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**Studies of the Relationship
of RNA/DNA Ratios
and the Rate of Protein Synthesis
to Growth in the Oyster,
*Crassostrea Virginica***

by

Alan K. Pease

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Studies of the relationship of RNA/DNA ratios and the rate of protein synthesis to growth in the oyster, *Crassostrea virginica*

by

Alan K. Pease

This is the fiftieth
Technical Report from the
Research and Development Directorate
Marine Ecology Laboratory
Bedford Institute of Oceanography
Dartmouth, Nova Scotia

Ceci est le cinquantième
Rapport Technique de la Direction de la
Recherche et Développement
Laboratoire d'Ecologie Marine
L'Institut Océanographique de Bedford
Dartmouth, Nouvelle-Ecosse

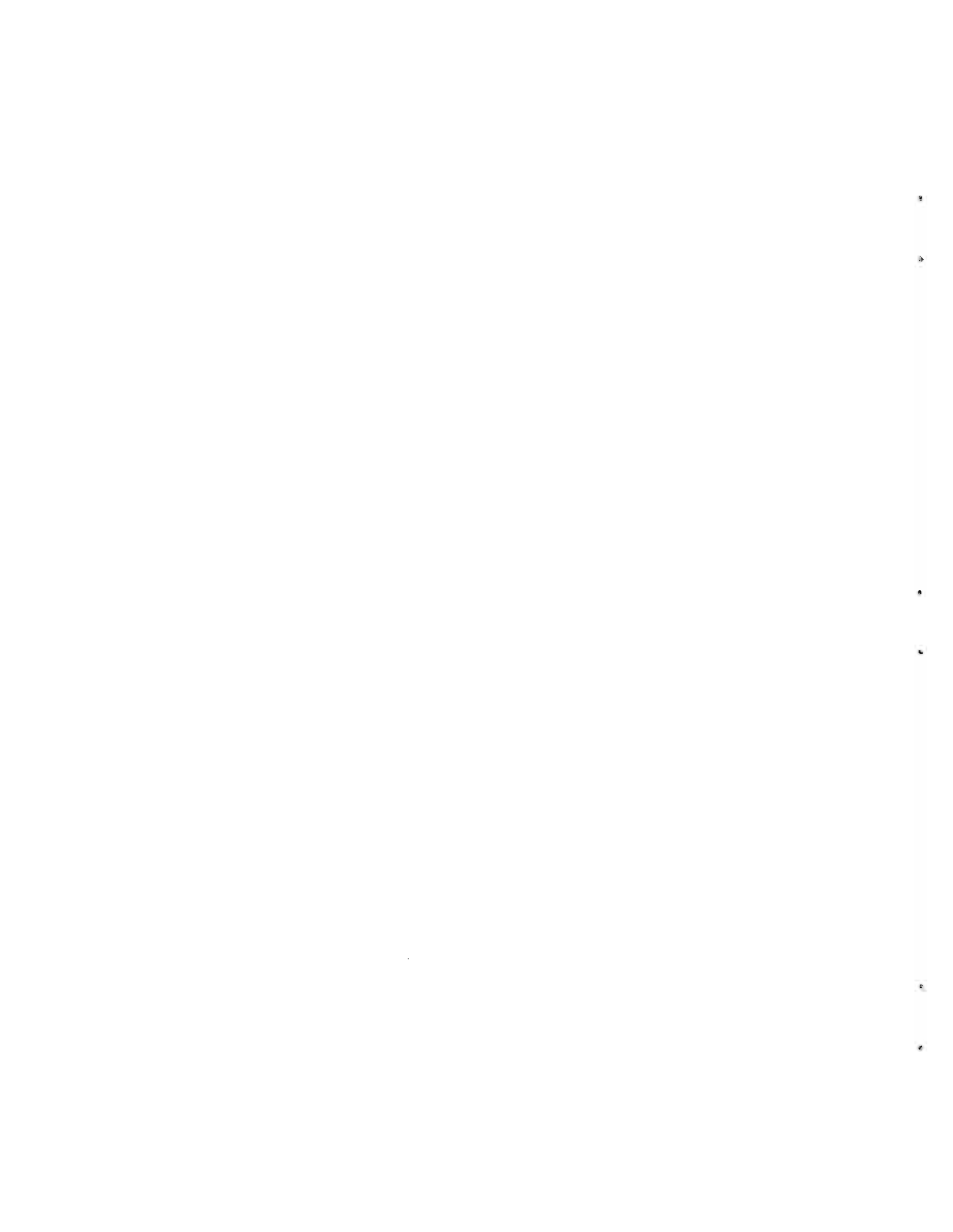


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ABSTRACT

Pease, Alan K. 1976. Studies of the relationship of RNA/DNA ratios and the rate of protein synthesis to growth in the oyster, Crassostrea virginica. Fish. Mar. Ser. Res. Dev. Tech. Rep. 622: 88 pp.

Collections were made of the oyster, Crassostrea virginica from the Bras d'Or Lakes, Nova Scotia between October 1974 and October 1975. Measurements were made of RNA, DNA, and protein synthesis in cell suspensions made from the oysters. An attempt was made to predict the growth of the oysters from the ratio of RNA/DNA and the rate of protein synthesis. A month's lag was found between changes in the ratio of RNA/DNA and the rate of protein synthesis. The ratio of RNA/DNA in each year class of oyster appeared to be related to its growth rate.

SOMMAIRE

Pease, Alan K. 1976. Studies of the relationship of RNA/DNA ratios and the rate of protein synthesis to growth in the oyster, Crassostrea virginica. Fish. Mar. Ser. Res. Dev. Tech. Rep. 622: 88 pp.

Des huîtres, Crassostrea virginica, ont été recueillies dans les lacs Bras d'Or (Nouvelle-Écosse) entre octobre 1974 et octobre 1975. On a mesuré la teneur en A.R.N. et en A.D.N. et enregistré la synthèse de protéines de cellules en suspension. On a essayé de prédire la croissance des huîtres en se basant sur le rapport A.R.N./A.D.N. et sur le rythme de synthèse de protéines. On a constaté dans ce rythme un retard d'un mois sur des changements dans le rapport A.R.N./A.D.N. Il semble y avoir relation entre le rapport A.R.N./A.D.N. et le taux de croissance des huîtres chaque année.

INTRODUCTION

Measurements may be made of the concentrations of the nucleic acids, RNA (ribonucleic acid) and DNA (deoxyribonucleic acid), and of the rate of protein synthesis in cell suspensions made from whole organisms (Pease, 1973). These measurements may allow an ecologist to assess the health and state of growth of an organism because these parameters are related to several fundamental biological processes, specifically, the transmission and translation of genetic information. The following report examines relations among these factors in the commercial oyster, Crassostrea virginica, taken from the Bras D'Or Lakes, Nova Scotia.

I. 1. NUCLEIC ACIDS AND PROTEIN SYNTHESIS AS MEASURES OF GROWTH OF ORGANISMS.

In multicellular organisms, growth consists of an increase in cell number, cell size, and even non-cellular material. This requires the synthesis of DNA, RNA, and protein. The total amount of DNA is proportional to cell number since most DNA is chromosomal. Protein synthesis involves DNA-dependent synthesis of RNA and a RNA-dependent synthesis of protein (Polypeptide). Because the bulk of the material synthesized is protein or requires protein (ie. -the enzymes), Brachet (1960) referred to growth as the rate of protein synthesis.

Van't Hof (1963) and Van't Hof and Sparrow (1964) pointed out that there is a relationship between DNA content, nuclear

volume, and minimum mitotic time in plants. Commoner (1964) presented some evidence that the amount of nuclear DNA is an important species specific characteristic inversely correlated with basic metabolic rate. Stebbins (1966) found that the amount of nuclear DNA in plants is inversely correlated with their rate of growth. Goin et al (1968) found that DNA is inversely correlated with rate of development in frogs; they emphasized observations of Beverton and Holt (1959) who showed in a number of fish there is a direct relationship between the characteristic rate of development of a species and metabolic rate.

Sutcliffe (1969) found that the RNA content of organisms ranging in size from bacteria to mammals was directly related to growth rate. Munro (1969a) reported relationships between total body protein synthesis, the DNA and RNA content of specific tissues of mammals, oxygen consumption, and their total body weight. Mattern (1969) determined that the rate of RNA synthesis in phytoplankton was almost linearly correlated with gross primary production as measured by the evolution of O_2 . Ramsammy (1969) found a relationship between RNA and the activity of the respiratory enzyme succinic dehydrogenase and postulated that such a relationship could be used to estimate production in zooplankton. Haines (1973) provided evidence that the ratio of RNA/DNA may be a reliable indicator of long term growth in fish populations and suggested that this method has potential for use in detecting responses of fish to environmental change before there is a change in growth rate.

Clearly the evidence points to strong links between the nucleic acids, protein synthesis and growth. However, it appears to the present

author that the ratio of RNA/DNA may measure the potential growth rate in an organism; that is, the ratio reflects the capacity of the cells for protein synthesis (Munro, 1969a). If this is the case, the ratio provides a measure of growth which is quite different from the actual growth rate which is reflected in the measured rate of protein synthesis. Such a relationship between nucleic acids and protein synthesis would be similiar to the relationship described by Ryther and Yentsch (1957) for chlorophyll and photosynthesis and would provide information on both the growth potential of organisms and how this is realized in a particular environment.

Some care must be taken in assembling information to distinguish genetic or long term potential growth characteristics from short term environmental aspects. The present author believes that the ratio of RNA/DNA is a direct measure of genetic factors in growth since the mechanism for protein synthesis must be more conservative to changes in the environment than the actual rate of protein synthesis. The actual rate of protein synthesis may respond to many factors, many of which represent short term changes in the environment. Further information on the usefulness of these indices in the oyster can be derived from studies of the nucleic acids and protein synthesis in relation to environmental factors.

I. 2. FACTORS WHICH AFFECT THE NUCLEIC ACIDS AND PROTEIN SYNTHESIS.

The levels of the nucleic acids and the rate of protein synthesis appear to respond to a number of different environmental factors. For example, growth may be interrupted by removal of a food supply. The rate of protein synthesis is thereby reduced and this reduction preceeds a decrease in RNA (Spiegelman, 1965). With the

development of techniques for studying ribosomal RNA it has been determined that imbalanced diets and acute nutritional stress affect polyribosomal profiles in mammalian tissue systems (Young and Alexis, 1968; Munro 1969b, Reid et al, 1970; von der Decken and Omstedt, 1970; Pronczuk et al, 1970). Crowding of organisms may affect protein synthesis through competition for food resources (Bolla and Roberts, 1971a, 1971b) except where stored mRNA (information RNA) delays the crowding effect.

Disease may affect both the levels of the nucleic acids and protein synthesis. Squibb et al (1968) found that virus infections resulted in highly significant depression of the levels of protein, DNA, and RNA. Changes in the levels of these compounds occurred in the very early stages of infection and were correlated with intensity of disease development.

Acclimation of animals to different temperatures affects the levels of the nucleic acids and proteins as well as their rates of synthesis. Rao (1962) found for several poikilotherms that the concentration of nucleic acid phosphorous was higher in certain tissues of cold adapted animals. Das (1967) and Das and Prosser (1967) found for goldfish that RNA concentrations in liver tissues increased during cold adaptation but gill tissue showed the reverse. Greater incorporation was also found for leucine into proteins of several tissues. The total amount of DNA in the tissues in these studies did not change due to thermal adaptation of the fish. Haschemeyer (1969) also found increased protein synthesis in a cold adapted fish. On the other hand Burr and Hunter (1969) found that the RNA content of a eurythermal species of fruit fly when grown in the cold was lower than flies grown

at warmer temperatures.

Indirect evidence has been found that alternations in salinity affect protein synthesis in marine organisms. Berger et al (1970) found that the application of actinomycin-D, which inhibits DNA-dependent synthesis of RNA, reduced the ability of veligers of a mollusc to adapt to waters of low salinity. Better established are the effects of certain ions (Mg^{++} and K^+) on cell free protein synthesis. CO_2 is known to stimulate protein synthesis in cestodes (Harris and Read, 1969). Protein synthesis usually has a pH optima.



MATERIALS AND METHODS

II. 1. CRASSOSTREA VIRGINICA.

Oysters were gathered in two locations in the Bras D'Or Lakes. Oysters whose spat settled on scallop shells during the years 1975, 1974, 1973 and 1972 were obtained from rafts in Portage Creek. Oysters whose spat settled during 1973 were also obtained from rafts near Seal Island. The oysters were transported to Dalhousie University where they were maintained at 2°C in a cold room of the Oceanography Department.

For each experiment the average wet wt of several oysters including the shells, the wet wt of the oyster meat excluding the adductor muscle, and the wet wt of the adductor muscle were obtained.

II. 2. INCUBATION WITH LEUCINE-4, 5-H³.

Protein synthesis was measured as the incorporation of the radioactive amino acid, leucine-4, 5-H³ into protein. To facilitate comparison of analyses between experiments whole oysters were reduced to cells and the concentration of cells in suspension was standardized. That is, the entire tissue excluding the adductor muscle was dispersed through a 500 μ metal sieve with a pestle. The separated cells were homogenized once with a 40 ml Ten Broeck glass homogenizer. The suspension was diluted to a constant density with sea water. Cell concentrations was determined at 600 nm wavelength in the turbidity position of a Unicam SP 8000 spectrophotometer. Cell suspensions which produced an absorbance of 0.6-0.7 units provided enough material for all analyses.

Duplicate one ml portions of the suspension of cells were pipetted into centrifuge tubes (designated series A) for analysis of

protein and DNA. Ten ml portions were pipetted into heavy walled tubes and centrifuged for 10 min at 5000 RPM in a Sorvall RC2-B centrifuge. The supernatant was discarded and the pellet resuspended in six mls of filtered sea water (20 o/oo) buffered with piperazine dihydrochloride at a pH of 7.2 (Smith and Smith, 1949) to which glucose had been added at a concentration of 0.001M. The cell suspension was homogenized again with eight strokes of a 15 ml Ten Broeck homogenizer. Two-2.5 ml portions were transferred into 25 ml erlyenmeyer flasks. To the flasks were added the following:

1. Antibiotics (0.1 ml) to a concentration of 100 μ g of penicillan-G and 50 μ g of streptomycin per ml of culture medium.
2. Enzymes (0.1 ml) to a concentration of 0.1% hyaluronidase (Sigma Chemical Co., Type I) and 0.05% collagenase (Sigma Chemical Co., Type II) to help keep the cells dispersed (Schreiber and Schreiber, 1973).
3. After 20 min pre-incubation, 1.72×10^{-4} μ mol of tritiated leucine (in 0.1 ml of filtered sea water).

The mixture of cells, buffer, glucose, antibiotics and enzymes (now 2.8 mls in volume) was placed in a Hetotherm waterbath and shaker. The samples were shaken at 130 strokes per minute at 20°C for two hours.

The adductor muscle was reduced to a suspension by sonification. Sea water was added and the turbidity adjusted to give a reading of 0.2-0.3 units. Two-one ml portions were pipetted into test tubes for analysis of RNA and two-one ml portions into centrifuge tubes (Series B) for analysis of DNA and protein. No analysis was made for the amount of incorporation of leucine by the homogenate of the adductor muscle.

