation. At present this is not possible but the data provided in the accompanying density-growth study using population C. gigas (B) is of interest in this context. At low initial biomass levels per tray water circulation is unimpeded by the presence of shellfish and food supply is not limiting. As growth proceeds, equilibrium is approached between available food supply and maintenance ration of the shellfish biomass. However, water flow is gradually further impeded by the growing shellfish until, as shell growth continues, food supply becomes inadequate and mortality rises. The estimates of optimal initial biomass

per tray must thus be compromised from predicted growth, mortality (a dead shell still impedes water movement), tray design and the economic aspects of handling and maintenance.

In the present study percentage biomass increment was markedly independent of density in positions 7 and 12 at 20°C indicating a situation of adequate water circulation but low food concentration. In position 1 at 20°C a decrease in percentage biomass increment with increasing density was evident illustrating the impediment of water circulation and decrease in food supply per animal at high densities. At 15°C maximum biomass levels were recorded between January and March 1976. A small decrease in percentage biomass increment was evident at higher densities in all positions although the range of increments (128-212% for the whole raceway, all densities included, suggests some food limitation at all positions. However it is relevant to note that biomass levels in excess of 4 kg per tray were recorded at the highest densities in all positions at 15°C, this approaching the maximum physical capacity of a "Nestier" tray.

Mortality data for the present study is superficially confusing. Populations at higher temperatures generally exhibited lower condition indices and meat growth. Surprisingly they also exhibited lower mortality. However, no consistent pattern of mortality was evident with time throughout the various positions in the raceway. A closer inspection of the mortality data at 15°C reveals a clear pattern; mortalities occurred earlier towards the front of the raceway

and were especially evident at higher densities. This pattern suggests the introduction of some toxic material into the raceway with the algal food source during a period early in the study when raceways were being supplied from separate culture ponds. The level of this toxicant would thus be decreased either by dilution or removal by filtration along the raceway, and would thus make its effect more clearly evident in those populations where food availability was highest. The animals toward the rear of the raceway would thus receive a lower dosage than those near the front and consequently take longer to exhibit any adverse effects. Confirming evidence for this hypothesis is lacking. However, on one occasion mechanical failure resulted in sediment from the facility sewage storage tanks being pumped into the algal culture pond supplying the 15°C raceway. The potential toxicity of this material has yet to be evaluated, but the coincidence is worthy of note.

In conclusion the present data for C. gigas, V. semidecussata and, to a lesser extent, O. edulis, indicate the feasibility of the basic concept of the production of animal protein through a combined tertiary sewage treatment-aquaculture system. More so, this end has been achieved within the limitation of the inability to control species predominance in mass cultures of marine phytoplankton in sewage-sea water mixtures. Although growth rate data per se in the present study may not be spectacular when compared to natural situations, the indications of promise for culture at high densities speak

for themselves. However, many questions of a biolgical, engineering and economic nature remain to be answered before a complete analysis of feasibility can be attempted.

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Throughout Tables I-VIII inclusive the following anotations have been used.

W: mean live weight (g)

M: mean dry meat weight (mg)

Ci: mean condition index = $\frac{\text{dry meat x 1 000}}{\text{dry shell}}$

Each table gives growth data for two temperatures, 20°C and 15°C, at three raceway positions 1, 7 and 12 (see text) and four dates, except where otherwise stated.

TABLE I

Growth of C. gigas (stock A) at 20°C for the period November 1975-May 1976.

Parameters	Date	20°C		
		1	7	12
W (g)	11-75	3.5		
	1-76	7.69	3.75	3.66
	3-76	13.45	5.75	6.00
	5-76	18.64	12.50	11.25
M (mg)	11-75	57.8		
	1-76	208.9	41.9	63.8
	3-76	260.0	39.0	63.8
	5-76	313.1	82.6	*
Ci	11-75	40.7		
	1-76	55.7	34.7	35.2
	3-76	54.1	39.0	42.2
	5-76	47.5	47.4	*
N	11-75	250		
	1-76	247	250	250
	3-76	209	156	172
	5-76	189	121	129

*No data: sample damaged in processing.

TABLE II

Growth of C. gigas (stock B) for the period November 1975-May 1976. The two values given for each position - sampling date combination of W represent mean values for the populations on top and bottom of the experimental stacks respectively. Significant differences are only evident between these two values in position 1 at 20°C.

Parameters	Date	20°C			15°C		
		1	7	12	1	7	12
W (g)	11-75	1.2					
	1-76	3.23	1.40	1.40	2.90	2.77	2.71
		2.36	1.48	1.48	3.25	3.03	2.61
	3-76	4.48	1.7	1.66	4.11	3.3	3.07
		3.47	1.69	1.66	3.73	3.24	3.07
	5-76	5.65	2.38	2.27	4.40	3.46	3.01
		3.76	2.37	2.16	4.34	3.69	3.68
M (mg)	11-75	38.0					
	1-76	100	28.9	29.5	147.0	124.0	109.0
	3-76	114.4	33.9	37.7	99.8	98.3	98.8
	5-76	105.1	40.5	54.4	111.8	111.7	98.0
Ci	11-75	54.3					
	1-76	47.8	27.6	27.2	75.4	65.6	58.9
	3-76	44.1	34.9	34.3	43.2	50.4	52.3
	5-76	36.9	30.7	32.5	49.2	43.7	40.0
N	11-75	600					
	1-76	600	591	598	600	598	600
	3-76	584	583	589	488	590	589
	5-76	534	551	565	407	458	455

TABLE III

Growth of V. semidecussata for the period November 1975-May 1976.

Parameters	Date	20°C			15°C		
		1	7	12	1	7	12
W (g)	11-75	4.0					
	1-76	5.11	4.3	4.05	6.17	5.99	5.53
	3-76	6.48	4.68	4.55	7.30	6.87	5.82
	5-76	7.95	4.79	5.47	8.70	8.01	6.56
M (mg)	11-75	86.5					
	1-76	103.0	87.9	83.0	500.1	300.5	237.5
	3-76	374.5	141.5	123.2	418.7	415.5	316.0
	5-76	440.2	303.4	214.3	654.0	*	352.3
Ci	11-75	65.5					
	1-76	37.3	49.8	56.3	163.0	137.2	102.8
	3-76	122.05	73.7	84.2	149.2	147.6	125.2
	5-76	130.1	128.3	116.6	166.8	*	130.5
N	11-75	420					
	1-76	414	418	418	416	405	412
	3-76	408	412	409	407	400	412
	5-76	402	392	398	397	348	398

*No data: sample damaged in processing.

TABLE IV

Growth of M. mercenaria for the period November 1975-May 1976.

Parameters	Date	20 °C			15 °C		
		11	7	12	1	7	12
W (g)	11-75	3.06					
	1-76	3.21	3.08	3.20	3.23	3.05	3.13
	3-76	3.50	3.25	3.44	3.95	3.21	3.36
	5-76	3.68	3.57	3.58	4.89	3.51	3.50
M (mg)	11-75	66.3					
	1-76	156.0	61.8	90.6	162.5	85.5	118.4
	3-76	137.0	92.6	73.0	136.1	118.1	101.3
	5-76	172.6	91.5	77.6	142.1	*	160.0
Ci	11-75	46.4					
	1-76	59.6	42.0	51.2	77.4	65.7	64.3
	3-76	63.4	53.3	48.4	80.4	65.8	53.7
	5-76	73.2	57.9	48.6	73.8	*	82.3
N	11-75	450					
	1-76	430	434	432	394	422	414
	3-76	400	420	400	270	367	337
	5-76	352	382	362	259	342	300

*No data: sample damaged in processing.

TABLE V

Growth of C. virginica for the period November 1975-January 1976.

Parameters	Date	20 °C			15 °C		
		1	7	12	1	7	12
W (g)	11-75	1.52					
	1-76	2.21	1.57	1.50	1.80	1.68	1.66
M (mg)	11-75	47.9					
	1-76	73.9	41.0	37.3	45.6	68.2	45.0
Ci	11-75	62.6					
	1-76	50.5	42.8	48.4	60.0	60.3	54.5
N	11-75	715					
	1-76	620	708	708	697	704	704

TABLE VI

Growth of M. edulis at 20°C for the period November 1975-January 1976.

Parameters	Date	20°C		
		1	7	12
W (g)	11-75	2.84		
	1-76	3.72	2.97	2.97
M (mg)	11-75	58.1		
	1-76	120.1	41.6	56.9
Ci	11-75	61.2		
	1-76	83.2	55.5	50.8
N	11-75	480		
	1-76	263	327	340

TABLE VII

Growth of O. edulis (stock A) for the period November 1975-May 1976.

Parameters	Date	20°C			15°C		
		1	7	12	1	7	12
W (g)	11-75	39.25	46.4	41.2	43.65	52.30	34.45
	1-76	49.65	45.65	40.2	53.85	54.75	36.6
	3-76	51.98	46.8	42.25	47.65	*	*
	5-76	52.2	48.25	42.45	52.5	49.05	35.15
N	11-75	31	32.5	31	30	30	41
	1-76	31	32.5	31	29.5	29.5	41
	3-76	29.5	32.5	31	28.5	*	*
	5-76	27.5	29.5	29.5	21.5	23.5	33

*No data recorded.

TABLE VIII

Growth of O. edulis (stock B) at dates March, April and May 1976.

Parameters	Date	20°C			15°C		
		1	7	12	1	7	12
W (g)	3-76	2.90					
	4-76	3.83	3.60	3.26	3.51	3.12	2.90
	5-76	4.44	3.47	3.11	3.34	2.96	2.75
M (mg)	3-76	--					
	4-76	64.0	58.0	53.0	78.4	54.7	68.8
	5-76	70.5	55.0	52.7	121.8	68.0	63.5
Ci	3-76	--					
	4-76	38.3	35.7	30.7	33.3	30.5	29.7
N	3-76	250					
	4-76	203	208	215	208	204	216
	5-76	178	182	186	179	181	191

TABLE IX

Biomass of populations of C. gigas (stock B) at a variety of initial densities (250, 500, 750, 1 000, 1 250 and 1 500/tray, mean weight 1.2 g) for the period November 1975-May 1976.

Date	20°C			15°C		
	1	7	12	1	7	12
11-75	300					
1-76	980	382	380	720	690	660
3-76	1 760	435	415	935	855	800
5-76	1 565	470	490	735	650	635
11-75	600					
1-76	1 565	740	715	1 505	1 419	1 370
3-76	2 250	850	791	1 765	1 585	1 565
5-76	2 240	960	910	1 470	1 145	1 315
11-75	900					
1-76	2 070	1 090	1 080	2 300	2 202	2 050
3-76	2 795	1 225	1 205	2 220	2 325	2 220
5-76	2 850	1 395	1 330	1 680	1 345	1 735
11-75	1 200					
1-76	2 325	1 380	1 455	3 105	2 896	2 790
3-76	2 930	1 550	1 610	2 500	2 848	2 955
5-76	3 165	1 830	1 825	2 060	1 390	2 310
11-75	1 500					
1-76	2 540	1 795	1 810	4 025	3 555	3 250
3-76	3 270	1 995	1 965	2 995	3 215	3 425
5-76	3 380	2 230	2 275	2 340	1 475	2 720
11-75	1 800					
1-76	3 230	2 144	2 205	4 760	4 170	4 170
3-76	4 045	2 375	2 420	3 740	3 790	4 310
		2 740	2 835	2 890	1 870	3 305

TABLE X

% cumulative mortality of populations of C. gigas (stock B) at a variety of initial densities for the period November 1975-May 1976.

Date	20 °C			15 °C		
	1	7	12	1	7	12
11-75	250=N					
1-76	0	1.6	0	0	0	0
3-76	2.0	4.8	2.0	17.6	2.8	1.2
5-76	18.4	14.4	4.8	26.4	13.8	20.0
11-75	500=N					
1-76	0	0.6	0	0	0.2	0.4
3-76	0.6	1.0	1.8	8.6	2.2	1.6
5-76	10.6	9.2	7.8	20.6	21.8	21.2
11-75	750=N					
1-76	0	0.6	0	0	0	0
3-76	0.4	1.7	1.5	15.2	2.3	1.73
5-76	8.3	10.9	8.4	31.1	27.2	34.5
11-75	1 000=N					
1-76	0	0.7	0.3	0	0	0
3-76	0.5	1.9	2.1	17.2	3.8	1.0
5-76	6.5	8.6	8.3	32.3	27.3	38.8
11-75	1 250=N					
1-76	0.6	0.8	0	0	0	0
3-76	1.8	1.5	2.6	22.9	5.4	0.9
5-76	8.6	8.9	7.3	40.6	33.8	36.6
11-75	1 500=N					
1-76	0.3	0.4	0	0	0	0
3-76	1.5	3.1	2.3	18.7	3.5	0.9
5-76	6.2	11.7	8.5	41.3	34.0	32.9

PRELIMINARY STUDIES OF THE EFFECT OF TEMPERATURE ON GROWTH AND AMMONIA EXCRETION IN THE MANILA CLAM *VENERUPIS SEMIDECUSSATA*[1]

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Running Head: Physiology of *Venerupis semidecussata*

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Authors' note:

Several different scientific names have been used for the species studied in the present work. These include Venerupis semidecussata, V. japonica, V. philippinarum, Tapes semidecussata, T. japonica, T. philippinarum, Paphia philippinarum and Venus philippinarum. For a more detailed account of these alternatives see Cahn (1951) and Ohba (1959). The first of the above has been used throughout the present text except in reference to the work of other authors where their own nomenclature has been followed.

INTRODUCTION

The waste-recycling aquaculture system proposed by Ryther et al. (1972) effects the removal of dissolved inorganic nutrients, predominantly nitrogen and phosphorus, from secondary sewage effluent with an accompanying production of animal protein in the form of edible bivalve molluscs. The combination of such a system with a thermal effluent source presents the prospect of year round culture of bivalve species that are usually environmentally restricted to only seasonal growth. However, the optimization of temperature regimes in such a situation is problematic in that cycles of growth, gonad production and spawning in bivalves are directly related to temperature. Thus any chosen temperature regime must compromise the individual goals of promoting meat growth, minimizing protein catabolism and surpressing gonad production. The present study reports data on one aspect of this problem, namely the effect of sustained elevated temperature on growth and ammonia excretion in the Manila clam, Venerupis semidecussata, which in recent, unpublished studies has exhibited considerable promise as a candidate for culture in waste-recycling systems.

METHODS

Growth experiment

Experimental animals were obtained from International Shellfish Enterprises, Moss Landing, California in November 1974 as 3 mm (14 mg) juveniles

and grown in the waste-recycling aquaculture system at Woods Hole Oceanographic Institution (Ryther et al., 1975) until the present study commenced in February 1976. Throughout the intervening period animals were fed on cultures of marine phytoplankton, predominantly the diatom Phaeodactylum tricornutum, grown in outdoor ponds (120,000 liters) in sea water enriched with either secondary sewage effluent or inorganic nutrients. Water temperature was maintained at 20° C throughout the winter months and at ambient when this exceeded 20° C during the summer months.

For the present study one hundred animals, matched by live weight, were selected from the parent stock. These were divided at random into five groups of twenty individuals. One group was sacrificed to estimate initial experimental values for dry meat and dry shell weight following drying for 24 hrs at 100° C. The remaining groups were placed in mesh-bottomed, wooden boxes in each of four fibreglass trays (57 x 45 x 9 cm). Each tray was supplied with sand-filtered sea water at a flow rate of 200 ml/min at an initial temperature of 20° C. The sea water was enriched with a mixed phytoplankton culture, obtained from the outdoor mass cultures described above, to a mean concentration of 80 cells/µl. The sea water temperature in the experimental trays was decreased at a rate of 1° C/day to obtain experimental temperatures of 18, 16, 14, and 12° C in the four respective trays. These temperatures were held constant (± 1° C) for the remainder of the growth experiment which from initial sorting to termination was of 77 days duration.

At the end of the experiment live weight, dry meat weight and dry shell weight were recorded for each group. A dry meat condition index, Ci, was calculated for each group by the following equation:

$$\text{Condition index, Ci} = \frac{\text{dry meat weight x 1000}}{\text{dry shell weight}}$$

Ammonia excretion

Dissolved ammonia was measured by the colorimetric method of Solorzano (1969). Static systems were used in these assays, in which ammonia accumulation was monitored in beakers of sand filtered or Gelman GF-A filtered sea water containing the experimental animals. A preliminary study indicated that a linear increase in dissolved ammonia concentration with time ($r = 0.97$, 7 d.o.f. $P < 0.001$) was recorded over a period of five hours at 20° C in beakers containing one, three or five animals plus 200 ml of water per animal For the present study incubations were of three hours duration in 250 or 800 ml beakers containing either one or three animals plus 150 mls of water/animal. Control beakers without animals were included in all incubations. During incubation beakers were immersed in the relevant growth tray to ensure temperature control.

No assays of ammonia excretion were attempted during the first two weeks of the growth experiment to allow the animals to acclimate to the experimental temperatures. Following this period assays

were carried out at frequent but irregular intervals throughout the course of the experiment, a minimum of eighteen assays per temperature being completed. During the final seven days of the growth period assays were carried out at all the experimental temperatures. For this latter period animals were numbered individually and subsequently sacrificed thus allowing calculation of excretion rate on both live weight and dry meat weight bases.

RESULTS

Growth

All initial values were recorded on pooled samples; final values were recorded on individual animals. Both are summarized in Table I.

Similar mean final values were recorded at all temperatures for both live weight and dry shell weight ($P < 0.05$), these being consistently higher than the mean initial values (as initial values were taken from pooled, rather than individual samples it is not possible to effect a statistical comparison of the inital and final mean values). A final mean dry meat weight of 337.5 mg was recorded at 12° C, this being greater than at all other temperatures ($P < 0.05$) and indicating a threefold increase in mean dry weight over the experimental period. Similar final dry meat weights were recorded at 14 and 16° C ($P < 0.05$), the value for 16° C being

TABLE I

The growth of Venerupis semidecussata at elevated temperature for a period of 77 days. Initial values are the mean of a pooled sample of twenty individuals. Final values at each temperature give the mean ± one standard deviation.

	Initial value	12° C	14° C	16° C	18° C
Live wt (g)	2.80	3.94 ± 0.08	3.88 ± 0.17	4.09 ± 0.31	4.00 ± 0.36
Dry meat (mg)	106.0	337.5 ± 8.70	263.3 ± 23.3	277.7 ± 27.3	221.2 ± 20.5
Dry shell (g)	1.30	1.89 ± 0.05	1.85 ± 0.09	2 16 ± 0.19	2.07 ± 0.20
Condition index, CI	81.54	179.4 ± 4.4	140.6 ± 9.2	129.2 ± 9.4	107.3 ± 5.1

greater than that for 18°C ($P < 0.05$). Decreasing final mean values of condition index, Ci, are evident with increasing experimental water temperature (Ci 12° C > Ci 14° C = Ci 16° C > Ci 18° C, $P < 0.05$).

Ammonia excretion

No significant differences were recorded in excretion rate (μg NH_4-N/g live weight/hr) thoughout the time course of the experiment within any one of the experimental groups, although differences did occur between the groups. Values obtained for static systems using either coarse or fine filtered sea water were similar for any one group of animals. Thus values collected throughout the experimental period have been pooled and are given in Table II, together with values recorded on a dry meat basis (μg NH_4-N/g dry meat/hr) during the final seven days of the experiment.

