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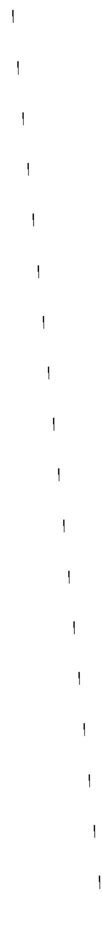

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A STUDY OF NUTRITIONAL REQUIREMENTS OF THE LARVAE OF THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)

The College of William and Mary in Virginia PH.D. 1982

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A STUDY OF NUTRITIONAL REQUIREMENTS OF THE LARVAE OF THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*)

A Dissertation

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Doctor of Philosophy

---

by

Fu-Lin E. Ko Chu

1982

APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Fu-Lin E. Ko Chu

Approved, May 1982

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Ko To, whose love I can always feel around me, though I am separated from them by the big Pacific Ocean.

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PREFACE

This dissertation consists of six chapters. Chapter I is a general introduction and Chapter VI is a general summary and conclusion. Chapters II through V deal with discrete topics considered appropriate for Journal publication. Chapter II concerns the fatty acid composition of five unicellular algal species commonly used as food sources for larvae of the American oyster *Crassostrea virginica*. Parts of Chapter II has been published in *Lipids*. The third chapter describes the polysaccharide composition of five algal species used as food for larvae of *C. virginica*; it has been accepted for publication in *Aquaculture*. The fourth chapter is about the amino acid composition of algal species used as food for the American oyster. The fifth chapter concerns aspects of acceptability and digestibility of microcapsules by larvae of *C. virginica*. It has been accepted for publication in the *Journal of Shellfish Research*.

ACKNOWLEDGEMENT

This work is a result of research supported in part by The Office of Sea Grant, NOAA, Department of Commerce under grants No. 04-8-MOI-85, NA-79AA-D-00055, and NA-80AA-D-00021 to the Virginia Institute of Marine Science and grant No. NA-81AA-00025 to the Virginia Graduate Marine Science Consortium, Sea Grant Program. I wish to express my sincere gratitude to my major professor Dr. Kenneth L. Webb for his patience, understanding and encouragement throughout the course of this work. His discipline in science I will always remember.

I wish to thank my committee members Drs. Maurice P. Lynch, William J. Wardle, Morris H. Roberts and Chih-Wu Su for their suggestions and criticism of this dissertation.

It is a pleasure to acknowledge those in our laboratory who have contributed their time and effort toward the completion of the dissertation. I am grateful to Ms. Beverly B. Casey, Ms. M. Bolus, Ms. Terri Stahl, Mr. Daniel A. Hepworth, Donald H. Abernathy and Mr. Jeffery Thompson for their valuable technical assistance.

I also would like to acknowledge Dr. John L. Dupuy for the valuable ideas which lead to the initiation of this project. The author is grateful to Mrs. Jeanne D. Joseph for her criticism and advice on the lipid work, and for letting me use some of her unpublished lipid data.

Finally, I would like to express my deep appreciation and gratitude to my husband Dr. William P. Chu without whose love, help, moral support and encouragement, this accomplishment would be only a dream.

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ABSTRACT

The purpose of this study is to obtain information for the formulation of artificial diets for larvae of the American oyster (Crassostrea virginica) and to test procedures related to presenting these diets to the oyster larvae. In order to determine the nutrient components usually provided in larval diets, biochemical analyses of lipids and fatty acids, proteins and amino acids and polysaccharide carbohydrates were performed on five algae species used as a food source for oyster larvae. These algae are Chlorella sp., Pyramimonas virginica, Pseudoisochrysis paradoxa, Pavlova (Monochrysis) lutheri and Isochrysis galbana. The latter two species are the traditional diet for oyster larvae, the former three species are the standard (CPP) algal diet currently used in the marine culture laboratory at the Virginia Institute of Marine Science.

The biochemical analyses indicate that the nutritional value of the algal species was not correlated with the total lipid or carbohydrate content of the algae, but higher concentration of total protein within the algal cells appears related to good food quality. Further, the total quantity of fatty acid of the w6 family in an alga may be important to determine the food quality.

The fatty acid patterns of these five algal species were qualitatively similar. The major fatty acid components of the total lipids of the five species were the C12, C14, C16, and C18 saturated fatty acids and the C16 and C18 mono-and polyunsaturated acids. They constituted 63-93% of the total lipids. There were modest amounts of C20 and C22 polyunsaturated acids. The total w6 fatty acids were found to be higher in our standard algal diet (CPP) than in the traditional diet. There were some differences in the proportion of some fatty acid components among the CPP diet species during the culture growth period.

The polysaccharide composition of these five algal species was similar. The principle sugar components in the polysaccharide of these five algal species are glucose, mannose, ribose, xylose, rhamnose and fucose. The major constituent was glucose which accounts for 28 to 86% of the total carbohydrate. Mannose was usually the second most abundant carbohydrate component. Carbohydrate composition was observed to change with age of the algal culture. Both the percentage and the amount of glucose increased with age in all species except Py. virginica.

In addition to the five algae (Py. virginica, Ps. paradoxa, Chlorella sp., Pa. lutheri and I. galbana), the protein and amino acid composition of four other algal species, Nannochloris oculata, Dunaliella tertiolecta, Phaeodactylum tricornutum and Tetraselmis

suecica were also investigated. The latter two algal species are used to condition adult oysters for spawning. The major free amino acid components were alanine, arginine, glutamic acid, lysine, proline, serine and taurine. The principal protein amino acids were alanine, aspartic acid, glutamic acid, glycine, leucine, lysine, proline, serine, threonine and valine. The protein amino acid composition of all the algae was remarkably similar. There were differences in distribution of free amino acid among these algae. Glutamine was found only in Tetraselmis suecica. The food quality appeared related to the concentration of total protein in the algal species studied.

Microcapsule particles have been shown to be suitable for presenting food to filter-feeding animals. In order to find a suitable type of microcapsule to utilize for oyster larval food, two types of microcapsules were tested for acceptability to and digestibility by oyster larvae. Gelatin-acacia and nylon protein capsules were fed to oyster larvae Crassostrea virginica. Larvae were observed to ingest and digest both types of microcapsules. It was found that both types of microcapsules supported growth of larvae. Larvae fed gelatin-acacia microcapsules incorporated with cod liver oil grew as much as larvae fed algae until they were eleven days old. Results also indicated that microcapsule concentration affected growth rate.

A STUDY OF NUTRITIONAL REQUIREMENTS OF THE LARVAE OF THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*)

CHAPTER I

INTRODUCTION

The food requirements of oyster larvae have received considerable attention in recent years (Helm et al., 1973; Holland and Spencer, 1973; Waldock and Nascimento, 1979; Langdon, 1980). Such research is important because hatchery culture may, in the future, play an important role in providing seed requirements of the shellfish industry. Despite the many earlier studies, the nutritional requirements of oyster larvae are still poorly understood. The research effort described in this dissertation is an attempt to provide detailed information on the nutritional requirements of oyster larvae at the biochemical level from which a defined artificial diet can be formulated.

Dissolved organic material, detritus and living phytoplankton are all natural food sources for bivalve larvae and adults. Nevertheless, phytoplankton species are believed to be the major food source which satisfies the basic nutritional requirements for survival, growth and reproduction.

As early as 1900 bivalve biologists reared animals using cultured algae as food. Martin (1927, 1928) demonstrated that oysters are capable of growing on a diet consisting solely of cultured algae. Cole (1937, 1938) first raised bivalve larvae on a diet of small naked flagellates and Bruce (1939) cultured larvae of *Ostrea edulis* on a large scale using an algal diet. Further studies on the use of phytoplankton by *Crassostrea virginica* larvae were carried out by Davis (1950, 1953).

Algae have proven to be the best and most widely used food source for rearing oyster larvae (Ukeles, 1971). The search for alternate (good) algal diets for better and faster growth of oyster larvae under culture has been a subject for investigation for several decades. There are several species of algae which have been successfully isolated, cultured and utilized as food for oyster larvae and spat at various marine laboratories and hatcheries. They include Pyramimonas virginica, Pseudoisochrysis paradoxa, Pavlova (Monochrysis) lutheri, Isochrysis galbana, Dunaliella teriolecta, Cyclotella sp., Skeletonema costatum, Chaetoceros calcitrans, Tetraselmis suecica, Phaeodactylum tricornutum, and several species of Chlorella..

Dean (1957) compared the food value of the diatom, Skeletonema costatum, to that of a mixture of algae dominated by Cryptomonas sp. and Chlamydomonas sp. He found that the mixture was a more valuable diet than Skeletonema alone. Since then there have been a number of investigations on the nutritional value of various specific algal diets for bivalves (Davis and Guillard, 1958; Loosanoff and Davis, 1963; Walne, 1963).

Walne (1970a) evaluated 25 species of algae as food for several juvenile (post-larval) bivalve species. He reported that both different species and concentrations of algae affected growth rates of juvenile bivalves. Further he concluded that the relationship between the quantity of algae fed and larval concentration is critical to the growth and survival of the larvae. The optimal food concentration varies with life history stage of bivalve larvae (Schulte, 1975; Windsor, 1977); thus optimal larval and food concentrations must be ascertained for each

species and stage cultured.

Results from feeding experiments using both larvae and juvenile bivalves indicate that those fed a diet consisting of more than one species of algae usually grow faster and are healthier than those fed only one species. Walne (1974) reported that oyster spat derived from larvae fed on mixed diets grew faster and had lower mortality than those fed single species. Helm (1977) also reported larval growth and spat productivity were significantly greater with a mixed diet. Diets composed of three or four species of algae generally support faster growth of juvenile and adult bivalves than diets consisting of one or two species (Mann and Ryther, 1977; Epifanio and Mootz, 1976; Loosanoff and Murray, 1974; Walne, 1970b). The standard diet for oyster larval culture in our laboratory is a combination of three algal species, *Chlorella* sp., *Py. virginica* and *Ps. paradoxa* (Dupuy et al., 1977; Windsor, 1977).

A mixed algal diet may provide a better balance in biochemical components such as amino acids, fatty acids and sugars as well as micronutrients, e. g. vitamins and minerals, than a single species diet. Thus some nutrient component deficient in one species may be made up by its presence in other species. Such an hypothesis would explain the improved nutritive value reported for mixed algal diets.

While phytoplankton constitute the principal food of bivalve larvae, not all phytoplankton species are equally suitable as food. The species must be digestible, within the acceptable size range for ingestion, nontoxic and contain essential components for growth.

The "good" food value of some algae is thought to be related to

their small size, absence of a thick cell wall and the production of little or no toxic metabolites (Ukeles, 1971; Owen, 1974). Both ingestion and digestion are affected by cell size and cell wall properties. Filtration efficiency of bivalves is affected by the size of food particles (Haven and Morales-Alamo, 1970; Hughes, 1969). The suitable size range of food particles may be different for different bivalve species and for different life history stages of the same species.

The composition and thickness of the cell wall are regarded as two factors affecting digestion. Walne (1974) measured larval growth of O. edulis fed with several species of Chlorella and Coccomyxa and reported that some species of these two genera supported little or no growth. It was suggested that the thick cell walls possessed by these species could not be digested by the larvae. Babinchak and Ukeles (1979) observed uptake, lysis and digestion or rejection of two algae, Pa. lutheri and Chlorella autotrophica by larvae of Crassostrea virginica, with epifluorescence microscopy. They found that larvae fed with C. autotrophica did not grow. Although C. autotrophica was taken up rapidly by the larvae, no lysis or digestion of this alga was observed. The diatom Ph. tricornutum was found to be a poor food for bivalves (Epifanio and Mootz, 1976; Epifanio et al., 1981). Similarly, Ph. tricornutum was found unsatisfactory as a food supplement for ripening adult oysters for spawning (Dupuy, personal communication). Epifanio and his colleagues (Epifanio et al., 1981, 1982) suggested that the nutritional inadequacy of Ph. tricornutum is possibly due to the indigestibility of this diatom or to its lack of tryptophan.

Some chlorophyte species such as Chlorella sp., Chlamydomonas sp., Stichococus sp. and one chrysophyte Prymnesium parvum were found to be toxic to oyster and clam larvae (Davis and Guillard, 1958). The toxicity of these algal species may be due to the production of toxic metabolites, or to the presence of certain toxic species of bacteria in the algal cultures. The poor food value of some species such as some Chlorella sp. has been suggested to be caused by settlement of nonmotile cells which results in reduced availability to the larvae. This hypothesis has been rejected by Babinchak and Ukeles (1979). Their study indicated that the uptake of C. autotrophica and the flagellate P. lutheri by larvae of C. crassostrea was the same. Consequently, they concluded that the poor food value of C. autotrophica was due to cell wall indigestibility rather than nonmotility. Some investigators, however, have reported that a mixed algal diet containing Chlorella sp. could serve as an adequate food for some species of bivalve larvae (Davis, 1953; Dupuy et al., 1977; Windsor, 1977).

In addition to cell wall composition and cell size, differences in food value could be due to different biochemical composition of the algae. Several attempts have been made to correlate the biochemical content (such as protein, carbohydrate and lipid) of the algae to their suitability as a food for herbivores (Parsons et al. 1961; Strickland 1965; Chau et al. 1967; Walne 1970b; Epifanio 1979; Waldock and Nascimento 1979; Langdon and Waldock 1981). Parsons et al. (1961) analyzed the chemical composition of eleven species of marine phytoplankton. They found the organic chemical compositions of these phytoplankton were very similar qualitatively, though there were

quantitative differences among some chemical components. They suggest that the high percentage of glucose in the readily hydrolyzable carbohydrate of Pa. lutheri make this alga nutritionally more important than other species. Strickland (1965) and Chau et al. (1967) also reported that there was little systematic difference in amino acid composition in the algae they studied. Walne (1970a) examined the amino acid composition of 25 algal species, including representatives of "good" foods and "bad" foods for larvae of O. edulis. He found no substantial differences in quantity or quality among them. Walne concluded that the cause of differences in food value of most algal species probably lies in an area other than their amino acid composition. But in a later study of growth rates of juvenile clams Saxidomus giganteus fed with three species of algae (Walne 1973). He speculated that differences in food value might be explained by variation in the relative assimilation of nitrogen from different algal protein.

Epifanio and his colleagues (Flaak and Epifanio 1978; Epifanio 1979; Epifanio et al. 1981) also attempted to correlate the food value of different algal species for post-larval bivalves with the biochemical composition of the algae, both qualitatively and quantitatively. By cultivating Thalassiosira pseudonana under light of different spectral distribution and by harvesting these algal cultures from either exponential or stationary phases they found that oysters grew more rapidly when fed with diets rich in carbohydrates than those rich in proteins (Flaak and Epifanio 1978). Later results involving feeding American oyster C. virginica and hard clams Mercenaria mercenaria with 15 diets composed of various mixtures of four species of algae (Carteria

chui, Isochrysis galbana, Tetraselmis suecica, and Thalassiosira pseudonana) were contradictory. Epifanio and his associates suggested that growth of hard and soft tissues was not correlated with amount of protein, lipid, carbohydrate or amino acid in the diet, but rather to the presence or absence of particular algal species in the diet. Similarly, the nutritive value of Ph. tricornutum and T. pseudonana did not appear related to their gross composition of lipid, carbohydrate and protein when varying proportions of these two diatoms were fed to juvenile oysters of C. virginica (Epifanio et al. 1981).

The results of recent studies on the nutritional status of lipid and fatty acids for the growth of bivalves and bivalve larvae have suggested that the lipid quality, not quantity, is more important in the diet (Waldock and Nascimento 1979, Langdon and Waldock 1981). The growth rate of oyster larvae of C. gigas was found to correlate with the neutral lipid content of the diet. The fatty acid composition of the neutral lipids in the larvae was similar to the fatty acid composition of the algal food; however, the fatty acid composition in the phospholipid fraction was less dependent on the diet composition (Waldock and Nascimento 1979). Langdon and Waldock (1981) also suggested that a deficiency of certain fatty acid components such as 20:5w3 and 22:6w3 in an algal food would limit the growth of C. gigas spat.

A review of the literature reveals that there are no detailed qualitative and quantitative analyses of amino acids, fatty acids and carbohydrates of algal species used as oyster larval food. Walne (1963, 1970a) examined only the protein and amino acid in algal diets of the larvae of O. edulis. On the other hand, Epifanio and his co-workers

(Epifanio and Mootz 1976; Flaak and Epifanio 1978; Epifanio et al. 1981) focused on nutritional studies of post-larval oysters. The algae (Ph. tricornutum and T. pseudonana) they analyzed for amino acid and fatty acid are more suitably used as foods for spat and adult oyster.

Currently, there is no information on the biochemical composition of any mixed algal diet which supports normal larval growth and development. Thus the first objective of this study was to determine the fatty acid, amino acid and carbohydrate content of a "good" algal diet (a combination of Py. virginica, Ps. paradoxa and Chlorella sp.), and the traditional algal (Pa. lutheri and I. galbana) diet for oyster larvae. The former three species of algae currently used as the standard diet in our laboratory produced faster growth, earlier setting and a higher percentage of setting success (Windsor, 1977) than Pa. lutheri and I. galbana. With the information obtained from the present study we might be in a better position to understand the nutritional requirements of oyster larvae which are thought to have more specific dietary requirements than other species of bivalve larvae (Ukeles, 1976). If quantitative and qualitative differences are found among these algal species, it will be possible for us to formulate the criteria for categorizing "good", "moderate", and "poor" food algae. Moreover, since these analyses will provide information on the biochemical constituents of algal diets which support normal growth of oyster larvae, they can serve as the basis for formulation of a complete biochemically balanced artificial diet.

In order to test formulated oyster larval foods for their ability to promote growth and survival, one must present these foods in an

acceptable and digestible form. If nonliving food particles are supplied directly, they are susceptible to leaching, disintegration and bacterial breakdown. One way to avoid these problems is to encapsulate the food particles. Therefore, the second objective of this study was to determine the acceptability and digestibility of gelatin-acacia and nylon-protein microcapsules to larvae of C.virginica. Gelatin-acacia microcapsules proved to be suitable for the presentation of dietary lipids to larvae and spat of C.gigas (Langdon 1980; Langdon and Waldock 1981). Jones and his colleagues (Gabbott et al. 1976; Jones et al. 1974, 1976, 1979a, 1979b) also successfully encapsulated artificial food particles in nylon-protein microcapsules to study the nutritional requirements of crustacean larvae. These capsules also were successfully used as food for both the brine shrimp, Artemia, and the Japanese oyster, C. gigas.