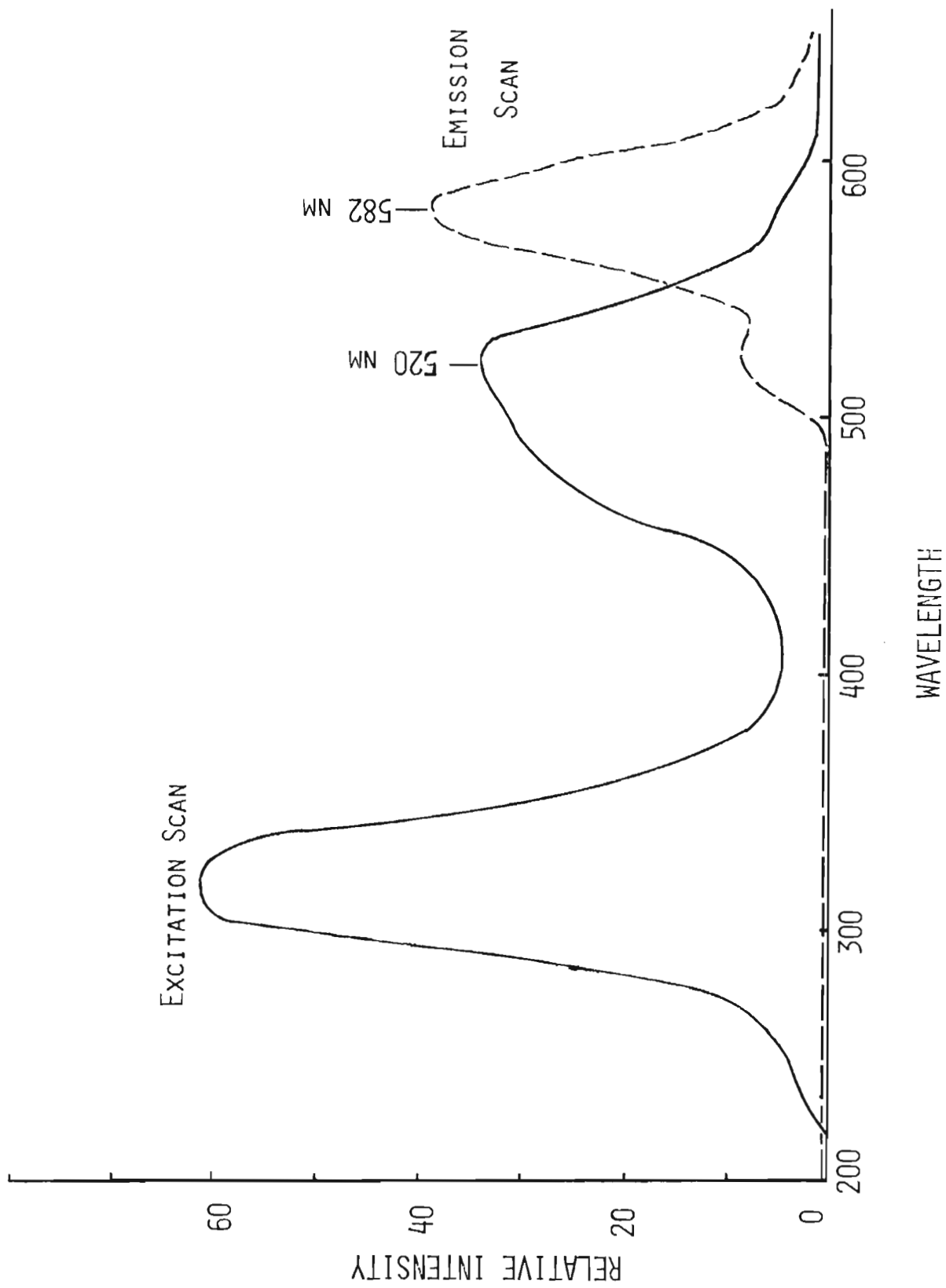
II. 3. ESTIMATION OF RNA.

RNA was measured by its fluorescence with ethidium bromide using the method of Le Pecq and Paoletti (1966). Immediately upon homogenization of the oysters the samples for analysis of RNA must be placed in an ice bath to prevent any degradation of RNA by the enzyme RNase. Fluorescence measurements were made in an Aminco Bowman spectrofluorimeter at an excitation wavelength of 520 nm and emission wavelength of 582 nm. Fig. 1 shows the excitation and emission spectra for RNA in this instrument. Although DNA may be measured after the samples have been incubated with the enzyme RNase it became apparent that this part of the method was unsatisfactory because of masking of the DNA due to the turbidity of the samples. The amount of RNA was determined from the difference between the fluorescence of undigested oyster extract and the fluorescence of the extract treated with RNase. The preparation of the reagents and the procedure is as follows.

II.3.1. Preparation of reagents.

Ethidium bromide was made up to a concentration of 20 µg/ml by dissolving the compound in 0.1 M Tris buffer (pH 7.8), 0.1 M NaCl in distilled water. The enzyme was prepared by dissolving 15 mg of RNase (Type II-A, Sigma Chemical Co.) in 25 ml of the TRIS buffer. To prepare the standard, 7 mg of RNA (Type XI, Sigma Chemical Co.) was dissolved in 25 ml of buffer. The solution is unstable and must be prepared immediately before use.

Fig. 1. Excitation and emission spectra for RNA



II. 3.2. Procedure.

One ml of the ethidium bromide solution was added to each tube containing the oyster homogenate. The fluorescence of this mixture was read at room temperature. 0.1 ml of RNase was added to the mixtures and the samples were incubated at 50°C for 1 hr. After the samples had cooled to room temperature the fluorescence was read again. The amount of RNA in each sample may be calculated according to the following expression:

$$\text{RNA } \mu\text{g/ml} = (F_1 - F_2) \times S_1 \times (F_{\text{RNA}} / C_{\text{RNA}} \times S_2 - F_B)$$

Where F_1 = fluorescence of undigested extract

F_2 = fluorescence of digested extract

S_1 = scale of fluorimeter used for measuring extract

F_{RNA} = fluorescence of RNA standard

C_{RNA} = concentration of RNA standard

S_2 = scale of fluorimeter used for measuring standard

F_B = fluorescence of sea water/ethidium bromide blank

II. 4. ESTIMATION OF PROTEIN, DNA, AND PROTEIN SYNTHESIS.

The Schmidt-Thannhauser assay used in this analysis has been discussed by Munro, (1969c). Protein was determined with the Lowry et al (1951) technique of Campbell and Sargent (1967). DNA was measured by the method of Ceriotti (1952). Protein synthesis was determined as per Pease (1973).

II. 4. 1. Reagents and standards.

1. Lowry method for protein. A primary standard was made up by dissolving 3 mg of egg albumen (Grade V, Sigma Chemical Co.) in 10 ml of 0.3N KOH. Reagents were as follows:

- a. Reagent A was a 2% solution of NaK-Tartrate.
- b. Reagent B was a 1% solution of CuSO_4 .
- c. Reagent C was a 50% mixture of reagents A and B plus 0.1 ml/ml of C of a solution of 2% Na_2CO_3 in 0.1 N NaOH.
- d. Reagent D was a mixture of 1.36 ml of distilled water plus 1 ml of phenol reagent (Folin-Ciocalteu; Fisher Scientific Co.)

Reagents C and D were prepared immediately before use.

2. Ceriotti method for DNA. A primary standard was made up by dissolving 4.5 mg of DNA (Type I, Sigma Chemical Co.) in 100 ml of 0.3N KOH. A 0.04% solution of indole was also prepared.

3. Measurement of radioactivity. A scintillation cocktail was made up to a concentration of 5 gm of PPO (2,5-diphenyloxazole; Packard Instrument Co.) and 0.1 gm of POPOP (1,4-bis-(2-(4-methyl-5-phenyloxazolyl))-benzene) per liter of scintillation grade toluene. A stock solution of leucine-4,5- H^3 was prepared by diluting 1 mCi to 10 ml with filtered sea water. Standards were made up by diluting 0.1 ml of the stock of radioactive substrate to 25 ml with filtered sea water. 0.5 ml was mixed with NCS solubilizer (Amersham-Searle), scintillation fluid, and a thixotropic agent, Cab-O-Sil (Packard Inst.). Triplicate-5 min counts were made in a Packard TRICARB Model 3880/544 liquid scintillation counter. Quenched standards (Packard Inst.) were used to determine H^3 counting efficiencies. This information was used to determine total and specific activities of the labelled compound. Between experiments the radioactive leucine was stored in a deep freeze.

II. 4. 2. Chemical analyses.

At the end of the incubation 1.0 ml samples were taken from the erylenmeyer flasks and mixed with 2 ml of cold (0°C) ethanol. The samples in series A tubes were treated in the same manner. The samples were centrifuged and the supernatent discarded. The pellets were then re-suspended in ethanol/ether (3:1) and centrifuged. The pellets were re-suspended in ethyl ether and centrifuged. The pellets were subsequently washed twice with cold (0°C) 10% TCA (trichloroacetic acid). Then the pellets were suspended in 1 ml of 0.3N KOH and placed in a waterbath at 37°C for 1 hr. The samples were then chilled in an ice bath and 0.6 ml of 1.2N PCA (perchloric acid) added. After 10 min had elapsed any precipitated protein was removed by centrifugation and the pellets washed twice with 0.3N PCA. The samples were again warmed to 37°C in 2.5 ml of 0.3N KOH to dissolve protein and DNA. 0.5 ml of each sample was taken for analysis of radioactivity. 0.8 ml was taken for analysis of protein and 1.0 ml for DNA.

Radioactivity.

To analyze for radioactivity 0.3 ml of NCS solubilizer was added to each scintillation vial containing the KOH extract and the sample mixed on a stirrer. 10 ml of scintillation fluid and a thixotropic agent were added. Triplicate-5 min counts were taken of the number of dpm/sample in the tritium channel of the TRICARB. The results were calculated according to the following expression:

Total incorporation (dpm/mg protein)

$$\frac{\text{dpm/sample} \times \underline{2.5 \text{ ml of KOH}}}{0.5 \text{ ml aliquot}}$$

protein in 2.5 ml of KOH

Since the protein content of the samples (Series B) taken for incubation with the isotope and analysis of radioactivity was lower than the protein content of the sample of oyster homogenate taken only for analysis of protein and DNA (Series A), the amount of radioactivity was additionally adjusted upward for the differences in the protein content of the two solutions.

Lowry procedure for protein.

In the Lowry procedure for protein 0.8 ml of reagent C was added to the centrifuge tube containing the protein dissolved in KOH. The mixture was stirred and allowed to stand at room temperature for 10 min. 0.1 ml of reagent D was added and the mixture was stirred and allowed to stand for an additional 30 min. The absorbance of the samples was read at 666 nm. The quantity of protein per sample was calculated according to the following expression:

Total protein (mg/sample)

$$\frac{\text{OD/ml}}{\text{OD/mg protein}} \quad \times \quad \frac{2.5 \text{ ml of KOH}}{0.8 \text{ ml aliquot}}$$

Cerioti procedure for DNA

In the Cerioti procedure 0.5 ml of the indole reagent and 0.5 ml of concentrated HCl are mixed with the 1.0 ml of sample containing DNA. The test tubes were then heated in a boiling water bath for 10 min. After the samples had cooled to room temperature their absorbance was read at 490 nm. The quantity of DNA/sample was calculated according to the following expression

Total DNA (μg /sample)

$$\frac{\text{OD/ml}}{\text{OD/ } \mu\text{g DNA}} \quad \times \quad \frac{2.5 \text{ ml of KOH}}{1.0 \text{ ml aliquot}}$$

II. 5 PRECISION OF THE ANALYSES

In the present study precision is defined in the manner used by Strickland and Parsons (1965); and at the 95% level the precision \underline{P} is 2σ . Therefore, if \underline{n} determinations are made on cell suspensions of the oysters the true results will lie in the range:

Mean of n determinations $\pm P/\sqrt{n}$ with 95% confidence.

The tables in the appendix report the results with an analysis of \underline{P} .

RESULTS

The data examined in this report were obtained on oyster tissues collected at Portage Creek and Seal Island from January to October 1975. Some collections were also made in October 1974 but this material was analyzed with methods which are not considered satisfactory today. The six month progress report (Pease, 1975) contains comments on this material.

III. 1. MONTHLY CHANGES IN ENVIRONMENTAL PARAMETERS AT PORTAGE CREEK AND SEAL ISLAND.

Portage Creek

Average temperatures and salinity at Portage Creek during the months of May to October are shown in Figs 2a and 2b. At the end of May temperatures rose above 10°C and remained at about 20°C for the next three months before they again dropped below 10°C in October. Salinity varied slightly around 20 o/oo during the same period.

Seal Island

Average temperatures and salinity at Seal Island during the month of January and from May to October are shown in Figs 2c and 2d. Temperatures were lower than at Portage Creek but the salinities were higher.

III.2. MONTHLY CHANGES IN THE RNA/DNA RATIOS, PROTEIN SYNTHESIS, AND THE WET WT OF OYSTERS AT PORTAGE CREEK AND SEAL ISLAND.

Portage Creek

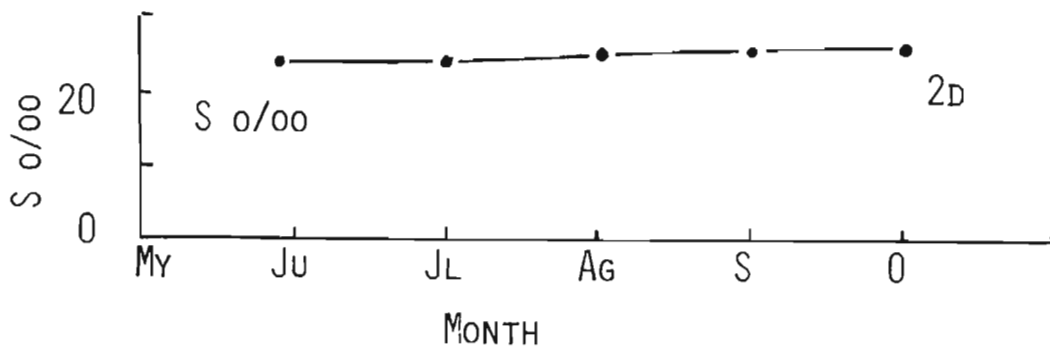
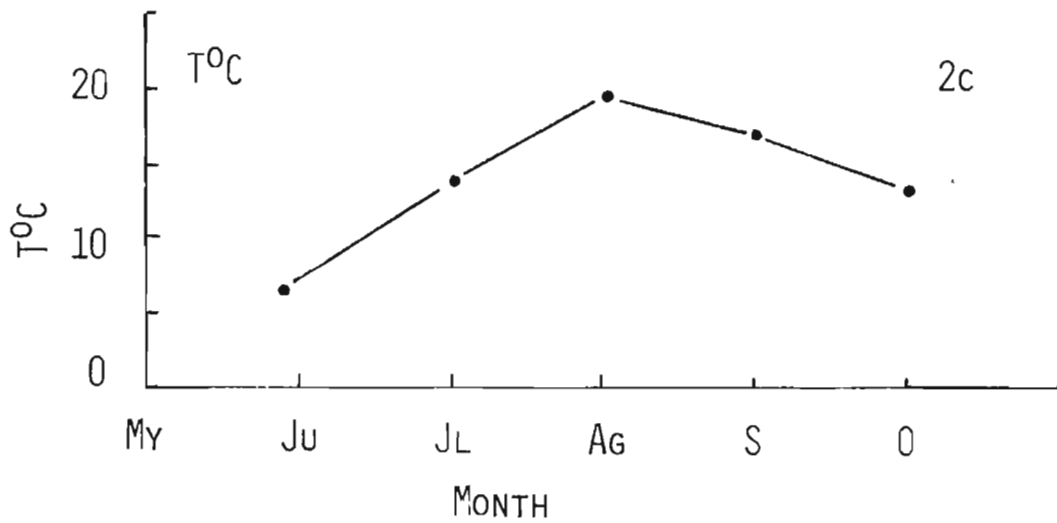
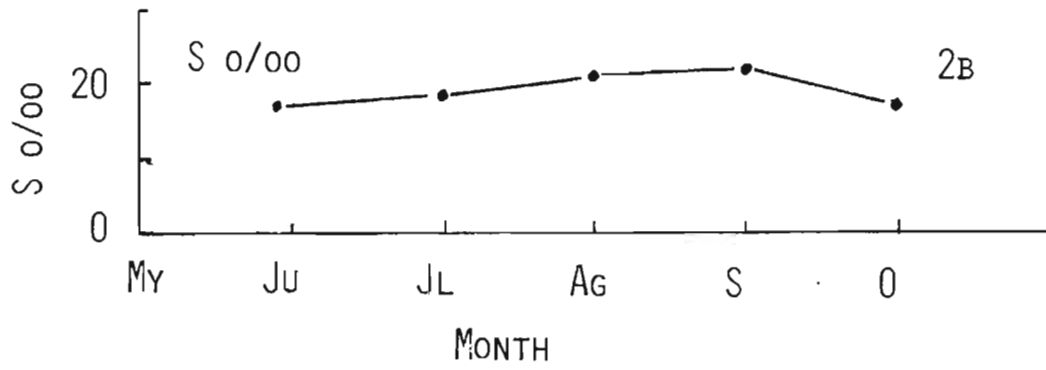
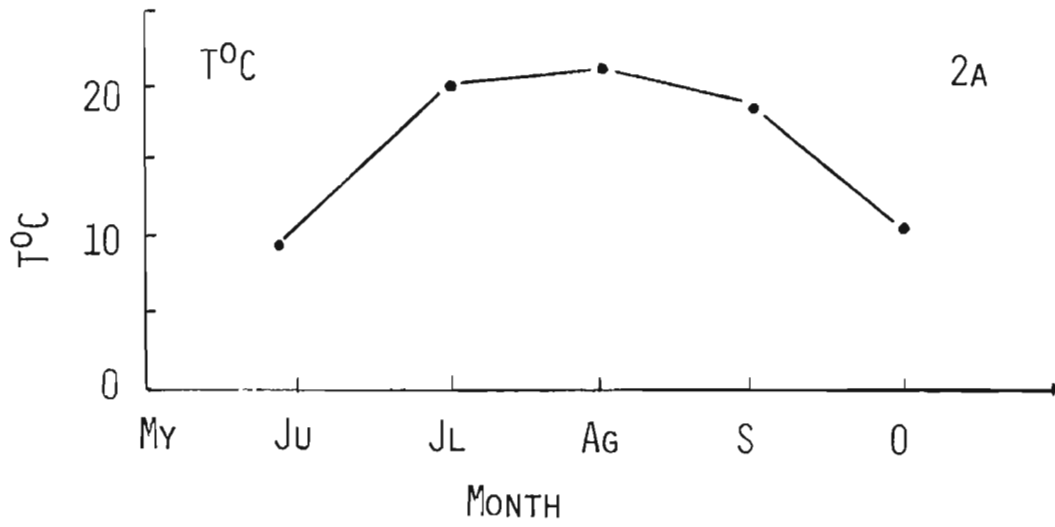
Seasonal pictures of the changes in the RNA/DNA ratios in the whole oyster and adductor muscle, protein synthesis, and the wet

