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DETERMINATION OF THE RELATIVE VALUE OF PHYTOPLANKTON FOR FEEDING THE JUVENILE OYSTER, *OSTREA EDULIS*

DEPARTMENT OF BIOLOGY

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF PH.D. AT DALHOUSIE UNIVERSITY, JUNE 1984.

DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled " Determination of the Relative Value of Phytoplankton for Feeding the Juvenile Oyster, Ostrea Edulis"

by Catherine Theresa Enright

in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Dedicated to Dr. J. S. Craigie,

Atlantic Research Laboratory

National Research Council of Canada

in appreciation for encouraging me

to pursue my doctoral degree.

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ALGAL CARBOHYDRATES

Abstract

The growth response of Ostrea edulis (L.) juveniles when fed various phytoplankton species at a range of feeding densities was examined. Chaetoceros gracilis, C. calcitrans, Skeletonema costatum, C. simplex, Rhodomonas sp. and Thalassiosira pseudonana, yielded higher oyster growth rates than that obtained with the reference species Isochrysis galbana (clone T-iso). When fed the best diet, C. gracilis, the daily oyster growth rate was 1.5 to 1.8 times higher than that of the reference diet.

The biochemical composition of C. gracilis was altered by varying the nutrient conditions of the culture. The cellular protein level of the control cultures (complete f/2 media; Guillard and Ryther, 1962) and the silicate limited cultures was similar, but the nitrogen limited cultures had ca. 60% less protein. There was little change in the amino acid composition of all three cultures. The total lipid per cell in the silicate depleted cultures was 212% and 205% higher, respectively, than the control and the nitrogen limited cultures. The predominant fatty acids were 14:0, 16:0, 16:1ω7 and 20:5ω3. The essential fatty acid 22:6 ω3 content of the control, silicate limited and nitrogen limited cultures was 0.23, 0.08 and 0.10 $\mu g(10^6 \text{ cells})^{-1}$, respectively. The carbohydrate levels of the silicate limited and nitrogen limited cultures were, respectively, 163% and 320% higher than the control culture.

The growth response of O. edulis juveniles to the manipulated cultures of C. gracilis depended on feeding density. In an optimal feeding density range, the highest oyster growth rates were obtained

with the control cultures. The relatively high and adequate level of the 22:6ω3 fatty acid in this diet is a possible explanation. C. gracilis grown under silicate limited conditions yielded the highest oyster growth rate of all three diets at the lowest feeding density where insufficient food was supplied. The relatively higher caloric value of the silicate limited algal diet, due to the enhanced lipid level, may explain this result. The growth response of juvenile oysters fed various ratios of the three C. gracilis diets was examined. The highest oyster growth rates were obtained when 25% of the control algal cells were replaced with an equal number of nitrogen deficient cells. This suggests that higher oyster growth rates are possible with additional carbohydrate, provided that adequate protein is supplied.

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Objectives

The goal of this research project was to vary the biochemical composition of selected algal species and evaluate the growth response of O. edulis juveniles when fed these algal diets. The first objective was to monitor the growth response of the oysters reared on various, untested phytoplankton food species and compare their growth response with that obtained by feeding with traditional algal diets. The second objective was to select a good algal diet for juvenile oysters, based on the results of the previous oyster growth trials, and grow this alga under culture conditions that would induce distinct differences in the biochemical composition of the diet. By manipulating one algal species to create different diets, as opposed to feeding the oysters a variety of different algae, fewer cell parameters are changed between diets. By feeding these defined algal diets to juvenile oysters and monitoring their growth response, critical components of the oyster diet can be identified.

PART I

Part I

Introduction

a) Significance

The lack of a complete and inexpensive food source for the Ostrea edulis (L.) juvenile is a major restriction in the pursuit of intensive cultivation of this species. Because the nutritional requirements of juvenile oysters are not known, it is difficult to develop an efficient feed. If a cost effective diet were available for juvenile oysters, the expense of nursery culture would be greatly reduced.

Oyster development after metamorphosis is greatly enhanced when oysters are transferred from hatchery to nursery, where they are nurtured preceding the grow out stage in the ocean. As a result of the nursery stage, the oysters reach market size sooner, which can potentially improve the economics of the industry. The nursery operation, unlike the hatchery, is designed to provide the large volumes of food and heated water that are necessary for accelerated juvenile growth rates. Such a facility is particularly important in the spring in temperate regions where ambient seawater is too cold for feeding. With a nursery operation, the oysters are not prematurely transplanted to the grow out site, where higher mortality may result due to insufficient food, low temperatures, predation, storms, and handling. As shown by a recent survey (De Pauw, 1981), the major food source for cultured juvenile oysters is provided by the mass culture of phytoplankton. Epifanio et al. (1975) and Pruder and Greenhaugh (1978) have demonstrated that juvenile oysters will develop and grow normally

on diets composed completely of phytoplankton in a non-axenic culture. The phytoplankton species that are routinely fed to juvenile oysters by various workers are catalogued in Enright and Newkirk (1982). Most laboratories lack the time and facilities to experiment with a large number of algal species and tend to retain a given feeding routine once it has proven adequate. Thus, the most commonly used species are not necessarily the best.

Persoone and Claus (1980) identified the expense of mass algal culture as the major restriction confronting the nursery culture of molluscs. Pruder and Bolton (1981) estimate that algal production in their controlled-environment system for rearing bivalves from egg to marketable size accounts for nearly 80% of their total costs; a substantial portion of this percentage covers the expense of feeding the juveniles. A more specific diet could be developed with extensive research directed towards examining a wider range of algal species, coupled with identifying the poorly understood nutritional requirements of juvenile oysters. With the massive volume of food presently fed to the juvenile oyster, even a small improvement in feeding efficiency would result in a substantial reduction of feed costs in the nursery.

b) Criteria for Good Algal Diets

There are many variables contributing to a good algal food for juvenile oysters.

Size may limit the usefulness of an algal species as a food organism. Oysters are believed to filter food particles of a narrow size range. Haven (1965), reported that oysters remove natural

particles from seawater that are as small as 1 to 2μ. The maximum particle size accepted by oysters is not clear. Ingle et al. (1981) report that ground corn meal particles of 50μ or less were retained by oysters. Loosanoff and Engle (1947) found that oysters ingested species of algae as large as 60μ. The maximum particle size accepted by oysters, however, was not examined. In the diets used by Castell and Trider (1974), the suspended particles ranged in size from less than 1 to ca. 50μ in diameter with over 90% of the particles in the 5 to 15μ diameter size range. The most commonly used algae (Isochrysis galbana, Pavlova lutheri, Tetraselmis suecica and Dunaliella tertiolecta) are of medium size and volume, usually measuring between 5 to 11μ and 32 to 335μ^3, whereas the less commonly used algal species tend to be either larger or smaller. The filtration efficiency of bivalves is affected by the cell size of the phytoplankton (Hughes, 1969; Haven and Morales-Almo, 1970). Adult C. virginica have been reported to filter particles efficiently from 3 to 12μ, with efficiencies which are reduced by 1/3 to 1/2 for particles which are between 1 and 3μ (Haven and Morales-Almo, 1970).

An acceptable algal diet for oysters must contain the nutrients for normal growth and development. These are: 1) nitrogen sources, amino acids and proteins; 2) reduced carbon sources; 3) lipids and sterols; 4) specific vitamins; and 5) inorganic anions and cations. The problem lies in identifying which specific nutrients are needed and the best way to provide them. The chemical composition of algal cells would seem to give the most obvious explanation for variations in the

nutritional value of algae. In general, however, no striking differences in the chemical compositions of good and poor algal oyster feeds have been found. Parsons et al. (1961) reported marine phytoplankton develop a similar organic composition when grown under like physical and chemical conditions, regardless of size or class. Cowey and Corner (1966), Walne (1970), Castell and Trider (1974) and Epifanio (1983) agree that attempts to correlate differences in oyster growth with the composition of the algal species have met with little success. When comparing the nutritional status of various algal species, there are numerous other non-nutritional aspects that will also affect the food value of the diet (cell size, digestibility and toxicity). One area which has been overlooked is the contribution made by algal exudates. Algal exudates are a part of the oyster diet which are not included in the biochemical analyses of the diet since the phytoplankton cells are typically separated from the media prior to analysis. A considerable amount of research must be conducted before reliable correlations can be made between diet suitability and chemical composition.

Many of the variations in the food value of phytoplankton are a reflection of the differences in toxicity of internal or external products of the algal cells. Prymnesium parvum, Stichococcus sp., Chlamydomonas sp., Amphidinium carterī and Gymnodinium sp. were considered by Guillard (1958) and Davis and Guillard (1958) to be toxic algae for oysters. Spoehr and Milner (1949) and Proctor (1957) have suggested that the toxicity of the three algae, Chlorella sp., Chlamydomonas sp. and Stichococcus sp., may be due to liberation of

unsaturated fatty acids. Ryther (1954) has explained the toxicity of Chlorella sp. as being due to the presence of senescent cells. Loosanoff et al. (1954) found that a concentrated filtrate of Chlorella sp. culture was relatively more harmful than a high concentration of living cells. Bayne (1965) demonstrated that the cell-free medium of Nannochloris sp., particularly at the stationary phase, inhibited the growth of Mytilus edulis. However, many of the algae mentioned above have been successfully used as feed organisms in oyster hatchery and nursery operations (Enright and Newkirk, 1982). Large differences in toxic levels may be common among various strains of these species. There does not appear to be any class or characteristic type of phytoplankton that is more toxic to oysters than any other. For example, the non-motile chlorophyte Chlorococcum sp. appeared to be as good an oyster larval food as Pavlova lutheri or Isochrysis galbana, while the non-motile species Stichococcus sp. was found to be highly toxic (Davis and Guillard, 1958).

The food source must be inherently non-toxic and care must be taken not to render it toxic during the manipulatory processes. Toxicity may result through the chelation of essential trace elements which are required for cilia movements and activation of digestive enzymes. Even algae that are normally satisfactory as oyster food may become toxic as a result of bacterial contamination. Interactions within a culture are complex and substances supplied by other organisms may affect the toxicity of a given algal species. Certain species of bacteria in algal feed cultures are believed to cause bivalve mortality (Guillard, 1959; Tubiash et al., 1965). There is also evidence to suggest that large

numbers of nontoxic bacteria may cause a normally good food to become ineffective (Ukeles and Sweeney, 1969). On the other hand, the toxicity of Prymnesium parvum has been decreased by the addition of a bacterial population (Shilo and Aschner, 1953). The condition of the culture must be carefully examined, as well as the algal food species used.

The nutritional substances of the algae must be accessible to the oyster. Digestion of the algal cell wall is determined by the oyster's enzymatic capability as well as the composition of the algal cell wall. There is little information on the susceptibility of algal cells to mechanical and enzymatic digestion. According to Owen (1975), the digestive enzymes of O. edulis are present in the style and the tissue of the digestive diverticula. The former type are released into the stomach when the style dissolves; the latter remain in the tissue where they act intracellularly. Phagocytes, which possess powerful digestive enzymes, are present throughout the digestive system. The main enzymes of an oyster are amylase and glycogenase, with substantially less amounts of lipase and proteinase (Purchon, 1971). Dean (1958), who stressed that the differences between good and poor foods may largely be due to the oyster's ability to digest algae, observed that Cryptomonas spp. disintegrated when swimming near an undissolved style. Pavlova lutheri behaved similarly, while on the other hand I. galbana came in contact with a style for more than 72 hours without any discernible effect. The enzymes in the tissue of the digestive diverticula of the oyster presumably digest I. galbana. Independent of the algal food species used, many physical and chemical factors affect the oyster's

ability to digest and absorb algal cells: gut enzyme level; the presence of the style; and feeding temperature.

It is critical that the algal food organisms selected for a bivalve nursery operation be easily cultured, preferably with a reliable culture technique already developed for the species. Although productivity is an important characteristic, it is critical that the yield of the algae is also predictable and consistent. Culturing the algae should require minimum labour and material costs.

Good quality seawater is fundamental for all nutritional studies since it is a source of nutrients in addition to its important role in the osmotic and respiratory systems of oysters. Seawater quality can determine the type of supplementary diet that is needed by the oysters, as shown by Walne (1970). He observed differences in oyster growth rate when feeding various algae in both filtered and unfiltered seawater. Poor quality seawater can inhibit oyster growth even if all the nutritional requirements are being fulfilled. The nutritional requirements of many marine animals are also affected by seawater temperature (NRC, 1983).

Walne (1970), in examining 18 different algal species, has conducted the most extensive series of juvenile oyster growth trials. His experiments helped distinguish potentially satisfactory algal feed organisms for juveniles from algae that are unsatisfactory. Unfortunately, his results were expressed in terms of the mean change in oyster size, with no reference to sample size or to the variation among the individuals within the group. In cases where two or three oyster trials were conducted with the same algal diet, there was a considerable

range between the means. In Walne's experiment (1970), there is no mention of the number of oysters nor the volume of water used. Therefore, the amount of food per oyster biomass or feeding ration cannot be calculated. While 1×10^5 cells ml^{-1} may be an adequate initial algal cell concentration for a few small oysters contained in a large volume of water, this same algal concentration may be insufficient for several larger juveniles contained in a small vessel.

Table 1. contains algal species ranked according to the growth rate obtained in feeding trials with juvenile oysters. The relative values which Walne (1970) obtained have been used as the basis for the ranking. Despite its short-comings, Walne's study (1970) is the most comprehensive of its type. The data of five other groups conducting feeding trials on juvenile oysters have also been examined and their findings have been considered in the determination of the ranked order. Distinctive characteristics of each of the algal species are also noted in Table 1.

Oysters increase in biomass throughout the experimental period - a fact that must be taken into account. In a study by Walne and Spencer (1974), where sufficient details are provided, a ration of Tetraselmis suecica fed to O. edulis decreased from 35 to 2% of the oyster fresh weight over a three week period. So that growth responses can be obtained from a well-defined feeding ration, oyster biomass increases must be compensated for by frequent increases in the amount of food provided.

It has been amply documented that diets consisting of more than one species generally promote more rapid growth (Matthiessen and Toner,

TABLE 1 Biochemical Information on Algal Species, Ranked With Respect To Food Potential For Juvenile Oysters.

(From Enright and Newkirk, 1982)

Rank	Algal Species [Class[1], Size (μ), Volume (μ^3)]	Oyster[2] Feeding Trial Ref.[3], Algal Feeding Density (10^4 cell/ml)	Distinctive Characteristics of Algal Species
1	Chaetoceros spp [B, 3, 35]	e [1, 3 - 1.5 opt.] g [2, 10]	70% of dry wt. is silica (8), possible grinding function; produces antibiotic (11), 35% protein, 7% carbohydrate, 7% lipid (8), excretes glycolic acid (10) & lipids (12).
2	Pavlova lutheri [Chr, 5, 58]	e [1, good, 2.5 - 10] g [1, fair, 2.5 - 20] l [1, poor, 2.5 - 15]	48% protein, 31% carbohydrate, 12% lipid (8), 20 - 60% protein (13), glycerol & cyclitols stored (14), releases glycolate & mannitol (10).
3.	Tetraselmis suecica [H; 11, 335 - 515]	e [1; >3] g [2; 1] v [3, 30, poor] v [15; 2]	Non-rigid theca of galactose, uronic acid (17) & a pectin-like calcium material (18), grows on glucose in the dark (16), 30% protein, 2% lipid, 52% carbohydrate (15), no fatty acids >20 carbons (2).
4.	Skeletonema costatum [B, 3 - 20, 203 - 350]	e [1; 5 - 8]	Low temp. adapted, opt. 15°C (19), high levels of 20:5W3 (9), mannose - main polysaccharide (22), excretes glycerol (10), produces anti-bacterial substances (21), releases thiamine and biotin when grown with a B_{12} source (20).
5.	Isochrysis galbana [H, 3, 46 - 74] I. aff galbana clone T-ISO	e, g, l [1, 1-3 opt., filtered S.W.] e [1, 8, unfiltered S.W.] v [15, 0.2] v [4, 23, high temp. tolerant stain]	Widely used (34), better oyster growth in unfiltered S.W. (1); 60% proteins, 17% lipid, 16% carbohydrates (15), releases glycolate, cyclohexanetetrol (26) & a bacterial inhibitor (24,25), glucose, galactose & arabinose - main polysaccharide (24)
6.	Thalassiosira pseudonana [B, 6, 36]	e, v [5, 20] v [15, 0.1]	Extracellular fibers made of chitin (27), 41% protein, 27% carbohydrate, 12% lipid (15), excretes large amounts of polysaccharides (26).

cont..../

7.	Dicrateria inornata [H; 4; 21]	e [1; 13]	Difficult species to rear (1).
8.	Cryptomonas spp. [Cr; 5; 46]	e [1; 1 opt.]	Oyster growth doubled when fed with unfiltered S.W. (1); intracellular parasites (28); naked cells possessing trichocysts (30); contains 20:5W3 & 22:6W3 (31,32).
9.	Phaeodactylum tricornutum [B; 12 - 32; 60]	e [1, 1 opt.] e, g [5, good; 20] v [5, poor; 20] e, g, [6, good; 10 - 30] v [6, poor; 10 - 30]	Apparent absence of trytophan (7), high levels of 20:5W3 & 22:5W3 (31).
10.	Dunaliella tertiolecta [Chl; 10, 300]	e [1, 0.5 - 5] e [2, 2.5]	Opt. salinity, 1.2 M; high glycerol (33); lacks fatty acid 22:6W3 (2); excretes biotin & thiamine & has no specific vitamin requirements (20).

1. B (Bacillariophyceae), Chr (Chrysophyceae), P (Prasinophyceae), H (Haptophyceae), Cr (Cryptophyceae), Chl (Chlorophyceae)

2. e (Ostrea edulis), v (Crassostrea virginica), g (C. gigas), l (O. lutaria)

3. (1) Walne, 1970; (2) Langdon & Waldock, 1981, (3) Epifanio, 1979; (4) Ewart & Epifanio, 1981, (5) Epifanio et al., 1976; (6) Mann & Ryther, 1977; (7) Epifanio et al., 1981; (8) Aaronson et al., 1980; (9) Chuecas & Riley, 1969; (10) Hellebust 1965; (11) Cauthier et al., 1978, (12) Bountry et al., 1977; (13) Taub, 1980, (14) Craigie, 1974; (15) Romberger & Epifanio, 1981; (16) Droop, 1974, (17) Lewin, 1958; (18) Manton & Parke 1965; (19) Soeder & Stengel, 1974; (20) Carlucci & Bowes, 1970; (21) Duff et al., 1966, (22) Handa, 1969, (23) Ewart & Pruder, 1981, (24) Marker, 1965, (25) Bruce et al., 1967; (26) Hellebust 1974, (27) Blackwell et al., 1967; (28) Ettl & Hoestrup, 1980; (29) Beach et al., 1970; (30) Evans, 1974; (31) Kates & Volcani, 1966, (32) Moreno et al., 1979; (33) Wegmann, 1971; (34) De Pauw, 1981.

1966; Gruffydd and Beaumont, 1972; Walne, 1974; Koganezawa, 1975; Epifanio, 1979; and Newkirk and Waugh, 1980). Epifanio (1983) speculates that improved balance of micronutrients such as vitamins and trace minerals accounts for increased oyster growth rates. Langdon and Waldock (1981) on the other hand, believe the key additive components in combined diets are related to the availability and balance of fatty acids. Epifanio (1979) suggests the combination of an easily digestible algal species such as I. galbana with a less readily digestible species such as T. suecica results in a more effective digestion of the less digestible species. Many oyster hatcheries are presently using a mixture of algal species. Given the cost of culturing many species, however, one would want to feed with algal species which produced the highest oyster growth rates per unit of culture effort.

The hypothesis to be tested in this section is as follows: phytoplankton species presently untested as food organisms for O. edulis juveniles yield higher growth rates than are obtained with traditional algal diets. The cell concentration which produces the highest growth rate in juvenile oysters varies, depending which algae is used (Walne, 1970). Thus a range of feeding concentrations must be examined for each algal species which is evaluated.

Part I

Materials and Methods

a) Seawater

The seawater used for both the algal cultures and the oyster growth trials was obtained from the Dalhousie University Aquatron system. The source of this water is the Northwest Arm of Halifax. It is pumped through a pipeline which is two meters above the sea floor. It then passes through a sand bed pressure filter (O'Dor et al., 1977). Before being used for the oysters, the seawater is heated and filtered through a 1.0. cartridge filter (Filterite, Brunswick Technetics, Timonium, Maryland). The seawater used for the algal cultures is autoclaved.

b) Algal Cultures

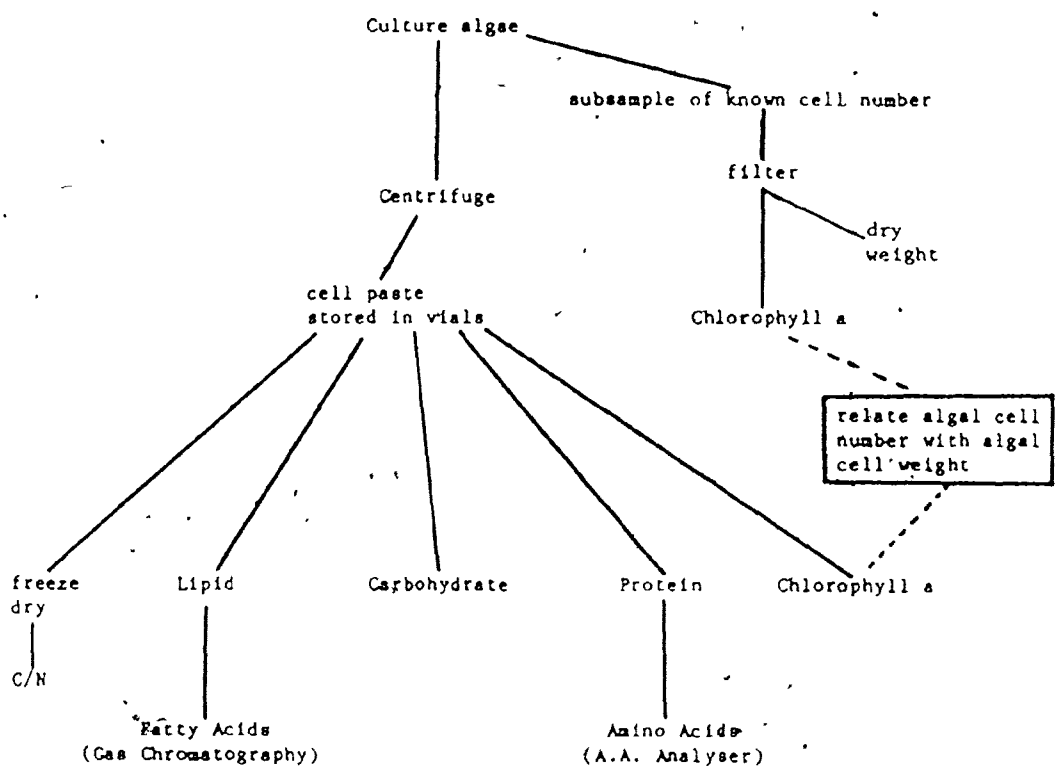
The algal species and the origin of the cultures are shown in Table 2. The algal cultures were reared under batch conditions. Axenic stock cultures were maintained in 250 ml flasks, plugged with cotton stoppers. They were kept at 15°C ± 2°C in a culture chamber (Percival, Model E54-U, Boone, Iowa). Using a 12h:12h light:dark cycle, the cultures were irradiated with cool white fluorescent lights (General Electric, F36 T12 CW HO), which delivered ca. 100 $\mu E\ m^{-2}\ s^{-1}$ (6.0×10^{19} photons) in the Photosynthetically Active Radiation (P.A.R.) spectrum between 400 and 700 nm. These stock cultures were subcultured between one and three times monthly in order to maintain actively growing cultures. Approximately 50 ml of a dense stock culture were used in the sterile inoculation of autoclaved, cotton-stoppered, one litre flasks, which contained ca. 300 mls of seawater. Periodically, both the stock

cultures and the one litre flask cultures were manually agitated. Within three to four days, a one litre flask culture was sufficiently dense to be used to inoculate an autoclaved 20 litre carboy. The one litre flask and 20 litre carboy cultures were maintained at 22°C - 1°C unless otherwise stated. The incident maximum irradiance at the culture vessel surface was ca. 300 E m^{-2} s^{-1} (18.1 x 10^{19} photons) in the P.A.R. spectrum between 400 and 700 nm. Aeration, carbon and pH control were supplied to the carboy cultures by a pressurized airstream which was supplemented with CO_2 from a gas cylinder. The pH of the algal cultures ranged between 7.9 and 8.4. The f/2 nutrient mix of Guillard and Ryther (1962) was supplied to all algal cultures. On the second day after inoculation, 40 ml of $Na_2SiO_3 \cdot 9H_2O$ (30 g/l) was added to each of the 20 litre carboy, diatom cultures. The total culture volume was ca. 18 litres. Algal cell counts of these cultures were conducted at the time of harvesting, prior to feeding the oysters. Cell counts were obtained for each culture using a Laborlux II microscope (Ernst Leitz Wetzlar GMBH, Type 020-435.026, Germany) with a hemacytometer (Improved Neubauer, Ultra plane, spot lite, 1/400 sq. mm, 1/10 mm deep) and/or a coulter counter (Model Z_B, Coulter Electronics, Hialeah, Fla.). Several replicate cultures of each species were available. Algal cultures were monitored for contamination using a microscope magnification of 1000x. Cultures in which bacteria were observed were discarded.

c) Algal Biochemical Analysis

The method used to obtain the biochemical data on the algal cultures is outlined in Fig. 1. Cell counts were obtained for each

Fig 1. Schematic diagram of the biochemical procedures used with the algal cultures.



algal culture. Culture subsamples, ranging from 5 to 50 ml, were collected, using a millipore filter apparatus, on pre-weighed glass microfiber filters (Whatman), 4.25 cm in diameter and used to determine the dry weight of the algae. Chlorophyll a determinations were also conducted on filter papers containing a known number of algal cells and a thin coating of $MgCO_3$ to neutralize any acidic compounds the cells may contact. The remaining culture was centrifuged (Sharples Super, Centrifuge, The Sharples Corp., Philadelphia). The resulting cell paste was either used immediately or placed in 2 ml plastic vials and stored at -60°C for a week to several months prior to analysis. Subsamples of the cells were then placed in tared test tubes for protein, lipid, carbohydrate and chlorophyll a determination.

Protein and amino acid analyses were determined by Dr. J. Craigie, National Research Council, using 10 to 40 mg of frozen algal cells and hydrolysing with 2 ml of 6N redistilled HCL in vacuum-sealed glass ampoules. The ampoules remained at 110°C for 45 to 69 hours and were manually shaken twice during that period. The ampoules were opened and evaporated to dryness with an air jet. The dry samples were dissolved in a citrate buffer, stirred with a magnetic stirrer, and centrifuged, prior to placement on an amino acid analyzer, set at AR 1.0. (Beckman 119 CL, Beckman Instruments Inc., Spinco Division, Palo Alto, California). Norleucine was the standard. Partial protein values were obtained by the addition of the amino acids shown in Appendix I.

Total lipid was analysed, making use of the techniques outlined by Folch et al. (1957) and Bligh and Dyer (1959). The algal samples were homogenized (Polytron, Type PT 10/35, Kinematica Gmbh, Switzerland) and

dried under a nitrogen evaporator. The extracted lipid was weighed and dissolved in hexane. Fatty acid analysis either followed immediately or the samples were frozen at -15 or -60°C for future analysis. Fatty acid methyl esters (FAME) were prepared from the lipid sample by transesterification, either with boron trifluoride in anhydrous methanol (Mehlenbacher et al., 1965) or by adding Christoperson's reagent, (Christoperson and Glass, 1969) to a 10% lipid in hexane solution. The FAME sample was passed through a column containing acid-washed Florisil retained on a glass wool plug (Caroll, 1963) in order to remove any remaining pigments, as pigments interfere in gas-chromatography. The fatty acids were analysed by two gas chromatographs. A Perkin-Elmer, model 3920 (Norwalk, CT), belonging to Dr. J. Castell, Department of Fisheries and Oceans, was used during 1982. These samples were analysed using a hydrogen flame detector and a Silar 5CP stainless steel column, 45m x 0.025 cm, which was coated with butanediol succinate. The injector temperature was 200°C and the oven temperature was 180°C. Helium was the carrier gas at an inlet pressure of 50 psi. A similar gas chromatograph (Perkin-Elmer, Model 900) and column, belonging to Dr. R. Ackman, Nova Scotia Technical University, was used during 1983. Thin layer chromatography was conducted on the samples analysed in 1983. The size of the algal lipid samples ranged from 1 to 3 µg. Tentative FAME identifications were based upon comparison with chromatograms of commercial fatty acid standards and the methyl esters of cod liver oil, analysed under the same chromatographic conditions (Ackman and Burgher, 1963). Semilog plots of retention time (relative to 18:0) vs. carbon chain length were constructed for the

algal FAME and standards to aid identification (Ackman, 1963). The algal FAME were hydrogenated (Appelqvist, 1972) to confirm the accuracy of identification and quantification of major components. The chromatograms were quantified by a computer program developed by Dr. Ackman, or by determining the area under each fatty acid peak.

Carbohydrates were analysed using a colorimetric phenol-sulfuric acid method (Dubois et al., 1956). These samples were initially hydrolysed with 2N H_2SO_4 for one hour in boiling water. A standard curve was developed using glucose. Colour development in the experimental and reference samples was quantified with a spectrophotometer, using a wavelength of 485 nm (UV Visible, Spectrophotometer, Varian Canada Inc., Ottawa, Ont.).

Chlorophyll a determinations were conducted on the weighed, frozen algal cell samples in accordance with the procedure outlined by Jensen (1978). The samples were extracted with methanol containing $MgCO_3$. They were read on the Varian spectrophotometer at 660 nm. These data, coupled with the relationship between algal cell number and the chlorophyll a concentration, enabled the number of algal cells for a given algal weight to be determined for each diet. Thus, the protein, lipid and carbohydrate content could be expressed in terms of algal cells.

The carbon and nitrogen ratio of the algal diets was measured by Microanalysis Laboratories Limited, Markham, Ontario. The analyses were conducted on freeze-dried algal samples.

d) Oyster Growth Trials

The Dalhousie University Hatchery provided the juvenile European Oysters (Ostrea edulis L.). Initially the oysters measured from 3 to 7 mm in diameter and weighed between 5.0 to 25.0 mg fresh weight. Prior to experimentation the oysters were held in a flowing seawater system (22°C ± 2°C) in the Dalhousie hatchery and were fed a mixture of the following algal species: Isochrysis galbana T-iso, Thalassiosira pseudonana, Chaetoceros gracilis and occasionally Pavlova lutheri and Dunaliella tertiolecta. The oysters were not fed for one to two days prior to the experiment.

The growth response of individual juvenile oysters was monitored using the following procedure. Individual oysters were blotted with an absorbent cloth to remove excess surface water and weighed on an analytical balance (Sartorius-Werke AG, Type 2442 Gottingen, Germany). After their individual oyster fresh weights were recorded a subsample was placed on a labelled foil-covered board and put in a drying oven (Lab-line Instruments, Inc., No. 3505, Melrose Park, Illinois) at 60°C ± 5°C until the oysters reached a constant dry weight. The remaining oysters were attached with a marine glue (Sea Goin' Heavy Duty Poxy, Permalite Plastics Co., Newport Beach, California) to a numbered Dymo tag. The oysters were positioned with the convex side of their lower valves adhering to the glue. Care was taken to keep the glue away from the edges of the shell as this would impair the feeding capability of the animal. In a preliminary experiment of five weeks duration, it was determined that there was no significant difference between tagged and untagged oysters. The numbered Dymo tag and attached oyster were tied

to a string. Each string contained 25 animals which were ca. 1.5 cm apart. The strings (previously held in a common tank) were arbitrarily assigned to labelled, eight litre, plastic pails. Three to four strings were loosely hung across the pail, suspended from open rings made of PVC plastic, which clipped to the rim of the pail. The pails were filled to the top with 23°C ± 1°C seawater. The seawater temperature remained at this temperature for the duration of the experiment. The pH of the seawater in the pails ranged between 7.7 and 8.1. Water circulation and oxygen was provided for the oysters in the initial experiments by airlift tubes made from CPVC plastic, and air stones in the final experiments. The source of the pressurized air was the Dalhousie University compressor system.

The duration of the oyster growth experiments was five weeks. Daily, each pail was manually emptied and refilled with fresh seawater and a known volume of a specific algal diet. The inside of the pail and the oyster strings were cleaned daily by spraying with fresh seawater. Weekly, the pails and airlift tubes or air stones were wiped with a cloth which had been soaked in a dilute bleach solution. The position of the pail in the oyster culture room was rotated weekly. Since the nutritional value of algal species with varying digestibilities was compared, kaolin (K3 Laboratory Grade, Fisher Scientific) was added daily to the pails at a concentration of ca. 30 mg/l. It was assumed that kaolin functioned as a grit or grinding substrate and increased the digestibility of those algae with a less digestible cell wall. The addition of kaolin marginally enhanced the growth response of the oysters when a mixed algal diet consisting of Isochrysis galbana, T-iso,

Dunaliella tertiolecta, Thalassiosira pseudonana and Pavlova lutheri was provided in a five week long preliminary oyster growth trial. There were two types of unfed control treatments; a daily change of fresh seawater either with or without the addition of kaolin. All remaining pails received an algal diet. The reference diet for all the oyster growth experiments was I. galbana T-iso. Unicellular algal diets were added to all pails except the unfed control treatments and the mixed algal diet treatment. The mixed algal diet was comprised of I. galbana T-iso, Chaetoceros gracilis, C. simplex, Pavlova lutheri and Phaeodactylum tricornutum. At the termination of the experiment, the oysters were removed from their numbered tags and blotted with an absorbent cloth. Any traces of glue were scraped off the oyster. The final fresh weight was obtained, maintaining the same method that was used to obtain the initial weight. Dry weights were obtained for the oysters using the procedure previously mentioned. The dried oysters were grouped according to their experimental treatment, placed in labelled and sealed plastic petri dishes and stored in desiccators at $-15^{o}C$.

