1977

Effect of Various Algal Diets and Larval Density in the Larviculture of the American Oyster, Crassostrea virginica (Gmelin)

Nancy T. Windsor
College of William and Mary - Virginia Institute of Marine Science

EFFECT OF VARIOUS ALGAL DIETS AND LARVAL DENSITY IN THE LARVICULTURE OF THE AMERICAN OYSTER, CRASSOSTREA VIRGINICA (GMELIN)

A THESIS

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

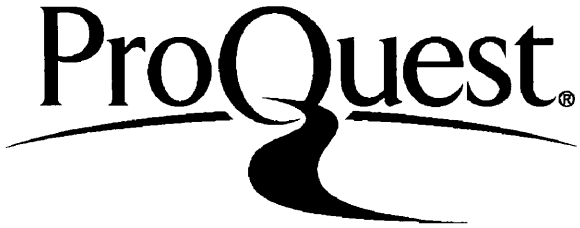
Master of Arts

---

by

Nancy T. Windsor

1977



ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Arts in Marine Science

Nancy T. Windsor

Approved, August 1977

John L. Dupuy, Ph.D.

John J. Manzi, Ph.D.

John M. Zeigler, Ph.D.

Morris H. Roberts, Ph.D.

George C. Grant, Ph.D.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The author wishes to express her appreciation to Dr. John L. Dupuy under whose guidance this study was conducted and for his support throughout the study. The author also wishes to thank Drs. John Zeigler, Morris Roberts, George Grant and John Manzi for their critical review of the manuscript. Additional thanks are due to Dr. Morris Roberts for assistance with statistics.

The author is also indebted to the entire Department of Marine Culture for providing excellent technical support with special thanks to Mr. C.E. Sutton and Ms. S.M. Blaylock. Thanks are also due to Ms. Peggy Peoples for the art work and to Ms. Laura Creekman for typing of the manuscript. My sincere gratitude and thanks to Ms. Jill Fredericksen for her interest and constructive criticism of this research.

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Larvae of the American oyster, Crassostrea virginica (Gmelin) were subjected to eighteen experimental algal diets. The test parameters in which the diets were assessed were growth rate, survival, pediveliger production and setting success. Additional experiments on the effect of larval density and algal density on the test parameters were also examined. Results indicated Pyramimonas virginica to be the most influential dietary component. The best diet was the combination of Pyramimonas virginica, Pseudoisochrysis paradoxa and Chlorella sp. An experiment on larval density recommended that for optimal culture success, larval density should not exceed five larvae per milliliter. The algal density experiment suggested that larval culture may benefit from an increase in algal density in the later larval period.

EFFECT OF VARIOUS ALGAL DIETS AND LARVAL DENSITY IN THE LARVICULTURE OF THE AMERICAN OYSTER, *CRASSOSTREA VIRGINICA* (GMELIN)

INTRODUCTION

The nutritional aspect of oyster larviculture has been studied by biologists for many years. Early investigators relied exclusively on naturally occurring food in seawater to raise their larvae (Wells, 1920; Prytherch, 1924; Cole, 1937; Hughes, 1940; and Wilson, 1941). Cole (1937) and Hughes (1940) and Wilson (1941) demonstrated that not all phytoplankton species were of equal nutritional value to the larvae of Ostrea edulis and aside from the importance of physical parameters (i.e. salinity and temperature), quantity and quality of the food available to the larvae were equally important. Oyster larviculture was greatly enhanced by the work of Bruce et al. (1940), who developed fundamental methodology using unialgal cultures as food for the larvae of Ostrea edulis. Their work added important evidence on the nutritional variability of the algal species as a food source.

These early investigations were limited to the natural spawning period of oysters and only when year-round conditioning of adults became possible did substantial advances occur in the artificial culturing of oysters (Loosanoff, 1945). Work performed by Davis (1953), Loosanoff (1945), Loosanoff et al. (1955), Walne (1956), Davis and Guillard (1958), and Loosanoff and Davis (1963) confirmed previous indications that naked flagellates were better rations for larvae, and that the

presence of cell wall thickness and/or the degree of toxicity of algal metabolites were found to be important factors in determining the suitability of such foods.

Experimenting with larvae of Mercenaria mercenaria, Loosanoff et al. (1953), showed optimal concentrations of algae used as larval food were proportional to cell densities. Walne (1965), agreed with Loosanoff and concluded that larvae of Ostrea became relatively satiated with food at much lower cell densities when cells were large than when they were small. Therefore, one must consider not only the effect of food concentration but also size of the food particle to get a realistic evaluation of the food value of an algal species for larval culture.

Though it is apparently agreed upon that phytoplankters are a major food source for larvae, many investigators have continued searching for other food sources. Imai et al. (1949, 1950) used the non-photosynthetic flagellate Monas to successfully rear oysters. Walne (1956), also experimented with a non-photosynthetic flagellate, Bodo sp. in his work with Ostrea. However, Davis (1950), reported C. virginica larvae did not appear to utilize either species. The nutritional value of bacteria is questionable. Davis (1953) found no evidence of bacteria being a food source for oyster larvae; while others suggest certain species may be used (Hidu and Tubiash, 1963). Davis (1950) evaluated several sources of detritus as possible diets and found none satisfactory. Addition of dissolved glucose or yeast extract to larval cultures proved unsatisfactory in low concentrations and greater amounts only promoted

dense bacterial blooms and high larval mortalities (Davis, 1950). Carriker (1956) reported larvae could be reared on cereal flakes but his results were inconclusive.

Of all the commercially valuable marine species the oyster has undoubtedly been the most extensively studied. Nevertheless with all the knowledge and impressive progress in culturing techniques, there are apparently still various gaps in our knowledge which hamper commercial application. An important factor determining the survival and development rate of any phytotrophic larvae is the food source which they are given. At the present time there are several species of phytoplankton being utilized as food sources at various hatcheries including: Monochrysis lutheri, Isochrysis galbana, Dunaliella sp., Cyclotella sp., Skeletonema costatum, Phaeodactylum tricornutum, and Chaetoceros sp. (Dupuy, personal communication). These species have been tested and widely accepted as "good" foods for larvae. However, more emphasis has been placed on the ease of culturing these food sources then on their nutritional value to oyster larvae. The development of a nutritionally complete diet for oyster larvae which yields a high percentage of spat would do much to improve the economic success of existing hatcheries.

The purpose of the present study was to determine the effects of various algal diets on growth, survival, production of pediveligers and setting success of oyster larvae. Four algal species have been successfully utilized as a standard diet for bivalve culture at the Virginia Institute of Marine Science

for several years (Dupuy, 1973, 1975). However, little quantitative work has been done on the nutritional value of the different algal species. All species were isolated from the York River, Virginia. The four species include two species of Chlorophyceae, Nannochloris oculata (2.0 μm) (isolated by F.D. Ott), Chlorella sp. (unidentified) (2.0 μm) (isolated by N.T. Windsor), a Prasinophyceae, Pyramimonas virginica nom. prov. (4.0 μm) (isolated by F.D. Ott) and a Haptophyceae, Pseudoisochyrsis paradoxa nom. prov. (5.0 μm) (isolated by F.D. Ott).

These four species singly and in various combinations, and Monochrysis lutheri (Droop) and Isochrysis galbana (Parke), traditional diets for oyster larvae (Davis, 1953; Davis and Guillard, 1958; Walne, 1965, 1970; Ukeles, 1969) were tested and compared.

Other objectives considered in this study included the effects of larval and algal densities on larval survival and growth. These two aspects should receive consideration when attempting to achieve "optimal" culturing conditions. Experiments were run to determine the spatial requirements of the larvae and the effect of various algal densities on the parameters of growth rate, survival, production of pediveligers and success of setting.

MATERIALS AND METHODS

Algal Culture

All algal cultures were grown in pasteurized, enriched filtered estuarine water contained in 40 liter Pyrex carboys. York River estuarine water was triple filtered through a series of Cuno Cotton® filters (10 µm, 1 µm, 1 µm). The filtered water was passed through an Aquafine Model MP-2PVC1 Ultraviolet water sterilizer. The water was heated to 82 C in a glass heat exchanger (Corning Model 135GRB) and passed directly into sterilized 40 liter carboys. The water was then enriched with N_2M medium and Guillard's vitamin mix [Table 1].

Algal cultures were held at 18 C under continuous illumination. Pyramimonas and Pseudoisochrysis were grown using a light source of 2000 lux, whereas Chlorella, Nannochloris, Monochrysis and Isochrysis grew best at 3300 lux. All cultures were aerated with gas dispersion fritted discs. Because of the enormous amounts of algal culture needed, no attempt was made to grow axenic algal cultures.

Depending on the cell counts, the amount of inoculum used to initiate the batch cultures was such as to obtain the latter part of log phase, usually reached within two weeks. Algal cells used as larval food were always harvested during log growth. If the algal culture showed any change in coloration, pH, or if frothing was noted, the culture was discarded

TABLE 1

N_2M Enrichment and Vitamin Mix for Algal Cultures

N_2M Enrichment is composed of six basic stock solutions which are prepared as follows:

(1) Sodium Silicate Solution
$Na_2SiO_3 \cdot 9H_2O$.....4.66 g
Distilled H_2O.....to 100 ml

(2) Ketchum and Redfield's Solution "A"
KNO_3.....20.2 g
Distilled H_2O.....to 100 ml

(3) Ketchum and Redfield's Solution "B"
$Na_2HPO_4 \cdot 7H_2O$.....3.0 g
$CaCl_2 \cdot 2H_2O$.....2.7 g
$MgSO_4$ (anhyd.)...2.9 g
$FePO_4$.....0.5 g
HCl (conc.).....2.0 ml
Distilled H_2O.....to 100 ml

(4) Sodium Molybdate Solution
$Na_2MoO_4 \cdot 2H_2O$.....0.0119 g
Distilled H_2O.....to 100 ml

(5) Arnon's Micronutrient Solution (modified)
H_3BO_3.....0.286 g
$MnCl_2 \cdot 4H_2O$.....0.181 g
$ZnSO_4 \cdot 7H_2O$.....0.022 g
$CuSO_4 \cdot 5H_2O$.....0.0079 g
$CoCl_4 \cdot 6H_2O$.....0.004 g
Distilled H_2O..to 100 ml

(6) Soil Extract
One kilogram of top soil was mixed with 2 liters of distilled water and sterilized in a large flask 1 hour at 15 lbs pressure. After allowing to settle, the liquid was decanted, passed through a Whatman No. 1 filter paper.

The N_2M Enrichment is made up of the above six stock solutions as follows:

Sodium Silicate Solution...100 ml
Ketchum and Redfield's Solution "A".....200 ml
Ketchum and Redfield's Solution "B".....100 ml
Sodium Molybdate Solution.....50 ml
Arnon's Micronutrient Solution (modified)...50 ml
Soil Extract.....200 ml

The Vitamin Mix is made up as follows:

Thiamine HCl.....200 mg
Biotin..... 1 mg
B_{12}..... 1 mg
Distilled H_2O.....to 100 ml

The N_2M Enrichment is distributed 2 ml per 1 liter of sea water and the Vitamin Mix is added at the rate of 1 ml per liter of sea water in preparing algal cultures.

and the cells were not used as larval food.

The feeding criteria were based on cell volumes. The cell volume for each species was calculated by using a Model TA Coulter-Counter [Table 2]. Mean algal densities were determined from replicate counting on a hemacytometer. Algal counts were done every other day on the cultures being used as food. Tables 3 and 4 show the feeding schedules for the experimental diets and the control.

For experimental treatments single species diets comprised the daily total cell volumes of 690×10^9 μm^3 and 1380×10^9 μm^3. With the multiple species the total cell volumes were equally divided amongst the species fed to a given larval culture. For example, in the diet consisting of the species P. paradoxa and P. virginica, each species contributed 345×10^9 μm^3 in Stage I and 690×10^9 μm^3 in Stage II out of a total of 690×10^9 μm^3 and 1380×10^9 μm^3 respectively.

All possible combinations of the four locally isolated species were tested as food for oyster larvae. In addition as previously mentioned, diets of Monochrysis and Isochrysis were compared to the VIMS' Protocol (control). The eighteen diets were divided into 4 groups which could be tested simultaneously with available facilities [Table 5]. The VIMS' Protocol was also run with each group as a control (Dupuy, 1975). This was done not only for comparison purposes but also to judge the quality of the batches of larvae used throughout the experiments.

TABLE 2

Volumes of Individual Algal Cells

Pyramimonas virginica (Va-17)	33.50 μm^3
Chlorella sp. (Va-52)	4.85 μm^3
Nannochloris oculata (Va-19)	5.58 μm^3
Pseudoisochrysis paradoxa (Va-12)	47.70 μm^3
Monochrysis lutheri	73.50 μm^3
Isochrysis galbana	57.80 μm^3

TABLE 3

Feeding Schedule for Experimental Diets

A sequential feeding program for all diets was used

LARVAL SIZE RANGE	TOTAL CELL VOLUME FED DAILY
Stage I - 70 μm--120 μm	690×10^9 μm^3
Stage II - 120 μm--300 μm	1380×10^9 μm^3

The algal culture volumes to be fed are then calculated with the equation:

$$F = \frac{V_f}{V_a \times C_s}$$

where:

F = milliliters of one species of algal culture to be fed per 250 liter larval tank per day

V_f = theoretical volume required of one algal species per larval tank

V_a = known volume of a single algal cell of one species

C_s = number of cells per ml of algal culture

TABLE 4

The Sequential Feeding Schedule for the Protocol Diet

LARVAL SIZE RANGE	SPECIES	TOTAL CELL VOLUME FED	TOTAL COMBINATION CELL VOLUME FED
70 µm -- 100 µm	Va-17*	300×10^9 µm^3	690×10^9 µm^3
	Va-19	390×10^9 µm^3	
100 µm -- 120 µm	Va-17	390×10^9 µm^3	690×10^9 µm^3
	Va-19	300×10^9 µm^3	
120 µm -- 300 µm	Va-17	390×10^9 µm^3	1380×10^9 µm^3
	Va-19	300×10^9 µm^3	
	Va-12	690×10^9 µm^3	

The algal culture volumes to be fed are then calculated with the equation

$$F = \frac{V_f}{V_a \times C_s} \quad **$$

* see Table 2

** see Table 3

TABLE 5

Experimental Diets

Group 1

Diet 1	Pyramimonas virginica
Diet 2	Pseudoisochrysis paradoxa
Diet 3	Nannochloris oculata
Diet 4	Chlorella sp.
Diet 5	P. virginica - P. paradoxa - N. oculata - Chlorella
	VIMS' Protocol

GROUP 2

Diet 6	P. paradoxa - N. oculata
Diet 7	P. paradoxa - Chlorella sp.
Diet 8	P. virginica - Chlorella sp.
Diet 9	N. oculata - Chlorella sp.
Diet 10	P. virginica - P. paradoxa
Diet 11	P. virginica - N. oculata
	VIMS' Protocol

GROUP 3

Diet 12	P. virginica - P. paradoxa - Chlorella sp.
Diet 13	P. virginica - P. paradoxa - N. oculata
Diet 14	P. paradoxa - Chlorella sp. - N. oculata
Diet 15	P. virginica - N. oculata - Chlorella sp.
	VIMS' Protocol

GROUP 4

Diet 16	Monochrysis lutheri
Diet 17	Isochrysis galbana
Diet 18	M. lutheri - I. galbana
	VIMS' Protocol

Larval Culture

Oyster broodstocks were obtained from the Rappahannock River. Six different broods were used to complete the experiments. During the months when ambient temperatures were too low for natural gonadal development oysters were conditioned in the laboratory over a 4-8 week period by temperature manipulation and supplementary feeding of cornstarch (Haven, 1965; Dupuy, 1975; Creekman, 1977) and Tetraselmis suecica (Helm et al., 1973).

Water pumped from the York River was heated by a Karbate heat exchanger (Union Carbide Corp; Material System Division, Cleveland, Ohio). The heated water was cascaded in a system of two flumes (4.1 meters by 0.6 meters) and aerated through a series of airstones to insure removal of excess gases from the heated water (Dupuy and Rivkin, 1972; Malouf et al., 1973). Flow rates were adjusted to give each oyster 10 liters of estuarine water per hour (Galtsoff, 1964).

Desired temperature regimes for the conditioning flumes were obtained by mixing heated water and ambient water. Oysters were gradually acclimated to 22 C by raising the flume water temperature 6-8 C per week (or 1 C/day). Oysters held at 22 C usually produced mature gametes within 4-6 weeks. A starch suspension (0.48 g/oyster/day) was metered by a Milroyal metering pump to the conditioning flume at the rate of 30 ml per minute. Tetraselmis suecica was also metered to the flumes at the same rate from an aerated stock bottle with a cell density of 1.0×10^6 cells/ml.

Three to six oysters from the broodstock were sacrificed

weekly to determine the stage of gonadal development. When histological samples of the oysters were found to contain sufficient mature gametes, spawning was initiated within a few days. The method used to induce spawning was similar to that described by Dupuy (1975). Approximately twenty-five oysters were placed in a conditioning flume with running unfiltered estuarine water. The water temperature was gradually increased from 22 C to 30 C during the first hour. This increase allowed the oysters to pump and eliminate fecal material before spawning commenced. As a further inducement to spawn, sperm stripped from a ripe male was added to the water. Spawning usually occurred within the first few hours. Spawning oysters were removed from the flume and placed into individual spawning jars (4 liters) containing 30 C estuarine water filtered to 1 µm. After spawning, all eggs were pooled and screened through a stainless steel sieve (100 µm) to remove feces and shell debris. Portions of sperm suspensions from each spawned male were also pooled and used for fertilization. Mass crosses of at least six oysters were used for each experiment to help insure a large gene pool and viable larvae. After fertilization, eggs were resuspended in 10 liters of filtered water from which two 1 ml samples were drawn, transferred to Sedgewick-Rafter cells and counts made. Once the zygote density was known, an appropriate volume of zygote suspension was placed into two 250 liter fiberglass larval tanks (conical shaped, 1.30 m x 0.55 m) to obtain a final concentration of 50 zygotes per ml. Holding zygotes at this density yielded straight-hinge larvae within 24 hours at 25 C. Straight-hinge larvae

larvae were collected on a 50 µm sieve and washed off the sieve with filtered water into a 10 liter calibrated container filled with filtered water. Larvae were counted by the same method used for zygotes except the samples were preserved with 5% formalin. If the total larval populations were below one million a 1 liter volumetric flask was used to achieve a more accurate count.

An initial population of 2.5×10^6 larvae (10 larvae/ml) was chosen for all diet and control experiments (Davis and Guillard, 1958; Loosanoff and Davis, 1963; and Dupuy, 1975). The culture water was changed three times a week using a sieve of an appropriate size to retain all the larvae. At each change, population counts were made on each culture. Samples of each culture were taken daily and 50 larvae were measured (anterior-posterior length) using an ocular micrometer. During the course of the experiments the salinity varied 14 to 19 $^{o}/oo$. All larval cultures were maintained at 27-28 C.

The available facilities necessitated the establishment of a time schedule to compare the performance of each diet in the production of pediveligers and the success of setting. Pediveligers were harvested for three days after they first appeared in a culture. They were removed by screening the entire population through a 280 µm sieve (which is the minimum size for pediveligers). The pediveligers were then placed in a 1 liter volumetric flask and replicate sample aliquots were removed and counted. The larvae were transferred to a setting tray module and allowed to set on frosted Mylar sheets

(Dupuy, 1975). The setting tray module consists of a fiberglass tray (25 1/2 inches x 20 1/2 inches x 4 inches), a fiberglass liner and a Mylar sheet. The liner aids in holding down the sheet and helps to prevent larvae from escaping the area designated for setting. The trays were filled with 15 liters of filtered estuarine water, aerated with two airstones and fed 100 ml of the appropriate larval culture diet daily. The trays were checked for set and the filtered estuarine water was changed daily. Sheets were changed if the set was heavy. Pediveligers from each experimental and control culture were given six days to set. The number of pediveligers that set from each culture container (i.e. experimental or control) was determined by subtracting the number of pediveligers remaining at the end of six days from the total number of pediveligers added at the beginning of the setting period.

Given the large initial population and the time required to evaluate each diet for the parameters chosen, it was decided that it would be adequate to test each diet only once since the repeatability of the VIMS' Protocol used as a control had been demonstrated many times (Dupuy, personal communication).

After determination of the "best" larval diet on a qualitative basis, experiments with larval and algal densities as variables were performed. Densities of one, three, five, seven, and ten larvae per ml were tested. The same test parameters used in the diet determinations were measured in these experiments. Once an optimal larval density was chosen various algal densities were fed to the oyster larvae (normal algal

density, double algal density, double algal density - 1/2 the volume per 12 hours, triple algal density, triple → 1/2 the volume per 12 hours, normal to double algal density after day 6 of culture and double to triple algal density after day 6 of culture). The effect on test parameters previously described was determined.

