RELATIONSHIPS BETWEEN GROWTH RATE AND RNA, DNA, PROTEIN AND DRY WEIGHT IN *ARTEMIA SALINA* AND *EUCHAETA JAPONICA*

by

MICHAEL J. DAGG

B.Sc., Mount Allison University, 1966

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
in the Department
of
BIOLOGY

We accept this thesis as confirming
to the required standard

Accepted for the Faculty of Graduate Studies
April 7, 1970.

MICHAEL J. DAGG
UNIVERSITY OF VICTORIA

September, 1969

UNIVERSITY OF VICTORIA
LIBRARY
Victoria, B. C.

Supervisor: Dr. J. L. Littlepage

ABSTRACT

The concentration of RNA, DNA and protein, and the dry weight of three cultures of *Artemia salina* were measured to investigate the usefulness of the RNA-growth relationship in estimating growth or productivity in other species. Similar analyses were done on copepodite stages 3 to 6 of *Euchaeta japonica,* collected periodically over seven months in Haro Strait and over four months in Saanich Inlet, B. C.

Changes in protein, DNA and RNA concentration, expressed against growth rate occurred in *A. salina* and *E. japonica.* DNA concentration appeared to be intrinsic to the stage in *E. japonica,* being high in the young stages and declining in the older stages. The range of RNA concentrations found within a given stage was greater than the range of DNA concentrations.

The RNA-growth relationship derived by Sutcliffe (1965) does not agree with the relationship found in this work for *A. salina.* Neither the equation derived herein, nor the one derived by Sutcliffe (1965) accurately predicts the growth rate of *E. japonica* stages.

The relationship between RNA concentration and growth rate is of little use in predicting or determining secondary production because wide ranges of growth rates were found associated with a single RNA concentration.

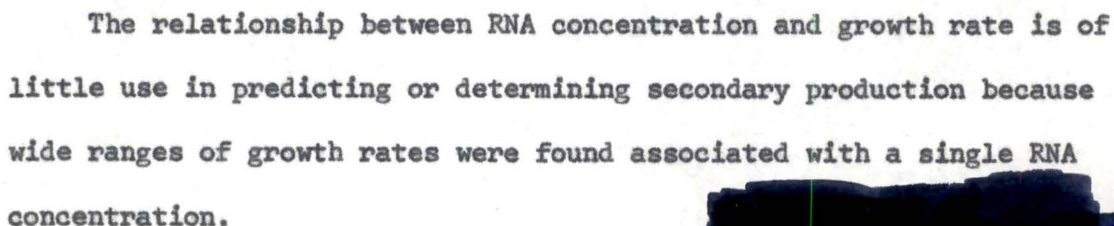


TABLE OF CONTENTS

PAGE

ABSTRACT.. i

TABLE OF CONTENTS... ii

LIST OF TABLES.. v

LIST OF FIGURES... vi

ACKNOWLEDGEMENTS.. viii

INTRODUCTION.. 1

METHODS AND MATERIALS....................................... 6

A. Collection of samples.................................... 6

(i) *Artemia salina*.. 6

(ii) *Euphausia pacifica*................................... 8

(iii) *Euchaeta japonica*................................... 8

B. Experimental procedures.................................. 9

(i) Standards... 9

(ii) Development of technique............................... 13

(iii) Analytical methods.................................... 17

C. Precision.. 18

RESULTS... 22

A. *Artemia salina*... 22

(i) Growth.. 22

(ii) Protein concentration.................................. 25

(iii) DNA concentration..................................... 25

(iv) RNA concentration and growth rate..................... 26

(v) Ribosome efficiency..................................... 29

B. *Euphausia pacifica*..................................... 29

PAGE

C. *Euchaeta japonica*.................................. 32

(i) Growth.. 32

(ii) Haro Strait.. 36

(iii) Saanich Inlet..................................... 41

(iv) Geographical distribution.......................... 45

(v) Biomass... 51

DISCUSSION.. 53

A. Growth and RNA concentration......................... 53

B. Geographical distribution of RNA, DNA, protein
and dry weight in *Euchaeta japonica*................ 61

C. The feasibility of secondary production
determination from RNA concentration................. 63

SUMMARY... 66

REFERENCES CITED.. 68

APPENDICES.. 74

I. Length range in some *Artemia salina* samples........ 74

II. RNA, DNA and protein concentration of
A. salina samples.................................. 75

III. Observed growth rates (percent per day)
of *A. salina* samples............................... 77

IV. Numbers of *E. japonica* caught per sample........... 79

V. RNA, DNA and protein concentrations of
E. japonica samples

(a) Haro Strait...................................... 80

(b) Saanich Inlet 82

(c) Geographical distribution......................... 83

PAGE

VI. Molting and Growth Records of Saanich Inlet

Euphausiids.................................. 84

LIST OF TABLES

TABLE		PAGE
I	Feeding schedule of *Artemia salina* cultures	7
II	Recovery of standard solutions of RNA and DNA with the technique of Wannemacher et. al. (1965).....	14
III	Effect of extraction time on DNA recovery, using the technique of Wannemacher et. al. (1965)..........	16
IV	Recovery of standards by the adopted technique	17
V	Precision of analysis	20
VI	RNA, DNA and protein concentration values for replicates of *E. japonica* samples	21
VII	Observed vs. expected percent growth (dry weight increase) per day for *A. salina*	30
VIII	Statistical significance of the relationships between RNA concentration and growth rate	32
IX	*A. salina* ribosome efficiency	33
X	Intermolt periods of Haro Strait *E. japonica*	34
XI	a. Dry weight (mg) per individual *E. japonica*	37
	b. DNA (μg) per individual *E. japonica*	38
XII	RNA concentrations and growth rate of *E. japonica* copepodites	41
XIII	Example of regional differences in *E. japonica* protein concentration	48
XIV	Biomass (mg wet weight/m^3) of *E. japonica* for station 6 and station 8 (Bute Inlet)	52

LIST OF FIGURES

FIGURE PAGE

1. *Euchaeta japonica* sampling areas 10
2. DNA standard curve 12
3. Chart of the adopted analytical procedure 19
4. Growth curves and RNA, DNA, and protein concentration changes in:
   (a) *A. salina* Culture 'A' 23
   (b) *A. salina* Culture 'B' 24
   (c) *A. salina* Culture 'C' 24
5. Regression analyses for *A. salina* of:
   (a) RNA/dry weight concentrations on the percentages of increase in dry weight per day 31
   (b) RNA/protein concentrations on the percentages of increase in protein per day 31
   (c) RNA/DNA concentrations on the percentages of increase in DNA per day 31
   (d) RNA/dry weight concentrations on the percentages of increase in dry weight per day ... 31
6. The relative frequencies of each *E. japonica* copepodite stage over the collection period 35
7. RNA concentration changes in Haro Strait *E. japonica* from December, 1968 to June, 1969:
   (a) RNA/protein concentrations 39
   (b) RNA/dry weight concentration 39