Fig 2a. Monthly changes in temperature at Portage Creek.

Fig 2b. Monthly changes in salinity at Portage Creek.

Fig 2c. Monthly changes in temperature at Seal Island

Fig 2d. Monthly changes in salinity at Seal Island.



wt of the whole oyster tissues are presented in the following figures. Figs 3a-c show the changes of these parameters in the 1974 and 1975 year class. Figs 4a-d show the changes in the 1973 year class; Figs 5a-d, the changes in the 1972 year class.

Seal Island

A seasonal picture of the changes in the RNA/DNA ratios in the whole oyster and adductor muscle, protein synthesis, and the wet wt of the whole oyster tissues is shown in Figs 6a-d for samples gathered at Seal Island. The 1973 year class is represented in these figures.

III. 3. ALLOMETRIC RELATIONSHIPS IN THE OYSTER C. VIRGINICA.

The seasonal cycle of the oyster C. virginica includes the appearance of gonads as a substantial part of the tissues during the months of June through August. Gonadal tissues would give rise to substantial changes in RNA/DNA ratios derived from analyses made on whole animals and complicate attempts to make predictions of growth based on these ratios. Therefore, an attempt was made to determine if the growth of whole oysters could be predicted from the growth and RNA/DNA ratio in one specialized tissue, the adductor muscle. Figs 7 and 8 respectively show the relationship of the whole oyster wet wt including the shell to the wet wt of the animal alone and the wet wt of the animal to the adductor muscle. The relationships appear to follow the equation $Y = aX^b$ and the correlation coefficients are respectively 0.96 and 0.93.

An examination of the seasonal data (Section III.2) suggested that there is a relationship between the RNA/DNA ratio of

Fig 3a. Monthly changes in the ratio of RNA/DNA in whole 1974 and 1975 oysters at Portage Creek.

Fig 3b. Monthly changes in protein synthesis in 1974 and 1975 oysters at Portage Creek.

Fig 3c. Monthly changes in weight of 1974 and 1975 oysters at Portage Creek.

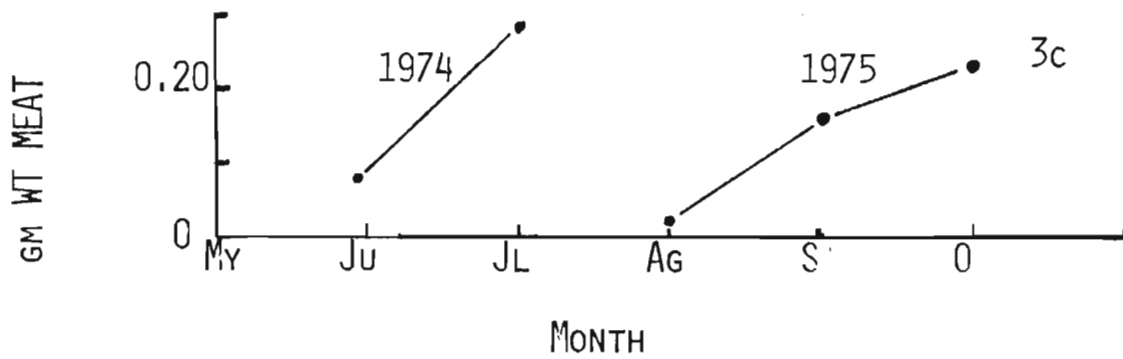
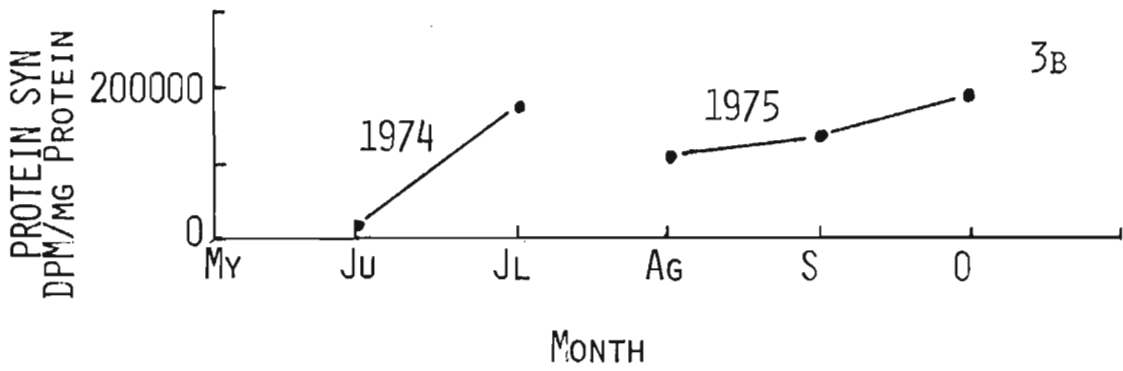
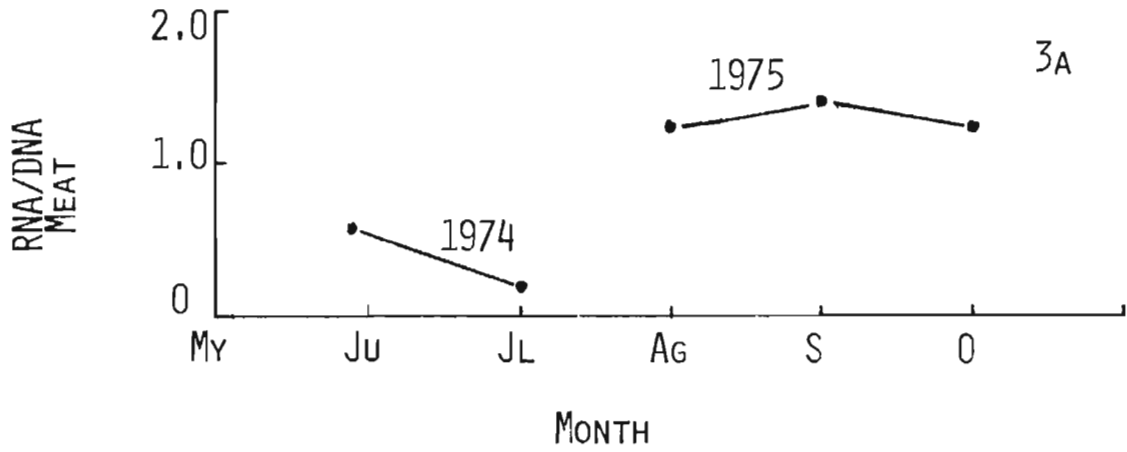


Fig. 4a. Monthly changes in the RNA/DNA ratio in whole 1973 oysters at Portage Creek.

Fig 4b. Monthly changes in the RNA/DNA ratio in the adductor muscle of 1973 oysters at Portage Creek.

Fig 4c. Monthly changes in protein synthesis in 1973 oysters at Portage Creek.

Fig 4d. Monthly changes in the wet wt of 1973 oysters at Portage Creek.

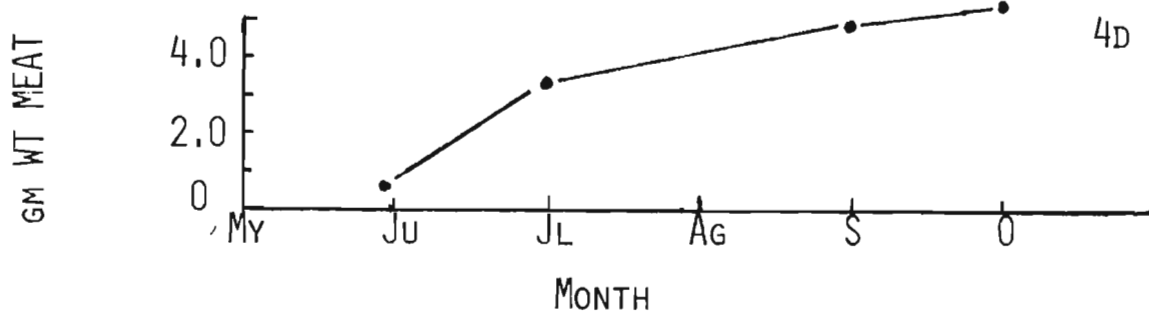
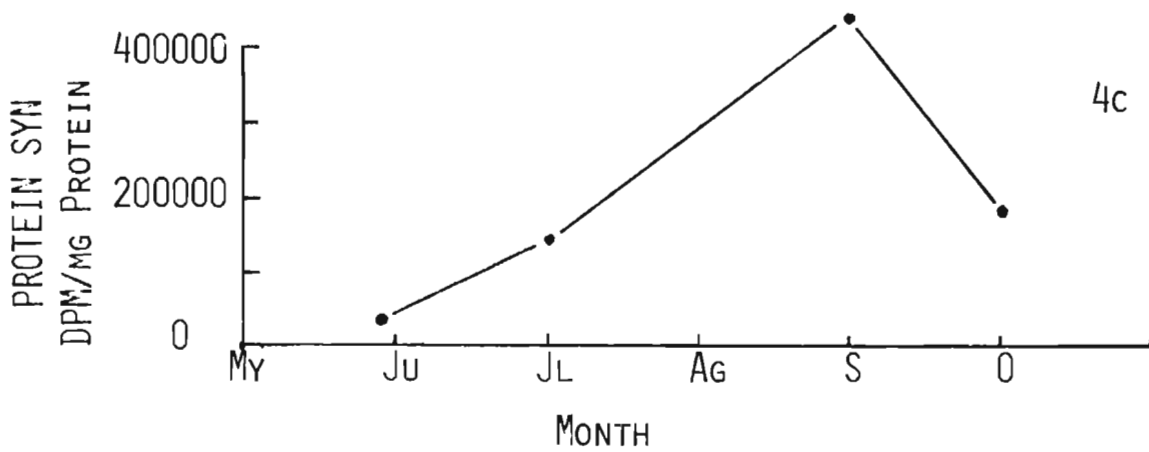
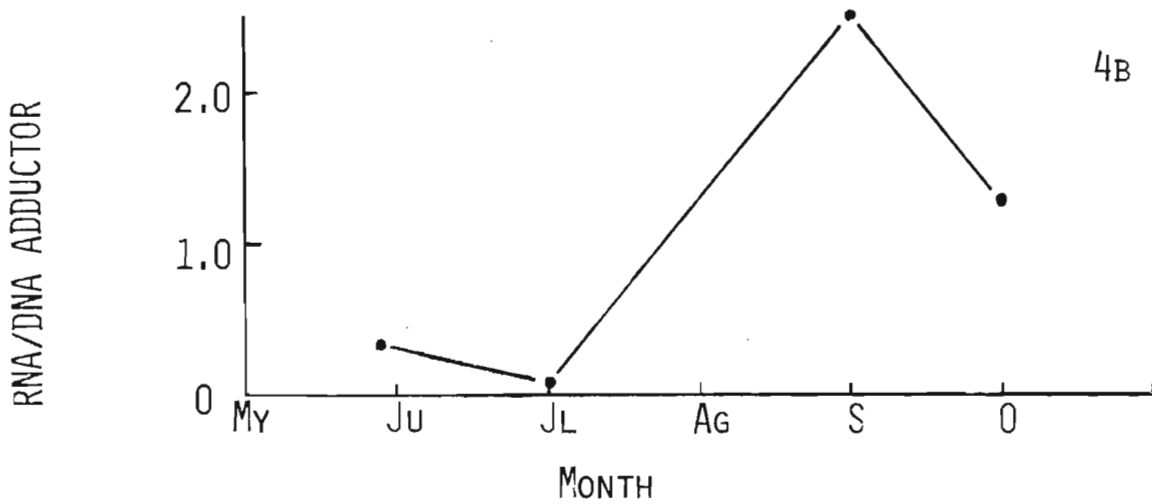
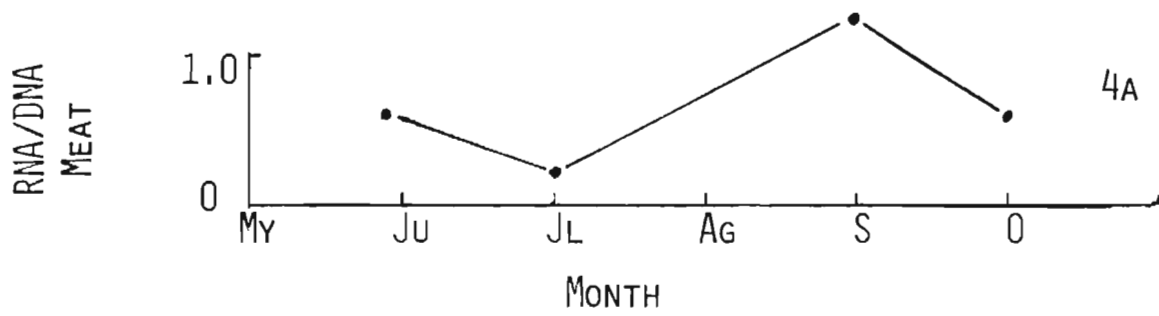


Fig 5a. Monthly changes in the RNA/DNA ratio in whole 1972 oysters at Portage Creek.

Fig 5b. Monthly changes in the RNA/DNA ratio in the adductor muscle of 1972 oysters at Portage Creek.

Fig 5c. Monthly changes in protein synthesis in 1972 oysters at Portage Creek.

Fig 5d. Monthly changes in the wet wt of 1972 oysters at Portage Creek.