RESULTS

Diet Experiments

Growth Curves

Figures 1 through 4 illustrate the effect of the different diets upon larval growth rates. Regression curves and their slopes for each experimental diet are also presented in Appendix A. Growth measurements were taken until the first pediveligers were removed from the larval cultures.

The data presented in Figure 1 shows that within the Group 1 series, the quadruple diet and the single diet of Pyramimonas yielded a growth rate almost identical to that of the VIMS' Protocol (Control). It should be noted that the curves for Nannochloris and Chlorella represent larval growth at one half the total cell volumes. When Nannochloris and Chlorella were fed to the larvae at the identical volume as the other experimental foods almost complete mortality was seen by day 6. Therefore, referring back to Table 3, the total cell volumes for Stage I and II were 345×10^9 μm^3 and 690×10^9 μm^3 respectively.

The diets within the Group 2 series reinforce the conclusion that Nannochloris and Chlorella are inadequate larval foods. It is interesting to note diets containing Pyramimonas in any combination gave growth curves similar to or better than the Protocol (Control). Diet combinations containing

FIGURE 1

Growth Curves of the Experimental Diets
and the VIMS' Protocol Diet
within Group 1 Series

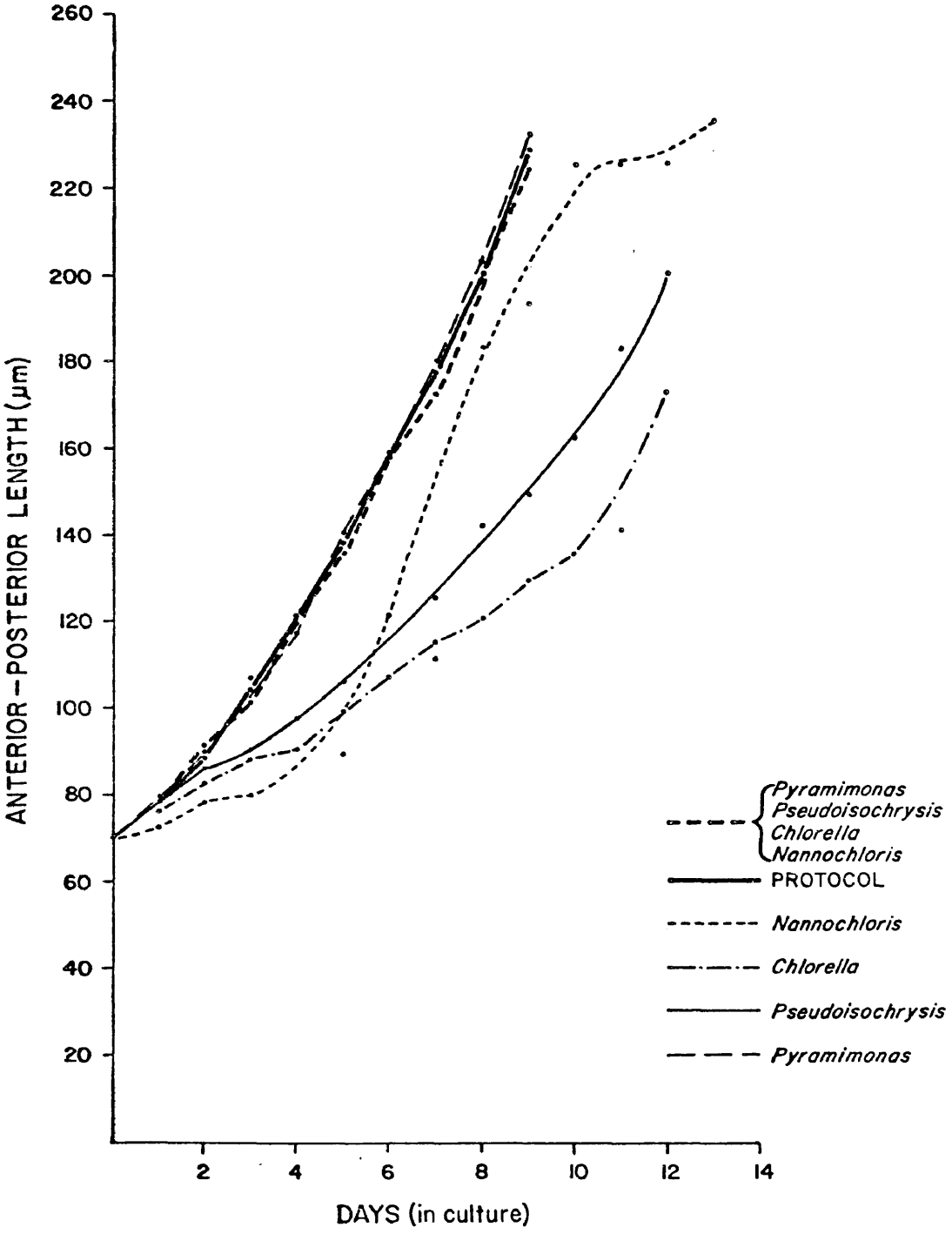

260
240
220
200
180
160
140
120
100
80
60
40
20
ANTERIOR-POSTERIOR LENGTH (μm)
2
4
6
8
10
12
14
DAYS (in culture)
Pyramimonas
Pseudoisochrysis
Chlorella
Nannochloris
PROTOCOL
Nannochloris
Chlorella
Pseudoisochrysis
Pyramimonas

FIGURE 2

Growth Curves of the Experimental Diets
and the VIMS' Protocol Diet
within Group 2 Series

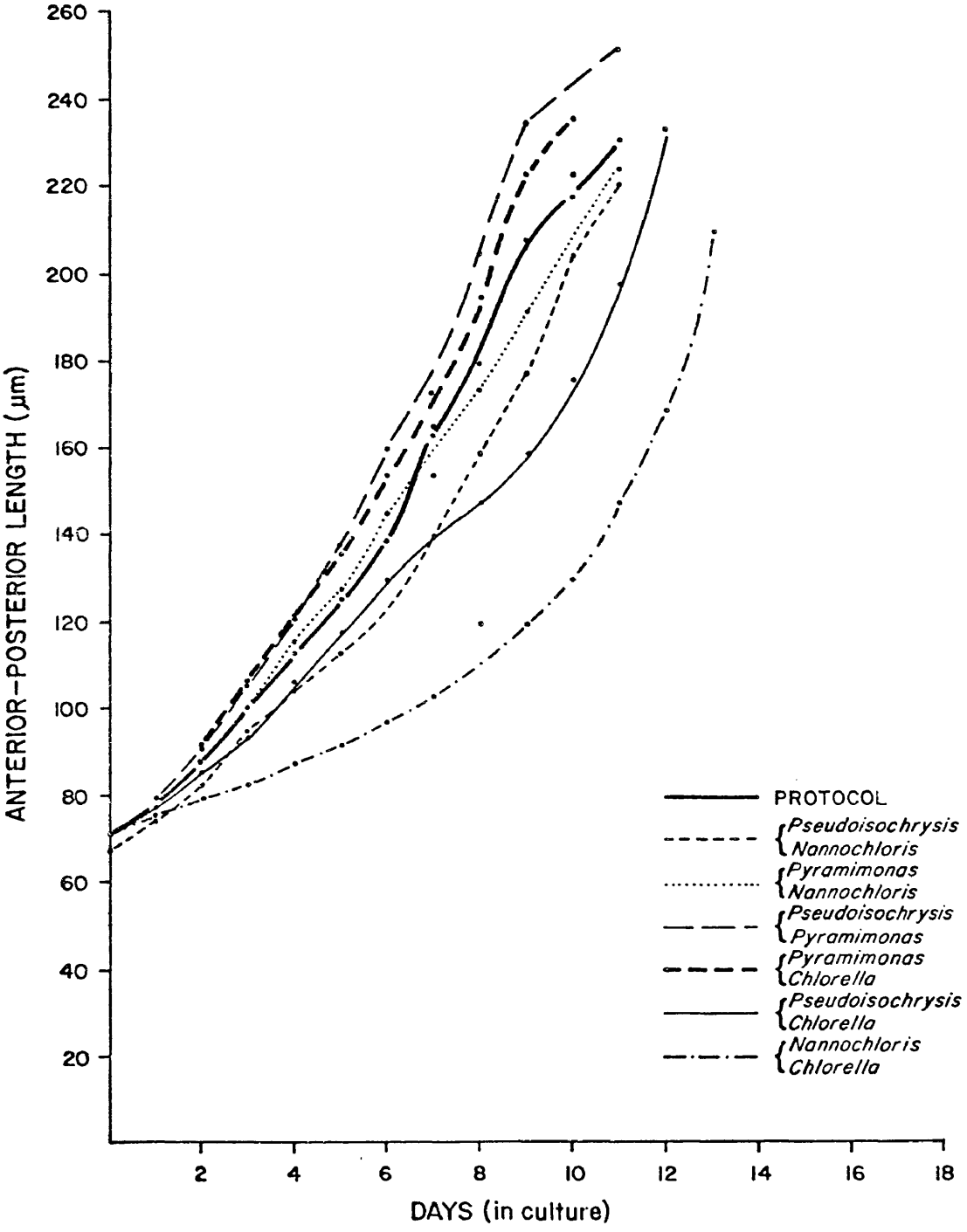

260
240
220
200
180
160
140
120
100
80
60
40
20
ANTERIOR–POSTERIOR LENGTH (μm)
2
4
6
8
10
12
14
16
18
DAYS (in culture)
PROTOCOL
Pseudoisochrysis
Nannochloris
Pyramimonas
Nannochloris
Pseudoisochrysis
Pyramimonas
Pyramimonas
Chlorella
Pseudoisochrysis
Chlorella
Nannochloris
Chlorella

FIGURE 3

Growth Curves of the Experimental Diets
and the VIMS' Protocol Diet
within Group 3 Series

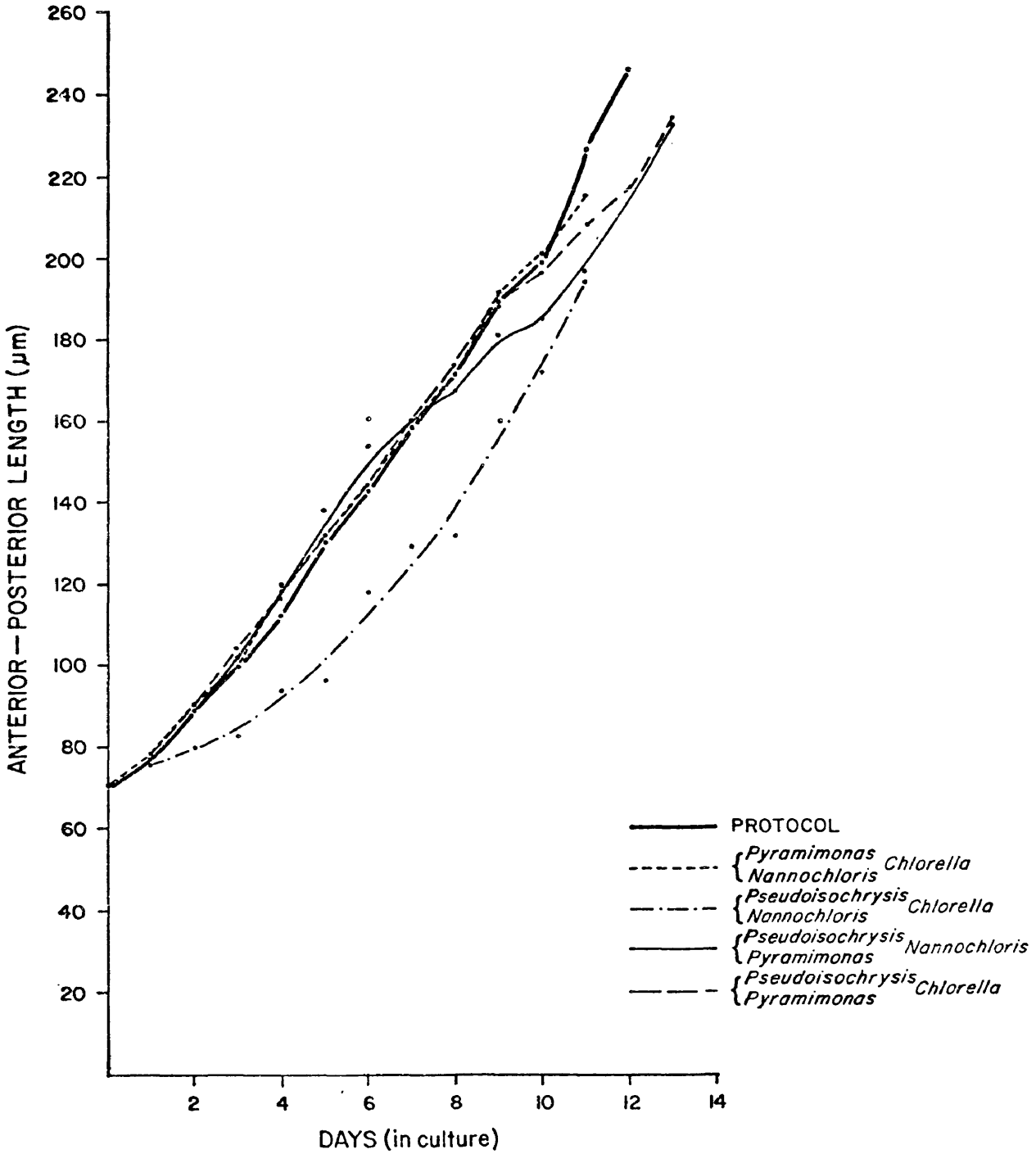
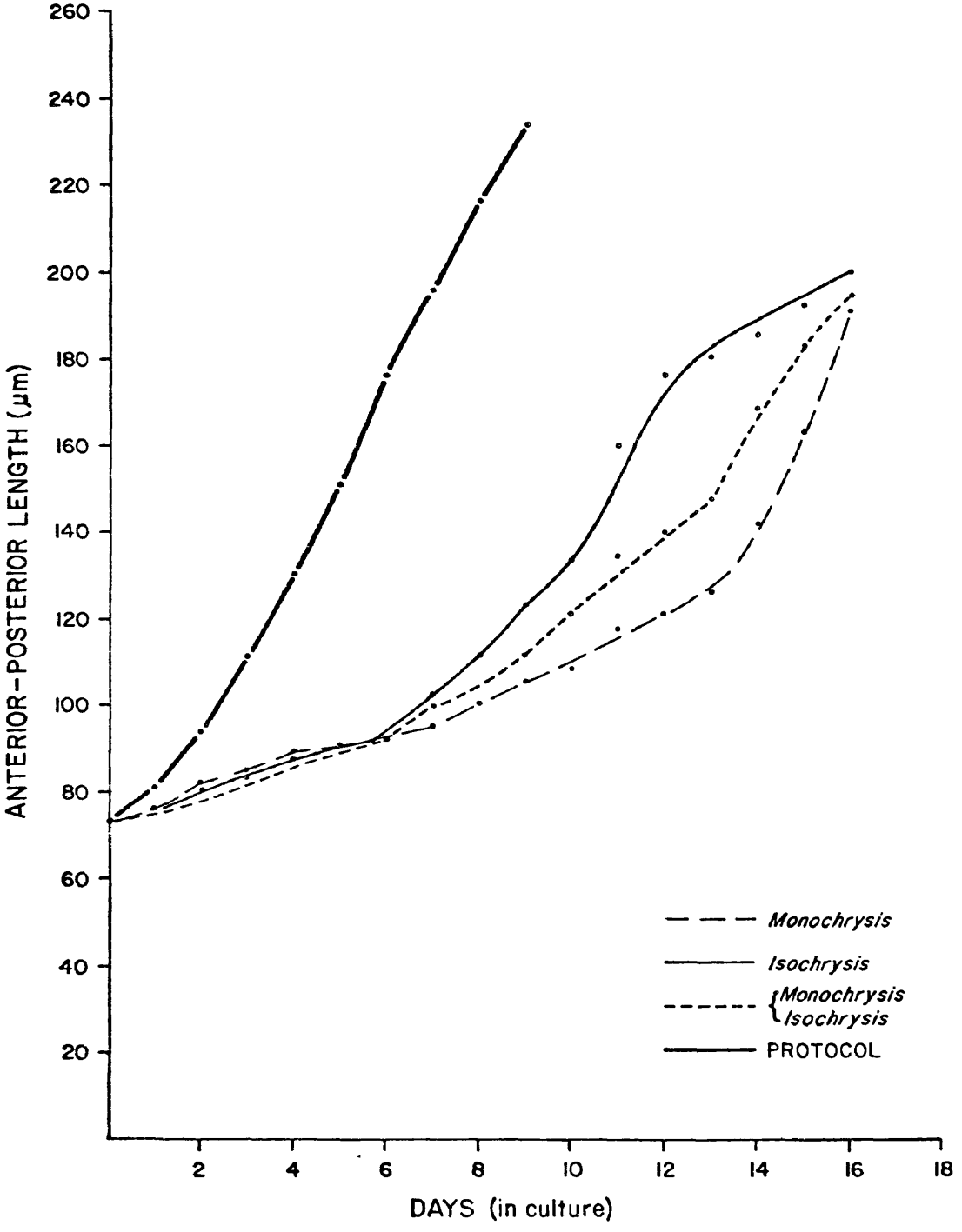

260
240
220
200
180
160
140
120
100
80
60
40
20
ANTERIOR—POSTERIOR LENGTH (μm)
2
4
6
8
10
12
14
DAYS (in culture)
PROTOCOL
Pyramimonas Nannochloris Chlorella
Pseudoisochrysis Nannochloris Chlorella
Pseudoisochrysis Pyramimonas Nannochloris
Pseudoisochrysis Pyramimonas Chlorella

FIGURE 4

Growth Curves of the Traditional Diets
and the VIMS' Protocol Diet
within Group 4 Series


260
240
220
200
180
160
140
120
100
80
60
40
20
ANTERIOR-POSTERIOR LENGTH (μm)
2
4
6
8
10
12
14
16
18
DAYS (in culture)
Monochrysis
Isochrysis
Monochrysis
Isochrysis
PROTOCOL

Pseudoisochrysis were only slightly poorer than those of Pyramimonas [Figure 2].

The series of triple diets within Group 3 support the conclusion that Pyramimonas is the best species of those tested as food for oyster larvae since the diet without Pyramimonas had a slower growth rate [Figure 3].

The data presented in Figure 4 shows the growth rates of the traditional larval diets, Monochrysis and Isochrysis singly and in combination versus the Protocol (Control). It is clearly shown that Monochrysis and Isochrysis are poor foods for larval growth. It is interesting that the growth rate of the combination of Monochrysis and Isochrysis is almost exactly half way between the single diets. It appears Isochrysis may be the determining factor in the combination.

Yield Parameters

Survival of the cultures to early pediveliger, production of pediveligers, and setting success within each group are compared graphically in Figures 5 through 8. These parameters are expressed as percentages from which the data can be seen in Appendix B.

Nannochloris and Chlorella gave poor results for survival and subsequently low percentages of pediveligers and set [Figure 5] Pyramimonas, Pseudoisochrysis and the "Quad" diets were similar in survival rates but differed in pediveliger production. Protocol (Control) had the highest survival rate but the Pyramimonas diet gave slightly better overall yields.