FIGURE PAGE

8. RNA/dry weight concentrations vs. the percentages of increase in dry weight per day for *E. japonica* ... 39

9. DNA/protein concentration changes in Haro Strait *E. japonica* from December, 1968 to June, 1969 42

10. DNA/dry weight concentration changes in Haro Strait *E. japonica* from December, 1968 to June, 1969 43

11. Changes in Haro Strait *E. japonica* from December, 1968 to June, 1969 in:

(a) RNA/DNA ratios 44

(b) percent protein 44

12. RNA concentration changes in Saanich Inlet *E. japonica* from March to June, 1969:

(a) RNA/dry weight concentrations 46

(b) RNA/DNA ratios 46

(c) RNA/protein concentrations 46

13. Changes in Saanich Inlet *E. japonica* from March to June, 1969 in:

(a) percent protein 47

(b) DNA/dry weight concentrations 47

(c) DNA/protein concentrations 47

14. Geographical variation for *E. japonica* in March, 1969 of:

(a) RNA/protein concentrations 49

(b) RNA/dry weight concentrations 49

(c) RNA/DNA ratios 49

(d) DNA/protein concentrations 50

FIGURE PAGE

14. (e) DNA/dry weight concentrations 50

(f) percent protein 50

ACKNOWLEDGEMENTS

I would like to thank Dr. J. L. Littlepage for advice and assistance received during the period of this study.

Miss Kathy Rauchert assisted with portions of the laboratory work and provided advice for which I am most grateful.

I would also like to thank the operator of the University of Victoria launch, Mr. D. Hartley, who assisted me with the collection of material.

Further collections were made from the C.S.S. Parizeau. Many thanks to Captain C. Angus and crew for an enjoyable collecting cruise.

CHAPTER I

Introduction

In the oceanic environment, the autotrophic algae (primary producers) are utilized by the herbivores of the zooplankton (secondary producers). As more information regarding primary production becomes available, it is only natural that interest increases in heterotroph energy flow and the utilization of net primary production.

Before 1952, primary production was typically estimated indirectly from changes in the standing crop over a period of time. Primary production determined by the oxygen method of Gaarder and Gran (1927) lacked sensitivity while measurements of nutrient depletion were often erroneous due to water movements. In 1952, a method for determining the carbon fixation rate of phytoplankton, using radioactive C^{14}, was introduced (Steeman Nielson, 1952). This provided a simple, direct and "instantaneous" method for measuring primary production.

There are no methods available for directly measuring secondary production. The most direct estimates of net herbivore production are based on standing crop measurements utilizing zooplankton live weight (biomass), dry weight, dry organic matter, displacement volume, calculated volume and others (Steeman Nielson, 1963). Secondary production is derived from measurements of one or more of these parameters over a period of time.

A number of indirect methods for predicting or determining secondary production have recently been devised. The model developed by Sverdrup (1953) to predict the spring phytoplankton bloom has been used to derive a measure of copepod growth rate from March to April at Weather Station "P"

(Parsons and LeBrasseur, 1968). A model for herbivore production has been outlined by Cushing (1959). There is a possibility of discussing metabolism and growth of zooplankton in terms of food concentration (Conover, 1968). He considers recent models for fish and their applicability to zooplankton. More complex models such as those proposed by Conover (1968) and Cushing (1959) are limited by the state of our physiological knowledge. It has recently been shown, for example, that a difference in the zooplankton grazing scheme on phytoplankton gives rise to small differences in primary production estimates. These, in turn, result in large differences in secondary production estimates when assimilation and respiration by zooplankton are taken into account (McAllister, 1969). Assimilation values vary considerably for different zooplankton species and assimilation values for a single species may vary depending upon food and environmental conditions (Corner & Cowey, 1968). Therefore, any model involving transfer coefficients between trophic levels soon encloses a wide range of possible values for secondary production. A direct method of secondary production determination is needed. It should be instantaneous and not based on changes in standing crop.

The concentration of ribonucleic acid (RNA) in some small organisms has been shown to be indicative of their growth rate (Sutcliffe, 1965). He constructed a growth curve for the amphipod, *Orchestia platensis*, using dry weight increase as a measure of growth. At different stages in the amphipod life cycle, the percentage of growth per day was determined and plotted against the RNA concentration of the corresponding stage. From this standard curve the percentage of expected growth per day for cultures of *Artemia salina* and *Nassarius*

obsoletus larvae was predicted from their RNA concentrations. Sutcliffe concluded that the RNA-growth rate relationship might be useful in estimating growth or production in other species or in mixed populations of animals.

Relationships between growth rate and RNA content have been reported by a number of authors. Kjeldgaard (1961) reported a constant relationship between cell content of ribosomes and rate of protein synthesis in *Salmonella typhimurium*. Neidhardt and Magasanik (1960) reported for *Aerobacter aerogenes* that the RNA/protein ratio of a culture is a direct function of the growth rate; and that these two variables are linearly related at growth rates greater than 0.6 doubling per hour. The RNA content of eye lenses of rats decreases with age (Dische et. al., 1961). RNA concentration has been used in growth studies of the mosquito (Lang et. al., 1965), the boll weevil (Vickers and Mitlin, 1964), the fruit fly (Church and Robertson, 1966), and numerous other insects.

There are three types of RNA. Messenger RNA (mRNA) serves as the template for protein biosynthesis. Transfer RNA (tRNA) translates mRNA information into the desired amino acid sequence. Ribosomal RNA (rRNA) is part of the actual site for mRNA translation and peptide bond formation. The rate of protein synthesis is determined by the number of ribosomes and therefore the quantity of rRNA gives a measure of the rate of protein synthesis. Since rRNA is a constant or nearly constant fraction of total RNA (Rosset et. al., 1966) the total RNA content is a measure of protein synthesis rate.

There are many ways to measure growth. Growth rate is derived from

changes with time in whatever parameter(s) are being measured. Since the RNA content is related to the protein synthesis rate, it would seem reasonable to use the rate of increase in protein content of an organism as an index of its growth rate. The use of an increase in dry weight to indicate growth rate is valid if the protein/dry weight ratio is uniform.

Deoxyribonucleic acid (DNA) content is commonly used as an index of enzyme activity and is often used to quantitatively measure growth because it gives an estimate of cell number (Lang et. al., 1965). The RNA content per cell can be determined from the RNA/DNA ratio and the amount of DNA per cell. Other relationships between RNA, DNA, protein and dry weight have value and can lead to valuable information concerning growth and development.

The purpose of this study was to investigate the relationships between RNA content and growth rate as measured by rate of increase in protein, DNA, and dry weight content. Cultures of *Artemia salina* were grown in the laboratory at different rates and the parameters followed for various lengths of time. A field study using the calanoid copepod *Euchaeta japonica*, was also done.