A range of feeding densities was examined for each algal species. Algal densities were expressed in terms of a feeding ration using the following equation:

$$\text{Feeding ration} = \frac{\text{number of algal cells}}{\text{live oyster weight (mg)}}$$

The amount of algal food supplied was adjusted daily to account for the estimated increasing oyster biomass, according to the procedure outlined

in Urban et al. (1984). Half-way through the experiment, the weight of arbitrarily selected oysters was obtained from each pail. This information was used to calculate an estimated growth rate for each experimental treatment. The daily instantaneous relative growth rate of the oysters was calculated for each treatment using the following equation (Brody, 1945). The rate is relative to the initial oyster weight.

$$\text{Growth rate } (k) = [(dW/dt)/Wo] = (2.303/t) \log (Wt/Wo)$$

where Wo = initial live weight (mg)
Wt = final live weight (mg)
t = 35 days
2.303 = conversion to natural log from $\log_{10}$
W = weight

Part I

Results and Discussion

a) Oyster Growth Trials

The histograms in Figs. 2 through 7 show the comparative growth response of juvenile European oysters fed a variety of algal cultures, compared to oysters fed the reference species I. galbana T-iso and the unfed control treatments. A range of algal cell densities was examined for each phytoplankton species. In experiments 1 and 2 (Figs. 2 through 5) the only common alga was the reference species, I. galbana. In experiment 3 (Figs. 6 and 7) several algal species previously examined in experiment 1 or 2 were retested at different densities. Daily increases of algal cells were required to compensate for the increase in oyster biomass, thereby maintaining a constant feeding ration.

In Table 2 the algal diets are ranked according to the highest oyster growth rate obtained with each algal species over the density range tested. The oyster growth rate k, based on fresh weight, which was obtained with each diet was divided by the k value obtained with the reference species, I. galbana T-iso.

b) Algal Diets

Several phytoplankton species, previously untested as food organisms for O. edulis juveniles, produced higher oyster growth rates than results with traditional diets. The algal species will be discussed as oyster diets, with reference to their biochemical composition which is presented in Appendix I, II and III and summarized in Table 3.

Fig. 2. The daily growth rate (k), based on fresh weight data of Ostrea edulis juveniles, when fed various phytoplankton diets at a range of feeding densities. 95% confidence intervals are shown, with the number of replicate oysters indicated above the histogram bars.

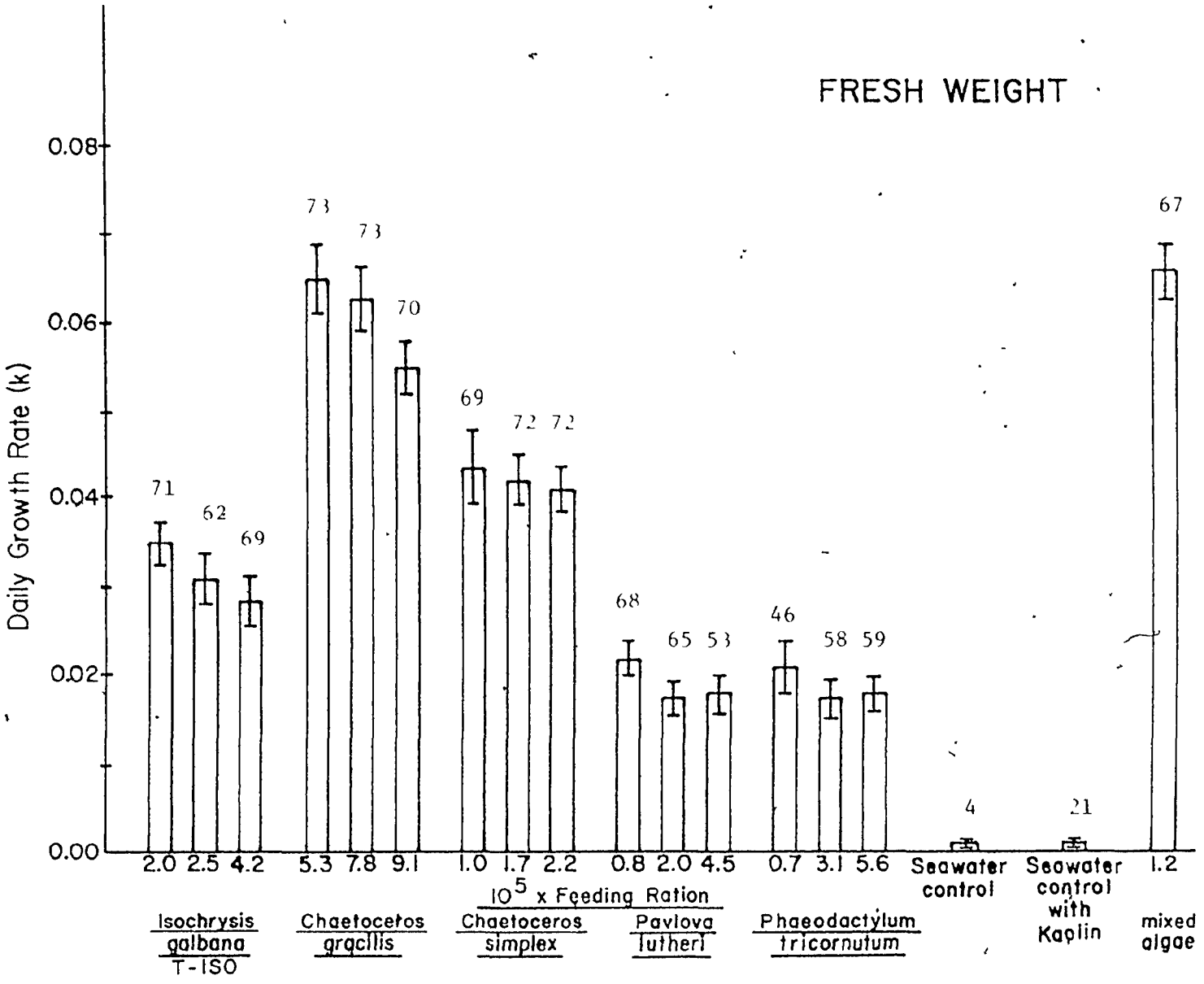

FRESH WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0.00
71
62
69
73
73
70
69
72
72
68
65
53
46
58
59
4
21
67
2.0 2.5 4.2
5.3 7.8 9.1
1.0 1.7 2.2
0.8 2.0 4.5
0.7 3.1 5.6
Seawater control
Seawater control with Kaplin
1.2
10^5 x Feeding Ration
Isochrysis galbana T-ISO
Chaetocetos gracilis
Chaetoceros simplex
Pavlova lutheri
Phaeodactylum tricornutum
mixed algae

Fig. 3. The daily growth rate (k), based on dry weight data of Ostrea edulis juveniles, when fed various phytoplankton diets at a range of feeding densities. 95% confidence intervals are shown, with the number of replicate oysters indicated above the histogram bars.

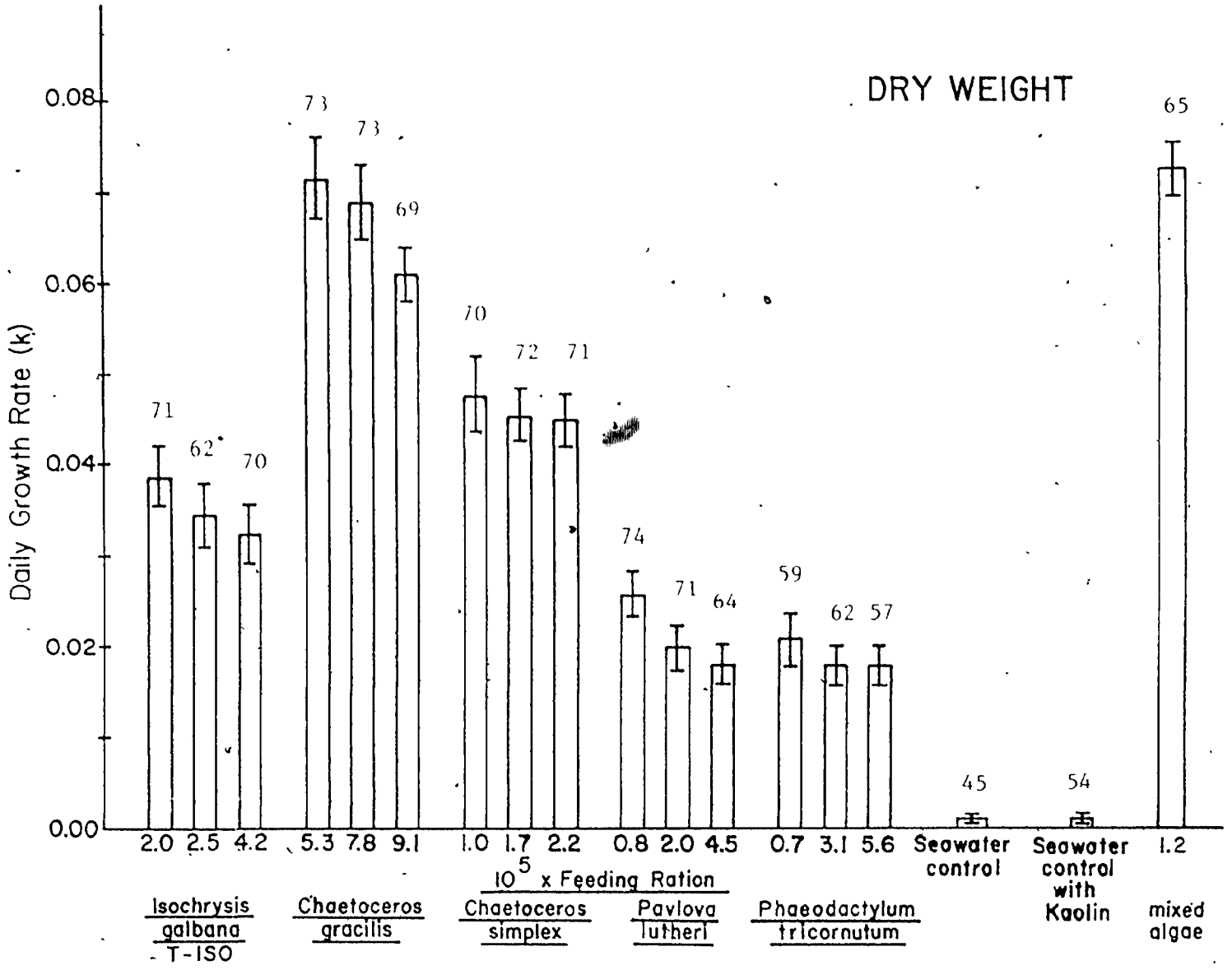

DRY WEIGHT
Daily Growth Rate (k)
0.00
0.02
0.04
0.06
0.08
71
62
70
73
73
69
70
72
71
74
71
64
59
62
57
45
54
65
2.0 2.5 4.2 5.3 7.8 9.1 1.0 1.7 2.2 0.8 2.0 4.5 0.7 3.1 5.6 Seawater control Seawater control with Kaolin 1.2
10^5 x Feeding Ration
Isochrysis galbana T-ISO
Chaetoceros gracilis
Chaetoceros simplex
Pavlova lutheri
Phaeodactylum tricornutum
mixed algae

Fig. 4. The daily growth rate (k), based on fresh weight data of Ostrea edulis juveniles, when fed various phytoplankton diets at a range of feeding densities. 95% confidence intervals are shown, with the number of replicate oysters indicated above the histogram bars.

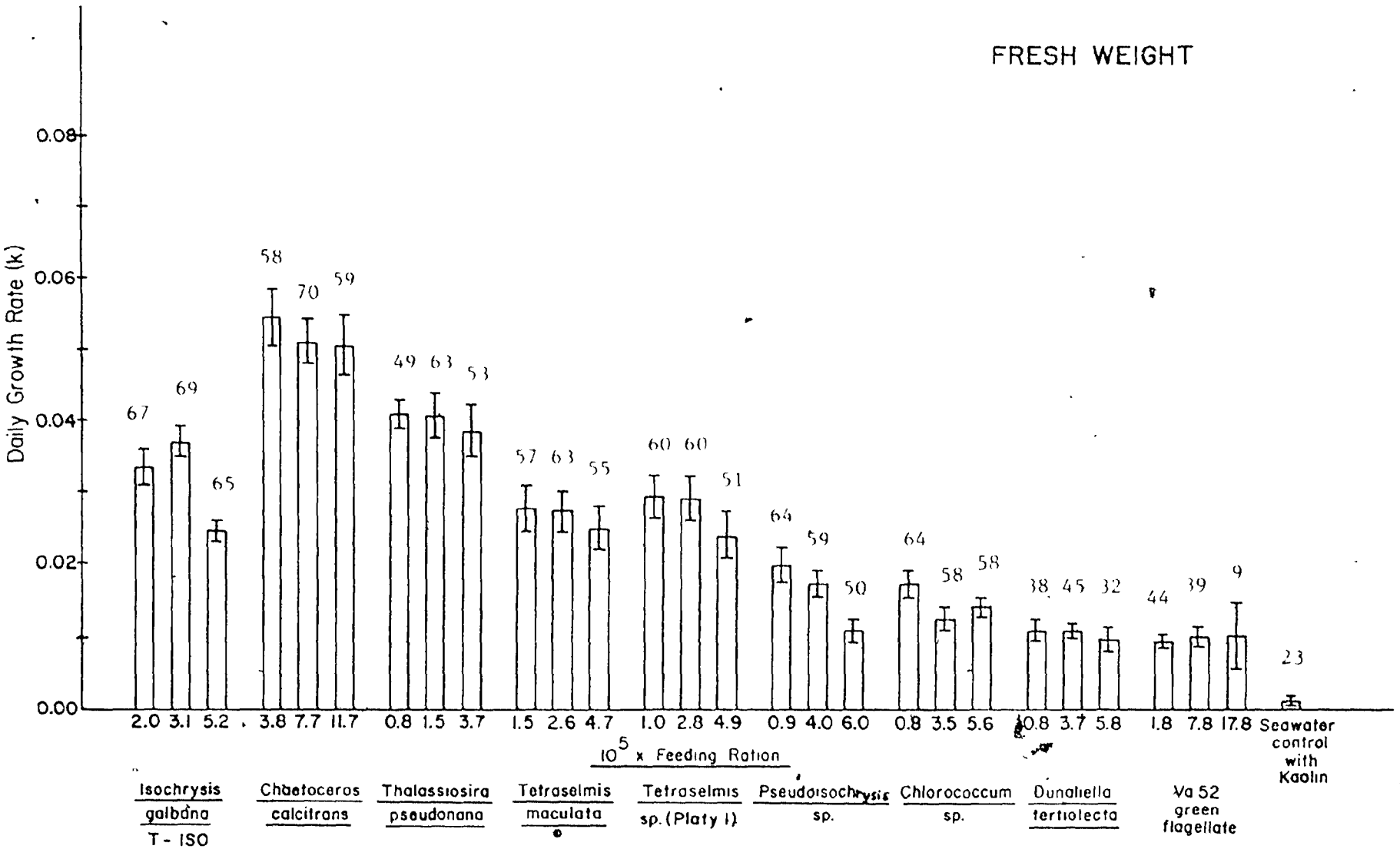

FRESH WEIGHT
Daily Growth Rate (k)
0.00
0.02
0.04
0.06
0.08
67 69 65
58 70 59
49 63 53
57 63 55
60 60 51
64 59 50
64 58 58
38 45 32
44 39 9
23
2.0 3.1 5.2 3.8 7.7 11.7 0.8 1.5 3.7 1.5 2.6 4.7 1.0 2.8 4.9 0.9 4.0 6.0 0.8 3.5 5.6 0.8 3.7 5.8 1.8 7.8 17.8
10^5 x Feeding Ration
Isochrysis galbana T-ISO
Chaetoceros calcitrans
Thalassiosira pseudonana
Tetraselmis maculata
Tetraselmis sp. (Platy I)
Pseudoisochrysis sp.
Chlorococcum sp.
Dunaliella tertiolecta
Va 52 green flagellate
Seawater control with Kaolin

Fig. 5. The daily growth rate (k), based on dry weight data of Ostrea edulis juveniles, when fed various phytoplankton diets at a range of feeding densities. 95% confidence intervals are shown, with the number of replicate oysters indicated above the histogram bars.

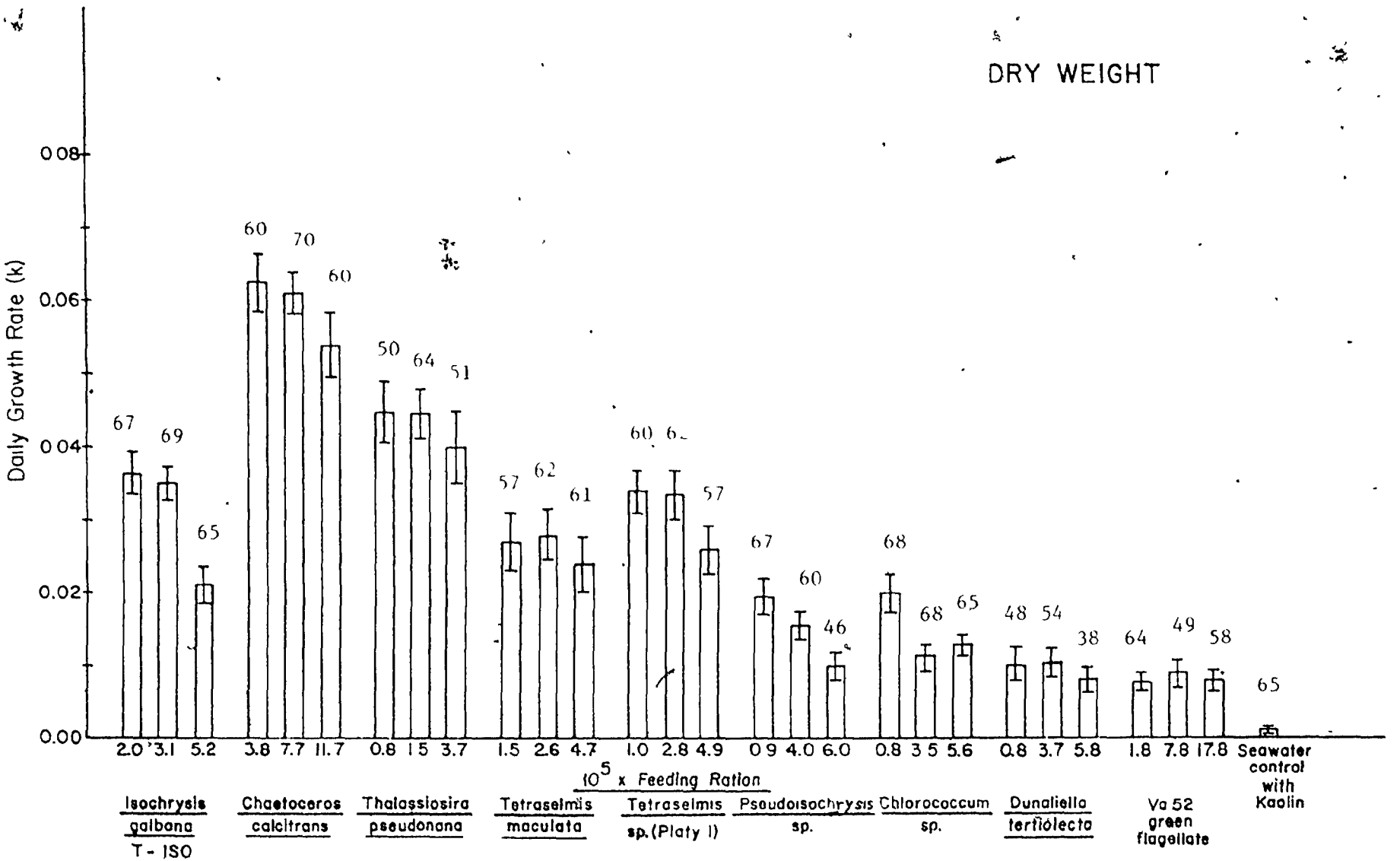

DRY WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0.00
67
69
65
60
70
60
50
64
51
57
62
61
60
62
57
67
60
46
68
68
65
48
54
38
64
49
58
65
2.0 3.1 5.2
3.8 7.7 11.7
0.8 1.5 3.7
1.5 2.6 4.7
1.0 2.8 4.9
0.9 4.0 6.0
0.8 3.5 5.6
0.8 3.7 5.8
1.8 7.8 17.8
Seawater control with Kaolin
10^5 x Feeding Ration
Isochrysis galbana T-ISO
Chaetoceros calcitrans
Thalassiosira pseudonana
Tetraselmis maculata
Tetraselmis sp. (Platy I)
Pseudoisochrysis sp.
Chlorococcum sp.
Dunaliella tertiolecta
Va 52 green flagellate

Fig. 6. The daily growth rate (k), based on fresh weight data of Ostrea edulis juveniles, when fed various phytoplankton diets at a range of feeding densities. 95% confidence intervals are shown, with the number of replicate oysters indicated above the histogram bars.

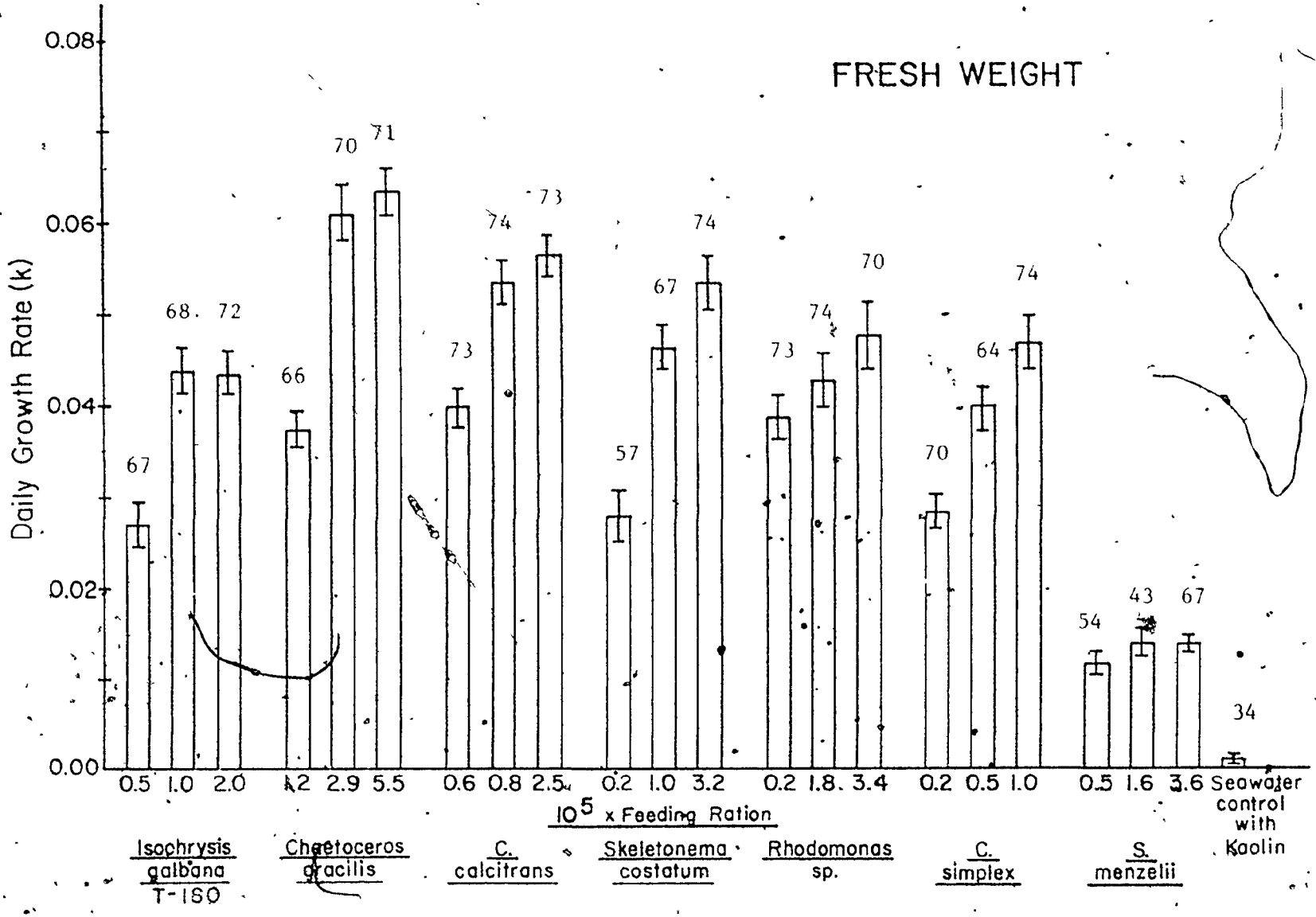

FRESH WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0.00
67
68
72
66
70
71
73
74
73
57
67
74
73
74
70
70
64
74
54
43
67
34
0.5 1.0 2.0 1.2 2.9 5.5 0.6 0.8 2.5 0.2 1.0 3.2 0.2 1.8 3.4 0.2 0.5 1.0 0.5 1.6 3.6 Seawater control with Kaolin
10^5 x Feeding Ration
Isochrysis galbana T-ISO
Chaetoceros gracilis
C. calcitrans
Skeletonema costatum
Rhodomonas sp.
C. simplex
S. menzelii

Fig. 7. The daily growth rate (k), based on dry weight data of Ostrea edulis juveniles, when fed various phytoplankton diets at a range of feeding densities. 95% confidence intervals are shown, with the number of replicate oysters indicated above the histogram bars.

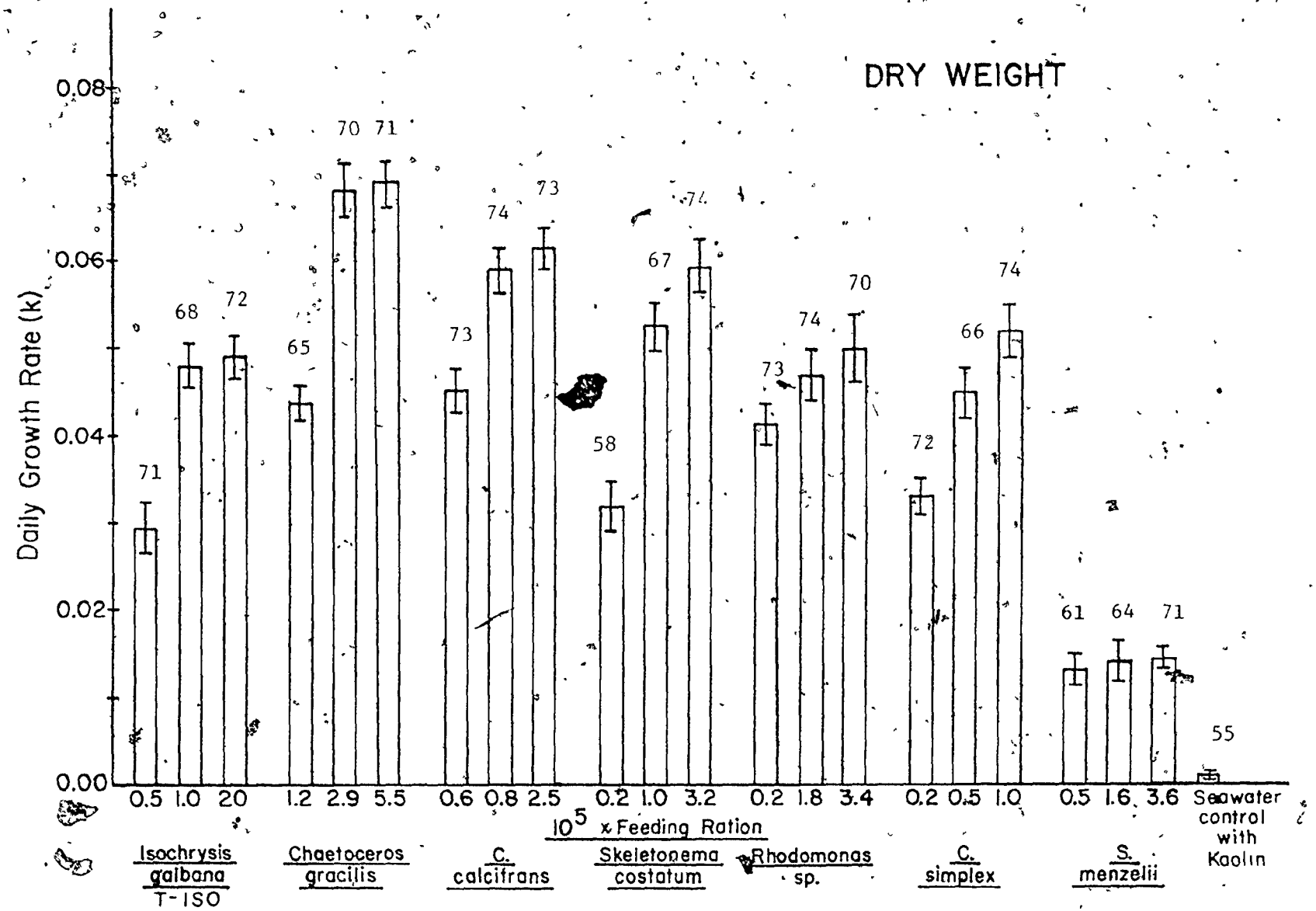

DRY WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0.00
71
68
72
65
70
71
73
74
73
58
67
74
73
74
70
72
66
74
61
64
71
55
0.5 1.0 20
1.2 2.9 5.5
0.6 0.8 2.5
0.2 1.0 3.2
0.2 1.8 3.4
0.2 0.5 1.0
0.5 1.6 3.6
Seawater control with Kaolin
10^5 x Feeding Ration
Isochrysis galbana T-ISO
Chaetoceros gracilis
C. calcitrans
Skeletonema costatum
Rhodomonas sp.
C. simplex
S. menzelii

Table 2. Algal Diets, Ranked According to the Growth Response Obtained with Ostrea edulis juveniles.

		Algal Cell Diameter, Volume (μ, μ^3)[d]	Relative Oyster Growth Response[+] Experiment No. 1	2	3
1.	Mixed Algae (Chgra, Bbsm, T-iso, Mono and Pet Pd)		1.9		
2.	Chaetoceros gracilis (Chgra)[b]	7	1.8*		1.5
3.	Chaetoceros calcitrans[b]	3		1.5	1.3
4.	Skeletonema costatum[c]	3-22,203-350			1.2
5.	Chaetoceros simplex (Bbsm)[a]	9	1.2		1.1
6.	Rhodomonas sp.[c]	4-13,46			1.1
7.	Thalassiosira pseudonana (3H)[a]	4,6-36		1.1	
8.	Isochrysis galbana T-iso (REFERENCE)[b]	3-4,46-74	1.0	1.0	1.0
9.	Tetraselmis maculata[b]	10		0.8	
10.	Tetraselmis sp. (Platy 1)[a]	11,335-515		0.8	
11.	Pavlova lutheri (Mono)[a]	5-6,32-58	0.6		
12.	Phaeodactylum tricornutum (Pet Pd)[a]	12-32,60	0.6		
13.	Pseudoisochrysis sp. (Va 12)[b]	6		0.5	
14.	Chlorococcum sp. (Chloro)[a]	7		0.5	
15.	Dunaliella tertiolecta (Dun)[a]	9-11,300		0.3	
16.	Skeletonema menzelii[a] (Men)	3-7			0.3
17.	green flagellate (Va 52)[b]	1-2		0.3	

Algal Culture Sources: [a] Guillard, R.; Bigelow Laboratory for Ocean Science, West Boothbay Harbour, Maine.
[b] Pruder, G.; College of Marine Studies, University of Delaware, Lewes, Delaware.
[c] Craigie, J.; Atlantic Research Laboratory, National Research Council of Canada, Halifax, Nova Scotia.
[d] Enright and Newkirk (1982).
[+] Growth Rate k, based on fresh weight for each algal diet relative to the reference diet Isochrysis galbana T-iso. ($k_{alga}/k_{I.\ galbana\ T\text{-}iso}$).
* On a fresh weight basis the change in biomass of the oysters fed this diet was 3.5 times higher than that obtained with oysters fed the reference diet for 35 days.

Table 3 Summary Table The key points presented in Appendices are summarized, with the results expressed in both $\mu g(10^6 \text{ cells})^{-1}$ and % dry weight

	Protein					Lipid			
	dry wt. of 10^6 cells	$\mu g(10^6 \text{ cells})^{-1}$	Reference (Appendix no., Table no.)	% dry wt.		$\mu g(10^6 \text{ cells})$	Reference (Appendix no., Table no.)	% dry wt	Reference (Appendix no., Table no.)
	(μg)								
Chaetoceros gracilis (control)	45	10.8	(I, 9)	24		9.2	(II, 9)	20	
(silicate limited)[5]	44	10.1	(I,10)	23		19.5	(II, 9)	44	
(nitrogen limited)[5]	45	4.3	(I,11)	10		9.5	(II, 9)	21	
Chaetoceros calcitrans	12	2.2	(I, 1)	18					
Skeletonema costatum	38	6.8	(I, 1)	18					
Chaetoceros simplex	50								

*whole cell

	Fatty Acid[4]				Carbohydrates		
	20:5ω3 % dry wt.		22:6ω3 % dry wt.	Reference (Appendix no., Table no.)	pg glucose equiv. $(10^6 \text{ cells})^{-1}$	Reference (Appendix no., Table no.)	% dry wt.
Chaetoceros gracilis (control)	11.5		2.5	(II,10)	8.3	(III, 2)	19
(silicate limited)[5]	5.6		0.4	(II,11)	13.5	(III, 2)	31
(nitrogen limited)[5]	7.1		1.0	(II,12)	26.6	(III, 2)	59
Chaetoceros calcitrans							
Skeletonema costatum	30,23		2,7	(II, 1)			
Chaetoceros simplex							

		Protein				Lipid			
	dry wt. of 10^6 cells (pg)	pg $(10^6 \cdot cells)^{-1}$	Reference (Appendix no., Table no.)	% dry wt.		pg $(10^6$ cells)	Reference (Appendix no., Table no.)	% dry wt.	Reference (Appendix no., Table no.)
Rhodomonas sp. (control)	95	33.0	(I, 1)	35		20.9	(II, 8)	22	
(nitrogen limited)[5]	206	26.9	(I, 8)	13		47.2	(II, 8)	23	
Thalassiosira pseudonana (control)	15								
(silicate limited)[5]									
Isochrysis galbana T-iso	31	7.1	(I, 1)	23		8.6	(II, 5)	28	
								17[2]	(II, 2)
								23[3]	(II, 2)
Tetraselmis sp.	100							7.8	
Pavlova lutheri	39								

	Fatty Acid[4]				Carbohydrates		
	20:5ω3 % dry wt.		22:6ω3 % dry wt.	Reference (Appendix no., Table no.)	µg glucose equiv. $(10^6$ cells$)^{-1}$	Reference (Appendix no., Table no.)	% dry wt.
Rhodomonas sp. (control)	13		5		29.7	(III, 3)	31
(nitrogen limited)[5]	(II, 3)				130.1	(III, 3)	63
Thalassiosira pseudonana (control)	9.0		1.4	(II, 6)			
	21		-	(II, 1)			
(silicate limited)[5]	6.5		0.7	(II, 7)			
Isochrysis galbana T-iso	0.7		10.8	(II, 5)	7.3	(III, 2)	23
	4,7		4	(II, 1)			
Tetraselmis sp.	4.0		-	(II, 4)[2]			
	8.0		0	(II, 1)			
Pavolva lutheri	14,19		8,0	(II, 1)			

	Protein					Lipid			
	dry wt of 10^6 cells	g$(10^6$ cells$)^{-1}$	Reference (Appendix no., Table no.)	% dry wt		µg$(10^6$ cells)	Reference (Appendix no., Table no.)	% dry wt.	Reference (Appendix no., Table no.)
	(µg)								
Phaeodactylum tricornutum	50								
Dunaliella tertiolecta	90	18.5	(I, 1)	21					
Skeletonema menzelii	45	10.9	(I, 1)	24					

	Fatty Acids[4]				Carbohydrates		
	20:5ω3 % dry wt.		22:6ω3 % dry wt.	Reference (Appendix no., Table no.)	µg glucose equiv. $(10^6$ cells$)^{-1}$	Reference (Appendix no., Table no.)	% dry wt.
Phaeodactylum tricornutum	18		1	(II, 1)			
Dunaliella tertiolecta	(0,10)		(0,6)	(II, 1)			
Skeletonema menzelii							

1. Numbers separated by commas represents the results from two different cultures, see table reference.
2. The culture was grown at 20°C ± 1°C
3. The culture was grown at 25°C ± 1°C
4. All other cultures were grown at 15°C ± 1°C
5. The results of the silicate and nitrogen limited cultures will be discussed in Part II.

i) Isochrysis galbana cloné T-iso (hence referred to as I. galbana T-iso)

I. galbana T-iso was selected as the reference species in each oyster growth trial (Figs. 2 through 7) because it is commonly used in bivalve hatcheries and nurseries. I. galbana T-iso of the I. galbana species is a high temperature-tolerant, tropical flagellate. It was isolated in the late 1970's by Dr. K. Haines, University of Texas at Austin, from a mixed species culture received from Dr. J. L. Martin, Centre Oceanologique du Pacific, Vairao, Tahiti. Ewart and Epifanio (1981) have shown that I. galbana T-iso is a suitable substitute for I. galbana as food for larval and juvenile C. virginica. I. galbana has a growth rate maximum of ca. 1 doubling per day (18 to 20°C and 350 to 750 W cm^{-2}). In comparison, I. galbana T-iso has a growth rate maximum of ca. 2.8 doublings per day (27°C with an irradiance of 1500 W cm^{-2}). I. galbana T-iso can adapt to a much broader range of temperature-light conditions than I. galbana, as shown by Ewart and Pruder (1981).