The survival rates for Group 2 were all low [Figure 6],

FIGURE 5

Percentages of Survival, Pediveliger Production, and Set of the Diets within Group 1 Series

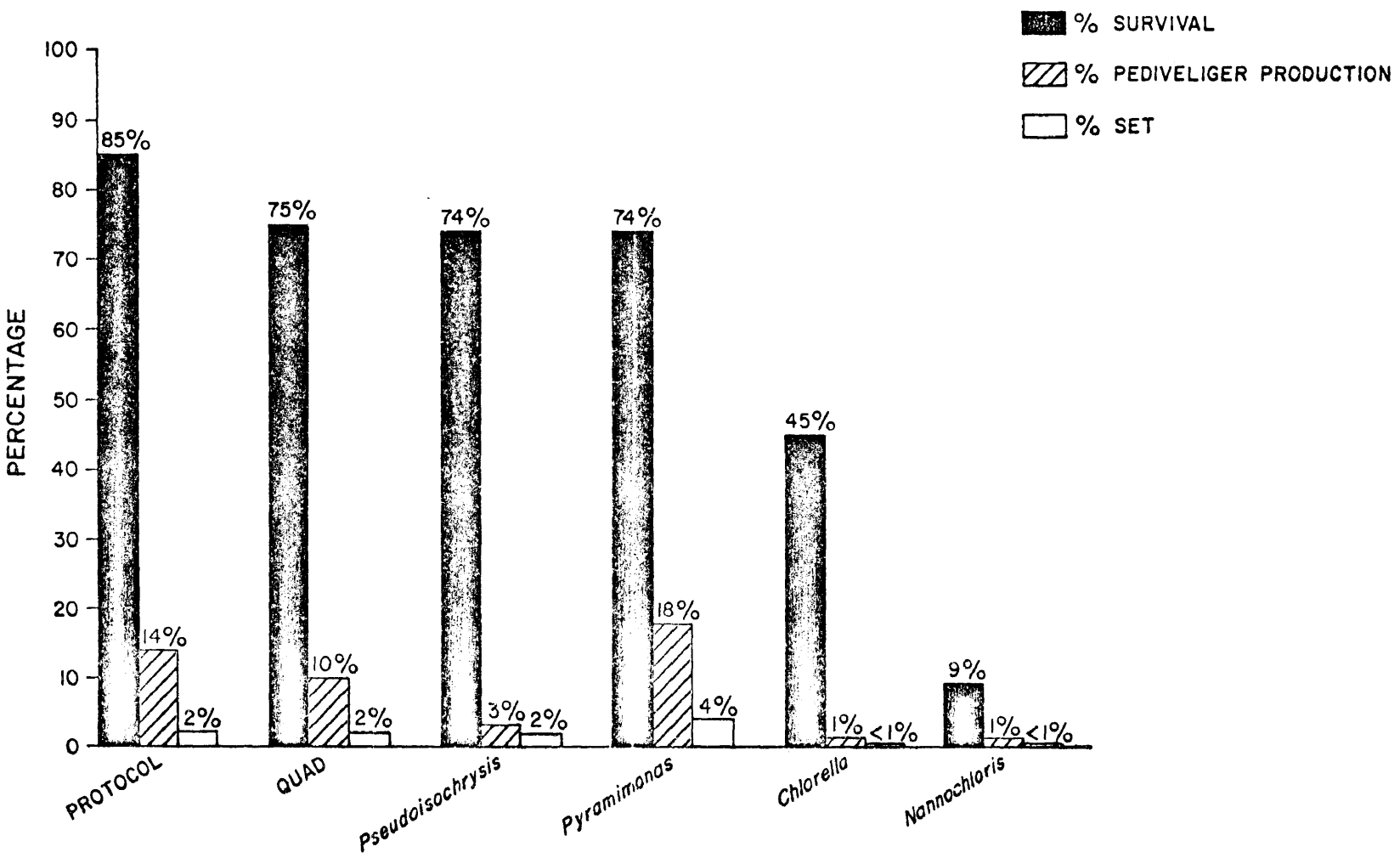

% SURVIVAL
% PEDIVELIGER PRODUCTION
% SET
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
85%
14%
2%
75%
10%
2%
74%
3%
2%
74%
18%
4%
45%
1%
<1%
9%
1%
<1%
PROTOCOL
QUAD
Pseudoisochrysis
Pyramimonas
Chlorella
Nannochloris

FIGURE 6

Percentages of Survival, Pediveliger Production, and Set of the Diets within Group 2 Series

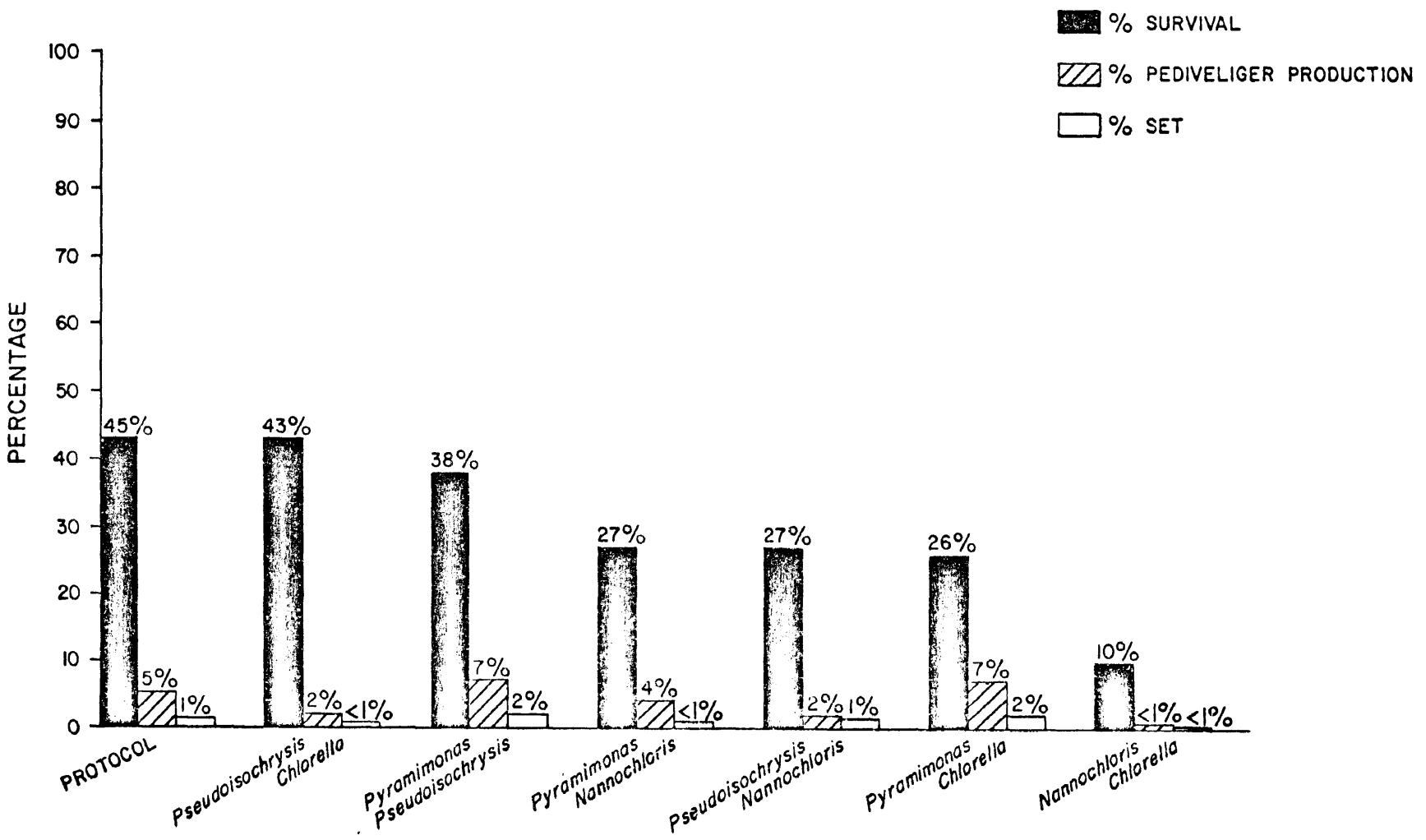

% SURVIVAL
% PEDIVELIGER PRODUCTION
% SET
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
45%
5%
1%
43%
2%
<1%
38%
7%
2%
27%
4%
<1%
27%
2%
1%
26%
7%
2%
10%
<1%
<1%
PROTOCOL
Pseudoisochrysis Chlorella
Pyramimonas Pseudoisochrysis
Pyramimonas Nannochloris
Pseudoisochrysis Nannochloris
Pyramimonas Chlorella
Nannochloris Chlorella

FIGURE 7

Percentages of Survival, Pediveliger Production, and Set of the Diets within Group 3 Series

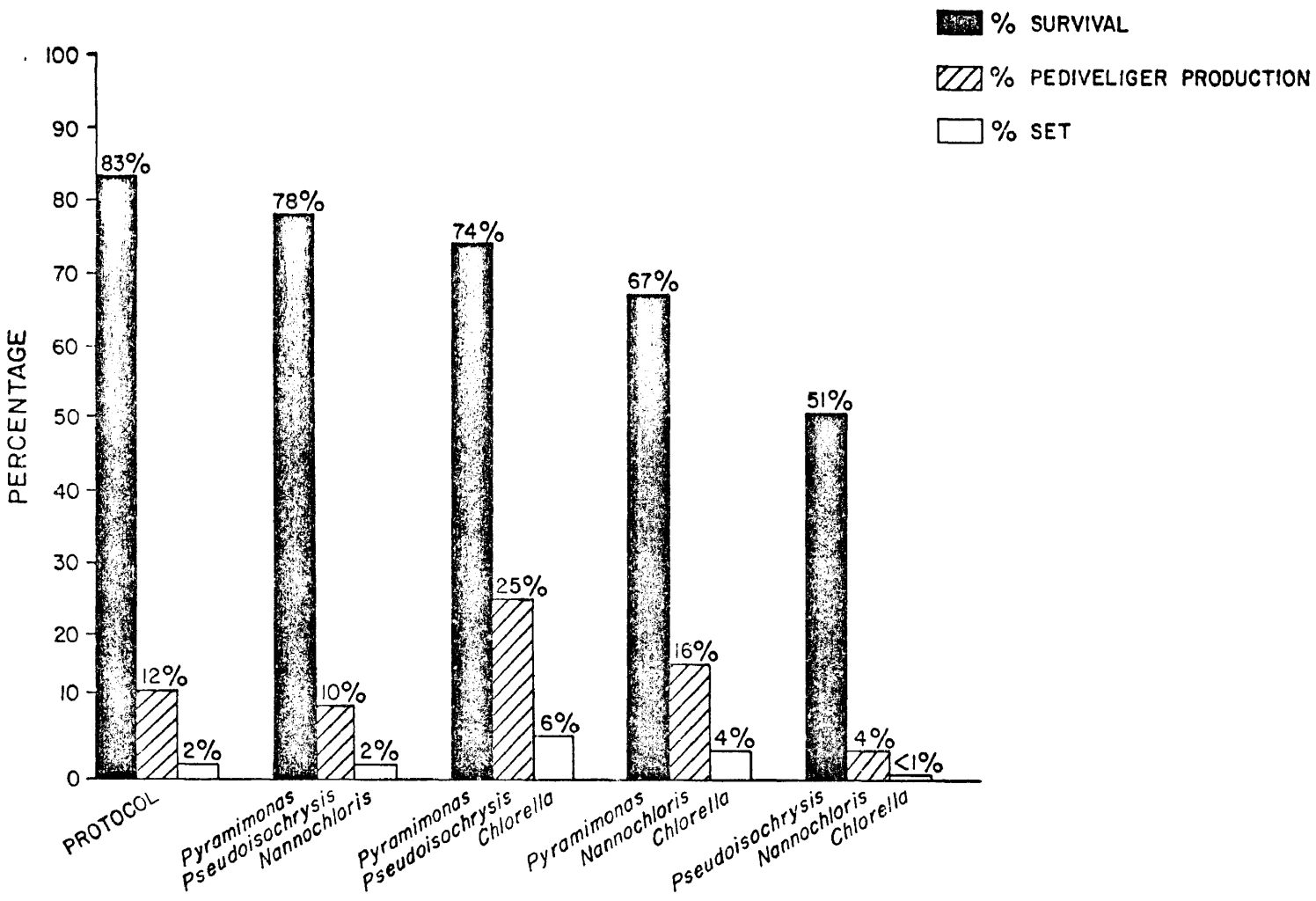
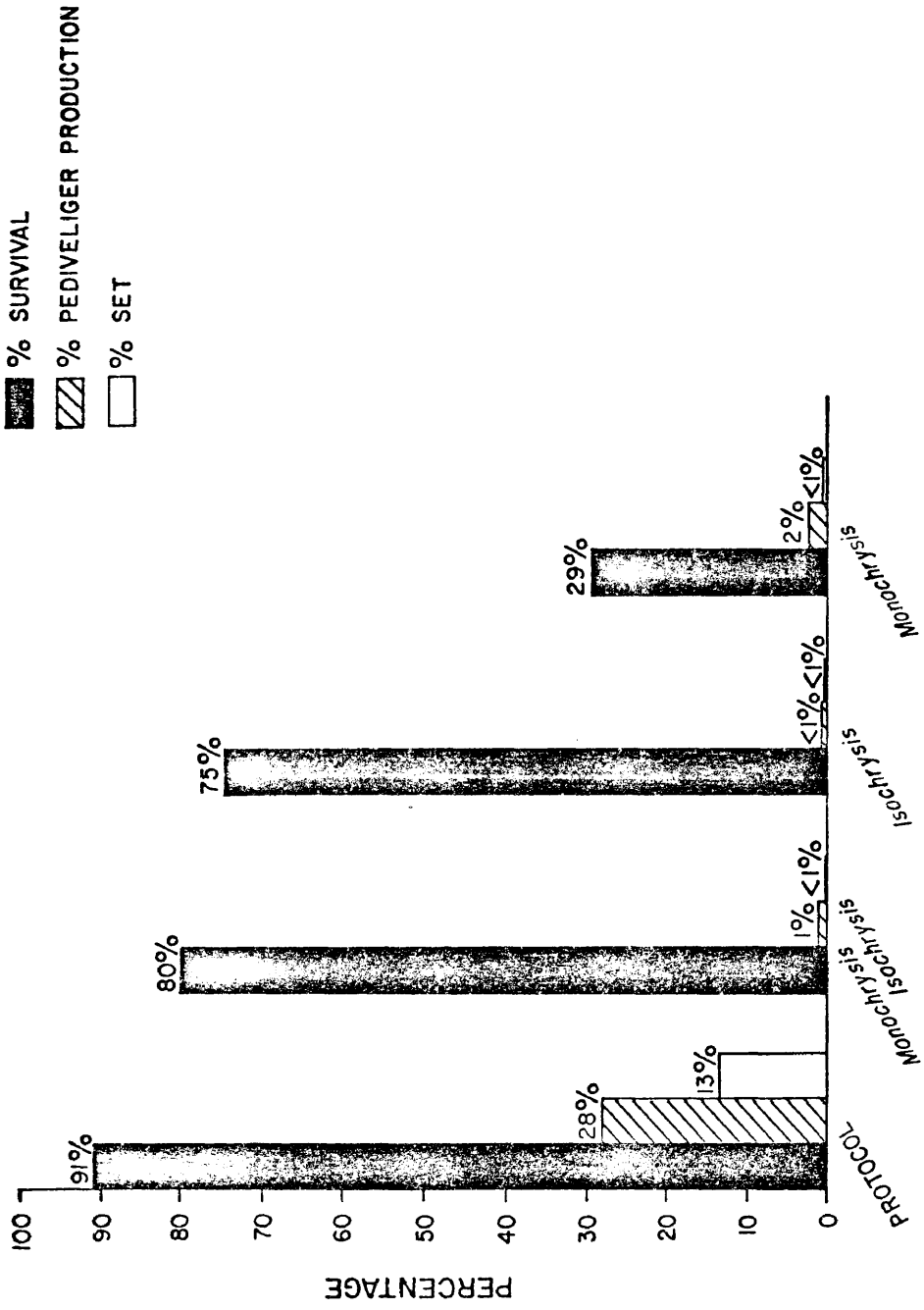

% SURVIVAL
% PEDIVELIGER PRODUCTION
% SET
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
83%
12%
2%
78%
10%
2%
74%
25%
6%
67%
16%
4%
51%
4%
<1%
PROTOCOL
Pyramimonas
Pseudoisochrysis
Nannochloris
Pyramimonas
Pseudoisochrysis
Chlorella
Pyramimonas
Nannochloris
Chlorella
Pseudoisochrysis
Nannochloris
Chlorella

FIGURE 8

Percentages of Survival, Pediveliger Production, and Set of the Diets within Group 4 Series

FIGURE 8

Percentages of Survival, Pediveliger Production, and Set of the Diets within Group 4 Series


% SURVIVAL
% PEDIVELIGER PRODUCTION
% SET
PERCENTAGE
100
90
80
70
60
50
40
30
20
10
0
91%
28%
13%
80%
1%
<1%
75%
<1%
<1%
29%
2%
<1%
PROTOCOL
Monochrysis Isochrysis
Monochrysis Isochrysis
Monochrysis

which may be a reflection of the quality of the larvae used in this experiment. It is apparent that diets which contained Pyramimonas rank highest in yields of pediveligers and set.

Another indication of the beneficial quality of Pyramimonas as a dietary component can be seen in Group 3. Althought the diet, Pyramimonas, Pseudoisochrysis and Chlorella did not have the highest survival rate, the diet yielded a significantly larger number of pediveligers and set when compared to the other diets within Group 3.

Monochrysis, Isochrysis and their combination gave low yields of pediveligers and set [Figure 8]. This supports the conclusion that they are poor foods for oyster larvae. The Protocol (Control) within this group had extremely high yields in all parameters. This indicates an exceptionally good larval brood compared to Groups 1, 2 and 3 Controls.

The results of Chi-Square Contingency Tests among treatments within each group are listed in Table 6. The poor results of Nannochloris and Chlorella as larval foods influence the degree of difference within Group 1 in all the parameters. In Group 2 the significant differences among treatments were effected by the poor survival rate of the entire series, and by the results of the diet Nannochloris and Chlorella. Group 3 results reflect the treatment differences of Pyramimonas, Pseudoisochrysis and Chlorella to the other triple diets and Protocol (Control). The results of Group 4 reflect the significant differences between treatments of the Protocol and the traditional diets, Monochrysis and Isochrysis.

TABLE 6

Chi-Square Contingency Tests For

Group 1, Group 2, Group 3, and Group 4

Experimental Diets

Diet Group	Degrees of Freedom	Total Larval Survival	Total Pediveliger Production From Survival Population	Total Set of Pediveligers
1	5	11.8×10^{6}**	1.9×10^{5}**	3.1×10^{3}**
2	6	4.7×10^{5}**	3.4×10^{5}**	4.8×10^{2}**
3	4	7.2×10^{5}**	3.2×10^{5}**	3.4×10^{4}**
4	3	2.5×10^{6}**	1.0×10^{6}**	6.7×10^{3}**

$\alpha = 0.05$

** = highly significantly different

The VIMS' Protocol diet gave the highest survival rate in all groups; however, in choosing the "best" diet all parameters (growth, survival, pediveliger production and setting success) had to be considered. Therefore, the "best" diet was that which within its group surpassed the performance of Protocol. Based on this criterion the triple diet, Pyramimonas, Pseudoisochrysis, and Chlorella was chosen. This diet was utilized for all subsequent larval and algal density experiments.

Larval Density Experiment

The data illustrated in Figure 9 show the effect of larval densities on growth rates. Growth rates differed towards the end of the larval period. The growth rate of the denser cultures began to tail off after day 6, while the cultures with the least amount of larvae showed very little lag in growth. Regression curves and their slopes for each larval density cultures can be seen in Appendix A.

Survival, pediveliger production and set percentages can be seen in Table 7. Parameter percentages were greatly influenced by larval density. with the lowest percentages in the cultures with the highest larval densities (7-10 larvae/ml). The data from which the percentages were obtained can be seen in Appendix B.

The results of Chi-Square Contingency tests among treatments (larval densities) are listed in Table 8. The significant differences among treatments were effected by the low percentages of the dense cultures.