E. japonica is a large, brightly coloured, copepod common to local waters (Campbell, 1934) and is widely distributed over the North Pacific (Davis, 1949). "All six copepodite stages are capable of a carnivorous diet but have been found to feed on large members of the phytoplankton" (Lewis and Ramnarine, 1969). Although omnivorous, this species was chosen because it reproduces all year round and the desired stages would

be present at all times. The RNA, DNA, protein and dry weight of *E. japonica* copepodite stages 3 to 6 from two different areas were followed. Geographical distribution of these parameters in *E. japonica* was studied for six areas in March of 1969. Biomass data was also obtained during this period.

The effects of the molting cycle in RNA concentration are of some importance as the cyclic secretion of a new epicuticle and endocuticle must cause some fluctuation in protein synthesis activity. *E. japonica* molts eleven times before reaching adulthood. The effect of molting on protein synthesis will decrease as the intermolt period increases. *Euphausia pacifica* molts every 3 to 8 days, depending on the temperature (Lasker, 1966) and the individuals are large enough to analyze singly. Laboratory studies were done with *E. pacifica* to determine whether the effects of molting on RNA concentration could be discerned from those of growth.

Methods and Materials

A. Collection of Samples

(i) *Artemia salina*

A number of cultures were started in 2 liter Pyrex dishes. The time of hatching was noted and 24 to 48 hours later most of the newly hatched nauplii were transferred to a 30 liter aquarium. This established uniformity of size and stage at the beginning of the culture. Growth was reasonably uniform throughout the culture period (Appendix I). Three *A. salina* cultures were grown consecutively in the 30 liter aquarium. The culture medium was sea water increased to a salinity of approximately 35 o/oo by the addition of NaCl and maintained at room temperature (21°C). Maximum feeding efficiency is obtained at this salinity for *A. salina* feeding on *Phaeodactylum tricornutum* (Reeve, 1963). The medium was changed at the beginning of each culture.

Monochrysis lutheri served as food but no effort was made to restrict bacterial growth. Detritus-bacteria-phytoplankton aggregates (marine-snow, Riley, 1963) may have served as food in part. About two weeks after culture initiation a small (adult length,<1 mm), unidentified cyclopoid copepod bloomed for a week to ten days. It is not known whether this cyclopoid originated with the *A. salina* eggs or with the sea water supply.

Different amounts of food were added to each culture (Table I) and samples were removed every 2 or 3 days for analysis.

TABLE I. Feeding Schedule of *Artemia salina* cultures

Culture	Duration	Feeding Schedule
A	66 days	day 0 only
B	29 days	day 1,7,10,13,18,22,25
C	15 days	daily

A subsample was anesthetized in $MgCl_2$ or killed in a dilute formalin solution. The remainder of the sample was separated from the sea water by a piece of fine mesh and placed in distilled water. This killed the cyclopoids, if present, but did not affect *A. salina*. It also rinsed away any remaining salt. The *A. salina* were removed and transferred in a small amount of distilled water to a labelled lyophilizer vial and frozen. The sample was then transferred to a Virtis Automatic Freeze Dryer (Model 10-010), dried, vacuum sealed and returned to the freezer for later analysis.

The length, from the anterior tip of the head to the base of the caudal furcae (Reeve, 1963) was determined with an eye piece micrometer for 20 to 50 individuals of the anesthetized subsample. The average length was computed and from a length to dry weight curve for *A. salina* (Reeve, 1963) the average dry weight per individual was determined. The subsample was assumed to be representative of the lyophilized sample and the aquarium culture. The age of the sample in days was calculated from the time of addition of the encysted embryos to the sea water medium. Dry weight per individual was plotted against the age of each sample to obtain the growth curve for each culture.

(ii) *Euphausia pacifica*

The collection and maintenance technique of Lasker and Theilacker (1964) was used with the following changes. First, the animals were maintained between 9.5°C and 10.6°C instead of 14.8°C to 16.4°C. This was done because the temperature was 9.5°C at the depth (75 m) from which the sample was collected. Second, a dark refrigerator was used for storage instead of a covered sea water table. Collection was made with a standard 1 meter net (Nitex #760) in Saanich Inlet, B.C. (Figure 1).

Animals were fed a mixture of newly hatched *A. salina* and phytoplankton. The samples were checked daily for molts. Uropod length was used as an indicator of growth (Lasker, 1966).

(iii) *Euchaeta japonica*

Collections were made with a standard 1 meter net (Nitex #760) in Haro Strait and Saanich Inlet (Figure 1). Tows were 5 to 10 minutes long at a depth of approximately 250 meters in Haro Strait and 125 meters in Saanich Inlet. The first 3 tows in Saanich Inlet were oblique from 150 meters to the surface. A Brown-McGowan "Bongo" net (Nitex #333) was used for the sampling in Jervis Inlet, Bute Inlet and Georgia Strait in March, 1969 (Figure 1).

Twelve 1-liter Erlenmeyer flasks were filled with surface water and placed in an ice chest containing 2 to 3 inches of ice and water. The net was brought up at approximately 20m/min. and the plankton sample was quickly placed in a polyethylene pan with a small amount of water. Aliquots were transferred to the Erlenmeyer flasks, which were then stoppered and returned to the ice chests. The samples were returned

to the lab and placed in a dark refrigerator (9.5 - 10.6°C) within 1.5 to 2.5 hours after collection. The contents of each flask were examined and copepodite stages 3,4,5,6♀ and 6♂ of *Euchaeta japonica* were placed in a separate dish of sea water. Dead individuals were discarded but mortality was low. The copepods were rinsed briefly in distilled water and each stage was counted, placed in a separate lyophilizer vial, and frozen. The animals were either frozen immediately over dry ice or over a period of a few minutes in a freezer. This procedure was repeated for each Erlenmeyer flask until a sufficiently large sample of each stage had been collected for analysis. The samples were freeze dried, vacuum sealed and stored in a freezer for later analysis.