A decrease in excretion rate is evident on increasing the experimental temperature from 12° C to 14° C ($P < 0.01$ on both live weight and dry meat basis). However a further increase in temperature is accompanied by a marked increase in excretion rate at 16° C ($P < 0.01$). A further increase is noted at 18° C when data are expressed on a live weight basis ($P < 0.01$) although this is not significant on a dry meat basis. Mean values for the present study vary in the range 1.16-3.01 μg NH_4-N/g live weight/hr (27.84-72.24 μg NH_4-N/g live weight/day). These compare well with values of 9.6-67.2 μg/g live weight/day recorded by Bayne (1973) for Mytilus edulis, and with values

TABLE II

Ammonia excretion in Venerupis semidecussata grown at elevated temperature for a period of 77 days. Data for (A) was collected during days 15-77 inclusive. Data for (B) was collected during days 70-77 inclusive. A mean value ± one standard deviation is given for each temperature, the accompanying bracketed number giving the number of assays completed.

	12° C	14° C	16° C	18° C
(A) μg NH_4-N/g live wt/hr	1.47 ± 0.06	1.16 ± 0.06	2.20 ± 0.24	3.01 ± 0.15
	(52)	(18)	(36)	(52)
(B) μg NH_4-N/g dry meat/hr	19.11 ± 0.69	14.21 ± 1.48	32.09 ± 3.57	29.48 ± 4.10
	(16)	(18)	(18)	(16)

of 42 and 25 μg/g live weight/day recorded for Modiolus demissus and Crassostrea virginica by Lum and Hammen (1964), and Hammen, Miller and Geer (1966) respectively.

DISCUSSION

The temperature related seasonal cycles of growth, gonad production and spawning in bivalves are usually accompanied by distinct cycles of accumulation and utilization of gross biochemical components (Ansell, 1972; Ansell et al., 1964; Ansell and Trevallion, 1967; Masumoto et al., 1934; Walne and Mann, 1975). The rapid production of gonad material in the warmer months is often accompanied by a depletion of metabolic reserves, usually in the form of glycogen, with a subsequent shift in the predominant respiratory substrate. The use of protein as such a substrate is indicated by a quantitative increase in ammonia excretion (see Bayne, 1973). The use of sustained elevated temperatures will obviously affect the aforementioned seasonal cycle, alternating periods of gametogenesis and spawning being possible if temperatures are maintained at too high a level. Such a situation is to be avoided in bivalve aquaculture systems in that such animals are both unsuitable for marketing and, during growth, are predominantly catabolizing the protein substrate that such aquaculture facilities were designed to produce.

In the present study it is clear that meat production is enhanced at the lower experimental temperature. It is also evident

that at temperatures above 14°C a marked increase in ammonia excretion occurs. Holland and Chew (1974) report that specimens of Venerupis japonica from Hood Canal, Washington have ripe gonad in May-June when water temperatures rise above 14° C, and subsequently spawn in July-October. Ohba (1959) summarizes much Japanese work on the biology of Tapes japonica; two annual periods of spawning are reported in spring and late autumn coincident with a minimum water temperature of 14° C. Thus, results from both the literature and the present study indicate that a change in metabolic emphasis is evident above this temperature, the implications of which must serve as a precautionary note in situations were elevated culture temperatures are envisaged. In the case of species with distinct seasonal growth cycles further investigations are required as to their physiological responses to a non-seasonal temperature regime. In bivalve molluscs such investigations must include certain basic parameters; oxygen consumption and nitrogen excretion as indices of physiological rate and stress, glycogen content as an index of available reserve material for use under stress conditions, an histological quantification of gonad production and the relative production of meat and shell for assessment of economic potential.

SUMMARY

The effect of sustained temperatures of 12, 14, 16 and 18° C on the growth and ammonia excretion of the Manila Clam, Venerupis

semideccusata, were measured over a 77 day period. Meat growth was highest at 12° C, less at 14° C and 16° C, and the lowest at 18° C. No significant differences were noted in shell growth at the four temperatures. Ammonia excretion decreased from 12° C to 14° C but increased markedly at 16° C and 18°C. The implications of these results on the use of sustained elevated temperature in bivalve aquaculture systems are discussed.

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Yields of the seaweeds *Neoagardhiella baileyi* and *Gracilaria foliifera* in a waste recycling-polyculture system[1]

by

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A pilot-plant scale aquaculture system was developed at the Woods Hole Oceanographic Institution's Environmental Systems Laboratory in 1973 and its various components as well as the system as a whole have been continuously tested and evaluated in the two-year period since its completion (Ryther, 1975).

Briefly, the system consists of a series of PVC-lined, 15 x 15 x 1 m, 120,000 l-capacity ponds in which marine phytoplankton are grown in continuous, flow-through cultures on mixtures of treated wastewater effluent and seawater. Overflow (harvest) from the ponds is fed to a series of 12 x 1.2 x 1.5 m cement channels or raceways containing trays of bivalve molluscs. Lobsters and flatfish are grown together with the shellfish to utilize their solid wastes and the small invertebrate fauna that live on these wastes. The phytoplankton remove the nutrients (nitrogen and phosphorus) from the wastewater effluent and the animals remove the phytoplankton from the water, thus providing a nutrient-free final effluent (i.e., a biological tertiary sewage treatment) and crops of commercially-valuable animals.

In order to accomplish the first of these objectives, however, a final polishing step is necessary to remove the nutrients regenerated by the excretion of the animals and the decomposition of their solid wastes, as well as any nutrients not initially removed from the wastewater by the unicellular algae. This "polishing step" consists of macroscopic algae or seaweeds grown in suspension by vigorous aeration in one of the aforementioned cement raceways, modified only by

insertion of a sloping plywood bottom. Effluent from the shellfish raceway passes through the seaweed culture prior to its final discharge. As previously demonstrated (Ryther, loc. cit.), when the complete system is in proper balance it is capable of removing over 90% of the nitrogen from the original wastewater effluent, rendering it incapable of sustaining further algal growth.

The seaweeds grown in the final stage of the aquaculture system are those that have commercial value for their contained hydrocolloids, agar or carrageenan, which are used as stabilizers and emulsifiers in the food, drug, and cosmetic industries.

Initially the macroscopic red alga Chondrus crispus (Irish moss) was grown, but the species was found to grow slowly, had a tendency to become epiphytized by other filimentous species of algae, and in particular did not do well at the relatively high water temperatures (ca. 25°C) at which the system operated in summer. Because of the latter problem, Chondrus was replaced by other species of red algae that are more southern in their distribution, appearing in the Woods Hole area as summer annuals. These were Gracilaria foliifera, an agar-producing plant, and Neoagardhiella baileyi which, like Chondrus, is a carrageenan-producer. Both were collected locally during the summer of 1974.

The nutrient removal capacity of these macroscopic algae has been discussed previously (Ryther, loc. cit.) and various aspects of their

physiology, nutrient uptake kinetics, and the relationship between their nutrition and hydrocolloid production are reported elsewhere (DeBoer et al., Unpubl. ms.). Presented here are the yield data for the two species of seaweeds grown in the complete waste recycling-aquaculture system during 1975.

Because of major problems with shellfish culture, a considerable research effort was devoted to that area in 1975 including experimentation with flow-rates, the proportions of algal pond harvest and diluting seawater fed to the molluscs, temperatures, shellfish stocking density, and other factors. For that reason, the effluent from the shellfish raceways (which is equivalent to the input to the seaweed cultures) was highly variable throughout the year in its chemical and physical characteristics. In spite of that, the flow of water and the supply of nutrients are believed to have been adequate for and not limiting to seaweed growth during the entire year (Table 1). The primary factors influencing growth of the plants are believed to have been incident solar radiation and water temperature, neither of which would be controllable in a commercial seaweed culture operation at the same latitude. Thus the yields observed in our system are thought to be representative for the algal species, the culture system, and the climate in question, with the possible exception that they may have been somewhat higher in early spring and late fall, due to use of heated seawater, than would have been the case in an unheated culture system.

Table 1. Yields of Neoagardhiella baileyi and Gracilaria foliifera in 1975 with mean chemical and physical data in raceways as final polishing step of waste recycling-aquaculture system.

Dates	Days	Radiation Langleys/day	Nutrients (μmoles/l) $NO_2^- + NO_3^- + NH_4^+$	Nutrients (μmoles/l) $PO_4^{\equiv}$	Influent water temperature °C	Water flow l/min*	Density (kg wet wt/m_2) N. baileyi	Density (kg wet wt/m_2) G. foliifera	Mean production (g dry wt/m_2/day) N. baileyi	Mean production (g dry wt/m_2/day) G. foliifera
Mar 20-Apr 15	21	366	37	12	16	48	3.9	2.8	22	4
Apr 15-May 6	22	400	14	8	19	48	9.5	3.9	33	8
May 6-May 20	15	543	21	7	22	72	8.9	5.4	41	16
May 20-May 28	8	548	11	2	22	72	12.3	6.4	23	18
May 28-Jun 5	9	519	27	7	22	72	10.1	8.1	28	44
Jun 5-Jun 12	8	430	16	7	21	72	11.5	8.4	14	8
Jun 12-Jun 27	16	566	34	9	23	96	8.2	10.2	36	7
Jun 27-Jul 16	20	518	75	13	23	138	5.5	5.9	20	6
Jul 16-Aug 14	30	496	58	10	24	96	6.6	6.3	22	18
Aug 14-Aug 27	14	467	81	14	24	48-144	5.9	6.5	35	16
Aug 27-Sep 18	23	423			19	48-144	4.2	4.0	22	15
Sep 18-Oct 6	19	361			18	48-144	3.1	2.6	14	21
Oct 6-Nov 7	33	246			16	70	2.6	1.7	6	1
Nov 7-Nov 21	15	181	44	8	17	72	3.1	2.8	6	21
Nov 21-Dec 31	38	130	37	5	15	60-80	---	---	0	0
Jan 1-Mar 19	78	269			14	60-80	---	---	0 (assumed)	0 (assumed)
Mean Mar 20, 1975-Mar 19, 1976**									15	9
Mean Jun 27-Dec 31, 1975**									14	11

*48 l/min = 8 hrs retention in raceway or 3 exchanges/day

**time weighted

These yields are reported in Table 1. They were obtained by dip-netting the algae from the raceways into cloth mesh bags from which loose water was allowed to drain prior to weighing. Yields are reported as dry weight, independently determined as 12% of drained wet weight for both species. After weighing, the population was cut back to its starting biomass by harvesting the incremental growth. Normally, the starting biomass was 3.0 kg (wet wt) per m^2, though this was varied experimentally to some extent since the optimal density for maximum growth rates in the raceways had not previously been determined.

The populations were weighed and harvested at intervals of 8-38 days during the period March 20, 1975, when they were first stocked in the raceways, to December 31, 1975 when the experiment was terminated due to lack of further growth and deterioration of the algae. Generally, the population was weighed and harvested at intervals of about 1 week during the period of most rapid growth in spring and early summer and at longer intervals in the early spring and late fall. Mean densities of the seaweeds for the periods between harvest are shown in Table 1, these varying of course with the rate of growth.

The seaweeds were maintained in the raceways throughout the winter and early spring of 1976 before the remnant populations were discarded, but their condition steadily deteriorated throughout that period despite the fact that heated water was used in the shellfish

raceways. Presumably, the lack of growth in winter could be attributed to insufficient solar radiation and/or low temperatures. Although the latter did not fall below 12°C in the water entering the seaweed raceways, the fact these were uncovered and vigorously aerated resulted in a significant drop in temperature, to as low as 8°C during cold spells in midwinter, within the raceway itself. Thus although growth was not monitored during the period January 1 - March 19, 1976, it can be assumed to have been essentially zero, in which case the mean annual production can be calculated (Table 1). Mean values of seaweed production for the entire year of March 20, 1975 - March 19, 1976, assuming zero growth for the unmonitored period of January 1 - March 19, 1976, were 15 g dry weight per square meter per day for Neoagardhiella baileyi and 9 g/m^2/day for Gracilaria foliifera. These values are equivalent to yields of 55 and 33 metric tons per hectare per year respectively.

If mean production is calculated for the period June 26 - December 31, 1976, half the calendar and very nearly half the solar year (and a period for which hard data exist and no assumptions need be made), the values are 14 and 11 g dry wt/m^2/day respectively, essentially the same as those estimated for the entire year. Since half-year mean values from summer to winter solstices should be roughly the same as the full year means, the similarity between the two sets of data lend some support to the validity of each.

The mean yields obtained in this study were surprisingly similar to those recently reported for Gracilaria sp. and Hypnea musciformis

grown in essentially the same way in Florida (Lapointe et al., 1976). Yields of Hypnea from February through June ranged from 4.5 to 17.6 g dry wt/m^2/day and for Gracilaria, from 7.7 to 16.9 g dry wt/m^2/day from April through October. In both cases, growth was measured in rather short-term experiments lasting from 14 to 42 days each, following which the seaweeds had to be discarded due to epiphytization, fragmentation, or other causes. Apparently, the greater solar radiation in Florida is compensated for by other unfavorable environmental conditions, such as high water temperatures in the warmer seasons. It is anticipated, however, that once techniques for continuous culture of these or other, perhaps better-adapted species of seaweeds have been worked out for the more semi-tropical climate of Florida, annual yields of seaweeds in that region will increase significantly over those reported here for the temperature latitudes, where essentially no organic production occurs in winter.

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Effects of culture density and temperature on the growth rate and yield of *Neoagardhiella baileyi*[*]

by

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INTRODUCTION

A combined tertiary sewage treatment-marine aquaculture system has been developed which has the capacity of 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, 1975). 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 algae 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 (lobsters, 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 removing the dissolved nutrients regenerated by the animal culture prior to discharge of the final effluent.

Consistent with the objective of growing commercially valuable organisms in the aquaculture system, the seaweeds used in the "polishing" step have been species of red algae (Rhodophyceae) that contain the hydrocolloids agar or carrageenan which are employed as stabilizers or emulsifiers in the food, drug, and cosmetic industries. The species initially used, Chondrus crispus and Rhodymenia palmata, grew slowly at

all times, had a tendency to become heavily epiphytized with other, filimentous species of algae, and they could not survive the summer water temperatures, which at times exceeded 25°C. Particularly for the last reason, the seaweeds were changed to warmer-water species that are summer annuals in the Woods Hole region, Hypnea musciformis, Gracilaria foliifera, and Neoagardhiella baileyi. Of these, Hypnea tended to fragment and disintegrate at irregular but rather frequent intervals and its use was discontinued. The other two species proved highly successful both with respect to their own growth and yields and their nutrient-removal capacity, and their use in the combined system has continued.

Of the two species currently in use, N. baileyi has proved the more successful with respect to growth and yield. Although the alga did not grow in the four winter months (December through March), its average yield throughout the year, including winter, was 15 g dry weight/m^2/day, which is equivalent to 55 m tons/hectare year (DeBoer et al., 1976). Maximum productivity during late spring was recorded as 41 g/m^2/day. Such yields make this plant one of the more productive of the many aquatic and terrestrial photosynthetic systems, cultivated natural, for which information on sustained yields has been documented (e.g., Westlake, 1963). Furthermore, N. baileyi is of special potential value to the hydrocolloid industry because it contains iota-carrageenan,

a colloid previously found only in the tropical red alga Eucheuma spinosum. Though there is now a small commercial culture operation for growing E. spinosum in the Philippines (Parker, 1974) and it is harvested from natural population wherever it occurs in sufficient quantity, the supply is severely limited and its commercial utilization is at present resource limited.

The commercial cultivation of Neoagardhiella baileyi by and for itself, in a one-step aquaculture system, therefore appears to be a distinct possibility. If the seaweed could also serve as a biological tertiary wastewater treatment system, removing the nutrients from treated sewage effluent, the economic benefits from its culture could be further enhanced.

Experience in growing this algal species is, however, extremely limited, and little or no information concerning its physiology exists in the published literature. It was therefore considered desirable to carry out some rather basic studies on the growth of this algae, particularly with respect to the effects of those environmental factors that might influence the yields of the plant when grown outdoors in mass culture. The following report describes the results of some preliminary studies of Neoagardhiella baileyi with some supplementary information concerning the other species of red algae presently in culture, Gracilaria follifera.

RESULTS AND DISCUSSION

Effects of culture density on growth and yield:

The specific growth rate (growth per unit standing crop) and the productivity or yield (weight increase/m^2/day) were both measured for

N. baileyi grown out of doors in six plywood tanks. These measured 2.4 m (L) x 1.0 m (W) x 1.2 m (D), and were fitted with sloping bottoms and with an air line which extended the length of the tank along its greatest depth. The tanks, painted with white epoxy, were located in a geodesic dome fitted in winter with a transparent vinyl cover to retain heat. They received seawater from Vineyard Sound which was passed through a 20 μm sand-filtration system and, in winter, through a heat exchanger to maintain desired temperatures. The tank system, shown in Fig. 1, is described in more detail in Lapointe et al., 1976.

To determine the effect of culture density on specific growth rate and yield in winter, an experiment was conducted during the period December 4, 1975-January 12, 1976. Each of the six tanks were stocked with a different initial density of N. baileyi, ranging from 180 to 3 000 g wet weight/m^2. Filtered seawater heated to 18.5-21.5°C was enriched with ammonium chloride (NH_4Cl) and sodium phosphate ($NaH_2PO_4 \cdot H_2O$) so as to give concentrations of 50 μM NH_4^+-N and 10 μM $PO_4^=$. The heated, enriched seawater was passed through the tank on a continuous-flow basis at a rate of 1 tank volume (1 825 liters) per day.

Three times per week the seaweed was removed from the tanks with dip nets, drained of excess water, weighed, and returned to the tanks. Samples of the alga were oven dried at 60°C to obtain the relationship between wet and dry weight. Solar radiation was measured during the period with an Epply pyroheliometer.

The results of this experiment are shown in Fig. 2. Specific growth (% increase/day) decreased with increasing density of the culture, from 10% at about 500 g wet wt/m^2 to zero at 3 800 g wet wt/m^2. Productivity, or yield, which is a function of both specific growth rate and density, was highest at the intermediate density of 2 000-2 500 g wet wt/m^2, at which a value of about 8 g dry wt/m^2/day was observed.

The results shown in Fig. 2 are averages over the entire 40-day experiment, during which time the mean solar radiation was 136 langleys/day. During brief periods (ca. 1 week) of sunny weather when solar radiation averaged 160 ly/day, productivity increased markedly to a peak of 22 g dry wt/m^2/day at a density of 2 900 g wet wt/m^2. During cloudy periods (85 ly/day) production decreased to less than 5 g dry wt/m^2/day at densities of 500-1 500 g wet wt/m^2 (Fig. 3).

During the spring of 1976 the culture of Neoagardhiella was lost and a new culture could not be collected locally until early summer. The companion experiment to that described above, in which specific growth rates and yields are determined during summer, was therefore not completed at the time this report was prepared. However, the results of a similar experiment with another species of red algae, Gracilaria foliifera, are shown (Fig. 4) to illustrate the contrast between growth and yields during winter and summer conditions. Although there is no reason to assume that the two algal species would respond seasonally in exactly the same way, their relative growth at different times of has been found to be closely similar (DeBoer et al., 1976).

The experiment with G. foliifera was conducted during the period June 3-28, 1976. Mean solar radiation for the 28 days was 549 ly/day with a range of 162-768 ly/day. Experimental temperatures ranged from 18° to 21°C. The filtered seawater in this experiment, enriched with the same nutrients at levels of 60 μM NH_4^+-N and 10 μM PO_4^{-3}, flowed through the tanks at a turnover rate of 2 tank volumes per day.