Densities of 3, 5, and 7 larvae per milliliter were re-

FIGURE 9

Growth Curves of Cultures at Various Larval Densities

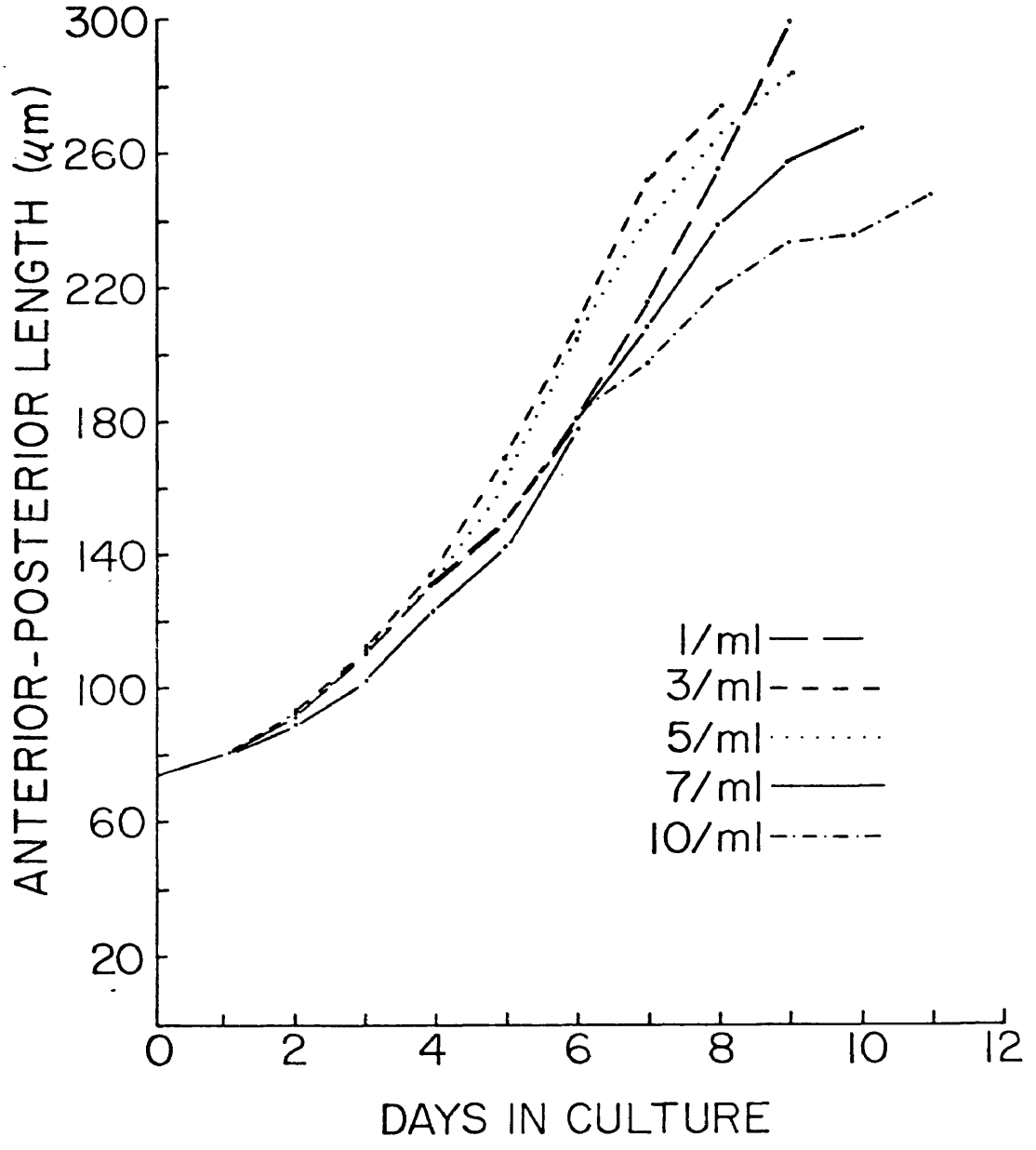

300
260
220
180
140
100
60
20
ANTERIOR-POSTERIOR LENGTH (μm)
0
2
4
6
8
10
12
DAYS IN CULTURE
1/ml
3/ml
5/ml
7/ml
10/ml

TABLE 7

Influence of Larval Density

Percent Survival, Percent Pediveliger Production, and Percent Set

Larvae/ml	1	3	3 Replicate	5	5 Replicate	7	7 Replicate	10
Total % Larval Survival	100.00	99.6	93.3	82.4	78.0	63.8	64.3	77.5
Total % Pediveliger Production From Survival Population	85.5	95.2	94.8	95.9	92.4	78.8	75.6	25.6
Total % Set of Pediveligers	99.8	91.2	89.6	89.2	87.4	45.5	43.1	19.1

TABLE 8

Chi-Square Contingency Tests For

Larval Density Experiment

Experiment	Degrees of Freedom	Total Larval Survival	Total Pediveliger Production From Survival Population	Total Set of Pediveligers
Larval Densities	4	4.6×10^{5}**	2.1×10^{6}**	1.2×10^{6}**

α = 0.05

** = highly significantly different

plicated therefore allowing analysis of variance tests to be performed on the yield parameters after transformation to arcsin [Table 9]. All F values ($\alpha = 0.05$) were significant, therefore Tukey's Multiple Comparison Tests were run on the treatment means [Table 10]. In percent survival, there was no significant difference between 5 and 7 larvae per ml. and 3 and 5 larvae per ml., however, there was significant difference between 3 and 7 larvae per ml. In percent pediveligers produced there was no significant difference between 3 and 5 larvae per ml., but 7 larvae per ml. was significantly different from 3 and 5 larvae per ml. Finally in percent set there was no significant difference between 3 and 5 larvae per ml. but significant difference was seen between 5 and 7 larvae per ml.

The population size distributions of the larval density experiment are shown in Figure 10. Larval densities appear to influence the size range of the larval population. The size distributions of the lower larval densities show more larvae in the upper size range than the more densely populated cultures.

Algal Density Experiment

The effect of various algal densities on larval growth can be seen in Figure 11. Regression curves and their slopes can be found in Appendix A. The larval density for this experiment was 3 larvae per ml. The growth rates were very similar until day 6, however, at this point the cultures receiving the normal density, and triple density began to lag

TABLE 9

Analysis of Variance on percent survival, percent pediveliger production, and percent set at the three different larval densities (3, 5, and 7 larvae/ml)

Analysis of Variance with Arcsin Transformation

% Survival	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Ratio
	Among Treatments	2	772.2	386.1	16.6*
	Within Treatments	3	69.7	23.2	
	Total	5	841.9		

% Pediveliger Production	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Ratio
	Among Treatments	2	306.0	153.0	38.6**
	Within Treatments	3	11.9	3.96	
	Total	5	317.9		

% Set	Source of Variation	Degrees of Freedom	Sums of Squares	Mean Square	F Ratio
	Among Treatments	2	1141.77	570.88	378.07**
	Within Treatments	3	4.54	1.51	
	Total	5	1146.31		

* significant at the 0.05 level

TABLE 10

Tukey's Multiple Comparison Test between treatment means of the three different larval densities (3, 5, and 7 larvae/ml)

$(\bar{x} - \bar{x}') \pm T\sqrt{MS_w}$ $\quad T = \frac{1}{\sqrt{N}} \cdot q_{(1-\alpha)(r-n)}$ $\quad T = 4.18$

$\alpha = 0.05$

% Survival

$T \cdot \sqrt{MS_w} = 20.13$

3 larvae/ml	5 larvae/ml	7 larvae/ml
$\bar{x} = 80.68$	$\bar{x} = 63.61$	$\bar{x} = 53.16$

% Pediveliger Production

$T \cdot \sqrt{MS_w} = 8.32$

3 larvae/ml	5 larvae/ml	7 larvae/ml
$\bar{x} = 77.08$	$\bar{x} = 76.16$	$\bar{x} = 61.49$

% Set

$T \cdot \sqrt{MS_w} = 5.13$

3 larvae/ml	5 larvae/ml	7 larvae/ml
$\bar{x} = 71.96$	$\bar{x} = 69.92$	$\bar{x} = 41.72$

FIGURE 10

Population Size Distribution of the Larval Density Experiment

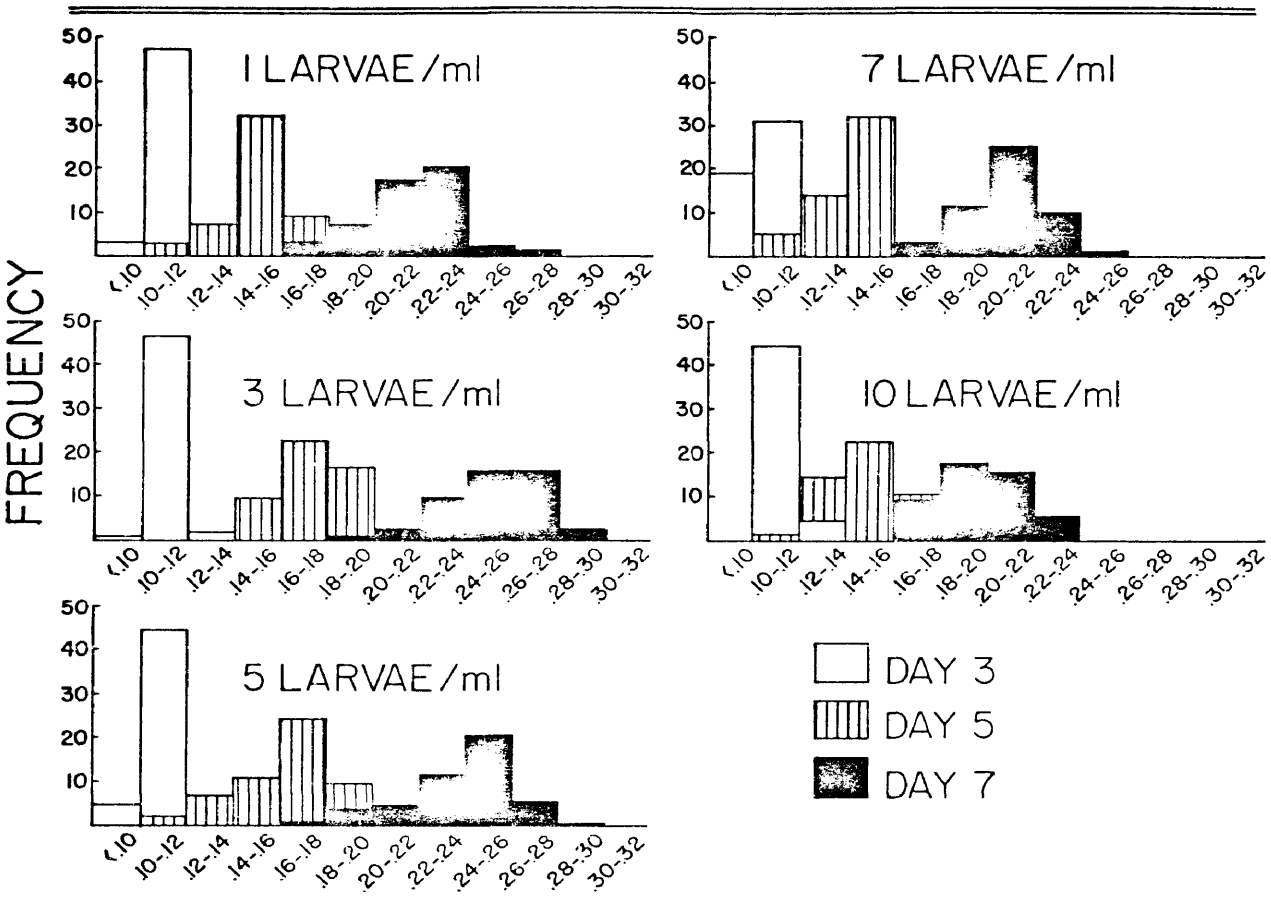
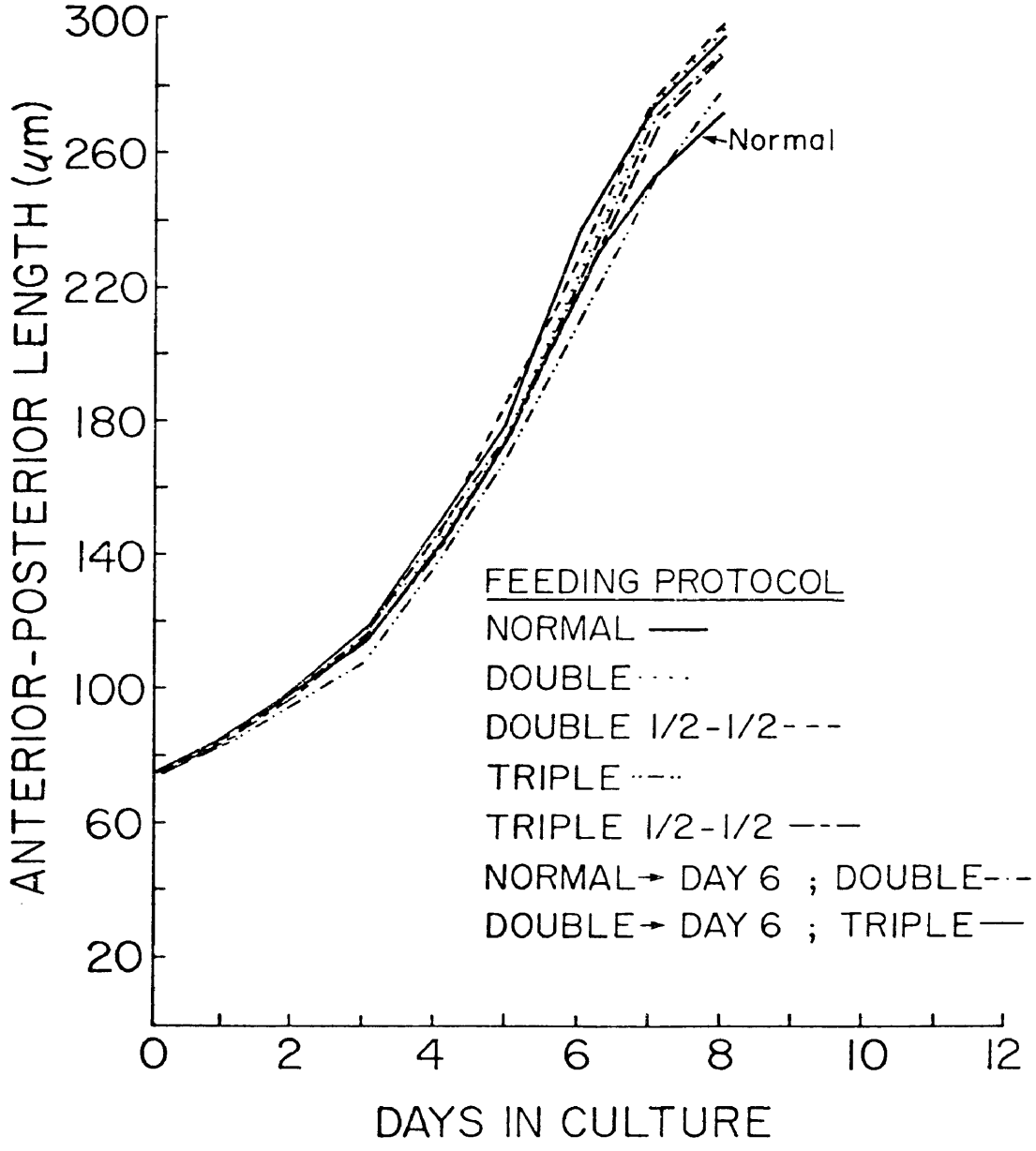

1 LARVAE/ml
7 LARVAE/ml
3 LARVAE/ml
10 LARVAE/ml
5 LARVAE/ml
FREQUENCY
50
40
30
20
10
<.10
.10-.12
.12-.14
.14-.16
.16-.18
.18-.20
.20-.22
.22-.24
.24-.26
.26-.28
.28-.30
.30-.32
DAY 3
DAY 5
DAY 7
ANTERIOR-POSTERIOR LENGTH (mm)

FIGURE 11

Growth Curves of Cultures at
Various Algal Densities


300
260
220
180
140
100
60
20
ANTERIOR-POSTERIOR LENGTH (μm)
Normal
FEEDING PROTOCOL
NORMAL ——
DOUBLE ····
DOUBLE 1/2-1/2 - - -
TRIPLE ·—·—·
TRIPLE 1/2-1/2 — — —
NORMAL→ DAY 6 ; DOUBLE- - -
DOUBLE→ DAY 6 ; TRIPLE——
0
2
4
6
8
10
12
DAYS IN CULTURE

behind the remaining cultures. Perhaps the larval culture receiving algal cells at the normal density was not receiving enough food and the culture being fed the triple density was getting too much thus interfering with the feeding rate. The cultures which were fed additional amounts of food (i.e. normal density to day 6 -- double density) showed better growth rates. This might indicate a need for additional food towards the end of the culturing period.

The percentages of the yield parameters are listed in Table 11 and the data from which the percentages were obtained can be seen in Appendix B. Setting was less successful in the experiment because larvae escaped under the setting tray inserts and were trapped under the Mylar sheets.

A Chi-Square Contingency test was also performed on the algal density experiment. Significant differences were found in all test parameters [Table 12].

Population size distributions of larval cultures at all algal densities can be seen in Figure 12. There is very little difference between the distributions.

TABLE 11

Influence of Algal Density on

Percent Survival, Percent Pediveliger Production,

and Percent Set

Diet Pyramimonas Pseudoisochrysis Chlorella	Total % Larval Survival	Total % Pediveliger Production From Survival Population	Total % Set of Pediveligers
Normal Algal Density*	96.8	90.0	70.0
Double Algal Density	92.3	93.9	65.2
Double Density - 1/2 per 12 hours	88.6	96.0	75.6
Triple Algal Density	82.2	92.9	74.8
Triple Density - 1/2 per 12 hours	95.6	95.4	72.8
Normal Density to day 6 - Double Density	96.7	97.0	67.5
Double Density to day 6 - Triple Density	100.0	95.5	67.1

TOTAL CELL VOLUME FED

* Normal Algal Density { 690×10^9 µm^3 Stage I; 1380×10^9 µm^3 Stage II } per 24 hours

Double Algal Density $2 \times$ { 690×10^9 µm^3 Stage I; 1380×10^9 µm^3 Stage II } per 24 hours

Double Density - 1/2 per 12 hours { 690×10^9 µm^3 Stage I; 1380×10^9 µm^3 Stage II } per 12 hours

TABLE 12

Chi-Square Contingency Tests For

Algal Density Experiment

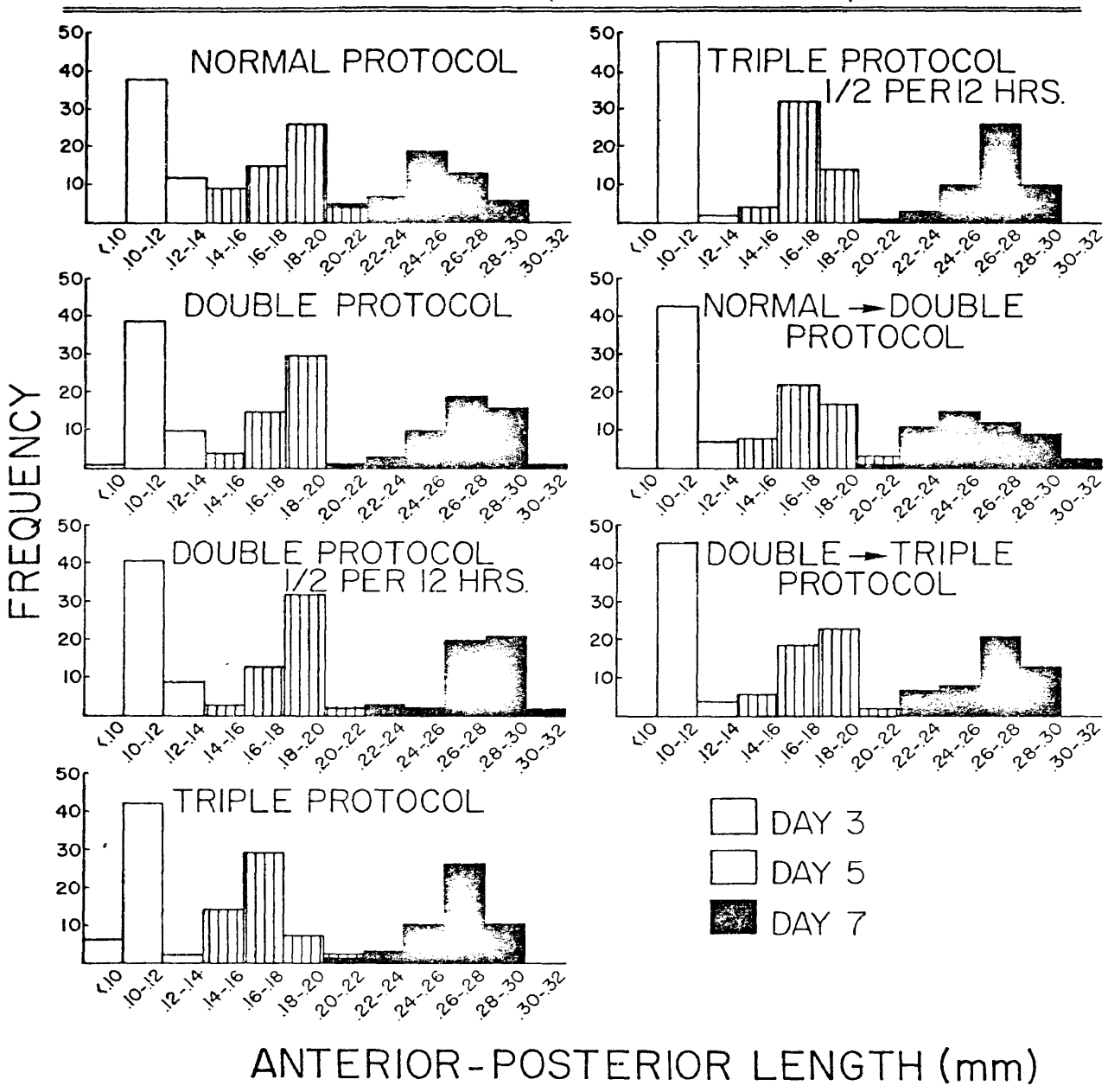
Experiment	Degrees of Freedom	Total Larval Survival	Total Pediveliger Production From Survival Population	Total Set of Pediveligers
Algal Densities	6	4.7×10^{4}**	4.7×10^{4}**	3.6×10^{6}**

$\alpha = 0.05$

** = highly significantly different

FIGURE 12

Population Size Distribution of the Algal Density Experiment


NORMAL PROTOCOL
TRIPLE PROTOCOL 1/2 PER 12 HRS.
DOUBLE PROTOCOL
NORMAL → DOUBLE PROTOCOL
DOUBLE PROTOCOL 1/2 PER 12 HRS.
DOUBLE → TRIPLE PROTOCOL
TRIPLE PROTOCOL
FREQUENCY
50
40
30
20
10
<.10
.10-.12
.12-.14
.14-.16
.16-.18
.18-.20
.20-.22
.22-.24
.24-.26
.26-.28
.28-.30
.30-.32
DAY 3
DAY 5
DAY 7
ANTERIOR-POSTERIOR LENGTH (mm)

DISCUSSION

The results of this investigation not only illustrated the effect of different algal diets on larval growth rates, but also showed the influence of diet on the production of pediveligers and success of setting.