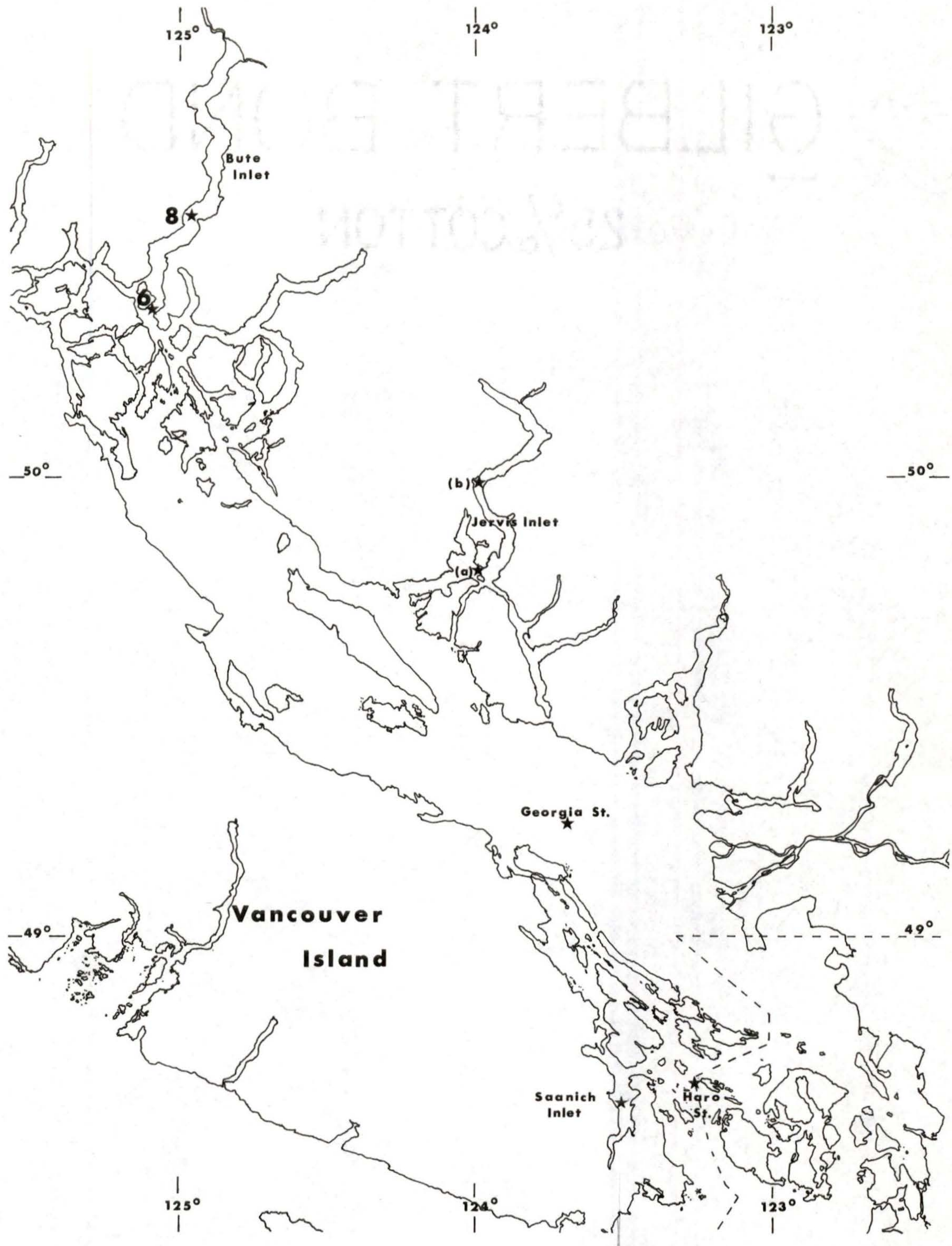
Collections for the biomass study in Bute Inlet were made with a Brown-McGowan "Bongo" net at a ship speed of 3k. The samples were split with a Folsom sample splitter and a conveniently sized aliquot was counted and sorted. Depths sampled were 40m, 175m, 250m and (station 6 only) 400m. The locations of station 6 and station 8 are shown in Figure 1. Wet weight/individual was determined from a prosome length-wet weight graph for copepods (Fulton, 1968). The total wet weight of a sample was determined by the direct weighing of an aliquot on a P 1200 N Mettler Balance.

B. Experimental Procedures

(i) Standards

(a) RNA. Arabinose (Calbiochem-A grade), used as recommended by Sutcliffe (1965), served as the RNA standard for the orcinol method

Figure 1. *Euchaeta japonica* sampling areas.



125°
124°
123°
Bute Inlet
8
6
50°
(b)
Jervis Inlet
(a)
Georgia St.
Vancouver
Island
49°
Saanich Inlet
Haro St.

(Schneider, 1957). After this technique for RNA measurement was discontinued, yeast RNA (Type X1, Sigma Chemical Co.) was used. A standard curve was determined for concentrations from 3 to 30μg RNA/ml .2N perchloric acid (PCA) at 260 mμ, on a Coleman 139 spectrophotometer. The line, with a slope of .0455 obeyed Beer's Law over this range.

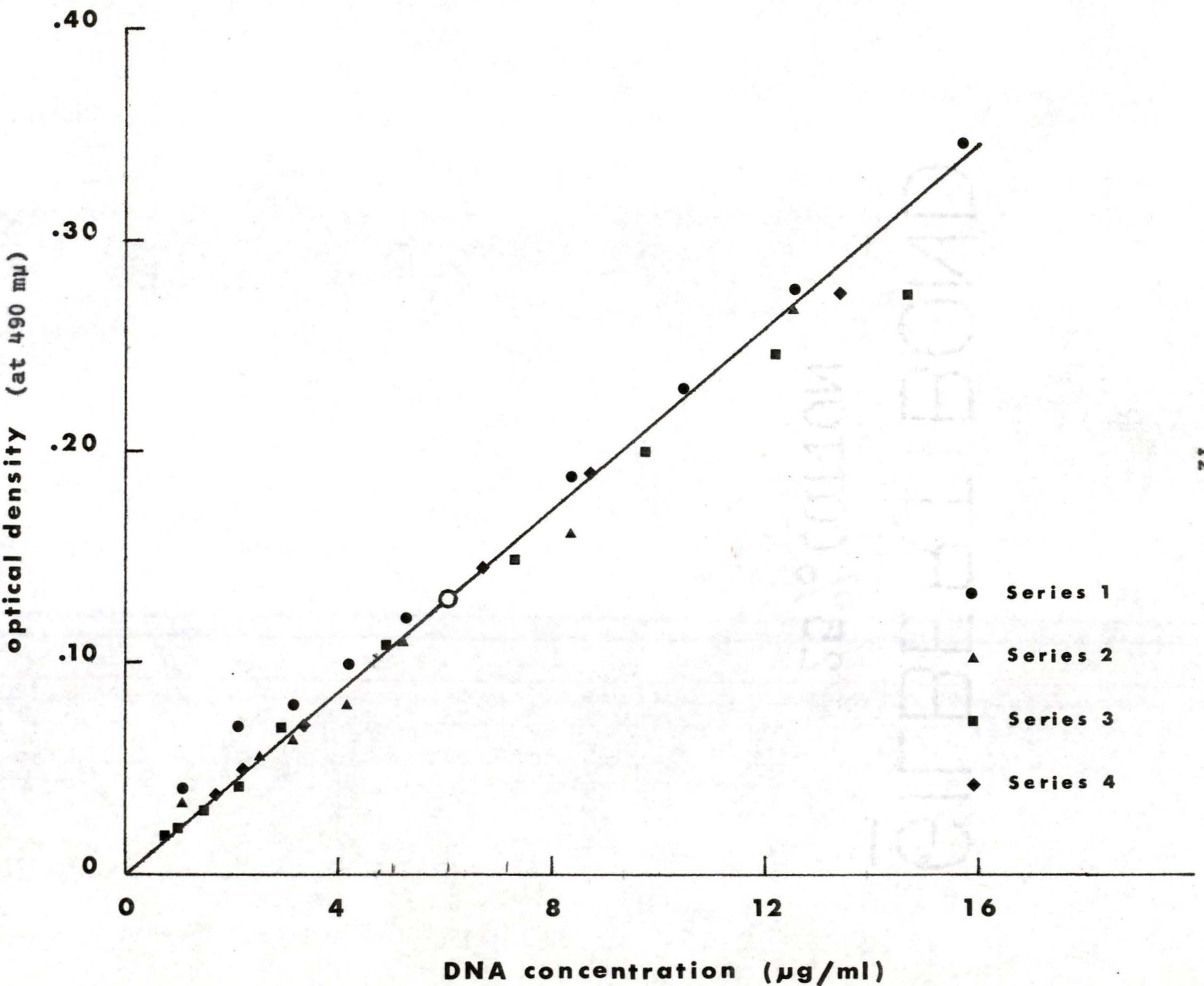
(b) DNA. Highly polymerized calf thymus DNA (type I, Sigma Chemical Co.) was used to prepare the DNA standard curve. Concentrations of DNA ranging from 1.46 to 14.64 μg DNA/ml .3N KOH were measured at 490 mμ on a Coleman 139 spectrophotometer. This curve obeyed Beer's Law between the tested ranges and had a slope of .046.