Initial (stocked) densities of Gracilaria were respectively 400, 800, 1 600, 2 400, 3 200, and 4 000 g wet wt/m^2 in the six tanks. The algal populations were weighed twice weekly and cut back to the initial crop at each weighing.

The results of this experiment are given in Fig. 4, which again shows that specific growth rate (% increase/day) was inversely proportional to density of the population, ranging from 13%/day at a density of 600 g/m^2 to 4% at 4 500 g/m^2. Production reached its peak of 26 g dry wt/m^2/day at a density of 2 600 g/m^2 and was again less at both lower and higher population densities.

Effects of temperature on yield

The six experimental tanks described above were maintained two each at temperatures of 7°, 12.5°, and 18°C by blending ambient and heated seawater. The water, enriched with 50 μM NH_4^+-N and 10 μM PO_4^{-3} was introduced at a flow rate equivalent to 1.5 tank volumes per day. N. baileyi was stocked in each tank at a density of 1 500 g wet wt/m^2 and the populations were weighed and cut back to the starting biomass

every three days. After 17 days (February 6-22, 1976), the temperatures were change to 19.5°, 21°, and 23.5°C in the three experimental pairs of tanks at which conditions growth continued to be monitored for an additional 11 days (February 23-March 5, 1976).

Mean production of the seaweed ranged from 0.4 g dry wt/m^2/day at 7°C to 12.4 g/m^2/day at 18°C. At the two lower temperatures (7° and 12.5°C) yields were relatively constant throughout the 17 day experiment. At 18.5°C, production varied much more, from 7.0 to 23.0 g/m^2/day. The variations appeared to be loosely if at all related to changes in solar radiation.

At the three higher temperatures (19.5°, 21°, 23.5°) production initially increased (to a maximum of 27 g/m^2/day at 21°C), but then rapidly fell to levels below 5 g/m^2/day by the end of the experiment. The latter may have resulted from failure of the alga to adapt to the sudden 5-10°C increase in temperatures, but it also coincided with a period of extremely low solar radiation (< 100 ly/day) during the last week of the experiment.

It may be concluded from the preliminary experiments described in this report that commercial cultivation of Neoagardhiella baileyi would be limited to regions or situations where water temperatures range approximately between 10° and 22°C. For maximum productivity of the alga, cultures should be maintained at a mean density of 2-4 kg/m^2.

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Figure Legends

Figure 1. Seaweed culture tanks inside geodesic dome.

Figure 2. Production (o) and specific growth rate (*) as a function of the density of Neoagardhiella baileyi during the period December 4, 1975-January 12, 1976.

Figure 3. Production as a function of the density of Neoagardhiella baileyi and solar radiation during the period December 4, 1975-January 12, 1976.

Figure 4. Production (•) and specific growth rate (o) as a function of the density of Gracilaria foliifera during the period June 3-28, 1976.

Figure 5. Effect of water temperature on the carbon/nitrogen by weight and dry weight production in Neoagardhiella baileyi during the period February 6-March 5, 1976 The temperature in the tanks was increased on February 22 as indicated by the arrow. The vertical bars denote the 90% confidence intervals.

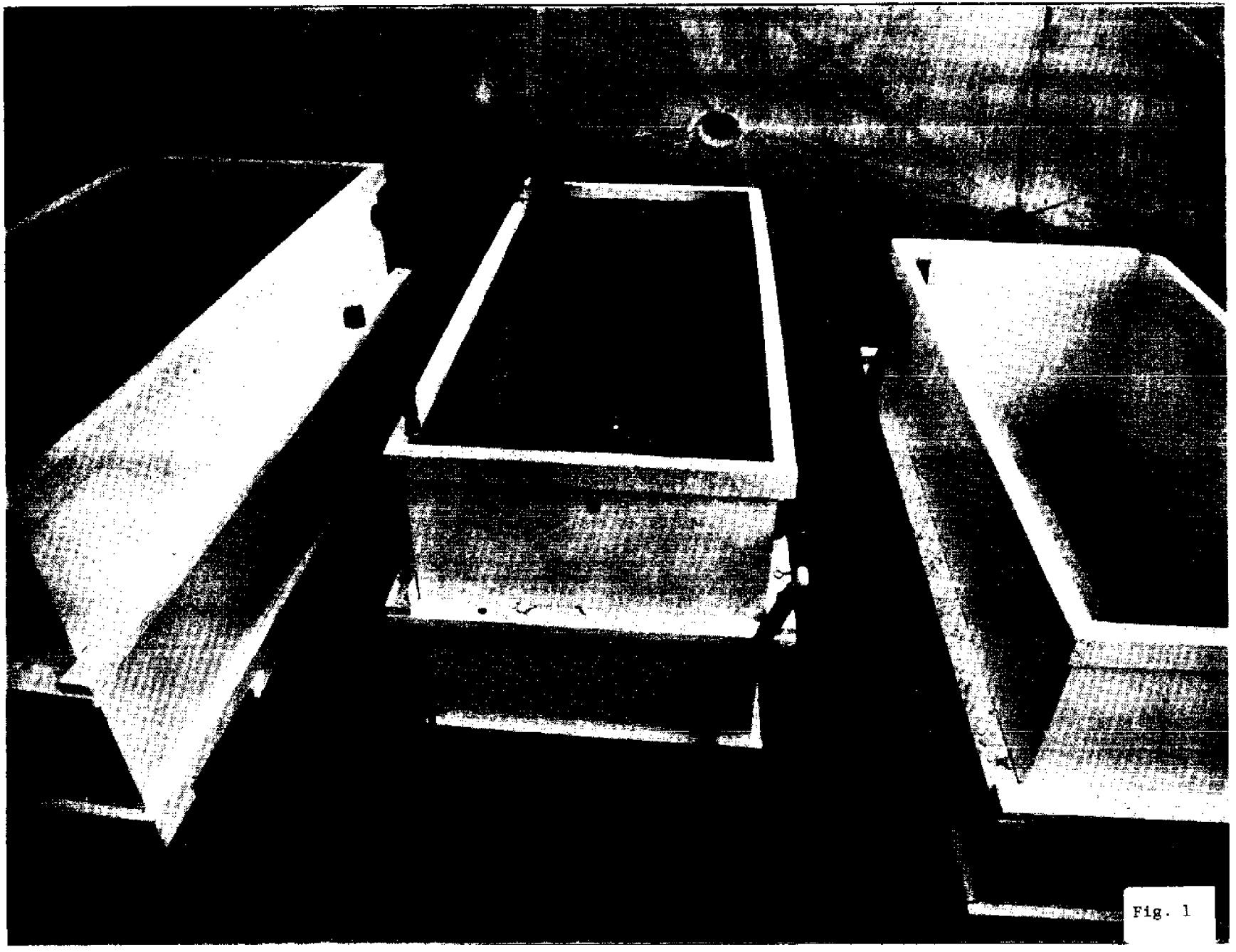


Fig. 1

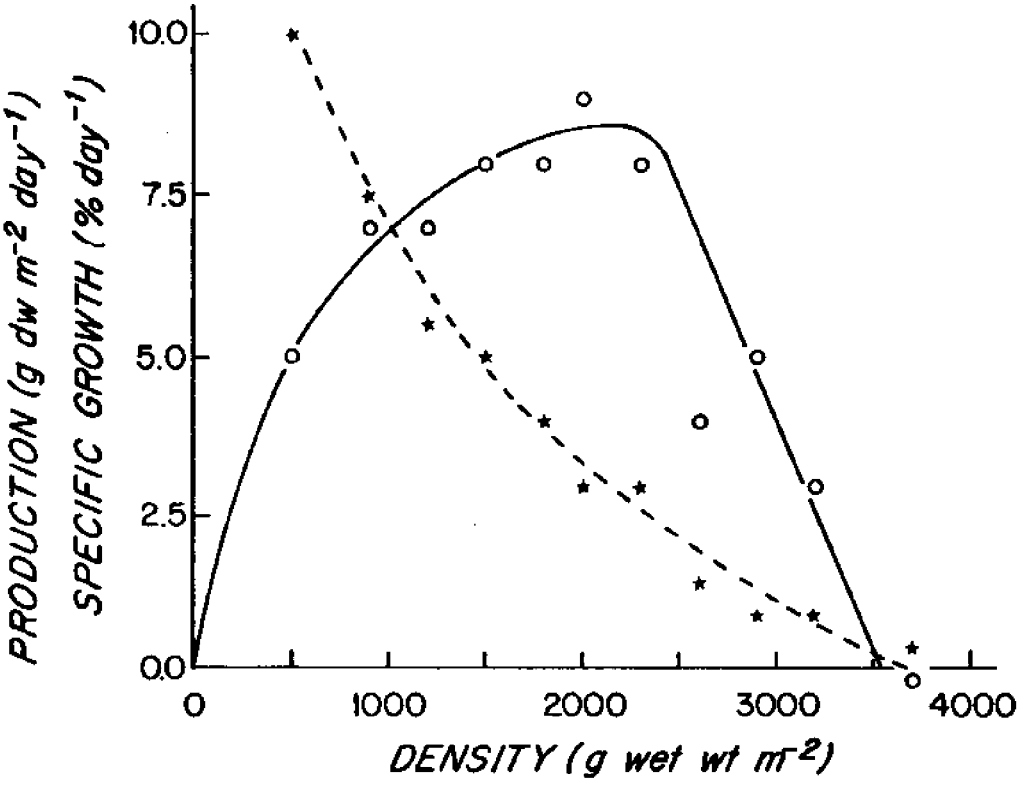

PRODUCTION (g dw m-2 day-1)
SPECIFIC GROWTH (% day-1)
10.0
7.5
5.0
2.5
0.0
0
1000
2000
3000
4000
DENSITY (g wet wt m-2)

Fig. 2

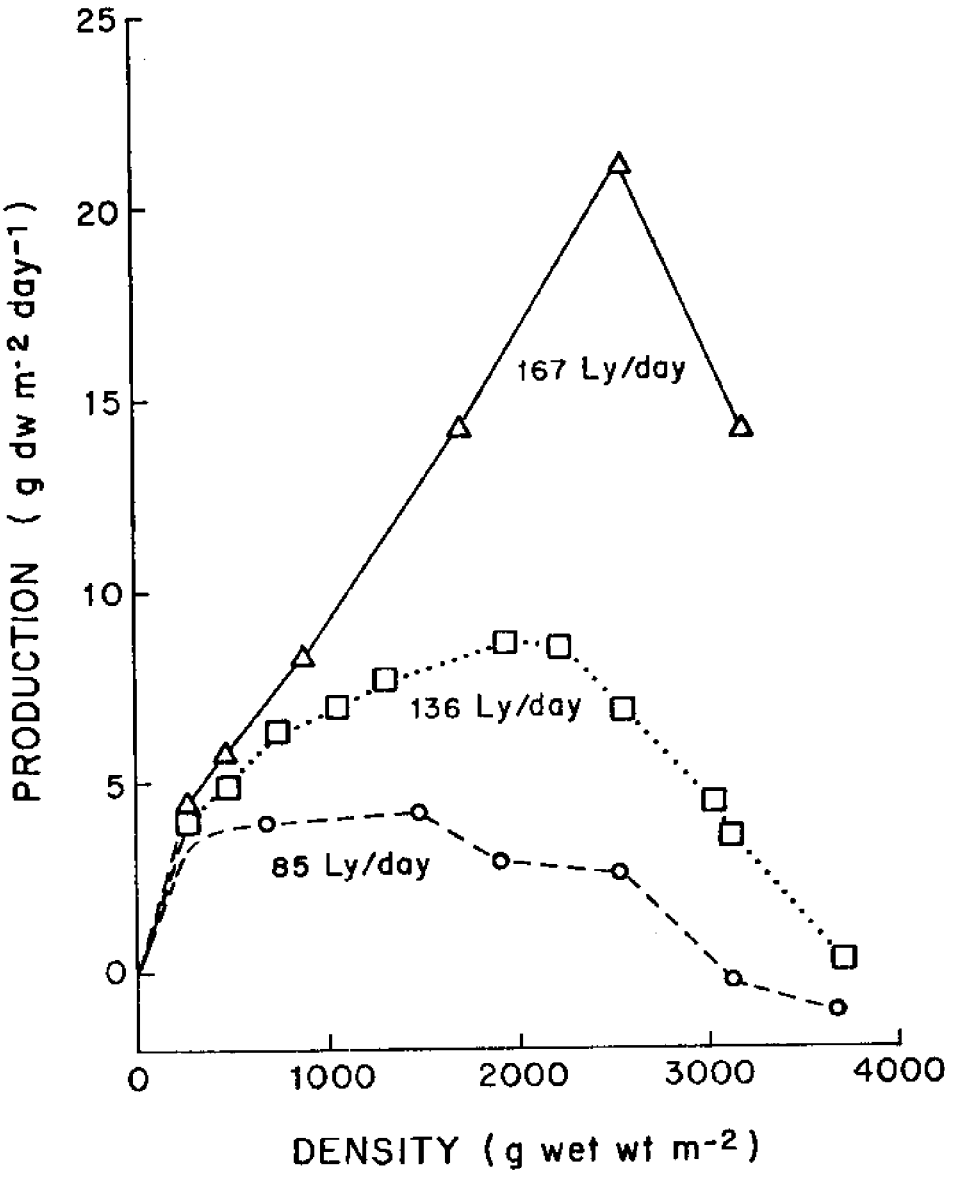

25
20
15
10
5
0
PRODUCTION (g dw m-2 day-1)
167 Ly/day
136 Ly/day
85 Ly/day
0
1000
2000
3000
4000
DENSITY (g wet wt m-2)

Fig. 3

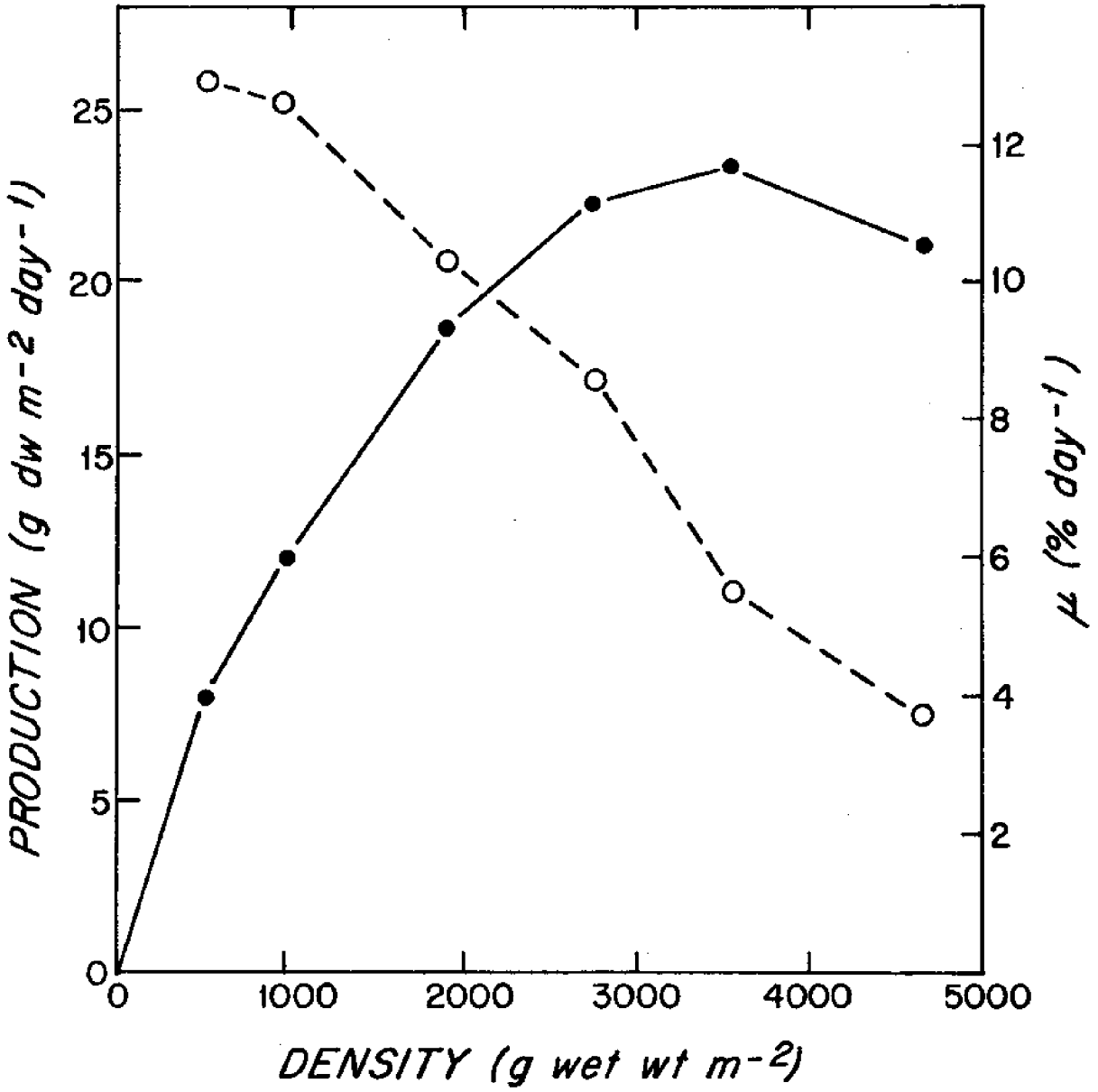
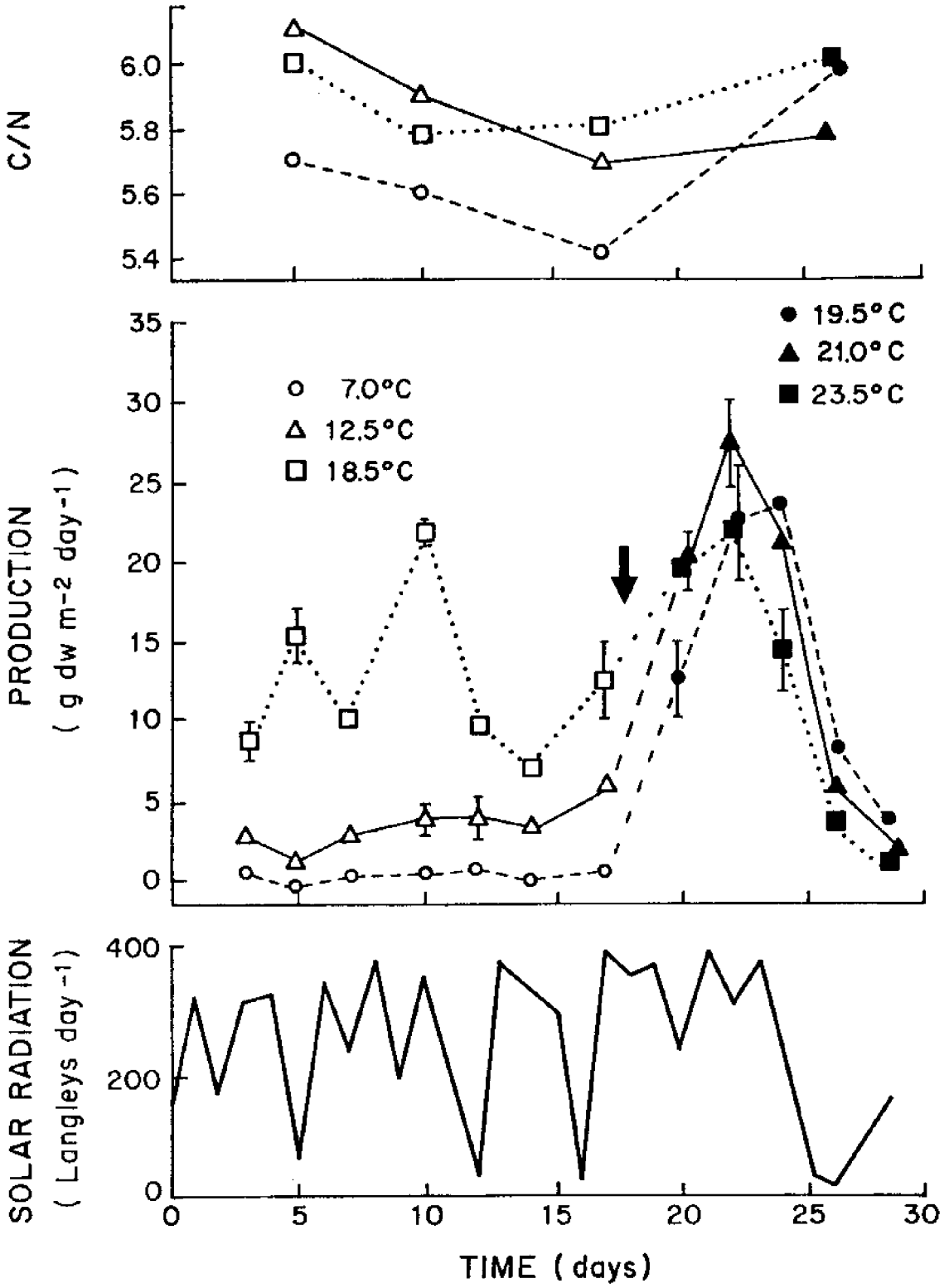