In summary, the purposes of this study were: (1) to understand the nutritional requirements of oyster larvae by analyzing the biochemical constituents of their diets, (2) to assess the acceptability and digestibility of microcapsules by larvae. It is anticipated that with the information obtained from this study, a defined artificial diet can be formulated. If artificial shellfish diets become economically feasible, it will be possible to predict shellfish growth more precisely than at present and the burden of uncertainty of cultivating living food will be eliminated. Further, with this defined artificial diet one can investigate the essential nutrients for oyster larvae.

CHAPTER II

THE FATTY ACID COMPOSITION OF FIVE UNICELLULAR ALGAL SPECIES USED AS FOOD SOURCES FOR LARVAE OF THE AMERICAN OYSTER (*CRASSOSTREA VIRGINICA*)

INTRODUCTION

In the past few decades a number of studies have examined the nutritional values of different phytoplankton species as food for molluscan bivalve adults and larvae (Bruce et al., 1940; Loosanoff and Davis, 1963; Walne, 1970a, 1973; Hartman et al., 1974; Loosanoff and Murray, 1974; Epifanio et al., 1976; Epifanio and Mootz, 1976; Ukeles, 1971; Dupuy, 1973). All these studies indicated that diets composed of three to four species of algae generally support faster growth than diets consisting of only one or two species. In the Virginia Institute of Marine Science (VIMS) marine culture laboratory, a combination of four algal species, *Nannochloris oculata*, *Chlorella* sp., *Pyramimonas virginica* and *Pseudoisochrysis paradoxa* was successfully utilized as a standard diet for oyster larvae culture (Dupuy, 1973, 1975).

Windsor (1977) fed the algal species *Ps. paradoxa*, *Py. virginica*, *Chlorella* sp., and *N. oculata* to oyster larvae (*Crassostrea virginica*) singly and in various combinations. The results were then compared with those for larvae fed with *Pavlova (Monochrysis) lutheri* and *Isochrysis galbana* (PI diet), which is the traditional diet for American oyster larvae. Her study indicated that *Py. virginica* was the most influential dietary component. The best diet, judged in terms of maximum growth

rate, survival, pediveliger production and setting success, was a combination of Ps. paradoxa, Py. virginica, and Chlorella sp. (CPP diet). The best diet was labeled as "good" food while the rest were labeled "mediocre" food.

There have been very few qualitative and quantitative analyses of the major chemical components (protein, lipid and carbohydrates) of algal species used as larval oyster foods. Most nutritional studies of oysters have been performed only on the adult stage (Flaak and Epifanio, 1978; Castell and Trider, 1974; Haven, 1965; Ingole, 1967; Dunathan et al., 1969). Only two papers (Holland and Spencer, 1973; Helm et al., 1973) have dealt with the needs of larvae during development, metamorphosis and early spat growth.

By culturing Thalassiosira pseudonana under light of different spectral distribution and by harvesting this algal culture from either exponential or stationary phases, Flaak and Epifanio (1978) successfully produced 6 diets of differing carbon to nitrogen ratios to feed to oysters. They concluded that oysters grew more rapidly when fed with diets rich in carbohydrates than diets rich in proteins. In adult oysters, polysaccharides have been found to serve as a major energy reserve (Giese, 1969). Castell and Trider (1974) fed oysters with formulated feeds of varying protein-carbohydrate ratios and observed that diets with 60% carbohydrate content gave rise to higher glycogen production in oysters than diets with 20% carbohydrate content, supporting previous oyster nutrition studies (Haven, 1965; Ingole, 1967; Dunathan, et al., 1969).

In view of the results of nutritional studies in both adult and

larval oysters, it seems likely that carbohydrate is important for growth and accumulation of glycogen in juvenile and adult oysters, while lipid could play a vital role in development and metamorphosis in oyster larvae.

This chapter describes the results of analyses for total lipid and fatty acid composition of *Py. virginica, Ps. paradoxa, Chlorella* sp., *Pa. lutheri* and *I. galbana* and provides a comparison to the results of lipid analyses of *Pa. lutheri* and *I. galbana* reported by Ackman et al. (1968), Watanabe and Ackman (1974), Chuecas and Riley (1969) and J. D. Joseph (personal communication). According to Otsuka and Morimura (1966), the fatty acid composition of *Chlorella ellipsoidea* cultures changes with age. Ackman et al. (1964) also observed changes in the relative proportions of fatty acids, including shifts in the biosynthetic pathway for acids belonging to either the linoleic or linolenic systems. Therefore the fatty acid composition of algal cultures of the CPP diet species of different ages was examined. The fatty acid composition of the other two species (PI diet) was determined only in log growth phase (10 to 15 day old algal culture). Algal cells used as larval food are usually reported to be harvested during the log growth phase.

The fatty acid composition of cod liver oil (CLO) was also examined. It has been reported to be rich in polyunsaturated fatty acids, such as 20:5w3, and 22:6w3 (Ackman and Burgher, 1964). CLO is generally available and inexpensive, and thus may be economical as a source of lipid in larval diets if it is similar in lipid composition to the algal diets.

METHODS AND MATERIALS

Since it is not economical to grow axenic mass cultures of algae, all algal species used as food to rear oyster larvae in the marine culture laboratory of VIMS are produced in xenic mass cultures. The algal cultures used for fatty acid analysis were grown under the same conditions as the mass cultures except that the scale was much smaller. The three algal species were cultured at 16 C to 19 C in 3 L fernbach flasks containing 1.5 to 2.0 L pasteurized and filtered estuarine water at 13 to 20 o/oo salinity enriched with N_2M medium (a mixture of Redfield's solutions A and B), Guillard's vitamin mix and a manure extract (Dupuy et al., 1977). _Py_. _virginica_ and _Ps_. _paradoxa_ were grown using a continuous light source of 2000 lux; _Chlorella_ sp. _Pa_. _lutheri_ and _I_. _galbana_ were grown at 3000 lux. A current of air provided circulation and aeration. _Py_. _virginica_ is a flagellated unicellular alga of the class Prasinophyceae, _Ps_. _paradoxa_, _Pa_. _lutheri_ and _I_. _galbana_ belong to the class Haptophyceae, and _Chlorella_ sp. is a representative of the class Chlorophyceae.

Three separate cultures of each species were grown for fatty acid analyses. Algal cells of the CPP diet were harvested by centrifugation and filtration on the 5th, 10th, 15th and 20th days of culture; cells of the PI diet were harvested between the 10th and 15th day of culture. Cell counts of algal samples were made at the time of harvesting. The algal pellets were drained to determine approximate cell wet weight. The Bligh and Dyer (1959) method was employed to extract the total lipids of

the pelleted cells. Lipids were subsequently transesterified with BF_3 in CH_3OH (14% V/V) with additional CH_3OH and benzene (Morrison and Smith, 1964).

Fatty acid methyl esters (FAME) of the algae were chromatographed on a Varian model 3700 gas chromatograph, equipped with dual hydrogen flame ionization detectors and a two-channel Omniscribe strip chart recorder. Both polar and nonpolar columns (8 ft., 4mm I.D. glass), 3% EGSP-Z (100/120 mesh, gas-chrom Q) and 3% SE-30 (gas-chrom Q 100/120 mesh) respectively were used to separate the algal FAME. Following a three minute hold at initial temperature, the columns were temperature programmed from 100 C to 170 C at 8 C/min. The flow rates of nitrogen, compressed air and hydrogen were 40 ml/min, 300 ml/min, and 30 ml/min, respectively.

Tentative identification of FAME were based upon comparisons with chromatograms of commercial fatty acid standards and with the methyl esters of cod liver oil which were used as a secondary reference standard (Ackman and Burgher, 1964), and analyzed under the same chromatographic conditions. Semilog plots of retention time (relative to 18:0) against the carbon chain length were constructed for the algal total lipid FAME and standards to aid identification (Ackman, 1963). Subsequently the method of hydrogenation of Appelqvist (1972) was also performed on the algal FAME mixture to confirm the accuracy of identification and quantification of major components. The chromatograms were quantitated by triangulation. The estimation of the total lipid in wet algal cells was based on the sulphophosphovanillin method (Barnes and Blackstock, 1973).

RESULTS AND DISCUSSION

Cell counts for the CPP diet are given in Table II.1. Algal cultures usually reached log phase in 10 to 15 days. The death phase of Py. virginica came before 15 days. After the 15th day, the number of cell count dropped. Interestingly, changes were observed in the total monoethylenic and polyethylenic acids. The total monoethylenic acid increased while total polyethylenic acid decreased on the 20th day. The results of the estimation of total lipid in terms of micrograms lipid x 10^{-7} per cell and ratio μg lipid to μm3 cell volume are also given in Table II.1. Ps. paradoxa and I. galbana showed a greater amount of total lipid per cell than the other three species. The yields of wet cell weights of algae in all our experiments were inconsistent; therefore, the cellular content of algae was not calculated as lipid per mg wet algae.

The results obtained from the analyses of fatty acid composition of algae of the CPP diet at different culture ages are shown in Table II.2 through II.4. The fatty acid composition of the PI diet species is shown in Table II.5. The major fatty acid constituents in the total lipids were the saturated fatty acids of the even chain lengths C12-C18, and mono and polyunsaturated C16 and C18 acids. These components comprised from 70 to 93% of the total lipids, whereas odd-numbered saturated and other saturated fatty acids accounted for 3 to 18%. There were modest to trace amounts of polyenic acids of the C20 and C22 chain length.

The fatty acid spectra of these five algal species were

Table II.1

Comparison of cell density to age of culture and total lipid content at 15 days for the algal species used as food for oyster larvae.

	CPP diet			PI diet	
	Ps. paradoxa	*Py. virginica*	*Chlorella* sp.	*Pa. lutheri*	*I. galbana*
	(Cell counts per ml x 10^6)				
Days after inoculation					
5	6.1	2.7	14	-	-
10	9.8	11.0	68	-	-
15	18.0	11.0	130	-	-
20	19.0	5.0	190	-	-
μg Lipid x 10^{-7}/cell	22.91	15.22	1.48	59.84	72.36
Mean individual cell volume (μm^3)	47.70	33.50	4.85	57.80	73.50
μg Lipid x $10^{-7}/\mu m^3$ cell volume	0.48	0.45	0.31	1.04	0.98

Table II.2

Fatty Acid Weight Percent Composition of *Pyramimonas virginica*

Fatty acid	Culture day			
	5	10	15	20
Saturated				
12:0	2.40	3.60	3.45	8.70
13:0	1.15	0.60	1.30	1.30
14:0	4.30	2.60	2.00	4.95
15.0	0.86	0.45	0.45	-
16:0	29.10	34.25	31.40	23.30
17:0+16:2	2.30	2.05	1.20	3.75
18:0	4.05	2.80	4.20	3.80
TOTAL	44.16	46.35	44.00	45.80
Monoethylenic				
14:1w5	2.10	1.65	4.15	6.90
16:1	2.60	2.00	2.50	5.75
18:1	3.10	3.10	3.90	15.70
20:1	-	Tr	Tr	Tr
22:1	0.20	0.30	-	-
TOTAL	8.00	7.05	10.55	28.35
Polyethylenic				
16:3w3	8.20	10.30	9.60	5.78
18:2w6	3.35	2.55	2.00	0.80
18:3w3	5.85	7.50	5.85	1.90
18:4w3	4.95	5.10	6.45	3.90
20:2w6	2.65	2.75	2.85	0.70
20:3w3	-	0.20	-	-
20:4w6	0.85	3.35	0.80	8.60
20:4w3	-	-	-	-
20:5w3	1.55	-	2.50	-
22:5w3	[a]Tr	0.90	0.95	0.50
22:6w3	2.60	3.05	2.75	1.40
TOTAL	30.00	35.70	33.75	23.58
Other Saturated				
7:0	3.90	0.85	2.10	2.85
8:0	0.45	-	-	-
10:0	1.10	0.25	0.45	-
11:0	2.50	1.05	0.85	1.20
14:0 iso	1.40	0.65	0.65	2.05
15:0 anteiso	0.80	0.45	0.45	-
16:0 iso	0.30	0.45	0.20	-
18:0 iso	2.70	1.50	1.30	-
TOTAL	13.15	5.20	6.00	6.10
Unknowns total	1.55	1.85	2.50	1.20

[a]Trace (less than 0.04%)

Table II.3

Fatty Acid Weight Percent Composition of Chlorella sp.

Fatty acid	Culture day			
	5	10	15	20
Saturated				
12:0	7.82	3.23	2.75	2.75
13:0	1.08	1.28	1.55	0.15
14:0	5.37	2.48	2.35	0.95
15:0	0.17	Tr	0.70	Tr
16:0	26.68	27.23	27.00	39.45
17:0+6:2	1.10	5.28	3.05	3.60
18:0	3.45	1.37	1.45	2.35
TOTAL	45.67	40.87	38.95	49.25
Monoethylenic				
14:1w5	0.63	Tr	0.30	0.10
16:1	2.70	3.65	5.15	-
18:1	9.18	7.57	7.20	15.00
20:1	0.57	0.30	-	0.95
22:1	Tr	Tr	6.40	-
TOTAL	13.08	11.52	19.05	16.05
Polyethylenic				
16:3w3	2.25	6.47	4.90	3.85
18:2w6	6.98	11.03	7.55	14.20
18:3w6	0.67	-	-	-
18:3w3	12.70	16.65	12.40	10.45
18:4w3	0.20	0.60	1.50	0.40
20:2w6	0.17	Tr	1.00	-
20:3w3	-	-	0.20	-
20:4w6	0.80	0.40	0	-
20:4w3	Tr	Tr	0.85	-
20:5w3	0.17	0.17	0.10	0.50
22:4w3	0.17	-	-	-
22:5w6	-	-	0.35	-
22:5w3	-	Tr	0.25	-
22:6w3	Tr	Tr	0.60	-
TOTAL	24.11	35.32	29.70	29.40
Other Saturated				
7:0	0.77	0.30	1.25	1.80
9:0	0.70	0.20	0.80	0.80
10:0	0.67	-	0.10	Tr
11:0	4.30	2.03	2.30	1.90
14:0 iso	1.37	1.87	1.55	0.15
15:0 anteiso	0.45	-	0.68	Tr
16:0 iso	1.10	Tr	0.70	0.80
18:0 iso	2.27	0.70	0.75	-
Total	11.63	5.10	8.13	5.45
Unknowns Total	3.93	3.28	2.35	1.25

Table II.4

Fatty Acid Weight Percent Composition of *Pseudoisochyrysis pardoxa*

Fatty acid	Culture day			
	5	10	15	20
Saturated				
12:0	4.10	2.80	0.90	2.65
13:0	0.97	0.80	0.45	0.20
14:0	26.00	23.77	23.10	22.10
15:0	0.47	0.47	0.20	0.15
16:0	17.53	18.40	15.65	18.25
17:0+16:2	1.45	0.67	0.25	0.50
18:0	1.40	1.47	0.95	1.10
TOTAL	51.92	48.38	41.50	44.95
Monoethylenic				
14:1w5	1.00	0.63	1.30	Tr
16:1	3.87	4.77	3.65	3.40
18:1	16.90	18.13	18.20	21.25
20:1	0.97	0.63	0.95	0.95
22:1	0.30	0.27	0.20	-
TOTAL	23.04	24.43	24.30	25.60
Polyethylenic				
16:3w3	1.97	1.27	1.05	1.40
18:2w6	3.27	4.23	3.00	3.85
18:3w3	1.90	2.13	2.00	2.20
18:4w3	1.40	1.63	2.20	2.20
20:2w6	0.57	0.73	0.35	0.65
20:3w3	0.30	0.27	-	Tr
20:4w6	0.10	0.17	[a]Tr	Tr
20:4w3	0.07	-	Tr	Tr
20:5w3	0.20	0.40	0.05	0.10
22:5w3	0.47	-	0.35	0.30
22:6w3	1.87	3.10	2.20	2.05
TOTAL	12.21	13.93	11.20	12.75
Other Saturated				
7:0	0.97	-	0.80	1.15
9:0	0.30	-	0.10	0.10
10:0	0.13	-	Tr	0.15
11:0	0.57	0.60	0.15	0.15
14:0 iso	-	-	0.45	0.20
15:0 anteiso	0.17	0.10	0.10	0.10
16:0 iso	0.67	0.33	0.15	Tr
18:0 iso	1.97	1.97	-	0.50
TOTAL	4.78	3.00	1.75	2.35
Unknowns Totals	3.48	7.26	19.85	14.25

[a]Trace (less than 0.04%)

qualitatively similar and the major fatty acids were those found by earlier investigators (Ackman, et al., 1968; Chuecas and Riley, 1969; Kates and Volcani, 1966). In Py. virginica and Chlorella sp., hexadecanoic acid (16:0) was dominant (23 to 39%). This result is similar to the findings of Ackman et al. (1968), Watanabe and Ackman (1974) and Kates and Volcani (1966). The presence of large quantities (from 22 to 26%) of tetradecanoic acid (C14) was characteristic of Ps. paradoxa, I. galbana and Pa. lutheri. Tetradecanoic acid has also been reported as a prominent fatty acid in the Chrysophyceae, Pa. lutheri (Ackman et al., 1968; Watanabe and Ackman, 1974) and I. galbana (Watanabe and Ackman, 19974), but the level was much lower (9 to 11%). The weight percentage of oleic acid (C18:1) was higher in both Ps. paradoxa and I. galbana than in the other species. Among the CPP diet species, Chlorella sp. had the highest proportion of a linolenic acid (18:3w3), reaching 17% of the total fatty acid in the 10th day culture, while gamma-linolenic acid (18:3w6) was found only in Chlorella sp. and then only in the 5th day culture . This suggests that the series of reactions, 18:2w6 $\longrightarrow$ 18:3w6 $\longrightarrow$ 20:4w6 might be active only up to the 5th day in this species. Comparatively higher levels of 22:6w3 were also observed in Pa. lutheri, I. galbana, Py. virginica and Ps. paradoxa . The presence of iso and anteiso fatty acids suggests contamination by bacteria (Kaneda, 1967, 1969) since the algal cultures were not bacteria free.

There were some variations in the proportion of certain fatty acid components during the growth period (Tables II.2 through II.4). Therefore, the culture age of algae may affect the synthesis of certain

fatty acids (Otsuka and Morimura, 1966; Ackman et al., 1964). For example in Chlorella sp. the proportion of 12:0 was highest in 5th day cultures while 16:1 disappeared on 20th day. In contrast, Py. virginica had the highest proportion of 12:0 and 16:1 on the 20th day. The proportion of 18:1 in latter stages (20th day) was highest in all three species of the CPP diet. The effect of age was also observed in the relative increase of total C18 acids in the three species; this effect could possibly be due to the accumulation of reserve fat for cell division (Otsuka and Morimura, 1966). However, in Chlorella sp. the pattern of increase was not linear in the total C18 acids, which showed a decrease on the 15th day and an increase on the 20th day culture.