(c) Protein. Albumin from bovine serum (Sigma Chemical Co.) was used to prepare the protein standard curve for use with the Biuret reagent. A standard curve was determined at 540 mμ on a Coleman 139 spectrophotometer for concentrations ranging from 0.5 to 10.0 mg protein/ml .3N KOH. The curve had a slope of 19.0 and obeyed Beer's Law over the tested range.

Casein (J. T. Baker Co.) was used to prepare the protein standard curve for use with the Lowry reagents. A standard curve was determined for concentrations ranging from 20 to 200 μg protein/ml .15N KOH. Standards were measured at 750 mμ on a Coleman 139 spectrophotometer. The curve did not obey Beer's Law and protein content of all samples were determined directly from this curve.

All replicate curves were similar, as illustrated by the DNA standard curve (Figure 2).

Figure 2. DNA standard curve.



.40
.30
.20
.10
0
optical density (at 490 mµ)
0
4
8
12
16
DNA concentration (µg/ml)
Series 1
Series 2
Series 3
Series 4

(ii) Development of Technique

A complete review of the methods avilable for nucleic acid determination in biological materials and the problems expected with each technique is available (Hutchinson and Munro, 1961). A supplement to this has recently been published (Munro and Fleck, 1966).

Sutcliffe (1965) used the orcinol method (Schneider, 1957) to measure the RNA content of his test animals. This reagent is not specific for RNA a given weight of DNA results in 0.3 to 19.0 percent of the colour formed by the same weight of RNA (Hutchinson and Munro, 1961). Sutcliffe did not separate RNA from DNA but assumed this source of error to be negligible. Although the orcinol method was initially attempted and appeared to work reasonably well, it was discontinued because it lacked the sensitivity of the uv method and was not specific for RNA.

A method for determining RNA, DNA and protein from the same extract was desired because the sample size would be small. The method of Wannemacher et. al. (1965) is a modification of the recommendations of Hutchinson and Munro (1961) for the Schmidt-Thannhauser (1945) technique. Wannemacher et. al. (1965) derived a method whereby protein, RNA, DNA and labelled amino acid incorporation could be determined from the same extract. It was easily adapted for my use. Numerous analyses were performed with standard solutions. The procedure involved precipitating RNA, DNA and protein from a homogenized sample with trichloroacetic acid (TCA) followed by the removal of lipids. The precipitate was incubated for one hour at 37 C in.3N KOH. This incubation sufficiently hydrolyzed the RNA for it to become acid soluble but did not affect the protein or

DNA. One ml. of KOH solution was used for protein analysis (Biuret). DNA and protein in the remaining KOH solution were precipitated by cold perchloric acid. The acid soluble RNA was measured by uv absorption (at 260 mμ) of the supernatent. The precipitate was hydrolyzed in PCA at 96° C for 45 minutes to dissolve the DNA. The supernatent was then read at 265 and 290 mμ and from the difference the DNA content was calculated.

In many cases DNA was incompletely precipitated by the PCA after alkali incubation and some would therefore appear in the RNA portion of the sample. This was attributed to an impure or partially degraded DNA standard. Even with this problem the amount of error due to DNA found in the RNA fraction was usually small (Table II). In all cases, however, DNA recovery was low (Table II). Wannemacher et. al. (1965) stated that peak DNA recovery was obtained after 45 minutes incubation at 96 C. To verify this 3 sets of samples, containing only DNA standard solution were treated and incubated for 40, 45 and 50 minutes at 96 C. This peak extraction at 45 minutes was apparently verified (Table III) but 100% recovery of DNA was not attained, nor were the DNA recoveries consistent. No DNA contamination of the RNA fraction was found. This apparent maximum extraction is a balance between incomplete extraction and partial destruction of DNA (Munro and Fleck, 1966). The modifications of Wannemacher et. al. (1965) to the recommendations of Hutchinson and Munro (1961) were therefore discontinued and the indole method (Ceriotti, 1952) was adopted for DNA determination. Two sets of standards were analyzed with results shown in Table IV.

TABLE II. Recovery of standard solutions of RNA and DNA with the technique of Wannemacher et. al. (1965).

I. RNA Measurement

Date	Test Tube Contents							
	RNA only			DNA only		RNA and DNA		
	measured	theoretical	% recovery	measured	theoretical	measured	theoretical	% recovery
11/6/68	44.2μg	48.0μg	92	3.2μg	0	25.7μg	24.0μg	107
13/6/68	47.2	48.0	98	7.0	0	30.5	24.0	127
18/6/68*	-	-	-	5.8	0	28.1	24.0	117
21/6/68*	46.4	48.0	97	3.3	0	24.8	24.0	103
25/6/68*	44.2	48.0	92	2.0	0	25.6	24.0	107
27/6/68	49.0	48.0	102	2.8	0	24.9	24.0	104
3/7/69*	48.9	48.0	102	1.2	0	27.3	24.0	114
5/7/69	47.1	48.0	98	2.4	0	20.4	24.0	85
9/7/69	48.4	48.0	101	2.2	0	23.3	24.0	97

* New DNA standards prepared on these days

II. DNA Measurement

Date	Test Tube Contents							
	DNA only			RNA only		RNA and DNA		
	measured	theoretical	% recovery	measured	theoretical	measured	theoretical	% recovery
13/6/68	10.9μg	19.6μg	55	-	-	6.0μg	9.8μg	61
18/6/68*	14.4	21.8	66	-	-	10.2	10.9	94
21/6/68*	11.0	21.1	52	0.4μg	0	7.4	10.6	70
25/6/68*	13.2	24.7	53	3.8	0	9.8	12.4	79
27/6/68	11.5	21.2	54	0	0	-	-	-
3/7/68*	15.3	21.7	71	0.5	0	6.7	10.8	62
5/7/68	13.1	16.8	78	1.3	0	6.4	10.8	60
9/7/68	17.3	21.6	80	6.5	0	13.5	10.8	125

* New DNA standards prepared on these days

TABLE III. Effect of extraction time on DNA recovery, using the technique of Wannemacher et. al. (1965).