PRODUCTION (g dw m-2 day-1)
μ (% day-1)
DENSITY (g wet wt m-2)
0
5
10
15
20
25
2
4
6
8
10
12
0
1000
2000
3000
4000
5000

Fig. 4


C/N
6.0
5.8
5.6
5.4
PRODUCTION
(g dw m-2 day-1)
35
30
25
20
15
10
5
0
7.0°C
12.5°C
18.5°C
19.5°C
21.0°C
23.5°C
SOLAR RADIATION
(Langleys day-1)
400
200
0
0
5
10
15
20
25
30
TIME (days)

Effects of nitrogen concentration on growth rate and carrageenan production in *Neoagardhiella baileyi*[1]

J. A. DeBoer, B. E. Lapointe[2]

and C. F. D'Elia

Running head:

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Introduction

The hydrocolloid, carrageenan, is a sulfated polysaccharide obtained from several species of red algae that is used commercially in the food, pharmaceutical and cosmetic industries (Mathieson, 1975). The discovery that different chemical species or blends of carrageenan from different algal species have different gelling, emulsifying or stabilizing properties has led to a large number of new applications of this product. This has steadily increased the demand for carrageenan during the past few years.

Initially, most carrageenan was obtained from *Chondrus crispus* (Irish moss) harvested in the Maritime Provinces of Canada, Northern New England and Europe. Populations of *Chondrus* are now harvested to capacity. Although other sources of raw material have been found, the carrageenan industry is still resource limited (Mathieson, 1975, and Silverthorne & Sorenson, 1971). As a result, there has been a recent interest generated in the mass culture of *Chondrus* and other carrageenan-producing seaweeds.

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 research on the culture of unattached *Chondrus crispus* in tanks containing flowing seawater

(Neish and Fox, 1971). One of their early observations was that plants in the same tank grew at considerably different rates. One clone (T-4) grew much faster than others and, in addition, was less susceptible to epiphytization by undesired algal species. Another important finding was that the chemical composition of the plants could be altered by manipulation of the culture environment. Neish and Shacklock (1971) 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 such as the types of nutrients, temperature, amount and type of circulation, and population densities which yield maximum production were also investigated (Neish and Fox, 1971; Neish & Shacklock, 1971; and Shacklock *et al.*, 1973).

Another major source of carrageenan is the tropical red algal genus *Eucheuma*. Over harvesting of the natural populations in Southeast Asia has led to *Eucheuma* farming in the area. Parker (1974) describes cultivation methods, productivity, and the economics of *Eucheuma* farming in the Philippines.

There have been a number of laboratory studies of *Chondrus* and other carrageenan-producing algae in which photosynthetic rates under various light and temperature regimes (Mathieson and Burns, 1971; Mathieson and Dawes, 1974; and Dawes *et al.*, 1976), growth and reproduction (Burns and Mathieson, 1972; Dawes *et al.*, 1974; and Mathieson and Burns, 1975) and carrageenan content

(Fuller and Mathieson, 1972; Dawes *et al.*, 1974; and Mathieson and Tveter, 1975) have been examined. However, the studies by Neish and his collaborators, Parker, and those of this group in Woods Hole, Mass. (Prince, 1974; Ryther *et al.*; 1975, and DeBoer, *et al.*, 1976) and in Ft. Pierce, Fla. (Lapointe *et al.*, 1976) are the only published reports of large-scale intensive, outdoor culture of carrageenan-producing algae.

During the past year our seaweed research has concentrated on *Gracilaria foliifera*, an agarophyte, and *Neoagardhiella* which produces carrageenan (DeBoer et al., 1976a,b). Ryther and Dunstan (1971) found that nitrogen is the chemical nutrient most likely to be limiting algal growth in coastal marine waters. Our seaweed studies also indicate nitrogen to be the critical limiting factor in a seaweed mariculture system. As a result, this investigation was undertaken to determine the concentration of nitrogen at which maximum growth rate and carrageenan content occurs in *Neoagardhiella baileyi*.

Methods

Neoagardhiella baileyi used in the experiment to be described below was harvested from large, outdoor mass cultures where it had been grown for over 16 months as part of a waste recycling-marine aquaculture system (DeBoer *et al.*,1976a). Four kilogram-aliquots of the seaweed were stocked in each of six 2.4 m (l) 1.0 (w) x 1.2 m (d) plywood tanks fitted with sloping bottoms,

as described by LaPointe *et al.* (1976) and DeBoer *et al.* (1976b). The tanks were equipped with an airline on the bottom along the deep side so that the algae could be maintained in suspension by vigorous aeration. Seawater was circulated through each of the tanks at a rate of one tank volume (1825 liters) per day.

In one experiment (Oct. 30 - Dec. 1, 1975) three of the cultures were enriched with ammonium chloride and sodium phosphate at concentrations of 50, 100, and 150 μM NH_4^+-N and 10, 20, and 30 μM PO_4^{-3}. Two tanks were enriched with 5% and 10% (by volume) wastewater effluent from the activated sludge secondary treatment plant at Wareham, Mass. (see DeBoer *et al.* 1976a for more details on the use of sewage effluent). The wastewater effluent contained, on an average, approximately 1800 μM inorganic nitrogen (about 75% NO_3^- and 25% NH_4^+) and 300 μM PO_4^{-3}, so that 5% wastewater effluent was roughly equivalent to the intermediate strength of inorganic nutrients used in the same experiment. The sixth tank received no enrichment of its seawater input.

In a second experiment (March 21 - April 8, 1976) the nutrient concentrations of the enriched seawater were 4, 10, 18, 35, and 70 μM NH_4^+-4 and 1, 2, 3,7, and 14 μM PO_4^{-3} respectively in the five experimental tanks, again with an unenriched seawater control in the sixth tank. Flow rates were equivalent to three tank volumes per day, and the starting inoculum of seaweed was 1.5 kg.

The seaweed was removed by dip net from the tanks, drained, and weighed every 5-7 days (Exp. 1) or 3 days (Exp. 2). Samples were oven dried at 60° C to obtain dry weights and organic carbon and nitrogen content of the dried material determined with a Perkin-Elmer C-H-N Elemental Analyzer. Ammonia and nitrate-nitrogen were determined in the seawater, the sewage effluent and the enriched culture media once or twice a week using the methods of Solórzano (1969) and Strickland and Parsons (1972) respectively.

Phycoerythrin, an accessary photosynthetic pigment in red algae, was measured in the second experiment. Fresh plant tissue (250 mg) was ground with a mortar and pestle in 25 ml phosphate buffer pH 7.0. Following an overnight aqueous extraction at 5°C the samples were centrifuged at 5°C for 30 minutes at 27,000 xg. Phycoerythrin concentration in the centrifugate was determined spectrophotometrically using the extinction coefficient $E/^{1\%}_{cm} = 81$ (Ó Carra, 1965).

The techniques for carrageenan extraction were developed with the assistance of Mr. Charles Allen of Marine Colloids, Inc., Rockland, Me. Fifty grams of dried plant material were added to 800 ml of hot water and placed in a boiling water bath for 30 minutes. The samples were then blended with 3.5 g of CaO for 30 minutes. The volume was brought up to 1000 ml and the samples returned to the boiling water bath for 3 hours. Diatomaceous

filter aid (65 g) was added 20 minutes prior to the end of the cooking period. The hot mixture was immediately filtered with a hot, pressurized filter bomb developed by Marine Colloids, Inc. The filtrate was adjusted to pH 8.5 and then poured in two volumes of 85% isopropyl alcohol per volume of extract. The carrageenan coagulum was then washed in 85% isopropyl alcohol, pressed free of excess liquid and dried overnight at 60°C. The carrageenan content, determined gravimetrically after drying, 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 tap water for 2 minutes, drying, and then reweighing.

Samples of *N. baileyi* were taken for carrageenan analysis three times during the first experiment (October 30, November 13, and December 1) and at the termination of the second experiment.

Results and Discussion

Figure 1 shows carregeenan production, the nitrogen:carbon ratio (by weight) of the dried plant material, and rate of production of the *Neoagardhiella baileyi* in the first experiment. Production was initially high in all six experimental tanks but decreased in every case during the course of the experiment, probably due to a decrease in incident solar energy.

Following the initial high level of production during the first week of the experiment, during which the algae were becoming acclimated to the experimental condition, the yields were subsequently highest in the tanks enriched with inorganic nutrients,

averaging 7 - 8.5 g/m^2/day (dry weight). Little difference was observed between the different concentrations of nutrients employed. The seaweeds grew less well in seawater enriched with sewage effluent, with yields of about 5 g/m^2/day in 5% effluent and 2 g/m^2/day in 10% effluent. Yields in the unenriched seawater after the first week of acclimatization averaged only about 1 g/m^2/day. These data are summarized in Figure 2, which also shows carrageenan production for the same period of time (November 13 - December 1, 1975).

The carrageenan content of the *N. baileyi* was approximately 31% of the salt-free dry weight at the beginning of the experiment. As seen in Figure 1, this hydrocolloid level remained roughly the same during the entire experiment in all of the cultures receiving nutrient-enriched medium, but it increased steadily to a final concentration of 43% in the unenriched culture. These results substantiate those of Neish and Shacklock (1971) who found that *Chondrus* grown in unenriched seawater has a higher carrageenan content than *Chondrus* grown in nitrogen-enriched media. It is noteworthy, however, that total carrageenan production, a function of biomass production and the carrageenan content in the plant is highest in the enriched cultures (Figure 2). In other words, if colloid production is the objective, it is better to produce more low-carrageenan plants than less colloid-rich material. This would be especially true if the enriched cultures could be "carrageenan-fattened" by transferring into unenriched seawater following their growth, a possibility that needs to be

examined.

The nitrogen:carbon ratio in the dried algal material followed roughly the same pattern as the carrageenan content (Figure 1). In enriched seawater, this ratio stayed between 0.1 - 0.2 while in the unenriched culture it fell to less than 0.05 during the course of the experiment. Since the measurement of organic carbon and nitrogen in dried algal material with an automated elemental analyzer is both rapid and simple compared with the extraction and measurement of carrageenan, the use of N:C ratios as an index of the hydrocolloid content is a possibility worth further evaluation.

The concentration of nutrients in the culture medium and in the effluent from the culture was highly variable (0 - 45 μM NH_4^+-N), depending upon both input and rate of uptake. It was not possible from that experiment to draw any firm conclusions concerning the effect of nitrogen content on growth rate, though there was some indication that yields were inversely proportional to nutrient concentrations between 50 and 250 μM NH_4^+-N (see Figure 2).

In the second experiment, lower concentrations of inorganic nutrients were used at a higher flow rate through the cultures (three volume exchanges/day vs. one/day in Experiment 1). In addition, the starting biomass of algae was 1.5 kg/tank as compared to 4.0 kg/tank in Experiment 1, and incremental growth was harvested and removed from the cultures every three days. The reason for the increased flow rate and reduced biomass was an

attempt to maintain more nearly constant levels of nutrients in the cultures. This objective was not, however, fully accomplished as may be seen in Figure 3, which shows specific growth rate (% increase/day) as a function of both influent and residual (effluent) nitrogen concentrations, the latter measured at noon. Although the two were still not the same, it is the residual concentration that is maintained in steady state in the culture media and is encountered by the algae. These residual concentrations of nitrogen ranged from less than 1 μM, when input concentrations were 0-10 μM, to as high as 25 μM at an input of 70 μM. Growth of *N. baileyi* was virtually constant at residual concentrations from 0.5 to 25 μM. Result of other experiments to be reported elsewhere have shown, however, that nutrient uptake and hence residual concentrations in the medium vary diurnally. Thus measurments taken at noon are not representative of daily levels of residual nutrients, which must lie somewhere between the noon residual and the influent, medium concentrations. Maximum growth rate thus can be said to occur at a nitrogen concentration between 0.5 and 10μM.

In Figure 4, the specific growth rates observed in Experiment 2 are plotted as a function of the N:C ratio of the dried plant material. Growth rates increased with increasing N:C ratios up to a plateau of 12-16%/day at N:C ratios of 0.1 and above. It would appear from this graph that N:C ratios of less than 0.1 in the plants, which is equivalent to a nitrogen content of about

5% of ash-free weight, are indications of growth-limiting nitrogen levels in the media.

Figure 5 shows both carrageenan content and phycoerythrin concentration in the *N. baileyi* cultures in Experiment 2, both plotted as functions of the N:C ratio. Carrageenan content was highest at N:C ratios of 0.06 - 0.10 but fell to low levels at ratios of .15 - .20. Conversely Phycoerythrin concentrations increased rather consistently with increasing N:C ratios. Thus phycoerythrin is another, relatively simple index of carrageenan content. In this connection, it should be pointed out that an obvious criterion of carrageenan content is simply the color of the plants; those with high concentrations of the red pigment, phycoerythrin, 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 to colloid concentration 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.

In both experiments, contaminating species of algae increased with increasing nitrogen concentration. Very few contaminants were present at nitrogen levels at or below the lowest concentration that supported maximum growth, while at higher concentrations, epiphytes became a problem. The contaminants consisted of species growing on the tank walls (primarily diatoms, blue-green algae and *Enteromorpha*), epiphytes (*Enteromorpha* and *Ceramium*) and free floating species (*Ceramium*). These contaminating species could

decrease profits in seaweed mariculture by lowering the productivity of the desired species or by decreasing the economic value of the crop.

Table I shows the results of hydrocolloid analyses of seaweed samples sent to Marine Colloids, Inc., Rockland, Me. This preliminary work showed that *Neoagardhiella baileyi* contains iota-carrageenan, a type of colloid currently obtained commercially primarily from *Eucheuma spinosum*, an alga that is in short supply. Although the gel properties of the carrageenan obtained from *Neoagardhiella baileyi* are variable, the quality of some samples is equal to the very best iota-carrageenan obtained from *Eucheuma spinosum*. Gel properties are dependent not only on the carrageenan in a particular plant but also on the physiological condition of the plant (Neish and Shacklock, 1971; Fuller and Mathieson, 1972; Dawes *et al.*, 1974; Mathieson and Tveter, 1975), method of drying, and on the methods used to extract the carrageenan. It is entirely possible, that, with additional experimentation, the yields and properties of the iota-carrageenan can be improved such that *Neoagardhiella baileyi* could be commercially grown for its hydrocolloid content.

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Table 1. Properties of carrageenan obtained from *Neoagardhiella baileyi*. See reference Marine Colloids (1974) for an explanation of the terms.

Sample	Carrageenan Type	Yield %	1.5% Viscosity cps	SPI Water Gel g	Desert Gel g	Desert Gel Penetration cm
Collected Waquoit Bay, Mass. Aug. 1976	iota	12	42	299	89	2.78
Collected Waquoit Bay, Mass. Aug. 1976 and kept in unenriched seawater for one month	iota	20	150	400	141	2.84
Collected at ESL from settling pond Aug. 1976.	iota	20	20	172	85	2.58
Harvested at ESL from seaweed raceway Aug. 1976.	iota	13	55	290	96	3.00
Harvested at ESL from seaweed raceway Aug. 1976 and kept in unenriched seawater for one month	iota	18	50	295	105	2.60
Good *Eucheuma spinosum*	iota	25	50-100	300-600	120-160	2.5-3.25

Figure 1. Effect of various nitrogen concentrations and sources on the carrageenan content (in % of the salt-free dry weight), nitrogen/carbon by weight and dry weight production in *Neoagardhiella baileyi*.

Figure 2. Effect of various nitrogen concentrations and sources on the dry weight production (open bars) and carrageenan production (shaded bars) in *Neoagardhiella baileyi*.

Figure 3. Specific growth rate (μ) of *Neoagardhiella baileyi* as a function of the ammonia concentration of the enriched seawater dilution medium (solid line) and the average ammonia concentration measured in the tanks at 12 noon during the experiment (broken line). The vertical bars denote the 90% confidence intervals.

Figure 4. Specific growth rate (μ) of *Neoagardhiella baileyi* as a function of the nitrogen/carbon (by weight) of the plants. The vertical bars denote the 90% confidence intervals.

Figure 5. Phycoerythrin content (broken line) and carrageenan content (solid line) as a percentage of salt-free dry weight of *Neoagardhiella baileyi* as a function of the nitrogen/carbon (by weight) of the plants. The vertical bars denote the 90% confidence intervals.