Limited comparisons can be made when contrasting this study to former investigations (Davis, 1950, 1953; Davis and Guillard, 1958; Loosanoff et al., 1963; and Walne, 1965, 1966, 1970). For the most part all previous work was in an assay type design where a diet was tested for a short period of time. Investigators used various culturing vessels and it has since been shown that vessel size influences growth rates (Dupuy, 1973). Parameters such as temperature, salinity and feeding regimes differed greatly. One can not always equate the British work involving the European oyster, Ostrea edulis to studies with Crassostrea virginica, because of the variations in methodology and culturing techniques. Therefore, only generalized statements can be made. These nutritional investigations relied almost entirely on growth rate to judge the value of algal species as food for oyster larvae. Although growth is an important factor this study suggests that it is not always an adequate indicator for setting success.

In determining whether or not an algal species is a satisfactory food for oyster larvae, all aspects must be considered. For example, in Figure 1, the diet Nannochloris

exhibits a fair growth rate, but further analysis of larval response (Figure 5) shows a low percentage of survival, pediveligers and set. In this case such a misleading growth curve was due to a mortality of the smaller larval class sizes towards the end of the larval period. This eliminates the small larvae from the daily measurements thus causing a large jump in the mean size of the remaining population.

There seems to be no correlation between the three yield parameters, however, they do influence one another. When considering the relationship between survival and pediveligers produced, survival only affects the number of larvae available to become pediveligers. The same can be said for setting success. In general a high survival rate yields a higher pediveliger count which in turn yields a higher set.

Ukeles *et al*. (1969) using an estimation of the mouth size of *Ostrea edulis* (Yonge, 1960) approximated the mouth size of a *Crassostrea virginica* larvae (measuring 78 µm x 67 µm) at less than 10 µm. Yonge (1926), Millar (1955) and Fretter and Montgomery (1968) proposed that there is no selection of food particles being swept into the mouth other than by size limitation. However, Mackie (1969) states that oyster larvae qualitively selected algal cells ranging from 1 µm to 30 µm. All algal species tested in the present study were within this size range. It is interesting to note that the smaller size foods (*Nannochloris* and *Chlorella*) gave poor results. Because the feeding protocol was based on total cell volumes the larval cultures were exposed to high densities of these species. Ukeles *et al*. (1969) concluded that larval feeding was con-

tinuous and retention time in the larval gut was inversely related to algal density. Therefore, considering the small cell size and the presence of a cell wall, the short retention time at high algal densities is inhibitive to cell digestion. By decreasing algal density the gut retention time was lengthened and algal cells were more readily utilized.

Another aspect of assimilation which may play a role in utilization of different algal cells is the digestibility of the cell (Dean, 1958). Since retention time of the larval gut is so short the better foods are those which are assimilated most quickly. This of course would explain the observation that naked flagellates are better foods than species which possess thick cell walls. The present study has shown that not only is this true but there is also a difference among the naked flagellates tested. The superior species is Pyramimonas. Pyramimonas is not a classical laboratory "weed organism". It is temperamental and its cells are quite fragile. There is difficulty in growing the species in mass culture if physical and chemical parameters such as light, pH, salinity and temperature are not closely controlled. And yet such fragility may be a dietary advantage which makes Pyramimonas more easily digested in the larval gut.

Aside from the physical attributes, each algal species may also contribute different chemical constituents to the diet. These nutrients may determine whether the species is a good or poor food. Biochemical examination of those algal species which promote fast growth and yield impressive set, would give necessary insight into the nutritional needs of

larvae in culture. The few analyses of phytoplankton composition may be misleading in their homogeneity (Parson et al., 1961). Analyses that consider only total protein, fats and carbohydrates are of little value in giving nutritional direction when other important factors such as pigments, vitamins, trace metals and most importantly the quality and ratios of cell constituents are disregarded.

The larvae may be exposed to the nutrients by the algae in two ways, intracellularly and extracellulary. The literature in this area is rather incomplete because much of the biochemical data deals with macroalgae. In the biochemical work that has been done on phytoplankton, methodology is not uniform, results are inconclusive and therefore it is difficult to obtain complete chemical information on a single species. Because very limited biochemical analysis has been done on the VIMS' algal species, the literature can only serve as a guideline.

Craigie et al. (1967), Craigie (1974) investigated the storage products of various algal species. Among the species examined were a Nannochloris sp., a Chlorella sp., a Pyramimonas sp. and Monochrysis lutheri. Nannochloris sp. was found to store mannitol, unusual in the Chlorophyceae where the normal storage product is sucrose (as was found in Chlorella sp.). Pyramimonas sp. also stores mannitol whereas Monochrysis was found to store glycerol. Monochrysis is considered unique because it accumulates a cyclitol, 1,4/2,5-cyclohexanetetrol. Isochrysis also produces this substance but does not retain it. Preliminary results indicate a significant difference in the

amount of protein among the VIMS' algal species but few differences were noted in the amino acid ratios making up the protein component (Chu and Dupuy, personal communication). Concerning free amino acids, Pyramimonas virginica has a higher mole percent of glycine than the other VIMS species. Pseudoisochrysis is relatively higher in arginine. Chlorella sp. contains trytophas whereas Pyramimonas and Pseudoisochrysis do not. Nannochloris has a high amount of glutamic acid. On-going lipid analyses of these species tentatively show different ratios of fatty acids which may prove to be an important dietary factor.

Extracellular products must also be considered since it has been established by Davis and Chanley (1956) that oyster larvae are capable of taking up dissolved organics from the culture water.

A Pyramimonas sp. gave the highest percent carbon excreted in a total of twenty-three species examined by Hellebust (1965). Seventy percent of the total percent carbon was released in the form of mannitol. Mannitol is then not only stored but also secreted by Pyramimonas. Pyramimonas also secretes peptides but no proteins. Hellebust (1965) also found that Chlorella sp. releases amino acids. Droop (1968) found Monochrysis to release vitamin B_{12} binding substances thus deactivating vitamin B_{12} and both Monochrysis and Isochrysis release 1,4/2,5-cyclohexanetetrol.

All cultures of algal species tested had some degree of bacterial contamination associated with them. Therefore a list of extracellular components would not be complete without

considering the bacteria. The food value of bacteria is questionable as mentioned previously. Whether or not the larvae gain any nutritional benefit from them is not known, however, Burkholder (1963), stated that many marine bacteria produce excess vitamins which perhaps enrich the algal culture. This in turn may be advantageous to the larvae. Pyramimonas virginica was noted (Sutton, personal communication) to have the richest bacterial flora and perhaps this increased the dietary value of this species. Bruce and Duff (1967) noted that Isochrysis galbana produced and released an antibacterial substance. This may have contributed to its rather poor performance as a larval diet.

Although it seems Pyramimonas alone is an adequate diet, most nutritionists believe that a more varied diet has a better chance of meeting the dietary needs. With this arrangement one species can compensate for the deficiencies of another. The combination containing Pyramimonas virginica, Pseudoisochrysis paradoxa and Chlorella sp. was chosen as the best diet from the results. The three species may differ enough chemically to include all necessary nutritional requirements for oyster larvae whereas each species alone may not. For example, tryptophan is found only in Chlorella, so although this species has been shown a rather poor food by itself, it might be of some benefit in a mixed species diet.

Effect of Larval Density and Algal Density

It has been well documented in the literature that the higher the larval density the lower the growth rate (Davis,

1950; Matthiessen and Toner, 1966). The present study supports this conclusion. Davis (1950) and Matthiessen and Toner (1966) believed there are three possible explanations for this phenomenon. Frequent collisions may occur within dense cultures, thus hampering feeding. Secondly, accumulation of larval excretory products may inhibit growth and thirdly, possible competition for food in the later larval stages may also retard growth. An experiment done in conjunction with this problem eliminates the third possibility. Two cultures, one containing 10 larvae per ml. and the other containing 3 larvae per ml. were run simultaneoulsy. The larval culture with the density of 3 larvae/ml. was fed the normal amount of algae (690×10^9 μm^3 - 1380×10^9 μm^3). To allow for the same amount of algal cells per larvae, the denser culture was given three times the amount of algae. Little improvement was seen in the growth rate (10 larvae/ml.), therefore it is believed that the tailing off in higher larval density cultures may be attributed to physical interference and/or a build up of excretory products.

It is evident from the results of the larval density experiment that densities not only influence larval vigor - growth but also affect the production of pediveligers and set. To insure high yields of pediveligers and set it is recommended that larval cultures be held to densities of five larvae or less per milliliter.

The experiments concerning algal densities were performed to determine whether an increase in food would enhance the yields of pediveligers and set. Little advantage can be seen in overfeeding. The results do suggest that a higher quantity

of algae is needed in the last few days of culture. Further work is necessary for an optimal feeding sequence.

The experiments done in this investigation were meant to improve upon existing larviculture methods. The results have produced an exceptional algal diet which consistently promotes high yields of set. The ancillary experiments have brought to light other factors influencing culture success, the most important of which is an optimal larval density.

Groups were tested separately because of space limitations. Therefore, between-group analysis was not possible. Future workers should endeavor to run all experimental work wimultaneously, if feasible, to eliminate such variables as salinity fluctuations and broodstock quality.

CONCLUSIONS

1. Algal diets not only affect growth rates of the larvae of *Crassostrea virginica* but also influence the production of pediveligers and the success of setting.
2. In the eighteen diets tested, *Pyramimonas virginica* was found to be the most important and successful dietary component.
3. The best diet was that consisting of the three species, *Pseudoisochrysis paradoxa*, *Pyramimonas virginica*, and *Chlorella* sp.
4. Larval densities of three to five larvae per milliliter are considered optimal.
5. Varying algal densities had little effect on culture success. However, results indicated additional food for larvae in the latter stages of culture could be beneficial.

APPENDIX A

Regression Curves and Slopes with 95% Confidence Intervals of the Larval Populations

Experimental Diets and Their Slopes

		Slope (log µm/day)
Group 1		
Diet 1	*Pyramimonas virginica*	0.055
Diet 2	*Pseudoisochrysis paradoxa*	0.035
Diet 3	*Nannochloris oculata*	0.051
Diet 4	*Chlorella* sp.	0.029
Diet 5	*P. virginica* - *P. paradoxa* - *N. oculata* - *Chlorella* sp.	0.053
Group 2		
Diet 6	*P. paradoxa* - *N. oculata*	0.045
Diet 7	*P. paradoxa* - *Chlorella* sp.	0.041
Diet 8	*P. virginica* - *Chlorella* sp.	0.050
Diet 9	*N. oculata* - *Chlorella* sp.	0.033
Diet 10	*P. virginica* - *P. paradoxa*	0.051
Diet 11	*P. virginica* - *N. oculata*	0.047

Experimental Diets and Their Slopes

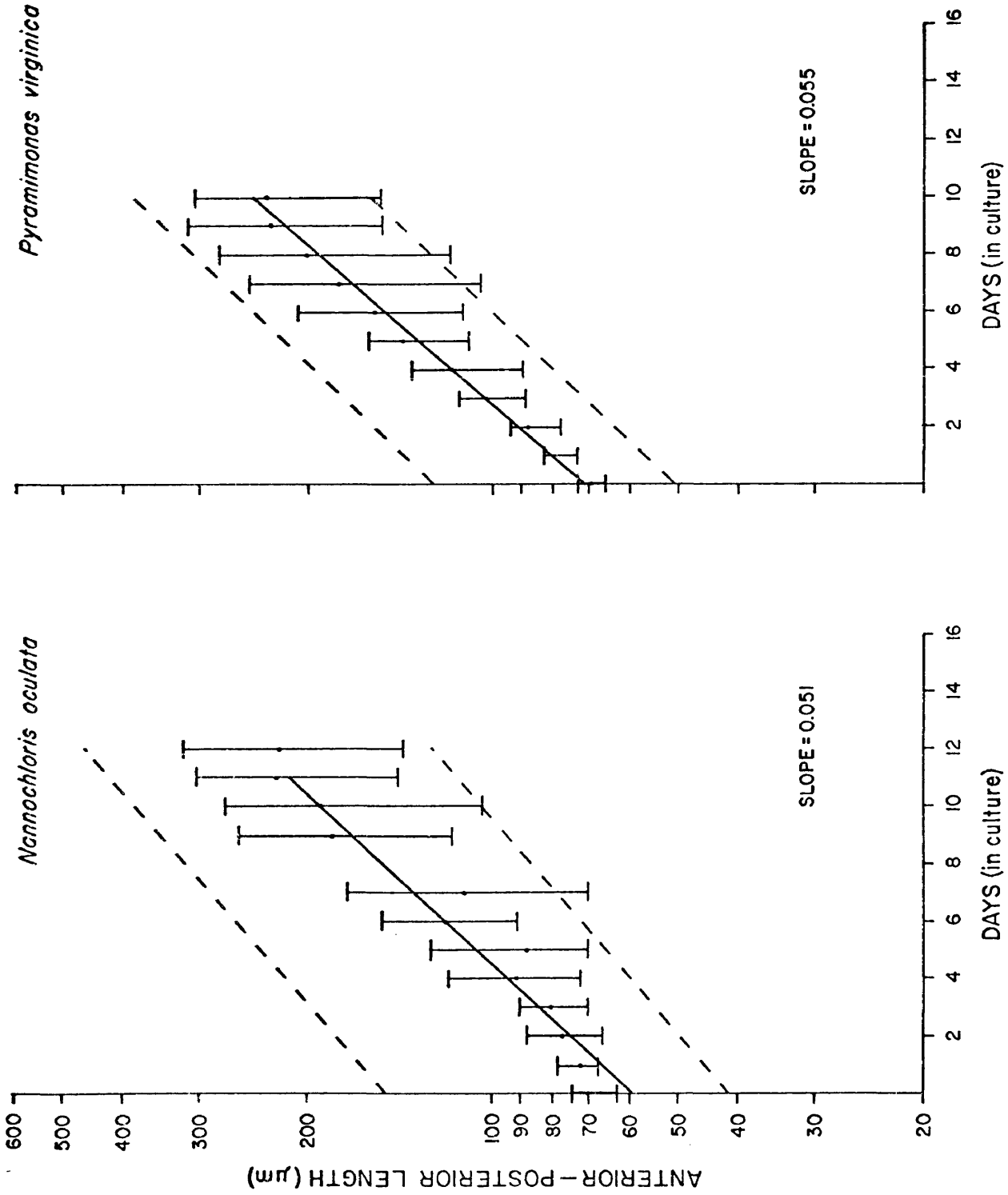
		Slope (log µm/day)
Group 3		
Diet 12	*P. virginica* - *P. paradoxa* - *Chlorella* sp.	0.040
Diet 13	*P. virginica* - *P. paradoxa* - *N. oculata*	0.037
Diet 14	*P. paradoxa* - *Chlorella* sp. - *N. oculata*	0.040
Diet 15	*P. virginica* - *N. oculata* - *Chlorella* sp.	0.043
Group 4		
Diet 16	*Monochrysis lutheri*	0.025
Diet 17	*Isochrysis galbana*	0.029
Diet 18	*M. lutheri* - *I. galbana*	0.028

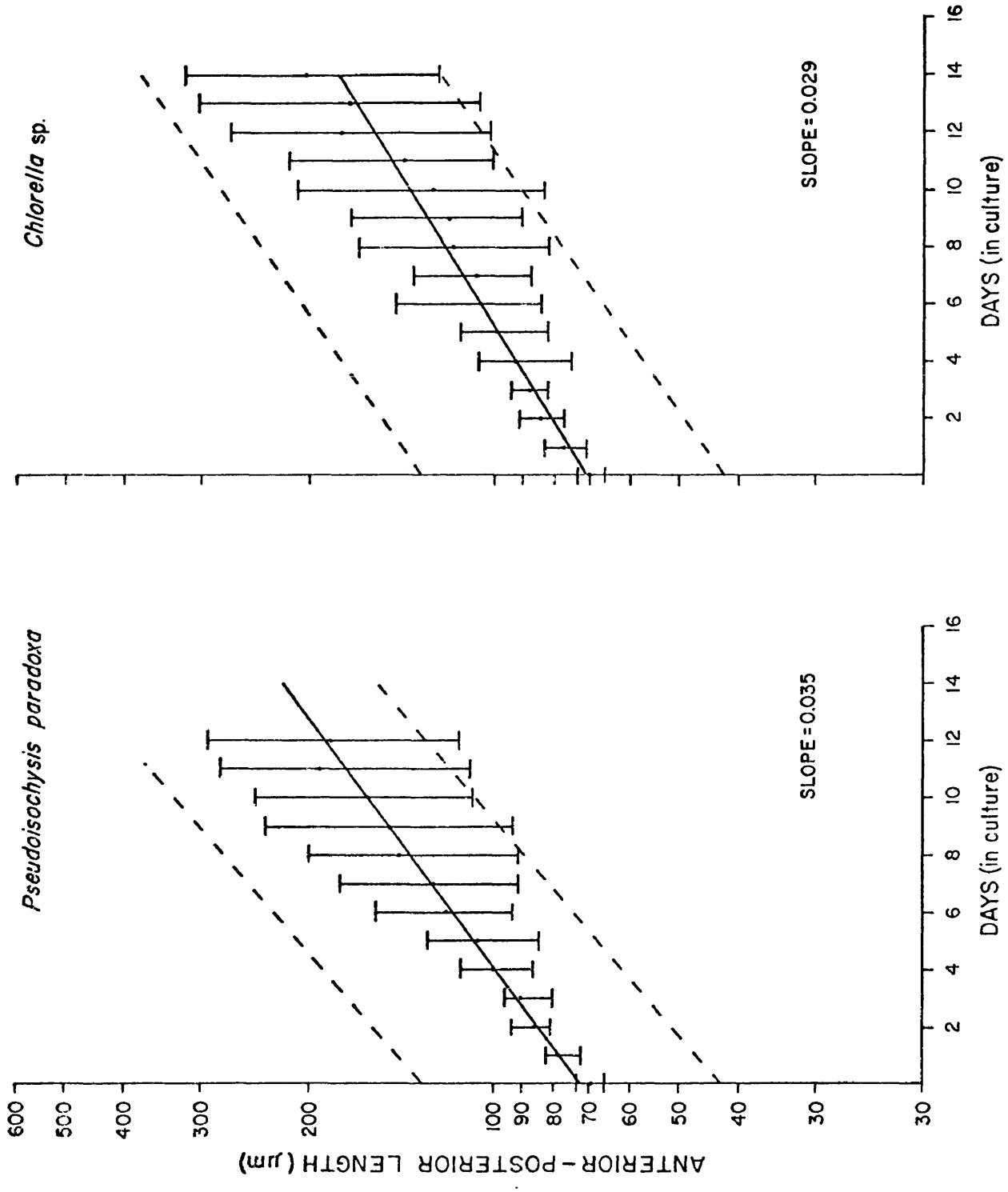
Spatial (Larval Density) Slopes

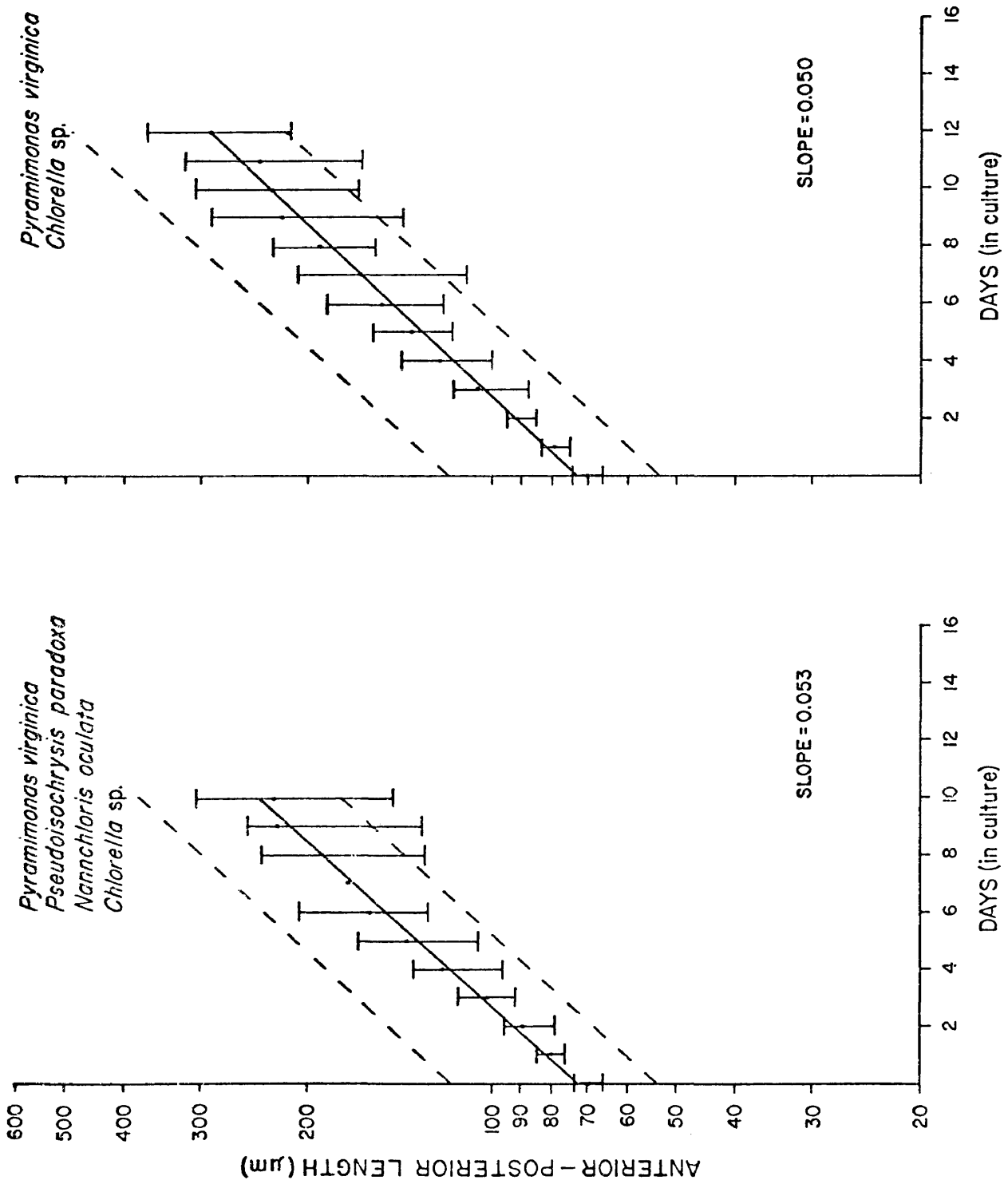
Larvae per milliliter	Slope (log μm/day)
1	0.070
3	0.081
5	0.076
7	0.063
10	0.052