Time incubated	Tube #	% recovery
40 min.	1	65
	2	73
	3	71
	4	64 $\overline{X}$ = 68
45 min.	5	-
	6	75
	7	75
	8	86 $\overline{X}$ = 79
50 min.	9	82
	10	77
	11	69
	12	78 $\overline{X}$ = 77

TABLE IV. Recovery of standards by the adopted technique

	Biuret (Protein)	260 mμ (RNA)	Indole (DNA)
1.	100%	85%	97%
2.	104%	98%	99%

Recovery of one of the RNA standards was only 85%. However, in previous tests (Table II) RNA recovery was good. For this reason the recommendations of Munro and Fleck (1966) for the Schmidt-Thannhauser (1945) technique were adopted.

The Biuret method for protein determination lacked sensitivity for the sample size being analyzed (2 to 12 mg usually). The method of Lowry et. al. (1951) was initiated. No problems were encountered.

(iii) Analytical Methods

The stored samples were placed on the freeze dryer for approximately half an hour to remove moisture residues. Each sample was weighed on a Cahn Gram Electrobalance, placed in a clean vial and returned to the freezer. Although the animals had previously been rinsed, some salt was usually found in the lyophilized samples. This was especially true for *E. japonica*. Possibly the salt originated in the body fluids of the animals. The salt was not included in the dry weight measurements because much was affixed to the vial walls. The samples (2 to 12 mg in dry weight) were homogenized in 4.0 mls of ice cold distilled water on a Cole-Parmer tissue grinder. Any residue was rinsed from the tissue grinder with 1.0 ml ice cold distilled water and added to the homogenate.

The homogenate was mixed on a Deluxe Mixer (Scientific Products No. S-8220) and a 1.0 ml aliquot removed for protein determination. The remaining 4.0 mls was placed in an ice jacket until all the samples, usually eight, had been similarly treated.

RNA was separated from DNA and protein by the Schmidt-Thannhauser (1945) method as modified by Munro and Fleck (1966) (Figure 3). The supernatent, containing RNA was read at 260 mμ on a Coleman 139 spectrophotometer.

The precipitate containing the DNA and protein was suspended in 4.0 mls of .3N KOH and maintained at room temperature for half an hour. The DNA content was determined by the indole method (Ceriotti, 1952) with one modification. The purification of chloroform originally recommended has been shown unnecessary and even disadvantageous (Hutchinson and Munro, 1961). Therefore, the small amount of ethanol added to analytical grade chloroform to prevent decomposition was not removed.

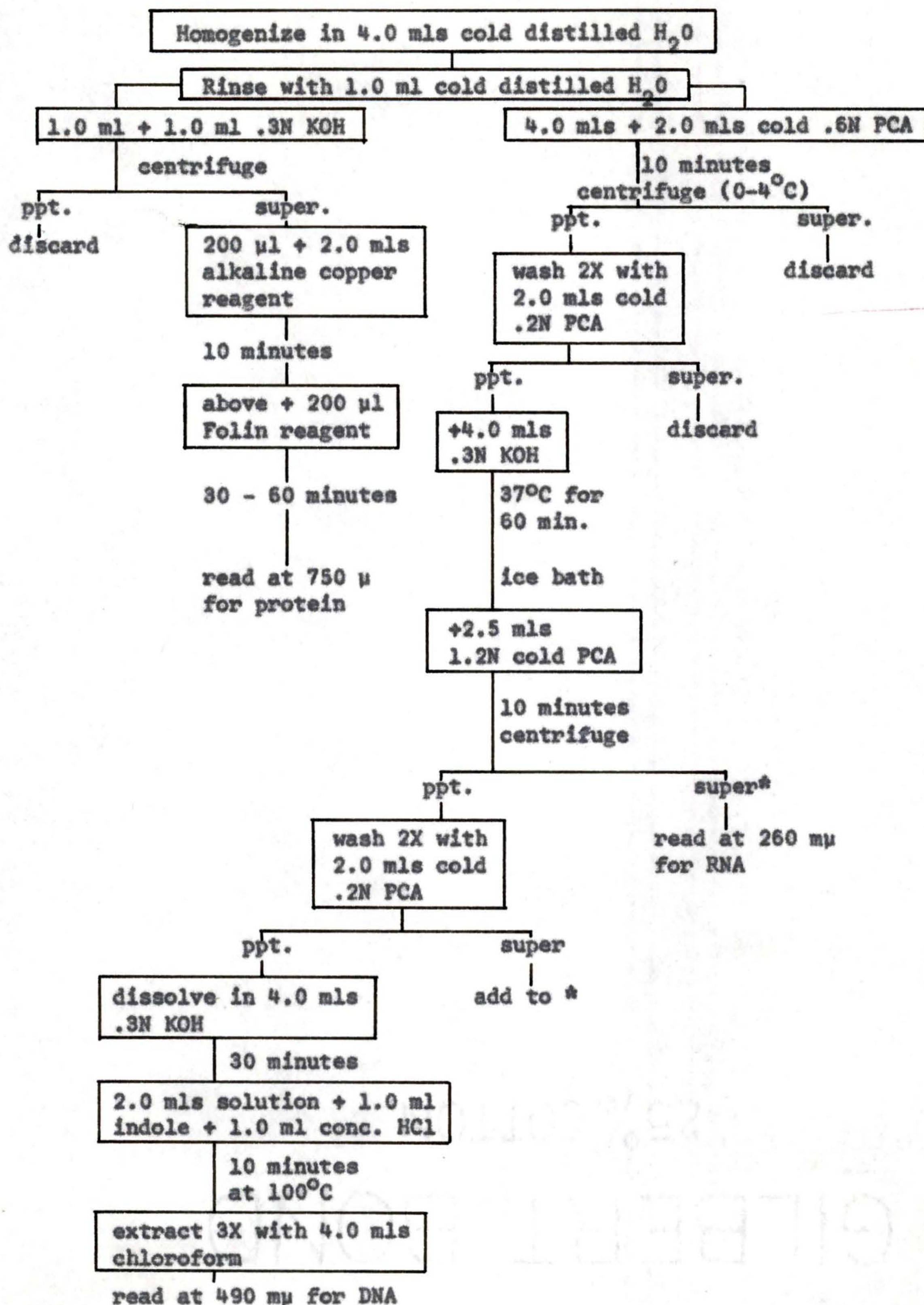
The protein aliquots were placed in an International Clinical Centrifuge (Model C-F, International Equipment Co.) and centrifuged for a few minutes. The precipitate was discarded and the protein content was determined from a 200 μl aliquot of the supernatent, in the manner described by Lowry et. al. (1951).