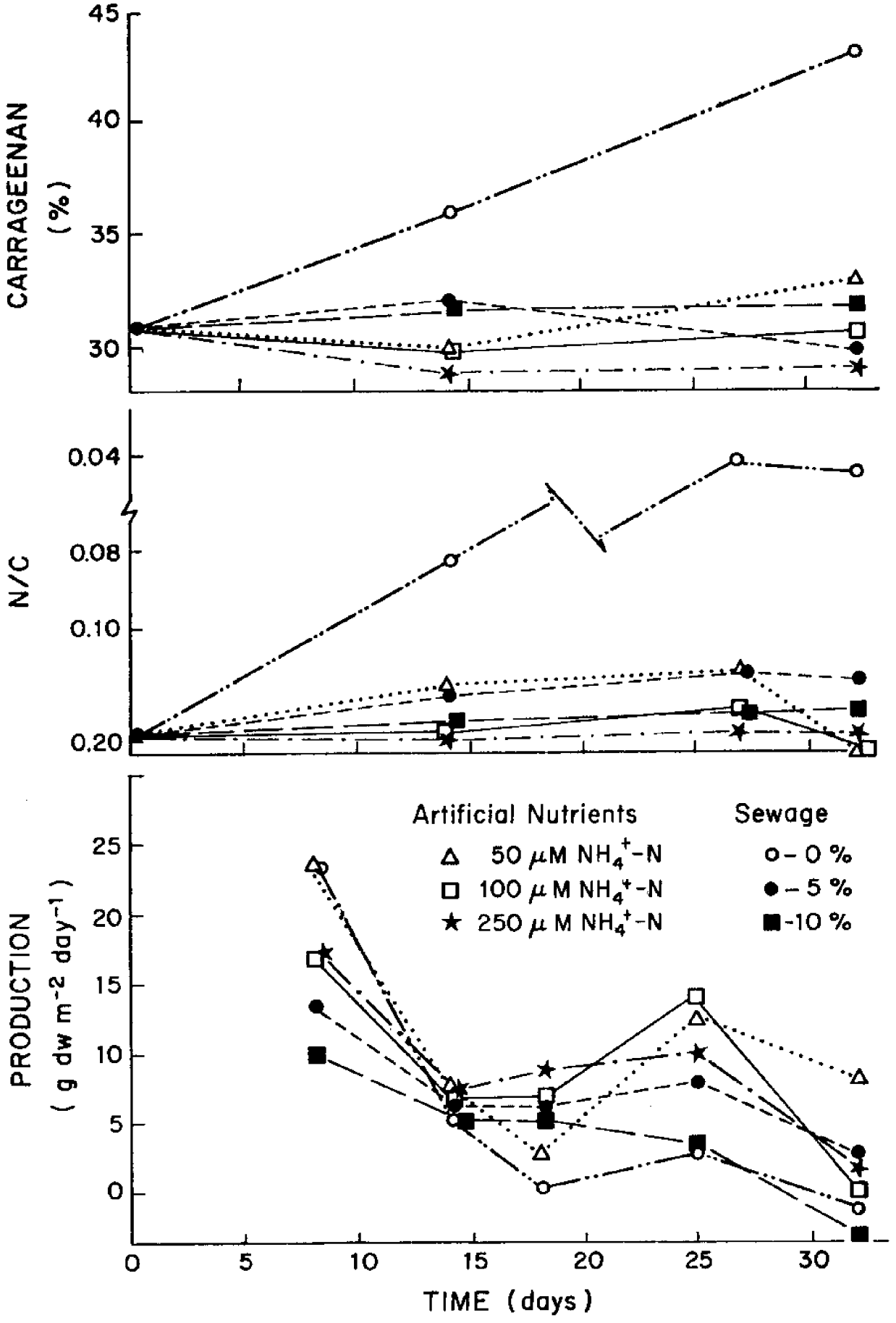
CARRAGEENAN (%)
45
40
35
30
N/C
0.04
0.08
0.10
0.20
PRODUCTION (g dw m^{-2} day^{-1})
25
20
15
10
5
0
0
5
10
15
20
25
30
TIME (days)
Artificial Nutrients
Δ 50 μM NH_4^+-N
□ 100 μM NH_4^+-N
★ 250 μM NH_4^+-N
Sewage
○ - 0 %
● - 5 %
■ -10 %

Fig. 1

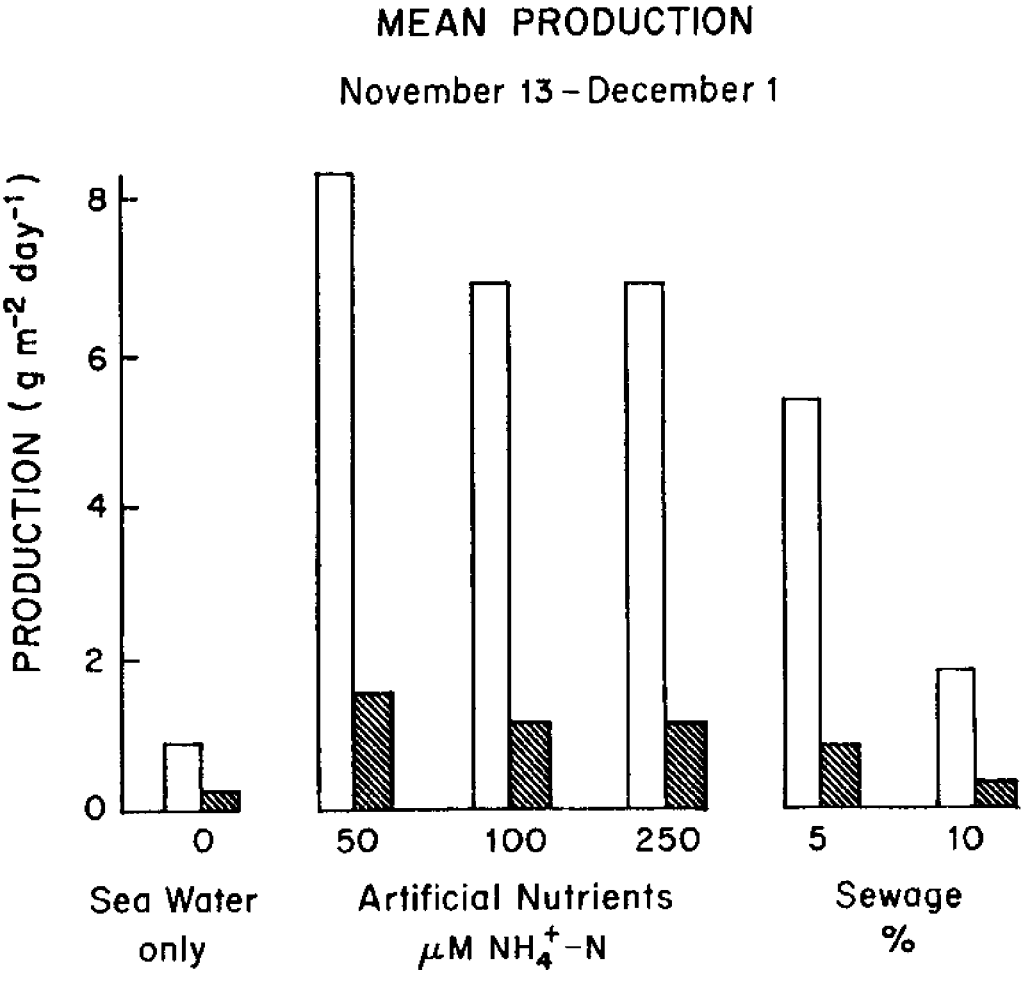
MEAN PRODUCTION
November 13 – December 1
PRODUCTION (g m-2 day-1)
8
6
4
2
0
0
50
100
250
5
10
Sea Water only
Artificial Nutrients μM NH4+-N
Sewage %

Fig. 2

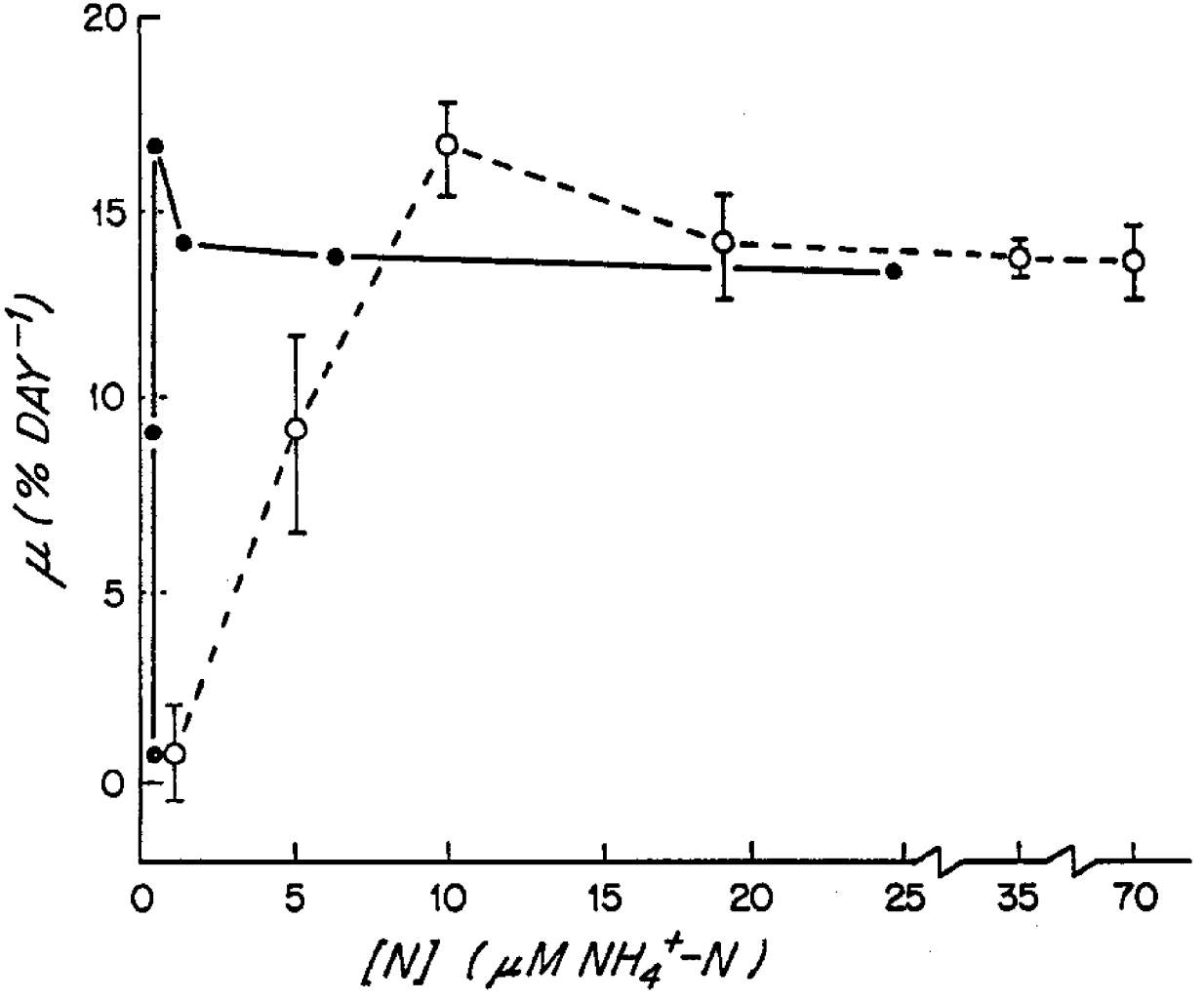

Fig. 3

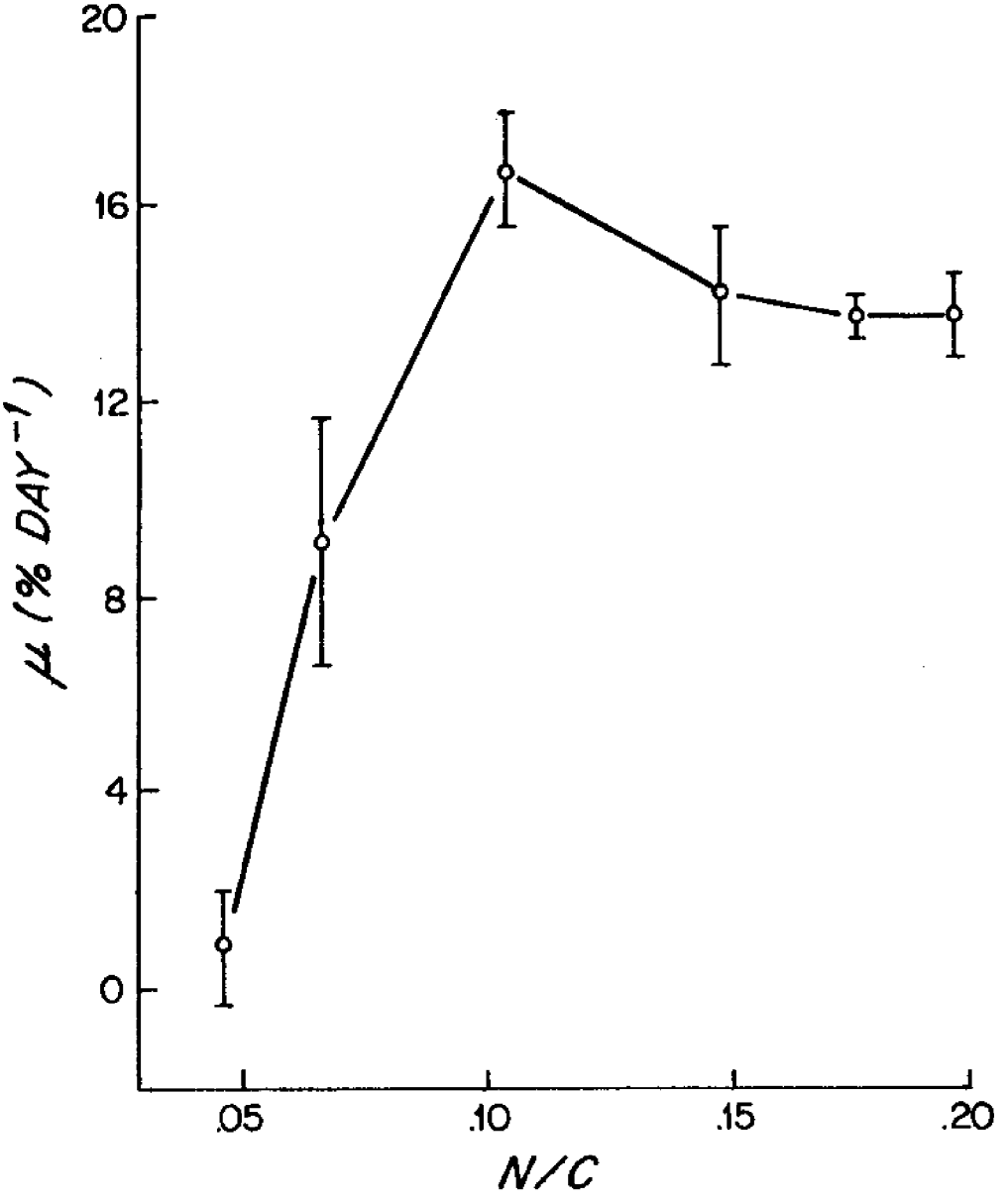

Fig. 4

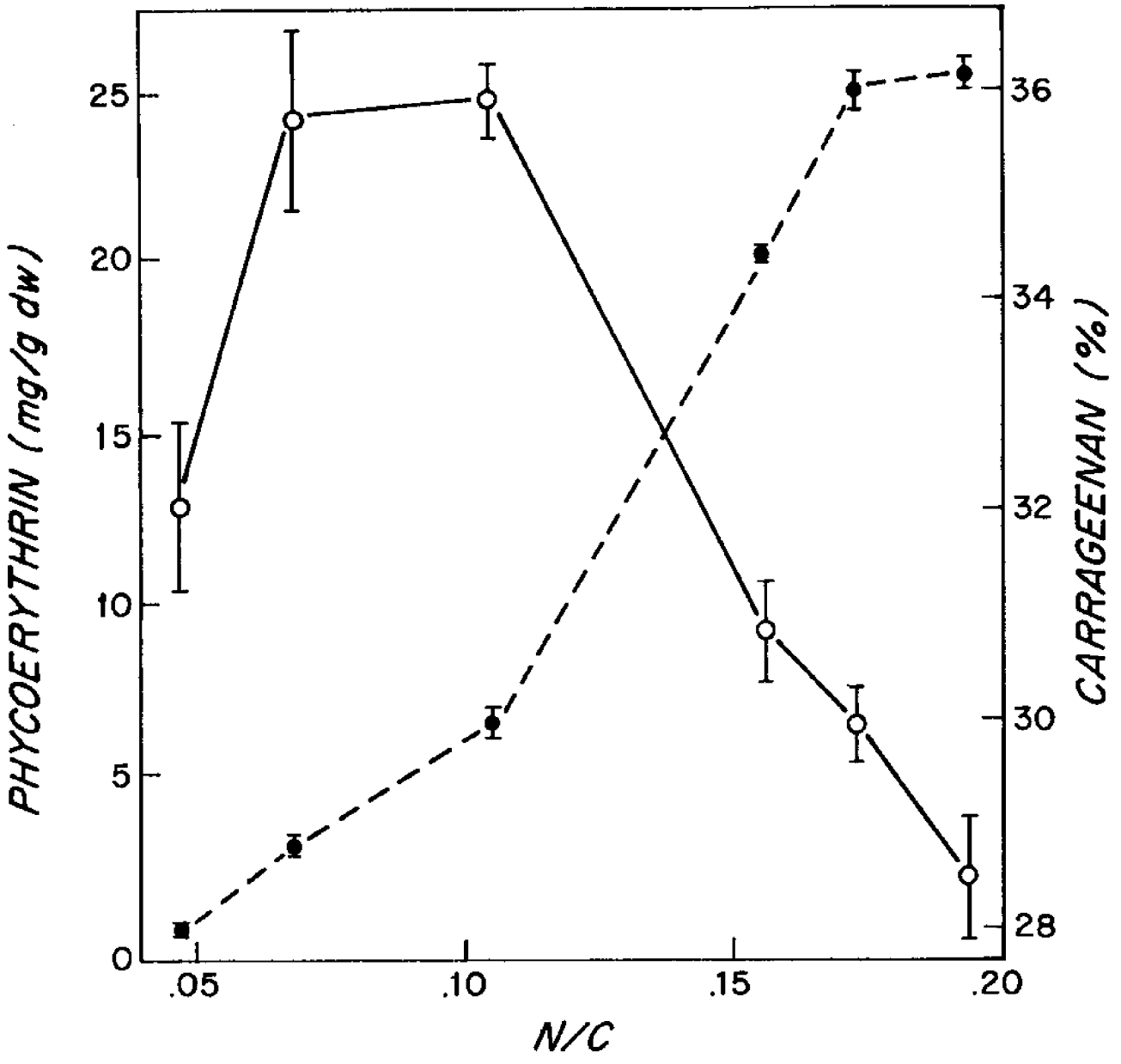
PHYCOERYTHRIN (mg/g dw)
CARRAGEENAN (%)
N/C
0
5
10
15
20
25
28
30
32
34
36
.05
.10
.15
.20

Fig. 5

Some aspects of the nutrient uptake kinetics of the macroscopic red alga, *Neoagardhiella baileyi*

by

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Waste recycling-marine aquaculture systems are being developed, tested and evaluated at the Woods Hole Oceanographic Institution (Woods Hole, Mass.) and the Harbor Branch Foundation (Fort Pierce, Fla.). In our systems, marine algae remove the nutrients from secondarily treated wastewater, performing a biological tertiary sewage treatment; the nutrients in turn provide for the growth of the algae. Macroscopic algae (seaweeds), in particular, are used either (1) as the sole component in a one-step tertiary treatment-aquaculture process, or (2) as a final "polishing step" at the end of a phytoplankton-animal polyculture food chain, in which the seaweeds incorporate nutrients not assimilated by the phytoplankton or regenerated by animal metabolism. In either case, the seaweeds used have been red algae (Rhodophyceae) which are commercially valuable in that they contain hydrocolloids such as agar and carageenan.

To date, studies of the seaweeds have concentrated on their growth and yields in the aquaculture systems (Lapointe et al., 1976; DeBoer et al., unpublished ms.). Less attention has been devoted to assessing and optimizing their nutrient-removal role in the biological tertiary treatment process. We have demonstrated that, under favorable working conditions, the plants are capable of almost complete nitrogen removal (e.g., Ryther, in press), and have calculated, from the rate of growth of the seaweeds and their composition, what their rate of nutrient removal must have been over the period of

their growth. We realize, however, that such estimates say nothing about short-term uptake characteristics of the algae that would be of critical importance if such a system were used to meet nutrient discharge standards.