The fatty acid compositions of the CPP diet, the PI diet and cod liver oil were compared by Ridit analyses (Fleiss, 1981). The statistical analyses indicated that there was no significant difference between the two algal diets ($p = 0.05$). However, there were some quantitative differences in some fatty acid components. For example, the weight percentages of 18:2w6, 18:3w3, 20:2w6 and 20:4w6 in the CPP diet were higher than in the PI diet, whereas the PI diet showed higher percentages of 18:4w3, 20:5w3 and 22:6w3.

The fatty acid compositions of the CPP and PI diets were compared individually to that of cod liver oil by Ridit analysis. Results from these analyses indicated a significant difference between algal diets and cod liver oil ($p = 0.0001$). The concentration of fatty acid components such as 16:1, 18:1, 20:1, 20:5w3 and 22:6w3 in cod liver oil are higher than in either algal diet. The ratio of w6/w3 for cod liver oil and the CPP diet (0.57 and 0.63 respectively) are higher than that

of the PI diet. Eicosapentaenoic acid (20:5w3) and 22:6w3 have been demonstrated to be essential fatty acids for growth of spat of C. gigas (Langdon and Waldock 1981). Jones et al. (1979) have also reported that both 20:5w3 and 22:6w3 may be required by the prawn, Penaeus japonicus, for maximum growth. Although the Ridit analyses indicated that the distributions of fatty acid of cod liver oil and algal diets are different, cod liver oil may prove to be a good fatty acid source for oyster larvae because it is rich in 20:5w3 and 22:6w3, 5 and 9 weight percent of the total respectively.

Results obtained from the fatty acid composition analyses of Pa. lutheri and I. galbana by other investigators (Giese, 1969; Chuecas and Riley 1969; Watanabe and Ackman, 1974; Joseph, personal communication) are also tabulated in Table II.5 and II.7 for comparison. The algal cultures of Pa. lutheri and I. galbana, which were analyzed by Joseph were supplied by our laboratory and were cultured under similar conditions. The results indicated that the amount of linoleic family (w6) fatty acid was higher in the CPP diet than in the PI diet (Tables II.5 and II.7). The amount of w3 fatty acid was lower in Ps. paradoxa, Pa. lutheri and I. galbana, (Table II.7). The amount of total polyethylenic acids of Pa. lutheri reported (Ackman et al., 1968; Chuecas and Riley, 1969) was higher than the present result, this may be due to differences in growth conditions and harvesting times.

In this present study, differences in weight percentages of fatty acids were also observed in different cultures of the same species. The weight percentage of some fatty acid components (e.g. 16:0, 16:1, 18:1, 20:5w3 and 22:6w3) in the present study of the PI diet also showed some

TABLE II.5

Fatty Acid Weight Percent Composition of Pa. lutheri and I. galbana

Algal species	Pa. lutheri				I. galbana		
(References)	(5)	(1)	(3)	(4)	(5)	(4)	(2)
Fatty Acid	Weight percent Composition						
Saturated							
12:0	5.88	0.2	0.6	1.2	5.31	2.9	0.6
13:0	3.20	-	0.2	0.7	1.27	0.7	0.2
14:0	21.36	11.2	9.2	13.8	25.57	9.7	10.6
15:0	-	0.2	0.4	0.9	-	1.2	1.3
16:0	19.27	15.1	10.1	28.9	15.19	21.3	22.0
17:0	1.31	-	0.4	-	0.68	-	-
18:0	2.29	-	0.4	0.8	1.52	2.5	2.3
20:0	0.78	-	-	0.7	0.84	Tr	-
TOTAL	54.09	26.5	21.3	47.0	50.38	38.3	37.0
Monoethylenic							
14:1	-	0.2	-	Tr	-	0.7	-
16:1	12.15	25.4	20.2	21.2	3.54	21.7	15.7
17:1	-	-	-	0.9	-	3.2	-
18:1	8.62	3.4	5.7	7.2	24.64	6.3	13.5
20:1	-	0.6	-	-	-	-	1.2
TOTAL	20.77	29.6	25.9	29.3	28.18	31.9	30.4
Polyethylenic							
16:2w7	-	0.2	2.5	1.3	-	2.0	-
16:2w4	-	1.7	4.6	-	-	-	-
16:3w4	-	0.5	14.8	-	-	-	-
16:3w3	-	0.1	-	-	-	-	0.4
16:4w1	-	0.3	1.5	-	-	-	-
18:2w6	1.83	0.7	1.6	-	3.38	1.3	2.3
18:3w6	0.78	0.1	-	-	Tr	-	0.2
18:3w3	1.63	0.2	-	1.4	3.38	1.4	0.4
18:4w3	3.59	4.0	0.6	1.1	8.02	3.8	8.0
20:2w6	-	0.3	-	0.6	-	-	-
20:3w6	-	0.1	1.7	-	-	0.6	-
20:3w3	-	0.1	-	-	-	-	-
20:4w6	-	0.3	-	0.8	-	0.5	0.1
20:4w3	-	0.1	0.5	-	-	1.2	-
20:5w3	5.88	16.3	18.9	9.9	1.01	12.3	7.2
22.2w6	-	0.5	-	-	-	-	-
22:5w6	-	1.2	-	1.4	-	1.0	-
22:5w3	-	0.4	0.5	-	-	-	-
22:6w3	2.94	13.1	3.3	3.9	3.54	5.7	4.3
TOTAL	16.65	40.2	50.5	19.8	19.33	29.8	32.9
other Saturated							
15:0 Branched	-	-	0.5	-	-	-	-
17:0 Branched	-	-	0.3	-	-	-	1.1
Total of w6	2.61	3.2	3.8	2.8	3.38	3.4	2.6
Total of w3	14.04	34.3	23.3	16.3	15.95	24.4	20.3
w6/w3	0.19	0.09	0.16	0.17	0.21	0.14	0.13

References 1) Ackman et al., 1968; 2) Watanabe and Ackman, 1974; 3) Chuecas and Riley, 1969; 4) J. D. Joseph, Personal communication; (5) This study.

TABLE II.6

Fatty acid weight percent composition of prominent or potentially interesting fatty acids of the CPP and PI diets and cod liver oil (CLO).

diet	CPP*	PI**	CLO
Fatty Acid	Weight % composition		
Saturated			
12:0	3.71	5.60	-
14:0	11.0	23.47	4.73
16:0	25.07	17.23	15.73
18:0	2.38	1.91	2.73
20:0	-	0.81	-
TOTAL	42.16	49.02	23.19
Monoethylenic			
16:1	3.40	7.85	10.93
18:1	11.91	16.63	26.54
20:1	0.48	-	12.88
22:1	1.75	-	-
TOTAL	17.54	24.48	50.35
Polyethylenic			
18:2w6	4.66	2.61	1.41
18:3w6	0.17	0.39	-
18:3w3	5.99	2.51	0.73
18:4w3	2.77	5.81	1.48
20:2w6	1.20	-	0.21
20:4w6	1.46	Tr	0.38
20:4w3	0.21	Tr	0.54
20:5w3	0.88	3.45	5.32
22.4w6	-	-	6.59
22:5w3	0.49	-	0.80
22:6w3	1.94	3.24	6.24
TOTAL	19.77	17.99	23.70
Total of w6	7.49	3.00	8.59
Total of w3	12.28	15.00	15.11
w6/w3	0.61	0.20	0.57
20:5w3 + 22:6w3	2.82	6.69	11.56

* lipid is 37% from P. virginica, 38% from P. paradoxa and 25% from Chlorella sp.
** lipid is 50% from each species.

Table II.7

Comparison of Total Weight percentage w6 and w3 Fatty Acid Families in Algae,

Ps. pardoxa, Py. virginica, Chlorella sp., *Pa. lutheri* and *I. galbana*

	Algal Species												
	Ps. paradoxa		*Py. virginica*		*Chlorella*		*Pa. lutheri*		*I. galbana*				
Fatty Acids	10th	15th	10th	15th	10th	15th	(5)[b]	(1)	(5)	(3)	(4)	(2)	(4)
Total of w6	5.13	3.45	8.65	5.65	11.48	8.90	2.61	3.20	3.38	3.80	2.80	1.10	3.40
Total of w3	8.80	7.95	27.05	25.35	23.89	20.80	14.04	34.30	15.95	23.30	16.30	20.30	24.40
w6/w3	0.58	0.43	0.32	0.22	0.48	0.43	0.19	0.09	0.21	0.16	0.17	0.13	0.13

[a] 10th, 15th days algal cultures

[b] references 1) Giese, 1969; 2) Ackman et al., 1968; 3) Chuecas and Riley, 1969; 4) J. D. Joseph, Personal communication; 5) This study.

difference from results reported by J. D. Joseph (personal communication). Many factors could have caused these differences even though cultures were grown under substantially the same conditions. Both total concentration of salts (i. e. salinity) and, to a certain degree, the ionic ratios varies, depending upon weather conditions and season. Similarly, the concentration of various chemicals in soil and manure extracts probably varied from location to location. Several investigators (Schlenk et al., 1960; Williams and McMillan, 1961; Klenk et al., 1963; Hulanicka et al., 1964; Erwin et al., 1964) reported that the fatty acid composition of algae may be altered by variables such as the concentration of available nitrate, etc.

Fatty acids of the w6 and w3 families have been shown to be essential in many animals. Some mammalian species such as the rat have a high requirement for w6 fatty acids and a low requirement for w3 fatty acids (Tinoco et al., 1978). For the rainbow trout the situation is reversed with w3 fatty acids being most important (Yu and Sinnhuber, 1972; Watanabe et al., 1974), However, German carp appear to require both w6 and w3 in the diet (Watanabe et al., 1975). Similar requirements could probably exist for the growth and metamorphosis of oyster larvae from free swimming form to settled spat. The high weight percentage of w6 in the CPP diet (Tables II.6 and II.7) strongly suggests that w6 fatty acids, in addition to w3 (Langdon and Waldock, 1981) are important for the growth and development of oyster larvae. It is interesting to note that for the adult C. virginica the ratio w6/w3 calculated from the data of Watanabe and Ackman (1974) is low (0.16). It is possible that the nutritional requirements for the growth of adult oysters and oyster

larvae are not the same. The chemical components which are considered to be essential for adult oysters might not be as important for the larvae. For example, the results of previous nutritional studies of the adult oyster (Castell and Trider, 1974; Haven, 1965; Ingole, 1967; Dunathan et al., 1969) showed that carbohydrate was important for the growth of oysters and was the major energy reserve. Flaak and Epifanio (1978) reported that _T. pseudonana_ cells with relatively high carbohydrate content have greater nutritional value for oysters and that the total protein requirement of oysters is relatively low. However, studies on biochemical changes in _O. edulis_ larvae by Holland and Spencer (1973) indicated that developing larvae accumulated neutral lipid which increased from 8.8% of the total organic matter (dry weight basis) in newly released larvae to 23.2% at metamorphosis. During metamorphosis neutral lipid decreased to 9.6% of the total organic matter and then remained constant up to 25 days after settlement. Additional studies on _O. edulis_ larvae by other investigators showed that lipid was not only the major energy reserve for growth but also during starvation periods (Helm et al., 1973; Millar and Scott, 1967), and that viability of larvae was not related to initial glycogen content (Collyer, 1957).

The present study has shown that the fatty acid composition is similar for the five algal species utilized as food for oyster larvae but there are some differences in the proportion of certain components. In addition, the proportion of total polyethylenic acids in _Ps. paradoxa_ is low. When comparing the total lipid and total protein of individual algal cells, _Pa. lutheri_ and _I. galbana_ had higher levels of total lipid per cell whereas _Py. virginica_ had more total protein per cell (Chapter

IV). Variations in relative proportions of certain amino acid components were also observed among these three algal species (Chapter IV). These results suggest that a diet consisting of several species of algae is required by oyster larvae to obtain an optimal balance of all chemical components during development and metamorphosis. Further, there are quantitative differences among certain fatty acids that are found in both the CPP diet and the PI diet which indicates a possible role of lipid in explaining the difference between "good" and "mediocre" algae as food for oysters.

CHAPTER III

POLYSACCHARIDE COMPOSITION OF FIVE ALGAL SPECIES USED AS FOOD FOR LARVAE OF THE AMERICAN OYSTER, CRASSOSTREA VIRGINICA

INTRODUCTION

In the past few decades, a number of studies (Davis and Guillard, 1958; Walne 1963, 1965, 1970a, 1970b; Helm, 1977) have been performed to examine the nutritional value of different phytoplankton species as food for larvae of bivalve molluscs. In most of these studies the food value of phytoplankters was judged by larval growth rate, percentage survival, pediveliger production, and setting success. It was suggested that various phytoplankters are not equal in nutritive value and that an algal diet composed of two or three species provides a better food source than any individual species.

At the Virginia Institute of Marine Science (VIMS), an algal diet (Chlorella sp., Py. virginica, and Ps. paradoxa) has been developed which is used successfully as food for oyster larvae. With this food one can optimize growth and reduce the time to setting for oyster larvae to 8-10 days (Dupuy et al., 1977). The traditional algal diet of Pa. lutheri and I. galbana (Davis and Guillard, 1958; Loosanoff and Davis 1963; Walne 1963, 1965, 1970a, 1970b; Ukeles, 1971) results in setting of larvae in 13 to 15 days. Py. virginica is a flagellated unicellular alga of the class Prasinophyceae, Ps. paradoxa, Pa. lutheri and I. galbana are members of class Haptophyceae, and Chlorella sp. belongs to the class Chlorophyceae.

Several attempts have been made to correlate the biochemical contents (such as protein, carbohydrate and lipids) of algae to suitability as food for herbivores (Parsons et al., 1961; Walne, 1970a; Epifanio, 1979). In each case the chemical compositions of the algal species were qualitatively similar, although there were some quantitative differences in certain chemical components. A more detailed analysis performed in this laboratory produced similar results (Chapters II, IV) showing differences in the quantity of some amino acids in the free amino acid fraction, total proteins, fatty acids and total fatty acids. In this chapter the results of analyses of carbohydrates (neutral sugar constituents) in the five algal species, *Py. virginica*, *Ps. paradoxa*, *Chlorella* sp., *Pa. lutheri*, and *I. galbana*, are reported.

METHODS AND MATERIALS

Algal culture

The five algal species were cultured at 16-19°C in 3 L fernback flasks containing 1.5-2.0 L of pasteurized and filtered estuarine water supplemented with N_2M medium, Guillard's vitamin mix and soil extract (Dupuy et al., 1977). The salinity range of the water was 13-23 parts per thousand during the period of the experiments. The algal species except _Py. virginica_ were grown under continuous illumination from one warm white and one gro-lux fluorescent lamps. _Py. virginica_ was illuminated under a light source of two gro-lux fluorescent lamps. Aeration provided circulation in the cultures.

Three separate batches of each species were cultured for carbohydrate analyses. The cultures were not bacteria free. Algal cells were harvested by centrifugation and filtration on the 4th, 10th, 15th and 21st days of culture. Cell counts of algae samples were made only at the time of harvesting. The algal pellets were drained to determine cell wet weight.

Analyses of carbohydrates

Figure III.1 shows a schematic representation of the sequence followed in the analyses of carbohydrates in polysaccharides of the algal culture sample.

The procedure of methanolysis described by Nozawa et al. (1969) was employed for the hydrolysis of the ethanol-insoluble materials. Before

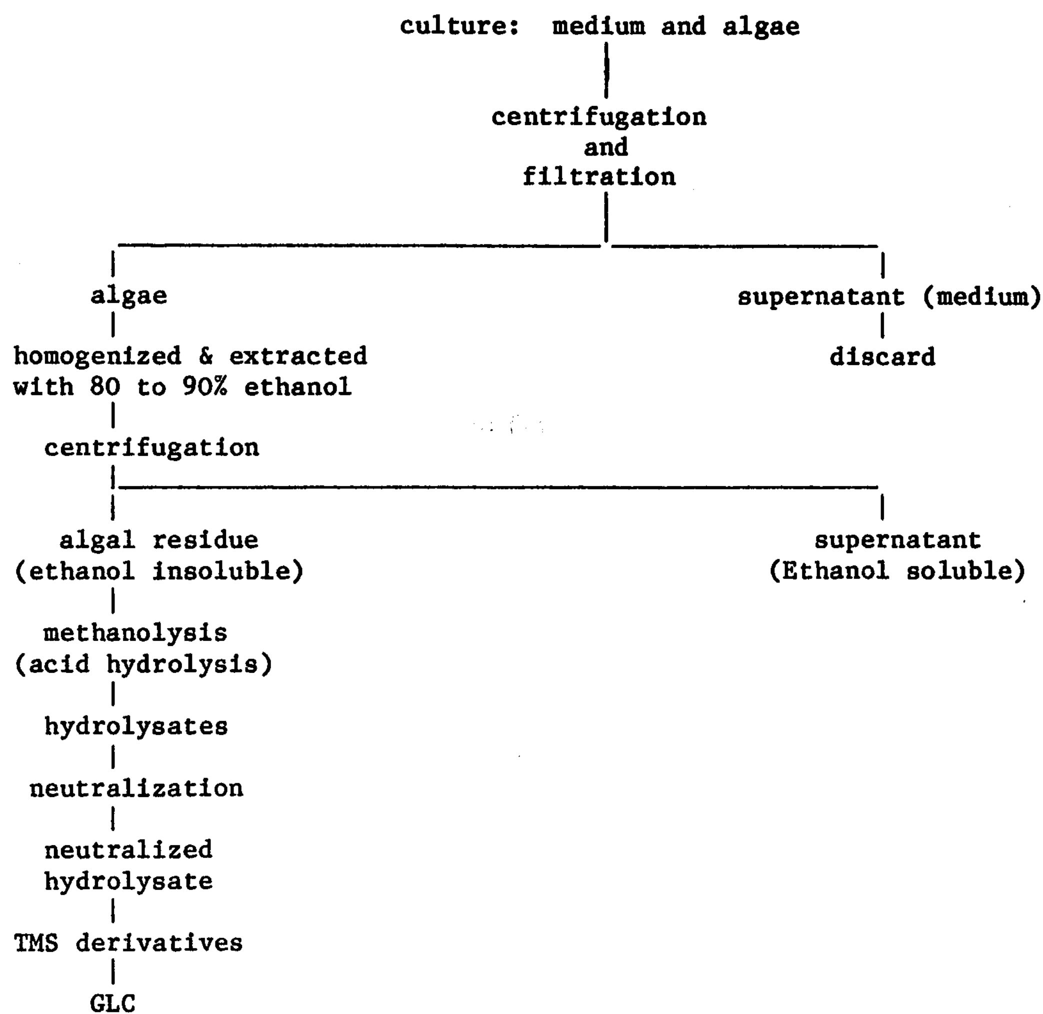


Figure III.1. Summary of fractionation and analysis procedure for carbohydrate analysis.

methanolysis, the algal polysaccharides of the ethanol-insoluble fraction (the colorless algal residue after extraction of soluble sugar and polyols with 80% hot ethanol) were dried and kept in a vacuum desiccator over KOH.