Although limited biochemical information is available on I. galbana T-iso, studies have been conducted on I. galbana. According to Romberger and Epifanio (1981), the food energy value of I. galbana is 20 J. mg^{-1}. This compares with 15 and 16 respectively for Tetraselmis suecica and Thalassiosira pseudonana (3H). All three algae were cultured under the same conditions. The percentage of protein for I. galbana is 60; double Tetraselmis suecica's value and 1.5 times the value for Thalassiosira pseudonana (Romberger and Epifanio, 1981). The amino acid composition of I. galbana T-iso is given in Appendix I, Tables 1 and 5. While the amino acid composition among phytoplankton is fairly uniform the arginine and leucine levels of both I. galbana and I. galbana T-iso are relatively

higher than those of the other algal species shown in Appendix I, Table 1. According to Romberger and Epifanio (1981), the lipid content of I. galbana at 20°C is 17% based on dry weight, which corresponds to the results of the present study for I. galbana T-iso (Appendix II, Table 2.). The fatty acid compositions of I. galbana and I. galbana T-iso is given in Appendix II, Tables 1, 2 and 5. The major saturated fatty acids are 14:0 and 16:0. The principal unsaturated fatty acids of both organisms are 18:1, 18:2, 18:3, and 18:4 and 22:6ω3. Compared with other algal species analysed I. galbana T-iso has a very high level of 22:6ω3 (10.8) and a very low level of 20:5ω3 (0.7), as shown in Appendix II, Table 5. Langdon and Waldock (1981) demonstrated 22:6ω3 and 20:5 3 are essential fatty acids in the diet of the Crassostrea gigas juveniles. The carbohydrate content of 7.3 pg glucose equivalent $(10^6 \text{ cells})^{-1}$ (Appendix III, Table 1) or 21% of the dry weight for I. galbana T-iso is low when compared to the other algal species examined (Appendix III, Tables 1 and 3). The carbohydrate concentration of I. galbana, according to Romberger and Epifanio (1981), is also relatively low; 16% compared to 27% and 52% of the dry weight respectively for Thalassiosira pseudonana (3H) and Tetraselmis suecica. Marker (1965) found that after acid hydrolysis, the carbohydrates of I. galbana contained mainly glucose and small amounts of galactose, arabinose, xylose and ribose. I. galbana excretes glycolate and cyclohexanetetrol (Hellebust, 1974), especially during the stationary growth phase (Guillard and Wangersky, 1958; and Marker, 1965). Wilson (1979) found that I. galbana cultures entering the stationary phase of growth, stimulated grazing in O. edulis larvae to a greater extent than cultures in other growth phases.

Compared to other phytoplankton species, I. galbana T-iso has a high protein level, an average lipid level and a low carbohydrate level. The low carbohydrate level may account for the consistent mediocre oyster growth response with this species (Figs. 2 through 7).

ii) Chaetoceros spp.

Chaetoceros species proved to be favourable diets for O. edulis juveniles (Table 2). In experiment 1 and 3 C. gracilis yielded respectively a 3.5 and 3.1 fold increase in oyster fresh weight gain compared to I. galbana T-iso over a five week period. The results, expressed in growth rate k based on fresh and dry weight values, are shown in Figs. 2, 3, 6 and 7. The C. gracilis culture was obtained originally from Dr. E. Laws, University of Hawa[redacted], via Dr. G. Pruder, University of Delawafe, upon our request for an untested algal culture with potential as an oyster diet. Chaetoceros species have been overlooked in bivalve culture in the past. Walne (1970) found C. calcitrans was an excellent food, and Langdon and Waldock (1981) used this alga in their studies (Table 1). DePauw (1981) lists C. calcitrans, C. simplex and C. curvisetus as algal food in bivalve nurseries; however, in most hatcheries and nurseries Chaetoceros species have been underutilized. Since C. calcitrans is ca. half the size of C. gracilis, one would expect this smaller alga to be a good larval diet. Scura et al. (1979) used Chaetoceros species isolated from cultures which developed naturally in their Hawaiian oyster ponds. The only literary reference to C. gracilis being used as one component of a mollusc diet is Ukeles (1976), who cites its use by Koganezawa in Japan.

As a result of this research, C. gracilis is routinely used in the Dalhousie University hatchery as a diet for larval, juvenile and spawning adult bivalves. C. gracilis is easy to grow and maintain in culture. Like I. galbana T-iso, this tropical strain grows well over a wide temperature range (10 to 30°C) with an optimal growing temperature of ca. 28°C. The high temperature tolerance of C. gracilis permits outdoor or greenhouse culture growth in subtropical and tropical climates and under summer conditions in temperate climates. Of the three Chaetoceros species, C. simplex was the most unreliable in culture due to frequent, premature senescence.

The biochemical analyses conducted on Chaetoceros species (Tables 3 and 4; Figs. 14, 15 and 16; Appendix I, Tables 1, 3, 9, 10 and 11; Appendix II, Tables 1, 9, 10, 11 and 12; and Appendix III, Table 2) show a typical C to N ratio and average levels of protein, carbohydrate and lipid. The small cells of C. calcitran (3μ) have a very low protein level of 2.2 $\mu g(10^6 \text{ cells})^{-1}$; however, C. gracilis, a medium sized alga (7μ), has a low protein level of 10.8 $\mu g(10^6 \text{ cells})^{-1}$ compared with comparable sized algal species. C. gracilis contains a moderate level, 9.2 $\mu g(10^6 \text{ cells})^{-1}$, of total lipid (Appendix II, Table 9). Although it is well established (Appendix II, Tables 1, 3, 6, 7, 10, 11 and 12) that long-chain polyunsaturated fatty acids are abundant in diatoms, Kayama et al. (1963) was unable to detect any fatty-acids with a carbon chain longer than 18 in C. simplex. Their lipid, however, was extracted with ether; a method which Chuecas and Riley (1969) have demonstrated to be inferior. The discussion of algal fatty acids in this report will be restricted to studies using a chloroform-

Table 4. The carbon and nitrogen content (% dry weight) of Chaetoceros gracilis and Isochrysis galbana T-iso.

Algal Diet	C%		N%		C/N
	$\bar{x}$ (n=3)	s.d.	$\bar{x}$ (n=3)	s.d.	
Chaetoceros gracilis					
Control	34.1	3.3	3.9	0.9	8.7
Silicate Limited[1.]	43.1	1.8	2.7	0.7	16.0
Nitrogen Limited[1.]	33.7	3.7	2.2	0.1	15.3
Isochrysis galbana T-iso	46.3	2.3	5.6	1.0	8.3

1. The results of the silicate and nitrogen limited cultures will be discussed in part II.

methanol extraction procedure. The 22:6ω3 fatty acid content in C. gracilis (2.5%) is very high compared with other diatom species. The only diatom with a comparable value (2.2%) appears to be Thalassiosira fluviatilis (Ackman and Tocher, 1968); however, this alga is of little use as an oyster diet because it has an inhibitory effect on O. edulis juveniles (Walne, 1970). The 20:5ω3 fatty acid content in C. gracilis (11.5%) is low relative to the other diatom species listed in Appendix II, Table 1. The biochemical composition of C. gracilis is discussed at length in Part II.

Based on the success in the oyster growth trials with C. gracilis, C. calcitrans and C. simplex, other Chaetoceros species were obtained from Dr. R. Guillard, Culture Collection of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine. These included C. pseudocrinitus, C. ceratosporum, C. affine, C. costatum, C. didymum and C. pseudocurvisetum. These species either did not grow well in our culture system or the cells tended to aggregate in clumps considered too large for the juvenile bivalves to ingest. Thus, we did not experiment further with these species.

iii) Skeletonema spp.

S. costatum (Table 2) was a favourable diet for the juvenile oysters. The culture used was obtained from Dr. J. Craigie, National Research Council of Canada. Several other S. costatum cultures, received from various collections, proved difficult to culture. Walne (1970) also encountered problems when attempting to grow this diatom. Craigie (pers. comm.) has suggested a viral infection may be the cause of the

problem with some S. costatum cultures. This species is considered a low temperature adapted species and is abundant in inshore, temperate waters. Nelson (1947) suggested that S. costatum supported the nutritional requirements of the natural oyster population in Delaware Bay. Soeder and Stengel (1974) found temperature adapted cultures of S. costatum grew only 50% better at 20°C than at 7°C. Perhaps only robust strains of this species are suitable for mass culture at temperatures near 22°C.

The chemical composition of S. costatum is typical of other diatom species (Sakshaug and Holm-Hansen, 1977). The amino acid composition is shown in Appendix I, Tables 1 and 4. Chuecas and Riley (1969) and Ackman et al. (1964) have shown that the major fatty acids in this species are 20:5ω3 and 16:1ω7. Glucose and oligosaccharides are its major storage products (Handa, 1969; and Allan et al., 1972).

S. menzelii yielded an unfavourable growth response when fed to O. edulis juveniles; however, it was reported to be a very good food for cultured clams (Mercenaria mercenaria) in New England (Petrovits, pers. comm.). Although S. menzelii is smaller (2.9μ ± 0.9) than S. costatum (4.7μ ± 1.2) the amino acid content of S. menzelii (Appendix I, Tables 1 and 2) is ca. twice that of S. costatum, with the exception of alanine which is comparable. S. menzelii was isolated in the Western Sargasso Sea (Guillard et al., 1974). S. menzelii tends to be a non-chain forming diatom, unlike S. costatum, which arranges itself in a chain-like form. On the basis of the oyster-feeding results algal chain formation does not appear to restrict bivalve feeding.

iv) Rhodomonas sp.

Rhodomonas sp. proved to be a good diet for the juvenile oysters. This cryptophycean culture was obtained from Dr. J. Craigie, National Research Council of Canada, and recommended for its high carbohydrate level. The carbohydrate level of Rhodomonas sp. was ca. four times higher than that of I. galbana T-iso or C. gracilis when cultures grown with the complete (f/2) nutrient medium are compared (Appendix III). Holland and Hannant (1974) suggest that carbohydrates are the main energy source for O. edulis juveniles.

Working with Cryptomonas sp., Walne (1970) suggested that Cryptophyceae species were unsuitable for bivalve diets as they did not form dense cultures. The contrary was found in this study, where the Cryptophyceae species, Rhodomonas sp., obtained cell densities in four litre carboys ranging from 2.0 to 6.0 x 10^6 cells ml^{-1} when harvested for oyster food three days after inoculation. Iwasaki et al. (1971) recorded cultures of Rhodomonas sp. averaging concentrations of 5.8 x 10^6 cells ml^{-1}.

The growth rate of O. edulis doubled when Cryptomonas sp. was supplied in unfiltered seawater compared to filtered seawater (Walne, 1970). The seawater must contain nutritional components for the oysters which were not present in the Cryptophyceae isolate used in Walne's study. Cryptomonas spp. do, however, contain the two fatty acids 20:5ω3 and 22:6ω3 (Kates and Volcani, 1966; Chuecas and Riley, 1969; Beach et al., 1970), which were shown to be growth limiting for juvenile bivalves (Langdon and Waldock, 1981). There is considerable variation in published reports between the various fatty acid spectra of

Cryptomonas species. Differing culture conditions, such as nitrogen limitation (Lichtle and Dubacq, 1984), may account for this. The analytical techniques used are also critical. For example, failure to extract lipids completely and to prevent autoxidation of the polyunsaturated components is a frequent cause of error in lipid analyses. Differences among gas chromatograph columns may account for some of the discrepancies. With the exception of the members of the Dinophyceae, *Cryptomonas* had the highest level of the 22:6ω3 fatty acid (10%) out of all the algal fatty acid spectrums reviewed by Pohl and Zurheide (1979). *Cryptomonas* species are also unique because they have a high concentration of the fatty acids 18:3ω3, 18:4ω3 and 20:5ω3. A mixed algal diet consisting of the unique fatty acids and high carbohydrate content of *Rhodomonas* sp., along with *Chaetoceros gracilis* with its abundant level of the fatty acids 22:5ω3 and 22:6ω3, could be expected to yield very high growth rates when fed to *O. edulis* juveniles.

v) *Thalassiosira pseudonana*

T. pseudonana is commonly used in hatcheries and nurseries. The results of the present study support its suitability as an algal food species for *O. edulis* juveniles. Protein (Epifanio, 1979; and Appendix I, Table 1) and fatty acid (Kates and Volcani, 1966; Orcutt and Patterson, 1975; and Appendix II, Tables 1, 3, 6 and 7) analyses have been conducted on this species. While the amino acid composition of *T. pseudonana* is similar to the other algae examined, a relatively higher level of isoleucine and tyrosine, along with a relatively lower level of

alanine are apparent (Appendix I, Table 1). The fatty acid spectrum of T. pseudonana (3H) cultured with a complete nutrient mix (f/2) is similar to that of Chaetoceros gracilis, except for lower levels of the essential fatty acids 20:5ω6 and 22:6ω3 and higher levels of saturated and monoethylenic fatty acids (Appendix II, Table 6). Ukeles (1976) found this alga difficult to maintain in culture, although our results do not concur.

vi) Tetraselmis spp.

The oyster growth response obtained using Tetraselmis sp. (Platy 1) and T. maculata as diets was disappointing. Based on the high position Tetraselmis spp. received both in Table 1, and Ukeles and Wikfors' report (1982), it was predicted this genus would rank higher than the reference species I. galbana T-iso in the present study. Despite the heavy use of this species (Table 1), some research shows it is a poor diet for oysters. Epifanio (1979) reported that T. suecica was a poor food for C. virginica juveniles, possibly due to digestibility problems. This alga is bound by a non-rigid theca composed of proteins and complex polysaccharides (Lewin, 1958). Digestion of this material may be largely an extracellular process (Owen, 1975). Presumably, the digestion rate of T. suecica cells would be slower than the rate for I. galbana or Thalassiosira pseudonana. Although Tetraselmis spp. have high carbohydrate levels (Romberger and Epifanio, 1981), they do not contain any of the essential fatty acid 22:6ω3 (Appendix II, Tables 1 and 4), which greatly restricts its usefulness as a juvenile oyster feed. This genus appears to have a typical amino acid composition

(Appendix I, Table 1). Tetraselmis sp. is not difficult to grow in culture. Droop (1974) reports that it grows on glucose in the dark. However, Tetraselmis spp. often cling to the culture vessel walls, reducing light penetration and thus making the species of less practical value.

vii) Pavlova lutheri

The oyster growth results were also disappointing when the oysters were fed with P. lutheri, as a better response was expected based upon the rating this alga received in Table 1. P. lutheri was a valuable algal species when combined with other phytoplankton (Dupuy, 1975). As a diet for Mytilus edulis larvae, Newkirk and Waugh (1980) found the combination of I. galbana and P. lutheri was preferable to a sole diet of P. lutheri, suggesting P. lutheri could not provide the complete nutritional requirements of mussel larvae. Newkirk and Waugh (1980) reported no progressive decrease in mussel larval growth rates with a mixed diet containing increasing amounts of P. lutheri, although Fretter and Montgomery (1968) reported P. lutheri was toxic for bivalves. Fretter and Montgomery (1968), however, also suggested that bacteria could metabolize the toxic substance and create an algal culture suitable for bivalves. The effects of bacterial contamination may explain the conflicting reports on the status of P. lutheri as a bivalve diet.

The biochemical composition of P. lutheri as reported by Aaronson, et al. (1980) is 48% protein, 31% carbohydrate and 12% lipid. Variation in algal composition under culture conditions can limit the value of such statistics. For example, Taub (1980) reports that the dry weight

protein yield of P. lutheri can be environmentally manipulated from 20 to 60%.

Qualitative information on the unique biochemical characteristics of the algae is more useful. The amino acid composition of P. lutheri as presented in Appendix I, Table 1, shows a relatively lower level of glutamic acid compared to other algal species. The major fatty acids of P. lutheri, determined by Langdon and Waldock (1981) and Chuecas and Riley (1969) are 16:1ω7, 20:5ω3, and 15:0 (Appendix II, Table 1). Langdon and Waldock (1981) and Ackman and Tocher (1968) found P. lutheri contained respectively 8.0 and 13.1% of 22:6ω3; however Chuecas and Riley (1969) did not identify this fatty acid in their culture. The major storage product is cyclohexane tetrol (Craigie, 1974). This research also identified the unusual accumulation of cyclitols (low molecular weight organic substances) in P. lutheri. Laycock and Craigie (1970) discovered cyclitols such as myo-inositol, 2-deoxy-myo-inositol and 1,4/2,5-cyclohexanetetrol in P. lutheri. Accumulation of the latter could account for 7% of the organism's dry weight. The levels of this material are regulated by the external osmotic environment (Craigie, 1969). Decreased salinity results in the rapid expulsion of cyclohexanetetrol while increased salinity lowers photosynthetic accumulation. P. lutheri is also known to release glycolate and mannitol (Hellebust, 1965). Droop (1974) and Kristensen (1956) showed that P. lutheri can release a high molecular weight, vitamin B_{12} binding substance.

viii) Phaeodactylum tricornutum

Tables 1 and 2 indicate the poor growth response of the juvenile

oysters when fed P. tricornutum. Epifanio et al. (1981) found the growth rate of C. virginica oysters over a five week period was inversely proportional to the amount of P. tricornutum in the diet. He also reported that P. tricornutum did not support good growth with C. gigas, O. edulis or Mytilus edulis (Epifanio et al., 1975). Walne (1970) reported that O. edulis juveniles developed an abnormal shape when fed high concentrations of P. tricornutum. The oysters became elongated due to reduced growth of the shell adjacent to the inhalent and exhalent margins. This was not observed in the present study. Walne (1970) suggested the poor growth recorded with the higher algal cell concentrations may be due in part to the physical irritation caused by the siliceous spines of P. tricornutum. The addition of kaolin to all of our algal diets may have assisted the oyster in grinding siliceous spines, if they exist. Reimann et al.(1966) suggest they do not exist.

In contrast to the findings of Walne (1970) and Epifanio (1983), Wilson (1979) demonstrated that P. tricornutum was a good feed organism. He found the growth rate of O. edulis and C. gigas larvae developed equally well on P. tricornutum and I. galbana. He also reported that local populations of P. tricornutum routinely used in Irish oyster hatcheries provided an ideal, low cost feed organism. Loosanoff and Murray (1974) and Goldman (1976) report that when mass cultures were inoculated with several species of algae, P. tricornutum normally became predominant. Fawley (1984) has shown that the maximum division rate for P. tricornutum is at 23^{0}C or less, depending on the irradiance level. Monas sp., a colorless flagellate, is a destructive contaminant of P. tricornutum cultures (Raymont and Adams, 1958; and Ansell et al., 1963).

However, Imai and Hatanaka (1949) have reared C. gigas juveniles on Monas sp., which suggests this contaminant may increase the nutritional value of the P. tricornutum diet.

Mann and Ryther (1977) grew six species of bivalve molluscs in a sewage waste-recycling aquaculture system in which P. tricornutum was the predominant feed organism. They found O. edulis, C. gigas and Tapes japonica grew well while C. virginica, Mytilus edulis and Mercenaria mercenaria grew poorly. This report suggests that bivalve species have a specific growth response to P. tricornutum which may explain the conflicting conclusions discussed above. The presence of other algae may compensate for the nutrient deficiencies of P. tricornutum.

The nutritional value of P. tricornutum for C. virginica juveniles was increased by the addition of silt (Ali, 1981). Nutrients and bacterial enzymes attached to the silt, or the enhanced grinding prompted by the silt, may account for the increased nutritional value. Insufficient grinding does not explain the poor oyster growth response obtained with P. tricornutum in the present study, since kaolin was added to all algal diets.

The gross chemical composition of P. tricornutum varies considerably, probably a function of environmental changes. According to Aaronson et al. (1980), the dry weight yield of this alga is 33% protein, 24% carbohydrate, 7% lipid and 4% nucleic acid. Strickland's review of similar analyses from four different labs (1960) reports ranges of 16 to 61% protein, 13 to 22% carbohydrate and 6 to 28% lipid. P. tricornutum is lower in lysine, sulphur amino acids, and histidine (the essential amino acids for mollusc growth) than Thalassiosira

pseudonana, for example. The absence of tryptophan convinced Epifanio et al.(1981) that P. tricornutum was a poor diet. Tryptophan, however, is readily destroyed in hydrolysis. Furthermore, Boudreau (1984) suggested that phenylalanine is often substituted for tryptophan, and P. tricornutum contains fairly high levels of phenylalanine (Appendix I, Table 1). Thus it is unlikely that trytophan is a growth limiting factor in this diet. The storage products of P. tricornutum include glucose, laminari-biose & -triose, sulphated mannan, myo-inositol, scyllo-inositol and laminitol (Ford and Percival, 1965). The mucilage covering of this species contains xylose, mannose, fructose, and galactose (Hellebust, 1974). The major fatty acids shown by both Chuecas and Riley (1969) and Kates and Volcani (1966) in Appendix II, Table 1 are 16:1ω3 and 20:5ω3. P. tricornutum produces and releases biotin which is used by other algae (Carlucci and Bowes, 1970). Sharp et al. (1979) have attempted to explain the allelopathic interaction between P. tricornutum and T. pseudonana in terms of vitamin competition. P. tricornutum produces a B_{12} binding factor which is believed to inhibit T. pseudonana. According to Ackman et al. (1966), P. tricornutum contains the antibacterial compound dimethyl- β -propiothetin (DMPT). Brown et al. (1977) have shown that C. gigas fed P. tricornutum contains reduced Escherichia coli levels, which has been attributed to the antibacterial effect of the DMPT.

ix) Pseudoisochrysis sp. (Va 12) and the Green Flagellate (Va 52)

Pseudoisochrysis sp. (Va 12) and the green flagellate (Va 52) are isolates which proved to be effective algal food species for larvae at

the Virgina Institute for Marine Science (Dupuy et al., 1977). The results were not the same with O. edulis juveniles in the present study. The carbohydrate level in these species may be insufficient to support the increased requirements of the juvenile stage oyster. The green flagellate (Va 52) may be too small to be efficiently filtered by oysters (Haven and Morales-Almo, 1970).

x) Chlorococcum sp. and Dunaliella spp.

Both Chlorococcum sp. and D. tertiolecta were very poor diets for the juvenile oysters. Little useful information is available on Chlorococcum species. Langdon and Waldock (1981) felt they could not explain the poor growth of Crassostrea gigas fed D. tertiolecta on the basis of chemical composition because adequate levels of protein, carbohydrate and a relatively high level of lipid was typical for this species. D. tertiolecta had the highest protein level of the species analysed in the present study. Relatively high levels of leucine, alanine, glutamic acid and aspartic acid were apparent (Appendix I, Table 1). Using microencapsulated fatty acids, Langdon and Waldock (1981) demonstrated that a deficiency of 22:6ω3 in this algae (Appendix II, Table 1) limited the growth of juvenile oysters. This fatty acid is believed to be critical for maintaining the bivalve's cell membrane fluidity (Langdon and Waldock, 1981).

Information on the chemical composition of D. salina is presented by Chapman (1970). He reports the following: 57% protein, 23% carbohydrate, and 6% lipid. When Dunaliella spp. are transferred to higher salinity a change in colour from green to red or brown occurs,

due to the synthesis of carotenoids and the decomposition of chlorophyll (Mironyuk and Einor, 1968).

The major storage product of D. tertiolecta is glycerol; the concentration of which is environmentally controlled. Craigie and McLachlan (1964) demonstrated that the glycerol level in D. tertiolecta increased as the culture salinity rose. Wegmann (1971) recorded a 13 to 65% increase in glycerol in the same species when the salt concentration was increased from 0.2 to ca. 2.7 M. Subsequent work with other species of Dunaliella supports these results (Ben-Amotz, 1975; Ben-Amotz et al., 1982; Borowitzka and Brown, 1974; and Borowitzka et al., 1977). Dunaliella spp. develop large amounts of glycerol to balance the high, external osmotic pressures caused by the increasing salinity (Enhuber and Gimmler, 1980). Extracellular products of Dunaliella spp. include peptides, polysaccharides, glycolate and glycerol, as well as the vitamins biotin and thiamine (Droop, 1974). Although Brown et al. (1982) demonstrated through NMR spectroscopy that Dunaliella sp. does not excrete significant amounts of glycerol into the media, there are reports of considerable leakage of this osmoregulatory compound with D. tertiolecta (Jokela and Tang, 1969) and D. parva (Ben-Amotz, 1975; and Enhuber and Gimmler, 1980).

xi) Mixed algal diet

The highest oyster growth rates were obtained with a mixed algal diet. This diet was an ancillary reference against which the single species diets could be compared. The five algal species comprising the mixed diet were added in approximately equal cell numbers daily so that

the total feeding ration was ca. 1.2. An adequate density range was not examined for the mixed diet; therefore a true comparison cannot be made between this diet and the others. The mixed diet was used in the first feeding trial (Figs. 2 and 3), prior to full realization of the influence of cell density on oyster growth response. As shown in Figs. 2 through 7 it is essential that an optimum density be determined for each algal diet. For the five algal species which were included in the mixed diet, the highest oyster feeding response obtained, when tested singly, occurred at the following feeding rations: P. tricornutum, 0.7; Pavlova lutheri, 0.8; Chaetoceros simplex, 1.0; I. galbana T-iso, 1.0 to 2.0 and C. gracilis, 3.0 to 3.5. The average of these values approaches a feeding ration of 1.2; however, to evaluate mixed algal diets properly it would be necessary to examine the component species in various ratio combinations and at various total densities. This was felt to be beyond the scope of this study.

xii) Controls

Consistently poor growth rates were obtained in the oyster feeding trials with the unfed control treatments (Figs. 2 through 7). Comparable oyster growth rates were obtained with both unfed control groups; with and without kaolin (Figs. 2 and 3). This suggests that the seawater used in the experiments was relatively free of particulate and dissolved material which could have accumulated on the surface of the kaolin and provided an ingestible source of nourishment for the oysters.

c) Oyster Mortality

Oyster mortality occurred for a variety of reasons. All of the oysters may not have been in a healthy state at the beginning of the experimental period. The valves of some oysters were inadvertently glued closed when attached to the tags and thus they could not feed properly. If the final fresh weight of the oyster was less than 3% greater than the initial weight, the oyster was considered unhealthy and was not included in the statistics. (The 3% margin accounts for any differences in moisture content between the initial and final weighing). Some oysters did not remain attached to their tags and were lost. Occasionally a section of one of the strings containing thetagged oysters would be tossed up on the rim of the pail by the motion of the circulating seawater. Also, on occasion the oysters were not all properly positioned below the water level of the pail, after daily routine maintenance. If the oysters were left exposed to the air for more than ca. 12 hours death resulted. The above mentioned causes of death are estimated to account for 5 to 10% of the total mortality. The remaining mortality is considered to be a function of the poor nutritional status of the oysters. In Figs. 2 through 7 the numbers above the histogram bars indicate the number of replicate oysters used in the analysis. If mortality or losses did not occur n equals 75. Mortality is higher among the poorer diets.

Part I

Conclusions

Out of the 16 phytoplankton species examined as diets for juvenile European oysters a greater growth response was obtained with the diatom species, in particular Chaetoceros species, when compared to flagellated species. C. gracilis was the highest ranking single algal diet. This alga is an excellent candidate for mass culture because it has a wide temperature tolerance range, is dependable in culture and multiplies as rapidly or more quickly than the other algal species examined. S. costatum and Thalassiosira pseudonana also yielded oyster growth responses which were greater than that obtained with the reference species I. galbana T-iso. Rhodomonas, a cryptomonad with a high carbohydrate content was also a high ranking diet. For reasons which were not identified, the two diatoms Pheodactylum tricornutum and S. menzelii were not good diets for O. edulis juveniles. Since the higher ranking diets of C. gracilis and Rhodomonas sp. have unexploited potential as oyster feed species relative to the more traditional diets these results will have great significance in an oyster nursery operation. The highest oyster growth rate was achieved with a mixed algal diet, although this diet was examined at only one feeding ration. A combination of the algal species which singly yield the highest oyster growth rate may further enhance oyster production.

As the algal species examined were of various sizes and morphologies each algal species was fed at a range of algal cell densities. Diets

Although carbohydrate is reported to be the main energy source for juvenile oysters (Holland and Hannant, 1974), this could not be demonstrated in the present study. The carbohydrate content of the Rhodomonas sp. diet was more than three times higher than that of C. gracilis or I. galbana T-iso, however, C. gracilis yielded the highest oyster growth rate. The inherent problem in attempting to explain nutritional requirements by feeding a variety of algal species is that several components changed simultaneously.

There are several possible reasons for discrepancies between the initial ranking of algal diets as based on literature reports (Table 1) and the results of the present study (Table 2). Firstly, several different oyster species were used in the total evaluation of Table 1. Secondly, the strains and physiological state of the algal cells used in different studies are not fully comparable. The preconditioning environment can greatly influence the biochemical composition of algae, as shown by the response of shade and sun adapted phytoplankton cultures (Yentsch and Lee, 1966) or phosphorus limited cultures (Perry, 1976). Thirdly, feeding rations varied considerably both within and among feeding trials. More research, involving large numbers of phytoplankton species under fully comparable, or at least well defined, culture conditions is required before the relative potential of various algal species for bivalve diets can be further defined.

In mollusc culture there is no commercial feed available to replace single celled algae. Although artificial diets may be used to feed oysters in the future, the industry will be based on natural feeds for

Were compared at the cell density yielding the highest oyster growth rate. All diets were examined over a reasonable cell concentration range. They were non-toxic, digestible and within the acceptable cell size range for the oyster. Thus, the nutritional quality of the algal diet is believed responsible for the differences in oyster growth response.

The fatty acid composition of the diet appeared important in determining a good diet for juvenile oysters. Diatoms have a unique fatty acid composition. The major fatty acids of diatoms are: 16:0, 16:1ω7, and 20:5ω3. These fatty acids account for more than 50% of the total fatty acids in diatoms. Thus, these fatty acids may be important nutritional components for juvenile oysters. The diatom species can be distinguished from other algae by the virtual absence of the fatty acids 18:2, 18:3 and 18:4, suggesting these are not critical in a juvenile oyster diet. Rhodomonas sp., a member of the Cryptophyceae, also was a good diet for the juvenile oysters. The Cryptomonadales contain a large percentage of 18:3, 18:4, 20:5ω3 and especially 22:6ω3. The above findings support the conclusions of Langdon and Waldock (1981) that 20:5ω3 and 22:6ω3 are essential fatty acids for juvenile oysters.

There was no trend in the protein level of the diets and the various growth responses obtained with the juvenile oysters. The total protein level of the diets varied somewhat. Dunaliella tertiolecta, which was one of the poorest diets tested, had a high protein level. The amino acid composition of the algal diets examined were similar.

many years before such a method becomes viable. The cost of culturing algae in a nursery operation may be significantly reduced as more suitable algal diets are located and better culture techniques are developed. For example, with dialysis culture, Jensen and Rystad (1973) reached cell densities of 1×10^7 and 4×10^7 cells per ml, respectively, with S. costatum and P. tricornutum, while Ney et al. (1981) maintained continuous cultures of T. pseudonana at a density of 5×10^8 cells per ml. The dialysis process may reduce the cost and space requirements for algal production while maintaining a high level of cell production in a controlled environment. Such techniques improve the commercial prospects for phytoplankton as food for bivalves.

Advances in culture technology, coupled with the selection of algal species which most efficiently satisfy the nutritional requirement of bivalves, will greatly improve the economics of shellfish aquaculture. In the present study only a very small proportion of the possible algal condidates were examined and yet substantial differences between diets were observed. As additional phytoplankton species are isolated from nature and successfully grown in mass culture, improved diets will eventually emerge. The combination of good algal species, when tested over a suitable cell density range, may yield better oyster growth responses compared to monculture diets. Such advances in the diet will greatly improve the economics of hatchery or nursery reared juvenile European oysters.

PART II

Part II

Introduction

The extent to which phytoplankton species are environmentally or genetically controlled is of great interest to those rearing algae. The protein, carbohydrate, lipid, total pigment and ash analysis of the 11 species of marine phytoplankton which were examined by Parsons et al. (1961) and which represent five classes, demonstrated that marine phytoplankton have a similar organic composition when grown under the same culture conditions, irrespective of the size of the alga or its class. Changes in macro- and micronutrient availability, culture age, salinity, temperature, irradiance level, light quality and pH can induce differences in the chemical composition and growth rate of phytoplankton (Lewin, 1962; Lewin and Guillard, 1963; and Stewart, 1974). Working with Dunaliella tertiolecta, Phaeodactylum tricornutum and Pavlova lutheri, Walne (1970, 1974) demonstrated that differences in chemical composition within a single species caused by environmental and chemical culture changes were greater than the differences observed among the three species when grown under the same conditions. Thus, culture manipulation within one species may produce a sufficient range of biochemically altered algae to be used as diets to determine the nutritional requirements of an organism such as a bivalve.