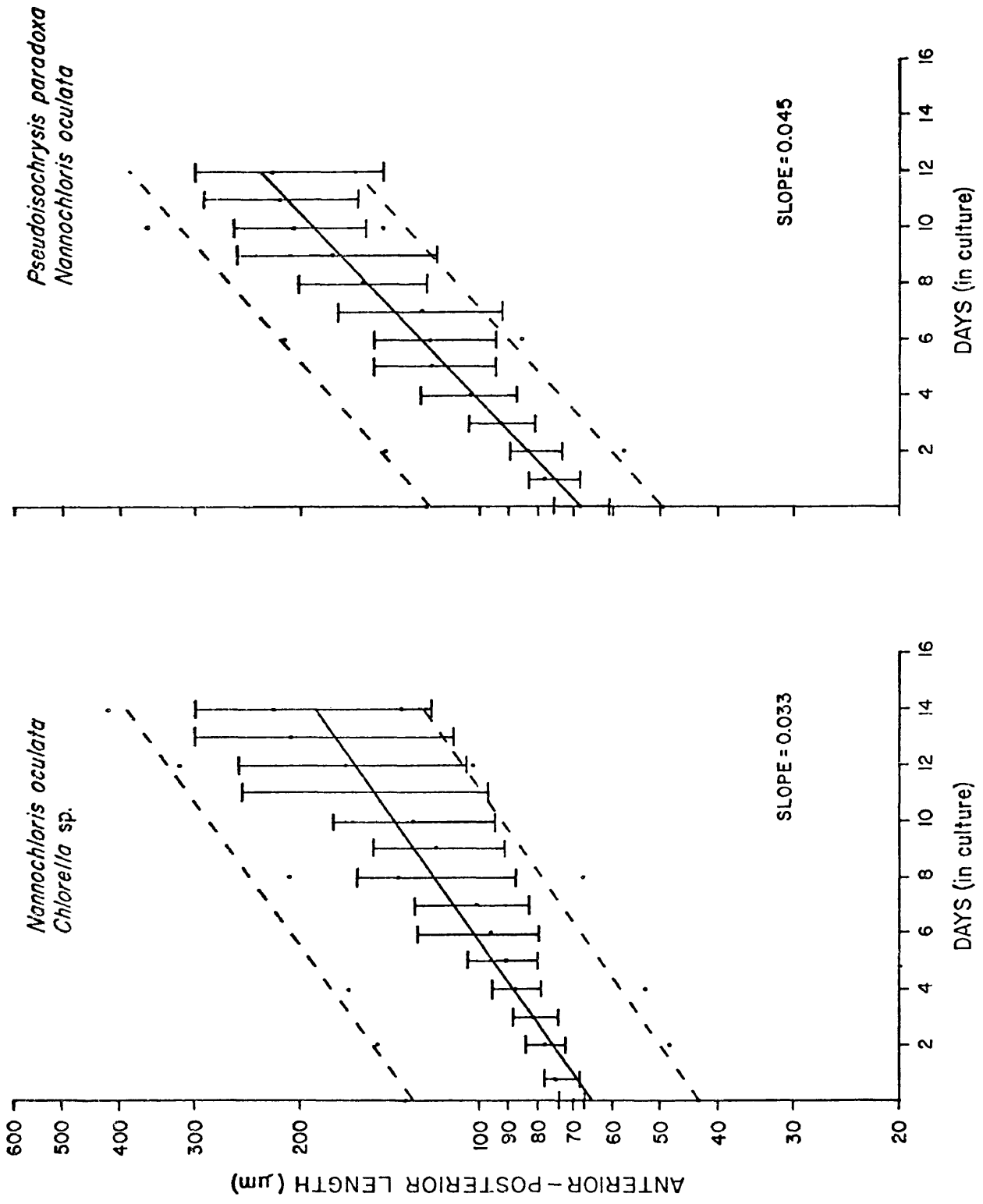
Nutritional (Algal Density) Slopes

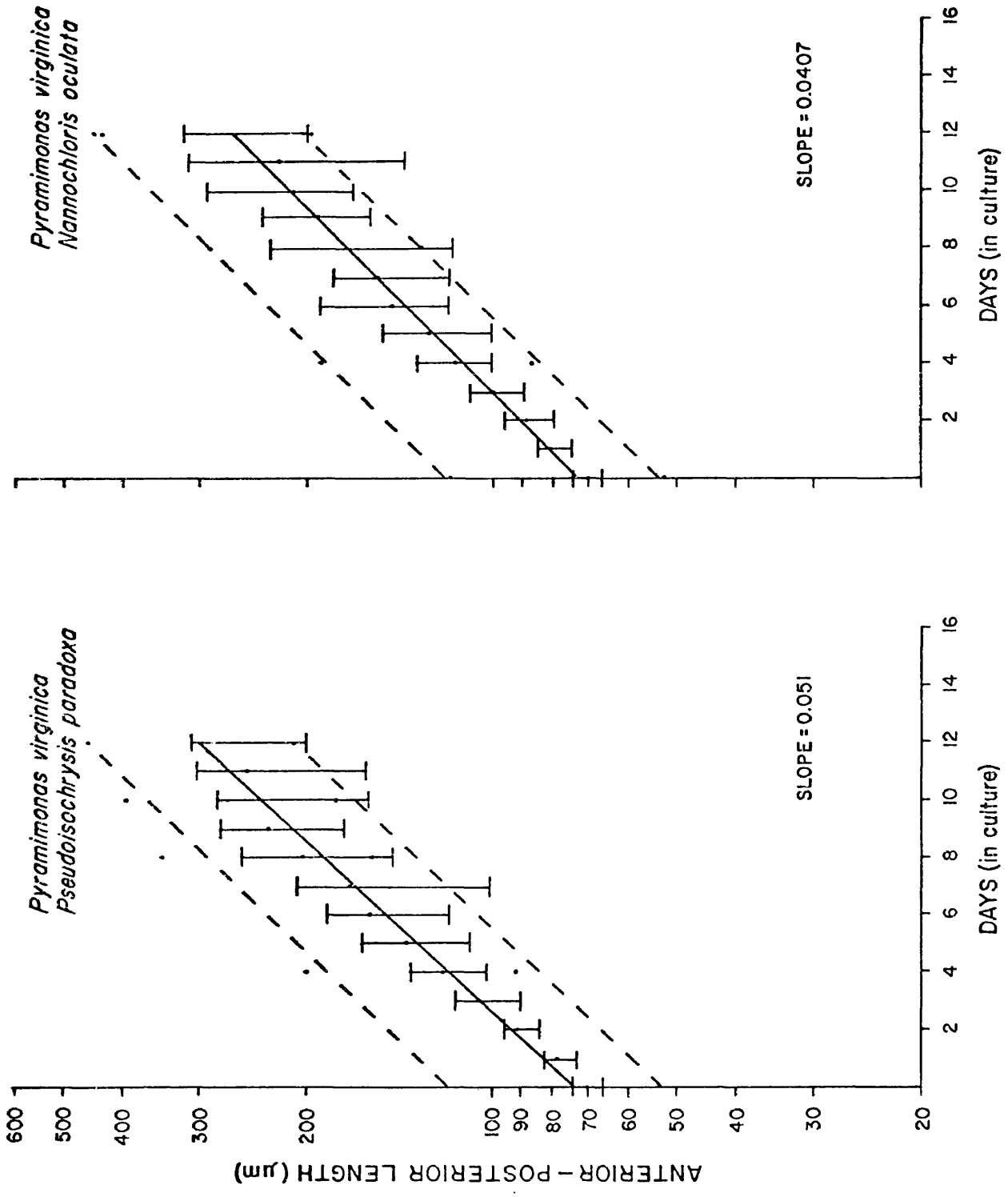
Algal Density	Slope (log µm/day)
Normal Algal Density	0.077
Double Algal Density	0.081
Double Density - 1/2 per 12 hours	0.082
Triple Algal Density	0.077
Triple Density - 1/2 per 12 hours	0.081
Normal Density to day 6 - Double Density	0.080
Double Density to day 6 - Triple Density	0.082

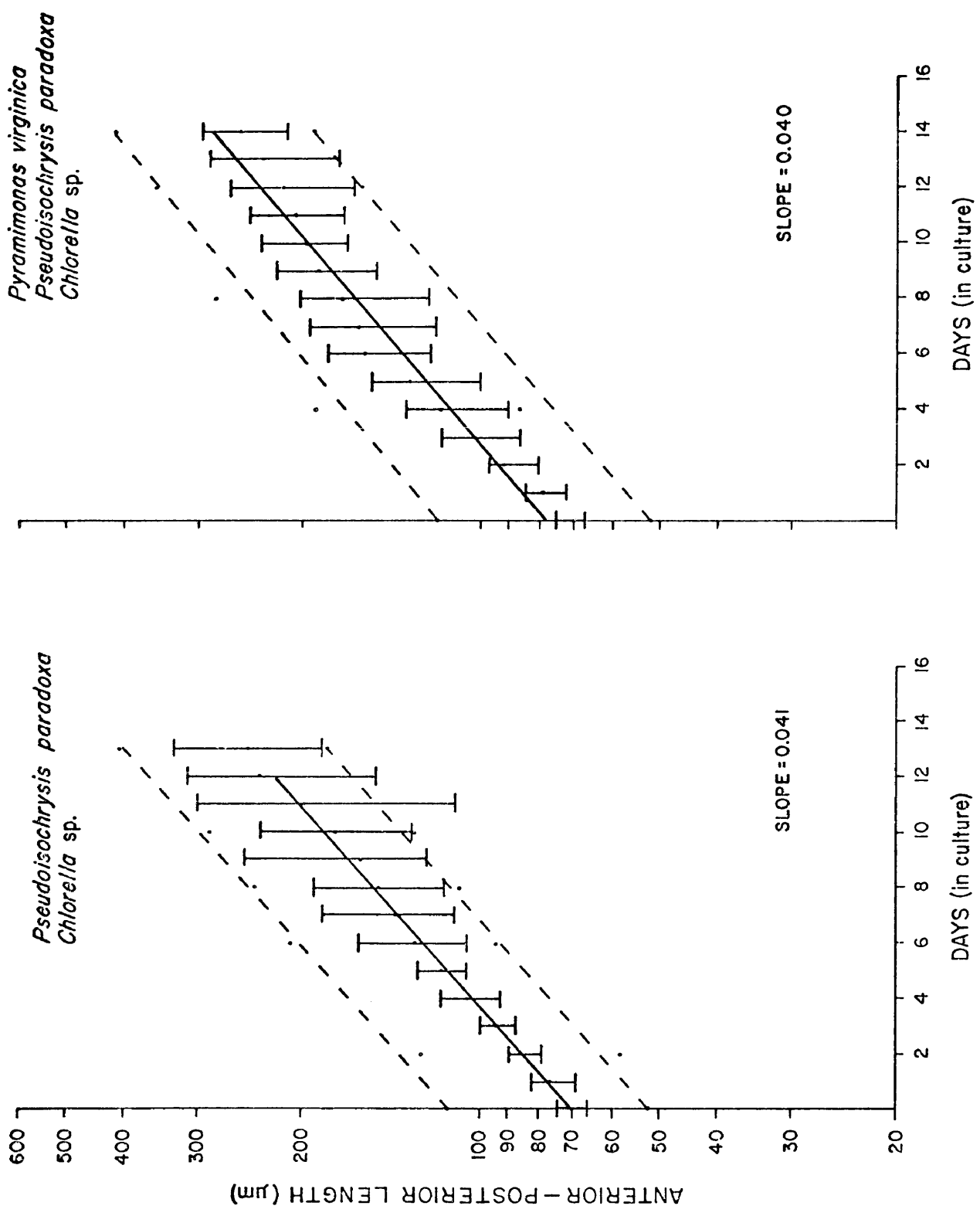

Pyramimonas virginica
SLOPE = 0.055
Nannochloris oculata
SLOPE = 0.051
DAYS (in culture)
DAYS (in culture)
ANTERIOR – POSTERIOR LENGTH (μm)
600
500
400
300
200
100
90
80
70
60
50
40
30
20
2
4
6
8
10
12
14
16

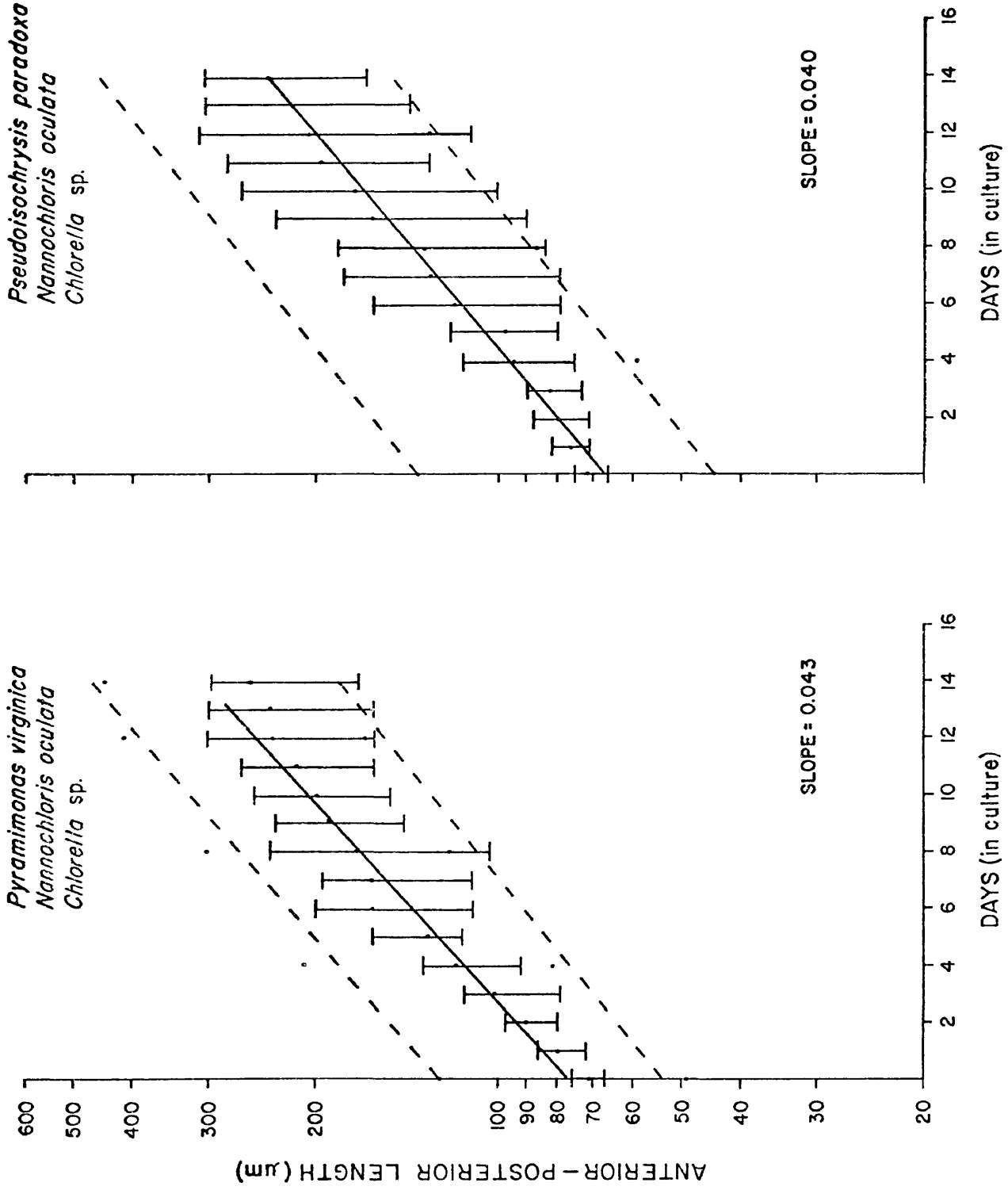

Pseudoisochysis paradoxa
Chlorella sp.
SLOPE = 0.035
SLOPE = 0.029
DAYS (in culture)
DAYS (in culture)
ANTERIOR - POSTERIOR LENGTH (μm)
600
500
400
300
200
100
90
80
70
60
50
40
30
2
4
6
8
10
12
14
16

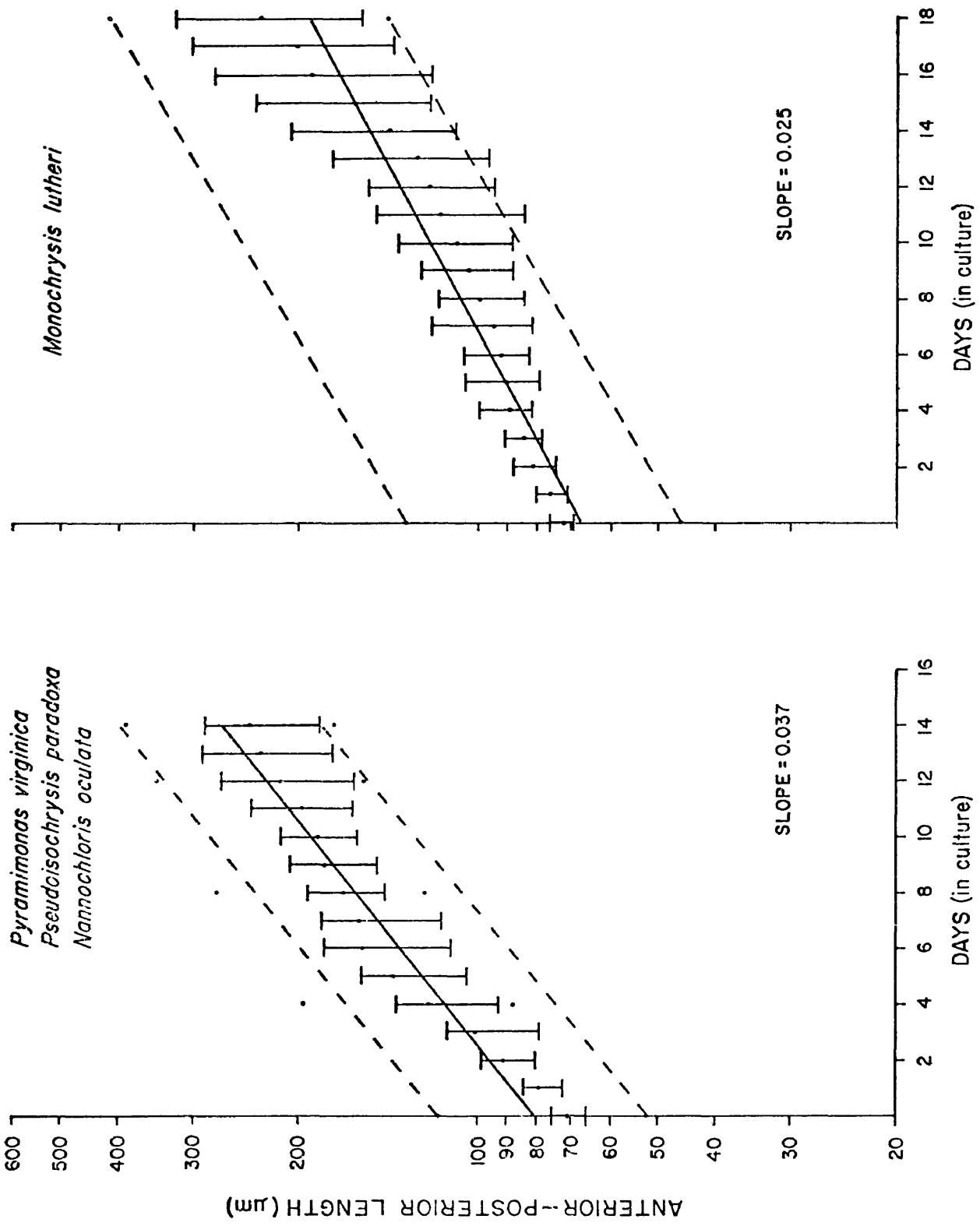

Pyramimonas virginica
Pseudoisochrysis paradoxa
Nannochloris oculata
Chlorella sp.
SLOPE = 0.053
Pyramimonas virginica
Chlorella sp.
SLOPE = 0.050
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)