The optimal hydrolytic conditions were determined by hydrolyzing samples of algal polysaccharides in 0.3, 0.5, 1, and 2 N methanolic hydrochloric acid for 3, 5, 8, and 16 hrs. These studies showed that the best conditions for all constituent sugars to achieve maximal release and minimal destruction were 0.3 N methanolic hydrochloric acid for 5 hrs. Both algal polysaccharide and sugar standards were hydrolyzed under these conditions. Before hydrolysis 2 or 5 mg of myo-inositol were added as an internal standard to the standard sugar and algal polysaccharide, respectively. The hydrolysates were neutralized by passage through a small column containing AG1-X8, acetate form, 100-200 mesh. The effluent collected from this column was introduced into another small column containing AG 50W-X8, hydrogen form, 100-200 mesh, to trap the amino acids and amino sugars. The effluent from this column was then dried under a stream of nitrogen and stored in a vacuum desiccator over KOH.

The method of Sweeley et al. (1963) was used for the trimethylsilylation of sugar standards, and hydrolysate of algal polysaccharides. Each dried sample was redissolved in 1 ml of pyridine, allowed to come to mutarotation equilibrium at room temperature for 2 hours, and then 200 µl of hexamethyldisilazane (HMDS) and 100 µl trimethylchlorosilane (TMCS) were added. The reaction mixture was shaken vigorously for about 1 minute. The sample was allowed to stand

overnight at room temperature in order for equilibrium to be attained. After centrifugation, aliquots of each reaction mixture were injected into the gas chromatograph.

Gas chromatography was performed on a Varian model 3700 equipped with dual hydrogen flame detectors and a two-channel Omniscribe strip chart recorder. The chromatographic columns were 6 foot, 4 mm i.d. glass columns.

Two columns were used to separate the algal TMS derivatives; the first was packed with 3% SE-30 on 80/100 mesh, acid washed, silanized chromosorb W; the second with 3% OV-225 on 80/100 mesh, gas-chrom Q. The first column was temperature-programmed from 120-230°C at 4°C/min. The second column was temperature-programmed from 140-270°C at 8°C/min. The flow rates of nitrogen, compressed air and hydrogen were 40 ml/min, 300 ml/min, and 33 ml/min, respectively.

Tentative identification of algal TMS derivatives was based on comparisons with chromatograms of commercial standard monosaccharide TMS derivatives analyzed under the same chromatographic conditions.

Quantification of chromatograms was based on the methods employed by Nozawa et al. (1969), Sawardeker and Sloneker (1965), and Reid et al. (1970).

Prior to the analysis of algal monosaccharide TMS derivatives, detector response factors (K) of the standard sugars relative to an internal standard (myo-inositol) were determined. Each sugar standard and a mixture of sugars was hydrolyzed, chromatographed and trimethylsilyation was performed under the conditions described previously for the algal polysaccharides.

RESULTS

Based on cell counts for the five algal species (Table III.1), the log growth phases for both Py. virginica and Ps. paradoxa were from 4th to 10th days. The log phase for Chlorella sp. ranged from the 4th to 15th days, while log phases of Pa. lutheri and I. galbana ranged from the 4th to 20th days or beyond. The relative size of these five algae as determined by a Coulter particle counter, ranged from 4.85 μm^3 for Chlorella to 73.5 μm^3 for Pa. lutheri (Table III.2).

The results obtained from the analyses of algal polysaccharides in the ethanol-insoluble fraction at different ages of algal cultures are shown in Tables III.3 to III.7. All values are means calculated from three experiments.

The sugars found in the polysaccharides of these five algal species were glucose, mannose, ribose/xylose, fucose, fructose, and rhamnose. Three sugar alcohols were found; glycerol, ribitol, and xylitol. Glucose was the major constituent and accounted for 28 to 86% of total carbohydrates. Mannose was an important component, while fucose, ribose/xylose, fructose, and glycerol were minor components.

Changes in carbohydrate composition with age of the algal cultures were observed. Both the percentage and amount of glucose increased with age in each species except Py. virginica. There was a marked increase in the percentage of glucose from day 4 to day 10 in Ps. paradoxa, Chlorella sp., Pa. lutheri, and I. galbana, and a less striking increase in Py. virginica. In contrast, the amounts of other sugar components declined as the algal cultures grew older. The percentage of mannose

Table III.1

Cell Counts of Five Algal Species

	Algal Species				
	Py. virginica	*Ps. paradoxa*	*Chlorella* sp.	*Pa. lutheri*	*I. galbana*
Day after inoculation	(Cell count per ml x 10^6)				
4th					
Experiment A	3.05	4.65	14.13	3.68	3.60
Experiment B	2.79	3.40	70.00	1.00	-
Experiment C	2.85	8.80	11.00	-	4.10
Experiment D	2.70	-	-	-	-
10th					
Experiment A	19.38	29.13	160.50	13.88	10.78
Experiment B	16.91	4.80	94.00	16.00	4.30
Experiment C	5.50	30.00	150.00	-	18.00
Experiment D	14.00	-	-	-	-
15th					
Experiment A	6.38	24.13	115.00	15.25	21.75
Experiment B	8.50	5.90	100.00	18.00	5.00
Experiment C	6.05	27.00	342.00	-	20.00
Experiment D	3.50	-	-	-	-
21st					
Experiment A	-	22.88	143.00	17.63	24.75
Experiment B	-	5.50	105.00	20.00	31.00
Experiment C	6.00	-	145.00	-	-
Experiment D	-	-	-	-	-

Table III.2

Comparison of age of culture to total carbohydrate content in ethanol-insoluble fraction for the algal species used as food for the larvae of *Crassostrea virginica*.

	Algal Species				
	Py. virginica	*Ps. paradoxa*	*Chlorella* sp.	*Pa. lutheri*	*I. galbana*
Day after inoculation					
4th					
$\mu g x 10^{-9}$/cell	2.28 ± 0.05	1.42 ± 0.80	0.34 ± 0.16	3.04 ± 0.63	1.53 ± 0.81
$\mu g x 10^{-6}$/µg WA*	4.07 ± 0.04	7.43 ± 4.12	1.60 ± 0.59	10.68 ± 2.19	4.50 ± 0.15
10th					
$\mu g x 10^{-9}$/cell	3.29 ± 1.19	1.05 ± 0.25	0.82 ± 0.15	3.00 ± 1.02	11.22 ± 3.07
$\mu g x 10^{-6}$/µg WA	12.81 ± 4.28	12.78 ± 3.90	27.24 ± 4.07	19.01 ± 3.76	128.57 ± 35.18
15th					
$\mu g x 10^{-9}$/cell	3.33 ± 1.57	3.10 ± 1.40	0.70 ± 0.41	5.08 ± 1.68	6.36 ± 1.80
$\mu g x 10^{-6}$/µgWA	8.99 ± 1.39	30.37 ± 19.00	36.78 ± 8.71	19.05 ± 3.95	63.85 ± 25.40
21st					
$\mu g x 10^{-9}$/cell	11.20 ± 8.60	4.24	1.00 ± 0.11	13.19 ± 1.10	17.80 ± 2.68
$\mu g x 10^{-6}$/µg WA	53.00 ± 44.54	32.06	32.80 ± 5.45	58.08 ± 14.96	50.60 ± 3.72
cell volume μm^3	33.50	47.70	4.85	73.50	57.80

* WA = wet algae

TABLE III.3

The Carbohydrate wt % Composition of Polysaccharide of

Pseudoisochrysis paradoxa

	Days After Inoculation			
Sugars	4th	10th	15th	21th
Glycerol	trace*	0.03 ± 0.01	1.65 ± 0.48	1.35
Rhamnose	7.29 ± 2.06	3.81 ± 0.73	3.91 ± 1.33	2.5 ± 1.88
Ribose/Xylose	7.61 ± 0.93	3.62 ± 1.29	3.41 ± 0.20	2.45 ± 2.10
Fucose	7.84 ± 1.12	4.46 ± 0.99	3.74 ± 0.66	3.61 ± 0.15
Fructose	-	0.88 ± 0.28	1.74 ± 0.96	0.82
Ribitol/Xylitol	7.24 ± 1.97	5.42 ± 1.49	4.24 ± 0.35	5.9 ± 1.48
Mannose	41.86 ± 3.23	27.88 ± 4.61	20.76 ± 3.38	30.25 ± 11.31
Glucose	28.15 ± 8.30	53.90 ± 5.36	60.12 ± 6.21	53.53 ± 3.20
Sucrose	-	-	0.89 ± 0.17	-

* Trace (less than 0.03%)

TABLE III.4

The Carbohydrate wt % Composition of Polysaccharide of

Pyramimonas virginica

	Days After Inoculation			
Sugars	4th	10th	15th	21th
Glycerol	trace*	trace	trace	trace
Rhamnose	2.84 ± 0.46	1.10 ± 0.83	trace	1.54
Ribose/Xylose	1.67 ± 0.87	1.16 ± 0.40	1.04 ± 0.15	1.34
Fucose	5.41 ± 3.46	1.13 ± 0.47	0.99 ± 0.55	1.02
Mannose	14.63 ± 1.65	8.06 ± 2.72	8.93 ± 0.74	12.56
Glucose	73.51 ± 1.24	82.59 ± 5.45	83.07 ± 3.95	82.29
Mannitol	trace	3.78 ± 1.38	3.5 ± 1.75	0.52
Galactose	-	2.84 ± 0.89	3.21	0.52

* Trace (less than 0.03%)

TABLE III.5

The Carbohydrate wt % Composition of Polysaccharide of

Chlorella sp.

Sugars	Days After Inoculation			
	4th	10th	15th	21th
Glycerol	trace*	trace	trace	trace
Rhamnose	3.00 ± 0.70	0.84 ± 0.37	0.94 ± 0.02	0.94 ± 0.21
Ribose/Xylose	10.93 ± 1.60	3.82 ± 0.35	2.62 ± 0.69	2.64 ± 0.64
Fucose	12.97 ± 1.87	3.21 ± 0.97	2.76 ± 0.65	2.72 ± 0.57
Fructose	4.80 ± 2.85	1.58 ± 0.55	1.29 ± 0.26	1.30 ±0.33
Ribitol/Xylitol	9.81 ± 1.55	2.63 ± 0.59	2.51 ± 0.25	1.78 ± 0.11
Mannose	24.46 ± 10.70	17.26 ± 4.50	16.37 ± 2.55	15.19 ± 1.90
Glucose	34.17 ± 11.92	71.21 ± 7.77	73.50 ± 4.21	75.10 ± 3.25

* Trace (less than 0.03%)

TABLE III.6

The Carbohydrate wt % Composition of Polysaccharide of

Pavlova lutheri

Sugars	Days After Inoculation			
	4th	10th	15th	21th
Glycerol	trace*	0.33 ± 0.25	2.60 ± 0.71	4.09 ± 1.47
Rhamnose	2.44 ± 0.43	1.74 ± 0.74	0.88 ± 0.69	0.59 ± 0.32
Xylose/Ribose	8.85 ± 1.91	6.52 ± 2.33	3.22 ± 1.21	2.49 ± 0.94
Fucose	6.17 ± 1.32	4.09 ± 2.17	2.71 ± 1.16	2.28 ± 0.56
Fructose	-	-	0.60 ± 0.14	trace
Ribitol/Xylitol	3.11 ± 1.43	2.23 ± 1.06	1.85 ± 1.06	1.13 ± 0.15
Mannose	21.31 ± 5.57	16.85 ± 6.08	12.33 ± 3.70	10.30 ± 0.84
Glucose	45.39 ± 16.29	67.76 ± 12.86	76.45 ± 7.81	78.99 ± 3.96

* Trace (less than 0.03%)

TABLE III.7

The Carbohydrate wt % Composition of Polysaccharide of

Isochrysis galbana

Sugars	Days After Inoculation			
	4th	10th	15th	21th
Glycerol	trace*	0.45 ± 0.07	0.43 ± 0.16	0.20 ± 0.04
Rhamnose	2.60 ± 0.87	0.86 ± 0.19	1.37 ± 0.10	0.71 ± 0.03
Xylose/Ribose	2.84 ± 0.89	1.20 ± 0.28	1.71 ± 0.48	1.34 ± 0.06
Fucose	5.16 ± 1.43	1.40 ± 0.28	2.02 ± 0.66	1.32 ± 0.17
Fructose	-	trace	-	-
Ribitol/Xylitol	-	0.86 ± 0.01	1.37 ± 0.42	0.96 ± 0.13
Mannose	25.34 ± 1.90	7.98 ± 1.28	11.91 ± 1.34	10.24 ± 0.79
Glucose	60.80 ± 0.29	85.98 ± 2.23	79.71 ± 3.55	83.09 ± 0.88
Mannitol	-	-	-	1.35 ± 0.25
Galactose	-	-	-	0.59 ± 0.12

* Trace (less than 0.03%)

dramatically declined in Pa. lutheri, I. galbana, Chlorella sp., and Ps. paradoxa. Glycerol usually first appeared in the algae in older stages. It is also interesting to note that in most of the algal species the total carbohydrate content increased with age of culture (Table III.2). Chlorella sp. is poor in total monosaccharides/cell at all times but comparable to Ps. paradoxa. After day 15, the total monosaccharides/cell in the other species increased dramatically.

The pattern of carbohydrate composition was found to be similar in the five species of algae studied in this investigation. However, some sugars occur in only a few species. Glycerol, rhamnose, ribose/xylose, fucose, ribitol/xylitol, mannose and glucose occur in all five species; mannitol and galactose were detectable only in Py. virginica and I. galbana; sucrose was observed only in Ps. paradoxa; fructose was not detected in P, virginica. The retention time of xylitol and ribitol, and ribose and xylose were the same.

DISCUSSION

The present findings are similar to the results reported by other investigators (Parsons, et al., 1961; Handa and Yanagi, 1969; Allan et al., 1972). Parsons and his colleagues (1961) reported that glucose, galactose, ribose, xylose, rhamnose, and mannose were the principle monosaccharides in eleven species of marine phytoplankton. Glucose was the principle sugar. Galactose was the next most abundant carbohydrate constituent; mannose was found in some species only in small quantity. In contrast, Handa and Yanagi (1969) found that glucose and mannose were the major components in 3 diatom species, Chaetoceros sp., Phaeodactylum tricornutum, and Skeletonema costatum, and in particulate matter from the North Pacific. Arabinose was the only sugar Handa and Yanagi (1969) found which we did not. They extracted first with ethanol and then with hot water. The percentage of the total extracted carbohydrate was 3.5-6.7, 40-56, and 37-56% for the ethanol, hot water, and the residue, respectively. The polysaccharide analyses we report are comparable to the hot water and residue from Handa and Yanagi's experiment. Allan et al., (1972) found that the polymers of the hot water extractable fraction were composed principally of glucose in 5 diatom species.

The increase of glucose and total carbohydrate concentration (with age of culture, Table III.2, 6 and 7) may be due to the change from production of growth metabolites to storage products. The polysaccharide glucan is considered to be the most common food reserve material in algae (Boney, 1966, pp. 14-18; Handa and Yanagi, 1969). A water-extractable beta-glucan, laminarin, or leucosin appeared to be the main

food reserve in Phaeophyceae and Chrysophyceae, whereas a water extractable alpha-glucan, floridean starch has been found to be the food reserve of Rhodophyceae. Perhaps glucan is the major food reserve in the 5 species investigated based on the predominance of glucose.

Handa and Yanagi (1969) noted that the maximum carbohydrate concentration was usually attained in the late stages of cultures of Skeletonema costatum. The protein composition of the algae was almost constant during growth while accumulation of carbohydrate and lipid was observed only in the stationary phase. The decline of mannose and other minor components during the log growth phase and stationary phase of culture could be explained by the conversion of these materials into storage glucan and an increase of photosynthetic activity. Parsons and his coworkers (1961) found no mannose in some algae and only very small amounts (0.41-3.7%) in other species. This difference in composition could be due to differences in growth stage of the cultures and analytical techniques.

The pattern of carbohydrate composition was found to be similar in the five species of algae studied in the present investigation. No major difference, qualitative or quantitative, in carbohydrate composition was found. Other investigators (Parsons et al. 1961; Strickland, 1965; Chau et al., 1967; Walne, 1970a) also noted the similarity of chemical composition of marine phytoplankters grown under the same physical and chemical conditions, although quantitative variations did exist in some components. For example, the amount of glucose found in the eleven species of marine phytoplankton studied by Parsons and his associates (1961) varied. Similarly, the amount of total

monosaccharide per cell and the weight percentage of sugar components in the five algae we analyzed were not the same. Moreover, fructose was found in only four of the five algae we studied. Parsons and his associates (1961) suggested that the higher percentage of glucose in the readily hydrolyzable carbohydrate of Pa. lutheri make this alga more nutritionally important, but the present results do not support this interpretation in reference to oyster larval nutrition. The total amount of sugar present in the algae was not correlated with the quality of the algae as food. The traditional diet (Pa. lutheri and I. galbana) have higher concentrations of sugar per cell than the three species mixture (Py. virginica, Ps. paradoxa and Chlorella sp.) currently used as the standard diet in our laboratory. These latter three species of algae produced faster growth, earlier setting and a higher percentage of setting success (Windsor, 1977) than Pa. lutheri and I. galbana. In our analyses, the weight percentages of glucose in Py. virginica and Chlorella sp. were similar to those of Pa. lutheri and I. galbana. It is possible that carbohydrates which have high nutritional value for juvenile and adult oysters (Flaak and Epifanio, 1978; Castell and Trider, 1974; Haven, 1965; Ingole, 1967; Dunathan et al., 1969) may not be as important as lipid in determining food quality of algae for the oyster larvae (Millar and Scott, 1967; Helm et al., 1973; Holland and Spencer, 1973; Holland, 1978; Chapter II).

Results of the present analyses of protein (Chapter IV), lipid (Chapter II) and carbohydrate show little correlation between the nutritional value of an algal species and its biochemical composition. Epifanio (1979) reported that the growth of hard and soft tissues in

juvenile American oysters (C. virginica) and hard clams, (Mercenaria mercenaria) was not correlated with the amount of protein, lipid, carbohydrates, or amino acids in the diets. Instead it was related to the presence or absence of particular algal species in the diet. Therefore it is possible that once the biochemical balance of protein, lipid, and carbohydrate has been achieved by the combination of several species of algae in a diet, other chemical components, and trace nutrients such as vitamins and minerals are then present in adequate amounts to promote growth.