When the growth rate of a phytoplankton culture is restricted because of an insufficient supply of nutrients, the algae often alter their metabolism and divert energy into the production of storage products. The effects of silicate and nitrogen depletion will be

discussed in terms of the chemical composition of algal cells.

a) Silicon Depleted Algal Cultures

With the exception of Phaeodactylum tricornutum (Nelson et al., 1984), diatoms have an absolute dependence on silicon (Darley, 1974), requiring it, generally in the form of soluble silicic acid, for their silicate cell walls (Lewin, 1962; and Darley and Volcani, 1969). The carbon to silicate ratio of silicate limited and starved diatoms is three to five times higher than the ratio for non-limited cells (Harrison et al., 1977). Cellular mechanisms requiring silicic acid and the alterations observed in the diatom when silicon metabolism is halted will be briefly reviewed.

In the presence of silicon, the cell's protoplasm doubles prior to cell division and silica wall formation. The carbon content of the cell increases as a result of nuclear division and cytokinesis (Coombs et al., 1967b). Cell wall formation typically follows mitosis while the two daughter protoplasts are still enclosed by the parent frustule. Using a radioisotope of silicon, Azam and Volcani (1974) studied the mobilization of silicic acid in Navicula alba. When silicic acid is available, it is removed from the media, polymerized by an unknown mechanism and deposited within sequentially-appearing pairs of joined vesicles. The vesicles are then bound by a unit membrane and form the new cell wall. This structure, believed to be derived from the Golgi-apparatus, is called the silicalemma (Reimann, 1964). The developing cell wall is surrounded by an organic casing (Reimann et al., 1966). Coombs and Volcani (1968b) found that amino acids were the first

compounds added to the newly formed cell walls of N. pelliculosa; xylose and fructose were added next, followed by mannose and gluconic acid. Kates and Volcani (1968) have shown that lipid accounts for 1 to 13% of the organic wall matrix. Following wall formation, the cell separates and the daughter cells are released.

Diatoms have a limited ability to store silicic acid. Cylindrotheca fusiformis (Coombs et al., 1967b; Darley and Volcani, 1969), Ditylum brightwellii (Eppley et al., 1967) and N. pelliculosa (Darley, 1969) have been reported to absorb silicic acid from the growth medium solely during cell wall formation and rapidly incorporate it into the developing frustule. According to Rueter and Morel (1981), silicic acid uptake is mediated by a zinc-dependent system which is inactivated by copper. They present only indirect evidence to support this claim, however, as the enzyme responsible for silicic acid uptake has not been isolated.

When silicon is depleted in a diatom culture, cell division ceases. One may hypothesize that silicon deprivation restricts cell development, due to an inadequate supply of building material for the new cell wall. However, silicic acid is also required for an earlier stage in cell division; the synthesis of DNA (Darley, 1969; and Darley and Volcani, 1969). DNA polymerase and thymidylate kinase are essential enzymes in DNA biosynthesis which require silicic acid (Sullivan and Volcani, 1973). When C. fusiformis was deprived of silicate for 12 hours in the light, the DNA content increased by only 4 to 10%, while the major cellular constituents increased by at least 75% (Darley and Volcani, 1969). Borowitzka and Volcani (1977) have shown that the ratio of the

AMP and GMP nucleotides in DNA is altered when cultures of C. fusiformis are deprived of silicate.

When cell division is blocked as a result of silicate limitation, the diatom alters its metabolism and chemical composition. The net synthesis of proteins, carbohydrates, and nucleic acids continues to increase for five to seven hours in silicate depleted cultures (Coombs et al., 1967a). The pool of L-glutamic acid, the main precursor of proteinogenic amino acids, was reduced to one-third of its original level prior to the inhibition of net protein synthesis, whereas the pool of the energy compounds, nucleoside triphosphate and glycerol-1-phosphate, remained unchanged (Werner, 1978). This suggests protein synthesis ceases due to a deficiency in the basic building compound, L-glutamic acid, rather than a lack of energy. In silicon-starved cultures of N. pelliculosa, the rates of photosynthetic carbon dioxide fixation and oxygen evolution decrease (Coombs et al., 1967d), as do the rates of chlorophyll a, fucoxanthin and diadinoxanthin synthesis (Healey et al., 1967).

The energy which was previously allocated for silicon uptake and deposition (Coombs et al., 1967a, 1967b, 1967c; and Azam and Volcani, 1974), may be diverted to lipid production in silicon-starved cultures. The diatom, Cyclotella cryptica, when starved of silicate, doubled its lipid mass per cell in six to nine hours (Werner, 1978), or 12 hours (Shifrin and Chisholm, 1981). Silicate deprivation may inhibit many cellular functions without directly affecting lipid production. Werner (1977), however, suggests there is a shift from carbohydrate to lipid accumulation in silicon-free diatom cultures. He has identified the

site of enhanced fatty acid synthesis in silicon-deficient cells as being between acetyl CoA and α-ketoglutarate in the citric acid cycle. Nothing more appears to be known about the mechanism. The quality of the lipid produced by silica starved cultures does not appear to have been examined.

At present, it may be concluded that silicon is not only an essential constituent of the cell wall in diatoms, but also plays a vital role in the regulation of cell metabolism. The absence of silicic acid in the growth medium prevents the synthesis of nuclear DNA and reduces the rate of biosynthesis of many other cell components, while greatly enhancing lipid production.

b) Nitrogen Depleted Algal Cultures

Carbon is diverted into carbohydrate or lipid synthesis in many algal cultures when nitrogen is unavailable. The level of nitrate reductase declines and the pathways for protein synthesis become blocked (Eppley et al., 1969).

The carbohydrate content is enhanced under conditions of nitrogen limitation in many algal species. The carbohydrate content of Chlorella sp. varies from 6 to 45% of the dry weight in response to nitrogen deficiency (Strickland, 1960). Specific storage products lacking nitrogen, such as glucan in Chaetoceros species, are synthesized when nitrogen levels limit culture growth (Myklestad and Haug, 1972; and Haug et al., 1973). If a nitrogen source is available, amino acids are produced before sugar phosphates, as shown by the short term inclusion of carbon dioxide with Chlorella pyrenoidosa (Holm-Hansen et al., 1959).

Edge and Ricketts (1977) have shown that the provision of nitrogen or ammonium ions to nitrogen starved Platymonas striata cells caused a drop in the abnormally high carbohydrate level. However, 16 hours after nitrogen supplementation, the average carbohydrate level in these cells was significantly greater than the carbohydrate level of cells grown under normal nutrition.

The lipid content is enhanced under conditions of nitrogen limitation in several algal species. Shifrin and Chisholm (1981) demonstrated that after four to nine days of nitrogen starvation many green algae increased their lipid fraction by two to three fold. Nannochloropsis salina, an Eustigmatophyte, reached a lipid level of 72% on a dry weight basis. In a flow-through system containing a low concentration of asparagine, Endomyces vernalis did not accumulate lipid, while in the absence of a nitrogen source lipid accumulated (Heide, 1939). An equimolar concentration of various inorganic and organic nitrogen compounds had similar effects on lipid accumulation in Heide's study. In contrast, Spoehr and Milner (1949) reported that lipid accumulation in C. pyrenoidosa was favoured by algae that were previously cultured with ammonium rather than nitrate nitrogen. The results of Spoehr and Milner (1949) are suspect, though, due to poor experimental design. The conclusions were based on the results of single cultures with pH values which varied from 3.5 to 5.9 between treatments. Lipid results were obtained from calculations based on the elemental composition of the algae instead of using extraction techniques.

Nitrogen deficiency does not affect all algae similarly. In some

algae, nitrogen deficiency retards cell division and protein synthesis without altering the production of non-nitrogenous reserve products. Collyer and Fogg (1955) concluded that members of Rhodophyceae and Myxophyceae differed from Chlorophyceae, Euglenophyceae, Xanthophyceae and Bacillariophyceae since they did not accumulate lipid as the cell nitrogen content fell. Thus, the effect of nitrogen upon the synthesis of lipids or carbohydrates may depend on a secondary product of nitrogen metabolism.

Werner (1978) notes that diatoms under nitrogen limitation initially store carbohydrate and shift to lipid storage only after extended periods of stress. In the study by Shifrin and Chisholm (1981), an increase in the lipid level of several marine diatoms was not clearly defined after nine days of nitrogen limitation. Fogg (1956) asserts that progressive nitrogen limitation caused a quantitative change in the enzyme content of microalgae, which changes the synthesis from carbohydrate to lipid. Furthermore, the enzymes responsible for lipid synthesis are slowly produced in response to the depletion of nitrogen (Fogg, 1956). An accumulation of carbohydrates may trigger the conversion of carbohydrates into lipid reserves, which are a more efficient storage material.

Some changes in lipid composition have been noted in cells cultured in nitrogen limited media. Algae grown in a nitrogen deficient medium under light saturation produce a high lipid content, rich in palmitic (16:0) and oleic acids (18:1ω9). Algae grown in a nitrogen rich medium generally have a low lipid content consisting predominantly of

polyunsaturated fatty acids (Schlenk et al., 1960; Hulanicka et al., 1964; and Pohl, 1974).

Nitrogen deficiency has other effects on the physiology of phytoplankton. An increase in extracellular carbohydrates in nitrogen depleted cultures has been reported by Marker (1965). Furthermore, in about half of the 29 algal species which Shifrin and Chisholm (1981) studied; the average cell mass decreased somewhat under conditions of nitrogen deficiencies. French (1980) noted that nitrogen depletion triggers resting spore formation in Chaetoceros diadema and a similar response was observed with Cryptomonas rufescens (Lichtle and Dubacq, 1984). An accumulation of lipid will increase the buoyancy of the cells and perhaps change their distribution in the water column.

The diatom, Chaetoceros gracilis, which yielded the highest oyster growth rates when fed to O. edulis juveniles (Part I), is cultured in this study under three nutrient regimes in an attempt to produce diets with altered chemical compositions. These cultures are biochemically analyzed and fed to juvenile European oysters to examine this bivalve's growth response to various levels of protein, lipid and carbohydrate. By feeding one algal species which has been environmentally manipulated to produce several diets of varying chemical composition, other components, such as vitamins and minerals, which may affect the nutritional status of the diet, would remain more constant than if a variety of algal species were used.

Part II

Materials and Methods

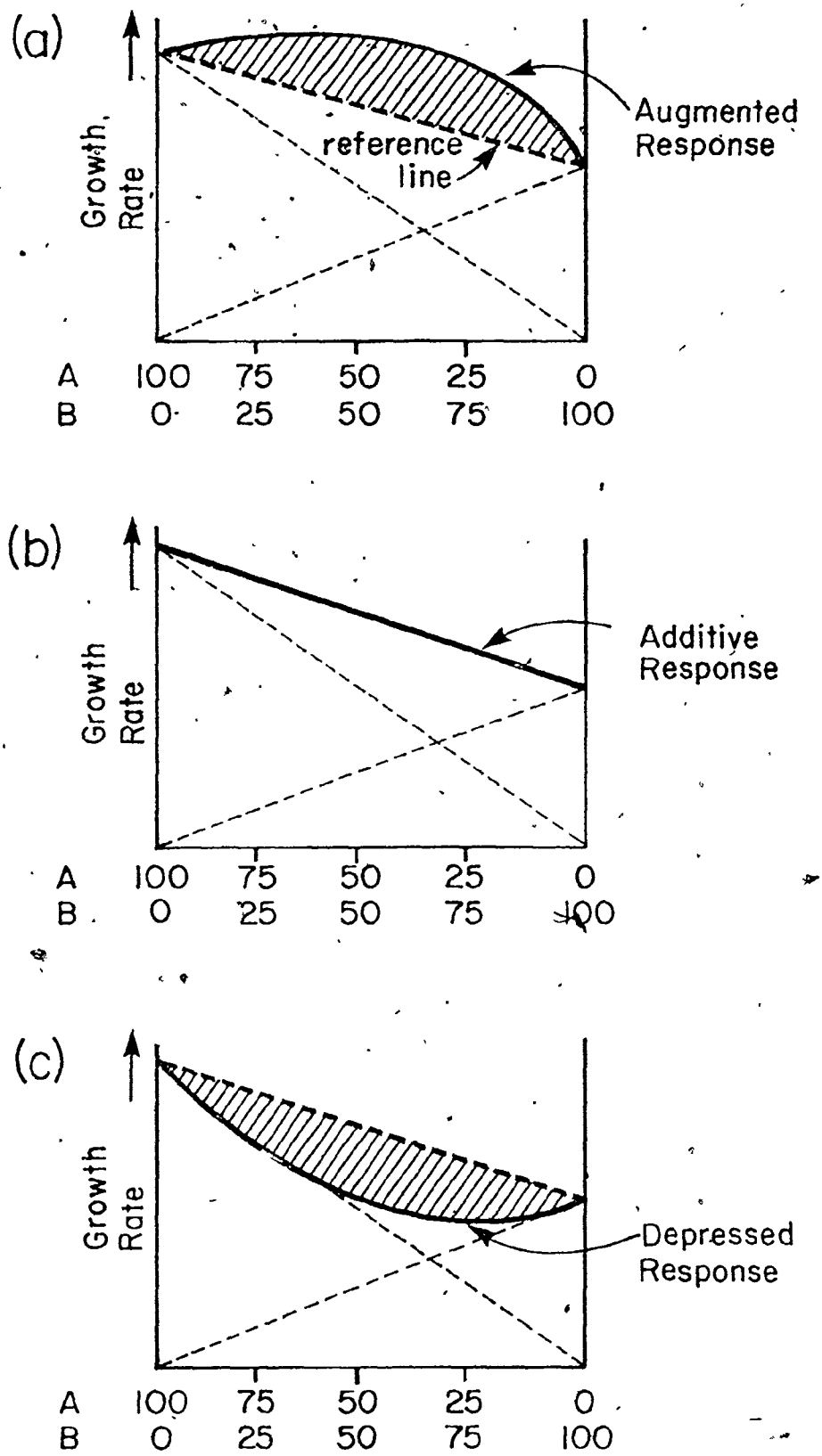
The methods used for the algal cultures, algal biochemical analyses and oyster growth trials in Part I were maintained in the following experiments. C. gracilis was cultured with the complete f/2 nutrient medium and with two nutrient limited media; f/2 lacking silicate and f/2 lacking nitrogen. On the day of inoculation, nutrients were added to the 20 litre carboys in the following proportions: 85 ml f/2 (control); 45 ml f/2 and 40 ml f/2 minus Si (silicate limited); or 20 ml f/2 and 65 ml f/2 minus N (nitrogen limited). On the second day after inoculation, the control cultures received 40 ml of $Na_2 SiO_3 \ 9H_2O$ (30 g/l), the silicate limited cultures received 15 ml of f/2 minus Si, and the nitrogen limited cultures received 15 ml of f/2 minus N. In preliminary experiments, the growth restricting factor of the limited treatments was verified by the addition of either nitrate or silicate to subsamples of the cultures. The cell division rate of these subcultures was compared to control subcultures. Monoculture diets of the control, silicate limited and nitrogen limited C. gracilis were fed to O. edulis juveniles in two separate experiments, both with a duration of five weeks.

Photographs were taken with a Carl Zeiss Photomicroscope II (West Germany) using the Nomarski Differential Interference Contrast Technique and High Speed Ektachrome, 160 ASA film.

a) Replacement Series Experiments

Two monocultures of either the control, silicate limited or nitrogen limited C. gracilis were combined in various rations, totaling

Fig. 8. Replacement series experiment; the possible growth responses of an individual fed varying proportions of diet A and diet B.


(a)
Growth Rate
reference line
Augmented Response
A 100 75 50 25 0
B 0 25 50 75 100
(b)
Growth Rate
Additive Response
A 100 75 50 25 0
B 0 25 50 75 100
(c)
Growth Rate
Depressed Response
A 100 75 50 25 0
B 0 25 50 75 100

either 3.5 or 6.0 x 10^5 cells ml^{-1}, and fed to *O. edulis* juveniles in a replacement series experiment which lasted five weeks.

Three possible growth rate responses may result when varying portions of two species, A and B, are used as diets (Fig. 8). If the growth response obtained with the combined diets equals the result expected from the summation of the two separate diets, represented by the reference line, then a simple additive response occurs (Fig. 8b). If the growth response is greater than the predicted response based on the addition of the component parts, then an augmented growth response results (Fig. 8a). A depressed growth response occurs when the resulting growth response from the combined diet is less than would be expected based on the response obtained from the diets fed separately (Fig. 8c).

Part II

Results

a) Oyster Growth Trials

The growth response of O. edulis juveniles to the test diets (Figs. 9, 10, 11 and 12) was a function of feeding density. At the lower feeding ration levels, the silicate limited algal diet yielded the highest oyster growth rate; whereas, at the higher ration levels, the control diet produced the highest growth response. A relatively poor growth response was obtained over the entire feeding density range with the nitrogen limited diet. A feeding ration of ca. 3.0×10^5 provided the highest oyster growth response when either nutrient limited algal culture was used. When fed the algal control diet, the growth rate of the oysters was saturated at a feeding ration of ca. 6.0×10^5.

b) Algal Analysis

i) Morphology

The algal cells constituting the three diets are shown in Fig. 13. Frustules of the silicate depleted C. gracilis culture did not have the normal morphology. These cells, and to a lesser extent the nitrogen limited cells, appeared rounder than the control cells.

ii) Biochemistry

Quantitative analyses were conducted on the carbon, nitrogen, protein, lipid and carbohydrate content for the three C. gracilis algal diets. The nitrogen limited cultures had the lowest nitrogen content while the silicate limited cultures contained ca. 30% more carbon than

Fig. 9. The daily growth rate (k), based on fresh weight data (n ranges between 66 and 72) of Ostrea edulis juveniles, when fed Chaetoceros gracilis at various feeding rations. C. gracilis was cultured with the complete f/2 nutrient medium (control) and with two nutrient limited media; f/2 lacking silicate (-Si) and f/2 lacking nitrogen (-N). 95% confidence intervals are shown.

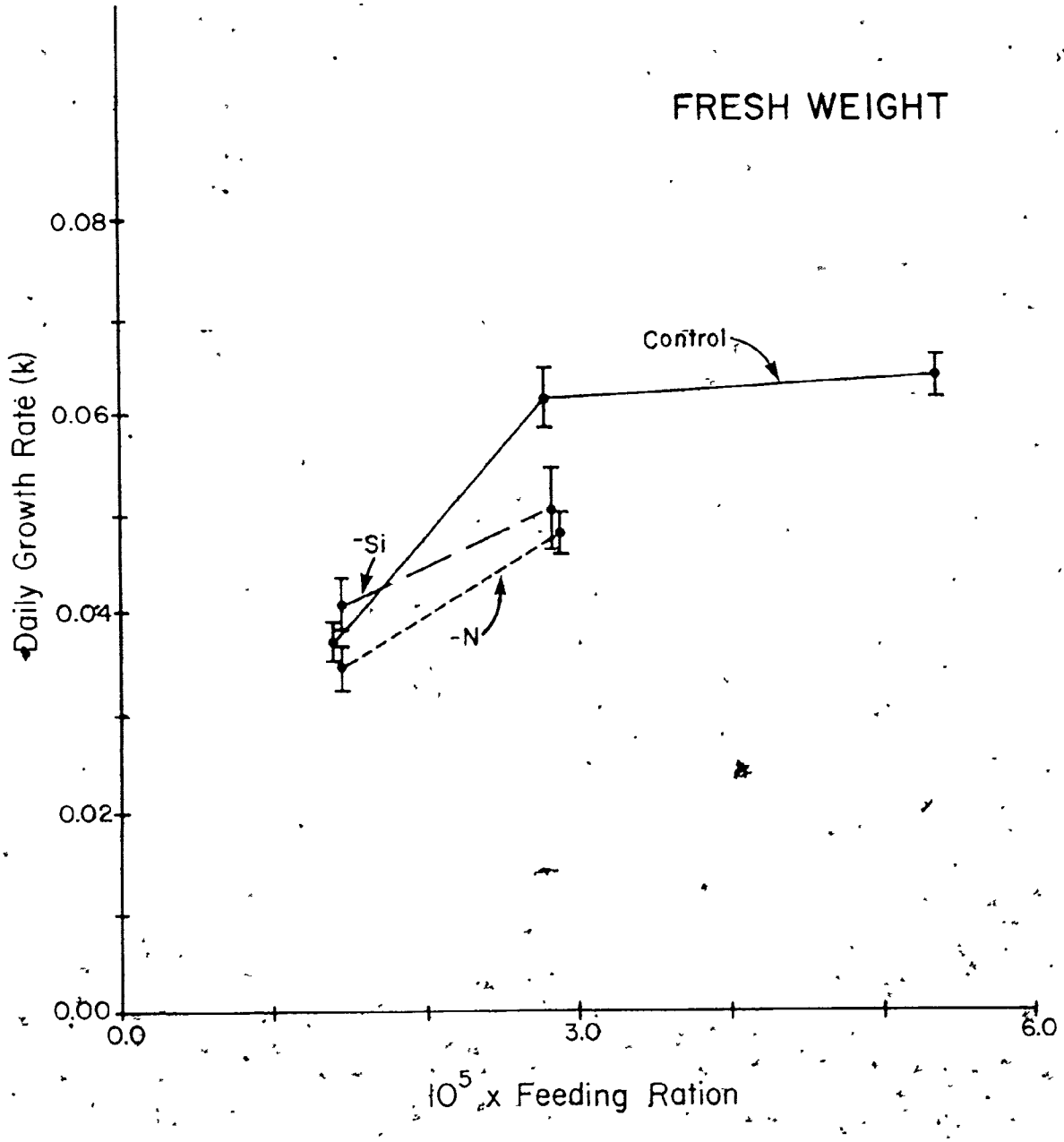

FRESH WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0.00
Control
-Si
-N
0.0
3.0
6.0
10^5 x Feeding Ration

Fig. 10. The daily growth rate (k), based on dry weight data (n ranges between 65 and 72) of Ostrea edulis juveniles, when fed Chaetoceros gracilis at various feeding rations. C. gracilis was cultured with the complete f/2 nutrient medium (control) and with two nutrient limited media; f/2 lacking silicate (-Si) and f/2 lacking nitrogen (-N). 95% confidence intervals are shown.

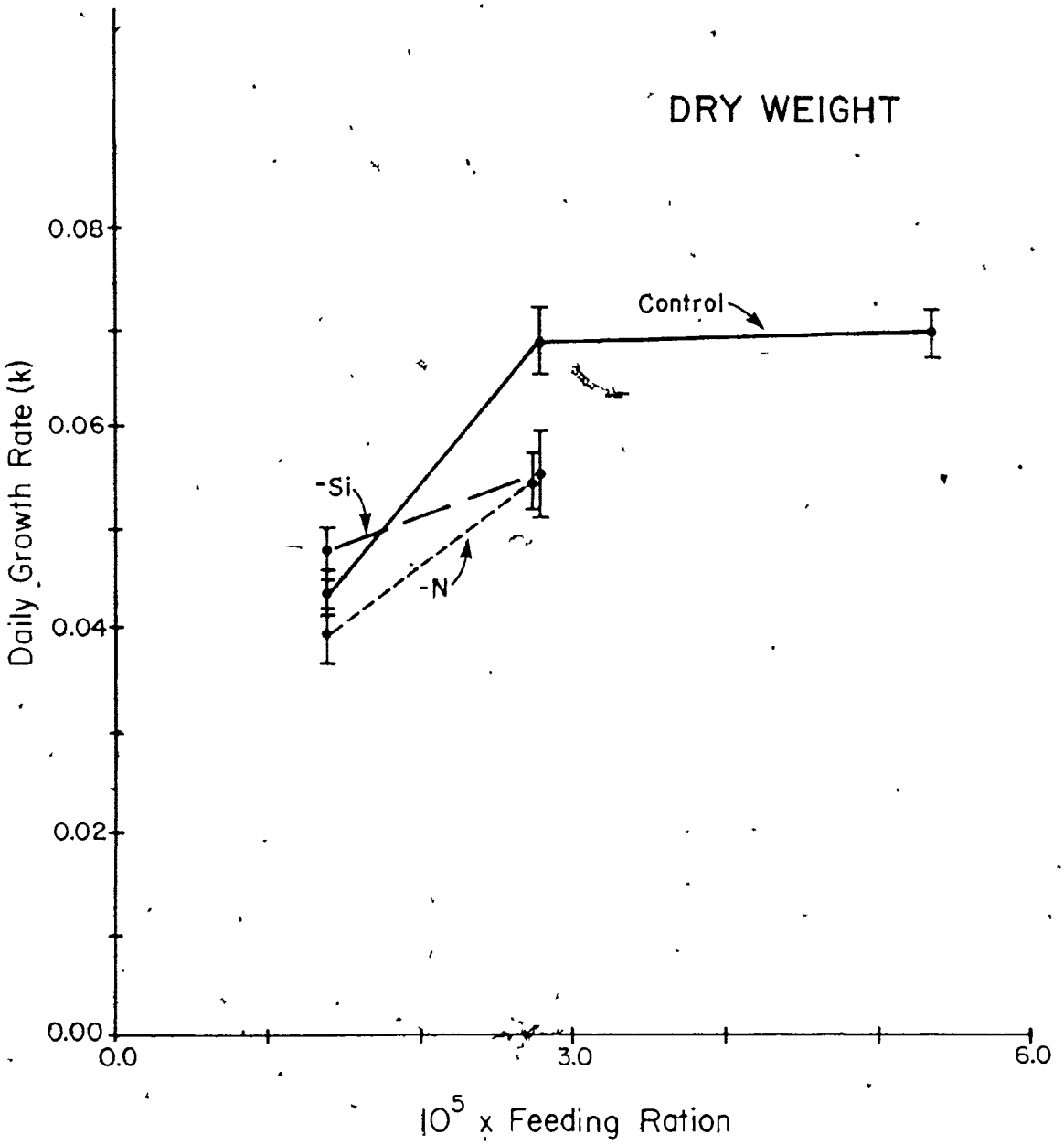

DRY WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0.00
Control
-Si
-N
0.0
3.0
6.0
10^5 x Feeding Ration

Fig. 11. The daily growth rate (k), based on fresh weight data (n ranges between 137 and 146) of Ostrea edulis juveniles, when fed Chaetoceros gracilis at various feeding rations. C. gracilis was cultured with the complete f/2 nutrient medium (control) and with two nutrient limited media; f/2 lacking silicate (-Si) and f/2 lacking nitrogen (-N). 95% confidence intervals are shown.

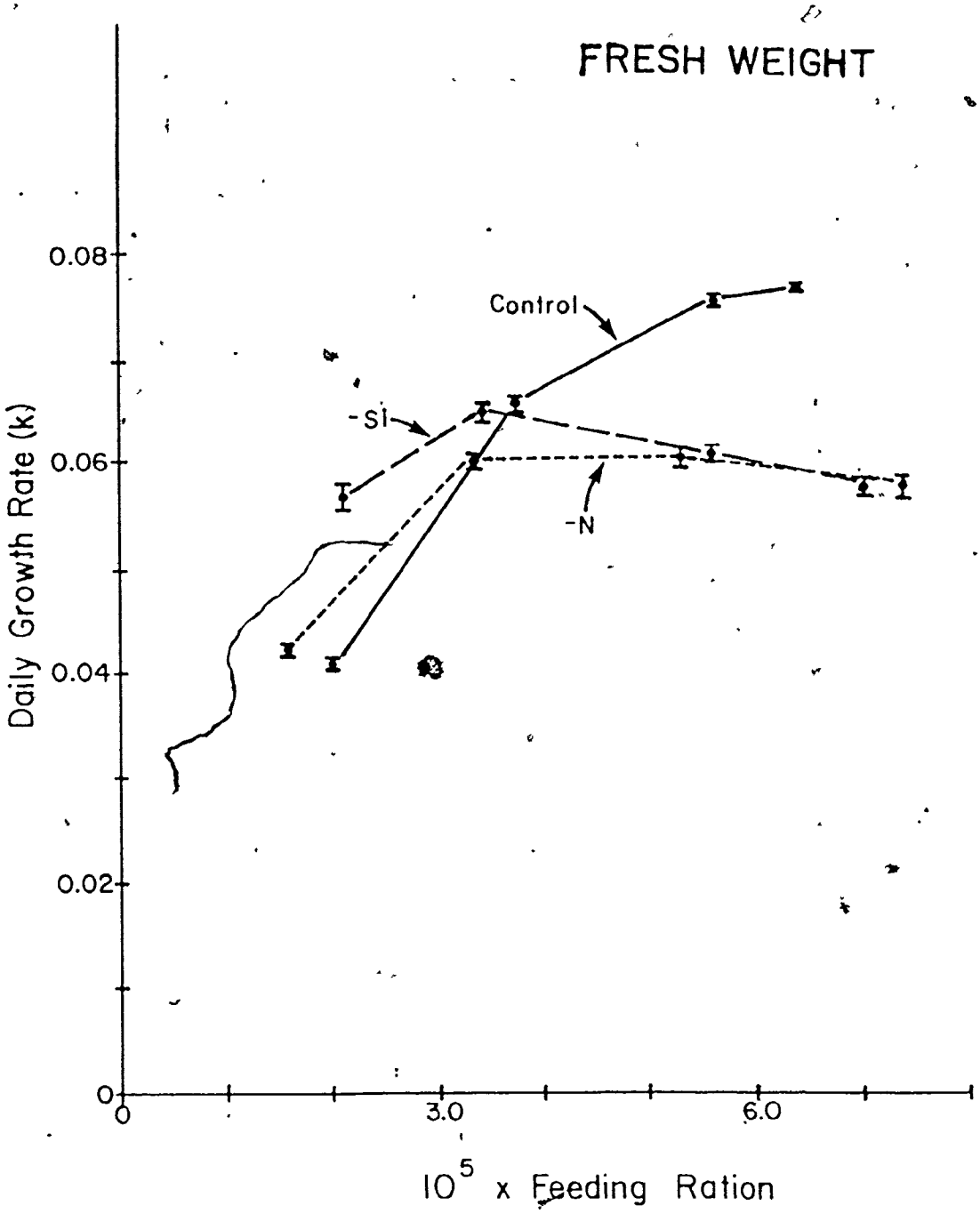

FRESH WEIGHT
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0
Control
-Si
-N
0
3.0
6.0
10^5 x Feeding Ration

Fig. 12. The daily growth rate (k), based on dry weight data of Ostrea edulis juveniles, when fed Chaetoceros gracilis at various feeding rations. C. gracilis was cultured with the complete f/2 nutrient medium (control) and with two nutrient limited media; f/2 lacking silicate (-Si) and f/2 lacking nitrogen (-N). 95% confidence intervals are shown; n ranges from 133 to 145 with the exception of the control diet at the lowest feeding concentration where n is 103 due to a handling accident during the drying process.

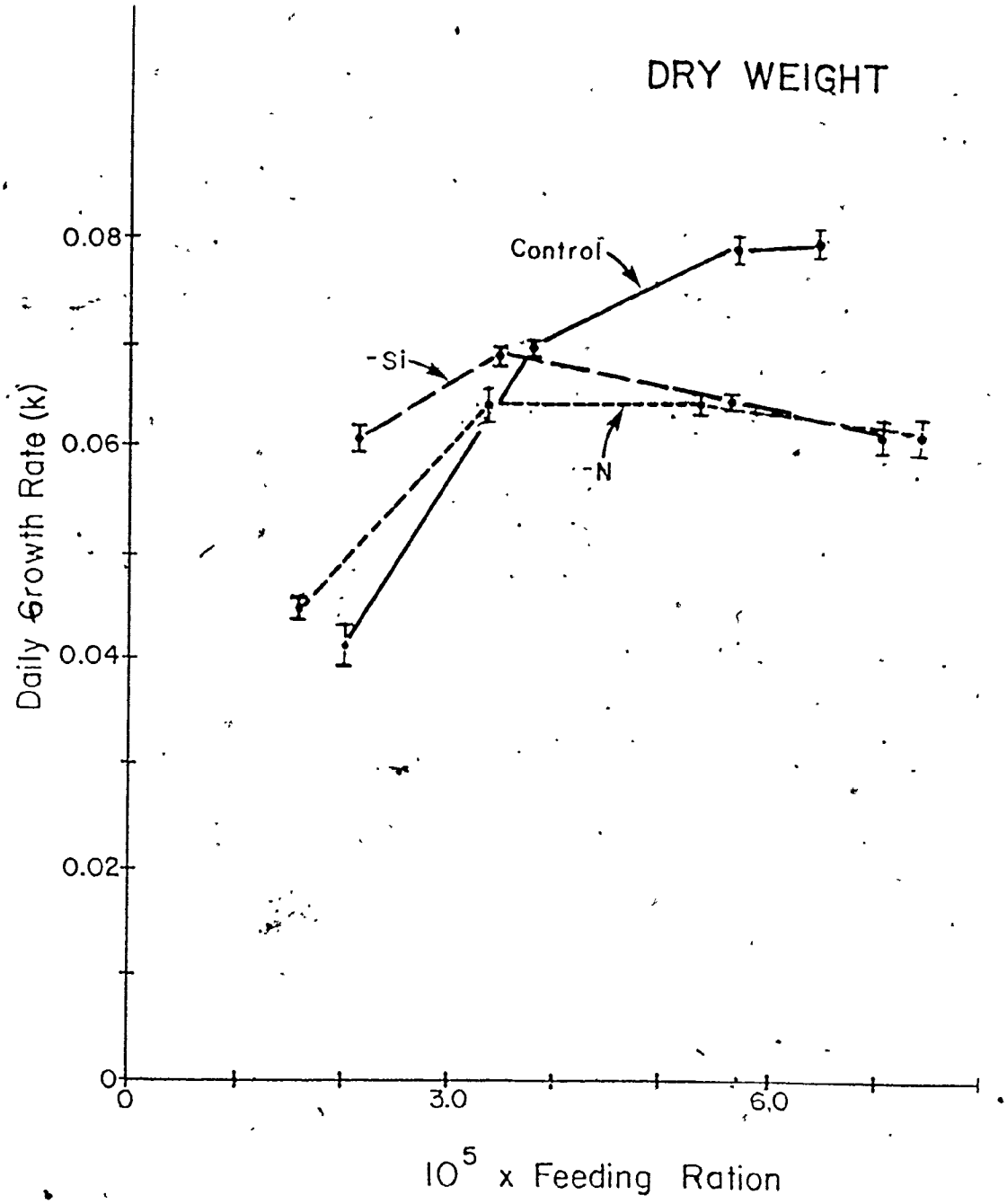

DRY WEIGHT
Control
-Si
-N
P
Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0
0
3.0
6.0
10^5 x Feeding Ration

Fig. 13. Chaetoceros gracilis cultured with: a) the complete f/2 nutrient medium (control); b) f/2 lacking silicate (-Si); and c) f/2 lacking nitrogen (-N).