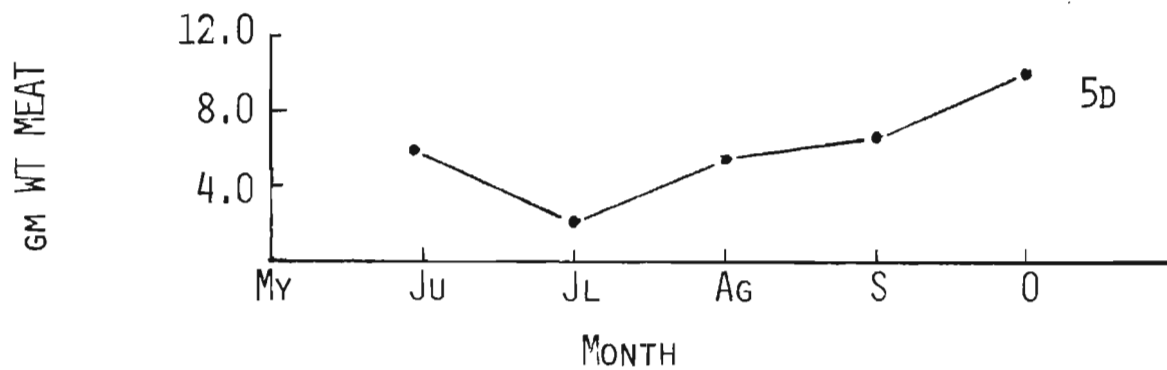
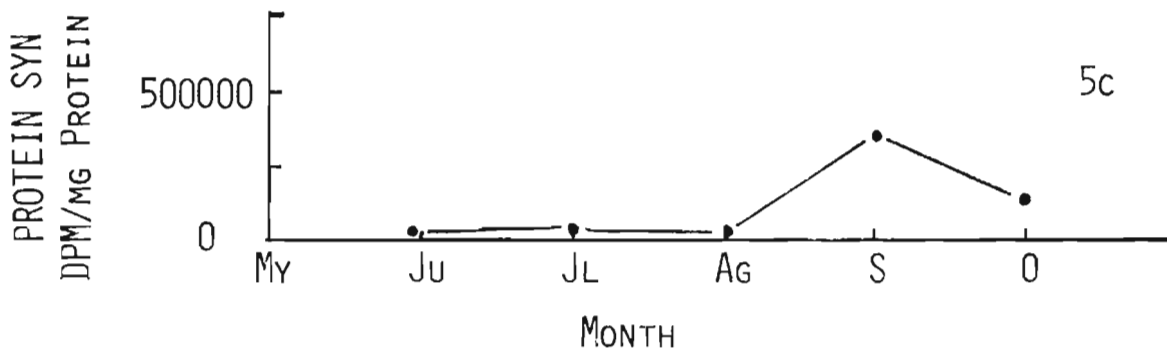
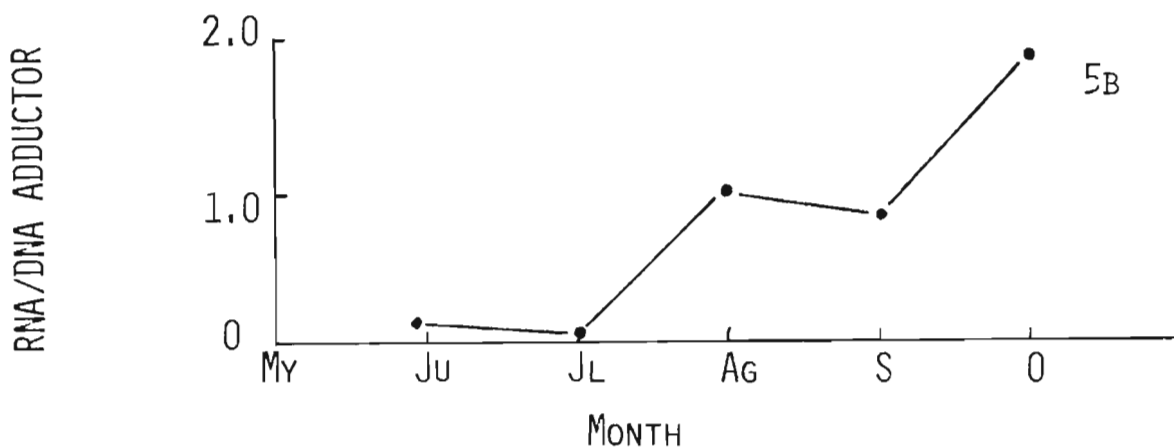
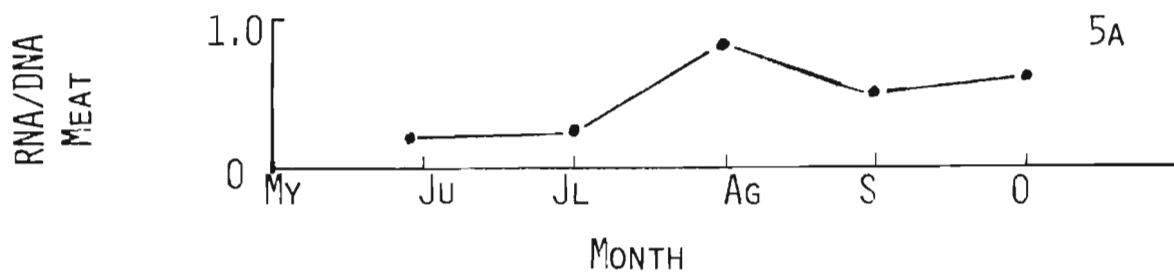


Fig 6a. Monthly changes in the RNA/DNA ratio in whole 1973 oysters at Seal Island.

Fig 6b. Monthly changes in the RNA/DNA ratio in the adductor muscle of 1973 oysters at Seal Island.

Fig 6c Monthly changes in protein synthesis in 1973 oysters at Seal Island.

Fig 6d. Monthly changes in the wet wt of 1973 oysters at Seal Island.

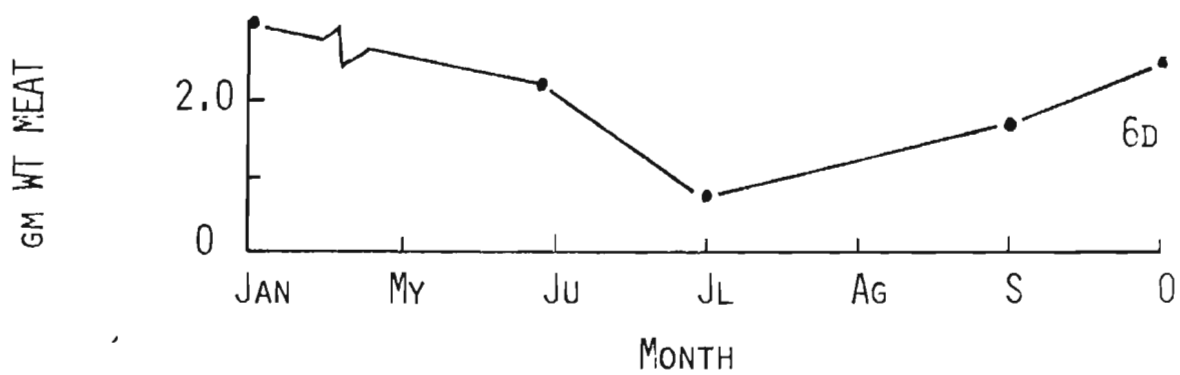
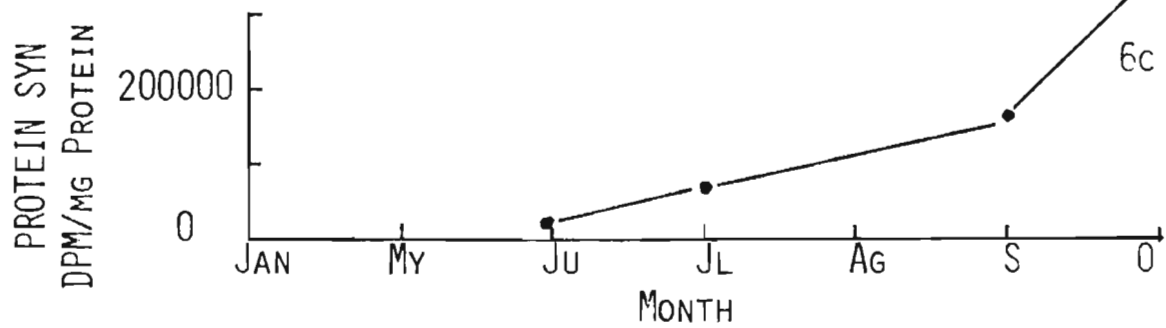
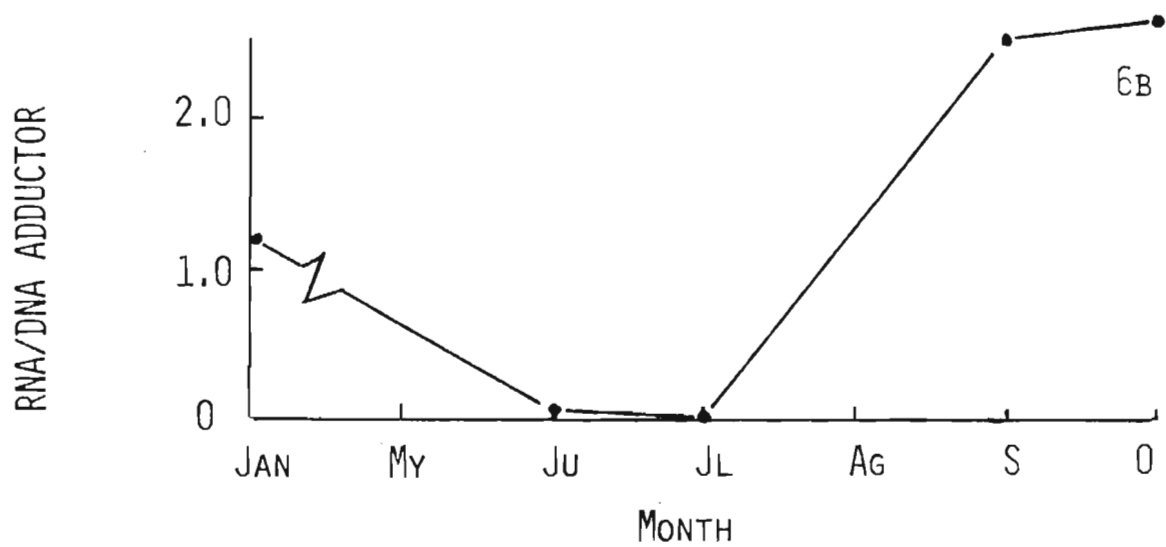
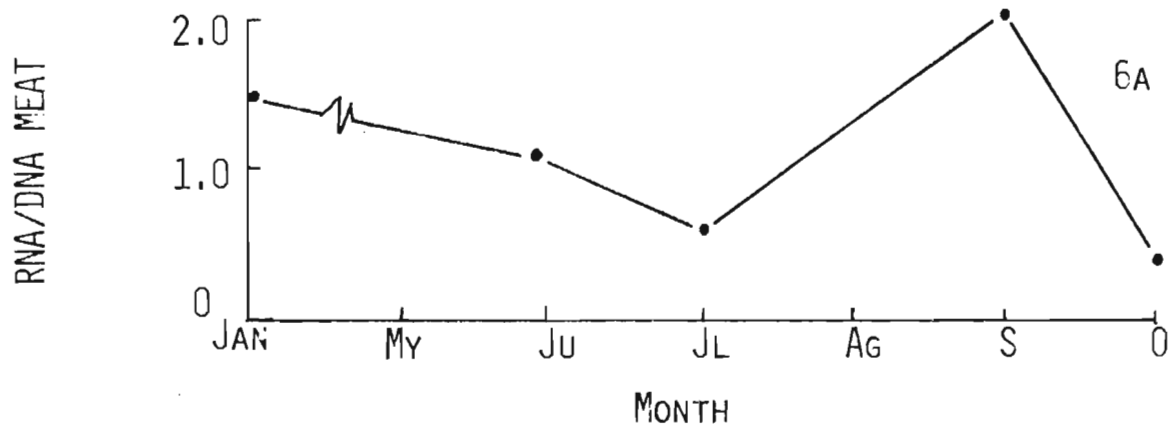


Fig. 7. Relationship of total oyster wt to the wet wt of the living tissues excluding the adductor muscle. The equation of the log linear fit to the data is $Y = 0.18X^{0.81}$ and the correlation coefficient is 0.96