It is generally accepted that the nutritional value of different phytoplankters is not the same. In addition to the biochemical composition of the algal cells, other factors such as the digestibility of the cell wall and cell size must be taken into account when evaluating the suitability of specific species for food.

CHAPTER IV

THE AMINO ACID COMPOSITION OF ALGAL SPECIES USED AS FOOD FOR THE AMERICAN OYSTER (CRASSOSTREA VIRGINICA)

INTRODUCTION

Research prior to 1971 (Davis, 1953; Davis and Guillard, 1958; Walne, 1965, 1970b; Ukeles, 1971) led to the use of algae as food for rearing oyster larvae with a mixture of Pa. lutheri and I. galbana becoming the traditionally accepted diet. In the past decade a number of studies have evaluated the suitability of other phytoplankton species as food for juvenile and adult bivalve molluscs (Mann and Ryther 1977; Epifanio et al. 1976; Loosanoff and Murray 1974; and Walne 1970a). These latter experiments have indicated that diets composed of three or four species of algae generally support faster growth than diets consisting of one or two species.

The diet used to culture larvae of C. virginica at The Virginia Institute of Marine Science (VIMS) was the traditionally used algae, I. galbana and Pa. lutheri, either singly or in combination, in the early 1970's (Dupuy, 1975). This diet was unsatisfactory for a number of reasons including slow larval growth and inconsistent setting success. This early diet was replaced with a sequential feeding protocol utilizing mixtures of Nannochloris oculata, and Py. virginica for larvae to 150 µm. Above 140 µm Ps. paradoxa was added and for larvae from 250 µm to pediveliger, N. oculata was omitted and Chrysophaeropsis planktonicus was added if available. This sequential protocol produced a

30% yield of "eyed" larvae within 11 to 14 days (Dupuy 1973, 1975). Windsor (1977) fed algal species *N. oculata, Ps. paradoxa, Py. virginica* and *Chlorella* sp. singly and in various combinations to oyster larvae, and compared her results to both the sequential protocol and the traditional diet. Growth curves adapted from her data are shown in Figure IV.1. Any diet combination of three of the four species which included *Py. virginica* resulted in faster growth and a shorter time to setting (Fig IV.1. curve A) than the similar diet without *Py. virginica* (Fig IV.1. curve B). The traditional diet of *I. galbana* and *Pa. lutheri* produced slower growth and a longer time to setting (Fig. IV.1, curve C) than the combinations containing *Py. virginica*. She concluded that *Py. virginica* was the most critical alga to the diet. The "good food" diet, in terms of growth rate, percentage survival, percentage pediveliger production, and setting success of oyster larvae was a combination of *Ps. paradoxa, Py. virginica*, and *Chlorella* sp. This has subsequently been used as the VIMS' protocol CPP diet (Dupuy, et al., 1977). Other diets were labelled as "mediocre" food (Windsor 1977).

Attempts have also been made to ripen adult oysters for spawning by supplying additional food, especially during winter months. This conditioning of brood stock may affect subsequent larval growth and vigor. *Tetraselmis suecica* has been used to ripen *Ostrea edulis* in Europe, producing larvae of higher quality and quantity than larvae from oysters receiving only natural phytoplankton (Helm et al. 1973). In contrast, *T. suecica* was a poor conditioning agent for producing vigorous progeny from *C. virginica* (Creekman 1977). She suggested that natural water enriched with cornstarch was a better conditioning agent than cultured

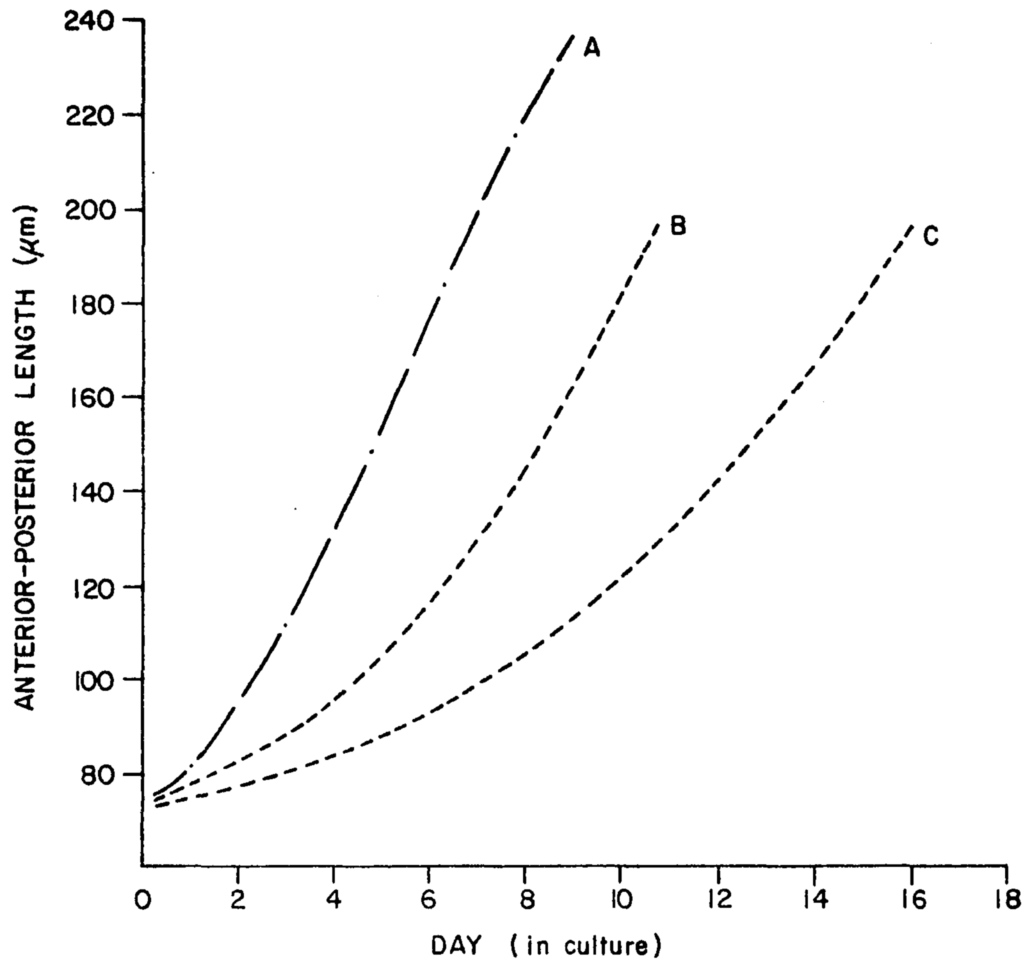


Figure IV.1. Growth of larvae of Crassostrea virginica on different algal diets. Curve A = any combination of three species of Pseudoisochrysis paradoxa, Pyramimonas virginica, Nannochloris oculata and Chlorella sp. which includes Pyramimonas virginica. Curve B = Pseudoisochrysis paradoxa, Nannochloris oculata and Chlorella sp. Curve C = Isochrysis galbana and Pavlova lutheri. Redrawn from Windsor (1977).

algae. In our laboratory, Phaeodactylum tricornutum and Dunaliella tertiolecta have also been tested and found unsuitable as food supplements for ripening adult oysters for spawning.

A possible index of the nutritional value of food is its content of lipid, protein, and carbohydrate. There have been few qualitative and/or quantitative analyses of these components of algae used as food for oysters. Analysis of lipid and fatty acid composition of Py. virginica, Ps. paradoxa, and Chlorella sp. (Chapter II) revealed that the fatty acid components of these algal species was similar. However, the amount of linoleic family (w6) fatty acid, essential for normal growth of many animals, was higher than in the traditional larval diet. It was also observed that Ps. paradoxa contained more total lipid per cell than did either Py. virginica or Chlorella sp. This result indicates that the amount of w6 fatty acids may be a criterion for categorizing good and mediocre food. In this chapter an attempt is made to relate free and protein amino acid composition, qualitatively and quantitatively, to algal suitability as food for oyster larvae.

METHODS AND MATERIALS

Algal Culture

All algal species were cultured in triple filtered (10 µm, 1 µm, 1 µm cotton filters), pasteurized (heated to 70-90°C and treated with UV light) York River water supplemented with N_2M medium, a Guillard's vitamin mix and horse manure extract (Dupuy et al. 1977). The salinity range of the estuarine water was 15 to 20 o/oo. Algal cultures were grown in 18 L Pyrex carboys held at 18 to 19°C under continuous illumination. Cultures were not axenic. _Py. virginica_, _Ps. paradoxa_, and _T. suecica_ were illuminated at 2000 lux, whereas _Chlorella_ sp., _Pa. lutheri_, _I. galbana_, _N. oculata_, _Ph. tricornutum_, and _D. tertiolecta_ were illuminated at 3000 lux. _N. oculata_, _Chlorella_ sp., _D. tertiolecta_ are representatives of the class Chlorophyceae; _Pa. lutheri_, _I. galbana_, and _Ps. paradoxa_ are members of the class Haptophyceae. _Py. virginica_ is a flagellated unicellular alga of the class Prasinophyceae. _Ph. tricornutum_ is a diatom of the class Bacillariophyceae.

For the purpose of this study algal cells were harvested at a time which corresponded to their use for larval food, i.e. during log phase of growth, 10 to 14 days after initiation of the culture. Cell counts were performed at the time of harvesting. The cells were harvested by centrifugation at 2000 g, and the supernatant media decanted.

Extraction of Free Amino Acids

Free amino acids (FAA) were extracted by homogenizing the algal

pellet in 80% ethanol; the pellets were re-extracted until they were colorless. The extracts were pooled and the volume determined. Plant pigments and lipids in the extract were removed with chloroform (2:1 v/v). Norleucine (0.25 µmol.) was added to an aliquot of the extract as an internal standard and the sample dried under a stream of nitrogen. The sample was re-dissolved in approximately 0.5 ml of sodium citrate buffer (pH 2.875) and injected onto a cation ion exchange column for amino acid analysis.

Hydrolysis of Protein

Approximately 0.5 mg. of the ethanol extracted colorless algal material was hydrolyzed with 2 ml of 6 N HCl in a sealed glass ampule for 20 hours at 110 $\pm$ 1°C. The hydrolysate was filtered and 0.25 µmol of norleucine added to an aliquot which was processed as for FAA analysis.

Estimation of Total Protein

Samples for total protein analysis (Lowry et al. 1951) were hydrolyzed with 2 ml of 1N NaOH in a boiling water bath for 2 hours.

Determination of Free and Protein Amino Acids

Qualitative and quantitative analyses of amino acids were performed with a Technicon amino acid analyzer. The chromatographic column was a 6 by 129 mm glass column packed with Chromobeads B. Amino acids were eluted with a citrate buffer gradient, pH 2.875 to 5.00, at a flow rate of 0.5 ml min.$^{-1}$. A ninhydrin reagent was used and color development was measured at 440 and 570 nm through continuous flow cuvettes with a 15 mm light path. Identification of amino acids was based on comparison

with chromatograms of commercial amino acid standards under the same conditions and by the addition of standards to samples for cochromatography. The amino acids were quantified on the basis of peak area on the chromatograms.

RESULTS

Free Amino Acids

The major free amino acid components (Table IV.1) were alanine, arginine, glutamic acid, lysine, proline, serine, and taurine. Differences in amino acid distribution were observed among the algal species. Glutamine was detected only in *T. suecica* and accounted for approximately 19% of this alga's FAA. The concentration of taurine was high in *Ps. paradoxa*, *Py. virginica*, *Pa. lutheri*, *I. galbana* and *Ph. tricornutum* accounting for 6.0 to 22.9% of the total.

Bound Amino Acids

The results of bound amino acid (BAA) analyses are presented in Table IV.2. The principle bound amino acids were alanine, aspartic acid, glutamic acid, glycine, leucine, lysine, proline, serine, threonine and valine. Only small variations were noted in the amino acid ratios of the bound amino acids. L-citrulline was detected in *Ps. paradoxa* and *T. suecica*. Gamma-amino butyric acid was present in five of the nine species of algae, and tryptophan was not detected in *Pa. lutheri*, *I. galbana*, and *Ph. tricornutum*.

Table IV.1. Free amino acids as mole % of total for algae. Unidentified amino acids accounted for 30.7 and 26.8% of the total for P. lutheri and I. Galbana respectively. Coefficient of variation was generally less than 5% for 3 to 5 analyses of components representing 5% or more of the total.

Free Amino Acid / Algal Species	Ps. paradoxa	Py. virginica	N. oculata	Chlorella sp.	Pa. lutheri	I. galabana	T. suecica	Ph. tricornutum	D. tertiolecta
Cysteic acid	0.07	1.0	0.20	0.16	0.69	1.1	0.45	0.22	-
Taurine	12.9	9.0	0.24	-	22.0	16.3	1.1	5.7	0.22
Aspartic acid	2.6	2.4	2.2	1.8	1.6	0.86	7.87	4.0	0.59
Threonine	3.1	3.0	2.0	1.8	3.5	1.5	1.4	0.89	6.00
Serine	3.0	3.1	7.20	5.1	1.9	6.3	2.3	6.50	8.60
Glutamic acid	31.0	23.2	52.2	49.7	16.2	17.6	27.0	24.0	22.7
Proline	2.2	4.4	2.23	6.20	-	1.8	5.7	1.8	0.91
Glycine	1.2	4.9	0.45	0.49	1.1	1.0	1.9	1.2	1.3
Alanine	8.60	19.3	20.1	21.3	12.9	9.6	8.23	7.80	36.5
Valine	2.8	3.8	1.2	1.2	2.2	6.0	0.76	0.57	2.2
Cystine	Trace	Trace	-	-	-	-	-	-	-
Methionine	1.7	1.9	0.21	0.27	-	-	-	-	0.24
Isoleucine	1.0	1.4	0.69	0.53	0.64	0.92	0.37	0.24	0.76
Leucine	1.5	3.2	1.1	0.69	1.1	1.7	0.55	0.30	1.3
Tyrosine	0.89	1.5	0.28	0.18	Trace	0.48	0.52	0.18	0.38
Phenylalanine	0.40	1.5	0.60	0.46	0.46	0.83	0.33	0.30	0.59
Ornithine	1.4	0.90	0.29	0.23	0.64	0.59	2.0	20.3	2.6
Lysine	4.2	3.6	1.4	1.7	2.0	4.1	2.00	5.3	4.1
Tryptophan	-	Trace	0.34	0.21	-	0.45	0.59	-	-
Histidine	1.1	1.1	-	-	0.46	0.95	1.2	0.29	0.29
Arginine	18.7	6.20	2.0	2.1	2.0	1.2	6.88	2.6	1.1
Aminomethylphosphonic acid	1.0	2.0	-	0.04	-	-	10.7	-	-
L Citruline	0.17	1.5	1.5	-	-	-	0.60	-	-
γ-Aminobutyric acid	0.75	0.62	3.6	6.00	-	-	0.40	-	-
Glutamine	-	-	-	-	-	-	18.4	-	-
Unknowns	-	-	-	-	30.7	26.8	-	7.80	9.70

Table IV.2. Bound amino acids (BAA) as mole % of total for algae. Coefficient of variation was generally less than 5% for 3 to 5 analyses of components representing 5% or more of the total.

Algal Species / Bound Amino Acid	Ps. paradoxa	Py. virginica	N. oculata	Chlorella sp.	Pa. lutheri	I. galabana	T. suecica	Ph. tricornutum	D. tertiolecta
Cysteic acid	0.03	0.04	0.25	0.02	1.30	1.30	0.15	1.10	0.72
Taurine	0.09	0.35	0.14	0.05	0.29	0.11	0.27	0.21	0.13
Aspartic acid	10.34	10.11	9.64	10.15	8.50	8.60	8.25	9.60	8.2
Threonine	5.85	5.95	5.56	5.89	5.00	5.20	5.75	5.70	5.20
Serine	5.77	5.94	5.95	5.35	5.90	6.20	6.70	6.70	7.70
Glutamic acid	10.07	11.60	9.60	10.85	9.00	9.10	8.90	9.60	9.90
Proline	5.27	4.90	5.53	5.07	4.60	4.60	7.05	2.50	4.60
Glycine	9.93	9.95	10.38	10.30	9.60	9.60	11.31	10.30	10.40
Alanine	11.42	11.25	11.03	9.70	11.80	11.70	10.56	10.40	10.20
Valine	5.92	6.54	5.88	6.86	6.40	5.80	5.17	6.50	5.90
Cystine	0.52	0.53	0.50	0.45	-	-	1.58	-	0.05
Methionine	0.40	0.55	0.30	0.21	1.60	0.94	0.31	1.70	0.94
Isoleucine	4.22	4.10	3.93	4.52	4.20	3.90	3.22	5.10	3.70
Leucine	9.34	9.04	9.48	9.24	10.30	9.90	8.52	9.20	9.20
Tyrosine	2.50	2.56	2.66	2.75	1.60	2.40	2.46	2.30	2.80
Phenylalanine	4.09	4.00	4.36	4.57	4.60	4.70	4.21	5.30	4.20
Ornithine	0.20	0.10	0.94	0.12	0.12	0.36	0.33	0.30	1.30
Lysine	5.14	4.90	5.63	6.11	6.00	5.60	5.43	5.50	6.20
Tryptophan	1.60	1.60	1.87	1.36	-	-	1.23	-	Trace
Histidine	1.87	1.60	1.84	1.78	2.00	2.00	1.70	1.50	2.00
Arginine	5.54	4.30	4.25	4.53	5.5	5.30	5.30	4.80	4.70
L Citruline	0.30	-	-	-	-	-	0.09	-	-
γ-Aminobutyric acid	0.12	0.04	0.99	0.04	1.70	2.70	2.71	-	-

Table IV.3. Characterization of algal species used in this study. Free amino acids = FAA; bound amino acids = BAA. Average BAA per μm^3 is 0.283 fmol.