(a)
(b)
(c)
3μ

the control or nitrogen limited cultures (Table 3). Protein levels of 10.8 μg $(10^6$ cells$)^{-1}$ and 10.1 μg $(10^6$ cells$)^{-1}$ were obtained, respectively, for the control and silicate limited algal cultures (Fig. 14 and Appendix I, Tables 9, 10 and 11). Nitrogen limited algal cells contained less than half the protein level of the other two cultures. The total lipid per cell in the silicate depleted cultures was 2.1 times higher than the control and the nitrogen limited cultures (Fig. 14, and Appendix II, Table 8). The carbohydrate levels of the nitrogen limited and silicate limited algal cells were, respectively, 3.2 and 1.6 times higher than the control algal cells (Fig. 14, and Appendix III, Table 1).

Qualitative analyses were conducted on the protein and lipid fraction of the three C. gracilis diets. With the exception of glutamic acid, the levels of the various amino acids were similar in the control and silicate limited cultures (Fig. 15, and Appendix I, Tables 9, 10 and 11). The level of each amino acid in the nitrogen limited cultures was ca. 40% that of the other two cultures. The fatty acids of the three diets have been grouped according to lipid class (Fig. 16 and Appendix II, Tables 10, 11 and 12). The levels of the total saturated and total monoethylenic fatty acids in the silicate limited culture were higher than the levels found in the other two diets. The control diet had the highest total polyethylenic fatty acid level. The level of 22:6ω3 in the control cultures was, respectively, five and two times higher than that of the silicate limited and nitrogen limited cultures.

Fig. 14. Chemical composition, expressed in $\mu g(10^6 \text{ cells})^{-1}$, of Chaetoceros gracilis culture with: the complete f/2 nutrient medium (control); f/2 lacking silicate (-Si); and f/2 lacking nitrogen (-N). 95% confidence intervals are shown.

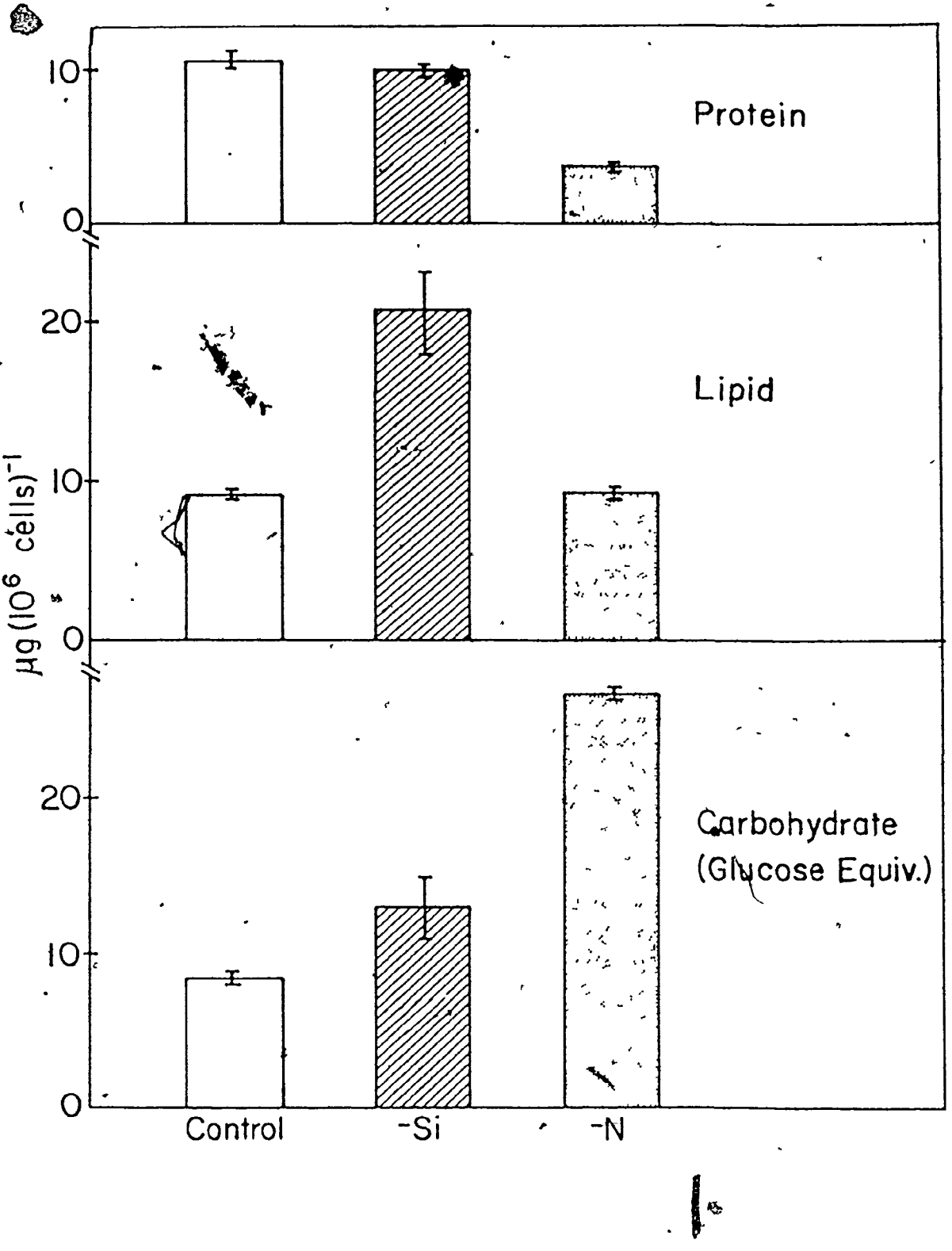

Protein
Lipid
Carbohydrate
(Glucose Equiv.)
μg (10⁶ cells)⁻¹
10
0
20
10
0
20
10
0
Control
-Si
-N

Fig. 15. Amino acid composition, expressed in $\mu g(10^6 \text{ cells})^{-1}$, of Chaetoceros gracilis cultured with: the complete f/2 nutrient medium (control); f/2 lacking silicate (-Si); and f/2 lacking nitrogen (-N). 95% confidence intervals are shown.

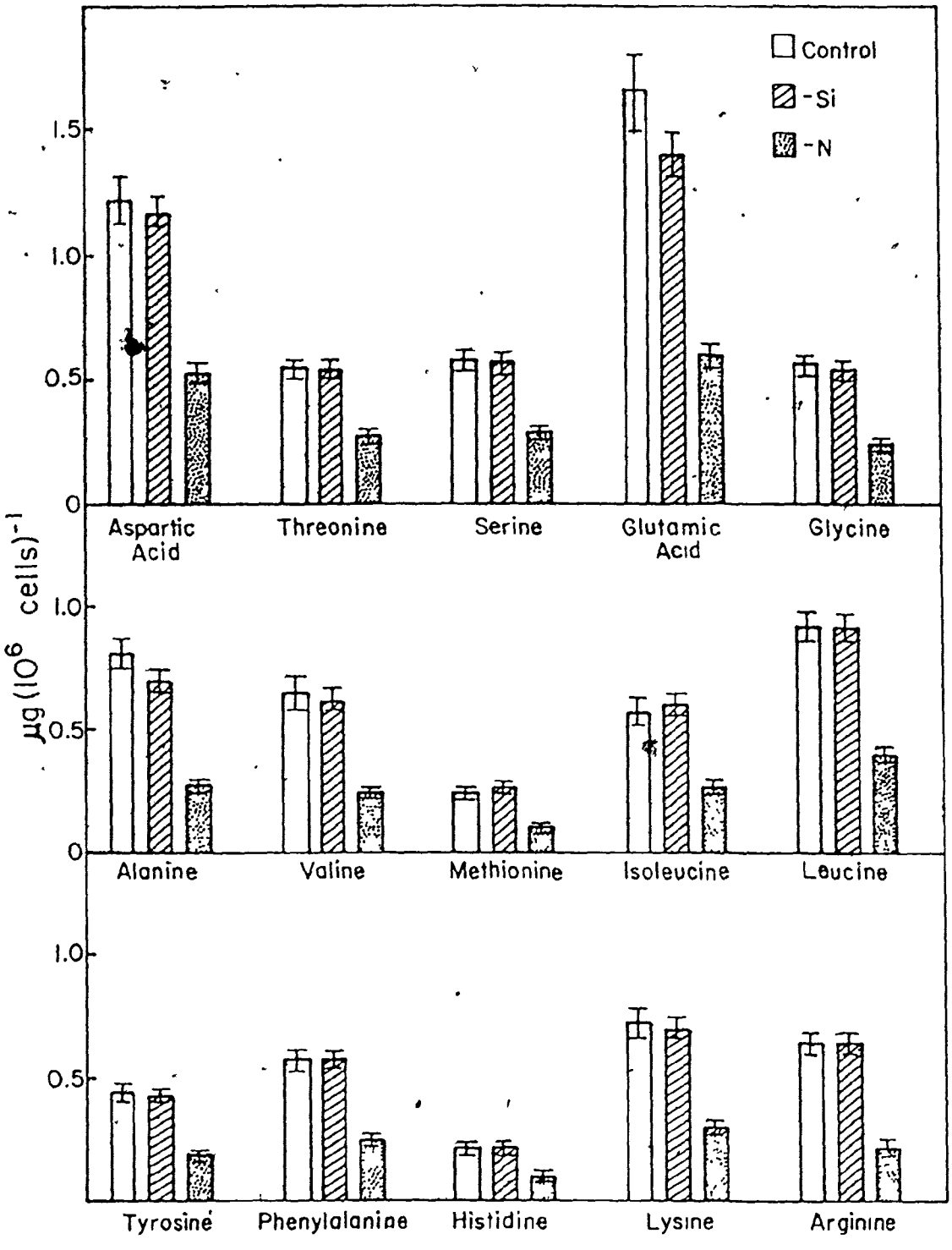

Control
-Si
-N
μg (10^6 cells)^-1
1.5
1.0
0.5
0
Aspartic Acid
Threonine
Serine
Glutamic Acid
Glycine
1.0
0.5
0
Alanine
Valine
Methionine
Isoleucine
Leucine
1.0
0.5
Tyrosine
Phenylalanine
Histidine
Lysine
Arginine

Fig. 16. Fatty acid composition, expressed in $\mu g(10^6 \text{ cells})^{-1}$, of Chaetoceros gracilis cultured with the complete f/2 nutrient medium (control); f/2 lacking silicate (-Si); and f/2 lacking nitrogen (-N). 95% confidence intervals are shown.

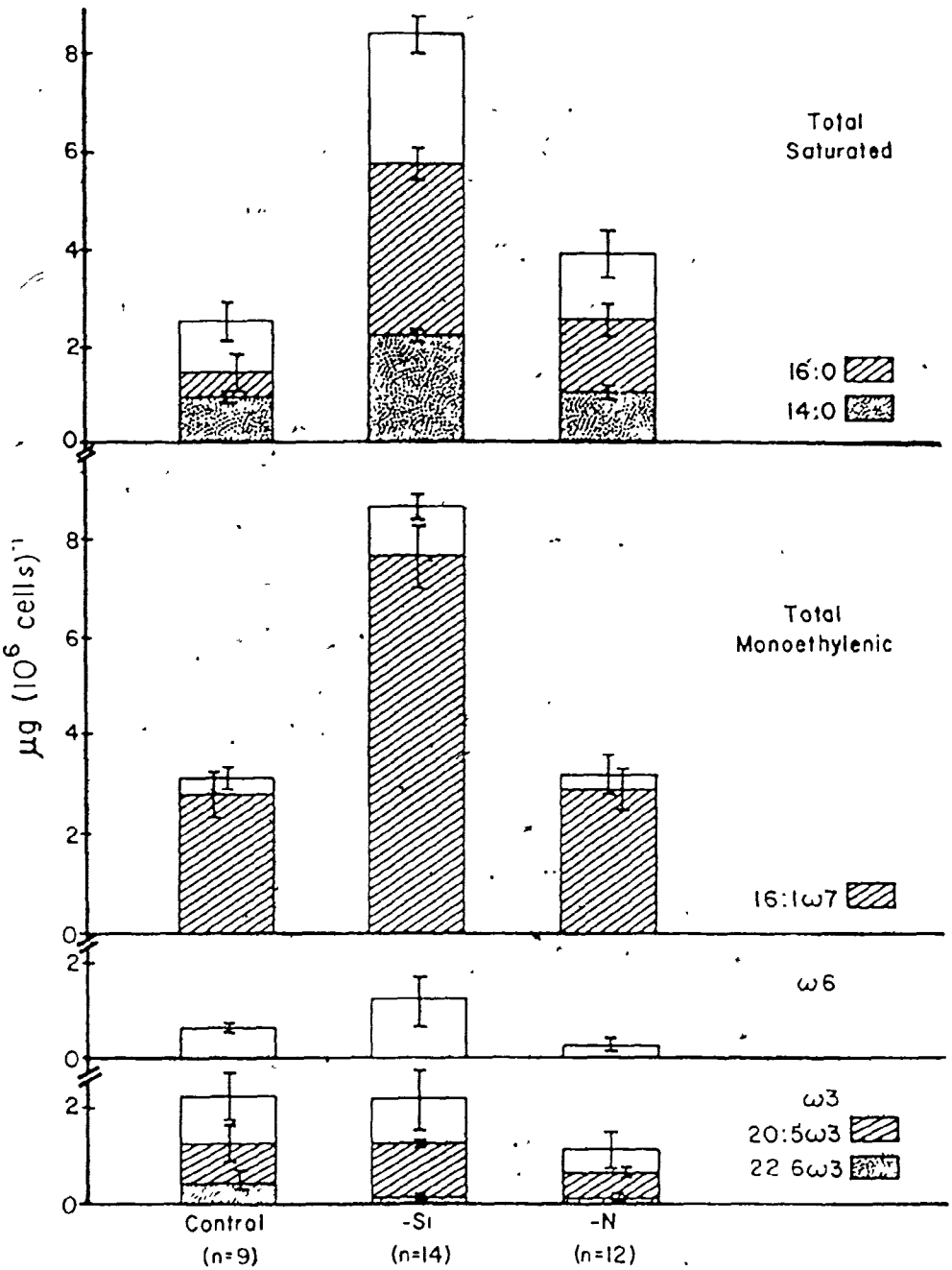

Total
Saturated
16:0
14:0
Total
Monoethylenic
16:1ω7
ω6
ω3
20:5ω3
22 6ω3
μg (10⁶ cells)⁻¹
Control
(n=9)
-Si
(n=14)
-N
(n=12)

Fig. 17. Replacement series experiment. The daily growth rate (k), based on fresh weight data of Ostrea edulis juveniles when fed various proportions of Chaetoceros gracilis cultured with the complete f/2 nutrient medium (control); f/2 lacking silicate (-Si); and f/2 lacking nitrogen (-N). 95% confidence intervals are shown; n ranges from 136 to 146.

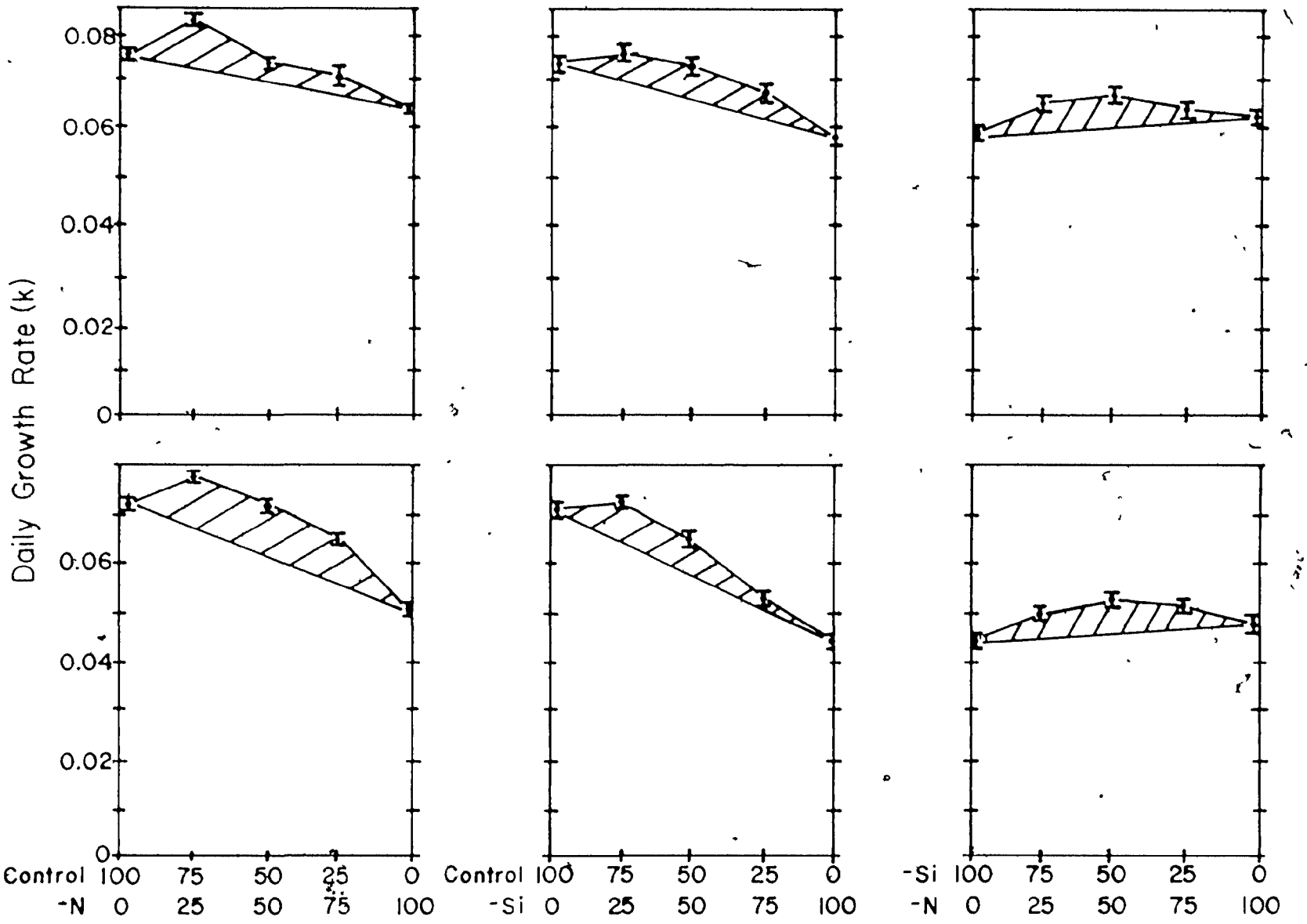
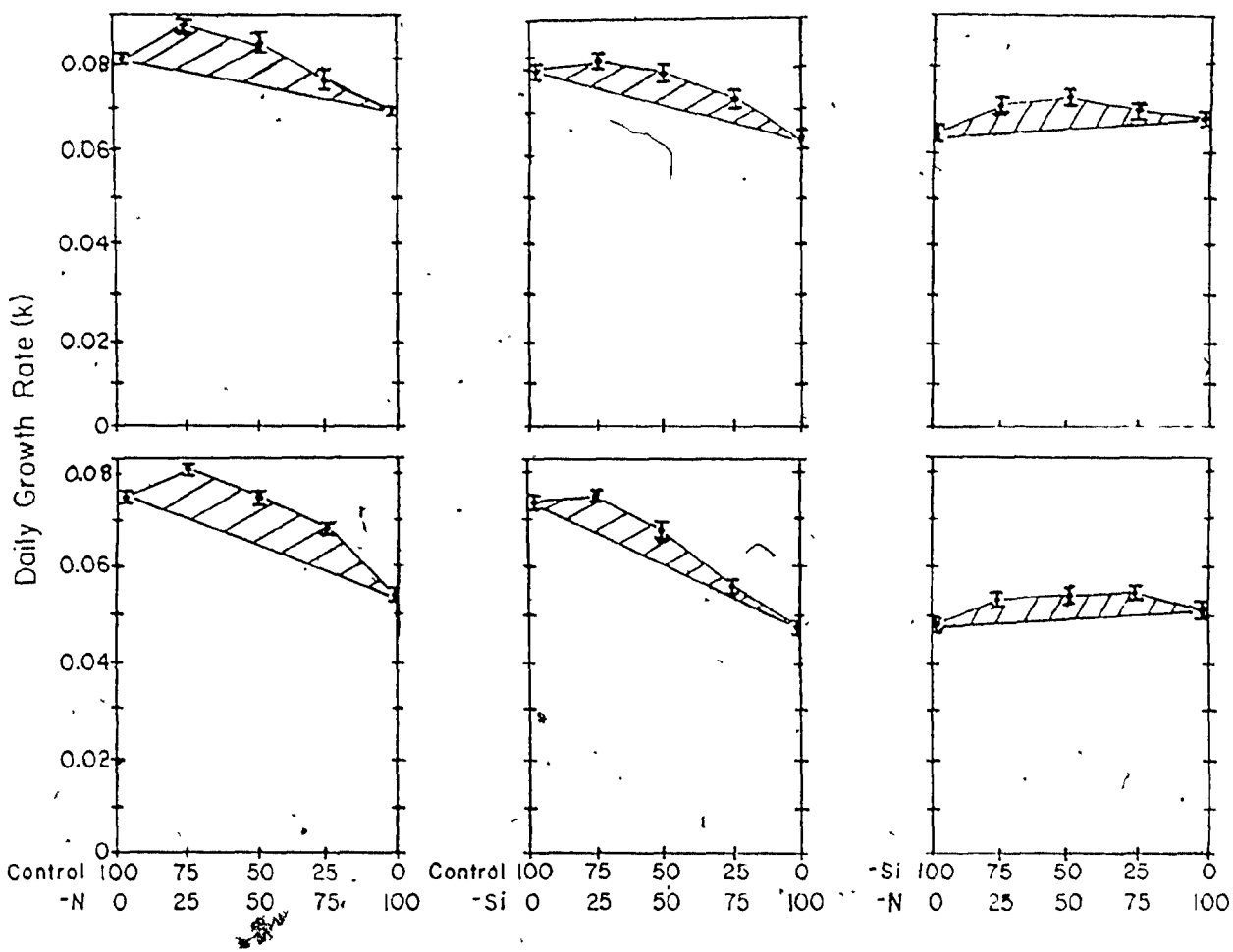

Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0
0.06
0.04
0.02
0
Control 100 75 50 25 0
-N 0 25 50 75 100
Control 100 75 50 25 0
-Si 0 25 50 75 100
-Si 100 75 50 25 0
-N 0 25 50 75 100

Fig. 18. Replacement series experiment. The daily growth rate (k), based on dry weight data of Ostrea edulis juveniles when fed various proportions of Chaetoceros gracilis cultured with the complete f/2 nutrient medium (control); f/2 lacking silicate (-Si); and f/2 lacking nitrogen (-N). 95% confidence intervals are shown, n ranges from 133 to 145.


Daily Growth Rate (k)
0.08
0.06
0.04
0.02
0
Control 100 75 50 25 0
-N 0 25 50 75 100
Control 100 75 50 25 0
-Si 0 25 50 75 100
-Si 100 75 50 25 0
-N 0 25 50 75 100

c) Diet Combinations

The oyster growth responses obtained with algal monocultures in the replacement series experiment (Figs. 17 and 18) support the results shown in Figs. 9, 10, 11 and 12. An augmented growth response resulted when the diets were combined in various ratios. The highest growth response at both feeding rations was obtained with 75% of the control diet and 25% of the nitrogen limited diet. A diet consisting of 75% of the control diet and 25% of the silicate limited diet yielded a growth response equal to that of the control monoculture diet. A mixture of the silicate limited and nitrogen limited cultures yielded higher oyster growth rates than were obtained using either of the single nutrient depleted monocultures.

Part II

Discussion

Under conditions of nutrient stress, the production of carbohydrate and lipid, relative to protein, may be a survival mechanism for phytoplankton. Lipids are the most efficient form of energy storage followed respectively by carbohydrate and protein (Lloyd et al., 1978). Amino acids are more valuable when directly involved in the structural synthesis of proteins, as opposed to energy storage when carbohydrates or lipids are available. Under adverse culture conditions, the photosynthetic process may favour the production of the higher energy compounds. On the other hand, under optimal conditions, the production of proteins and nucleic acids may be enhanced, meeting the requirements of cell division and growth.

The morphological differences between the control, silicate limited and nitrogen limited C. gracilis cultures, apparent in Fig. 13, were not thought to affect the food quality of the diets. The oysters ingested all three diets. The silicate frustule of the control and nitrogen limited algal cells may provide additional grinding material in the diet, compared to the silicate limited cells where the frustule is reduced. However, since kaolin was added to all diets to provide additional grit material it is unlikely there were any differences in digestibility among the three diets.

Trends in the juvenile oyster growth rates, as a function of feeding densities, with the C. gracilis diets (Figs. 9, 10, 11, 12, 17 and 18), will be discussed using the biochemical data presented (Tables 3,

4, 5; Figs. 14, 15, and 16; Appendix I, Tables 9, 10, and 11; Appendix II, Tables 9, 10, 11 and 12; and Appendix III, Table 2).

a) Carbon to Nitrogen Ratio

The change in a carbon to nitrogen ratio of the phytoplankton culture is an indication of alteration in the chemical composition of the algae. The carbon to nitrogen ratio of the silicate limited culture is 16.0 on a dry weight basis compared to 8.7 for the control culture, (Table 3). Depriving C. gracilis of nitrogen increased the carbon to nitrogen ratio from 8.7 to 15.3. As reviewed by Craigie (1984), these values are comparable to other carbon to nitrogen ratios reported for algal species cultured under similar conditions. Eppley and Renger (1974) maintained Thalassiosira pseudonana under steady state growth conditions in a continuous culture, at three different levels of nitrogen limitation. The carbon to nitrogen ratio for the cultures in their study increased from 5.0 to 12.0 with increasing nitrogen limitation. The carbon to nitrogen ratio of exponentially growing Skeletonema costatum and P. lutheri cultures increased from 6.0 to 35.0 as a result of nitrogen deficiency (Sakshaug and Holm-Hansen, 1977). In these reports, the partitioning of the additional carbon into various compounds was not examined, as was done in the present study.

b) Protein

The absence of a nitrogen source inhibits amino acid synthesis. In algae, the level of the enzyme, nitrate reductase, declines and the synthetic pathways for proteins become blocked (Eppley et al., 1969). Hence, the control and silicate limited C. gracilis diets, which have a

nitrogen source, contain more than twice the protein level of the nitrogen limited algal diet (Fig. 15).

In order to obtain growth with juvenile oysters, residual protein must be available in the diet after catabolic nitrogen losses have been replaced. The poor growth response obtained with the nitrogen limited algal diet (Figs. 9, 10, 11 and 12) correlates with the low protein level of the diet (Figs. 15 and Appendix I, Table 11). The silicate limited and control diets have protein levels, respectively, of 10.1 and 10.8 $\mu g(10^6 \text{ cells})^{-1}$ while the nitrogen limited diet has a protein level of 4.3 $\mu g(10^6 \text{ cells})^{-1}$.

Protein deficient diets typically result in reduced growth rates and depressed appetite among animals (NRC, 1983). Langton et al. (1977) found the gain in dry total weight, shell weight and the protein of juvenile clams correlated with the total amount of algal protein available. Webb and Chu (1983) found that the nutritional value of an algal species for oyster larvae was correlated with the total protein level of the alga as opposed to the total lipid or carbohdrate content.

Working with a limited number of animals, Flaak and Epifanio (1978) in contrast found that a marginally higher oyster growth rate (0.9% vs. 0.3% d^{-1}) resulted when Crassostrea virginica was fed a 21% protein diet on a dry weight basis compared to diets containing protein levels several fold higher. Furthermore, they found that a diet of 12.9% resulted in a growth rate of 0.7% d^{-1}. The diets used by Flaak and Epifanio (1978) were obtained by culturing T. pseudonana under several light regimes and harvesting at various growth phases. The results of the above studies are intriguing, because the protein content on a dry

weight basis of most of the algae which juvenile oysters feed upon is above 40% (Strickland, 1960). Also, the protein content of oysters, which may reflect the dietary requirements of the animal, has been estimated at ca. 50% of the dry weight (Quayle, 1969; and Walne and Mann, 1975).

Oysters must consume protein to produce a continual supply of amino acids. Upon digestion, the protein is hydrolyzed and free amino acids are released. The free amino acids are used to synthesize new proteins in various organs and tissues for either growth or tissue repair (NRC, 1983). Molluscs require a dietary source of arginine, histidine, methionine, cysteine, leucine, isoleucine, valine, lysine, tryptophane, threonine and proline (Boudreau, 1984; Harrison, 1975). Rice et al. (1986), used radiotracers to measure a net influx of dissolved alanine and glycine in juvenile O. edulis. Dissolved amino acids may be an important source of nitrogen for bivalves (Manahan et al., 1982). With the exceptions of cysteine, tryptophan and proline, all of the above mentioned amino acids have been analysed (Fig. 15). Methionine can be converted to cysteine, while phenylalanine can be converted to tyrosine in several fish species (NRC, 1983). The presence of non-essential amino acids in the diet have a sparing effect on the animal, in that the oyster does not have to produce them. The level of glutamic acid in the silicate limited diet was lower than that of the control diet (Fig. 15 and Appendix I, Tables 9 and 10). However, since glutamic acid is a non-essential amino acid, little significance has been attributed to this finding. Similar levels of all other amino acids were contained in the control diet and the silicate limited diet. This suggests that the protein quality of the silicate limited diet was not the cause of the

depressed growth response of the oysters at the higher feeding rations.

In both feeding densities in the diet combination experiments (Figs. 17 and 18) the highest growth response was obtained with a mixture of 75% of the control diet and 25% of the nitrogen limited diet, with a total protein level of 8.8 $\mu g(10^6 \text{ cells})^{-1}$ (Table 5). Although diets with higher protein levels were tested they also differed in other components such as lipids and carbohydrates. Thus it is impossible to determine the effects of higher protein levels.

c) Lipid

The higher levels of saturated and monoethylenic fatty acids in the silicate limited diet relative to the other two diets (Fig. 16; and Appendix II, Tables 10, 11 and 12) may be beneficial at the lower feeding rations where the caloric content of all diets is insufficient (Figs. 9, 10, 11 and 12). Lipids are the most energy rich class of nutrients, supplying 8 to 9 kcal/g (Lloyd et al., 1978). Saturated and monoethylenic fatty acids provide more energy than other fatty acids due to their hydrogen bonding arrangement. As lipids provide a rich supply of energy, the more valuable proteins can be directed towards growth. Takeuchi et al. (1978) found that when the lipid content of the rainbow trout diet was increased to 15 or 20% the protein in the diet was more efficiently utilized and could be reduced from 48% to 35% with no loss in fish weight gain. The higher caloric content of the lipid-rich silicate limited diet may account for the relatively higher growth rate obtained with this diet at the lower feeding range.

At the higher feeding densities, the silicate limited culture was a

Table 5. Chemical Composition of the control, silicate limited, and nitrogen limited Chaetoceros gracilis diets fed in the Replacement Series experiment and the resulting oyster growth response, ranked in order of highest growth rate.

Algal Diet	Oyster Growth Rates (k)		Algal Biochemistry							
	Feeding Ratio 3.5×10^5	Feeding Ratio 6.0×10^5	Protein $\mu g(10^6 \text{ cells})^{-1}$	Lipid $\mu g(10^6 \text{ cells})^{-1}$	Carbohydrate $\mu g(10^6 \text{ cells})^{-1}$	Protein :	Lipid :	Carbo-hydrate	Fatty Acid 22:6ω3 weight % in diet [1]	Possible Limiting Component
Control 75%, -N 25%	0.084	0.081	8.8	9.2	12.9	1.00 :	1.05 :	1.47	0.19	-
Control 75%, -S 25%	0.077	0.075	10.2	12.1	9.6	1.00 :	1.19 :	0.94	0.19	carbohydrate
Control 100%	0.077	0.074	10.3	9.1	8.3	1.00 :	0.88 :	0.81	0.23	carbohydrate
Control 50%, -N 50%	0.075	0.074	7.3	9.4	17.6	1.00 :	1.29 :	2.41	0.16	protein, 22:6ω3
Control 50%, -Si 50%	0.074	0.065	10.1	15.2	10.9	1.00 :	1.51 :	1.08	0.16	22:6ω3
Control 25%, -N 75%	0.072	0.066	5.8	9.4	22.2	1.00 :	1.62 :	3.83	0.12	protein, 22:6ω3
Control 25%, -S 75%	0.068	0.052	10.0	18.1	12.2	1.00 :	1.81 :	1.22	0.12	22:6ω3
-Si 50%, -N 50%	0.068	0.051	7.0	15.4	20.1	1.00 :	2.20 :	2.87	0.08	protein, 22:6ω3
-Si 25%, -N 75%	0.066	0.048	5.7	12.4	23.3	1.00 :	2.18 :	4.09	0.08	protein, 22:6ω3
-Si 75%, -N 25%	0.064	0.050	8.5	18.2	16.8	1.00 :	2.14 :	1.98	0.08	protein, 22:6ω3
-N 100%	0.064	0.048	4.2	9.5	26.8	1.00 :	2.26 :	6.38	0.08	protein, 22:6ω3
-Si 100%	0.057	0.042	9.8	21.1	13.4	1.00 :	2.15 :	1.37	0.08	22:6ω3

1. $\mu g(10^6 \text{ cells})^{-1}$ of lipid in the diet $\bar{x}$ weight % of 22:6ω3 in the lipid for each component, summed.

poorer diet for the juvenile oyster relative to the control diet, despite the higher total lipid level. Several possible explanations related to the quality of the lipid are suggested.

The high lipid level of the silicate limited diet may restrict the growth rate of the juvenile oysters as a result of contaminants in the lipid. Lipids can accumulate poorly biodegradable components such as polychlorinated biphenyls, pesticides, herbicides, crude oil and other hydrocarbon contaminants (NRC, 1983). There is no evidence in the present study to suggest that contaminants are present in the lipid of the silicate limited cultures. A decline in oyster growth rate corresponds with increasing food ration in the higher feeding density range with the silicate limited diet (Figs. 11 and 12). The opposite trend, however, occurs at the lower feeding densities range (Figs. 9, 10, 11 and 12). Thus the growth results are probably not in response to an accumulation of a toxic substance.

There may be an improper balance in the composition of the fatty acids in the silicate limited diet. The high levels of saturates and monoethylenic acids may have a diluting effect on the essential $\omega 6$ and $\omega 3$ fatty acid levels. The ratio of total saturate plus total monoethylenic fatty acids to total $\omega 6$ and $\omega 3$ fatty acids for the three diets are as follows: control, 2.3; silicate-limited, 4.9 and the nitrogen limited, 4.7. The higher growth rate obtained with the control diet, at the higher feeding densities may be a result of a better balanced diet, characterized by a lower value for the above ratio.