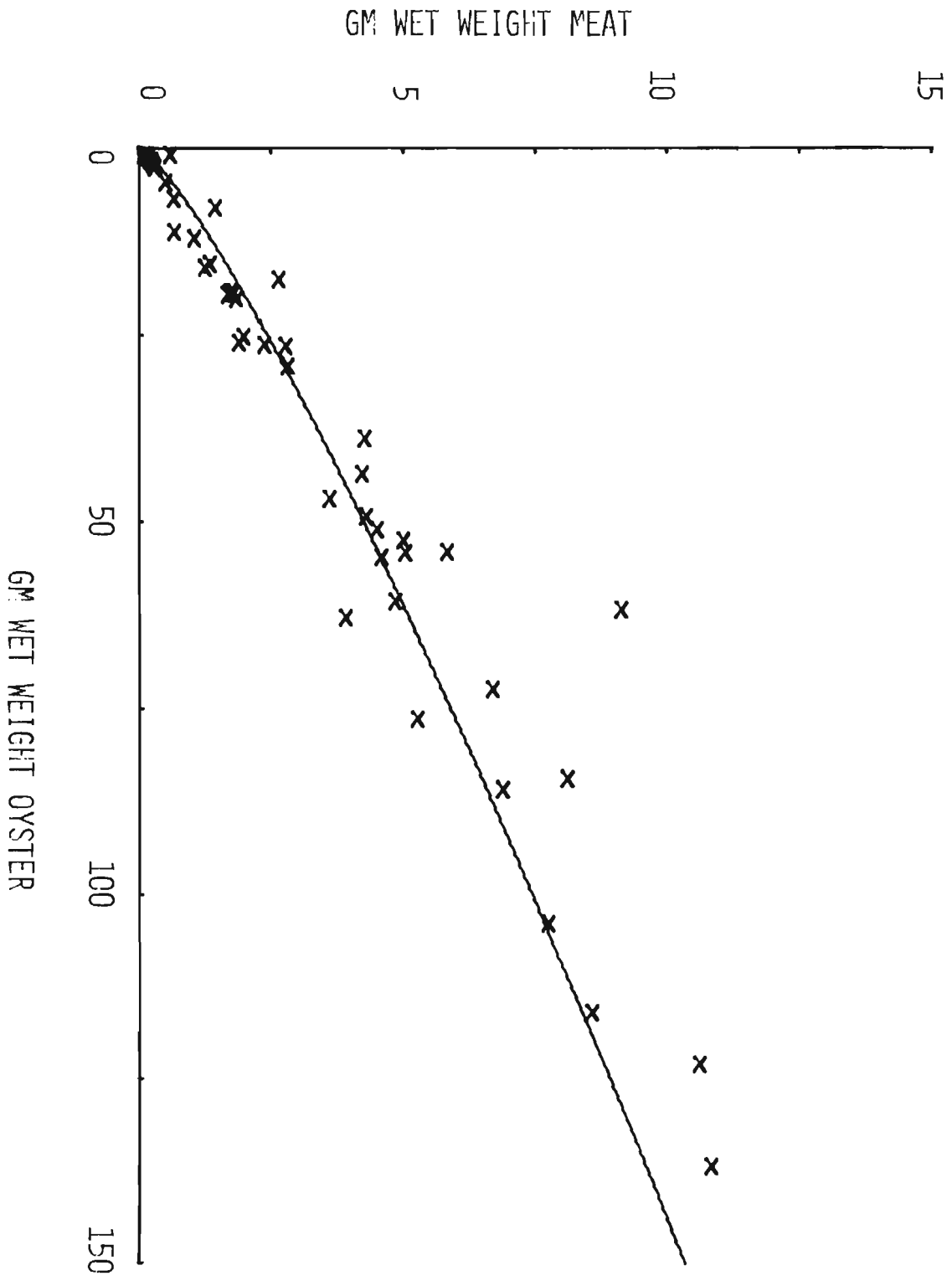
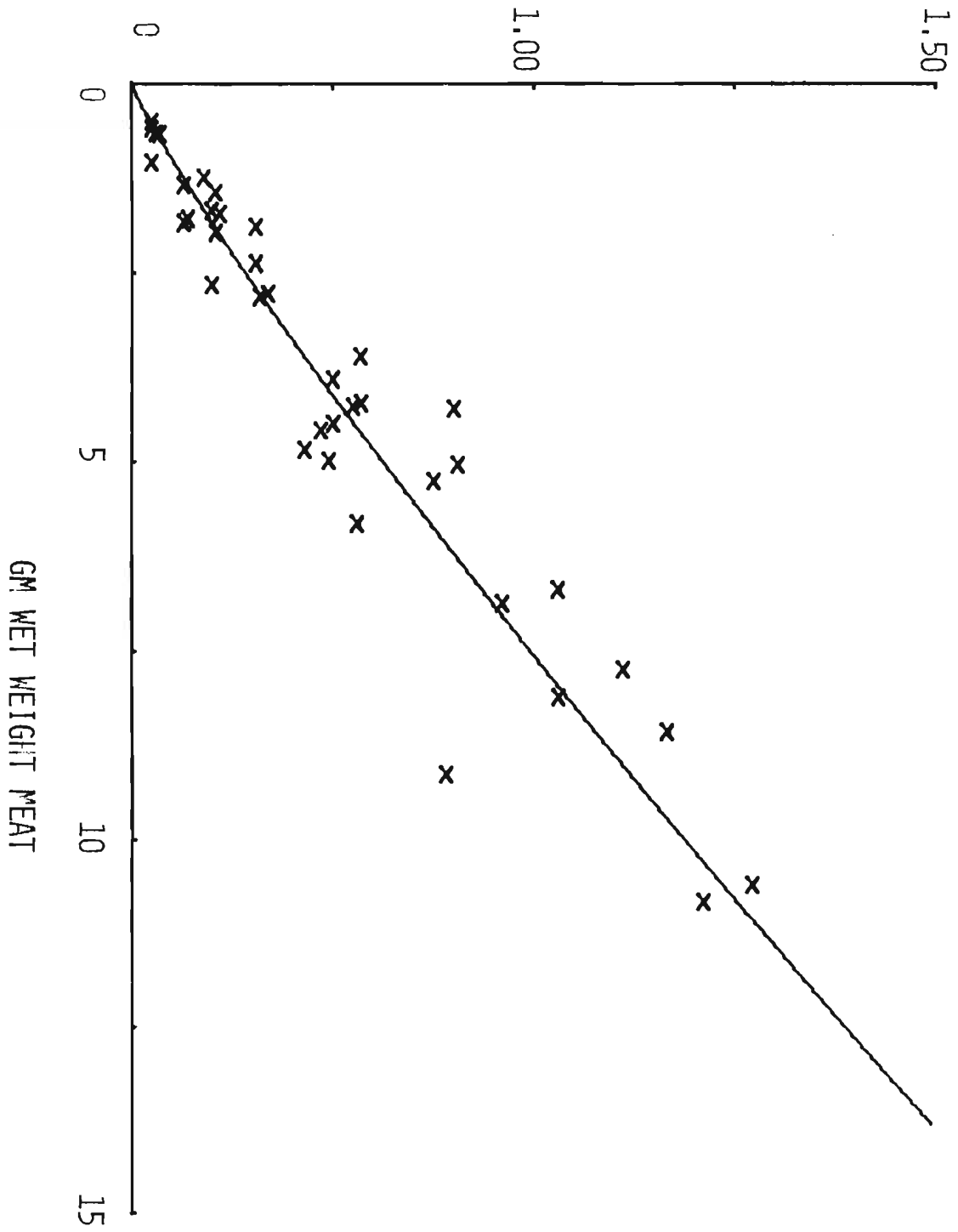


Fig 8. Relationship of the wet wt of the oyster tissues to that of the adductor muscle. The log linear fit to the data follows the equation $Y = 0.098X^{1.14}$ and has a correlation coefficient of 0.93.

GM WET WT ADDUCTOR



the animal and that of its adductor muscle. Fig. 9 examines the degree of correlation between the two for samples collected at Portage Creek. The best fit was again for the equation $Y=X^b$ and the correlation was 0.71. For the Seal Island data, however, the correlation coefficient was only 0.01 (Fig. 10) indicating that variability in the ratios here was unrelated to body size in these samples. In subsequent analyses we have avoided pooling the data for the two areas.

Although we have shown that it is possible to use the RNA/DNA ratio in the adductor muscle to study the growth of the oysters it is still necessary to use information derived from the RNA/DNA ratios in the whole animal because it was not possible to dissect the adductor muscle from the 1974 and 1975 oysters. In any case it is not believed that the two ratios in these year classes differ widely because there is no contribution of gonad.

III. 4. SIGNIFICANCE OF THE RNA/DNA RATIOS AND PROTEIN SYNTHESIS FOR DIFFERENT YEAR CLASSES AND BETWEEN SAMPLING AREAS.

An analysis of variance was applied to the RNA/DNA data for each year class and between the areas of sampling using the ANOVA program of a Hewlett-Packard calculator. Table Ia and Ib report the results for the analysis of the adductor muscle. There are no significant differences between year classes or between sampling areas. For the analyses of the RNA/DNA ratios in the whole animal (Table IIa,b) there are significant differences between year classes but not between areas. Therefore RNA/DNA ratios in Portage Creek and Seal Island appear to belong to one gene pool. If the RNA/DNA ratios measured on the whole animal are corrected for the weight of the animals (Table IIc) there are no significant differences between

Fig 9. Relationship of the RNA/DNA ratio in the whole animal and that of its adductor muscle for samples obtained at Portage Creek. The fit to the data follows the equation $Y = 0.64X^{0.70}$ and the correlation coefficient is 0.71.

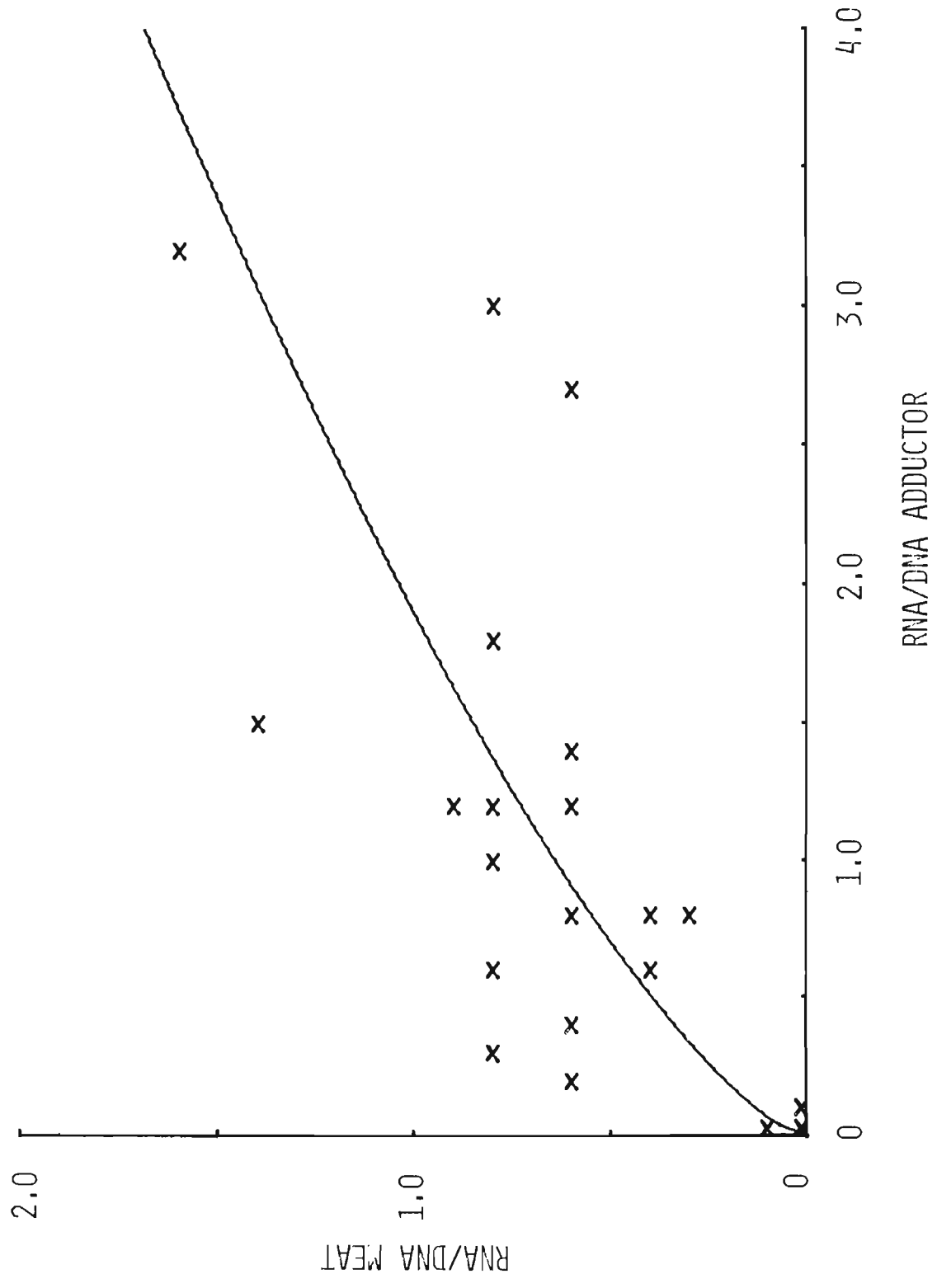


Fig 10. Relationship of the RNA/DNA ratio in the whole animal to that of the adductor muscle for samples collected at Seal Island. The fit to the data follows the equation $y = 0.79x^{0.03}$ and the correlation coefficient is 0.01.

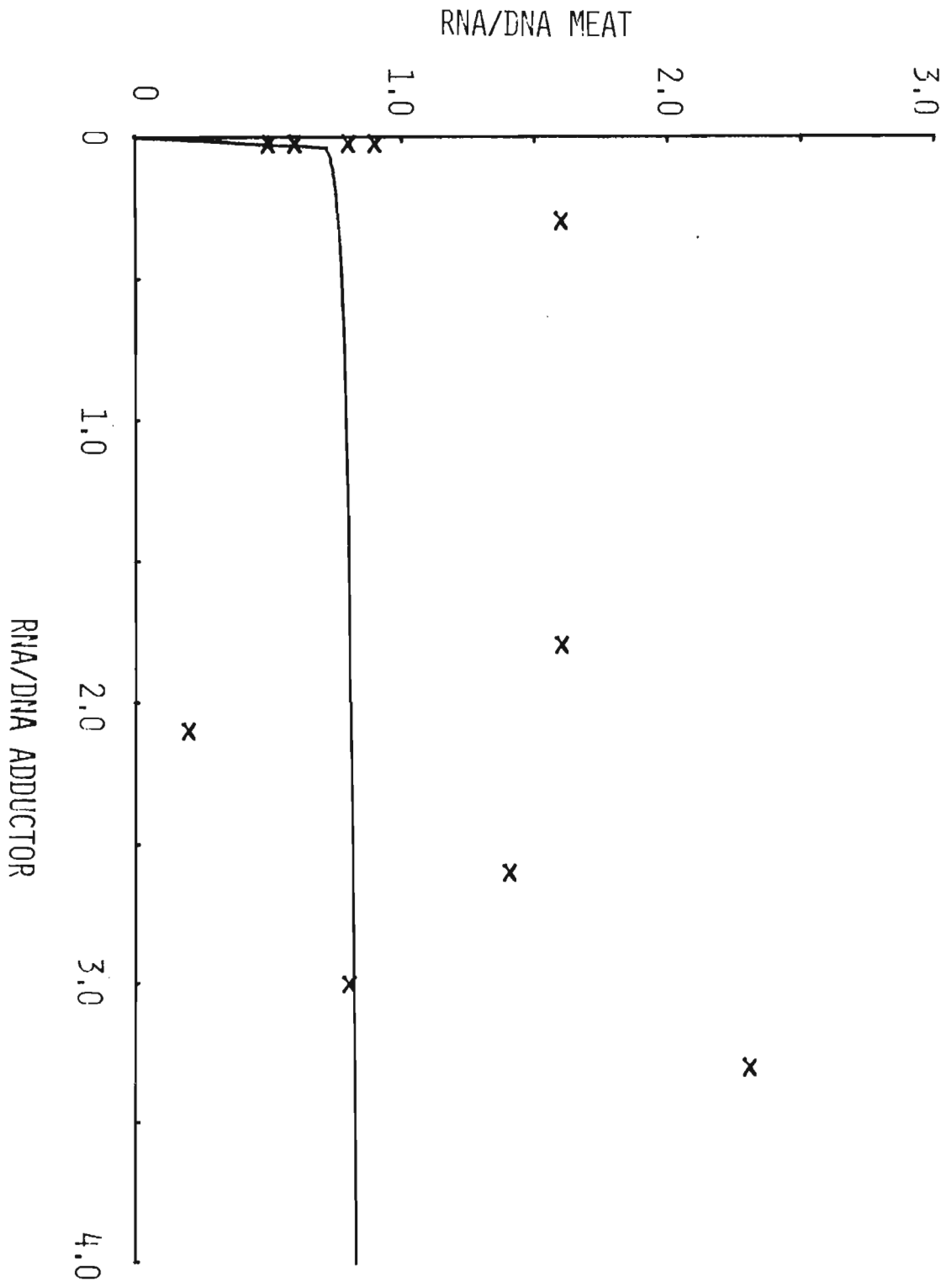


Table Ia. ANOVA between the different year classes
for the RNA/DNA ratios in the adductor muscle.

Source	df	SS	MS	F	
Between	3	2.9	1.0	1.4	NS
Within	30	20.4	0.7		
Total	33				

Table Ib. ANOVA between sampling areas for the RNA/DNA
ratios in the adductor muscles of the 1973 oysters

Source	df	SS	MS	F	
Between	1	0.1	0.1	0.1	NS
Within	21	32.7	1.6		
Total	22				

Table IIa. ANOVA for the RNA/DNA ratios in the whole animal between the different year classes at Portage Creek.

Source	df	SS	MS	F
Between	3	3.2	1.1	7.7*
Within	30	4.2	0.1	
Total	33			

Table IIb ANOVA between sampling areas.

Source	df	SS	MS	F	
Between	1	0.4	0.4	1.4	NS
Within	21	6.8	0.3		
Total	22				

Table IIc ANOVA for the RNA/DNA ratios in the whole animal corrected for weight ($\text{RNA/DNA} / W^{-0.26}$)

Source	df	SS	MS	F	
Between	3	1.4	0.5	1.5	NS
Within	30	9.8	0.3		
Total	33				

year classes indicating that the differences observed in Table IIa are due to the size of the animals, not year class.

An analysis of variance was also applied to the data for protein synthesis. Table IIIa and b shows that there are significant differences between year classes but not sampling areas.

III. 5. RELATIONSHIP OF RNA/DNA TO PROTEIN SYNTHESIS.

The graphs of the data on a seasonal basis indicate a general similarity in the seasonal trends of the ratio of RNA/DNA and protein synthesis. Accordingly, we have plotted the monthly data for these two parameters as correlation diagrams and provided measures of similarity by curve fittings.

Fig 11 and 12 show data representing RNA/DNA ratios and the rate of protein synthesis measured in the whole animal for oysters respectively collected at Portage Creek and Seal Island. Also shown are the fitted curves. For Portage Creek the linear fit of the data produces a correlation coefficient of 0.10 and the exponential, 0.06; for Seal Island the correlation coefficients are 0.03 (linear) and 0.02 (exponential). As might be expected, there is clearly little indication that these two indices are reflecting the same aspects of production at the same time.