Species	Cell Volume (μm^3)	FAA/cell (fmol.)	FAA/Protein (nmol./µg)	BAA/cell (fmol.)	fmol. BAA/μm^3	Deviation from mean fmol. BAA/μm^3 for all species	Total fg Protein/cell	BAA/ Protein (nmol./µg)
Chlorella sp.	4.87	0.21	-	1.71	0.35	+0.067	300	5.8
N. oculata	5.58	0.26	0.256	2.77	0.50	+0.22	300	9.3
Py. virginica	33.5	0.84	-	18.3	0.55	+0.27	2,190	9.8
Ps. paradoxa	47.7	0.81	0.377	9.88	0.21	-0.073	1,530	6.4
I. galbana	57.8	-	0.273	4.48	0.08	-0.20	528	8.48
Pa. lutheri	73.5	-	0.134	2.80	0.04	-0.24	368	7.57
T. suecica	390	20.2	-	96.7	0.25	-0.033	11,900	7.77
Ph. tricornutum	-	-	0.626	-	-	-	-	10.2
D. tertiolecta	-	-	0.704	-	-	-	-	7.04

Other Cell Parameters

Cell volumes, total free and bound amino acids are shown on both a per cell and per unit protein basis as well as total protein per cell (Table IV.3). Free amino acids averaged about 7% of the combined total of free and bound amino acids, ranging from 1.7 to 17.3% on a molar basis. The log of total bound amino acid per cell shows a linear relationship with the log of cell volume, with the exception of *I. galbana* and *Pa. lutheri* which have much less bound amino acid per unit cell volume.

DISCUSSION

There was little variation among the bound amino acid compositions of the nine algal species. The present findings agree quite well with results of other investigations (Chau et al. 1967; Cowey and Corner 1966) for Pa. lutheri, Ph. tricornutum and other algae. L-citrulline, not a typical protein amino acid, was found in the bound amino acid fraction of T. suecica and N. oculata. It is not readily apparent whether differences in ratios of bound amino acids are of any significance in determining the dietary quality of these algae for oyster larvae.

The best diet for oyster larvae should optimally provide the essential nutrients for rapid growth and early metamorphosis. Although Py. virginica was found to be the key component of a three species diet mixture (Windsor 1977), it has virtually the identical proportions of bound amino acids as the other two algae in the mixture as well as other algae which have been classified as mediocre foods (Windsor, 1977). Py. virginica does, however, possess the highest concentration of bound amino acids per unit of cell volume (Table IV.3), whereas the mediocre food organisms I. galbana and Pa. lutheri have the lowest concentration. Pa. lutheri and I. galbana have the highest total lipid per cell of several algal species tested (Chapter II). These results indicate that both concentration and component balance of nutrient constituents may be key factors making one phytoplankton species a better oyster larval food than another algal species.

Free amino acids of the algal species might be expected to be less

critical than bound amino acids in the diets because they are only 1 to 16% of the total amino acids. The free amino acids could also be expected to vary in concentration and proportion as a result of environmental factors, e.g. a change of salinity (Webb et al. 1971; Raymont et al. 1968) or the physiological state at the time of harvest or predation (Fowden 1962). Nevertheless, they may be more available than bound amino acids because they can be readily absorbed and utilized by predator organisms without prior digestion. Ps. paradoxa appears to be intermediate in free amino acid concentration among the algae tested. It does contain by far the highest relative proportion of arginine. Taurine is a free amino acid which varies considerably among the algae tested, and, although it is present in oyster tissue (Lynch and Wood 1966), it appears to be equally available in the PI diet (I. galbana) as it is in the CPP diet (Ps. paradoxa).

Dietary requirements at various life stages may be different, e.g. larval growth may require different dietary components than does conditioning of adults for optimal gonad development. Studies conducted by feeding C. virginica with Thalassiosira pseudonana containing different protein:carbon ratios (Flaak and Epifanio 1978) have demonstrated that the total protein requirement of adult oysters is relatively low compared to the requirement for cabohydrates. Carbohydrate analyses of the algae used for oyster larval food indicates that about 80% of the carbohydrate in Py. virginica is glucose and that the amount per unit volume is greater than for other algae analyzed (Chapter III).

Different species of organisms might be anticipated to be as different in dietary requirements as are different life history stages.

Although _T. suecica_ is a good conditioning agent for _O. edulis_ (Helm et al. 1973),this was not true for _C. virginica_ (Creekman 1977). Free amino acid composition of _T. suecica_ is quite different from that of the other species tested. _T. suecica_ was the only species in which glutamine was detected. Test diets of free amino acids fed to various fish have shown that while mixtures of amino acids imitating casein and gelatin can support growth in rainbow trout, the same diets retard the growth of young carp (Aoe et al. 1970).

CHAPTER V

THE ACCEPTABILITY AND DIGESTIBILITY OF MICROCAPSULES BY LARVAE OF *CRASSOSTREA VIRGINICA*.

INTRODUCTION

A major difficulty in the development of commercial culture systems for molluscan and crustacean larvae is the dependence upon supplies of live organisms for food. This dependence has also obstructed investigations into the nutritional requirements of many bivalve molluscs and crustaceans during their planktonic larval life, although some valuable information about larval nutrition has been gained in the last decade.

Artificial food particles are known to be acceptable to a wide range of filter feeders (Ling, 1969; Paffenhofer and Strickland, 1970; Jones, et al., 1972). These particles are susceptible to disintegration and associated bacterial contamination. One solution to these problems is to use an encapsulated diet. Moreover, if a diet can be defined biochemically, the technique of microencapsulation can be used to investigate the exact nutritional requirements of the animals under culture conditions.

The type of microcapsule that can be used successfully in feeding experiments will be dependent on the mode of feeding of the animals. Bivalve larvae and adults are filter feeders and ingest their food intact. Selection of food particles depends on size, surface properties and weight of the particle (Ukeles, 1971; Owen, 1974). Therefore, the

test of the acceptability of different types of microcapsules to the animal is important to justify future experiments with encapsulated diet components to evaluate growth and survival. It is also important to demonstrate that microcapsules, which are acceptable to the bivalves in terms of ingestion and retention, can be digested.

Gelatin-acacia microcapsules have proven to be suitable for the presentation of dietary lipids to larvae of *Crassostrea gigas* (Langdon, 1980). Previous investigators suggested that lipids play a significant role in the metamorphosis and development of the oyster larvae (Helm, et al., 1973; Holland and Spencer, 1973; Holland, 1978; Chapter II). Consequently, gelatin-acacia microcapsules filled with cod liver oil were used in these feeding experiments. Cod liver oil is rich in highly unsaturated fatty acids (Ackman and Burgher, 1964) and has a fatty acid composition quite similar to that found in the protocol algal diet (a combination of *Chlorella* sp., *Py. virginica* and *Ps. paradoxa*) used in this laboratory as a standard food source for larvae of *C. virginica* (Chapter II).

Jones and his colleagues (Jones et al., 1974; Gabbott et al., 1976; Jones and Gabbott, 1976; Jones et al., 1979a, 1979b) successfully encapsulated artificial food particles in nylon-protein microcapsules to study the nutritional requirements of crustacean larvae. It thus appeared likely that nylon-protein walled microcapsules could be used to provide protein, lipid and carbohydrates to oyster larvae. In this paper results of studies to assess the acceptability and digestibility of microcapsules by larvae of *C. virginica* are reported. Microcapsules tested included those with gelatin-acacia walls and nylon-protein walls.

METHODS AND MATERIALS

Microcapsules and diet.

Gelatin-acacia microcapsules were prepared by the method described by Green and Schleicher (1957). Cod liver oil containing vitamins A and D (E. R. Squibb and Sons Inc. Princeton, N.J.) was encapsulated for feeding experiments. The mean diameter of these microcapsules was 6.0 $\pm$ 1.8 µm ($\bar{x} \pm$ SD, n = 25). Stained gelatin-acacia microcapsules were prepared by dissolving Sudan Red in lipid before encapsulation (approximately 1-2 mg/ml lipid). Vitamins B_1, B_2, and B_{12} were supplied in the diet by mixing B_1 and B_2 with the lipid and dissolving the B_{12} in the solution of gelatin-acacia prior to microencapsulation. All gelatin-acacia microcapsules were autoclaved at 121°C (15 lbs pressure) for 15 minutes and stored in the refrigerator except those which were fed to the larvae immediately after manufacture. The autoclaving may have somewhat reduced the vitamin content due to heat lability; vitamin A is considered heat labile at 121°C while vitamins B_1, B_2, B_{12} and D are not.

Nylon-protein microcapsules were prepared with a modification (Jones and Gabbott, 1976) of the polymerization procedure described by Chang et al. (1966). The mean diameter of these microcapsules was 6.1 $\pm$ 1.95 µm (n = 25). Whole chicken egg homogenate mixed with an equal volume of 15% dextrose and 5% cholesterol in distilled water was incorporated into the nylon-protein microcapsules. Nylon-protein microcap-

sules were stained by adding Blue dextran to the egg-water mixture (8 mg of Blue dextran for 15 ml of mixture). A summary of the general characteristics of these two types of microcapsules is shown in Table 1.

Larval and algal culture.

Methods used to induce spawning and for embryo culture were the same as those described by Dupuy et al. (1977). After spawning, all eggs were pooled and counted before fertilization. About 12.5×10^6 fertilized eggs were placed in each 250 liter fiberglass larval tank. Eighteen to 24 hours after fertilization the larvae reached the straight-hinge stage when the cultures were maintained at temperatures of 27-28°C. The methodology for rearing oyster larvae and the procedure for feeding described by Dupuy et al. (1977) were used.

The 3 algal species, *Chlorella* sp., *Py. virginica* and *Ps. paradoxa*, used for the protocol diet, were cultured at 16 to 19°C in 40 liter carboys containing filtered and pasteurized estuarine water enriched with N_2M medium (a mixture of Ketchum & Redfield's solution A and B, sodium molybdate solution, Arnon's micronutrient solution) and a horse manure extract mixture (Dupuy et al. 1977). *Chlorella* sp. and *Ps. paradoxa* were grown under continuous illumination from one warm white and one Gro-Lux fluorescent lamp whereas *Py. virginica* was illuminated with 2 Gro-Lux lamps. Continuous aeration provided circulation in the cultures.

Feeding experiment.

Two feeding experiments were carried out in the laboratory with larvae of *C. virginica*. Larval density in all feeding experiments was 5-

6 larvae per ml. Larvae fed Ps. paradoxa (the other two species of the protocol diet were unavailable) and starved larvae served as controls for all feeding experiments. Seawater filtered through 10 and then 1 μm Cuno cotton filters was used throughout these experiments.

I. Feeding and digestion activity.

Stained gelatin-acacia microcapsules and nylon-protein microcapsules were fed to 2 day old larvae in 300 ml glass beakers. Microcapsules were fed to larvae each day for 2 days after which the larvae were held for an additional 3 days in clean water. The seawater in the beakers was changed every other day, prior to feeding if the larvae were fed. Beakers containing larvae were covered and held at room temperature (26-27°C). Twenty-four and 48 hours after the last feeding, larvae were sampled to observe the contents of the digestive system with a Zeiss standard UPL inverted microscope. Photographs were taken of the same sample after preservation in a 0.5% formalin solution. A Leitz Ortholux microscope with variable phase contrast optics and a Reichert camera with Ektachrome film (ASA 160, tungsten) were used to photograph representative larvae.

II. Growth experiment.

The purpose of the growth experiments was to determine a suitable range of microcapsule concentration to use in future experiments. Growth was the definitive indicator of digestion and utilization of microcapusles.

1. Straight hinge oyster larvae were grown in 250 liter larval tanks with 3 different concentrations of gelatin-acacia microcapsules

containing cod liver oil; 500, 1600, and 5000 microcapsules/ ml. Starved larvae and larvae fed with Ps. paradoxa were used as controls for this experiment. Some gelatin-acacia microcapsules containing cod liver oil were supplemented with vitamin B_1, B_2, and B_{12}. The ratio of microcapsules without vitamins to those with vitamins arbitrarily was 6:1. Microcapsules were added to the tanks every day and the seawater was changed every 2nd day. The number and size of the larvae was determined on days 3, 5, 11, 13, and 17. Larvae were concentrated (50-250/ml) for counting; the anterior to posterior length of twenty of these larvae were measured.

2. Straight hinge larvae were cultured in 300 ml glass beakers with different concentrations (50, 100, 200, 500, 1000, and 5000) of microcapsules per ml. Cod liver filled microcapsules were added every day and the seawater changed every 2nd day. Size of 20 larvae was measured at 16 days.

RESULTS

Feeding and digestion activity.

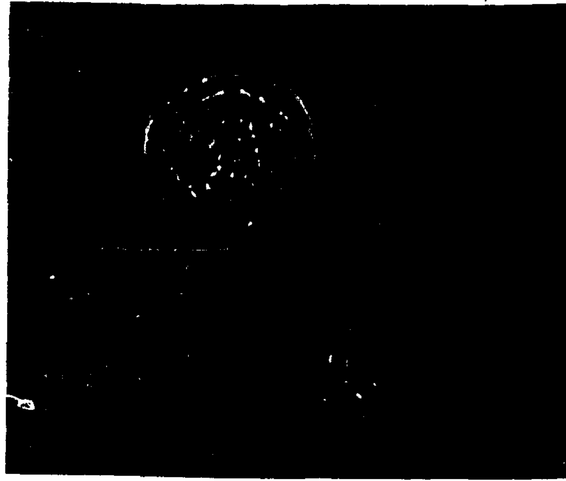
Larvae were observed to ingest and digest both gelatin-acacia and nylon-protein microcapsules. Sudan Red stained gelatin-acacia microcapsules in the position of the stomach and digestive diverticular were observed to fade during the first 24 hours after feeding was terminated and completely disappeared within 48 hours. Approximately 72 hours elapsed for the larvae to completely digest the nylon-protein microcapsules. Microcapsule-fed larvae appeared healthy and vigorous throughout the test. In this feeding experiment, both types of microcapsules supported some growth. The "starved" larvae stayed in the straight hinge stage throughout the experiment while the microcapsule fed larvae developed to umbo stage (Fig V.1a and V.1b).

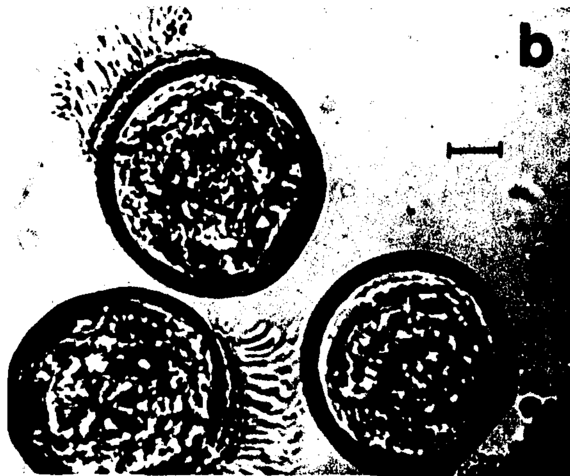
Growth experiment.

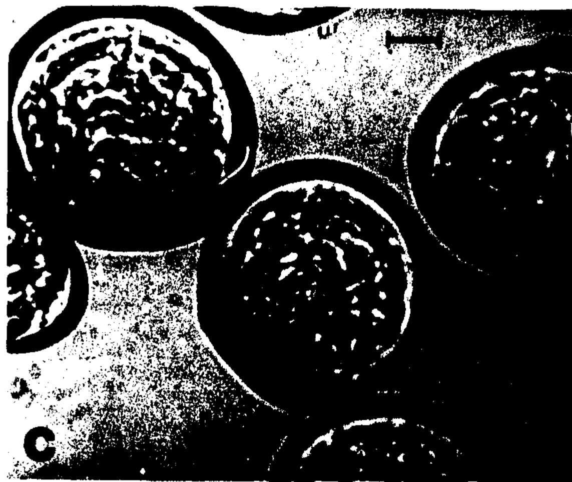
Growth rates of oyster larvae cultured in hatchery sized larval tanks on several diets are shown in Figure V.2. Microcapsule-fed larvae grew as rapidly as those fed with the algal, Ps. paradoxa, until about day 11 and grew much better than the "starved" control larvae. The percentage survival was 33% for all treatments with the exception of the 5000 microcapsule/ml concentration which had less than 10% survival at day 13.

There appears to be some growth at every concentration of microcapsules supplied; growth was reasonably constant above 500 microcapsules / ml (Fig. V.3) based on results for 16 day old larvae grown in

Figure. V.1. Photomicrographs of 4 day old oyster larvae. "Starved" larval controls (Fig. V.1a), larvae fed with gelatin-acacia microcapsules (Fig V.1b), and larvae fed with nylon-protein microcapsules (Fig V.1c). Note that the fed larvae progressed to the umbo stage. Bar = 20 um.




b


c

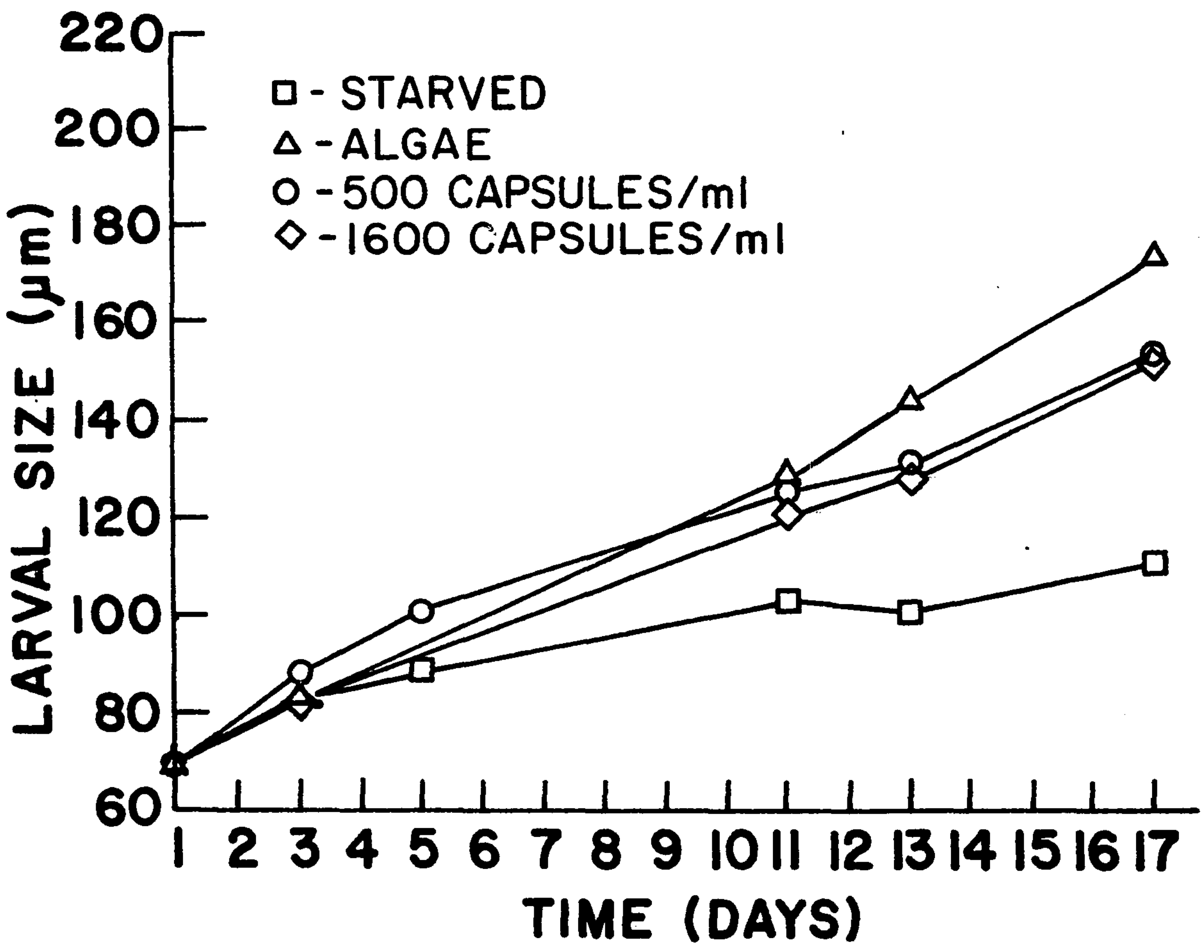


Figure V.2. Growth of oyster larvae on different diets. Larvae were raised in hatchery-size tanks.