Several studies have demonstrated that growth inhibition is the

result of a highly saturated or short-chain fatty acid content in the diet. Feeding a rich source of saturated fatty acids, such as hydrogenated coconut oil, resulted in poor growth with C. virginica (Trider and Castell, 1980). For several species, growth decreased when saturated fatty acids were added to an essential fatty acid deficient diet (Aaes-Jorgensen and Dam, 1954a and b). Increased fluid consumption was also observed (Aaes-Jorgensen and Dam, 1954c). An increase in the 20:3ω9 to 20:4ω6 ratio of the animal, and adverse dermal characteristics were evident (Evans and Lepkovsky, 1931). A 20% supplement of triolein (18:1ω9) to the algal diets of Langdon and Waldock (1981) had an adverse effect on the growth rate of juvenile oysters. High lipid levels may depress the growth rate of juvenile European oysters due to an imbalance in the diet's fatty acid composition.

With the possible exception of the land snail (Van der Horst and Oudejans, 1973), animals are not capable of de novo synthesis of fatty acids with double bonds in the ω6 or ω3 position. These essential fatty acids are synthesized by plants and are obtained through the diet (Ackman, 1983). Inadequate levels or dilution of these important fatty acids can be detrimental to the animal. Burr and Burr (1929, 1930) showed that homeothermic animals such as the rat have an essential dietary requirement for ω6. Castell et al. (1972a, b and c) were the first to demonstrate that poikilothermic animals like the rainbow trout have an essential fatty acid requirement for ω3 rather than ω6 fatty acids.

Castell (1983) suggests that the ω3 structure in cold-blooded animals permits a greater degree of unsaturation, which may be critical

in the membrane phospholipids for maintaining the required flexibility and permeability characteristics at low temperatures. Cell membranes require adequate levels of essential fatty acids to form a normal permeability barrier around the cells and subcellular particles. Additional functions of the ω3 fatty acids include the transportation of other lipids such as cholesterol, the activation of several specific enzymes and the regulation of various vital processes by prostaglandins (Castell, 1970).

The essential fatty acid requirements in fish have since been shown to differ considerably from species to species. Carp (Watanabe et al., 1975; Takeuchi and Watanabe, 1977), eels (Takeuchi et al., 1980), and chum salmon (Takeuchi and Watanabe, 1982) require 18:2ω6 in addition to 18:3ω3.

Castell and co-workers have pioneered the studies on the essential fatty acid requirements of oysters. Castell and Trider (1974) and Trider and Castell (1980), using a semi-purified test diet, found that cod liver oil, high in 20:5ω3 and 22:6ω3, promoted better growth and reproduction in the adult oyster, C. virginica, than diets with corn oil which are high in 18:2ω6. A 1:2 mixture of corn oil (ω6) and cod liver oil (ω3), in the diet of C. virginica adults produced only marginally higher growth rates than the cod liver oil diet. Thus, the ω3 requirements appear to be greater than the ω6 requirements. Ackman (1981) asserts that ω6 and ω3 cannot be interconverted. These fatty acids are either degraded for energy or elongated (eg. 18:3ω3 to 20:5ω3 and 22:6ω3). Severe ω6 and ω3 fatty acid deficiencies may occur in an oyster hatchery or nursery operation where a limited number of

algal species are routinely used. On the basis of the above evidence, it appears that C. virginica adults have a requirement for both ω6 and ω3 fatty acids, with the requirement for ω3 being much greater.

Waldock and Nascimento (1979) found that the ratios of 18:2ω6/18:3 ω3 and 20:4ω6/20:5ω3 plus 22:6ω3 in the triglycerides (the fatty acid energy storage component) of C. gigas juveniles were very similar to the ratios in the algal feed organism. Since the fatty acids of phospholipids (eg. structural components in membranes) can be derived in an unchanged form from the triglycerides (Ackman, 1983), fatty acids can be transferred directly from the diet, to a storage mode, into a structural mode. However, since the 20:4ω6 level in bivalve phospholipids appears higher than the level supplied in the algal diet, Ackman (1983) suggests this isomer may also be formed from the supply of 18:2ω6 stored in the triglyceride fraction within the oyster's cells. When comparing the ω6/ω3 fatty acids value in phospholipids, O. edulis appears to be somewhat unique among oysters. Both the wild C. virginica and hatchery-reared C. gigas spat have a ω6/ω3 fatty acid value of 0.25, while the value for wild O. edulis is 0.07. Other bivalves such as Mytilus edulis (0.07), Pecten maximus (0.05) and Arctica islandica (0.07) share similar values with O. edulis. The ω6/ω3 ratio may have species specific characteristics (Ackman, 1981). Furthermore, it is also possible the different ratios reflect species specific differences in polyene requirements or the types of food which the animal has been feeding upon.

The ω6/ω3 fatty acid values for the three C. gracilis diets are: control, 0.3; silicate limited, 0.8; nitrogen limited, 0.7. Webb and

Chu (1983) report that most algal diets which are good for C. virginica oyster larvae have a ω6/ω3 value of 0.3 to 0.5, while poor algal diets have a ω6/ω3 value of ca. 0.2. The present study supports the finding of Webb and Chu (1983) that a ratio of 0.3 is characteristic of a good bivalve diet and also suggests that values higher than 0.5 may indicate a poorer algal diet for juvenile European oysters.

The importance of the linolenic fatty acids (ω3), relative to the linoleic fatty acids (ω6), for several other marine animals has been reported. The studies of both Nicolaides and Woodall (1966) and Lee et al. (1967) suggest that ω3 fatty acids are more valuable in fish diets than the ω6 fatty acids. In their study, raising the ω3 fatty acid content in the diet clearly increased the growth response of the fish. Sinnhuber and Hendricks (1979) found that the weight gain and feed conversion efficiency were greater when rainbow trout were fed a diet containing 1% of 18:3ω3 compared to a diet of 1% 18:3ω3 plus 1.5% 18:2 ω6. Cowey et al. (1976) demonstrated that turbot not only required the ω3 structure but specifically needed 20:5ω3 or 22:6ω3 to satisfy its essential fatty acid requirements. Webb and Chu (1983) report that C. virginica larvae require ω6 to a lesser extent than ω3 fatty acids.

The relatively low level of 22:6ω3 in the silicate and nitrogen depleted diets may be a growth limiting factor for the juvenile oysters. Langdon and Waldock (1981) demonstrated that a deficiency in 22:6ω3 in D. tertiolecta was a growth-limiting factor in C. gigas juveniles. When a T. suecica diet was supplemented with 22:6ω3, the growth rate of these juveniles improved.

A requirement for 22:6ω3 in young or developing stages of other

marine animals is recognized. The dietary levels of 22:6ω3 significantly affect egg hatchability in the common carp (Shimeno et al., 1977). The major phospholipid during the development of the rainbow trout was 22:6ω3 (Ando, 1968). The shrimp, Penaeus setiferus, would not produce eggs unless the diet contained 22:6ω3 and 20:5ω3 (Middleditch et al., 1980). Morris (1973) pointed out that juvenile marine crustaceans typically have higher levels of essential fatty acids than adults of the same species.

The 22:6ω3 fatty acid may be a key component of the diets in the present study. The Chaetoceros gracilis control diet which has more than six times the 22:6ω3 level than the silicate limited diet (Appendix II, Tables 10 and 11), yielded a growth response that was ca. 25% greater at the higher feeding ration (Figs. 9, 10, 11 and 12). The principal signs of essential fatty acid deficiencies, reported in studies with warm water fishes, are reduced growth rate and reduced feed efficiency (NRC, 1983). The observed differences between the control and the silicate limited diet cannot be explained by the level of the total ω6 or 20:5ω3 as similar levels of these fatty acids were obtained in both diets. This study suggests, and Langdon and Waldock (1981) present evidence for, a 22:6ω3 fatty acid requirement in the diet of juvenile oysters.

The fatty acid composition of a second diatom, Thalassiosira pseudonana, cultured under the control and silicate limited conditions was also analysed (Appendix II, Table 6 and 7). While the differences between the control and the silicate limited cultures were not as striking with this diatom when compared to C. gracilis, the trends are

the same. The saturated and monoethylenic fatty acids are more abundant in the silicate limited culture, while the polyethylenic fatty acids are more prominent in the control culture. The level of the 22:6ω3 fatty acid was twice as high in the control culture as it was in the silicate limited culture. Thus, the changes in chemical composition as a function of silicate concentration may be common among diatom species.

The findings presented in this report and those reviewed above will help in establishing a good basal diet for microencapsulation studies. Such studies can verify the nutritional contribution of single dietary components for oysters. However, the economic viability of routinely using microencapsulation in commercial hatcheries and nurseries is doubtful.

There is a potential problem in satisfying the essential fatty acid requirement of the juvenile bivalve. Fish oils, a common source of ω3 in animal feeds, can easily become oxidized and render the diet rancid. Ko et al. (1975) showed that oxidized lipids reduced the digestibility of proteins and the availability of lysine. Sakaguchi and Hamaguchi (1979) found that oxidized oils were less digestible than fresh oils and caused hyperglycemia in red sea bream. Hung et al. (1980), however, showed that vitamin E protects rainbow trout from the toxic effects of oxidized oils. Castell et al. (1984) recognize that antioxidants and proper storage conditions are more critical in artificial diets when the ω3 fatty acid requirement is satisfied by the addition of fish oil.

While the costs involved in culturing phytoplankton for diets is high, a live food is still the most reliable and affordable way of providing a high quality source of the essential fatty acids for

several years to come. The present study has demonstrated the effect that nutrient culture status can have on the various fatty acid components and especially the 22:6 ω3 level in algal species. With better management and further investigation phytoplankton cultures can yield a higher percentage of the nutritionally essential components required by our target organisms.

d) Carbohydrate

C. gracilis cultured under conditions of nitrogen limitation accumulates carbohydrate (Fig. 14; and Appendix III, Table 1). A similar response occurs with other species (Shifrin and Chisolm, 1981, Appendix III, Table 3). The rate of excretion or release of carbohydrates is normally greater under stressful conditions such as nitrogen depletion (Marker, 1965). The carbohydrate level shown in Fig. 14 and Appendix III, Table 1 may underestimate the total carbohydrate provided by the diet, as only algal cells were analysed; however, both cells and media were fed to the oysters.

Carbohydrates are the main energy source for both juvenile and adult oysters (Haven, 1965; Ingle et al., 1981; and Dunatham et al., 1969). In adult oysters, polysaccharides serve as a major energy reserve (Giese, 1969). Castell and Trider (1974) varied the protein-carbohydrate ratios of formulated feeds and observed that diets with a 60% carbohydrate content resulted in higher glycogen production in oysters, than diets with a 20% carbohydrate content. High carbohydrate levels in the tissue characterize healthy spat and larvae, while depleted levels are typical of starved or stressed animals (Bayne, 1973;

Gabbott and Bayne, 1973; and Mann, 1979).

Glycogen, the D-glucose storage polysaccharide, is the major carbohydrate in juvenile oysters, representing 40-50% of the total carbohydrate content. Glycogen levels change readily during development while other polysaccharides and free-reducing sugars remain constant during larval development, metamorphosis and spat development (Holland and Spencer, 1973). Holland and Hannant (1974) have shown that glycogen replaces triglycerides as the major energy storage compound in O. edulis at an age of 3 to 5 months. In their study, reduced glycogen levels and higher triglyceride levels were evident among 3 to 5 month old oysters set in June compared to oysters set in January or March.

In the present study, qualitative carbohydrate analyses have not been conducted on the algal diets but the importance of various sugars can be determined from the literature. L-Fando et al. (1972) examined glycogen synthesis from fructose, glycerol, pyruvate, l-leucine, and hydroxybutyrate in the gill tissue of O. edulis, and found that glycerol produced the highest glycogen level. Glycerol is a component in fats which may suggest that fat metabolism plays some role in gluconeogenesis in oysters. L-Fando et al. (1972) indicated that glycogen behaves as a negative feedback inhibitor regulated by its own synthesis in O. edulis, as suggested by Danforth (1965) for mammals. The metabolism of glycogen and trehalose are closely related. Trehalose contains two D-glucose residues and is the major sugar found in the hemolymph of many insects (Lehninger, 1970). Badman (1967) found substantial amounts of trehalose in Crassostrea virginica, yet Whyte and Englar (1982) found no evidence of this carbohydrate in C. gigas.

Galactogen, another key mollusc polysaccharide, is a polymer of galactose found only in the albumin gland (May, 1932). L-Fando et al. (1972) speculate that inter-conversion between galactogen and glycogen occurs.

The high carbohydrate level in the nitrogen limited algal culture appeared to be of little use to the juvenile oysters (Figs. 9, 10, 11 and 12) because of the low protein level of this diet, as previously discussed limits its usefulness as a complete food. When the protein level of the nitrogen limited diet is increased, by replacing a portion of this diet with the control diet, a higher oyster growth response was obtained (Figs. 17 and 18). This suggests that while surplus protein is available in the control diet, the carbohydrate level or quality limits the growth rate of the juvenile oysters.

Carbohydrates may have a protein-sparing action, serving as energy sources so proteins may be utilized for growth instead of energy. In addition, carbohydrates are the least expensive source of dietary energy. The desirable, cohesive characteristics of carbohydrates may also be an important factor in the development of artificial foods for bivalves.

Part II

Conclusions

The chemical composition of C. gracilis was greatly altered when cells were cultured under various nutrient regimes. Diets of the altered C. gracilis produced profound differences in the growth rate of O. edulis during five week growth trials. The growth response of the oysters to the diets was a function of feeding ration. Examination of the diets and growth response gives some insight into the diet requirements of O. edulis. C. gracilis grown in a silicate limited culture had 30% more carbon. Part of this excess carbon was lipid, as these cultures had a lipid level twice that of the control or nitrogen limited cultures. The silicate limited diet enhanced the growth rate of the oysters, relative to the control diet, only at low feeding densities where total food energy was restricted. Most of the increase in lipid of the silicate limited algae was in the energy-rich saturated and monoethylenic fatty acids. Thus, the oysters responded positively to the higher energy diet when the food supply was limited.

At the higher feeding densities where ample energy is available as indicated by the higher growth rates, the growth response of the oysters fed the silicate limited diet was poorer than that of the control. An improper balance in the composition of the fatty acids in the silicate limited diet was suggested. The high levels of saturated and monoethylenic fatty acids may have a diluting effect on the essential ω6

and ω3 fatty acids. Diets consisting of high levels of saturated and monoethylenic fatty acids have shown to be detrimental to the growth of oysters (Trider and Castell, 1980 ; and Langdon and Waldock, 1981).

The higher oyster growth response obtained with the control diet was considered to be the result of a better balanced diet or the five times higher 22:6ω3 level; as similar levels of total ω6 and 20:5ω3 were present in both the control and silicate limited diets. While long chain polyunsaturated fatty acids have previously been shown to be essential in the diet of C. gigas juveniles (Langdon and Waldock, 1981), this study provides evidence that 22:6ω3 is essential for O. edulis. Once the caloric level of the diet is adequate the level of 22:6ω3 may greatly influence the growth rate of the juvenile oyster.

Although there was a two to three fold increase in the carbohydrate content of the nitrogen limited diet relative to the other two diets, the protein content of the nitrogen limited diet was less than half of the level of the other two diets. The low protein content appears to be the cause of the poor growth response with the nitrogen limited diet. Increasing the feeding ration did increase the growth response of the oyster over a narrow feeding range, however an increase in the feeding ration above 3.5 did not further increase the growth rate. The reasons for this are not clear; however, it could be a result of a need for an excessive amount of energy required to obtain the necessary protein.

The amino acid composition of all three diets were similar, with the exception of a relatively higher glutamic acid level in the silicate limited diet. Since glutamic acid is considered to be a non-essential

amino acid in the bivalve diet, little significance has been associated with this finding.

Since the control, silicate limited and nitrogen limited diets have different levels of either protein, lipid or carbohydrate, the oysters were fed these diets in various combinations. The augmented growth response which resulted with each diet combination suggests that the oysters responded to a better balanced diet in the combined diets. The highest oyster growth rate was obtained with a diet consisting of a 1.0:1.1:1.5 protein to lipid to carbohydrate ratio, which was obtained by replacing 25% of the control diet with an equal cell number of the carbohydrate rich, nitrogen limited diet.

The nutritional status of the algal cultures fed to juvenile oysters has tremendous impact on the growth response. It is, therefore, important that strict attention be paid to the various levels of essential nutrients in an algal culture, as the nitrogen level can greatly alter the protein content of the cells and the silicate level may profoundly change the carbohydrate and lipid content, as well as the fatty acid composition. Other components in the algae such as vitamins or sterols also quite likely change as a function of nutritional status and may well account for some of the different oyster growth responses observed in the present study. At present very little is known about changes in algal vitamin or sterol content in phytoplankton as a function of environmental conditions; thus this would be an excellent topic for future study. The present study has monitored changes in biochemical composition of the algae and correlated these changes with differences observed in the oyster growth rates. While components, other than those

identified, may also affect the growth rate of the oyster, studies using a technique such as microencapsulation, in which one component can be varied at a time, would be necessary to identify the impact of an individual component definitively. Such experiments would comprise a very interesting future study project. The techniques required for supplying a sufficient number of the required kinds of microcapsules must be available, such that oyster growth trials of adequate duration can be conducted. While advances have recently been made with the development of microencapsulated diets for bivalves, considerable refinement of this technique will be required before it can be used to identify the complete nutritional requirements of the juvenile bivalve. The present study demonstrates the importance of carefully monitoring the nutritional status of the algal cultures used to feed juvenile oysters and identifies some of the biochemical components and their levels which should be included in the basal diets. Such information will be necessary to begin microencapsulation studies.

With the type of data generated from this study, we enter an era in which selected phytoplankton species are domesticated on a production scale to fulfill very precise specifications, such as the nutritional requirements of bivalves. The ability of phytoplankton to convert light energy through photosynthesis to carbohydrates, proteins and lipids is being harnessed in a variety of specialized industries. These industries produce algae as food for various aquatic and domestic land animals, as well as for human consumption (Shelef and Soeder, 1980). The mass culture of lipid-rich phytoplankton for industrial chemicals, vegetable oils and energy-rich hydrocarbons is rapidly developing (Shifrin and Chisholm, 1980). Because of the diversity of phytoplankton

a range of potential commercial applications exist. The wide response of phytoplankton to various environmental parameters must be understood to produce the desired algal compounds efficiently.

This study demonstrates the importance of environmental parameters such as nutrient conditions, on the biochemical composition of phytoplankton. Considering the number of species available, the variety of possible culture conditions and the developing technology to produce mass cultures, it may be that the economic viability of controlled oyster production based on algal diets can be vastly improved.

APPENDICES

APPENDIX I

Algal Protein and Amino Acids

Appendix I

Appendix I, Table 1. Amino Acid Composition of Selected Phytoplankton Species. The data represent the specified amino acid as a percent of the total 15 amino acids shown.

	Skeletonema menzelii	Chaetoceros calcitrans	Skeletonema costatum	Isochrysis galbana T-iso	Isochrysis galbana
	1.	1.	1.	1.	3.
Aspartic Acid	14.4	11.3	11.2	11.1	10.8
Threonine	5.3	5.9	5.5	5.8	4.0
Serine	6.1	5.9	5.5	5.4	4.8
Glutamic Acid	17.2	14.9	16.8	15.2	13.3
Glycine	5.3	5.4	5.5	5.2	6.8
Alanine	6.3	7.7	9.2	8.1	9.0
Valine	6.3	5.9	5.5	6.5	7.7
Methionine	1.7	2.3	1.3	2.1	2.4
Isoleucine	4.9	5.4	5.9	7.1	5.7
Leucine	8.1	9.0	8.4	9.6	10.3
Tyrosine	3.8	4.5	3.8	4.0	3.2
Phenylalanine	5.0	5.9	5.5	5.4	5.8
Histidine	2.1	2.3	2.1	2.3	2.2
Lysine	7.4	7.2	7.0	6.7	6.2
Arginine	6.1	6.3	7.8	8.1	7.9

Sources: 1. Present Study, mean values, based on information in Tables 2 through 6; 2. Covey and Corner (1966); 3. Epifanio (1979(; 4. Chau (1987; 5. Walne (1970).

continued...

Appendix I, Table 1. (continued)

	Thalassiosira pseudonana		Phaeodactylum tricornutum	Pavlova lutheri		Rhodomonas sp.
	3.	4.	2.	4.	2.	1.
Aspartic Acid	11.0	10.8	11.4	10.5	10.1	10.7
Threonine	4.5	6.3	5.3	5.2	5.3	6.0
Serine	5.3	3.7	5.7	3.8	5.6	6.0
Glutamic Acid	12.0	15.4	14.2	11.9	11.5	13.7
Glycine	7.5	6.1	6.1	5.4	6.3	6.0
Alanine	7.3	12.3	7.6	11.5	9.1	9.2
Valine	7.2	5.6	8.1	4.1	7.3	6.8
Methionine	2.5	2.4	2.3	3.5	2.9	3.0
Isoleucine	6.0	4.9	5.3	5.1	4.8	4.5
Leucine	10.3	8.2	9.0	9.9	10.8	7.7
Tyrosine	3.8	4.0	4.0	3.7	4.9	5.1
Phenylalanine	6.4	9.3	6.1	7.1	5.7	5.1
Histidine	2.6	0.8	2.1	1.9	2.3	2.1
Lysine	6.7	5.3	7.4	7.9	7.4	7.1
Arginine	7.1	4.9	5.7	8.5	6.2	7.1

Appendix I, Table 1. (continued)

	Dunaliella tertiolecta		*Chaetoceros gracilis*	*Carteria chui-*		*Tetraselmis suecica*
	1.	4.	1.	2.	3.	5.
Aspartic Acid	10.7	10.8	12.2	10.2	10.5	9.7
Threonine	5.9	5.0	5.5	4.1	3.8	6.7
Serine	5.5	5.1	5.7	4.7	4.5	6.3
Glutamic Acid	14.0	14.2	16.3	13.9	14.2	10.1
Glycine	5.7	7.3	5.4	7.0	7.8	11.0
Alanine	8.7	12.5	7.8	8.7	9.1	14.1
Valine	6.6	4.3	6.1	6.9	7.4	6.8
Methionine	1.9	0.6	2.3	2.5	2.1	2.7
Isoleucine	4.4	3.9	5.9	5.0	5.0	4.4
Leucine	9.7	11.0	8.4	10.2	10.2	9.0
Tyrosine	4.3	3.6	4.0	3.5	3.4	2.3
Phenylalanine	6.0	8.3	5.4	6.9	6.2	4.3
Histidine	2.3	2.0	1.9	2.5	2.3	1.8
Lysine	7.4	5.1	7.1	6.9	6.9	6.2
Arginine	6.9	6.5	5.8	7.1	6.7	4.5

continued...

Appendix I, Table 2. Amino acids of Skeletonema menzelii.

Sample No.	77	78	79	76			
No. of cells in sample (x 10^6)	48.0	48.0	42.0	61.0	$\bar{x}$	s.d.	95% C.I.
	Amino Acids $\mu g(10^6 \text{ cell})^{-1}$						
Aspartic Acid	1.64	1.63	1.49	1.50	1.57	0.08	0.08
Threonine	0.61	0.61	0.53	0.55	0.58	0.04	0.04
Serine	0.67	0.71	0.60	0.60	0.65	0.05	0.05
Glutamic Acid	1.96	1.95	1.75	1.80	1.87	0.11	0.11
Glycine	0.61	0.61	0.54	0.55	0.58	0.04	0.04
Alanine	0.73	0.73	0.63	0.68	0.69	0.05	0.05
Valine	0.73	0.72	0.63	0.67	0.69	0.05	0.05
Methionine	0.20	0.23	0.19	0.10	0.18	0.06	0.06
Isoleucine	0.56	0.55	0.50	0.51	0.53	0.03	0.03
Leucine	0.92	0.92	0.82	0.84	0.88	0.05	0.05
Tyrosine	0.43	0.43	0.38	0.39	0.41	0.03	0.03
Phenylalanine	0.56	0.56	0.50	0.52	0.54	0.03	0.03
Histidine	0.23	0.24	0.22	0.22	0.23	0.01	0.01
Lysine	0.96	0.80	0.73	0.75	0.81	0.10	0.10
Arginine	0.71	0.70	0.63	0.65	0.67	0.04	0.04
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	11.52	11.39	10.14	10.33	10.85	0.71	0.70

Appendix I, Table 3. Amino acids of Chaetoceros calcitrans.

Sample No.	87	84	85	86			
No. of cells in sample (x 10^6)	507.0	680.0	547.0	374.0	$\bar{x}$	s.d.	95% C.I.
Amino Acids	$\mu g(10^6$ cells$)^{-1}$						
Aspartic Acid	0.24	0.24	0.25	0.26	0.25	0.01	0.01
Threonine	0.12	0.13	0.13	0.13	0.13	0.01	0.01
Serine	0.13	0.13	0.13	0.14	0.13	0.01	0.01
Glutamic Acid	0.32	0.33	0.32	0.35	0.33	0.01	0.01
Glycine	0.11	0.12	0.11	0.12	0.12	0.01	0.01
Alanine	0.16	0.17	0.17	0.17	0.17	0.01	0.01
Valine	0.13	0.13	0.13	0.13	0.13	0.00	0.00
Methionine	0.04	0.05	0.05	0.04	0.05	0.01	0.01
Isoleucine	0.12	0.12	0.12	0.13	0.12	0.01	0.01
Leucine	0.19	0.20	0.20	0.21	0.20	0.01	0.01
Tyrosine	0.10	0.10	0.10	0.10	0.10	0.00	0.00
Phenylalanine	0.12	0.13	0.13	0.13	0.13	0.00	0.00
Histidine	0.05	0.05	0.05	0.05	0.05	0.00	0.00
Lysine	0.15	0.17	0.16	0.17	0.16	0.01	0.01
Arginine	0.13	0.14	0.14	0.14	0.14	0.00	0.00
Partial Protein $\mu g(10^6$ cells$)^{-1}$	2.11	2.21	2.19	2.27	2.20	0.07	0.06

Appendix I, Table 4. Amino acids of Skeletonema costatum.

Sample No.	89	90	91	88			
No. of cells in sample (x 10^6)	132.0	115.0	96.0	111.0	$\bar{x}$	s.d.	95% C.I.
	Amino Acids $\mu g(10^6$ cells$)^{-1}$						
Aspartic Acid	0.73	0.75	0.77	0.77	0.76	0.02	0.02
Threonine	0.36	0.37	0.37	0.38	0.37	0.01	0.01
Serine	0.35	0.36	0.38	0.37	0.37	0.01	0.01
Glutamic Acid	1.11	1.13	1.14	1.16	1.14	0.02	0.02
Glycine	0.36	0.37	0.39	0.37	0.37	0.01	0.01
Alanine	0.60	0.62	0.64	0.62	0.62	0.02	0.02
Valine	0.36	0.37	0.37	0.37	0.37	0.01	0.01
Methionine	0.08	0.11	0.08	0.10	0.09	0.02	0.02
Isoleucine	0.32	0.33	0.43	0.50	0.40	0.09	0.08
Leucine	0.55	0.57	0.57	0.57	0.57	0.01	0.01
Tyrosine	0.25	0.26	0.27	0.27	0.26	0.01	0.01
Phenylalanine	0.36	0.37	0.38	0.38	0.37	0.01	0.01
Histidine	0.13	0.14	0.14	0.14	0.14	0.01	0.01
Lysine	0.51	0.52	0.53	0.54	0.53	0.01	0.01
Arginine	0.40	0.41	0.42	0.42	0.41	0.01	0.00
Partial Protein $\mu g(10^6$ cells$)^{-1}$	6.47	6.68	6.88	6.96	6.75	0.22	0.21

Appendix I, Table 5. Amino acids of Isochrysis galbana T-iso.

Sample No.	81	83	82	80			
No. of cells in sample ($x \cdot 10^6$)	305.0	239.0	159.0	118.0	$\bar{x}$	s.d.	95% C.I.
Amino Acids	$\mu g(10^6 \text{ cells})^{-1}$						
Aspartic Acid	0.73	0.73	0.75	0.89	0.78	0.08	0.08
Threonine	0.39	0.39	0.40	0.47	0.41	0.04	0.04
Serine	0.36	0.35	0.37	0.43	0.38	0.04	0.04
Glutamic Acid	1.01	0.96	1.03	1.22	1.06	0.11	0.11
Glycine	0.35	0.34	0.36	0.42	0.37	0.04	0.04
Alanine	0.55	0.53	0.56	0.65	0.57	0.05	0.05
Valine	0.45	0.44	0.44	0.52	0.46	0.04	0.04
Methionine	0.17	0.13	0.13	0.17	0.15	0.02	0.02
Isoleucine	0.32	0.32	0.33	0.39	0.34	0.03	0.03
Leucine	0.65	0.63	0.66	0.77	0.68	0.06	0.06
Tyrosine	0.28	0.26	0.27	0.32	0.28	0.03	0.03
Phenylalanine	0.37	0.36	0.36	0.43	0.38	0.03	0.03
Histidine	0.15	0.15	0.16	0.19	0.16	0.02	0.02
Lysine	0.45	0.44	0.45	0.54	0.47	0.05	0.05
Arginine	0.53	0.51	0.54	0.63	0.55	0.05	0.05
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	6.76	6.54	6.81	8.04	7.04	0.68	0.66

Appendix I, Table 6. Amino acids of Dunaliella tertiolecta.

Sample No.	75	73	74	72			
No. of cells in sample ($\times 10^6$)	78.0	128.0	78.0	94.0	$\bar{x}$	s.d.	95% C.I.
	Amino Acids $\mu g(10^6 \text{ cells})^{-1}$						
Aspartic Acid	1.86	2.01	2.05	1.95	1.97	0.08	0.08
Threonine	1.02	1.13	1.13	1.08	1.09	0.05	0.05
Serine	0.95	1.03	1.05	1.00	1.01	0.04	0.04
Glutamic Acid	2.48	2.61	2.65	2.60	2.59	0.07	0.07
Glycine	1.00	1.07	1.11	1.05	1.06	0.05	0.05
Alanine	1.52	1.63	1.66	1.58	1.60	0.06	0.06
Valine	1.13	1.28	1.25	1.21	1.22	0.07	0.07
Methionine	0.30	0.34	0.33	0.41	0.35	0.05	0.05
Isoleucine	0.77	0.84	0.86	0.82	0.82	0.04	0.04
Leucine	1.68	1.79	1.88	1.79	1.79	0.08	0.08
Tyrosine	0.74	0.82	0.84	0.81	0.80	0.04	0.04
Phenylalanine	1.02	1.11	1.15	1.10	1.10	0.05	0.05
Histidine	0.40	0.43	0.45	0.43	0.43	0.02	0.02
Lysine	1.28	1.33	1.43	1.38	1.36	0.06	0.06
Arginine	1.18	1.26	1.34	1.30	1.27	0.07	0.07
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	17.33	18.68	19.18	18.51	18.43	0.78	0.77

Appendix I, Table 7. Amino acids of Rhodomonas sp.

Sample No.	92	93	94	95	$\bar{x}$	s.d.	95% C.I.
No. of cells in sample (x 10^6)	29.1	50.7	52.2	56.2			
	Amino Acids $\mu g(10^6 \text{ cells})^{-1}$						
Aspartic Acid	3.44	4.02	3.39	3.34	3.55	0.32	0.31
Threonine	1.98	2.15	1.80	1.91	1.96	0.15	0.15
Serine	1.67	1.79	3.03	1.57	2.02	0.68	0.67
Glutamic Acid	4.91	5.10	4.19	4.21	4.60	0.47	0.46
Glycine	2.02	2.32	1.93	1.71	2.00	0.25	0.25
Alanine	2.72	3.12	2.56	3.82	3.06	0.56	0.55
Valine	2.26	2.52	2.12	2.27	2.29	0.17	0.17
Methionine	1.07	1.01	0.84	0.92	0.96	0.10	0.10
Isoleucine	1.64	1.66	1.39	1.44	1.53	0.14	0.14
Leucine	2.93	2.87	2.42	2.25	2.62	0.33	0.32
Tyrosine	1.77	1.84	1.54	1.67	1.71	0.13	0.13
Phenylalanine	1.84	1.80	1.52	1.65	1.70	0.15	0.15
Histidine	0.79	0.75	0.62	0.64	0.70	0.08	0.08
Lysine	2.69	2.55	2.21	2.11	2.39	0.27	0.26
Arginine	2.67	2.51	2.16	2.29	2.41	0.23	0.23
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	32.50	36.01	31.72	31.80	33.01	2.03	1.99

Appendix I, Table 8. Amino acids of Rhodomonas sp. cultured under nitrogen limited conditions.

Sample No.	97	98	99	$\bar{x}$	s.d.	95% C.I.
No. of cells in sample (x 10^6)	36.8	36.8	39.6			
	Amino Acids $\mu g(10^6 \text{ cells})^{-1}$					
Aspartic Acid	3.43	2.91	2.68	3.01	0.38	0.43
Threonine	1.97	1.68	1.64	1.76	0.18	0.20
Serine	1.44	1.29	1.20	1.31	0.12	0.14
Glutamic Acid	4.34	3.85	3.40	3.86	0.47	0.53
Glycine	1.60	1.33	1.37	1.43	0.15	0.16
Alanine	2.43	2.12	2.02	2.19	0.21	0.24
Valine	1.99	1.72	1.58	1.76	0.21	0.24
Methionine	0.93	0.74	0.81	0.83	0.10	0.11
Isoleucine	1.36	1.15	1.08	1.20	0.15	0.16
Leucine	2.45	2.08	2.05	2.19	0.22	0.25
Tyrosine	1.71	1.44	1.50	1.55	0.14	0.16
Phenylalanine	1.52	1.27	1.60	1.46	0.17	0.19
Histidine	0.63	0.53	0.62	0.59	0.06	0.06
Lysine	2.10	1.79	2.10	2.00	0.18	0.20
Arginine	1.88	1.61	1.88	1.79	0.16	0.18
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	29.78	25.51	25.53	26.94	2.46	2.78

Appendix I, Table 9. Amino acids of Chaetoceros gracilis cultured with the complete f/2 nutrient media (control).