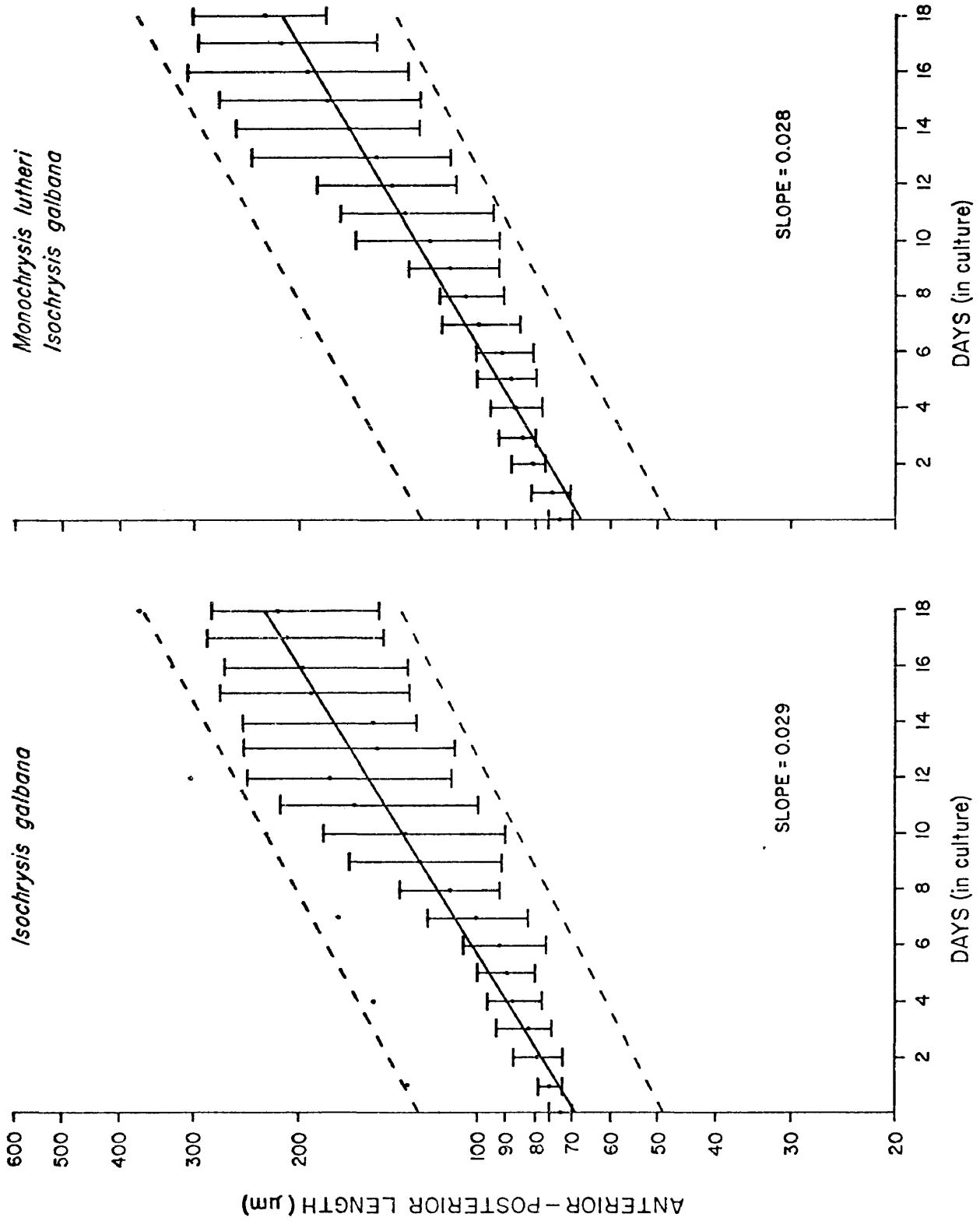

Nannochloris oculata
Chlorella sp.
SLOPE = 0.033
Pseudoisochrysis paradoxa
Nannochloris oculata
SLOPE = 0.045
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)

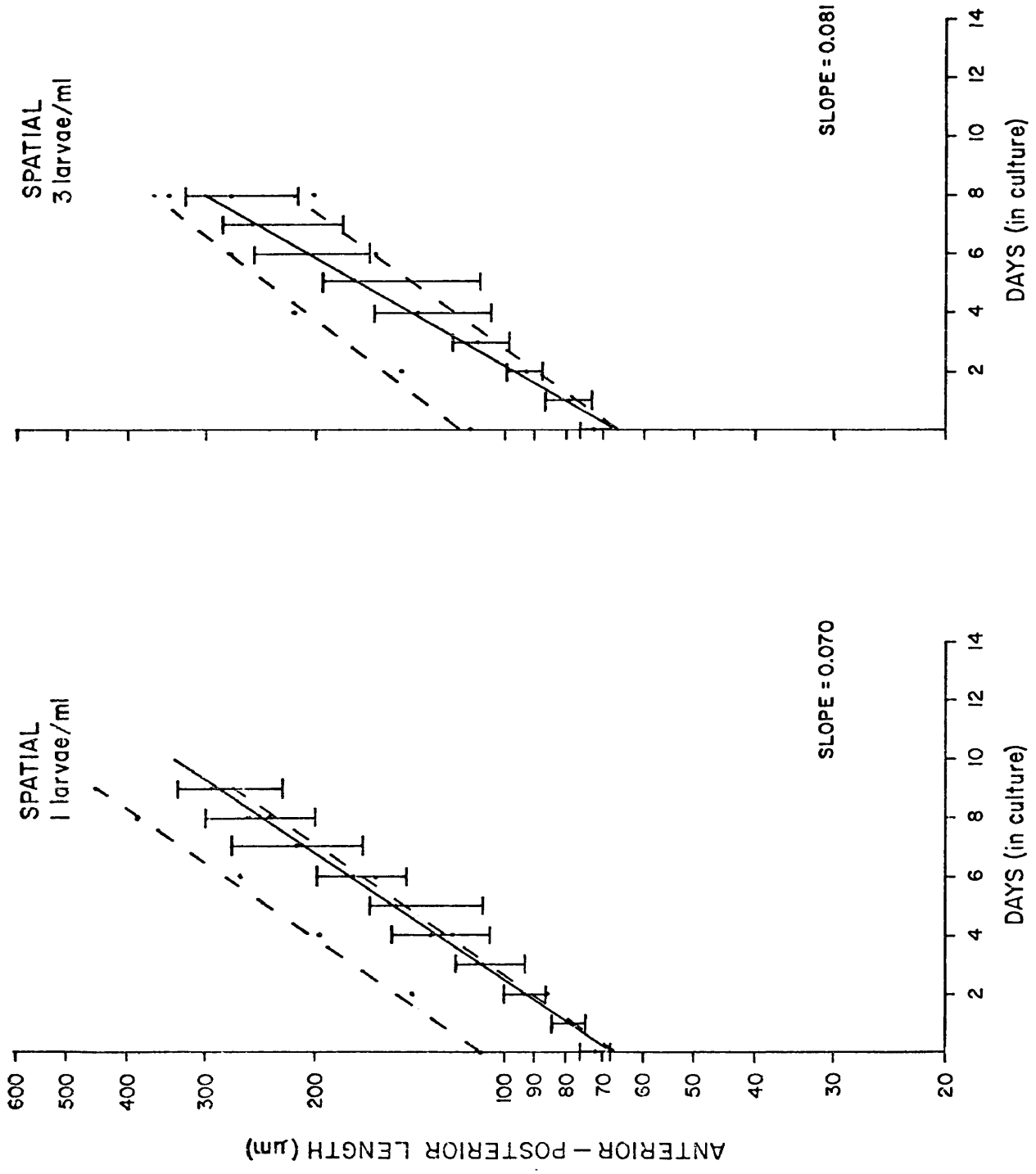

Pyramimonas virginica
Pseudoisochrysis paradoxa
SLOPE = 0.051
Pyramimonas virginica
Nannochloris oculata
SLOPE = 0.0407
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)

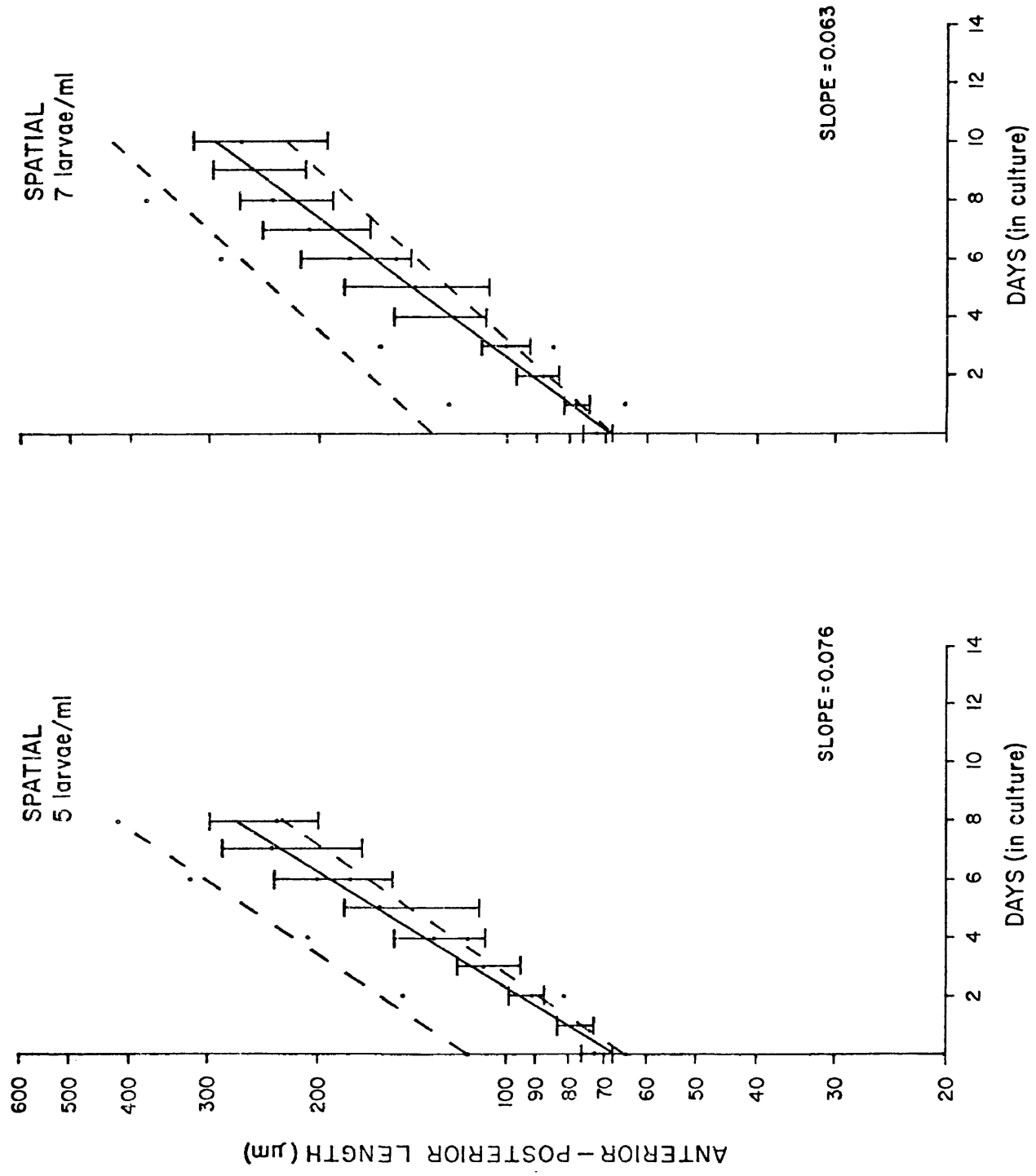

Pyramimonas virginica
Pseudoisochrysis paradoxa
Chlorella sp.
SLOPE = 0.040
Pseudoisochrysis paradoxa
Chlorella sp.
SLOPE = 0.041
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)

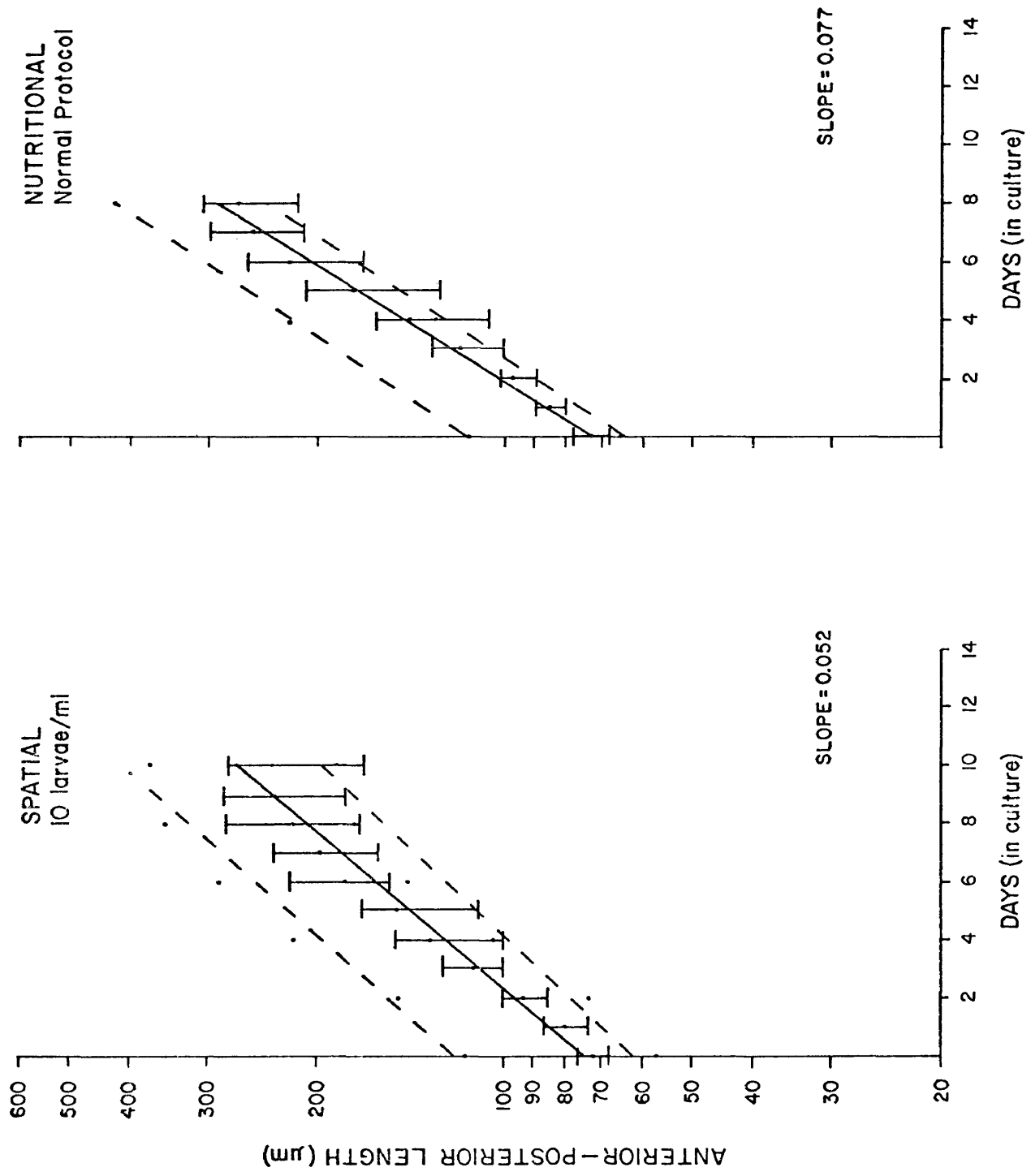

Pyramimonas virginica
Nannochloris oculata
Chlorella sp.
SLOPE = 0.043
Pseudoisochrysis paradoxa
Nannochloris oculata
Chlorella sp.
SLOPE = 0.040
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)

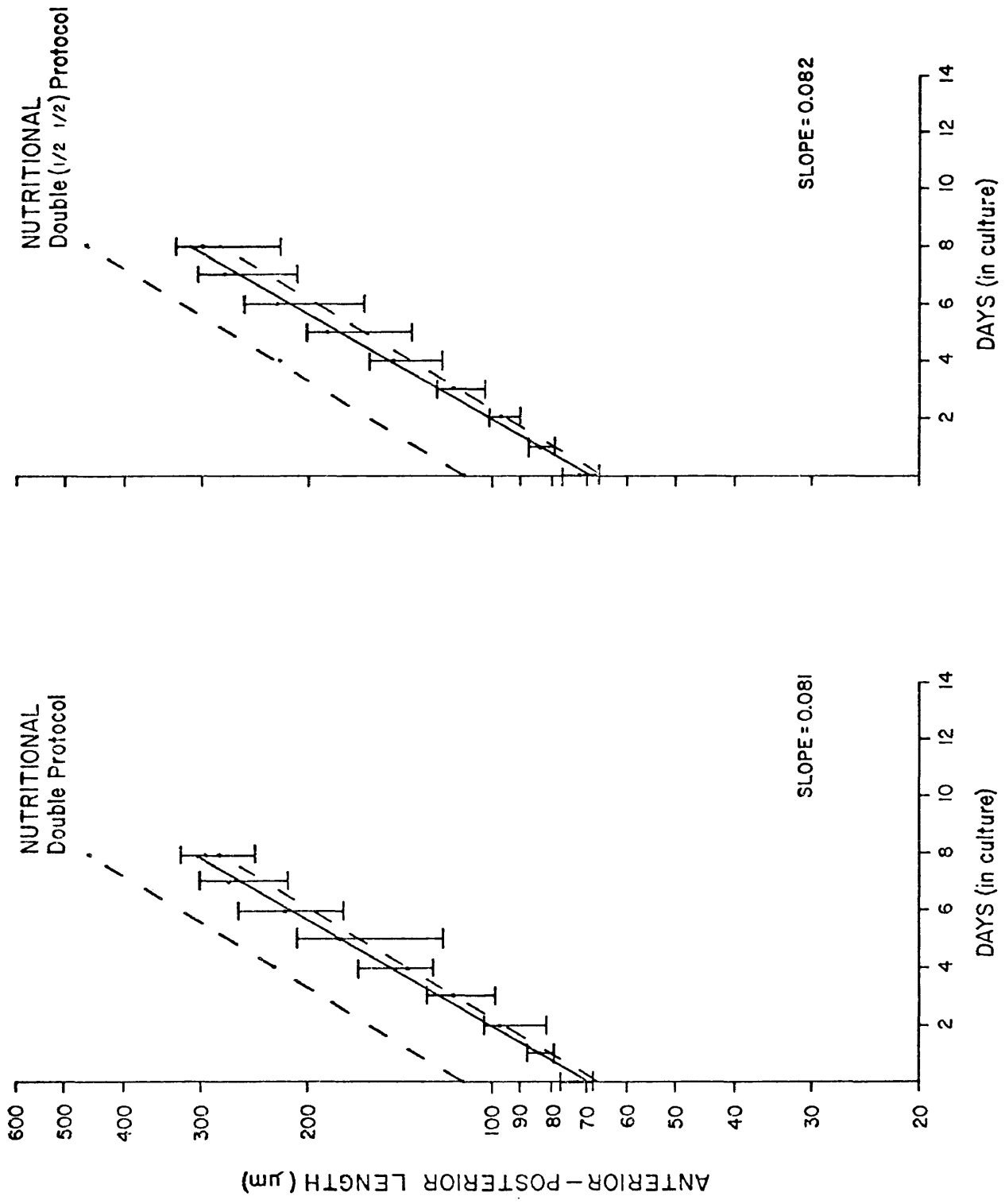

Monochrysis lutheri
SLOPE = 0.025
DAYS (in culture)
Pyramimonas virginica
Pseudoisochrysis paradoxa
Nannochloris oculata
SLOPE = 0.037
DAYS (in culture)
ANTERIOR--POSTERIOR LENGTH (μm)