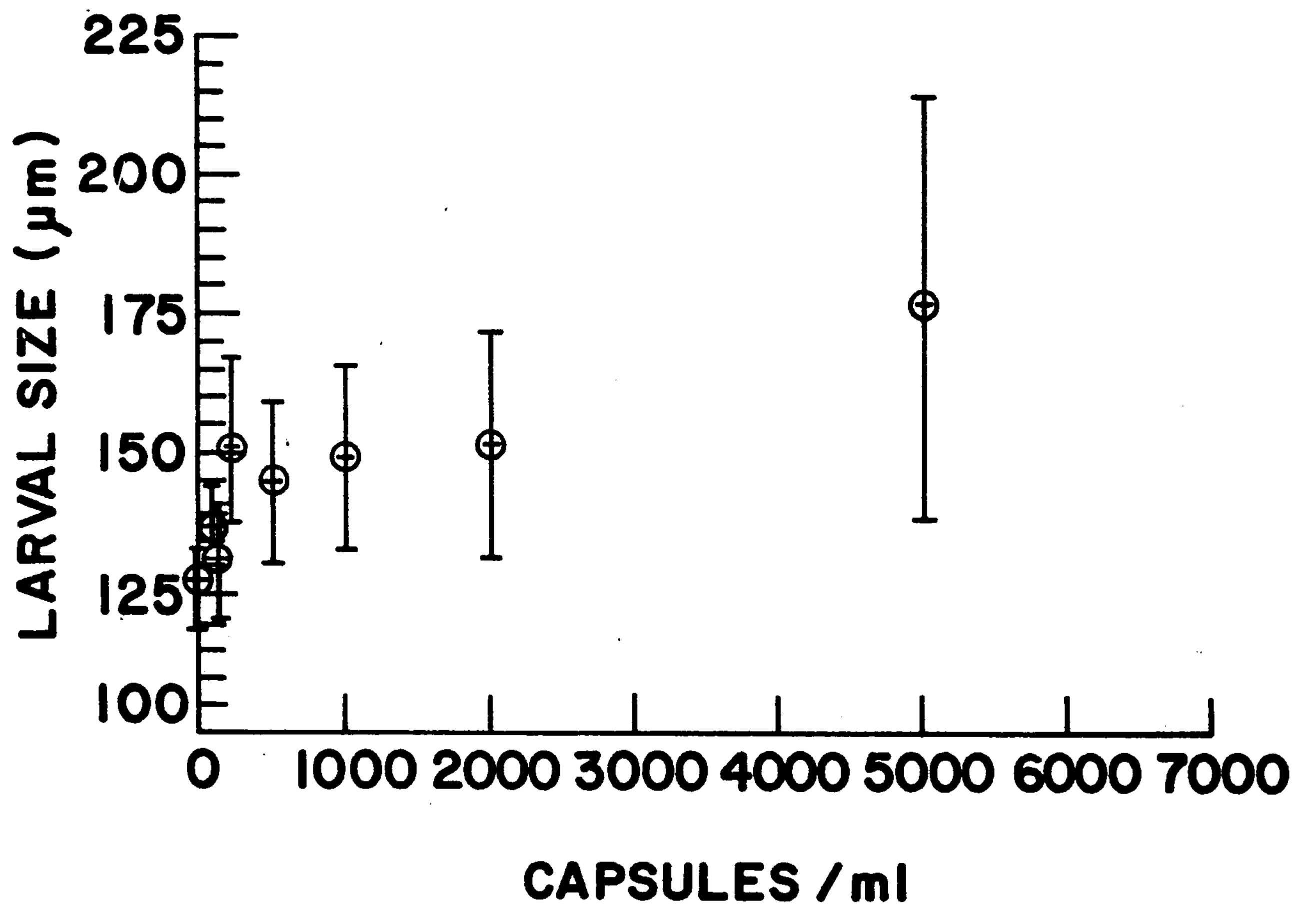


Figure V.3. Size of 16 day larvae in 300 ml beakers versus concentration of microcapsules. Standard deviation is indicated by the vertical bars, n = 20.

300 ml beakers. Least squares analysis of length of 16 day old larvae and capsule concentration gives a correlation coefficient of 0.72. Larvae grown in the larval tanks showed a similar trend in response to microcapsule concentrations below 2000 microcapsule / ml (Fig V.4). The reduced growth rate at 5000 microcapsules / ml is unexplained.

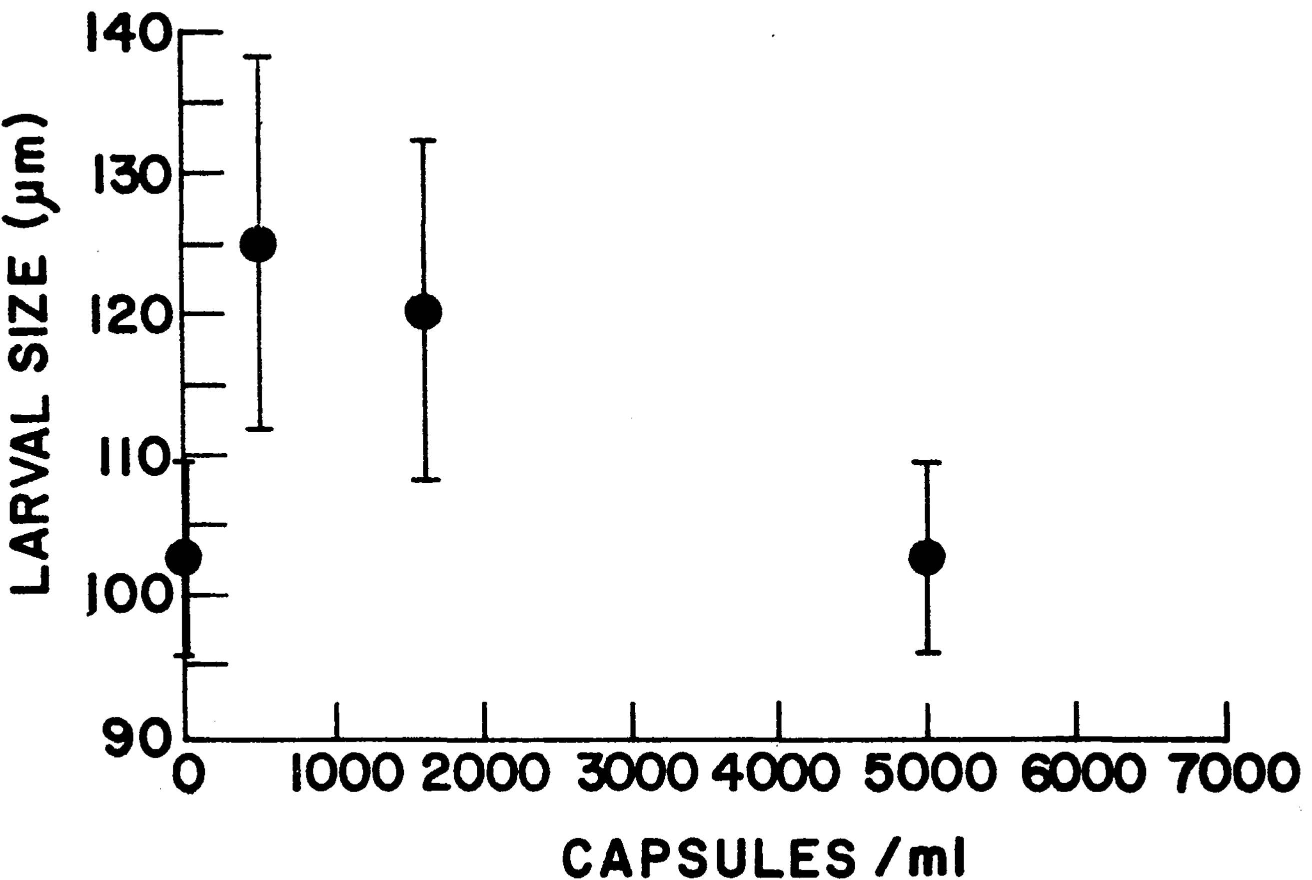


Figure V.4. Size of 11 day larvae in hatchery-size tanks versus concentration of microcapsules. Standard deviation is indicated by the vertical bars, n = 20.

DISCUSSION

Both the gelatin-acacia and nylon-protein microcapsules were acceptable to larvae of C. virginica. Nylon-protein walled microcapsules were not as digestible as the gelatin-acacia walled microcapsules presumably because the nylon-protein wall is formed by cross-linkage between nylon and protein. The nylon-protein wall is therefore less susceptible to attack by digestive enzymes than the gelatin-acacia wall. Jones and Gabbott (1976) have shown that if the nylon content is decreased, the wall becomes more susceptible to proteolytic breakdown. The nylon content can be diminished by reducing the concentration of 1,6-diaminohexane during the preparation of the capsules.

It should be emphasized that these experiments were set up primarily to test the acceptability and digestibility of these two types of microcapsules and detailed consideration was not given to requirements for optimal growth. It was interesting, therefore, to find that gelatin-acacia microcapsules filled with cod liver oil were supportive of larval growth and development. Other investigators (Jones et al., 1974; Gabbott et al., 1976; Jones and Gabbott, 1976; Jones et al., 1979a, 1979b) also reported that nylon-protein capsules containing protein, starch, and cholesterol supported growth of both the brine shrimp, Artemia and the Japanese oyster, Crassostrea gigas. In our experiments, gelatin-acacia capsules contained only lipid, with the exception of the small amount of protein in the gelatin and carbohydrate in the acacia. We anticipate better growth when optimal proportions of lipid, protein, and carbohy-

drate are encapsulated. There are indications that fatty acids may play a significant role in the metamorphosis and development of oyster larvae (Helm, et al., 1973; Holland and Spencer, 1973; Holland, 1978; Waldock and Nascimento, 1979; Chapter II). Increasing the supplement of lipid which contains high amounts of long chain polyunsaturated fatty acids (e.g. 22:5w3 and 22:6w3) in the diet could be a promising approach.

Since it is unlikely that vitamins would be present in sea water in sufficient quantity for growth, supplements of B_1, B_2, and B_{12} were provided. B_{12}, which is water soluble and may leach out during encapsulation, was observed to be retained in part by the gelatin-acacia capsules. It is bright red in color and the capsules with B_{12} were slightly pink.

Gelatin is quite susceptible to bacterial attack and bacteria may be attached to the capsule walls. Although bacterial contamination could not have been the source of the bulk nutrients, they may have been the source of trace materials.

It is a disadvantage that the gelatin-acacia wall is permeable and the nylon-protein wall is semi-permeable to small molecules. Only water insoluble and macromolecular components of the diet can be contained within such capsule membranes without loss. It would be ideal to produce a capsule with double walls since this type of capsule might be suitable for the encapsulation of both low molecular weight and water soluble components (e.g. amino acids and vitamins) as well as lipids. In this approach the aqueous solution would be encapsulated within the lipid before the second outer wall is formed.

CHAPTER VI

CONCLUSIONS

In general the fatty acid composition of the five algal species analyzed in this study do not show qualitative differences but there are quantitative differences in some fatty acid components (see Table VI.1). For example, both *Pa. lutheri* and *I. galbana* have a higher percentage of saturated fatty acids than the other species. The presence of large quantities of tetradecanioc (C-14) and oleic acid (C18:1) was characteristic of *Ps. paradoxa* and *I. galbana* (Table VI.1). The major fatty acid constituents in the total lipids were the saturated fatty acids of chain lengths C12-C18, and mono-and poly-unsaturated C16 and C18 acids. For the same species of algae, variation in chemical composition can occur when the culture conditions are varied. When data from this study are compared to those from other laboratories (Table VI.1) a great variation in fatty acid composition is observed; e. g. there are marked differences in total saturated and polyethylenic fatty acids for the algal species *Py. virginica*, *Pa. lutheri* and *I. galbana* under different environmental conditions. The ranges of variation of total saturated fatty acids (TSFA) and total polyethylenic fatty acids (TPFA) for the above three algal species are 26-45% and 31-46% respectively for *Py. virginica*, 21-64% and 8-51% for *Pa. lutheri* and 19-61% and 12-53% for *I. galbana* (Table VI.1). These differences are likely to be caused by different culture conditions. One should not, however, exclude the possibility that these differences might arise from differences in the analytical techniques. It is also interesting to note that the total

Table VI.1. Fatty acid composition of some algal species used as food for larvae, spat and adult oysters. The data from Chapter II for Chlorella sp., P. virginica and P. paradoxa are means of the data from 4, 10, 15, and 20 day old batch cultures.

	Chlorella Sp.	Pyramimonas virginica		Pseudoisochrysis paradoxa			Pavlova lutheri			Isochrysis galbana		Chaetoceros calcitrans	Dunaliella tertiolecta	Tetraselmis suecica
	(1)	(1)	(2)	(1)	(3)	(4)	(5)	(1)	(2)	(1)	(6)	(2)	(3)	(3)
Fatty Acids														
Saturated														
8:0	-	-	-	-	-	-	-	-	-	trace	-	-	-	-
10:0	-	-	-	-	-	-	-	trace	-	trace	-	-	-	-
12:0	4.14	4.54	-	2.61	-	0.20	0.60	5.88	-	5.31	0.60	-	-	-
13:0	1.02	1.09	-	0.61	-	-	0.20	3.20	-	1.27	0.20	-	-	-
14:0	2.78	3.46	1.50	23.74	11.20	11.20	9.20	21.36	7.80	25.57	10.60	1.30	1.9	5.20
15:0	0.22	0.59	0.50	0.32	0.30	0.20	0.40	-	1.60	-	1.30	0.30	0.80	0.40
16:0	30.09	29.51	19.90	17.46	22.80	15.10	10.10	19.27	8.80	15.19	22.00	6.40	16.0	25.40
17:0+16:2	3.26	2.33	-	0.71	-	-	0.40	1.31	-	0.68	-	-	-	-
18:0	2.16	3.71	3.6	1.23	1.50	-	0.40	2.29	-	1.52	2.30	2.20	0.70	1.80
19:0	-	-	-	-	-	-	-	-	0.70	-	-	-	-	-
20:0	-	-	-	-	-	-	-	0.78	-	0.84	-	-	-	-
22:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	43.69	45.08	25.50	46.69	35.80	26.50	21.30	54.87	18.90	50.63	37.00	10.20	19.40	32.80
Monoethylenic														
14:1	0.34	3.70	1.40	0.97	-	0.20	-	-	0.20	-	-	1.90	0.4	trace
16:1	2.88	3.21	1.50	3.92	25.80	25.40	20.20	12.15	0.80	3.54	15.70	15.40	4.60	3.80
18:1	9.74	6.45	9.60	18.62	5.80	3.40	5.70	8.62	10.40	24.64	-	11.40	7.00	22.70
20:1	0.61	trace	0.90	0.88	-	0.60	-	-	-	-	13.50	0.90	-	2.85
22:1	6.40	0.13	0.60	0.26	-	-	-	-	1.00	-	1.20	0.90	-	-
Total	14.93	13.49	14.00	24.34	31.60	29.60	25.90	20.71	12.40	28.18	30.40	30.50	12.00	29.35
Polyethylenic														
16:2w7	-	-	-	-	-	0.20	1.30	-	-	-	-	-	-	-
16:2w6	-	-	1.5	-	0.50	-	-	-	-	-	-	5.80	-	-
16:2w4	-	-	-	-	-	1.7	4.60	-	-	-	-	-	-	-
16:3w6	-	-	0.4	-	trace	-	-	-	1.20	-	-	4.70	1.70	trace
16:3w4	-	-	-	-	-	0.5	14.80	-	-	-	-	-	-	-
16:3w3	4.37	8.47	1.10	1.42	trace	0.1	-	-	trace	-	0.40	7.50	1.80	0.60
16:4w1	-	-	-	-	-	0.3	1.50	-	-	-	-	-	-	-
16:4w3	-	-	3.80	-	trace	-	-	-	-	-	-	1.90	12.4	11.00
18:2w6	9.94	2.18	2.60	3.59	2.80	0.70	1.60	1.83	4.30	3.38	2.30	0.20	8.80	1.20
18:3w6	0.67	-	0.90	-	trace	0.10	-	0.78	0.20	trace	0.20	1.40	3.50	trace
18:3w3	13.05	5.28	10.60	2.06	0.60	0.20	-	1.63	11.0	3.38	0.40	3.00	31.10	9.80
18:4w3	0.68	5.10	4.40	1.86	3.60	4.00	0.60	3.58	9.20	8.02	8.00	0.50	0.50	3.50
20:2w6	0.58	2.24	-	0.58	trace	0.30	-	-	-	-	-	-	0.30	trace
20:3w6	-	-	-	-	-	0.10	1.70	-	-	-	-	-	-	-
20:3w3	0.20	0.20	-	0.29	-	0.10	-	-	-	-	-	-	-	-
20:4w6	0.60	3.40	-	0.14	0.70	0.40	-	-	0.20	-	0.10	-	-	0.70
20:4w3	0.85	-	1.60	0.07	-	0.10	0.10	-	0.50	-	-	-	-	0.60
20:5w3	0.23	2.03	1.30	0.18	13.80	16.30	18.00	5.88	3.60	1.01	7.20	15.40	-	7.70
22:2w6	-	-	0.40	-	-	0.50	-	-	-	-	-	-	-	-
22:4w6	-	-	0.50	-	-	-	-	-	-	-	-	-	-	-
22:4w3	0.17	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5w6	0.35	-	1.8	-	1.10	1.20	-	-	4.30	-	-	3.30	-	-
22:5w3	0.25	0.78	1.4	0.37	trace	0.40	0.5	-	trace	-	-	-	-	-
22:6w3	0.60	2.45	13.70	2.31	7.90	13.10	3.30	2.94	18.90	3.54	4.30	1.90	-	-
Total	29.63	30.76	46.00	12.52	31.00	40.20	50.50	16.65	53.40	19.33	22.90	45.60	60.01	35.10

References: 1) Chapter II; 2) Waldock and Nascimento 1979; 3) Langdon and Waldock 1981; 4) Ackman et al. 1968; 5) Chuecas and Riley 1969; 6) Watanabe and Ackman 1974

polyethylenic fatty acid was comparatively lower for algal species grown in our laboratory than for algae grown in other laboratories, while the percentage of total saturated fatty acid was higher. In fact, there is evidence to indicate that differences in composition of culture medium, time of harvesting and experimental conditions greatly influenced the fatty acid composition. For example, algae grown under light saturation in a nitrogen deficient medium have an abundance of lipids which are rich in 16:0 and 18:1. On the contrary, the lipid concentration in algae grown in a nitrogen rich medium is low and consists predominantly of polyunsaturated fatty acids (Schlenk et al. 1960; Williams and McMillian 1961; Klenk et al. 1963; Hulanika et al. 1964).