Fig 13 and 14 show data on RNA/DNA ratios in the adductor muscle plotted against the rate of protein synthesis in the whole animal for oysters collected at these same two locations. The linear fit of the data for Portage Creek produces a correlation coefficient of 0.33 and the exponential, 0.29. For Seal Island the coefficients were 0.62 and 0.66. In contrast to the data of Fig 11 and 12 these

Table IIIa. ANOVA for protein synthesis between the different year classes at Portage Creek.

Source	df	SS	MS	F
Between	3	1.15×10^{11}	3.82×10^{10}	3.19*
Within	29	3.47×10^{11}	1.20×10^{10}	
Total	32			

Table IIIb. ANOVA for protein synthesis between sampling areas.

Source	df	SS	MS	F	
Between	1	1.32×10^{10}	1.31×10^{10}	0.5	NS
Within	20	5.28×10^{11}	2.64×10^{10}		

Fig. 11. Relationship of RNA/DNA ratios and rate of protein synthesis measured in whole oysters for animals collected at Portage Creek. The linear fit follows the equation $Y = 82464 + 103359X$ and has a correlation coefficient of 0.10. The exponential fit to the data follows the equation $Y = 56436e^{0.64x}$ and has a correlation coefficient of 0.06.

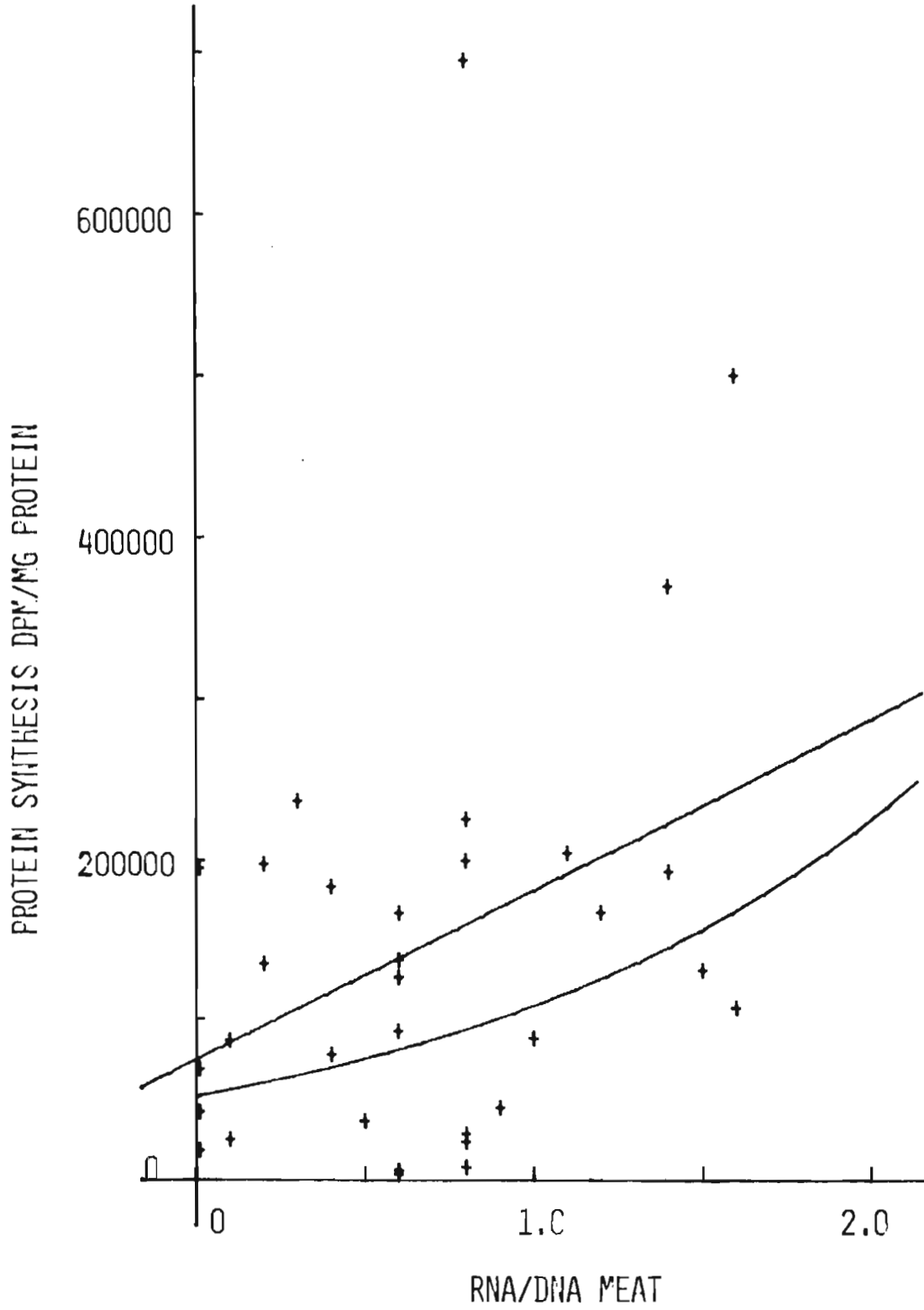


Fig 12 Relationship of the RNA/DNA ratios measured in whole oysters and the rate of protein synthesis for oysters collected at Seal Island. The linear fit to the data follows the equation $Y = 207514 - 45442X$ and has a correlation coefficient of 0.03. The exponential fit follows the equation $Y = 110859 e^{-0.34X}$ and has a correlation coefficient of 0.02.

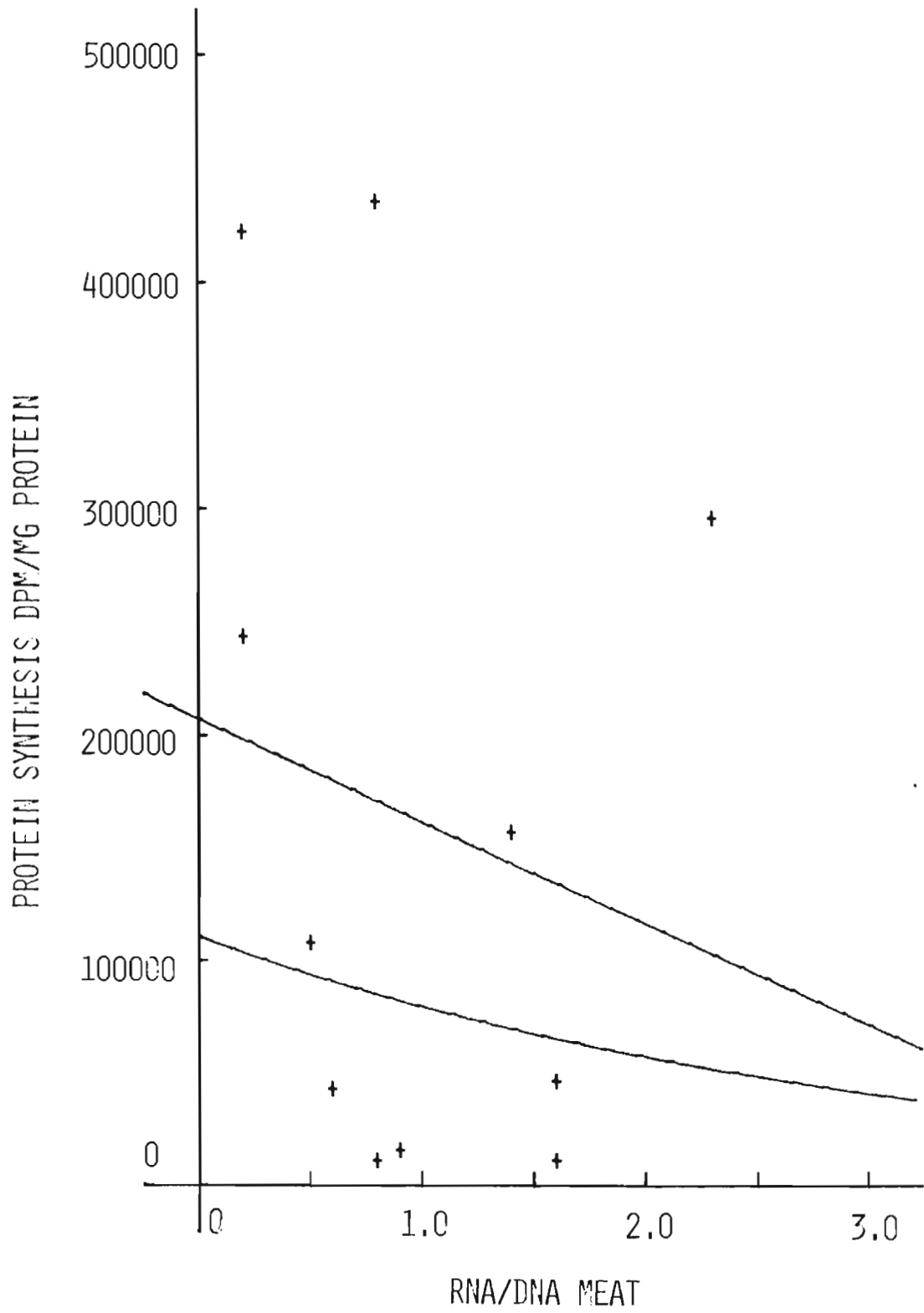


Fig 13. Relationship of the RNA/DNA of adductor muscles and protein synthesis for oysters collected at Portage Creek. The linear fit to the data follows the equation $Y = 61210 + 107337X$ and has a correlation coefficient of 0.33. The exponential fit has the equation $Y = 41278e^{0.75X}$ and a correlation coefficient of 0.29.

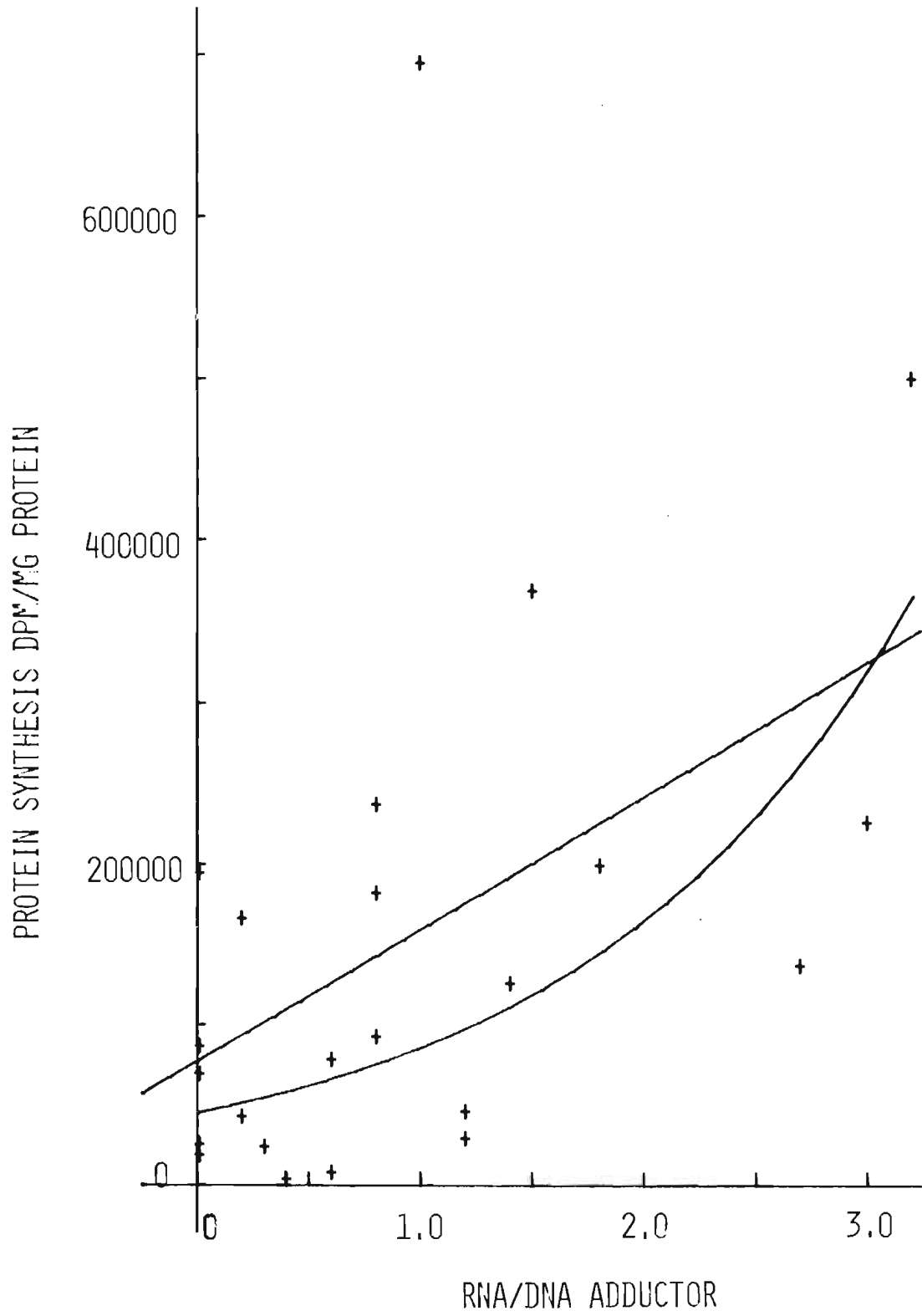
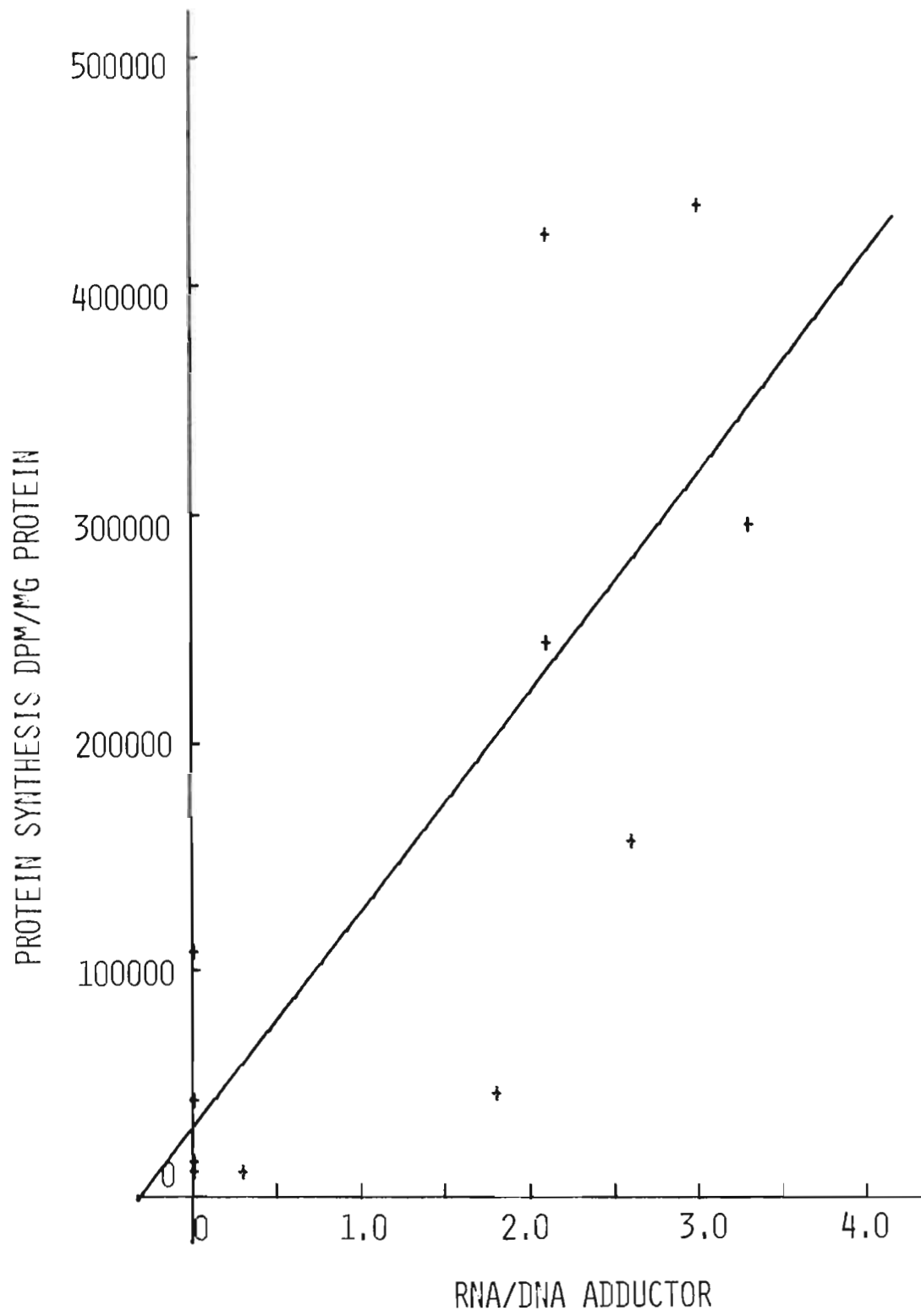


Fig. 14. Relationship of the RNA/DNA ratio of adductor muscles and protein synthesis for oysters collected at Seal Island. The linear fit follows the equation $Y = 29512 + 96174X$ and has a correlation coefficient of 0.62. The exponential is $Y = 23762e^{0.87X}$ and a coefficient of 0.66.



coefficients suggest that it is possible to use RNA/DNA ratios derived from the adductor muscle to reflect aspects of protein synthesis in the oyster. The differences between them may well reflect effects of development relative to the gonads which form a variable fraction of the tissue of the whole animal from time to time and between oysters.