Because phosphorus is present in wastewater at higher concentrations than nitrogen relative to the needs and utilization of these elements in plants, nitrogen becomes depleted before phosphorus and becomes the growth-limiting nutrient in seawater enriched with treated sewage effluent (Ryther and Dunstan, 1971). Complete nitrogen removal from wastewater therefore renders it incapable of supporting further growth of algae (Goldman and Ryther, 1975). For that reason, emphasis in the studies to be reported below has been devoted exclusively to nitrogen.

Surprisingly little has been published on the nitrogen uptake kinetics of the macroscopic algae, in contrast to the wealth of information that exists in this field with respect to the unicellular algae. Yet plant physiologists recognize that these larger algae are more highly organized, differentiated, and polarized than their single-celled relatives, and in this respect, perhaps more analogous to the higher plants. Thus, nutrient uptake kinetics of the seaweeds is of considerable interest to us in terms of basic scientific research as well as for the use of these plants in mariculture or waste treatment. The following report gives the results of some preliminary studies of the uptake kinetics of nitrogenous compounds by the red alga, Neoagardhiella baileyi.

Ammonium uptake kinetics

The relationship between nutrient concentration and its rate of uptake by unicellular algae may be described by the so-called Michaelis-Menten equation

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

where S = substrate or nutrient concentration and K and V_{max} are constants representing the half-saturation constant and maximum rate of uptake, respectively. There is some evidence that these constants are species specific among phytoplankton and may be used to characterize organisms or groups of organisms with respect to the nutrient environment in which they live or to which they have become adapted (Eppley et al., 1969b). Within limits (i.e., under nutrient-limited growth over long-term, steady-state conditions) nutrient uptake may correlate with growth and the above constants may be used in mathematical expressions of nutrient limited growth.

The following experiment was carried out to assess the applicability of using this approach to characterize nutrient uptake and perhaps ultimately growth characteristics of seaweeds as functions of substrate concentration. Approximately 35 grams (wet weight) of N. baileyi that had been maintained for several weeks in running seawater with a low concentration of nitrogen (0-1.0 μM) were placed in an eight-liter plexiglas growth chamber fitted with a circulating water pump to provide vigorous mixing. The chamber was illuminated with cool white fluorescent lamps providing an intensity of approximately 12,000 lux. Temperature of the seawater was maintained at 22°C.

After a brief, usually 15 minute period of acclimatization of the algae to the incubation conditions, the seawater was enriched with ammonium chloride so as to give a nitrogen concentration of 10 to 40 μM. This was allowed to mix thoroughly for about five minutes after which 25 ml samples were withdrawn at 5 minute intervals for 100 minutes. These were analyzed for ammonium-nitrogen using the method of Solórzano (1969).

Figure 1 shows a typical time course of ammonium-nitrogen uptake by N. baileyi. The curve shown was obtained by using a computer program that performed a least squares fit of the data to the integrated form of the Michaelis-Menten equation (cf. Caperon and Meyer, 1972).

Table 1 summarizes the results obtained in several such experiments with N. baileyi. The mean K value obtained from these experiments was 10.48 μM, a value considerably higher than has been reported for most marine phytoplankton, which typically have K values less than 1 μM (e.g., Eppley et al., 1969). This suggests that the seaweed has low affinity for NH_4^+ relative to phytoplankton; thus, higher nitrogen concentration may be required for optimal growth of the macroscopic than for the unicellular algae.

Diel uptake of ammonium-nitrogen by N. baileyi

Monitoring of the nitrogen removal from wastewater-enriched seawater medium by N. baileyi and the other seaweeds grown in the waste recycling-aquaculture system has given equivocal results, sometimes

with complete removal efficiences observed and other times with little or no evidence of uptake (Ryther, in press). Such experiences have suggested the possibility that uptake by the seaweeds is irregular and may be periodic.

To determine whether there is diel periodicity, an experiment was conducted in which N. baileyi was grown out-of-doors in large (1825 liter) wooden tanks that have been described in detail elsewhere (Lapointe et al., 1976; DeBoer et al., unpublished ms.). Approximately four kg of the seaweed were inoculated in the tank which received seawater enriched with ammonium chloride and sodium phosphate so as to provide input concentrations of 50 μM NH_4^+-N and 5 μM PO_4^{-3}-P respectively at a flow rate equivalent to one culture tank volume per day. Samples were withdrawn at three hour intervals for 27 hours and were analyzed for ammonium nitrogen as described above. The residual concentration of nitrogen in the tank (= output concentration) ranged from 6.5 μM at 0600 hrs to about 1.0 μM at 1500 hrs, showing clear evidence of diel periodicity (Fig. 2). Although it is difficult to calculate precisely, the increase in the residual nitrogen concentration from 1.0 to 6.5 μM relative to the input rate and concentration and an eight-hour night (the experiment was conducted June 29-30) is consistent with virtually zero uptake in the dark. Such a phenomenon was not observed in the marine phytoplankton ponds in our system, which have a constant, light-independent rate of nitrogen uptake (Goldman and Ryther, 1975). It also has important implications in any application of seaweeds

for advanced (nutrient removal) wastewater treatment purposes. However, the preliminary results reported here with N. baileyi must be repeated with that and other species of seaweeds before any generalization can be made.

The relative preference of N. baileyi for ammonium- and nitrate-nitrogen

To determine whether there is preferential utilization of NH_4^+-N or NO_3^--N by N. baileyi a series of experiments were carried out in which six-gram (wet weight) aliquots of the seaweed were incubated in vigorously-stirred 850 ml cultures of seawater in 1-liter beakers which were exposed to constant illumination at 12,000 lux and held at 20°C. One portion of the algae had been preconditioned for several weeks to running seawater enriched with ammonium chloride at a concentration of 100 μM of nitrogen. The other population was held for the same period of time in unenriched running seawater in which the nitrogen concentration was typically less than 1 μM.

Two each of the high-nitrogen and low-nitrogen preconditioned cultures were inoculated with approximately 210 μM of ammonium- and nitrate-nitrogen respectively. Samples were withdrawn at 15-minute intervals for analysis for ammonium (Solórzano, 1969) and nitrate (Strickland and Parsons, 1972) for a total period of 2.5 hours. Ammonia was rapidly assimilated by the alga in clear preference to nitrate, the concentration of which showed no reduction in the course of the experiment (Fig. 3) There was some indication that the algae preconditioned to a low nitrogen concentration (i.e., unenriched

seawater) removed ammonia more rapidly than did the plants that had been exposed to high levels of nitrogen, but the uptake of ammonia was rapid in both populations.

In another experiment, N. baileyi was preconditioned for three weeks in running seawater enriched with 80 μM nitrate-nitrogen (as sodium nitrate). A 50-gram (wet weight) sample of this conditioned material was then placed in an eight-liter plexiglas chamber as described above.

Initially, the culture was enriched with 65 μM nitrate-nitrogen and samples were removed every 10 minutes. After 55 minutes, the culture was further enriched with 65 μM ammonium-nitrogen and the sampling resumed at 10-minute intervals for another 85 minutes.

The results of this experiment (Fig. 4) show that nitrate was utilized at a slow rate when it was the sole form of nitrogen available (i.e., for the first 55 minutes of the experiment) and in plants that had been preconditioned for three weeks in nitrate (in contrast to the preceding experiment showing no nitrate uptake in algae preconditioned in ammonia; see Fig. 3). However, when ammonium-nitrogen was added to the culture, nitrate uptake ceased in response to a rapid preferential uptake of ammonium.

Although the two above experiments would appear to establish the fact that N. baileyi prefers ammonium to nitrate as a nitrogen source, growth of these algae in nitrate, in the absence of ammonium, is not precluded.

Although the nitrate-conditioned plants did, at best, assimilate nitrate very slowly in the absence of ammonium, it is possible that conditioning of the plants over much longer periods of time may result in much more efficient utilization of nitrate. Furthermore, different species, or even different strains of the same species, may differ in their nitrogen utilization.

What is believed to be the same species of alga (but referred to as Agardhiella tenera) and several other species of macroscopic algae, have been grown routinely in Florida over extended periods of time and at very high yields, both in an inorganic chemical enrichment with sodium nitrate as a sole nitrogen source and in seawater enriched with treated effluent in which virtually all of the nitrogen was in the form of nitrate (Lapointe et al., 1976).

Little if anything is known about the need for, existence, and development of a nitrate reductase system in the seaweeds. If nitrate must be reduced to ammonia before it can be assimilated by these plants, and if the enzyme system is not present in plants that have grown in ammonia, as is the case with marine phytoplankton (Eppley et al., 1969a) then it would seem quite possible that development of a nitrate reductase system may be a very long and slow process in these larger and more complex plants than in the case of the unicellular algae.

Table 1. K and V_{max} values for "N starved" (i.e., C/N ratio >20) N. baileyi.

K	V_{max}
(μM)	(μgat N . g dwt . min^{-1})
21.48	0.937
11.0	1.38
7.7	1.12
1.8	0.698
13.7	1.33
8.4	0.728
9.3	0.967
± 10.48	1.022
s 5.62	0.249

Figure Legend

Figure 1 Time course of NH_4^+-N depletion during an incubation of N. baileyi. Curve fitted by computer program incorporating the integrated form of the Michaelis-Menten equation.

Figure 2. Influent and residual (= effluent) NH_4^+-N concentrations over the diel cycle in tanks containing N. baileyi.

Figure 3. NO_3^--N and NH_4^+-N concentrations in the medium during incubations of N. baileyi grown on high nitrogen and low nitrogen media.

Figure 4. NH_4^+-N inhibition of NO_3^--N uptake by N. baileyi.

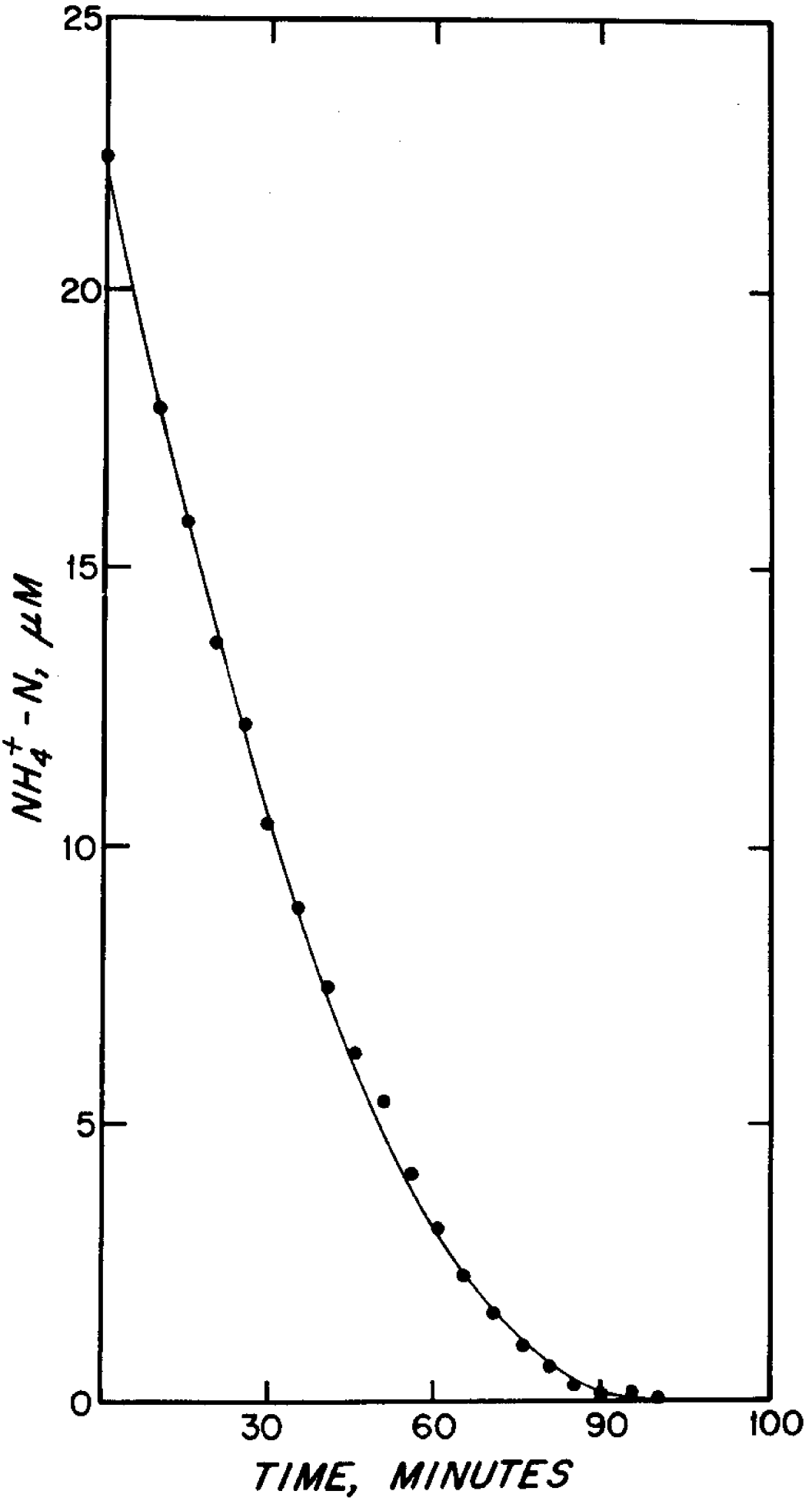

Fig. 1

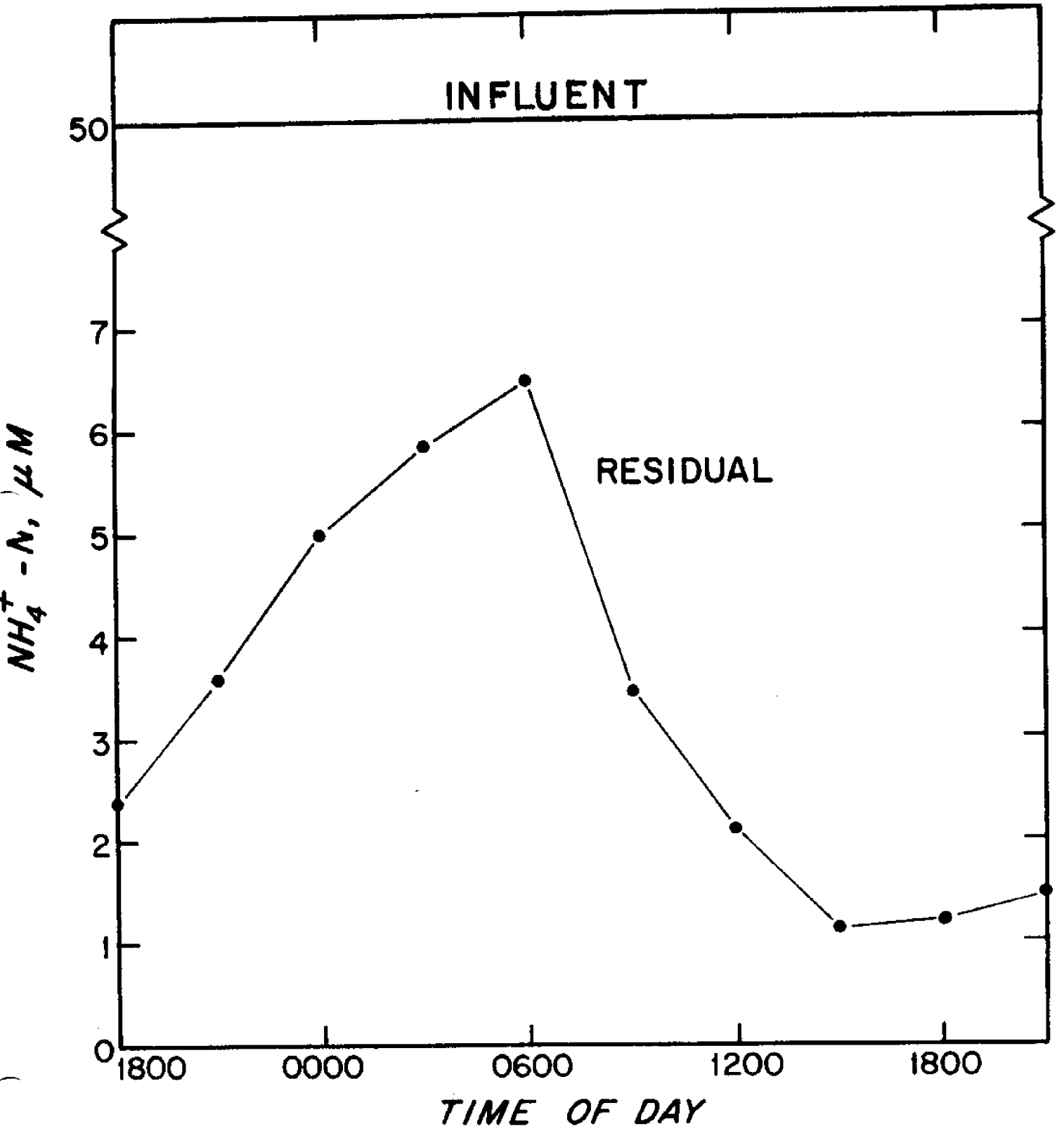
INFLUENT
50
7
6
5
4
3
2
1
0
RESIDUAL
NH_4^+ - N, μM
1800
0000
0600
1200
1800
TIME OF DAY

Fig.