The fatty acid composition of Chlorella sp., Py. virginica and Ps. paradoxa varies only to a small degree with the age of the culture. These variations form no consistent pattern and do not appear to affect food quality.

In most cases, 14:0 and 16:0 are the dominant saturated fatty acids of algal species used as bivalve food and the total saturated fatty acid is 15-64% (for 37 of 38 species for which data are reported). It was found that 20-50% of the total fatty acids of bivalves and bivalve larvae are also saturated (Ackman et al. 1974; Watanabe and Ackman 1974; Gardener and Riley 1972; Langdon and Waldock 1981). This is true for most marine animal lipids (Ackman 1980). Thus, the quantity of saturated fatty acids in algae and in marine animals is similar.

The nutritional quality of the algal diet was found not to be related to the total amount of lipid. This agrees with the results of other studies (Waldock and Nascimento 1979; Langdon and Waldock 1981).

The quantity of total w6 fatty acids in the CPP diet is higher than in the traditional PI diet of Pa. lutheri and I. galbana (Table II.6). Thus oyster larvae may be similar to german carp in that both w6 and w3 fatty acids are required in the diet. Most algae (three of five species) which have been reported as "good" food for oyster larvae have ratios of w6:w3 approximately 0.5 whereas algae labeled as "moderate" food have a ratio of about 0.2 (Table VI.2). D. tertiolecta has a high ratio of w6/w3 but may be a poor food as a result of deficiencies of 20:5w3 and 22:6w3 fatty acids. Trider and Castell (1980) also suggested that the adult oyster may have a dual essential fatty acid requirement, i.e. w6 and w3, although the latter seems to play a more significant role. They found a higher percentage dry weight in oysters fed a diet containing w6 and w3 fatty acids than those fed with a diet containing only w3 fatty acids.

A summary of carbohydrate weight percent composition of 8 algal species which are utilized as food sources for bivalves is shown in Table VI.3. Results of the analyses of polysaccharide for the five algae agree with those obtained by other investigators (Handa and Yanagi, 1969). The principal sugars are glucose, mannose, ribose, xylose, rhamnose and galactose. Glucose and mannose are the major components. No major difference, qualitative or quantitative, in carbohydrate composition was found. It would seem that there was no correlation between the nutritional value of an algal species and its carbohydrate content. Parsons et al. (1961) suggested that the high percentage of glucose in the readily hydrolyzable fraction of an alga is nutritionally important, but the present results do not support this

Table VI.2. Lipid and fatty acid content of algal species utilized as food for larvae and spat of *Crassostrea virginica* or *Crassostrea gigas*.

Algal diet	Food value	Total Fatty Acids (% dry wt)	Total Fatty Acids μg x 10^{-7} per cell	Total Lipid μg x 10^{-7} per cell	Total Lipid μg per mg dry wt	Total w3 Wt. %	Total w6 Wt. %	w6:w3 ratio	Presence of Fatty Acid 20:5w3	Presence of Fatty Acid 22:6w3
CPP diet										
Chlorella sp.	good[5]	-	-	1.48[2]	-	22.35[2]	10.19[2]	0.46	+[2]	+[2]
P. virginica	moderate[1]	4.2[1]	0.15[1]	15.22[2]	-	40.2[1]	8.1[1]	0.20	+[1]	+[1]
	good[5]					27.55[2]	7.15[2]	0.26	+[2]	+[2]
P. paradoxa	good[5]	-	-	22.91[2]	-	8.38[2]	4.29[2]	0.51	+[2]	+[2]
PI diet										
P. lutheri	moderate[3]	-	-	59.85[2]	-	6.44[2]	1.40[2]	0.22	+[2]	-[2]
						25.90[3]	5.10[3]	0.20	+[3]	+[3]
I. galbana	moderate[1]	12.5[1]	0.36[1]	72.36[2]	174[3]	43.3[1]	10.3[1]	0.24	+[1]	+[1]
						10.52[2]	1.31[2]	0.12	+[2]	+[2]
Other species										
T. suecica	good[3]	-	-	-	61.8[3]	33.2[3]	2.1[3]	0.06	+[3]	-[3]
D. tertiolecta	poor[3]	-	-	-	167[3]	45.8[3]	15.8[3]	0.35	-[3]	-[3]
C. calcitrans	good[1,3]	9.7[1]	0.21[1]	-	-	32.1[1]	16.5[1]	0.51	+[1]	+[1]

References: 1) Waldock and Nascimento 1979; 2) Chapter II; 3) Langdon and Waldock 1981

Table VI.3. The monosaccharide (including those hydrolyzed from polysaccharide) weight percentage composition of several algal species used as food for bivalves. Only the principal sugars are tabulated.

Sugar	*Pyramimonas virginica* (1)	*Ps. paradoxa* (1)	*Chlorella* sp. (1)	*Pavlova lutheri* (1)	*I. galbana* (1)	*Chaetoceros* sp. (2)	*S. costatum* (2)	*Ph. tricornutum* (2)	CPP*	PI**
Glycerol	trace	1.01	trace	1.76	0.27	-	-	-	0.15	1.02
Rhamnose	1.83	4.38	1.43	1.41	1.39	1.90	5.10	6.70	2.06	1.41
Ribose	1.30	4.28	5.00	5.27	1.77	-	0.60	2.80	3.35	3.53
Fucose	2.14	4.91	5.42	3.81	2.48	0.90	5.80	2.30	3.98	3.15
Fructose	-	1.15	2.24	0.60	trace	-	-	-	1.13	trace
Arabinose	-	-	-	-	-	-	3.30	3.10	-	-
Xylose	-	-	-	-	-	4.60	4.30	10.90	-	-
Ribitol/xylitol	-	5.70	4.18	2.08	0.80	-	-	-	2.65	1.44
Mannose	11.05	30.19	18.32	15.20	13.87	27.2	30.30	33.20	17.18	14.54
Galactose	2.19	-	-	-	0.59	9.10	2.00	5.50	-	0.30
Glucose	80.37	48.93	63.50	67.15	77.40	54.50	48.60	35.10	69.10	72.30
Manitol	2.60	-	-	-	-	-	-	-	1.12	-
Sucrose	-	0.89	-	-	-	-	-	-	0.13	-
µg x 10-9/cell	5.03	2.45	0.72	6.08	9.23	-	-	-	2.84	7.66
µg x 10-6/µg wet algae	19.71	20.66	24.61	26.71	61.88	-	-	-	22.18	44.30

(1) Chapter III. The tabulated values are the means of individual monsaccharides hydrolyzed from polysaccharide (percent weight composition), total per cell (µg x 10^{-9}/cell) and total per µg wet algae (µg x 10^{-6}) of 4, 10, 15 and 21 day old algal cultures.
(2) Handa and Yanagi 1969. The tabulated values are monosaccharide weight percentage composition of the whole algal cells.
* 43% of monosaccharide provided by *Chlorella* sp., 43% by *Py. virginica*, and 15% from *Ps. paradoxa*
** 50% of monosaccharided provided by each species.

this interpretation for oyster larval nutrition. In these analyses, the weight percent of glucose in *Py. virginica* and *Chlorella* sp. was similar to that of *Pa. lutheri* and *I. galbana*. *Chaetoceros* sp. was also found to be "good" food for larvae and spat of *C. gigas* (Walne, 1970b, 1974; Waldock and Nascimento, 1979; Langdon and Waldock, 1981) although the weight percent of glucose in this diatom species was the lowest of those algal species examined (Table VI.3). Only *Ph. tricornutum*, which was reported to be inadequate food for bivalves (Epifanio and Mootz, 1976) had a much lower concentration of glucose than the other algal species (Table VI.3). It is possible that carbohydrates which have high nutritional value for juvenile and adult oysters (Flaak and Epifanio, 1978; Castell and Trider, 1974; Haven, 1965; Ingole, 1967: Dunathan et al., 1969) may not be as important as lipids in determining food quality of algae for larvae (Millar and Scott, 1967; Helm et al., 1973; Holland and Spencer, 1973; Holland, 1978; Waldock and Nascimento, 1979).

The amino acid composition of algal protein is remarkably similar, although differences in the quantity of total protein and free amino acids in the different algal species were observed. The principal amino acids were those amino acids which had molar percentages of about 5 or greater, i. e. alanine, aspartic acid, glutamic acid, glycine, leucine, lysine, proline, serine, threonine, valine, arginine, and taurine. The result of analyses of protein amino acids (Table VI.4) agreed with the findings reported by earlier investigations (Parsons et al. 1961; Chau et al. 1967; Cowey and Corner 1966; Epifanio 1979). The difference in nutritional quality of phytoplankton species may be due to the total amount of amino acids within the algae (Table IV.3)

Table VI.4. The total (protein + free amino acid) amino acid molar percentage composition of algal species used as food for bivalves. The data are recalculated from the listed references. + = detected but not determined, .00 = below detectability.

	Pavlova lutheri				*Isochrysis galbana*		*Pp*	*Pv*	*Chlorella* sp.
	(1)	(2)	(3)	(4)	(1)	(5)	(1)	(1)	(1)
Cysteic acid	1.29	+	.00	.00	1.29	.00	.08	.08	.04
Taurine	.67	.00	.00	.00	.62	.00	.75	.75	.04
Aspartic acid	8.38	17.07	9.36	9.33	8.36	9.49	9.74	9.76	9.24
Threonine	4.97	3.58	5.12	4.89	5.08	3.91	5.81	5.81	5.44
Serine	5.83	+	4.32	5.16	6.20	5.37	5.80	5.81	5.32
Glutamic acid	9.13	3.98	9.53	10.64	9.37	10.59	12.11	12.13	15.10
Proline	4.52	+	5.97	4.19	4.51	5.75	4.87	4.88	5.19
Glycine	9.45	5.80	8.47	5.77	9.33	10.62	9.70	9.72	9.23
Alanine	11.82	17.54	15.30	8.41	11.63	11.84	11.60	11.62	10.97
Valine	6.33	2.50	4.10	6.65	5.81	7.64	6.40	6.41	6.24
Cystine	.00	.00	.00	1.47	.00	.41	.51	.51	.40
Methionine	1.57	+	2.78	2.71	.91	1.89	.61	.61	.22
Isoleucine	4.14	2.70	4.55	4.37	3.81	5.05	3.97	3.98	4.08
Leucine	10.14	.00	8.91	10.01	9.64	9.17	8.76	8.77	8.30
Tyrosine	1.57	+	2.44	4.54	2.34	2.10	2.51	2.51	2.47
Phenylalanine	4.53	1.01	5.11	5.27	4.58	4.08	3.88	3.89	4.12
Ornithine	.13	.00	.49	.00	.37	.00	.14	.14	.13
Lysine	5.93	6.61	6.39	6.82	5.55	4.95	4.83	4.84	5.63
Tryptophan	.00	.00	.00	2.03	.01	.18	1.53	1.53	1.23
Histidine	.01	.00	1.42	2.10	1.97	1.66	1.57	1.58	1.59
Arginine	5.44	.00	5.74	5.65	5.17	5.28	4.38	4.39	4.26
γ-Aminobutyric acid	.00	.00	.00	.00	2.62	.00	.07	.07	.69
Total	95.84	60.80	100.00	100.00	99.18	100.00	99.61	99.77	99.93

1) Chapter IV; 2) Parsons, et al. 1961; 3) Chau et al. 1967; 4) Cowey and Corner 1966; 5) Epifanio 1979; 6) Walne 1970a.

Table VI.4. cont.

	Nannochloris oculata	Phaeodactylum tricornutum				Tetraselmis suecica			Dunaliella tertiolecta
	(1)	(1)	(2)	(3)	(4)	(1)	(5)	(6)	(1)
Cysteic acid	.25	1.05	+	.00	.00	.20	.00	1.17	.65
Taurine	.15	.53	.00	.00	.00	.41	.00	.00	.14
Aspartic acid	9.08	9.28	10.13	9.53	9.90	8.18	9.34	9.24	7.51
Threonine	5.29	5.42	5.95	6.24	5.21	5.00	3.76	6.41	5.27
Serine	6.00	6.69	+	4.14	6.39	5.93	5.09	6.04	7.78
Glutamic acid	12.44	10.43	8.83	12.27	11.22	12.03	11.41	9.61	11.06
Proline	5.27	2.46	+	5.45	6.62	6.82	3.99	3.45	4.26
Glycine	9.65	9.77	10.67	9.55	9.40	9.68	12.25	10.47	9.57
Alanine	11.58	10.25	11.63	16.25	9.95	10.16	12.14	13.43	12.59
Valine	5.53	6.16	4.38	5.58	8.08	4.41	7.53	6.53	5.56
Cystine	.46	.00	.00	.00	.79	1.31	.21	.00	.05
Methionine	.29	1.60	.00	1.89	1.81	.26	1.67	2.59	.88
Isoleucine	3.69	4.82	4.38	4.39	4.68	2.73	4.55	4.19	3.43
Leucine	8.86	8.69	.00	7.32	8.01	7.14	9.19	8.63	8.48
Tyrosine	2.48	2.18	.00	2.62	2.53	2.12	2.20	2.22	2.58
Phenylalanine	4.08	5.01	7.53	6.59	4.26	3.54	4.44	4.07	3.87
Ornithine	.89	1.46	.00	.00	.00	.62	.00	.00	1.42
Lysine	5.31	5.49	2.46	4.29	5.84	4.84	5.61	5.91	6.01
Tryptophan	1.76	.00	.00	.00	.00	1.12	.30	.00	.05
Histidine	1.70	1.43	.00	.58	1.54	1.62	1.76	1.73	1.84
Arginine	4.07	4.67	.00	3.31	3.79	5.57	4.57	4.31	4.37
γ-Aminobutyric acid	1.16	.00	.00	.00	.00	2.31	.00	.00	.00
Total	100.00	97.38	65.96	100.00	100.00	95.99	100.00	100.00	97.39

Both gelatin acacia and nylon-protein microcapsules were acceptable to larvae of C. virginica . Gelatin-acacia microcapsules were more digestible than the nylon protein microcapsules. Larvae fed cod liver oil encapsulated by gelatin-acacia walls grew as much as larvae fed algae until about day 11 and grew much better than the "starved" control larvae. It must be kept in mind, however, that the algal diet used was suboptimal, consisting only of Ps. paradoxa. It was found that microcapsule concentration affected growth rate. Although the fatty acid composition of cod liver oil (CLO) was found to be dissimilar to that of the CPP diet (Table II.6), CLO is rich in long chain polyunsaturated fatty acids such as 20:5w3 and 22:6w3 and thus may be desirable as a supplement for larvae cultured under hatchery conditions.

Although little correlation between nutritional value of the algae and their composition has been reported, it seems likely that lipid composition is more critical than protein or carbohydrate. The nutritional value of lipids and fatty acids for bivalves has been examined by several groups of investigators (Millar and Scott 1967; Helm et al. 1973; Holland and Spencer 1973; Holland 1978; Waldock and Nascimento 1979; Swift et al. 1980; Langdon and Waldock 1981). Researchers have demonstrated the importance of lipid in larval growth and development of oysters. Creekman (1977) reported that the lipid content of the egg and that of the resulting larvae of C. virginica were correlated and that a greater larval lipid content significantly increased larval growth, vigor, set and successful metamorphosis. Similarly, Helm et al. (1973) reported that the viability of O. edulis larvae was related to their lipid content, particularly the neutral

lipid, at the time of liberation. Holland and Spencer (1973) also indicated that accumulation of neutral lipid, from 8.8 to 23.2%, occurred during development of O. edulis larvae. Collyer (1957) indicated that initial larval glycogen content was not related to larval viability. These results support the findings that lipid supplies most of the energy requirements of larvae during periods of both growth and starvation (Millar and Scott 1967; Holland 1978). Langdon and Waldock (1981) have concluded that the w3 fatty acids (20:5w3 and 22:6w3) are essential for spat of Crassostrea gigas. Waldock and Nascimento (1979) also reported that the growth rate of C. gigas larvae was correlated with the neutral lipid content of the algal diet. Moreover, in the present study it was found that microcapsules which contained CLO rich in polyunsaturated fatty acids supported some growth of larvae of C. virginica.

Our knowledge of specific nutritional requirements of oyster larvae is still not sufficient. The optimal amount and proportions of w3 and w6 fatty acids required for larvae and the appropriate proportions of the individual fatty acids, carbohydrates, amino acids and micronutrients is uncertain. Much further work is needed to determine the essential nutrients for growth of oyster larvae and their availability in algal species. This information will not only reduce the uncertainty related to formulating a biochemically balanced artificial diet, but also will help us in understanding the metabolism of the three basic nutrients (amino acids, carbohydrates and fatty acids) in bivalve larvae.

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Born in Hong Kong, May 15, 1941. Graduated from The Chinese University of Hong Kong in July, 1965 with a major in biology, and from the University of Rochester, Rochester, New York, with a M. S. degree in Radiation Biology in 1971.

In September 1979, I enrolled in the Ph.D. program of the School of Marine Science of The College of William and Mary in Virginia. I have been employed as a Marine Scientist at the Virginia Institute of Marine Science (VIMS) from October 1974 to the present with the exception of September 1979 through December 1980 when I held a graduate assistantship in the Department of Marine Culture, (VIMS).