Since there was no direct correlation between the RNA/DNA ratio and the rate of protein synthesis in the whole animal it was decided to determine if there were any lags in the relationship between these two parameters. Using the average RNA/DNA ratios of each months data with the subsequent month rate of protein synthesis Fig 15 was produced. The RNA/DNA ratios and the lagged protein synthesis increase together except for the last month when a decrease in the environmental temperatures may have led to a decrease in the rate of protein synthesis.

III. 6. RELATIONSHIPS OF PROTEIN SYNTHESIS AND THE RATIO OF RNA/DNA TO THE GROWTH RATE OF OYSTERS.

The growth rate of the oysters between each month of sampling was calculated from Winberg's (1956) expression:

$$C_m = 10^{1/n(\log W_n - \log W_o)} - 1 \times 100$$

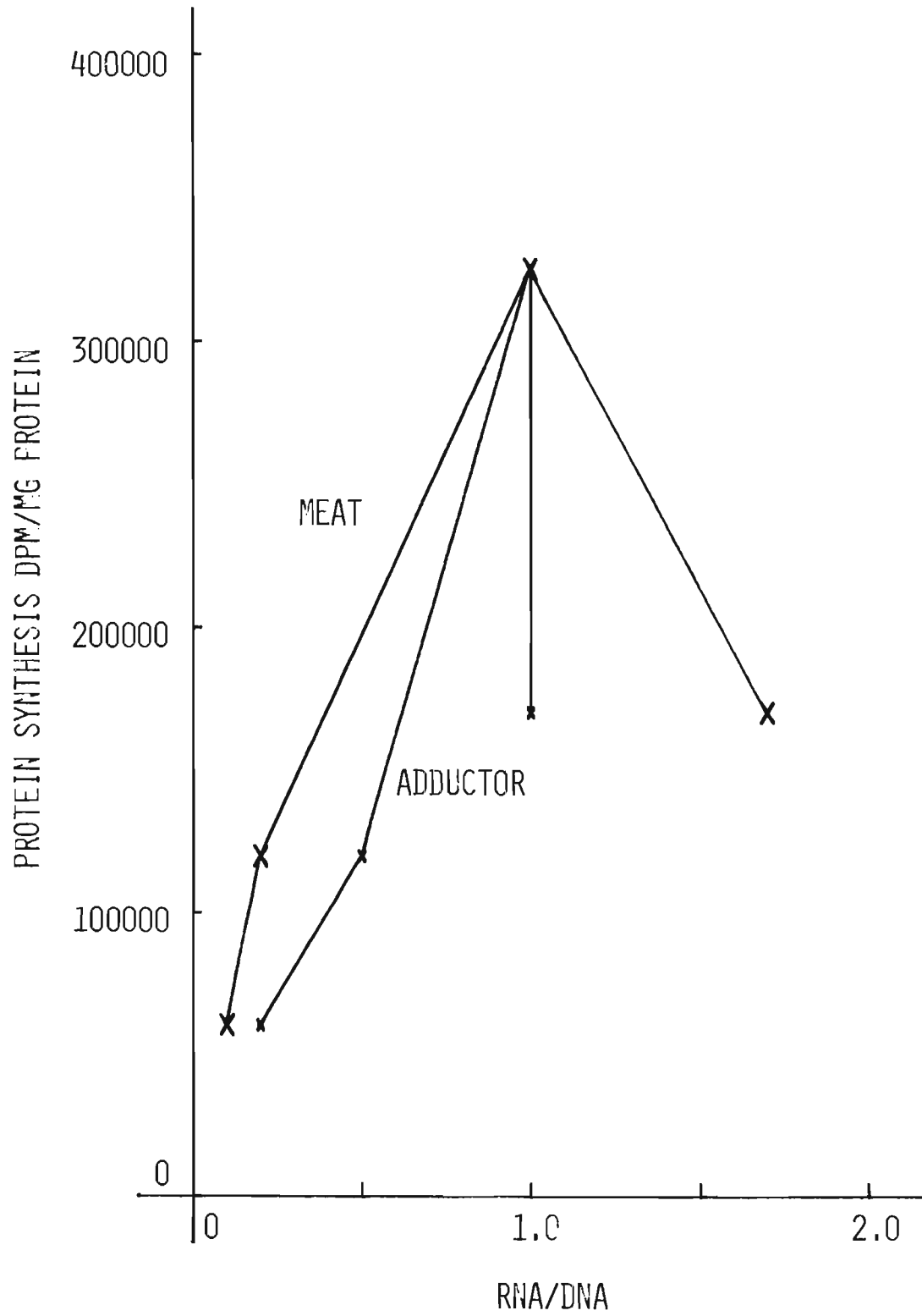
where C_m - % increase in wet wt/day

n = no. of days

W_n = wt at time \underline{n}

W_o = initial wt

Fig 15. Lags between the average RNA/DNA ratio of each month and the subsequent months rate of protein synthesis. Both the RNA/DNA ratios of the whole animal and the adductor muscle are shown.



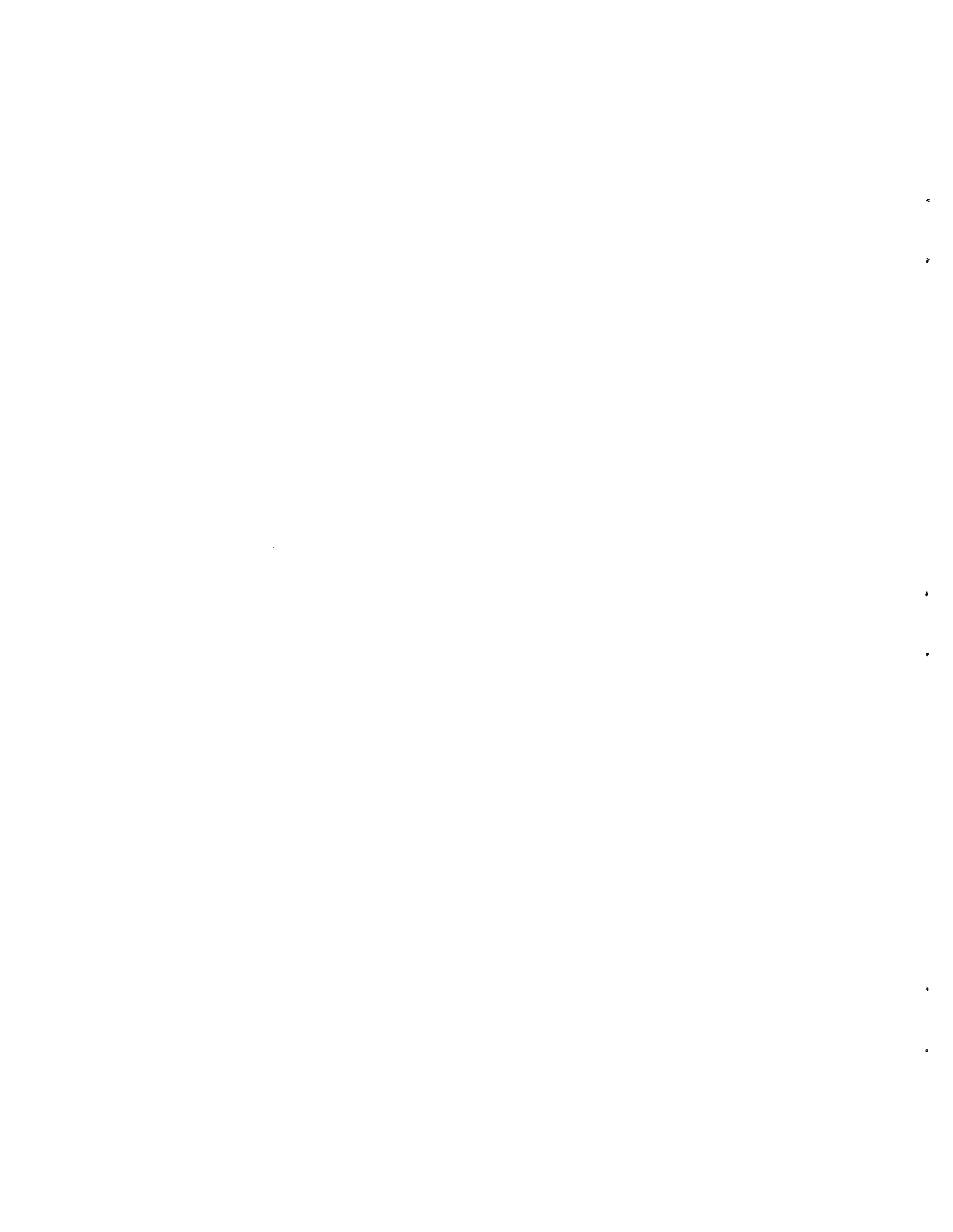


Fig 16 shows that the relationship of RNA/DNA to growth follows a slightly negative trend with a correlation coefficient of 0.01. The relationship of protein synthesis to growth (Fig 17) is also negative with somewhat higher correlation coefficients of 0.23 (linear) and 0.29 (exponential).

However, if the average RNA/DNA ratio for each year class are plotted against their growth rate a trend can be discerned (Fig 18). The exception is the 1974 oysters and this may be due to the fact that these averages represent data collected early in the sampling period when there was little growth.

Fig. 16. Relationship of RNA/DNA to growth (individual values).
The linear fit is $Y = 0.50 - 0.02X$ with a correlation coefficient
of 0.01. No exponential fit was possible.

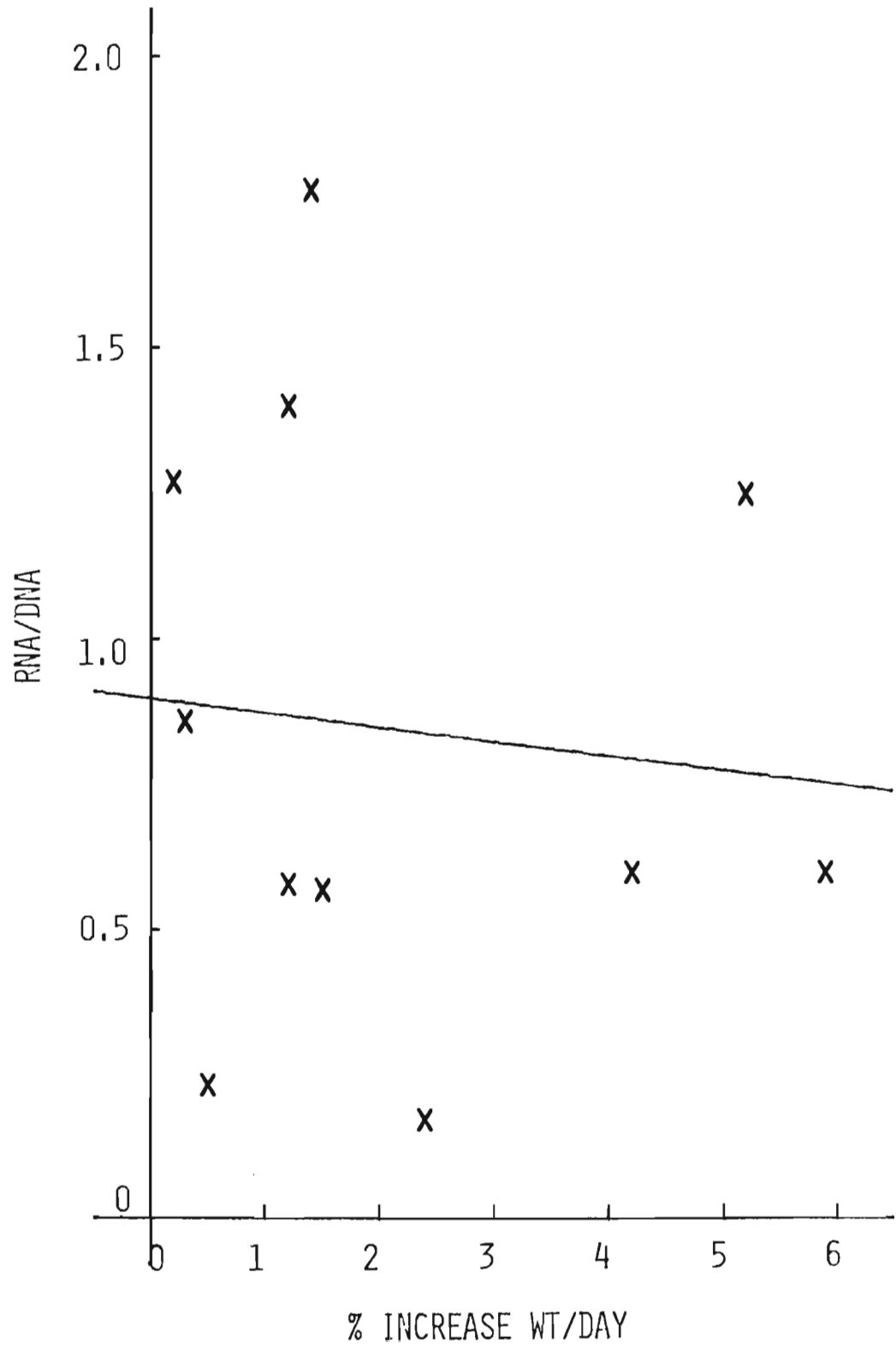


Fig 17. Relationship of protein synthesis to growth (individual values). The linear fit is $Y = 195198 + 28005X$ and the correlation coefficient is 0.23; the exponential fit is $Y = 162236e^{-0.33X}$ and a correlation coefficient of 0.29.