Sample No.	27	30	18	21	24	33	45	46	47	51	52
No. of cells in sample (x 10^6)	86.0	87.0	96.0	113.0	103.0	87.0	52.0	64.0	56.0	76.0	122.0
				Amino Acids	$\mu g(10^6$ cells$)^{-1}$						
Aspartic Acid	0.97	0.97	0.94	0.94	1.01	1.07	1.42	1.43	1.45	1.45	1.06
Threonine	0.47	0.47	0.45	0.50	0.47	0.44	0.63	0.50	0.57	0.71	0.51
Serine	0.44	0.45	0.46	0.49	0.49	0.52	0.57	0.58	0.59	0.72	0.51
Glutamic Acid	1.21	1.18	1.29	1.28	1.31	1.32	1.92	1.88	1.94	2.02	1.50
Glycine	0.49	0.47	0.46	0.45	0.46	0.54	0.58	0.59	0.63	0.69	0.50
Alanine	0.66	0.64	0.66	0.66	0.68	0.78	0.83	0.81	0.90	1.01	0.73
Valine	0.51	0.51	0.51	0.51	0.52	0.58	0.62	0.65	0.64	0.77	0.57
Methionine	0.18	0.17	0.21	0.19	0.20	0.16	0.20	0.25	0.25	0.30	0.23
Isoleucine	0.54	0.54	0.49	0.48	0.49	0.59	0.61	0.64	0.63	0.71	0.52
Leucine	0.80	0.79	0.74	0.72	0.74	0.87	0.97	1.01	1.01	1.10	0.81
Tyrosine	0.36	0.35	0.34	0.33	0.34	0.38	0.46	0.47	0.48	0.53	0.40
Phenylalanine	0.51	0.49	0.47	0.47	0.47	0.54	0.61	0.63	0.63	0.70	0.53
Histidine	0.18	0.18	0.15	0.17	0.17	0.21	0.20	0.24	0.21	0.28	0.19
Lysine	0.62	0.59	0.57	0.57	0.58	0.67	0.67	0.71	0.69	0.94	0.68
Arginine	0.57	0.53	0.54	0.50	0.52	0.58	0.66	0.69	0.70	0.79	0.58
Partial Protein $\mu g(10^6$ cells$)^{-1}$	8.51	8.33	8.28	8.26	8.45	9.25	10.95	11.08	11.32	12.72	9.32

continued...

Appendix I, Table 9. (continued)

Sample No.	53	63	64	65	69	71	70	100	101	102
No. of cells in sample (x 10^6)	100.0	49.0	46.0	72.0	67.0	69.0	62.0	37.1	28.9	28.1
				Amino Acids	$\mu g(10^6$ cells$)^{-1}$					
Aspartic Acid	1.09	1.45	1.41	1.25	1.44	1.34	1.37	1.43	1.57	1.72
Threonine	0.52	0.65	0.62	0.57	0.69	0.64	0.65	0.64	0.71	0.80
Serine	0.53	0.68	0.65	0.58	0.69	0.64	0.66	0.68	0.76	0.84
Glutamic Acid	1.34	2.04	1.99	1.75	2.10	1.94	1.93	1.91	2.09	2.24
Glycine	0.51	0.63	0.60	0.56	0.67	0.62	0.63	0.57	0.63	0.58
Alanine	0.74	0.85	0.82	0.81	1.00	0.93	0.93	0.90	0.96	1.35
Valine	0.57	0.70	0.67	0.61	0.75	1.13	0.70	0.66	0.70	1.06
Methionine	0.22	0.32	0.28	0.27	0.33	0.30	0.31	0.27	0.27	0.27
Isoleucine	0.53	0.67	0.65	0.58	0.69	0.65	0.66	0.67	0.74	0.97
Leucine	0.82	1.04	1.01	0.89	1.08	1.00	1.02	0.98	1.10	1.16
Tyrosine	0.39	0.48	0.47	0.42	0.51	0.48	0.50	0.43	0.49	0.45
Phenylalanine	0.52	0.66	0.63	0.57	0.67	0.63	0.64	0.59	0.64	0.63
Histidine	0.20	0.26	0.23	0.22	0.25	0.23	0.24	0.22	0.26	0.19
Lysine	0.70	0.88	0.80	0.74	0.88	0.85	0.87	0.83	0.94	1.02
Arginine	0.60	0.72	0.67	0.61	0.78	0.71	0.73	0.60	0.67	0.64
Partial Protein $\mu g(10^6$ cells$)^{-1}$	9.28	12.03	11.50	10.43	12.53	12.09	11.84	11.38	12.53	13.92

continued...

Appendix I, Table 9. (continued)

Sample No.	103	112	113	114	115	$\bar{x}$	s.d.	95% C.I.
No. of cells in sample ($\bar{x}$ 10^6)	69.4	20.3	30.5	28.1	38.0			
	Amino Acids $\mu g(10^6 \text{ cells})^{-1}$							
Aspartic Acid	1.42	1.72	1.49	1.47	1.34	1.32	0.23	0.09
Threonine	0.66	0.77	0.68	0.66	0.62	0.60	0.10	0.04
Serine	0.68	0.84	0.75	0.71	0.67	0.62	0.11	0.04
Glutamic Acid	1.95	2.21	1.93	1.90	1.70	1.76	0.34	0.13
Glycine	0.60	0.72	0.63	0.60	0.56	0.58	0.07	0.03
Alanine	0.92	0.90	0.81	0.73	0.72	0.84	0.15	0.06
Valine	0.76	-	0.61	0.54	0.59	0.66	0.15	0.06
Methionine	0.30	0.32	0.21	0.29	0.28	0.25	0.05	0.02
Isoleucine	0.69	0.79	0.70	0.67	0.62	0.64	0.11	0.04
Leucine	1.04	1.20	1.04	1.01	0.93	0.96	0.13	0.05
Tyrosine	0.47	0.47	0.44	0.42	0.40	0.43	0.06	0.02
Phenylalanine	0.65	0.70	0.63	0.59	0.57	0.59	0.07	0.03
Histidine	0.15	0.28	0.24	0.20	0.20	0.21	0.04	0.01
Lysine	0.87	0.96	0.84	0.80	0.75	0.77	0.13	0.05
Arginine	0.69	0.61	0.58	0.54	0.54	0.63	0.08	0.03
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	11.85	12.49	11.58	11.13	10.49	10.83	1.63	0.62

Appendix I, Table 10. Amino acids of Chaetoceros gracilis cultured under silicate limited conditions.

Sample No.	23	26	29	32	35	41	36	37	38	39	40	48
No. of cells in sample (x 10^6)	48.0	48.0	57.0	48.0	47.0	59.0	80.0	65.0	115.0	83.0	107.0	42.0
Amino Acids $\mu g(10^6 \text{ cells})^{-1}$												
Aspartic Acid	1.24	1.18	1.09	1.19	1.31	1.21	1.08	1.18	1.08	1.40	1.46	1.33
Threonine	0.56	0.52	0.52	0.52	0.59	0.55	0.44	0.46	0.42	0.70	0.51	0.66
Serine	0.61	0.59	0.52	0.57	0.63	0.53	0.48	0.53	0.45	0.71	0.65	0.72
Glutamic Acid	1.42	1.35	1.18	1.27	1.41	1.37	1.23	1.33	1.36	1.83	1.86	1.71
Glycine	0.53	0.51	0.51	0.56	0.62	0.52	0.50	0.52	0.49	0.63	0.67	0.65
Alanine	0.64	0.60	0.62	0.65	0.69	0.65	0.63	0.63	0.65	0.87	0.90	0.77
Valine	0.60	0.55	0.57	0.59	0.66	0.57	0.53	0.56	0.54	0.70	0.75	0.70
Methionine	0.23	0.22	0.19	0.22	0.27	0.24	0.24	0.25	0.22	0.28	0.32	0.32
Isoleucine	0.60	0.55	0.58	0.62	0.83	0.55	0.52	0.55	0.50	0.66	0.70	0.70
Leucine	0.93	0.86	0.86	0.94	1.02	0.90	0.83	0.89	0.79	1.04	1.10	1.11
Tyrosine	0.38	0.38	0.38	0.40	0.44	0.41	0.38	0.42	0.37	0.48	0.50	0.53
Phenylalanine	0.56	0.53	0.53	0.58	0.63	0.55	0.52	0.55	0.50	0.64	0.69	0.69
Histidine	0.21	0.21	0.20	0.23	0.20	0.21	0.19	0.21	0.18	0.23	0.28	0.29
Lysine	0.70	0.67	0.66	0.71	0.77	0.64	0.60	0.62	0.48	0.68	0.71	0.94
Arginine	0.62	0.60	0.59	0.63	0.68	0.61	0.57	0.60	0.58	0.78	0.81	0.80
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	9.83	9.32	9.00	9.68	10.75	9.51	8.74	9.30	8.61	11.63	11.91	11.92

continued...

Appendix I, Table 10. (continued)

Sample No.	49	50	54	55	56	60	61	62	105	107	120
No. of cells in sample (x 10^6)	57.0	78.0	120.0	111.0	97.0	80.0	113.0	95.0	40.4	63.4	49.9
					Amino Acids $\mu g(10^6 \text{ cells})^{-1}$						
Aspartic Acid	1.31	1.32	0.93	0.95	1.05	1.11	1.06	1.00	1.87	1.76	1.77
Threonine	0.64	0.64	0.46	0.46	0.51	0.55	0.52	0.49	0.90	0.84	0.85
Serine	0.69	0.69	0.46	0.47	0.52	0.56	0.53	0.50	0.95	0.88	0.91
Glutamic Acid	1.60	1.59	1.14	1.15	1.29	1.42	1.33	1.26	2.30	2.18	2.23
Glycine	0.63	0.63	0.45	0.45	0.50	0.51	0.49	0.47	0.96	0.84	0.82
Alanine	0.81	0.82	0.58	0.59	0.66	0.64	0.67	0.63	1.21	1.03	1.13
Valine	0.70	0.70	0.52	0.52	0.58	0.80	0.59	0.54	1.13	0.97	0.94
Methionine	0.29	0.31	0.23	0.24	0.26	0.29	0.26	0.23	0.49	0.47	0.39
Isoleucine	0.66	0.65	0.48	0.48	0.54	0.55	0.54	0.49	1.12	0.91	0.87
Leucine	1.03	1.03	0.74	0.75	0.83	0.88	0.84	0.78	1.72	1.43	1.32
Tyrosine	0.48	0.49	0.35	0.35	0.39	0.40	0.40	0.36	0.79	0.68	0.62
Phenylalanine	0.65	0.65	0.47	0.47	0.53	0.54	0.54	0.49	1.10	0.50	0.87
Histidine	0.26	0.27	0.19	0.18	0.21	0.21	0.21	0.19	0.37	0.38	0.33
Lysine	0.83	0.87	0.60	0.61	0.70	0.71	0.68	0.62	1.47	1.18	1.09
Arginine	0.73	0.75	0.52	0.53	0.59	0.64	0.62	0.56	1.21	1.04	0.84
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	11.31	11.41	8.12	8.20	9.16	9.81	9.28	8.61	17.58	15.09	14.98

continued...

Appendix I, Table 10. (continued)

Sample No.	121	123	124	125	126	127	$\bar{x}$	s.d.	95% C.I.
No. of cells in sample (x 10^6)	48.0	40.7	127.3	105.5	81.7	161.6			
	Amino Acids $\mu g(10^6 \text{ cells})^{-1}$								
Aspartic Acid	1.61	1.75	0.63	0.64	0.65	0.65	1.20	0.34	0.12
Threonine	0.80	0.84	0.30	0.30	0.32	0.32	0.56	0.17	0.06
Serine	0.83	0.89	0.34	0.42	0.43	0.39	0.60	0.17	0.06
Glutamic Acid	2.12	2.16	0.73	0.69	0.70	0.73	1.45	0.46	0.16
Glycine	0.81	0.79	0.28	0.25	0.27	0.26	0.56	0.18	0.06
Alanine	1.09	1.11	0.36	0.35	0.35	0.37	0.71	0.23	0.08
Valine	0.88	0.91	0.32	0.35	0.31	0.37	0.64	0.20	0.07
Methionine	0.43	0.39	0.15	0.12	0.12	0.14	0.27	0.09	0.03
Isoleucine	0.84	0.86	0.30	0.33	0.32	0.32	0.61	0.19	0.07
Leucine	1.30	1.29	0.46	0.46	0.46	0.46	0.93	0.29	0.11
Tyrosine	0.62	0.59	0.21	0.19	0.20	0.20	0.43	0.14	0.05
Phenylalanine	0.88	0.85	0.30	0.28	0.28	0.31	0.58	0.18	0.07
Histidine	0.28	0.30	0.11	0.10	0.11	0.10	0.22	0.07	0.03
Lysine	1.08	1.06	0.38	0.39	0.39	0.38	0.73	0.25	0.09
Arginine	0.87	0.82	0.31	0.28	0.28	0.30	0.65	0.21	0.08
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	14.44	14.61	5.18	5.15	5.19	5.80	10.13	3.11	1.12

Appendix I, Table 11. Amino acids of Chaetoceros gracilis cultured under nitrogen limited conditions.

Sample No.	42	44	43	25	19	31	28	22	58	57	59	66
No. of cells in sample (x 10^6)	26.0	44.0	48.0	104.0	103.0	107.0	114.0	111.0	73.0	70.0	53.0	52.0
					Amino Acids $\mu g(10^6 \text{ cells})^{-1}$							
Aspartic Acid	0.66	0.56	0.53	0.50	0.49	0.51	0.45	0.49	0.59	0.60	0.67	0.42
Threonine	0.32	0.26	0.21	0.33	0.25	0.24	0.20	0.22	0.33	0.32	0.40	0.19
Serine	0.31	0.27	0.25	0.27	0.26	0.26	0.23	0.24	0.32	0.33	0.37	0.23
Glutamic Acid	0.75	0.64	0.59	0.57	0.56	0.54	0.45	0.56	0.68	0.70	0.76	0.46
Glycine	0.28	0.25	0.23	0.23	[illegible]	0.24	0.21	0.22	0.27	0.27	0.30	0.18
Alanine	0.32	0.23	0.27	0.27	0.27	0.29	0.25	0.27	0.31	0.33	0.33	0.20
Valine	0.28	0.21	0.19	0.25	0.24	0.27	0.23	0.24	0.27	0.28	0.30	0.15
Methionine	0.09	0.08	0.10	0.10	0.09	0.06	0.09	0.10	0.12	0.13	0.13	0.09
Isoleucine	0.29	0.26	0.24	0.25	0.25	0.27	0.24	0.25	0.28	0.30	0.32	0.20
Leucine	0.48	0.42	0.39	0.39	0.37	0.40	0.35	0.38	0.45	0.46	0.50	0.31
Tyrosine	0.19	0.18	0.17	0.18	0.17	0.16	0.16	0.17	0.21	0.22	0.23	0.14
Phenylalanine	0.26	0.25	0.23	0.24	0.23	0.25	0.22	0.24	0.28	0.29	0.32	0.18
Histidine	0.09	0.08	0.08	0.09	0.09	0.09	0.08	0.08	0.12	0.13	0.15	0.08
Lysine	0.33	0.29	0.28	0.29	0.28	0.30	0.26	0.29	0.35	0.37	0.40	0.25
Arginine	0.23	0.23	0.21	0.25	0.24	0.24	0.22	0.24	0.28	0.29	0.31	0.17
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	4.88	4.21	3.97	4.21	4.02	4.12	3.64	4.00	4.86	5.02	5.49	3.25

continued...

Appendix I, Table 11. (continued)

Sample No.	67	68	108	109	110	111	128	130	131	$\bar{x}$	s.d.	95% C.I.
No. of cells in Sample (x 10^6)	63.0	72.0	54.0	39.7	27.8	22.4	382.2	475.0	469.0			
						Amino Acids $\mu g(10^6 \text{ cells})^{-1}$						
Aspartic Acid	0.49	0.46	0.61	0.77	0.73	0.88	0.37	0.33	0.33	0.54	0.14	0.06
Threonine	0.22	0.22	0.28	0.35	0.31	0.41	0.18	0.16	0.16	0.26	0.07	0.03
Serine	0.27	0.25	0.32	0.40	0.44	0.47	0.19	0.16	0.17	0.29	0.08	0.04
Glutamic Acid	0.54	0.53	0.67	0.84	0.72	0.99	0.45	0.43	0.42	0.61	0.15	0.06
Glycine	0.22	0.20	0.26	0.32	0.32	0.41	0.16	0.16	0.16	0.24	0.06	0.03
Alanine	0.24	0.25	0.32	0.39	0.36	0.46	0.26	0.20	0.20	0.29	0.07	0.03
Valine	0.20	0.20	0.22	-	-	-	0.22	0.19	0.19	0.23	0.04	0.02
Methionine	0.11	0.09	0.13	0.15	-	0.19	0.08	0.08	0.08	0.10	0.03	0.01
Isoleucine	0.23	0.22	0.29	0.36	0.33	0.44	0.19	0.17	0.17	0.26	0.06	0.03
Leucine	0.37	0.34	0.47	0.59	0.54	0.72	0.27	0.26	0.26	0.42	0.11	0.05
Tyrosine	0.17	0.16	0.19	0.25	0.18	0.25	0.12	0.13	0.12	0.18	0.04	0.02
Phenylalanine	0.22	0.21	0.27	0.34	0.25	0.38	0.18	0.19	0.19	0.25	0.05	0.02
Histidine	0.10	0.08	0.07	0.08	-	0.17	0.07	0.07	0.07	0.09	0.03	0.01
Lysine	0.28	0.27	0.37	0.46	0.36	0.58	0.22	0.20	0.20	0.32	0.09	0.04
Arginine	0.21	0.21	0.24	0.31	0.24	0.63	0.18	0.18	0.18	0.25	0.10	0.04
Partial Protein $\mu g(10^6 \text{ cells})^{-1}$	3.87	3.69	4.71	5.61	4.78	6.98	3.14	2.91	2.90	4.30	0.99	0.42

APPENDIX II

Algal Lipid and Fatty Acids

APPENDIX II

Appendix II, Table 1. Fatty acids of selected phytoplankton species. (From Enright and Newkirk, 1982)

		12:0	14:0	15:0	16:0	16:1	16:1 ω7	16:1 ω9	16:2 ω4	16:2 ω6	16:2 ω7	16:3 ω3	16:3 ω4
Pavlova lutheri	Langdon + Waldock, 1981		11	T	23		26			1	1	T	
	Chuecas + Riley, 1969		9		10		20		5		2		15
Tetraselmis suecica	Langdon + Waldock, 1981		5	T	25		2	2		T	T	1	
Dunaliella tertiolecta	Langdon + Waldock, 1981	1	2	1	16		4	1		2	2	2	
	Chuecas + Riley, 1969	1	6	1	13		5	5	3			5	5
Prymnesium parvum	Chuecus + Riley, 1969		6		16		10		2		T		1
Isochrysis galbana	Chuecas + Riley, 1969		6		16		10		2		T		1
	Watanabe + Ackman, 1974		11	1	22		14	1				T	
Dicrateria inornata	Chuecas + Riley, 1969		1		16		8		1		T		2
	Watanabe + Ackman, 1974		12	4	30		10	2				6	
Cricosphaera carterae	Chuecas + Riley, 1969		9		9		21		6		4		15
Thalassiosira pseudonana	Kates + Volcani, 1966		5		24		30		3		3		4
Cryptomonas sp.	Beach et al., 1970		6		4		2		T		T		T

Appendix II, Table 1. (continued)

		16.4 ω1	16:4 ω3	17:0	18:0	18:1	18:1 ω7	18:1 ω9	18:2 ω3	18:2 ω4	18:2 ω6	18:3 ω3	18:3 ω6
Pavlova lutheri	Langdon + Waldock, 1981		T		2		3	3			3	1	T
	Chuecas + Riley, 1969				T			6			2		
Tetraselmis suecica	Langdon + Waldock, 1981		11		2		T	23				10	T
Dunaliella tertiolecta	Langdon + Waldock, 1981	7	12	T	1		T	7			9	31	4
	Chuecas + Riley, 1969	7						8			6	8	1
Prymnesium parvum	Chuecas + Riley, 1969							25	11		18		T
Isochrysis galbana	Chuecas + Riley, 1969							25	11		18		T
	Watanabe + Ackman, 1974			1	2		9	4	13		2	T	T
Dicrateria inornata	Chuecas + Riley, 1969				T			17			5		T
	Watanabe + Ackman, 1974			T	2		5	10			3	3	T
Cricosphaera carterae	Chuecas + Riley, 1969				T			3			3		
Thalassiosira pseudonana	Kates + Volcani, 1966				2	3					2		T
Cryptoronas sp.	Beach et al., 1970	T										7	

Appendix II Table 1. (continued)

		18:4 ω3	20:1	20:1 ω7	20:1 ω9	20:1 ω11	20:2	20:2 ω6	20:2 ω9	20:3 ω3	20:3 ω6	20:3 ω3
Pavlova lutheri	Langdon + Waldock, 1981	4						T				T
	Chuecas + Riley, 1969	1									2	
Tetraselmis suecica	Langdon + Waldock, 1981	4		1	1	1		T				1
Dunaliella tertiolecta	Langdon + Waldock, 1981	1						T	1			
	Chuecus + Riley, 1969	.8							1		2	4
Prymnesium parvum	Chuecas + Riley, 1969	2							T		1	1
Isochyrsis galbana	Chuecas + Riley, 1969	2							T		1	1
	Watanabe + Ackman, 1974	8		1	T	T						
Dicrateria inornata	Chuecas + Riley, 1969	20							T		1	T
	Watanabe + Ackman, 1974	T		T	4	T						
Cricosphaera carterae	Chuecas + Riley, 1969	2										2
Thalassiosira pseudonana	Kates + Volcani, 1966	T										
Cryptomonas sp.	Beach et al., 1970	44										

Appendix II, Table 1. (continued)

		20:5 ω3	22.1	22:1 ω7	22:1 ω9	22:1, ω13	22:2	22:3	22:4 ω6	22:5 ω3	22:5 ω6	22:6 ω3	24:0
Pavlova lutheri	Langdon + Waldock, 1981	14								T	1	8	
	Chuecas + Riley, 1969	19								3			T
Tetraselmis suecica	Langdon + Waldock, 1981	8											
Dunaliella tertiolecta	Langdon + Waldock, 1981												
	Chuecas + Riley, 1969	10								6			
Prymnesium parvum	Chuecas + Riley, 1969	5								1			T
Isochrysis galbana	Chuecas + Riley, 1969	4								1			T
	Watanabe + Ackman, 1974	7		T		T						4	
Dicrateria inornata	Chuecas + Riley, 1969	8								3			
	Watanabe + Ackman, 1974	1		T	1	1						T	
Cricosphaera carterae	Chuecas + Riley, 1969	20											1
Thalassiosira pseudonana	Kates + Volcani, 1966	21											
Cryptomonas sp.	Beach et al., 1970	16										10	

Appendix II, Table 1. (continued)

		12:0	14:0	15:0	16:0	16:1	16:1 ω7	16:1 ω9	16:2 ω4	16:2 ω6	16:2 ω7	16.3 ω3	16:3 ω4
Rhodomonas sp.	Chuecas + Riley, 1969		18		13		5						
Skeletonema costatum	Chuecas + Riley, 1969		6	T	11		22		4		3		11
	Ackman et al., 1964 (2 day old)		10	T	9		18		9		1		5
	Ackman et al., 1964 (10 day old)		33	1	7		17		7		1		6
Chaetoceros septentrionale	Chuecas + Riley, 1969	1	6	1	11		19		3		2		9
Phaeodactylum tricornutum	Chuecas + Riley, 1969		9	T	11		27		9		5		10
	Kates + Volcani, 1966		3	T	15		30		6		3		7

Appendix II, Table 1. (continued)

		16:4, ω1	16:4 ω3	17.0	18:0	18:1	18:1 ω7	18:1 ω9	18:2 ω3	18:2 ω4	18:2 ω6	18:3 ω3	18.3 ωb
Rhodomonas sp.	Chuecas + Riley, ,1969				T			10		2	2	16	
Skeletonema costatum	Chuecas + Riley, 1969	1		T				2		2			
	Ackman et al., 1964 (2 day old)	3			T			5		1		T	T
	Ackman et al., 1964 (10 day old)	5			T			T		1		T	T
Chaetoceros septentrionale	Chuecas + Riley, 1969	1		1	T			3		1		1	
Phaeodactylum tricornutum	Chuecas + Riley, 1969	3		T	T			5		1		T	
	Kates + Volcani, 1966				2			2		1		1	

Appendix II, Table 1. (continued)

		18.4 ω3	20 1	20:1 ω7	20.1 ω9	20 1 ω11	20;2	20.2 ω6	20:2 ω9	20.3 ω3	20 3 ω6	20:4 ω3	20 4 ω6
Rhodomonas sp.	Chuecas + Riley, 1969	13											1
Skeletonema costatum	Chuecas + Riley, 1969	1							T			2	2
	Ackman et al., 1964 (2 day old)	2			1				T		T	T	1
	Ackman et al., 1964 (10 day old)	3			T				T		T	T	T
Chaetoceros septentrionale	Chuecas + Riley, 1969	T			1				1		1	1	1
Phaeodactylum tricornutum	Chuecas + Riley, 1969	T										1	T
	Kates + Volcani, 1966												

Appendix II, Table 1. (continued)

		20:5 ω3	22:1	22.1 ω7	22:1 ω9	22:1 ω13	22:2	22:3	22:4 ω6	22:5 ω3	22:5 ω6	22:6 ω3	24 0
Rhodomonas sp	Chuecas + Riley, 1969	13							1			5	
Skeletonoma costatum	Chuecas + Riley, 1969	30	T							2			1
	Ackman et al., 1964 (2 day old)	23							T	7	T		
	Ackman et al., 1964 (10 day old)	13								1			
Chaetoceros Septentrionale	Chuecas + Riley, 1969	21	1				6	3		4	T		1
Phaeodactylum tricornutum	Chuecas + Riley, 1969	18	T							1			T
	Kates + Volcani, 1966	30											

Appendix II, Table 2. The fatty acid composition (weight %) of Isochrysis galbana T-iso at various temperatures.

Fatty Acid	8°C	15°C	20°C	25°C	25-30°C
14:0	17.52	17.03	14.54	21.16	22.98
14:1ω7 + ω5	0.51	0.46	0.24	0.26	0.20
15:0	0.67	0.32	0.19	0.27	0.34
16:0	15.74	8.60	12.24	10.89	13.35
16:1ω9	0.86	0.44	1.25	0.22	0.30
16:1ω7	7.12	5.08	2.76	5,81	4.61
16:2ω6	0.47	0.69	0.47	0.69	1.01
16:3ω6	1.04	1.07	0.78	2.10	0.49
16:4ω3	0.06	0.01	0.07	0.11	0.01
18:0	1.84	0.41	0.46	0.15	0.53
18:1ω9	36.16	21.55	22.66	14.44	17.10
18:2ω6	5.60	5.18	5.06	4.19	6.10
18:3ω3	2.34	6.26	6.75	5.11	6.49
18:4ω3	6.16	20.62	20.20	21.39	18.24
20:0	0.92	3.56	2.03	1.38	0.73
20:5ω3	1.23	0.47	0.32	0.26	0.49
22:6ω3	1.74	8.30	9.97	8.56	7.02
Total Lipid					
(% dry wt.)	14.0	12.0	17.0	23.0	28.0
(% fresh wt.)	5.1	3.2	4.6	6.2	7.6

Note: The data presented on tables 2 through 4 were obtained in 1982 through collaboration with Mr. D. Jackson, a high school, who was working on a science project in Dr. J. Castell's laboratory.

Appendix II, Table 3. The fatty acid composition (weight %) of Thalassiosira pseudonana (3H) at various temperatures.

Fatty Acid	8°C	15°C	20°C	25°C	25°-30°C
14:0	9.59	7.13	5.25	8.13	7.41
14:1ω7 + ω5	0.51	-	-	-	0.02
15:0	2.13	1.75	0.60	0.93	0.63
15:1	0.17	-	-	0.13	0.04
16:0	7.16	17.02	27.99	17.47	26.11
16:1ω9 + ω7	22.74	27.91	33.51	28.12	30.37
16:2ω6	11.89	1.24	1.26	3.71	1.25
16:3ω6	2.09	4.42	2.25	5.58	8.01
16:3ω3	15.54	15.21	4.70	9.75	4.36
16:4ω3	6.61	4.93	0.06	0.47	0.14
18:0	0.42	0.54	0.47	0.17	0.50
18:1ω9	0.20	-	-	0.65	0.36
18:1ω7	1.16	0.86	0.60	1.83	0.85
18:1ω5	0.11	0.04	0.01	-	0.02
18:2ω6	0.11	0.29	0.52	0.65	0.55
18:3ω3	0.06	0.02	0.03	0.23	0.04
18:4ω3	7.14	9.47	8.63	4.94	3.96
20:4ω6	0.07	-	0.09	1.59	0.12
20:5ω3	10.88	7.95	13.37	14.16	15.95
22:6ω3	1.33	1.68	0.67	1.44	1.28
Total Lipid					
(% dry wt.)	23.0	42.0	22.0	11.0	6.0
(% fresh wt.)	6.9	13.0	6.8	3.4	1.9

Appendix II, Table 4. The fatty acid composition (weight %) of Tetraselmis sp. at various temperatures.

Fatty Acid	15°C	20°C	25-30°C
12:0	0.04	0.05	0.03
14:0	0.68	1.48	1.87
14:1	0.06	1.11	0.76
15:0	0.09	0.64	0.41
16:0	24.05	20.37	21.54
16:1ω9 + ω7	3.73	1.22	2.12
18:2ω6	1.15	1.33	0.53
17:0	0.20	0.62	3.00
16:3ω6	5.46	4.03	0.99
16:3ω3	0.24	0.45	0.31
16:4ω3	17.89	18.90	13.85
18:0	0.17	0.33	0.57
18:1ω9	6.00	5.52	18.87
18:1ω7	4.04	4.17	0.03
18:2ω6	2.49	2.73	6.37
18:3ω3	16.74	17.01	20.71
18:4ω3	9.00	14.14	2.96
20:0	1.24	0.72	0.12
20:1	1.40	0.77	0.97
20:4ω3	0.78	4.46	0.25
20:5ω3	4.48	3.95	3.73
Total Lipid (% dry wt.)	3.2	7.8	8.0

Appendix II, Table 5. The fatty acid composition (weight %) of Isochrysis galbana T-iso cultured at 23°C.

	1	2	3	4	$\bar{x}$	s.d.	n	95% C.I.
Saturated								
12:0	0.06	0.06	0.11	0.37	0.15	0.15	4	0.15
13:0	0.02	0.49	0.06	0.36	0.23	0.23	4	6.22
14:0	22.40	11.90	24.42	12.87	17.90	6.43	4	6.30
15:0	0.26	0.20	0.27	0.17	0.23	0.05	4	0.05
16:0	15.42	19.75	17.40	17.59	17.54	1.77	4	1.73
17:0	0.67	0.30	0.71	0.30	0.50	0.23	4	0.22
18:0	0.41	1.36	0.38	0.85	0.75	0.46	4	0.45
Total	39.24	34.06	43.35	32.28	37.23	5.03	4	4.93
Monoethylenic								
14:1ω7	0.02	-	-	0.03	0.03	0.01	2	0.01
14:1ω5	0.34	-	0.59	0.02	0.32	0.29	3	0.32
15:1ω6	0.05	0.17	-	0.11	0.11	0.06	3	0.07
16:1ω9	0.18	-	0.21	0.12	0.17	0.05	3	0.05
16:1ω7	3.09	2.16	3.92	1.44	2.65	1.08	4	1.06
18:1ω9	19.94	29.10	22.81	23.75	23.90	3.83	4	3.75
Total	23.62	31.43	27.53	25.47	27.01	3.35	4	3.28
Polyethylenic								
16:2ω6	0.49	0.19	0.47	0.23	0.35	0.16	4	0.15
16:3ω3	0.81	0.18	1.21	0.17	0.59	0.51	4	0.50
16:3ω6	0.01	1.05	-	0.93	0.66	0.57	3	0.64
18:2ω6	4.45	8.81	4.60	8.15	6.50	2.30	4	2.25
18:3ω6	-	-	0.14	0.11	0.13	0.02	2	0.03

continued...

Appendix II, Table 5. (continued)

	1	2	3	4	$\bar{x}$	s.d.	n	95% C.I.
Polyethylenic								
18:3ω3	6.10	3.17	5.31	3.66	4.56	1.38	4	1.35
18:4ω3	17.03	9.14	15.66	12.01	13.46	3.58	4	3.50
20:2ω6	0.63	-	0.97	-	0.80	0.24	2	0.33
20:4ω6	0.06	0.06	-	-	0.06	0.00	2	0.00
20:5ω3	0.26	1.19	-	0.54	0.66	0.48	3	0.54
22:6ω3	5.33	10.74	-	16.37	10.81	5.52	3	6.25
Total	35.17	34.53	28.36	42.17	35.06	5.65	4	5.54
Total ω6	5.69	10.11	6.18	9.53	7.88	2.26	4	2.22
Total ω3	29.53	24.42	22.18	32.58	27.18	4.74	4	4.64
Total ω9	20.12	29.10	23.02	23.87	24.03	3.74	4	3.67
Total ω6/ω3	0.19	0.41	0.28	0.29	0.29	0.09	4	0.09
Total Lipid % fresh wt.	6.7	6.6	7.1	5.9				
	5.9	6.9	6.9	5.5				
	6.3	6.5	6.8	5.7				
$\bar{x}$ =	6.3	6.7	6.9	5.7				
s.d. =	0.4	0.2	0.2	0.2				
95% C.I. =	0.5	0.2	0.2	0.2				
$\mu g(10^6 \text{ cells})^{-1}$	9.8	8.4	8.7	8.5				
	8.5	8.8	8.5	8.1				
	9.1	8.3	8,4	8.2				
$\bar{x}$ =	9.1	8.5	8.5	8.3				
s.d. =	0.6	0.3	0.2	0.2				
95% C.I. =	0.7	0.3	0.2	0.2				

Appendix -II, Table 6. Fatty acid composition (weight %) of Thalassiosira pseudonana (3H) cultured with the complete f/2 nutrient media (control).

	5	6	9	10	11	12	$\bar{x}$	s.d.	n	95% C.I.
Saturated										
12:0	-	-	-	-	-	0.16				
13:0	0.37	-	0.61	0.67	0.33	0.65	0.53	0.16	5	0.14
14:0	9.10	9.38	10.59	10.41	10.27	14.00	10.63	1.76	6	1.41
15:0	1.22	0.99	0.95	0.94	1.08	1.15	1.06	0.11	6	0.09
Iso 16:0	0.07	-	0.20	0.26	-	-	0.18	0.10	3	0.11
16:0	27.35	27.06	17.63	17.88	22.90	19.11	21.99	4.46	6	3.57
Transiso	-	-	0.17	0.28	-	-	0.23	0.08	2	0.11
17:0	1.80	1.57	3.19	3.33	1.94	2.14	2.33	0.75	6	0.60
18:0	0.29	-	0.43	0.61	0.29	0.48	0.42	0.14	5	0.12
Total	40.20	39.00	33.77	34.38	36.81	37.69	36.98	2.53	6	2.03
Monoethylenic										
14:1ω9	0.14	0.34	0.33	0.45	0.35	-	0.32	0.11	5	0.10
14:1ω7	0.31	0.14	0.04	-	-	-				
14:1ω5	0.14	-	-	-	-	-				
15:1ω8	0.09	-	0.14	0.19	-	-	0.14	0.05	3	0.06

continued...