Nucleic acids are especially susceptible to degradation by heat or chemicals (Geise, 1967). RNA and DNA extraction and separation was done, as much as possible, at low temperatures (0-4°C).

C. Precision

Few samples contained sufficient animals for replicate analyses.

Figure 3. Chart of the adopted analytical procedure.


Homogenize in 4.0 mls cold distilled H_2O
Rinse with 1.0 ml cold distilled H_2O
1.0 ml + 1.0 ml .3N KOH
centrifuge
ppt.
discard
super.
200 µl + 2.0 mls alkaline copper reagent
10 minutes
above + 200 µl Folin reagent
30 - 60 minutes
read at 750 µ for protein
4.0 mls + 2.0 mls cold .6N PCA
10 minutes centrifuge (0-4°C)
ppt.
super.
discard
wash 2X with 2.0 mls cold .2N PCA
ppt.
super.
discard
+4.0 mls .3N KOH
37°C for 60 min.
ice bath
+2.5 mls 1.2N cold PCA
10 minutes centrifuge
ppt.
super*
read at 260 mµ for RNA
wash 2X with 2.0 mls cold .2N PCA
ppt.
super
add to *
dissolve in 4.0 mls .3N KOH
30 minutes
2.0 mls solution + 1.0 ml indole + 1.0 ml conc. HCl
10 minutes at 100°C
extract 3X with 4.0 mls chloroform
read at 490 mµ for DNA

The *E, japonica*, CVI♀, sample from Saanich Inlet on March 7, 1969, was sufficiently large for five separate analyses. The Students "t" test (Speigel, 1961) was used to derive the range value of each parameter (Table V).

TABLE V. Precision of analysis

	µg RNA / mg drywt	µg RNA / mg protein	µg DNA / mg drywt	µg DNA / mg protein	µg RNA / µg DNA	Protein / drywt %
Values	7.4	16.6	5.9	11.7	1.40	44.7
	6.6	12.8	6.3	11.2	1.12	51.9
	7.1	12.5	5.1	10.5	1.26	56.3
	6.4	13.2	5.8	11.0	1.14	48.3
	6.6	12.5	5.2	11.3	1.14	52.9
$\bar{X}$	6.8	13.5	5.7	11.1	1.21	50.8
Range	6.8±.6	13.5±2.2	5.7±.6	11.1±.5	1.21±.15	50.8±5.4

Several samples contained sufficient animals for 2 to 3 analyses and values are given in Table VI.

The individuals of a sample are not necessarily identical. Two or three adult *E. japonica* were sufficient for analysis and individual differences could have contributed to the differences shown in Table V and Table VI.

TABLE VI. RNA, DNA and protein concentration values for replicates of *E. japonica* samples.

Sample date	*E. japonica*					
	µg RNA / mg dry wt	µg RNA / mg prot	RNA/DNA	µg DNA / mg dry wt	µg DNA / mg/prot	prot / dry wt
Dec. 23/68	9.3	-	.92	10.1	-	-
	9.1	-	.96	9.5	-	-
Jan. 8/69	7.6	16.9	.99	7.7	17.0	45.3
	8.1	19.0	1.23	6.6	13.2	42.9
Jan. 8/69	8.9	17.9	1.22	7.3	15.5	48.7
	10.3	20.1	1.53	6.7	14.7	51.1
Mar. 11/69	16.2	27.7	1.39	11.6	20.0	58.5
	17.0	29.0	1.61	10.4	17.8	58.5
Mar. 11/69	9.4	14.1	1.23	7.7	11.5	66.8
	9.7	12.4	1.11	8.8	11.2	78.4
	9.3	13.3	1.12	8.2	11.8	69.4
Mar. 14/69	7.6	13.1	1.27	6.0	10.3	58.2
	10.2	16.8	.92	11.4	18.8	60.8
Apr. 3/69	14.0	21.5	2.38	9.0	13.8	65.2
	13.4	20.8	2.19	8.0	12.4	64.2
Apr. 14/69	8.3	12.1	1.50	6.3	11.4	69.0
	9.6	17.3	1.12	7.5	10.9	55.6
Apr. 14/69	6.3	10.2	1.37	4.6	7.6	62.3
	8.6	11.5	.83	10.4	14.0	74.3
May 1/69	10.2	15.0	1.90	5.3	7.9	67.8
	10.8	18.8	2.21	4.8	8.4	57.7
May 12/69	10.4	18.2	1.53	6.8	9.0	59.8
	10.4	16.8	1.87	5.6	9.8	61.7
	6.4	10.1	1.03	6.2	11.9	63.6

CHAPTER III

Results

A. *Artemia salina*

(i) Growth

From the dry weight per individual, the DNA/dry weight and the protein/dry weight values, the amount of DNA and protein per individual can be determined. Growth rate can be expressed as the rate of increase in dry weight, protein or DNA per individual. If these growth indices do not change relative to each other, they will exhibit the same growth patterns.

In culture 'A', dry weight changes were irregular prior to exponential growth (Figure 4a) and individuals of two of the samples showed "negative growth" (i.e. weighed less than individuals of the previous sample). The short exponential phase was followed by a period of slow and irregular growth during which time two more samples showed negative growth. In culture 'B', the dry weight per individual initially increased slowly (Figure 4b). After a period of no growth an exponential increase in dry weight occurred. In culture 'C', individual dry weight increased rapidly until day 5 and then the growth rate slowed (Figure 4c).