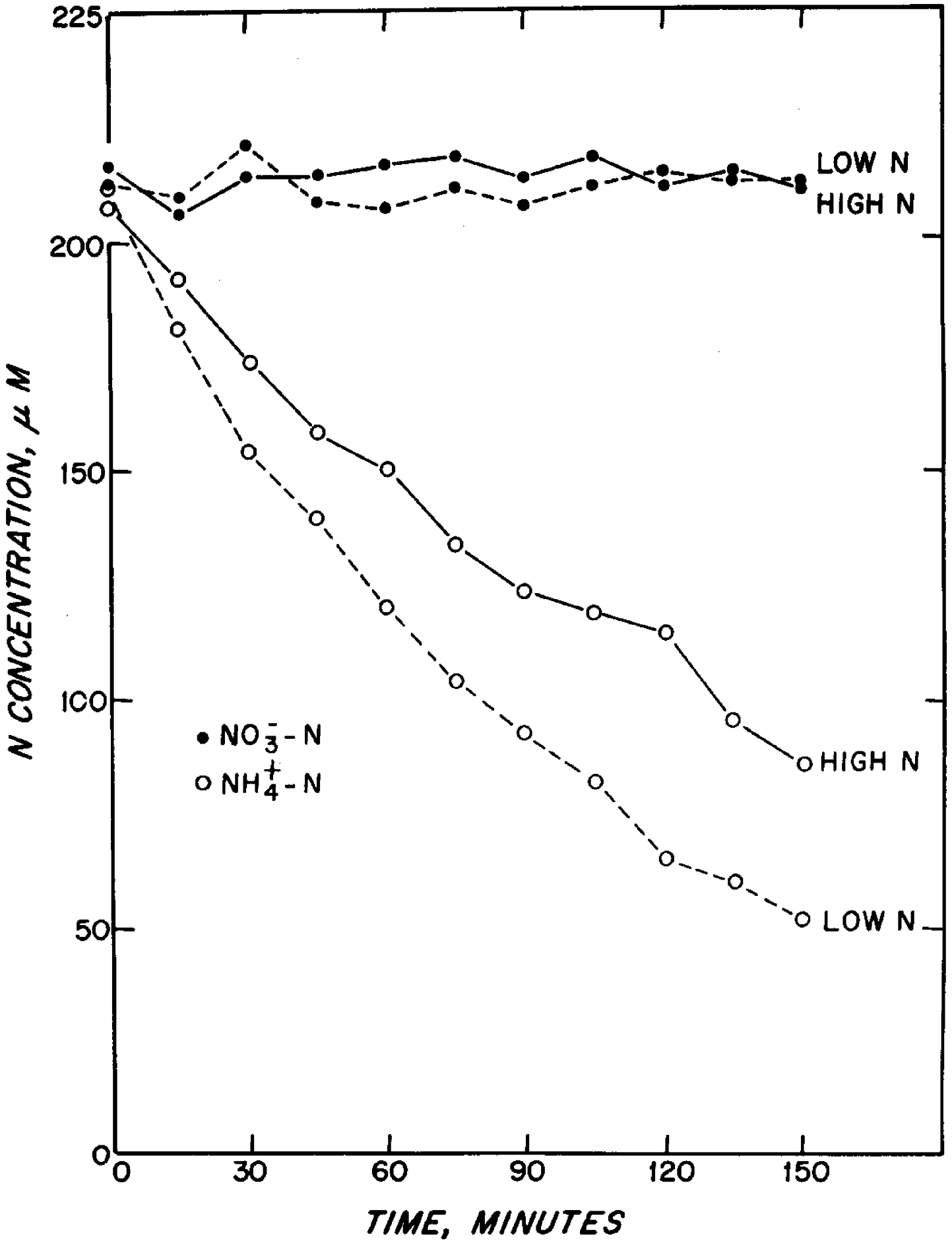
225
200
150
100
50
0
0
30
60
90
120
150
N CONCENTRATION, μ M
TIME, MINUTES
LOW N
HIGH N
• NO_3^- - N
o NH_4^+ - N
HIGH N
LOW N

Fig. 3

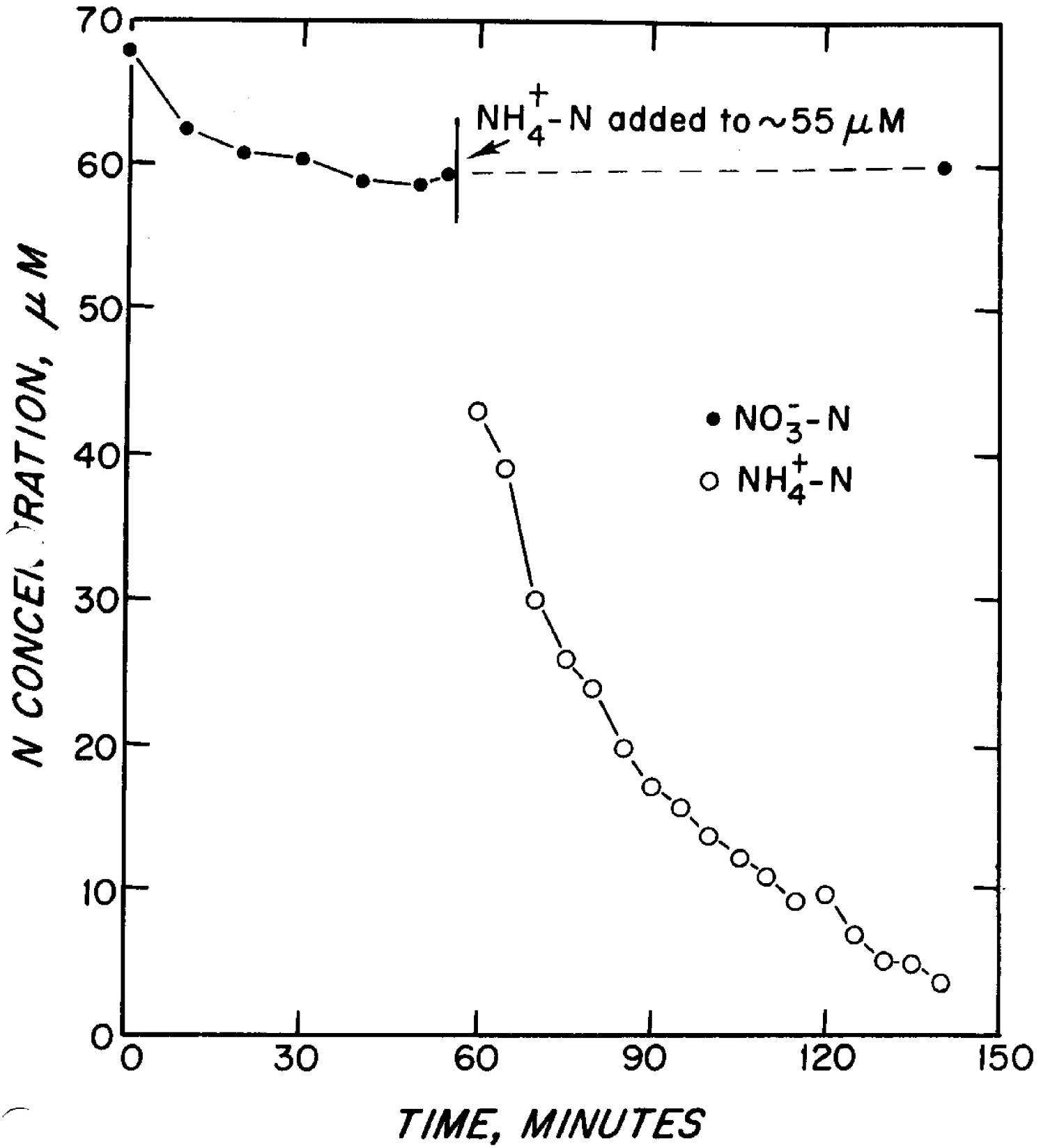
70
60
50
40
30
20
10
0
0
30
60
90
120
150
NH_4^+-N added to ~55 μM
• NO_3^--N
○ NH_4^+-N
N CONCEN RATION, μM
TIME, MINUTES

Fig. 4

THE IMPACT OF CRUSTACEAN HERBIVORES ON CULTURED SEAWEED POPULATIONS[1]

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[1]Contribution No. from the Woods Hole Oceanographic Institution.

ABSTRACT

The impact of two herbivorous crustaceans on seaweeds raised in an aquaculture system has been estimated from laboratory determined grazing rates and estimated crustacean densities in the system. Grazing losses are generally very small in comparison to algal productivity but may be significant when crustacean populations are abundant and algal productivity is low.

INTRODUCTION

A tertiary sewage treatment-marine aquaculture system has been developed (Ryther et al., 1972; Ryther, 1975) in which secondary sewage effluent is used as a nutrient source on which to grow phytoplankton; these algae remove nutrients from the water and, in turn, are themselves used as a food source for several species of shellfish. The final stage of nutrient removal consists of suspended seaweed cultures which assimilate nutrients not initially taken up by the phytoplankton as well as those reintroduced by shellfish excretion. The objective of this whole procedure is to reduce levels of nutrients in final effluent waters and at the same time to produce commercially valuable crops.

Several species of algae have been used in the final "polishing" step (Ryther et al., 1975) but current research has focussed on two potentially commercial species of red algae, *Gracilaria foliifera* and *Neoagardhiella baileyi*, which yield agar and carrageenan respectively.

Several species of crustacean herbivores have become abundant in these seaweed cultures. The isopod *Idotea baltica* and the tube dwelling amphipod *Ampithoe valida* are the most important of these grazers and potentially could be affecting algal productivity. Two other amphipod species are abundant, *Hyale plumosus* which occurs almost entirely on *Enteromorpha* growing attached to the side walls and *Jassa falcata* which is a suspension feeder eating mainly copepods abundant in the algal

cultures; neither of these species has much influence on the macroalgae of interest here.

The present study was undertaken to investigate the impact herbivores such as *Idotea* and *Ampithoe* have on cultured seaweed populations.

METHODS

The seaweeds were grown in two cement raceways, 1.2 m x 12.2 m x 1.5 m deep, and kept in suspension by circulation driven by an air line set along the bottom. Over most of the study period both *Gracilaria* and *Neoagardhiella* were each kept in half of each raceway, separated by a plastic mesh barrier which allowed animals to move freely from one algal species to the other. Over the winter months one of the raceways (#4) was heated to about 20°C and the other raceway (#2) was kept at 15°C; during extremely cold periods temperatures dropped below this, but remained above 11°C.

Grazer densities and size distributions were periodically sampled by taking 6 portions of the tumbling seaweed (each sample weighing approximately 30-60 gm wet wt) from each culture; these were treated with 3% formalin and hand sorted to separate the animals from the algae. Animals were counted and total body length of each was measured to the nearest mm (amphipods were stretched against a ruler to straighten them, resulting in some small error).

Growth rates were estimated from individually laboratory reared animals fed on *Gracilaria* at 20°C (rates for animals fed *Neoagardhiella* were also obtained for *Idotea*). Body length was measured weekly by immobilizing animals on a dissecting microscope stage with a piece of transparent plastic; amphipods were carefully straightened against a rigid edge. Measurement was done with an ocular micrometer calibrated to the nearest 0.1 mm. Repeated measurements showed accuracy to be within 0.2 mm. Molting frequency proved difficult to estimate but several molts/week occur for both *Idotea* and *Ampithoe*.

Grazing rates were obtained from laboratory experiments carried out at 12°C. Six species of algae were offered to isopods and amphipods: *Gracilaria foliifera, Neoagardhiella baileyi, Hypnea musciformis, Chondrus crispus, Fucus vesiculosus,* and *Corallina officinalis* (the former three are economically valuable species obtained from the mass outdoor cultures already described and the latter three are very abundant, readily encountered algae collected from natural field populations). Three grams (wet weight) of each of the above species were placed in each of 12 containers; four isopods (7-12 mm) were added to each of 4 of these containers and 4 amphipods (7-9 mm) were added to each of 4 others. The remaining 4 containers/alga were left without grazers, to serve as controls These were all left in a well-lighted water table for one week; at the end of this time, the herbivores were removed and the algae reweighed. Corrections were made for weight changes in the controls and for animal deaths during the experiment to arrive at the amount of each alga

consumed per individual grazer per week. From calculations based on these rates, the density of grazers, and on algal standing crop and productivity (DeBoer et al., 1976), the impact of *Idotea* and *Ampithoe* on seaweed cultures can be estimated.

RESULTS

The mean number of isopods per 100 gm (wet wt) of algae is shown in Table 1 and the same information for *Amphithoe* in Table 2. The numbers for *Idotea* are underrepresentative of true densities for two reasons. First, *Idotea* is a highly mobile animal and some individuals undoubtedly avoid the sampling net, although the majority tend to cling to the seaweed and so are sampled. Secondly, there is a portion of the population that clings to the side walls and to algae such as *Ulva* or *Ceramium* attached there. Sampling has shown that densities on attached *Ceramium* may be as much as 2 1/2 times those on the suspended seaweed (258 on *Ceramium* versus 101 on *Gracilaria*). Distribution is patchy, however, and sampling animals on the side walls is very difficult; this portion of the population remains unrepresented in the following analysis.

Such problems for *Ampithoe* are unimportant since it is rather sedentary. Animals build tubes and rarely leave them (Skutch, 1926). Therefore, few avoid the sampling net. Although amphipods are abundant in *Ulva* on side walls, due to their sedentary habits, this portion of the population has very little effect on the tumbling cultures.

There is a tendency for isopod densities to decrease in winter, even in the 20°C raceway. If a two-way analysis of variance is performed on densities in December (comparing raceways and algal species), it is found that densities are significantly greater on *Gracilaria* than on *Neoagardhiella* ($F_{1,20} = 6.40$, $P < 0.025$) and greater in raceway #2 (15°C) than #4 (20°C) ($F_{1,20} = 4.35$, $P = 0.05$). The cause of the difference between raceways is obscure, and probably unrelated to heating regimes, since it is evident in October before heat differences between raceways came into effect.

Ampithoe densities show a strong tendency to decrease over the period of study; the reason for this is again unknown. In December, densities are significantly greater on *Gracilaria* ($F_{1,20} = 53.76$ $P < 0.001$) although no difference between algae is evident in October ($F_{1,20} = 3.15$, $P = 0.08$). No temperature effects are seen and densities in raceways #2 and #4 are of similar magnitude ($F_{1,20} = 0.88$, $P > 0.75$).

Size distributions of *Idotea* (Fig. 1), as well as the presence of brooding females, reveal that reproduction is continuous throughout the year, although the percentage of very small animals present is lower in December. Size distributions are very similar on *Gracilaria* and *Neoagardhiella*.

Reproductive potential for *Idotea* is great since growth and development are fast and brood sizes relatively large. The life cycle is similar to that described for other species of the same genus (Naylor, 1975 a&b). Reproduction begins at a size of 7-9 mm; a maximum size of 23 mm for females and 30 mm for males has been observed. Eggs are

brooded in a thoracic pouch and brood size increases rapidly with female size (Fig. 2); a large female may produce more than 200 young. Development is direct and eggs are brooded for about 2 weeks, after which 2 mm juveniles are released. Females reproduce more than once but the actual number of broods per female is unknown. Growth is rapid, averaging 1 mm/week on *Gracilaria* and 0.33 mm/week on *Neoagardhiella*. At this rate, it takes 5-15 weeks to reach sexual maturity. Total life span is unknown but is probably less than 1 year, as seen from comparing observed maximum sizes with sizes at 1 year predicted from growth information.

Ampithoe life cycles are similar. Sample size distributions for September are shown in Fig. 3. Juveniles dominate the population and distributions on *Gracilaria* and *Neoagardhiella* are very similar. Due to the scarcity of animals in December and July, seasonal patterns are difficult to see; however, it is clear that reproduction is continuous. A maximum size of 12 mm was observed for both males and females. Young are again brooded although brood sizes are smaller and more variable than for *Idotea* (Fig. 4). Development takes about 2 1/2 weeks. Growth is rapid, again about 1 mm/week on *Gracilaria*. Reproduction begins at 6 mm; there may be several broods per female (Bousfield, 1973).

Grazing rates for similar sized isopods and amphipods are not significantly different on any alga except *Fucus*, which *Ampithoe* ignores (Table 3). The rates for *Idotea* on the 6 species of algae are not significantly

different from each other ($F_{5,16} = 2.24$, $P > .10$) but *Ampithoe* does graze *Hypnea* significantly faster than any of the other algae ($F_{5,16} = 6.31$, $P < 0.005$). The *Hypnea* used here was relatively unhealthy, however, and rates on robust *Hypnea* have not been determined.

DISCUSSION

It is possible to use the type of information discussed above to estimate the macroscopic algal losses to grazers, although only very approximate answers can be obtained. Given the density of algae (data from DeBoer et al., 1976), densities of grazers given in Tables 1 and 2 can be converted to an areal basis. Since most of the animals will be smaller than the 7-12 mm individuals used to obtain grazing rates, and hence will eat less, the calculated density must be reduced. An approximate correction factor of 0.5 has been used to produce a corrected density (an approximate density if all biomass were concentrated in 7-12 mm sized animals). When this density is multiplied by the grazing rates given in Table 3, a rough estimate of the amount of algal biomass consumed per week per m^2 is obtained. The results of such calculations for *Idotea* are shown in Table 4 and for *Ampithoe* in Table 5. It is evident that very little of the standing crop is being consumed by grazers; isopods have slightly more of an effect on *Gracilaria* and amphipods on *Neoagardhiella*.

This degree of grazing could be significant if it were concentrated on the growing tips of the plants. Observations of grazing damage does not support

this hypothesis, however, since damage is generalized throughout the plant.

What impact does this amount of grazing have on productivity? Algal dry weight is approximately 12% of wet weight (DeBoer et al., 1976); in Tables 6 and 7 the amounts of algae eaten by *Idotea* and *Ampithoe* respectively have been converted to a dry weight/m^2/day basis and compared with observed productivity values (data from DeBoer et al., 1976). These figures show that isopod grazing generally causes only a very small reduction in productivity of *Neoagardhiella* but that it may be significant, especially on *Gracilaria*, during times of low algal productivity. *Ampithoe* may have a significant impact on *Neoagardhiella* and *Gracilaria* during times when amphipods are abundant but such densities are uncommon.

These calculations have several implications. First, moderate grazer densities in actively growing summer cultures pose very little problem to the aquaculturist. A potential problem may arise if crustacean densities stay high when cultures are growing slowly, as in winter at temperate latitudes. Secondly, grazers may have different impacts on various algal species in culture. The easiest way to avoid the grazer problem is to emply a grazer resistant algal species like *Neoagardhiella*.

Control measures are difficult for small, highly mobile animals. Hand picking of algae is tedious, inefficient, and time consuming. Specific arthropod poisons such as Sevin are a possibility but are expensive and should be avoided in a polyculture system attempting to

produce a clean effluent. Fish predators could possibly be included in the system, although large *Idotea* are not favored by fish; *Ampithoe*, although readily eaten when found, is tubicolous and generally unavailable. Presently employed control measures consist of the removal of large isopods encountered during algal sorting and periodic drainage and cleaning of raceway walls; these appear to be keeping grazer populations within acceptable limits.

ACKNOWLEDGEMENTS

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TABLE I

Densities of *Idotea baltica* (mean #/100 gm wet wt of algae ± 1 standard deviation; N = 6).

Algal substrate	September	October	December	July
		Raceway #2 (15°C)		
Gracilaria	81.5±28.2	100.6±34.9	39.5±20.3	135.7±48.3
Neoagardhiella	-	30.4±17.3	15.3± 3.8	73.3±47.2
		Raceway #4 (20°C)		
Gracilaria	-	20.5±34.1	11.1± 3.8	61.2± 7.5
Neoagardhiella	23.1± 8.7	25.3± 7.8	15.8± 5.7	-

TABLE II

Densities of *Ampithoe valida* (mean #/100 gm wet wt of algae ± 1 standard deviation; N = 6).

Algal substrate	September	October	December	July
		Raceway #2 (15°C)		
Gracilaria	42.9±34.7	108.6±20.5	49.5±22.4	6.7± 2.3
Neoagardhiella	-	171.3±15.0	0.5± 1.6	28.1±26.1
		Raceway #4 (20°C)		
Gracilaria	-	83.8±43.6	47.9±11.2	9.8± 3.2
Neoagardhiella	304.0±93.8	62.2±24.7	9.2±5.5	-

TABLE III

Laboratory determined grazing rates (mean of 4 samples ± 1 standard deviation).