Appendix II, Table 6. (continued)

	5	6	9	10	11	12	$\bar{x}$	s.d.	n	95% C.I.
Monoethylenic										
15:1ω6	0.44	-	0.52	0.60	0.21	0.11	0.38	0.21	5	0.18
16:1ω7	41.04	46.29	33.30	33.91	35.92	27.76	36.37	6.48	6	5.19
18:1ω9	0.31	-	0.45	0.51	0.40	0.42	0.42	0.07	5	0.06
18:1ω7	0.35	-	0.40	0.50	0.40	0.42	0.41	0.05	5	0.05
24:1	-	-	-	0.35	-	-				
Total	42.82	46.77	35.18	36.51	37.28	28.71	37.88	6.28	6	5.02
Polyethylenic										
16:2ω6	1.18	0.83	1.71	1.82	1.15	1.74	1.41	0.41	6	0.32
16:3ω6	0.53	0.42	0.80	0.90	0.88	1.59	0.85	0.41	6	0.33
16:3ω3	2.92	3.51	5.94	4.96	4.05	4.62	4.33	1.08	6	0.86
16:4ω3	0.14	-	0.49	0.35	0.34	0.32	0.33	0.12	5	0.11
18:2ω6	0.20	-	0.36	0.59	0.38	2.71	0.85	1.05	5	0.92
18:3ω6	0.08	-	0.09	-	0.16	-	0.11	0.04	3	0.05
18:4ω3	4.13	4.06	5.46	4.09	5.19	3.81	4.46	0.69	6	0.55
20:5ω3	5.43	3.48	11.99	11.04	10.40	11.88	9.04	3.65	6	2.92
22:6ω3	0.29	-	0.94	1.43	1.62	2.71	1.40	0.90	5	0.79
22:5	-	-	-	0.17	0.10	0.32	0.20	0.11	3	0.13

continued...

Appendix II, Table 6. (continued)

	5	6	9	10	11	12	$\bar{x}$	s.d.	n	95% C.I.
Total	14.90	12.30	27.78	25.35	24.27	29.70	22.38	7.11	6	5.69
Total ω6	2.48	1.25	3.48	3.91	2.78	6.15	3.33	1.66	6	1.33
Total ω3	12.91	11.05	24.82	21.87	21.60	23.34	19.27	5.79	6	4.63
Total ω9	0.45	0.34	0.78	0.96	0.75	0.42	0.62	0.25	6	0.20
Total ω6/ω3	0.19	0.11	0.14	0.18	0.13	0.26	0.17	0.06	6	0.04
Total Lipid (% fresh wt.)	5.8	5.1	4.0	4.8	–	3.9	4.7	0.79	5	0.69

Appendix II, Table 7. Fatty acid composition (weight %) of Thalassiosira pseudonana (3H) cultured under silicate limited conditions.

	1	2	3	4	7	8	$\bar{x}$	s.d.	n	95% C.I.
Saturated										
12:0	-	-	0.09	-	-	0.04	0.07	0.04	2	0.05
13:0	0.33	0.08	0.27	-	0.15	0.06	0.18	0.12	5	0.10
14:0	10.16	10.33	9.14	10.95	10.57	11.26	10.40	0.74	6	0.59
15:0	1.02	0.99	1.05	1.34	1.32	1.48	1.20	0.21	6	0.16
16:0	22.88	23.05	24.26	27.45	34.86	36.58	28.18	6.09	6	4.87
17:0	2.18	2.32	2.10	1.53	0.96	0.79	1.65	0.66	6	0.53
18:0	0.29	0.23	0.51	0.54	0.63	0.55	0.46	0.16	6	0.13
Total	36.86	37.00	37.42	41.81	48.49	50.76	42.06	6.18	6	4.95
Monoethylenic										
14:1ω9	0.36	-	0.12	-	-	0.12	0.20	0.14	3	0.16
14:1ω7	-	0.08	-	0.89	0.51	0.96	0.61	0.40	4	0.40
14:1ω5	-	-	-	-	0.45	0.36	0.55	0.45	3	0.11
15:1ω8	-	-	0.10	-	-	- -				
15:1ω6	0.27	0.10	0.38	0.48	0.39	0.47	0.35	0.14	6	0.11

continued...

Appendix II, Table 7. (continued)

	1	2	3	4	7	8	$\bar{x}$	s.d.	n	95% C.I.
Monoethylenic										
16:1ω7	33.63	39.24	35.67	37.57	38.91	39.20	37.37	2.29	6	1.83
18:1ω9	0.40	0.50	0.66	0.50	0.36	0.29	0.45	0.13	6	0.10
18:1ω7	0.41	0.47	0.48	0.21	1.00	1.09	0.61	0.35	6	0.28
24:1	-	-	0.61	0.30	0.11	0.11	0.28	0.24	4	0.23
Total	35.07	40.39	38.02	40.40	41.64	42.79	39.72	2.78	6	2.22
Polyethylenic										
16:2ω6	1.14	1.39	1.11	0.50	0.38	0.34	0.81	0.46	6	0.36
16:3ω6	0.65	0.50	0.54	0.56	0.14	0.14	0.42	0.22	6	0.18
16:3ω3	4.04	4.61	4.13	2.28	1.29	0.92	2.88	1.59	6	1.27
16:4ω3	0.33	0.44	0.32	0.24	0.19	0.12	0.27	0.11	6	0.09
18:2ω6	0.39	0.75	0.78	0.56	0.20	-	0.54	0.25	5	0.21
18:3ω6	0.16	-	0.14	0.09	-	-	0.13	0.04	3	0.04
18:4ω3	5.19	5.74	4.65	3.63	2.53	1.49	3.87	1.63	6	1.31
20:5ω3	10.39	8.32	9.29	6.37	2.99	1.49	6.48	3.57	6	2.86
22:5	0.09	-	0.14	0.11	0.10	0.09	0.11	0.02	5	0.02
22:6ω3	1.55	0.77	0.84	0.59	0.20	0.40	0.73	0.47	6	0.37
Total	23.93	22.52	21.94	14.93	8.02	4.99	16.06	8.08	6	6.47

continued...

Appendix II, Table 7. (continued)

	1	2	3	4	7	8	$\bar{x}$	s.d.	n	95% C.I.
Total ω6	2.61	2.74	2.95	2.19	1.11	0.95	2.09	0.86	6	0.69
Total ω3	21.50	19.88	19.23	13.11	7.20	4.42	14.22	7.17	6	5.73
Total ω9	0.76	0.50	0.78	0.50	0.36	0.41	0.55	0.18	6	0.14
Total ω6/ω3	0.12	0.14	0.15	0.17	0.15	0.21	0.16	0.03	6	0.03
Total Lipid (% fresh wt.)	5.3	5.7	5.2	3.2	4.4	4.5	4.7	0.89	6	0.71

Appendix II, Table 8. Lipid level of Rhodomonas sp. cultured with the complete f/2 nutrient media (control) and under nitrogen limited conditions.

Control			Nitrogen Limitation		
Culture Dates	$\mu g(10^6 \text{ cells})^{-1}$	% fresh wt.	Culture Dates	$\mu g(10^6 \text{ cells})^{-1}$	% fresh wt.
130	20.1	6.6	130	48.8	9.9
130	22.8	7.5	130	46.3	9.9
130	19.9	6.6			
			133	48.6	9.2
			133	44.9	8.5

Appendix H, Table 9. Lipid level of Chaetoceros gracilis cultured under three nutrient treatments.

Control					Silicate Limited					Nitrogen Limited				
Culture Dates	µg(10^6 cells)$^{-1}$	Culture Average µg(10^6 cells)$^{-1}$	% fresh wt.	Culture Average % fresh wt.	Culture Dates	µg(10^6 cells)$^{-1}$	Culture Average µg(10^6 cells)$^{-1}$	% fresh wt.	Culture Average % fresh wt.	Culture Dates	µg(10^6 cells)$^{-1}$	Culture Average µg(10^6 cells)$^{-1}$	% fresh wt.	Culture Average % fresh wt.
67	7.3		3.1		67	18.8	18.8	7.4	7.4	75	6.9	6.9	2.4	2.4
67	10.2	8.8	2.1	2.6	63	18.3	18.3	6.1	6.1	59	12.2	12.2	2.2	2.2
77	9.6	9.4	2.7	2.7	77	19.0	19.0	5.7	5.7	154	10.9		4.5	
75	10.1	10.1	2.4	2.4	80	16.5	16.5	5.2	5.2	154	6.8		3.0	
63	6.7	6.7	3.6	3.6	154	31.5		10.9		154	12.5	10.1	5.5	4.3
154	10.1		4.1		154	42.5		11.6		168	8.8		4.4	
154	10.6	10.4	2.6	3.4	154	30.9	34.8	8.3	10.3	168	8.7		4.4	
168	10.0		2.6		175	22.5		7.5		168	12.3	9.9	6.1	5.0
168	9.1		2.3		175	26.8		8.9		178	8.6		5.4	
168	8.3		2.1		175	23.8	24.4	7.9	8.1	178	8.9		5.6	
168	11.4	9.7	2.9	2.5	178	14.4		9.6		178	8.3	8.6	5.2	5.4
235		$\bar{x}$=9.2 s.d.=1.3 n=6	1.3		178	12.7		8.5		67		$\bar{x}$=9.5 s.d.= 2.0 n=5	2.4	2.4
235			2.1	1.7	178	10.3		6.9		245			3.4	
237			3.5		178	19.4	14.2	6.3	7.8	245			3.2	
237			1.9	2.7	59	10.0	10.0	7.3	7.3	245			3.1	3.2
245			2.2		235		$\bar{x}$=19.5 s.d.= 7.4 n= 8	5.7						$\bar{x}$=3.6 s.d.=1.3 n=7
245			2.4	2.3	235			5.9						
				$\bar{x}$=2.7 s.d.=0.6 n=9	235			5.2	5.6					
					237			6.0						
					237			7.1	6.6					
									$\bar{x}$=7.0 s.d.=1.5					

Appendix II, Table 10. The fatty acid composition (weight %) of Chaetoceros gracilis cultured with the complete f/2 nutrient media (control).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	$\bar{x}$	s.d.	95% C.I.
Saturated																	
12:0	0.20	0.24	0.20	0.26	0.13	-	-	-	-	-	-	1.66	-	-			
13:0	1.31	2.33	1.34	1.20	0.53	-	-	-	-	-	-	-	-	-			
14:0	9.52	9.33	7.89	10.57	10.04	11.08	14.78	9.11	7.92	3.37	11.22	9.11	7.74	9.76	9.39	2.49	1.31
15:0	0.43	0.30	0.35	0.43	0.62	1.35	0.06	0.18	0.42	1.00	0.05	0.81	2.38	15.52	1.71	4.02	2.11
iso 16:0	0.09	-	0.18	0.25	0.40	0.28	0.90	0.04	0.98	-	-	-	-	-			
16:0	13.22	9.15	11.20	13.11	11.40	17.50	20.75	28.36	17.93	22.07	18.22	13.22	21.34	18.10	16.83	5.27	2.76
18:0	0.72	0.72	0.68	0.83	0.65	0.45	1.40	1.34	1.40	1.88	0.95	1.09	1.85	0.89	1.06	0.45	0.24
24:0	-	-	-	-	-	0.50	-	0.01	3.26	0.52	-	-	0.07	-			
Total	25.49	22.07	21.84	26.65	23.77	31.16	37.89	39.04	31.91	28.84	30.44	25.89	33.38	44.27	30.19	6.09	3.50
Monoethylenic																	
14:1ω9	0.45	0.75	0.54	0.71	0.49	-	-	-	-	-	-	-	-	-			
14:1ω7	0.22	0.06	0.31	0.24	0.08	-	-	-	-	-	-	-	-	-			
14:1ω5	0.04	-	0.06	0.05	0.02	-	-	-	-	-	-	-	-	-			
15:1ω8	0.39	0.16	0.37	0.41	0.28	-	-	-	-	-	-	-	-	-			
15:1ω6	0.83	0.59	0.77	0.91	0.86	-	-	-	0.34	-	-	-	-	-			
16:1ω9	0.09	-	0.19	0.27	0.84	-	-	-	-	-	-	-	-	-			

continued...

Appendix II, Table 10. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	$\bar{x}$	s.d.	95% C.I.
Monoethylenic																	
16:1ω7	31.51	21.15	23.05	32.32	31.99	43.11	33.91	30.54	21.17	37.94	37.61	29.50	37.85	22.31	31.00	6.97	3.65
16.1ω5	0.41	4.51	0.58	0.58	0.04	-	-	-	-	-	-	-	-	-			
18.1ω11	-	-	-	-	-	-	-	-	0.51	0.08	-	-	-	-			
18:1ω9	1.59	6.42	1.33	1.39	0.70	1.55	1.75	1.53	0.29	0.38	0.63	2.49	0.66	0.19	1.49	1.56	0.82
18.1ω7	3.88	0.06	4.27	3.79	2.38	1.17	1.04	0.24	0.18	0.22	0.44	0.43	0.62	0.19	1.35	1.55	0.81
Total	39.41	33.70	31.41	40.67	37.68	45.83	36.70	32.31	22.49	38.62	38.68	32.42	39.13	22.69	35.12	6.56	3.43
Polyethylenic																	
16:2ω4	3.53	2.16	2.20	3.65	3.75	3.95	4.07	2.55	1.03	9.07	-	3.99	5.45	5.68			
16:3ω4	2.25	2.09	1.40	2.28	2.67	0.09	0.69	0.64	1.14	4.15	1.58	3.11	2.62	3.70	2.03	1.18	0.62
16:3ω3	0.05	0.07	0.06	0.06	0.15	0.02	0.06	1.88	0.16	-	0.76	-	0.28	-			
16:4ω3	7.02	6.52	3.55	6.78	6.29	-	-	-	3.06	5.00	3.75	7.59	4.49	6.45			
18:2ω7	0.09	0.02	0.05	0.09	0.05	0.02	0.02	0.07	-	-	-	-	-	-			
18.2ω6	3.05	1.22	0.04	2.51	0.64	0.12	0.08	1.14	0.18	-	0.23	0.76	-	0.66			
18 3ω6	0.49	0.94	0.04	0.45	0.75	-	-	-	1.01	1.58	1.61	1.74	1.44	1.85			
18:3ω4	0.12	0.80	0.23	0.14	0.02	0.14	0.06	0.03	0.60	0.36	1.55	0.65	0.62	0.89	0.41	0.45	0.23

continued...

Appendix II, Table 10. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	$\bar{x}$	s.d.	95% C.I.
18:3ω3	0.11	0.19	-	0.11	0.10	0.01	1.54	1.24	0.49	-	-	0.22	-	0.49			
18:4ω3	0.25	2.89	-	0.19	0.35	0.53	0.53	0.53	1.11	0.71	0.30	1.06	0.47	0.15			
20:2ω6	-	-	-	-	-	0.03	0.12	-	0.20	-	-	0.02	0.10	0.12			
20:3ω6	-	-	-	-	0.04	0.09	0.10	0.18	0.33	0.16	-	0.01	0.15	0.02			
20:3ω3	-	-	-	-	-	0.01	-	0.09	0.33	0.04	-	-	0.07	0.07			
20:4ω6	3.65	6.10	3.77	3.50	4.96	5.00	4.85	4.04	7.90	1.68	5.72	5.77	2.11	4.41	4.53	1.62	0.85
20:4ω3	-	-	-	-	-	0.54	0.43	0.16	0.82	0.25	-	0.33	0.11	0.21			
20:5ω3	13.74	16.32	9.73	12.32	17.53	7.30	7.05	8.33	24.09	6.64	10.37	13.86	7.40	6.00	11.48	5.20	2.73
22:5ω6	0.14	0.38	-	0.06	0.95	0.13	-	-	-	-	-	-	-	-			
22:6ω3	0.61	4.53	*25.95	0.54	0.62	1.14	2.71	4.64	3.35	2.90	5.00	2.50	2.19	2.34	2.54	1.55	0.84
Total	35.10	44.23	45.02	32.68	38.60	19.12	22.31	25.52	45.80	32.54	30.87	41.61	27.50	33.04	33.85	8.47	4.44
Total ω6	8.16	9.23	4.62	7.43	8.20	5.37	5.15	5.36	9.96	3.42	7.56	8.30	3.80	7.06	6.69	2.05	1.08
Total ω3	21.78	30.52	39.29	20.00	25.04	9.55	12.32	16.87	30.41	15.54	20.18	25.56	15.01	15.71	21.48	8.47	4.44
Total ω9	2.13	7.17	2.06	2.37	2.03	1.55	1.75	1.53	0.[illegible]	0.38	0.63	2.49	0.66	0.19	1.80	1.74	0.91
Total ω6/ω3	0.37	0.30	0.12	0.37	0.33	0.56	0.42	0.32	0.30	0.22	0.37	0.32	0.25	0.45	0.34	0.11	0.06

* not included in $\bar{x}$

Appendix II, Table II. Fatty acid composition (weight %) of Chaetoceros gracilis cultured under silicate limited conditions.

	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$	s.d.	95% C.I.
Saturated															
12:0	0.21	0.24	0.49	0.18	0.35	0.34	0.08	0.12	0.08	0.50					
13:0	0.34	0.50	0.95	0.33	0.53	1.08	0.27	0.61	0.08	1.06					
14:0	10.68	11.07	9.47	8.14	9.58	9.58	11.33	10.67	10.35	8.86	11.03	11.65	10.20	1.07	0.61
15:0	0.93	0.99	0.92	0.69	0.83	1.00	1.37	1.07	0.98	0.84	1.21	0.65	0.96	0.20	0.11
Iso 16:0	0.12	0.12	-	0.08	-	-	-	0.05	-	-	0.55	0.29			
16:0	24.10	26.66	24.26	27.03	25.46	27.12	27.86	27.87	22.64	25.79	35.64	27.59	26.84	3.23	1.83
18:0	2.05	2.12	1.48	1.41	1.37	-	0.54	1.76	0.43	1.48	1.20	1.08			
Total	38.43	41.70	37.57	37.86	38.12	39.12	41.45	42.15	34.56	38.53	49.63	41.26	40.03	3.73	2.11
Monoethylenic															
14:1ω9	-	-	0.17	0.25	0.07	-	0.06	0.19	-	0.17	-	-			
14:1ω7	0.10	0.13	0.05	0.56	0.54	0.35	0.92	-	0.08	0.31	-	-			
14:1ω5	-	-	0.02	0.05	0.05	-	0.46	-	-	0.04	-	-			
15:1ω8	0.32	0.40	0.08	0.14	-	-	0.08	0.10	-	0.08	-	-			
15:1ω6	0.47	0.48	0.34	0.33	0.11	-	0.49	0.43	0.10	0.28	-	-			

continued...

Appendix II, Table II. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$	s.d.	95% C.I.
Monoethylenic															
16:1ω9	-	-	-	-	-	-	-	-	-	0.03	-	-			
16:1ω7	37.82	41.32	34.98	31.46	27.92	45.95	37.75	37.27	38.17	31.63	35.26	37.74	36.44	4.74	2.68
16:1ω5	-	-	-	-	-	-	-	0.19	-	-	-	-			
18:1ω11	-	-	-	0.14	-	-	0.15	-	0.22	0.06	-	-			
18:1ω9	0.94	0.88	1.37	1.75	1.44	-	0.50	0.80	0.48	0.72	0.76	0.80			
18:1ω7	-	-	3.45	10.65	4.89	-	0.21	2.09	0.45	4.85	0.69	-			
18:1ω5	3.14	2.22	-	-	5.89	-	-	-	-	-	-	-			
22:1ω7	-	0.03	0.01	0.38	0.11	-	-	-	-	0.02	-	-			
24:1	0.02	0.04	-	-	-	-	0.28	-	-	-	-	-			
Total	42.81	45.50	40.47	45.71	41.02	46.30	40.90	41.07	39.50	38.19	36.71	38.54	41.65	3.13	1.77
Polyethylenic															
16:2ω4	0.94	0.93	1.65	1.20	1.09	0.81	0.50	1.61	1.34	1.65	1.65	1.11	1.21	0.38	0.22
16:3ω4	0.71	0.70	1.28	1.41	1.37	1.54	1.53	1.26	2.24	1.40	1.07	0.58	1.26	0.46	0.26
16:3ω3	-	-	-	1.42	-	0.41	0.55	-	0.48	-	0.15	-			
16:3ω6	-	-	-	-	-	-	-	-	-	-	-	-			
16:4ω3	4.08	1.93	3.07	2.37	2.37	3.42	2.24	2.27	5.39	3.07	0.74	-			

continued...

Appendix II, Table II. (continued)

Polyethylenic	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$	s.d.	95% C.I.
18:2ω7	-	-	-	-	-	-	-	-	-	-	-	0.05			
18:2ω6	0.99	0.52	1.03	1.02	0.90	-	0.56	0.67	0.71	1.21	0.50	0.64			
18:3ω6	1.42	0.93	1.29	0.68	1.20	-	0.08	1.12	-	1.08	1.40	1.66			
18:3ω4	-	-	0.05	-	-	-	0.09	-							
18:3ω3	0.18	-	0.02	0.17	-	-	0.04	-	-	0.03	-	-			
18:4ω3	0.56	0.11	0.34	0.06	0.10	4.16	3.76	0.13	5.76	0.14	0.40	0.43	1.33	2.01	1.13
20:3ω6	0.24	-	0.40	-	-	-	-	0.36	-	0.18	0.26	0.22			
20:4ω6	3.85	3.30	5.13	4.25	7.70	-	-	4.29	-	7.41	3.70	6.13			
20:4ω3	-	-	0.04	-	0.09	-	-	0.03	-	0.02	0.11	0.15			
20:5ω3	5.03	3.81	6.81	3.53	6.03	4.28	7.83	4.52	9.90	6.24	3.53	5.72	5.60	1.92	1.09
22:5ω6	0.16	0.23	0.35	0.28	0.44	-	0.15	0.33	-	0.39	-	-			
22:6ω3	0.64	0.36	0.47	0.01	0.55	0.01	0.88	0.19	0.12	0.47	0.37	0.75	0.40	0.28	0.16
Total	18.75	12.82	21.93	16.40	21.84	14.58	18.21	16.78	25.94	23.29	13.88	17.44	18.49	4.03	2.28
Total ω6	7.13	5.46	8.54	6.56	10.35	-	1.28	7.20	0.81	10.55	5.86	8.65	6.58	3.19	1.89
Total ω3	10.44	6.21	10.75	7.56	9.14	12.23	15.30	7.14	21.65	9.97	5.15	7.05	10.22	4.58	2.59
Total ω9	0.94	0.88	1.54	2.00	1.51	-	0.56	0.99	0.48	0.92	0.76	0.80			
Total ω6/ω3	0.68	0.88	0.79	0.87	1.13	-	0.08	1.01	0.04	1.06	1.14	1.23	0.81	0.40	0.24

Appendix II, Table 12. Fatty acid composition (weight %) of Chaetoceros gracilis cultured under nitrogen limited conditions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	$\bar{x}$	s.d.	95% C.I.
Saturated																	
12:0	0.10	0.04	1.12	0.05	0.05	0.04	0.25	0.62	0.91	0.05	–	0.27	0.06	–			
13:0	0.52	0.07	0.21	0.64	0.70	0.16	0.04	0.50	0.57	–	–	–	–	–			
14:0	9.36	11.67	21.28	10.90	10.73	10.92	10.76	11.36	19.15	10.85	11.69	11.25	9.05	9.66	12.05	3.89	2.04
15:0	1.13	1.52	1.14	0.97	0.96	1.35	0.79	0.78	1.25	1.08	1.41	0.29	0.77	0.28			
Iso 16:0	–	–	–	0.20	0.27	0.01	–	0.10	–	–	–	–	–	–			
16:0	24.36	37.19	33.76	17.80	18.08	35.31	20.05	23.40	38.61	23.98	28.77	23.68	31.57	22.57	27.08	7.07	3.70
18:0	0.50	0.55	2.01	0.43	0.61	0.62	1.56	2.25	2.45	0.41	1.36	1.18	1.51	1.40	1.20	0.70	0.37
20:0	–	–	4.69	–	–	–	6.18	0.18	4.92	–	–	–	–	–			
24:0	–	–	–	–	–	0.33	–	–	–	–	–	–	–	–			
Total	35.97	51.04	64.21	30.99	31.73	48.41	39.63	39.19	67.86	36.37	43.23	36.67	42.96	33.91	43.01	11.35	5.95
Monoethylenic																	
14:1ω9	0.12	0.12	–	0.34	0.46	0.05	0.13	0.08	–	–	0.06	–	–	–			
14:1ω7	0.07	1.00	0.30	0.04	0.06	0.53	1.05	0.69	1.42	–	0.94	–	–	–			
14:1ω5	0.02	0.54	1.33	–	–	0.37	0.23	0.19	–	–	0.48	–	–	–			
15:1ω8	0.10	0.05	–	0.14	0.19	0.04	–	–	–	0.01	0.01	–	–	–			
15:1ω6	0.38	0.47	–	0.53	0.60	0.40	0.09	0.14	–	0.27	0.49	0.06	0.04	0.04			

continued...

Appendix II, Table 12. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	$\bar{x}$	s.d.	95% C.I.
Monoethylenic																	
16:1ω9	-	-	-	0.17	0.29	-	0.05	-	-	0.70	-	-	-	-			
16:1ω7	35.46	39.46	22.93	33.28	33.95	39.02	28.72	20.38	7.90	42.33	44.64	34.29	35.18	22.16	31.41	10.02	5.25
16:1ω5	-	-	-	-	-	-	0.37	2.71	-	-	-	-	-	-			
18:1ω9	0.65	0.29	5.02	0.44	0.50	0.35	1.53	3.40	3.34	0.50	0.51	0.72	0.64	0.67			
18:1ω7	0.40	1.08	-	0.40	0.49	0.98	8.92	4.86	2.78	0.42	0.22	0.65	0.64	0.67			
24:1	0.58	0.10	-	-	-	0.10	0.05	0.07	-	-	0.28	-	-	-			
Total	37.78	43.11	29.58	35.34	36.54	41.84	41.14	32.52	15.44	44.23	47.63	35.72	36.50	23.54	35.78	8.54	4.48
Polyethylenic																	
16:2ω4	-	1.09	0.34	-	1.69	1.80	0.38	2.24	2.06	12.50	1.17	0.05	1.71	3.88	19.21		
16:3ω4	2.07	0.79	0.51	3.16	3.30	0.95	1.76	2.26	0.67	2.24	1.57	1.08	0.81	1.14			
16:3ω3	0.53	0.14	-	0.79	0.89	0.13	0.04	0.34	-	4.11	0.57	0.22	0.08	0.68			
16:3ω6	-	0.92	-	-	-	-	-	-	-	0.11	2.30	-	-	-			
16:4ω3	4.02	0.12	0.62	5.82	4.87	1.27	7.16	1.93	-	0.30	0.56	3.69	1.77	3.57			
18:2ω7	-	-	3.25	-	-	-	0.06	4.36	-	-	-	0.01	0.04	0.11			
18:2ω6	0.76	-	0.51	0.35	0.58	0.20	0.83	2.07	0.23	0.42	0.59	0.77	1.29	0.78			
18:3ω6	0.14	-	-	0.09	-	0.04	0.63	0.52	-	0.19	0.01	1.90	1.61	1.65			
18:3ω4	0.07	-	-	0.03	0.07	-	-	0.15	-	0.06	0.10	-	-	-			
18:3ω3	0.06	-	-	0.07	0.08	0.03	0.97	0.24	-	0.09	0.05	0.01	-	0.01			

continued...

Appendix II, Table 12. (continued)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	$\bar{x}$	s.d.	95% C.I.
Polyethylenic																	
18:4ω3	4.77	1.55	-	5.63	4.23	2.62	-	0.51	-	5.61	3.90	0.69	0.45	0.51			
20:2ω6	-	-	-	-	-	-	-	-	-	-	-	0.01	-	-			
20:3ω6	-	-	-	-	-	-	-	-	-	-	-	0.14	0.23	0.15			
20:3ω3	-	-	-	-	-	-	-	-	-	-	-	0.08	0.04	0.06			
20:4ω6	0.02	-	0.67	-	-	-	3.20	6.31	0.29	-	-	5.99	3.09	5.01			
20:4ω3	0.08	-	-	-	-	-	-	-	-	-	-	0.20	0.06	0.20			
20:5ω3	11.30	1.81	0.64	14.66	13.52	3.67	1.63	5.37	0.69	13.20	8.08	9.81	6.61	8.20	7.09	4.95	2.59
22:5ω6	0.19	0.13	-	-	0.24	0.13	0.72	1.59	2.32	-	-	-	-	-			
22:6ω3	1.24	0.05	-	1.40	2.13	0.30	0.07	0.67	-	2.40	0.09	1.32	0.72	1.03	0.95	0.79	0.44
Total	26.34	5.85	6.20	33.69	31.71	9.72	19.31	28.38	16.70	29.90	17.87	27.63	20.68	42.29	22.59	10.77	5.64
Total ω6	1.49	1.52	1.18	0.97	1.42	0.77	5.47	10.63	2.84	0.99	3.39	8.32	6.26	7.61	3.78	3.28	1.72
Total ω3	22.00	3.67	1.26	28.37	25.72	8.02	9.87	9.06	0.69	25.71	13.25	10.68	9.73	14.26	13.02	9.14	4.78
Total ω9	0.77	0.41	5.02	0.95	1.25	0.40	1.71	3.48	3.34	1.20	0.57	0.72	0.64	0.67	1.51	1.41	0.74
Total ω6/ω3	0.07	0.41	0.94	0.03	0.06	0.10	0.55	1.17	4.12	0.04	0.26	0.78	0.64	0.53	0.69	1.05	0.55

APPENDIX III

Algal Carbohydrates

APPENDIX III

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ALGAL CARBOHYDRATES

Appendix III. Table 1. The carbohydrate content, expressed in µg glucose equiv. $(10^6 \text{ cells})^{-1}$ of Isochrysis galbana T-iso.

Culture Date	Carbohydrate µg glucose equiv. $(10^6 \text{ cells})^{-1}$	Culture $\bar{x}$
67	7.1	
67	6.7	
67	6.9	
67	7.0	6.9
75	8.2	
75	7.3	
75	7.9	
75	7.9	7.8
79	7.7	
79	7.5	
79	7.9	7.7
130	6.8	
130	6.5	
130	7.0	6.8
133	6.9	
133	7.1	
133	6.5	6.8
168	8.0	
168	7.9	
168	7.5	7.8

$\bar{x}=7.3$
s.d.=0.51
95% C.I.=0.41
n=6

Appendix III, Table 2. The carbohydrate content, expressed in µg glucose equiv. $(10^6 \text{ cells})^{-1}$, of Chaetoceros gracilis cultured under various nutrient regimes.

Control			Silicate Limited			Nitrogen Limited		
Culture Date		Culture $\bar{x}$	Culture Date		Culture $\bar{x}$	Culture Date		Culture $\bar{x}$
67	5.7		77	14.2		75	26.6	
67	5.8		77	17.2		75	33.9	
67	9.2		77	23.3	18.2	75	34.0	31.5
67	8.5	7.3	63	14.0		63	38.0	
80	9.6		63	13.2		63	33.9	
80	12.6		63	11.8		63	33.6	
80	14.2	12.1	63	15.4	13.6	63	39.7	36.3
77	6.3		75	22.2		59	19.0	
77	5.8		75	15.3		59	23.9	
77	8.2		75	19.1		59	22.7	
77	6.9	6.8	75	21.3		59	18.0	
77	-		75	23.3	20.2	59	17.4	
75	5.1		80	19.1		59	17.5	
75	4.9		80	15.0		59	36.7	
75	6.9		80	17.0		59	13.9	21.1
75	7.2		80	15.1		80	33.0	
75	6.5		80	16.9		80	34.2	
75	7.7		80	16.6		80	39.1	
75	8.2		80	16.3		80	31.5	
75	7.8	6.8	80	21.0	17.1	80	35.8	

continued...

Appendix III, Table 2. (continued)

Control			Silicate Limited			Nitrogen Limited		
Culture Date		Culture $\bar{x}$	Culture Date		Culture $\bar{x}$	Culture Date		Culture $\bar{x}$
63	3.1		154	13.5		80	28.6	33.7
63	5.7		154	20.5		67	26.9	
63	6.1		154	15.9		67	24.6	
63	7.6		154	11.2		67	28.7	
63	10.4		154	21.2		67	25.7	
63	5.8		154	18.1		67	33.2	27.8
63	6.5		154	15.8	16.6	154	18.4	
63	5.5	6.3	175	9.0		154	24.4	
154	8.8		175	6.7		154	28.6	
154	12.5		175	8.1		154	36.4	
154	7.7		175	8.0		154	26.5	
154	14.8		175	9.4		154	16.4	
154	9.0		175	7.8		154	15.9	23.8
[illegible]4	14.6		175	8.7		168	29.9	
[illegible]4	8.4		175	10.8	8.5	168	28.7	
154	8.4	10.5	178	4.4		168	12.2	
168	8.0		178	5.3		168	14.2	
168	5.7		178	8.9		168	29.7	
168	7.4		178	4.9		168	21.3	
168	12.2		178	4.8		168	12.1	21.2

continued...

Appendix III, Table 2. (continued)

Control			Silicate Limited			Nitrogen Limited		
Culture Date		Culture $\bar{x}$	Culture Date		Culture $\bar{x}$	Culture Date		Culture $\bar{x}$
168	8.5		178	6.0		178	15.9	
168	10.6		178	5.8		178	15.1	
168	7.8		178	4.8	5.6	178	15.7	
168	7.6	8.5	59	7.0		178	16.0	
		$\bar{x}$=8.3	59	10.2		178	17.7	
		s.d.=2.2	59	8.8		178	17.7	
		n=7	59	7.0		178	15.7	
		95% C.I.= 1.6	59	6.4		178	25.9	17.5
			59	6.7	7.7			$\bar{x}$=26.6
					$\bar{x}$=13.5			s.d.= 6.8
					s.d.= 5.5			n= 8
					n= 8			95% C.I.= 4.7
					95% C.I.= 3.8			

Appendix III. Table 3. The carbohydrate content, expressed in µg glucose equiv. $(10^6 \text{ cells})^{-1}$ of *Rhodomonas* sp. cultured with the complete f/2 nutrient media (control) and under nitrogen limited conditions.

Control		Nitrogen Limited	
Culture Date	Carbohydrate µg glucose equiv. $(10^6 \text{ cells})^{-1}$	Culture Date	Carbohydrate µg glucose equiv. $(10^6 \text{ cells})^{-1}$
130	26.5	133	92.2
130	31.5	133	144.2
130	31.8	133	130.0
130	28.9	133	84.7
		133	155.8
		133	173.6
$\bar{x}$	29.7		130.1
s.d.	2.5		35.4
95% C.I.	2.4		28.3
n	4		6

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