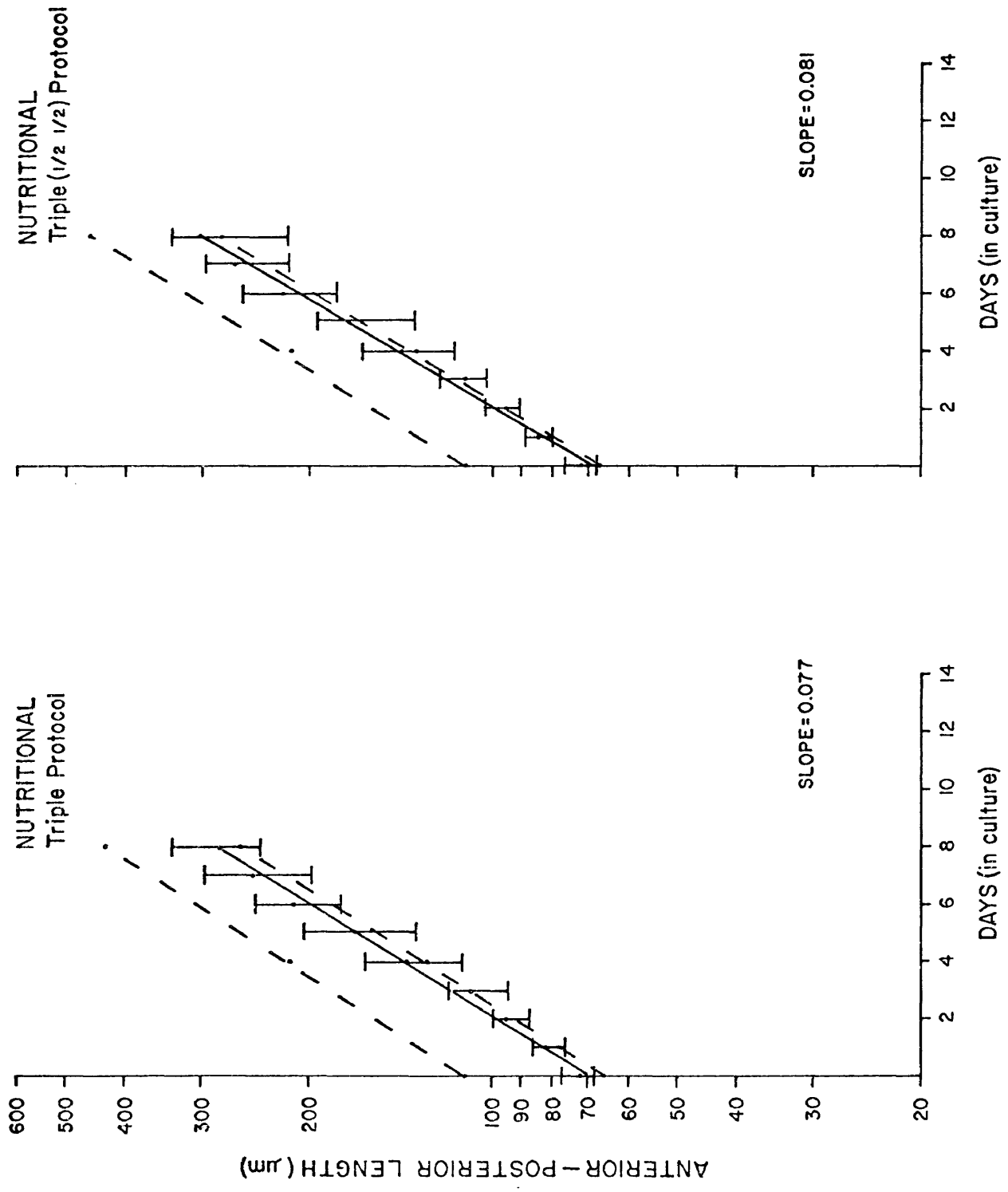

Isochrysis galbana
SLOPE = 0.029
Monochrysis lutheri
Isochrysis galbana
SLOPE = 0.028
DAYS (in culture)
DAYS (in culture)
ANTERIOR – POSTERIOR LENGTH (µm)
600
500
400
300
200
100
90
80
70
60
50
40
30
20
2
4
6
8
10
12
14
16
18

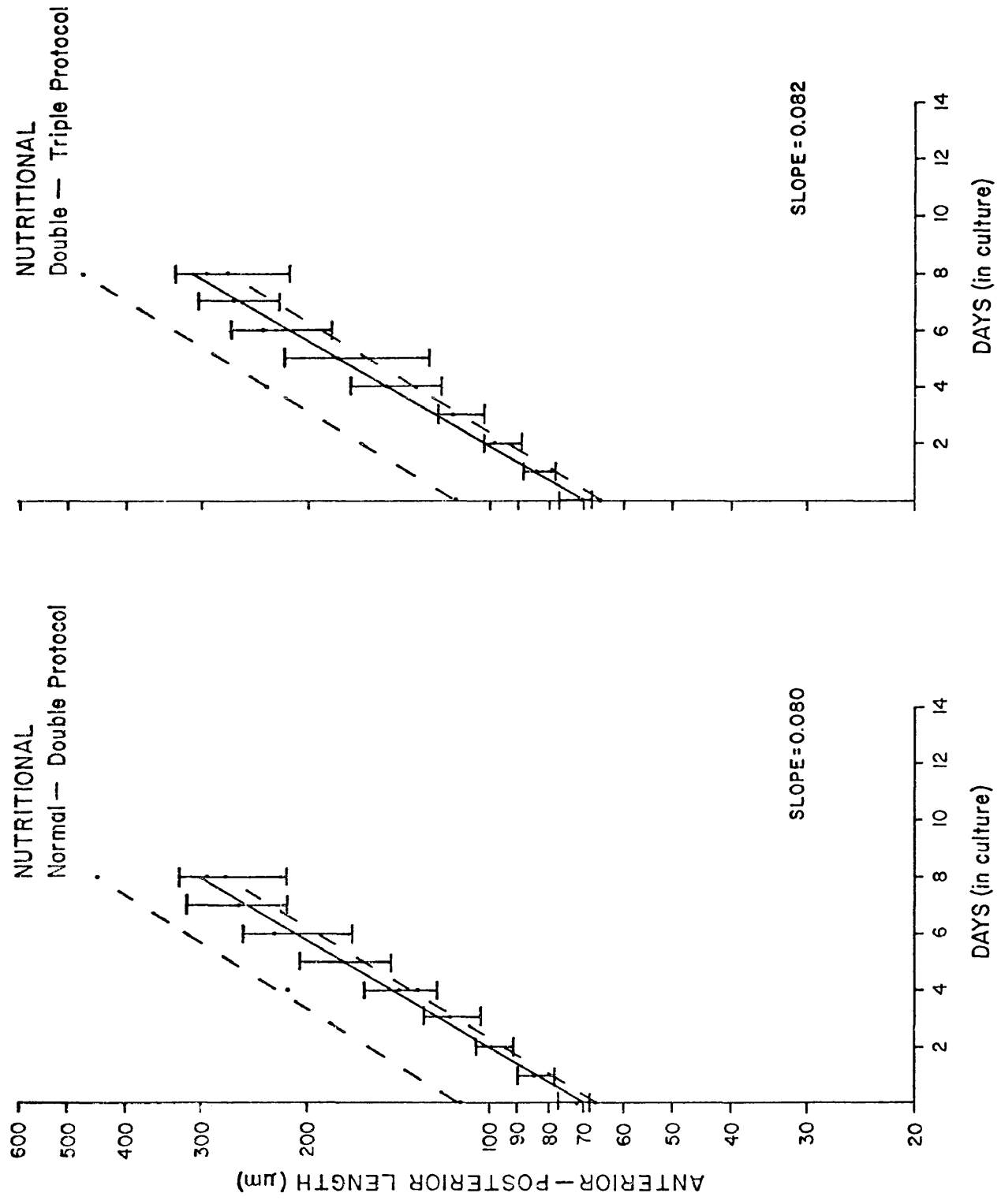

SPATIAL
1 larvae/ml
SLOPE = 0.070
SPATIAL
3 larvae/ml
SLOPE = 0.081
ANTERIOR – POSTERIOR LENGTH (µm)
DAYS (in culture)
DAYS (in culture)


SPATIAL
5 larvae/ml
SLOPE = 0.076
SPATIAL
7 larvae/ml
SLOPE = 0.063
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)


NUTRITIONAL
Normal Protocol
SLOPE=0.077
SPATIAL
10 larvae/ml
SLOPE=0.052
ANTERIOR–POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)


NUTRITIONAL
Double Protocol
SLOPE = 0.081
NUTRITIONAL
Double (1/2 1/2) Protocol
SLOPE = 0.082
ANTERIOR–POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)


NUTRITIONAL
Triple Protocol
SLOPE=0.077
NUTRITIONAL
Triple (1/2 1/2) Protocol
SLOPE=0.081
ANTERIOR – POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)


NUTRITIONAL
Normal — Double Protocol
SLOPE=0.080
NUTRITIONAL
Double — Triple Protocol
SLOPE=0.082
ANTERIOR—POSTERIOR LENGTH (μm)
DAYS (in culture)
DAYS (in culture)

APPENDIX B

Yield Parameter Data for Experimental Diets and Larval and Algal Density Experiments

Diet	Initial Population ($\times 10^6$)	Surviving Population ($\times 10^6$)	Number of Pediveligers Harvested	Number of Metamorphosed (Set) Pediveligers
Group 1				
Pyramimonas virginica	2.40	1.77	318,500	12,750
Pseudoisochrysis paradoxa	2.30	1.71	51,300	1,000
Nannochloris oculata	2.64	0.25	2,500	22
Chlorella sp.	2.48	1.11	10,025	100
P. virginica - *P. paradoxa* - *N. oculata* - *Chlorella* sp.	2.33	1.75	172,954	3,632
VIMS' Procotol	2.25	1.91	287,500	5,433

Diet	Initial Population ($\times 10^6$)	Surviving Population ($\times 10^6$)	Number of Pediveligers Harvested	Number of Metamorphosed (Set) Pediveligers
Group 2				
P. paradoxa - *N. oculata*	2.67	0.72	14,400	144
P. paradoxa - *Chlorella* sp.	2.51	1.08	21,600	200
P. virginica - *Chlorella* sp.	2.22	0.57	40,425	800
N. oculata - *Chlorella* sp.	2.58	0.25	2,250	20
P. virginica - *P. paradoxa*	2.44	0.92	65.000	1,300
P. virginica - *N. oculata*	2.81	0.75	30,250	275
VIMS' Protocol	2.40	1.08	54,000	540

Diet	Initial Population ($\times 10^6$)	Surviving Population ($\times 10^6$)	Number of Pediveligers Harvested	Number of Metamorphosed (Set) Pediveligers
Group 3				
P. virginica - *P. paradoxa* - *Chlorella* sp.	2.49	1.84	460,500	27,625
P. virginica - *P. paradoxa* - *N. oculata*	2.39	1.86	186,500	3,750
P. paradoxa - *Chlorella* sp.- *N. oculata*	2.35	1.20	47,900	500
P. virginica - *N. oculata* - *Chlorella* sp.	2.54	1.70	272,500	10,900
VIMS' Protocol	2.40	2.00	240,000	4,800

Diet	Initial Population ($\times 10^6$)	Surviving Population ($\times 10^6$)	Number of Pediveligers Harvested	Number of Metamorphosed (Set) Pediveligers
Group 4				
Monochrysis lutheri	2.35	0.68	15,650	125
Isochrysis galbana	2.27	1.70	16,000	150
M. lutheri - *I. galhana*	2.11	1.69	25,000	225
VIMS' Protocol	2.41	2.20	610,000	78,675

	Initial Population ($\times 10^6$)	Surviving Population ($\times 10^6$)	Number of Pediveligers Harvested	Number of Metamorphosed (Set) Pediveligers
Larval Density				
1	0.25	0.25	215,750	215,250
3	0.71	0.71	680,000	620,000
5	1.23	1.01	972,500	867,500
7	1.43	0.91	728,000	331,250
10	2.40	1.86	477,000	91,000

Algal Density	Initial Population	Surviving Population	Number of Pediveligers Harvested	Number of Metamorphosed (Set) Pediveligers
Normal Density	787,000	761,750	686,000	480,000
Double Density	692,000	638,750	600,000	390,000
Double Density - 1/2 per 12 hours	676,000	599,000	575,000	435,000
Triple Density	702,000	577,000	536,000	401,000
Triple Density - 1/2 per 12 hours	782,000	747,500	714,000	520,000
Normal Density to day 6 - Double Density	690,000	667,000	647,000	436,750
Double Density to day 6 - Triple Density	780,000	780,000	745,000	500,000

LITERATURE CITED

Bruce, D.L. and D.C.B. Duff. 1967. The identification of two antibacterial products of the marine planktonic alga Isochrysis galbana. J. Fen. Microbiol. 48: 293-298.

Bruce, J.R., M. Knight and M.W. Parke. 1940. The rearing of oyster larvae on an algal diet. J. Mar. Biol. Ass. U.K. 24: 337-74.

Burkholder, P.R. 1963. Some nutritional relationships among microbes of sea sediments and waters. In: Symposium on marine microbiology. Ed. by C.H. Oppenheimer, Thomas, Springfield, Illinois. pp. 133-150.

Carriker, M.R. 1956. Biology and propagation of young hard clams, Mercenaria mercenaria. J. Elisha Mitchell Sci. Soc. 72: 57-60.

Creekman, L.L. 1977. The effects of conditioning the American oyster, Crassostrea virginica with Tetraselmis suecica and cornstarch on the growth, vigor and survival of its larvae. M.S. Thesis, University of Virginia, Charlottesville, Va. 58 pp.

Cole, H.A. 1937. Experiments in the breeding of oysters (Ostrea edulis) in tanks, with special reference to the food of the larva and spat. Fish. Invest. Min. Agric. Food, London. Ser. II. 15: 1-24.

Craigie, J.S., J. Mclachlan, R.G. Ackman and C.S. Tocher. 1967. Photosynthesis in algae. III Distribution of soluble carbohydrates and dimethyl-B-propiothetin in marine unicellular Chlorophyceae and Prasinophyceae. Canadian J. Bot. 45: 1327-34.

Craigie, J.S. 1974. Storage Products. pp. 206-235. In: Ed. by W.D.P. Stewart. Algal physiology and biochemistry. University of California Press, Berkeley and Los Angeles.

Davis, H.C. 1950. On food requirements of larvae of Ostrea virginica. Anat. Rec. 108: 132-133.

Davis, H.C. 1953. On food and feeding of larvae of the American oyster, Crassostrea virginica. Biol. Bull. 104: 334-350.

Davis, H.C., P.E. Chanley. 1956. Effects of some dissolved substances on bivalve larvae. Proc. Natl. Shellfish. Ass. 46: 59-68.

Davis, H.C. and R.R. Guillard. 1958. Relative value of ten genera of micro-organisms as foods for oyster and clam larvae. Fish. Bull. 136, 58: 293-304.

Dean, D. 1958. New property of the crystalline style of Crassostrea virginica. Science 128: 837.

Droop, M.R. 1968. Vitamin B_{12} and marine ecology. IV. The kinetics of uptake, growth, and inhibition in Monochrysis lutheri. J. Mar. Biol. Ass. U.K. 48: 689-733.

Dupuy, J.L. and Samuel Rivkin. 1972. The development of laboratory techniques for the production of cultch-free spat of the oyster, Crassostrea virginica. Chesapeake Sci. 13: 45-52.

Dupuy, J.L. 1973. Translation of mariculture research into a commercial oyster seed hatchery. Marine Technology Society, 9th Annual Conference, Proceedings. pp. 677-685.

Dupuy, J.L. 1975. Some physical and nutritional factors which affect the growth and setting of the larvae of the oyster, Crassostrea virginica, in the laboratory. pp. 319-331. In: Ed. by F. John Vernberg Physiological Ecology of Estuarine Organisms. Univ. South Carolina Press.

Fretter, V. and M.C. Montgomery. 1968. The treatment of a food by prosobranch veligers. J. Mar. Biol. Ass. U.K. 48: 499-520.

Galtsoff, P.S. 1964. The American oyster, Crassostrea virginica Gmelin. Fish. Bull. 64: 1-480.

Haven, D.S. 1965. Supplemental feeding of oysters with starch. Chesapeake Sci. 6: 43-51.

Hellebust, J.A. 1965. Excretion of some organic compounds by marine phytoplankton. Limnol. Oceanogr. 10: 192-206.

Helm, M.M., D.L. Holland and R.R. Stephenson. 1973. The effect of supplementary algal feeding of a hatchery breeding stock of Ostrea edulis L. on larval vigor. J. Mar. Biol. Ass. U.K. 53: 673-684.

Hidu, H. and H.S. Tubiash. 1963. A bacterial basis for the growth of antibiotic-treated bivalve larvae. Proc. Natl. Shellfish. Ass. 54: 25-39.

Hughes, E. 1940. The breeding of oysters in tanks. J. Mar. Biol. Ass. U.K. 24: 543-547.

Imai, T. and M. Hatanaka. 1949. On the artificial propagation of the Japanese common oyster, Ostrea gigas Thun., Agric. Res. Tohoku Univ. 1: 33-46.

Imai, T., M. Hatanaka, R. Sato, S. Sakai, and R. Yuki. 1950. Artificial breeding of oysters in tanks. Tohoku J. Agric. Res. 1: 69-86.

Loosanoff, V.L. 1945. Precocious gonad development in oysters induced in midwinter by high temperature. Science. 102: 124-125.

Loosanoff, V.L., H.C. Davis and P.E. Chanley. 1953. Behavior of clam larvae in different concentrations of food organisms. Anat. Rec. 117: 586-587.

Loosanoff, V.L., and H.C. Davis. 1963. Rearing of bivalve mollusks. Adv. Mar. Biol. 1: 1-136.

Loosanoff, V.L., H.C. Davis and P.E. Chanley. 1955. Food requirements of some bivlave larvae. Proc. Natl. Shellfish. Ass. 45: 66-83.

Mackie, G. 1969. Quantitative studies of feeding in the oyster, Crassostrea virginica. Proc. Natl. Shellfish. Ass. 59: 6-7.

Malouf, R., R. Keck, D. Maurer, and C. Epifano. 1973. Occurrence of gas-bubble disease in three species of bivalve molluscs. J. Fish. Res. Bd. Canada. 29: 588-589.

Matthiessen, G.C. and R.C. Toner. 1966. Possible methods of improving the shellfish industry of Martha's Vineyard, Duke's County, Massachusetts. Marine Research Foundation, Inc. 138 pp.

Millar, R.H. 1955. Notes on the mechanism of food movement in the gut of the larval oyster Ostrea edulis. Quart. J. Mic. Sci. 96: 539-544.

Parsons, T.R., K. Stephens, and J.D.H. Strickland. 1961. On the chemical composition of eleven species of marine phytoplankters. J. Fish. Res. Bd. Canada. 18: 1001-1016.

Prytherch, H.F. 1924. Experiments in the artificial propagation of oysters. U.S. Bur. Fish. App. 11 to Rep. U.S. Comm. Fish., Fiscal Year 1923. 14 pp.

Ukeles, R. and B.M. Sweeney. 1969. Influence of dinoflagellate trichocysts and other factors on the feeding of Crassostrea virginica larvae on Monochrysis lutheri. Limnol. Oceanogr. 14: 403-410.

Walne, P.R. 1956. Experimental rearing of the larvae of Ostrea edulis L. in the laboratory. Fish. Invest. Min. Agric. Fish. Food, London. Ser. II. 20: 1-23.

Walne, P.R. 1965. Observations on the influence of food supply and temperature on the feeding and growth of the larvae of Ostrea edulis L. Fish. Invest. Min. Agric. Fish. Food, London. Ser. II. 24: 1-45.

Walne, P.R. 1970. Present problems in the culture of the larvae of Ostrea edulis. Helgolander wiss. Meeresunters. 20: 514-525.

Wells, W.F. 1920. Growing oysters artificially. Conservationist. 3: 151.

Wilson, D.P. 1941. Oyster rearing on the River Yealm. J. Mar. Biol. Ass. U.K. 25: 125-127.

Yonge, C.M. 1926. Structure and physiology of the organs of feeding and digestion in Ostrea edulis. J. Mar. Biol. Ass. U.K. 14: 295-386.

Yonge, C.M. 1960. Oysters. Collins, London and Glasgow. 209 pp.

VITA

Nancy T. Windsor

Born on 21 May 1948 in Clearwater, Florida. Graduated from James Island High School, Charleston, South Carolina, May 1966; B.A. in Chemistry, Newberry College, Newberry, South Carolina, May 1970.

Employed by the Virginia Institute of Marine Science, August 1970; entered into the School of Marine Science, College of William and Mary, February 1975.