Algal species	Amount eaten by *Idotea* (gm wet wt/isopod/week)	Amount eaten by *Ampithoe* (gm wet wt/amphipod/week)
Gracilaria	.03±.03	.03±.02
Neoagardhiella	.01±.03	.02±.01
Hypnea	.09±.03	.09±.03
Chondrus	.01±.02	.03±.03
Fucus	.05±.02	0.00±0.04
Corallina	.07±.07	.03±.02

TABLE IV

Impact of *Idotea* grazing on algal standing crops

Alga	Month sampled	Density of seaweed (gm wet wt/m^2)$^{\Delta}$	Corrected density of *Idotea* (#/m^2)	Grazing rate (gm wet wt eaten/ grazed/week	Amount of algae eaten (gm wet wt/ m^2/week)	% of standing crop eaten
	Sept.	3100	358		3.6	0.1
Neoagardhiella	Oct.	2600	395	0.01	4.0	0.2
	Dec.	3100*	237		2.4	0.1
	July	3100*	1136		11.4	0.4
	Sept.	2600	1060		3.18	1.2
Gracilaria	Oct.	1700	855	0.03	25.7	1.5
	Dec.	2800*	553		16.6	0.6
	July	2800*	1900		57.0	2.0

$^{\Delta}$Data from DeBoer et al., 1976.

*Biomass estimated.

TABLE V

Impact of *Ampithoe* grazing on algal standing crops.

Alga	Month sampled	Density of seaweed (gm wet wt/m^2)$^{\Delta}$	Corrected density of *Idotea* (#/m^2)	Grazing rate (gm wet wt eaten/grazed/ week	Amount of algae eaten (gm wet wt/ m^2/week)	% of standing crop eaten
Neoagardhiella	Sept.	3100	4712	0.02	94.2	3.0
	Oct.	2600	2227		44.5	1.7
	Dec.	3100*	14		0.3	0
	July	3100*	436		8.7	0.3
Gracilaria	Sept.	2600	558	0.03	16.7	0.6
	Oct.	1700	923		27.7	1.6
	Dec.	2800*	693		20.8	0.7
	July	2800*	94		2.8	0.1

$^{\Delta}$Data from DeBoer et al., 1976

*Biomass estimated

TABLE VI

Impact of *Idotea* grazing on algal productivity.

Alga	Month sampled	Observed productivity (gm dry wt/m^2/day)	Amount of algae eaten (gm dry wt/m^2/day)	% of total productivity eaten
Neoagardhiella	Sept.	14.2	0.1	0.7
	Oct.	5.9	0.1	1.7
	Dec.	55.7*	0.04	0.7
	July	19.4*	0.2	1.0
Gracilaria	Sept.	20.9	0.5	2.3
	Oct.	0.8	0.4	33.3
	Dec.	21.5*	0.3	1.4
	July	6.1*	1.0	14.1

$^{\Delta}$Data from DeBoer et al., 1976

*Estimated

TABLE VII

Impact of *Ampithoe* on algal productivity.

Alga	Month sampled	Observed productivity (gm dry wt/m^2/day)$^{\Delta}$	Amount of algae eaten (gm dry wt/m^2/day)	% of total productivity eaten
Neoagardhiella	Sept.	14.2	1.6	10.1
	Oct.	5.9	0.8	11.9
	Dec.	5.7*	0.01	0.2
	July	19.4*	0.2	1.0
Gracilaria	Sept.	20.9	0.3	1.4
	Oct.	0.8	0.5	38.5
	Dec.	21.5*	0.4	1.8
	July	6.1*	0.04	0.7

$^{\Delta}$Data from DeBoer et al., 1976

*Estimated

FIGURE LEGENDS

Figure 1. Size distribution for *Idotea*.

Figure 2. The relation between *Idotea* female size and brood size ($r = 0.92$, $t = 6.52$, d.f. = 8).

Figure 3. Size distributions for *Ampithoe* in September. Distributions at other times of year are similar.

Figure 4. The relation between *Ampithoe* female size and brood size ($r = 0.62$, $t = 5.82$, d.f. = 53).

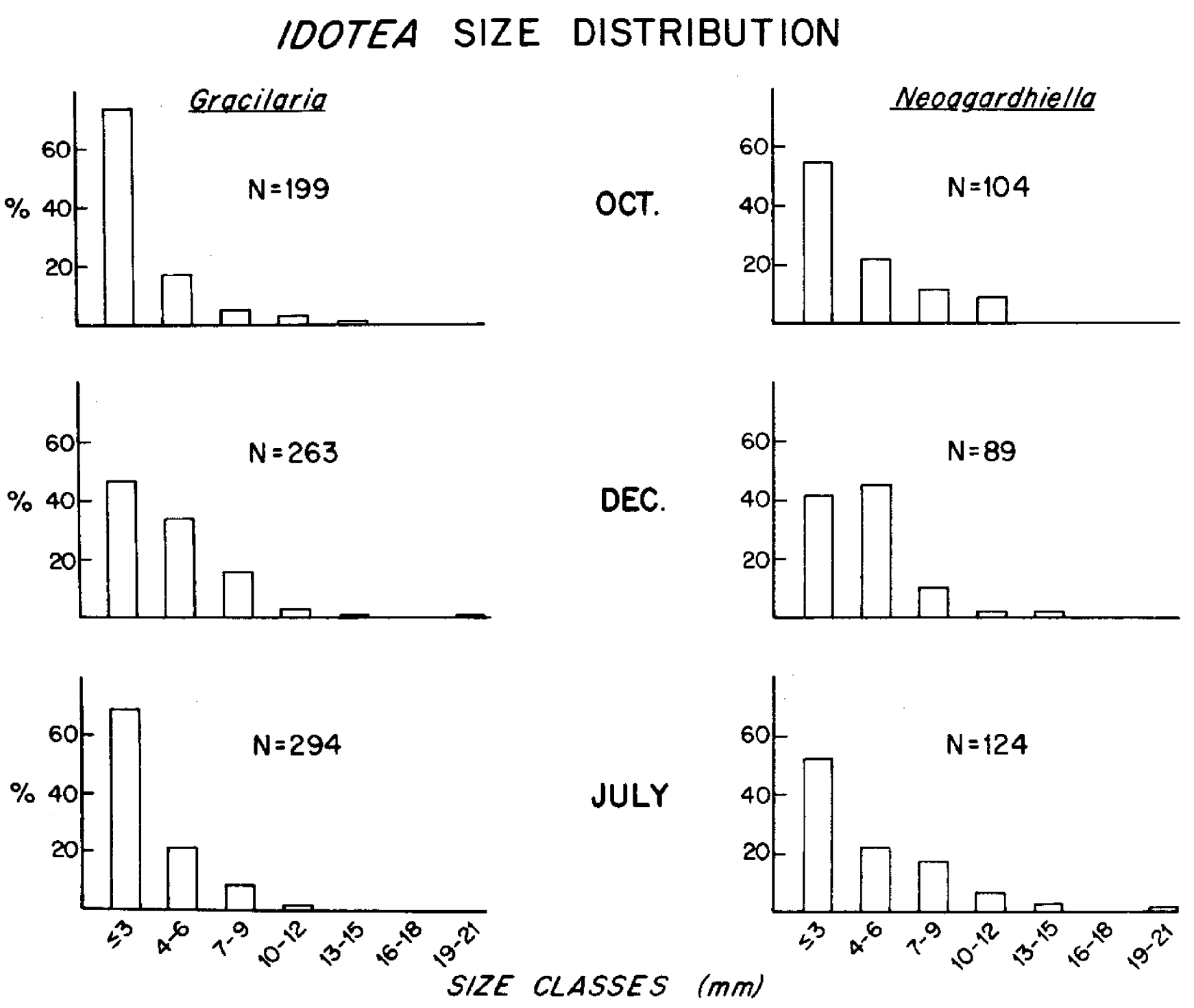
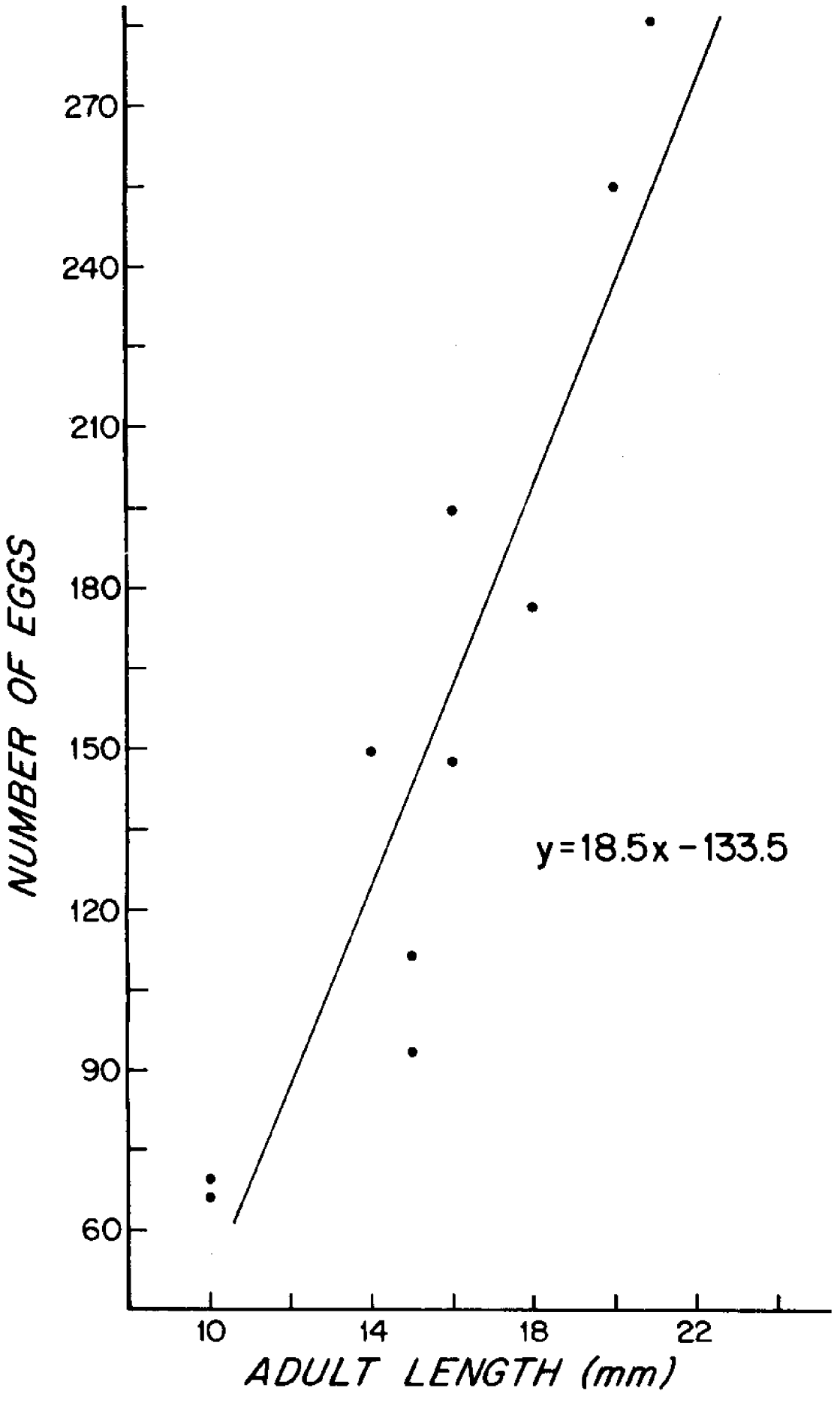

IDOTEA SIZE DISTRIBUTION
Gracilaria
Neoagardhiella
N=199
N=104
OCT.
N=263
N=89
DEC.
N=294
N=124
JULY
%
60
40
20
≤3
4-6
7-9
10-12
13-15
16-18
19-21
SIZE CLASSES (mm)

Fig. 1


NUMBER OF EGGS
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240
210
180
150
120
90
60
y=18.5x-133.5
10
14
18
22
ADULT LENGTH (mm)

Fig. 2

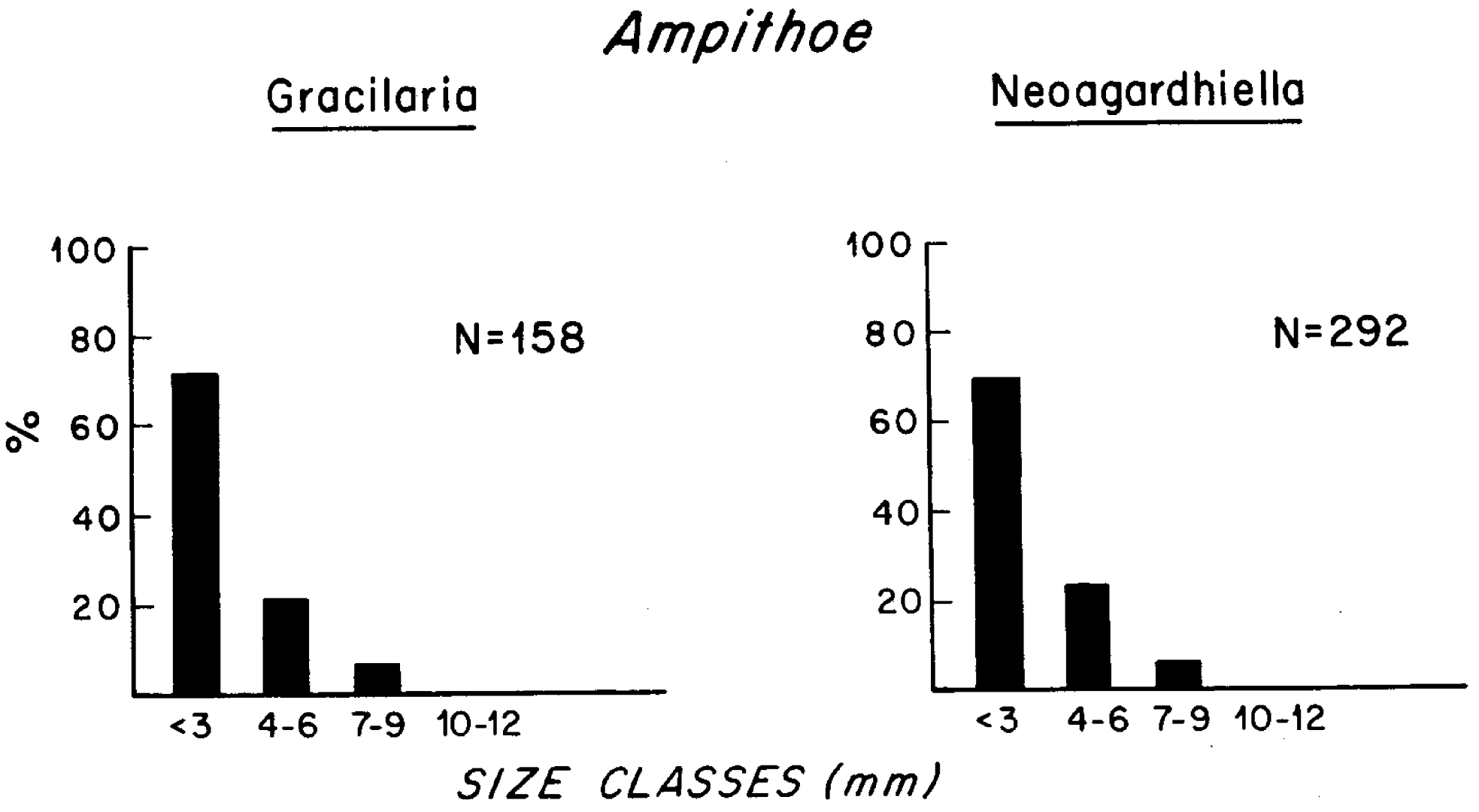
Ampithoe
Gracilaria
Neoagardhiella
100
80
60
40
20
%
N=158
N=292
<3
4-6
7-9
10-12
SIZE CLASSES (mm)

Fig. 3

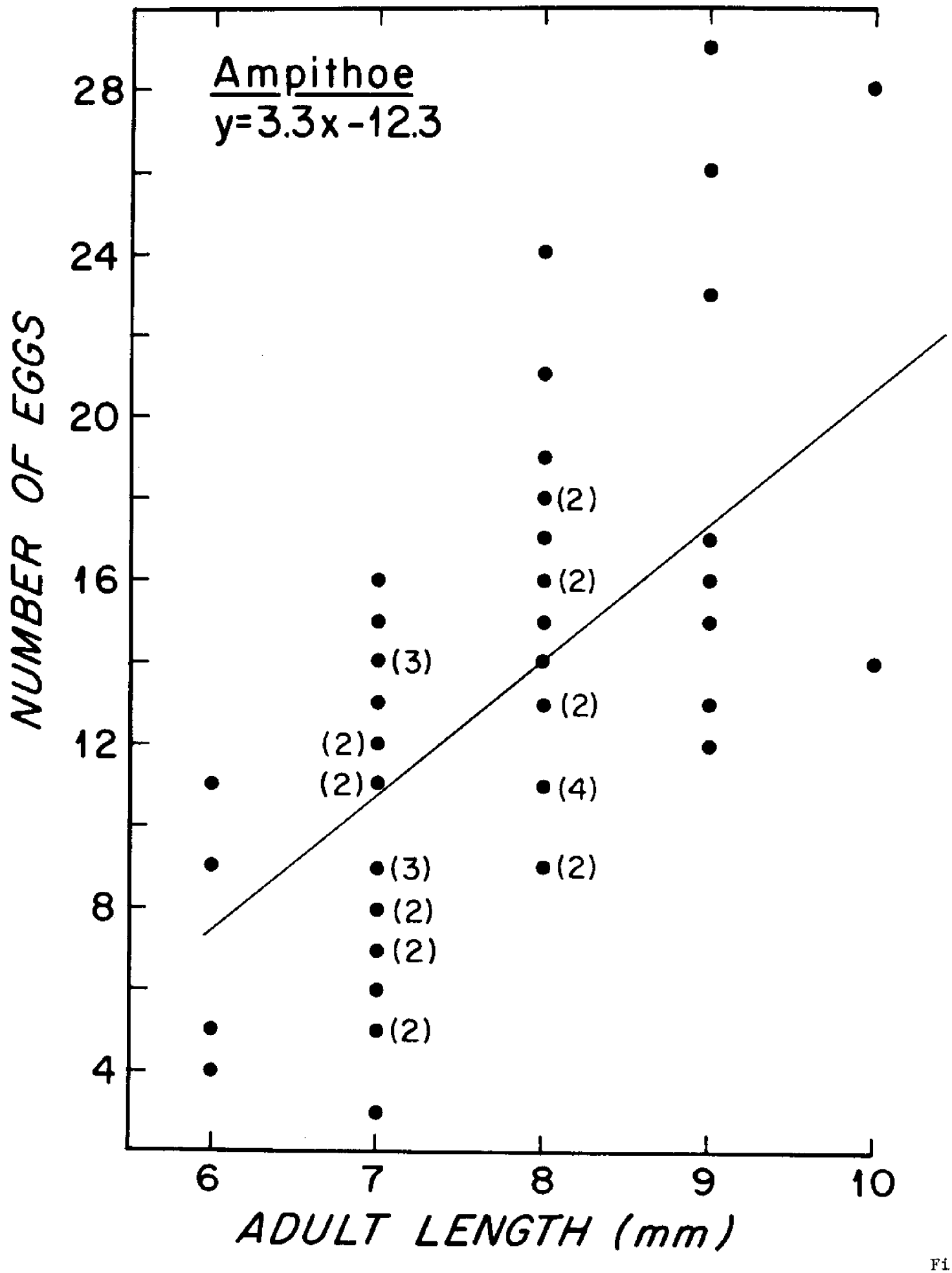
Ampithoe
y=3.3x-12.3
NUMBER OF EGGS
28
24
20
16
12
8
4
(2)
(2)
(3)
(2)
(2)
(4)
(2)
(3)
(2)
(2)
(2)
6
7
8
9
10
ADULT LENGTH (mm)

Fig. 4