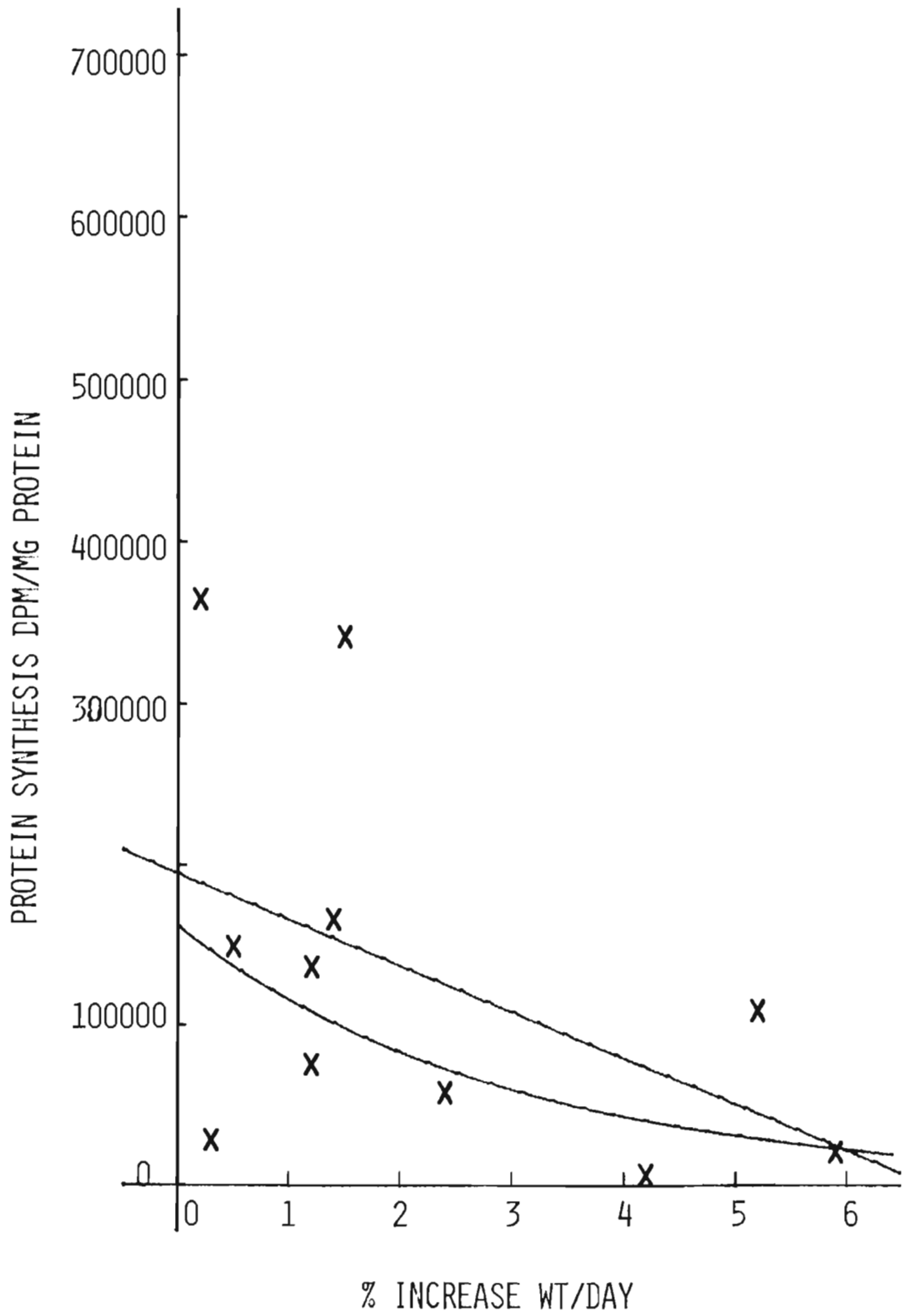
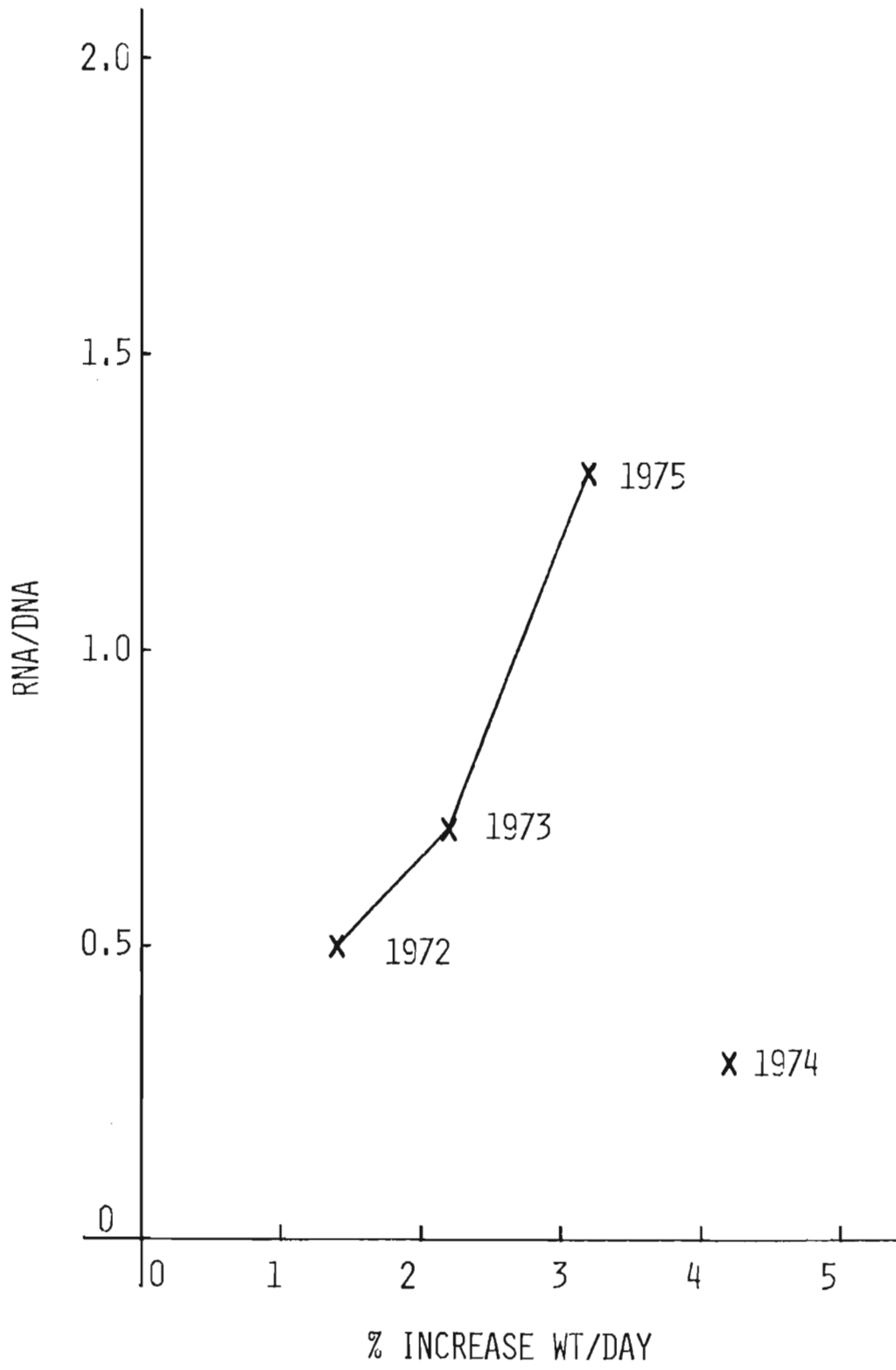


Fig. 18. Average RNA/DNA ratios for each year class vs average growth rates for each year class.



DISCUSSION

Some attention must be made to improving the methods of producing cell suspensions from both the adductor muscle and the whole oyster. The process of dispersing the oyster tissue through a sieve leaves a residue that won't reduce to cells. Sonification won't work if the goal is to produce a suspension in which protein synthesis may be studied. This technique destroys cells and even disrupts the machinery for protein synthesis. Sonification does not even reduce all of the adductor muscle to a suspension. The striated muscle of the adductor muscle is especially resistant to sonification.

The RNA/DNA ratio in the whole tissue and the adductor muscle appear related to tissue composition and function. RNA/DNA ratios in the adductor muscle showed no relationship to year class or body size when an analysis of variance was applied to the data. However, when such an analysis was applied to the RNA/DNA ratio in the whole tissue differences were observed. But when the RNA/DNA ratio of the whole animal was corrected for the effects of weight no differences in the ratios were again observed. Also no differences were found between sampling areas. This information suggests that the RNA/DNA ratio in the whole animal is a function of the presence of gonadal tissue. The amount of this tissue is probably a function of the age and size of the oysters. The uniformity of the RNA/DNA ratios in the adductor muscle in relation to age and size of the oyster suggests that this muscle is functioning at a constant level and that the level of the ratio is related to the metabolic function of the adductor muscle tissue.

The protein synthesis results suggest a similar conclusion. Differences were observed when the analysis of variance was applied to the data representing various year or size classes of the whole oysters. The data showed no difference in the rate of protein synthesis between the areas of sampling even though the salinities at Seal Island were higher and the temperatures lower than at Portage Creek. It appears that the rate of protein synthesis was again most affected by the proportions of gonadal tissue, the amount of tissue being a function of the age and size of the oysters.

No direct relationship was found between the RNA/DNA ratio and the rate of protein synthesis measured on the same oyster. However there appears to be a relationship between average RNA/DNA ratios in one sample and protein synthesis in the following month. Such a lag might be expected where the RNA/DNA ratio is a measure of the capacity of the cells for protein synthesis but actual realization in the form of protein synthesis takes place over a period of some weeks. The environment may also play a role in this lag. Where there was an increase in the temperature or the temperature remained constant the relationship between RNA/DNA and protein synthesis appeared strong. Where there was a sudden drop in temperature during a month the relationship broke down.

There was no direct relationship between protein synthesis and growth rate; the correlation coefficient is insignificant. Definite differences in size were noted at Portage Creek and Seal Island; the Portage Creek oysters were larger. However, rates of protein synthesis

were not significantly different between these areas. The fact that whole animal protein synthesis and growth were unrelated in this study may be due to the lack of an adequate method to measure protein synthesis. The best method would be to determine the maximum rate of protein synthesis using the Michaelis Menten relationships as has been done by Dugdale (1967) and others. This didn't work because diffusion kinetics were obtained rather than the expected saturation kinetics for the incorporation of amino acid into protein. Further study is required. The results may equally indicate that protein synthesis is a reflection of the general metabolic efficiency of the organism per unit weight but not of its overall growth efficiency. Under such circumstances protein synthesis would be affected by environmental parameters such as temperature but not directly by factors such as food supply (Lloyd Dickie, personal communication).

RNA/DNA ratios measured in the whole animal and on the adductor muscle did not predict growth as was expected. However, the average for the whole animal RNA/DNA ratios for each year class showed a significant relationship to growth. This is similar to the results obtained by Haines (1973) and may be due to the fact that the averages more closely represent a population estimate than individual values as was found in the case of Haines study. The season averages for RNA/DNA may reflect the rates at which the animals grow over a year but not for shorter periods of time such as a month. In this sense the ratio of RNA/DNA may well represent growth potential in an organism.

A lack of genetic drift is indicated by the data since

there are no differences in the levels of RNA/DNA and protein synthesis between the areas of sampling. This is probably because the oysters represent the same genetic stock introduced into the different areas at the same time and this introduction was quite recent. Longer periods of isolation have been observed in near and offshore populations of lobsters (Tracey et al, 1975). However, there was little genetic variation in enzyme concentration between lobster populations even though the populations have been isolated for a longer time. It would be interesting to examine other stocks of oysters to see if the same thing holds for this animal.

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APPENDIX

Tables A and B summarize the data for Portage Creek and Seal Island. Included is a measure of the precision from replicate analyses for RNA/DNA and protein synthesis.

TABLE A: Summary of data collected at Portage Creek

Date	n	GM Wet Weight		Adductor	Meat	Adductor	Protein Synthesis DPM/MG Protein	% Increase wet wt/day
		Oyster	Meat					
1975	Year	Class						
Aug. 11	50	50	0.064	0.023	-	1.0±0.5	87767 ± 41206	5.2
		50	0.072	0.028	-	1.5±0.4	130081 ± 79949	
Sept. 15	35	35	1.30	0.17	-	1.2±0.0	166312	1.2
		35	0.89	0.14	-	1.6±0.1	106764 ± 47196	
Oct. 13	30	30	1.74	0.24	-	1.1±0.2	204190 ± 63749	-
		30	1.73	0.22	-	1.4±0.5	192319 ± 21795	
1974	Year	Class						
My 28	38	38	0.47	0.08	-	0.6±0.1	6195 ± 732	4.2
Jl 4	9	9	2.65	0.30	-	0.2±0.0	134881 ± 610	-
		9	2.23	0.27	-	0.2±0.1	197726 ± 21542	
1973	Year	Class						
My 28	9	9	6.93	0.66	0.06	0.5±0.2	36822 ± 56610	5.9
		9	4.67	0.50	0.05	0.6±0.3	3686 ± 606	
Jl 4	2	2	51.12	4.50	0.50	0.0±0.0	194744 ± 45506	0.5
		2	49.38	4.30	0.80	0.6±0.1	166389 ± 32676	
Sept. 15	2	3	16.07	1.25	0.18	0.1±0.2	86510 ± 18589	0.2
		2	54.19	5.04	0.81	0.8±0.3	225467 ± 41726	
		2	54.73	4.59	0.47	1.4±0.0	368972 ± 239702	-
		2	60.69	4.85	0.43	1.6±0.4	499286 ± 245282	
Oct. 13	2	2	62.91	3.91	0.50	0.6±0.3	125970 ± 25766	-
		2	76.50	5.27	0.25	0.8±0.1	199415 ± 28137	
		2	86.00	6.88	0.92	0.4±0.1	182765 ± 67220	
1972	Year	Class						
My 28	2	2	54.10	5.83	0.56	0.8±0.2	23689 ± 10289	-
		2	61.85	9.13	0.78	0.0±0.1	42394 ± 9432	
		2	17.65	2.66	0.20	0.0±0.1	18434 ± 11057	2.4
Jl 4	3	3	19.69	1.69	0.20	0.1±0.0	24920 ± 14207	
		3	19.34	1.73	0.22	0.4±0.1	77859 ± 6987	-
		3	26.55	2.78	0.34	0.0±0.0	68860 ± 40189	
Aug 11	2	2	116	8.57	1.33	0.8±0.1	8027 ± 972	0.3
		3	43.68	4.22	0.57	0.8±0.0	28884 ± 1641	
		3	38.90	4.27	0.55	0.9±0.2	45276 ± 511	1.5
Sept 15	2	2	52.55	5.00	0.49	0.6±0.1	92489 ± 20839	
		2	72.45	6.70	1.06	0.3±0.2	237153 ± 164324	-
		2	103.85	7.74	1.22	0.8±0.9	694640 ± 461699	
Oct. 13	2	2	123	10.60	1.54	0.6±0.1	-	
		2	84.50	8.11	1.06	0.6±0.7	137002 ± 2595	-
		2	137	10.82	1.42	-	154276 ± 11839	

TABLE B: Summary of data collected at Seal Island

Date	n	GM Wet Weight		Adductor	RNA/DNA		Protein Synthesis		% Increase wet wt/day
		Oyster	Meat		Meat	Adductor	DPM/MG	Protein	
1973	Year	Class							
My 28	3	26.37	2.38	0.31	0.8±0.5	0.0±0.0	10969 ± 4265		
	2	29.23	2.82	0.32	0.9±0.2	0.0±0.0	15365 ± 673		-
	3	18.12	1.45	0.21	1.6±0.1	0.3±0.2	10818 ± 3874		
Jul 4	3	8.89	0.60	0.05	0.6±0.4	0.0±0.1	- -		
	3	11.33	0.67	0.07	0.5±0.2	0.0±0.1	108113±107184		1.2
	3	12.17	1.05	0.05	0.6±0.0	0.0±0.0	42270 6190		
Sept. 15	3	20.20	1.85	0.13	1.6±0.0	1.8±0.1	45485 ±33113		
	3	15.58	1.35	0.13	1.4±0.7	2.6±0.6	157338 ±40529		1.4
	3	19.42	1.80	0.14	2.3±0.8	3.3±0.4	295463 ±21197		
Oct. 13	3	25.28	1.98	0.21	0.2±0.1	2.1±0.4	422387 ±21718		
	3	86.08	1.90	0.31	0.2±0.1	2.1±0.4	243786 ± 741		-
	2	47.10	3.61	0.57	0.8±0.3	3.0±3.5	435338±102834		