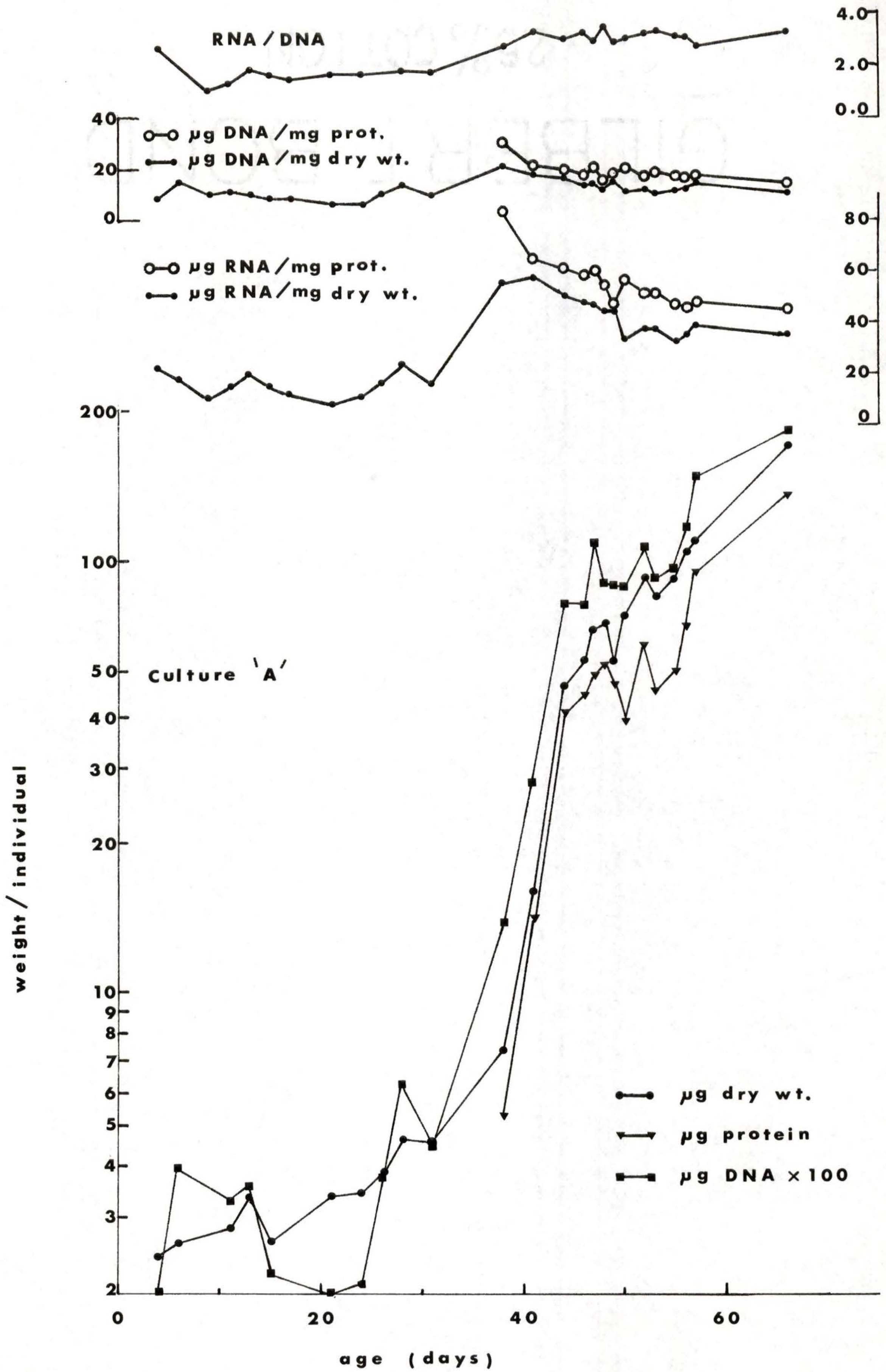
The weight of protein per individual (Figure 4) was not available for all samples. The growth curves derived from increases in protein per individual usually showed similar patterns to the growth curves derived from dry weight increases. However, protein per individual increased more rapidly than dry weight per individual in the early exponential phase (Figure 4a, 4b). Dry weight per individual increased more rapidly than protein per individual for a short period immediately after exponential

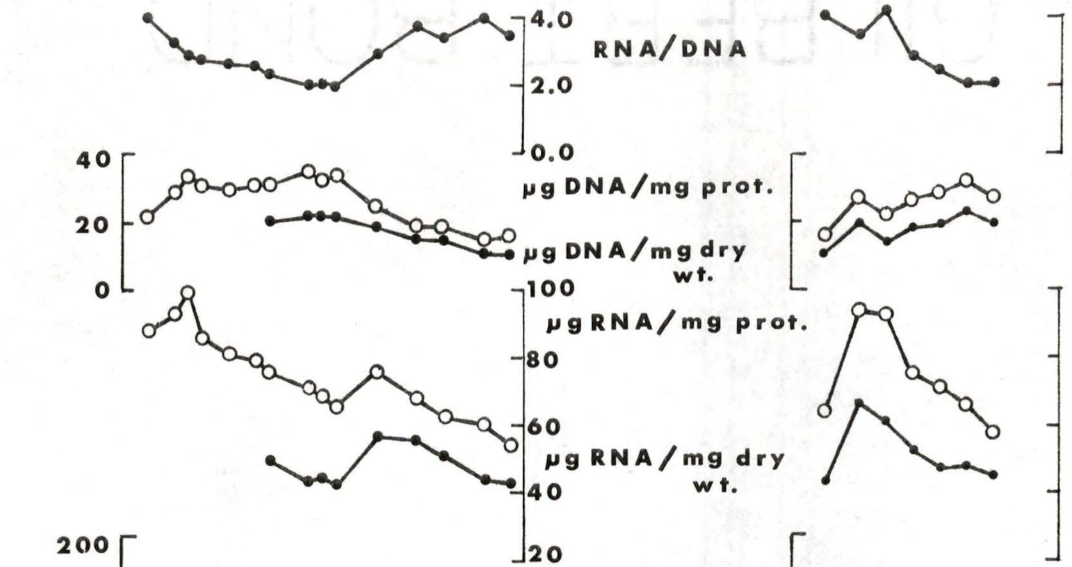
Figure 4. Growth curves and RNA, DNA and protein concentration changes in:

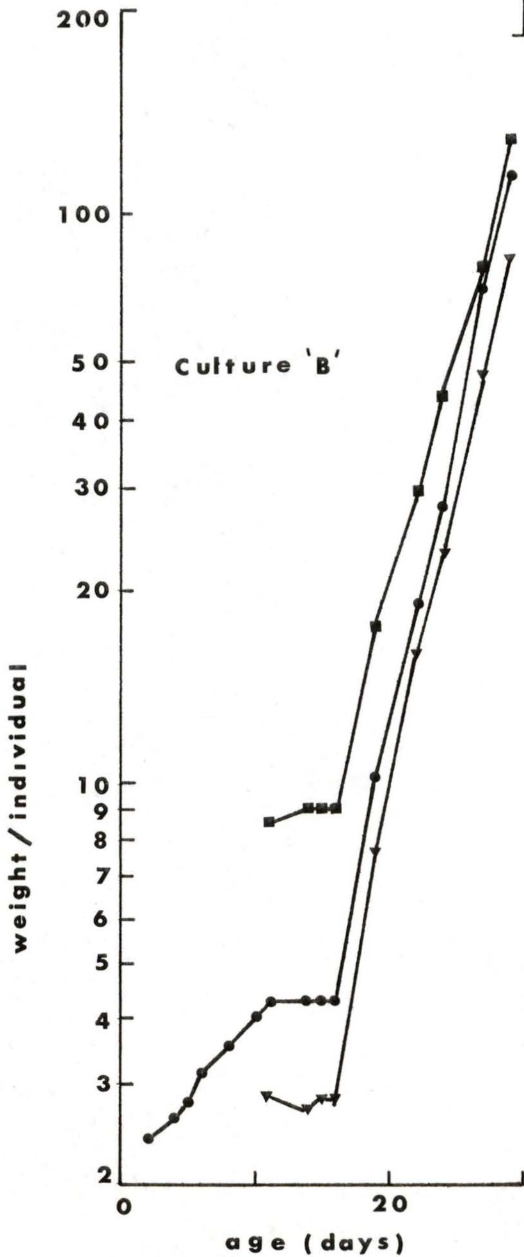
(a) *A. salina* culture 'A'

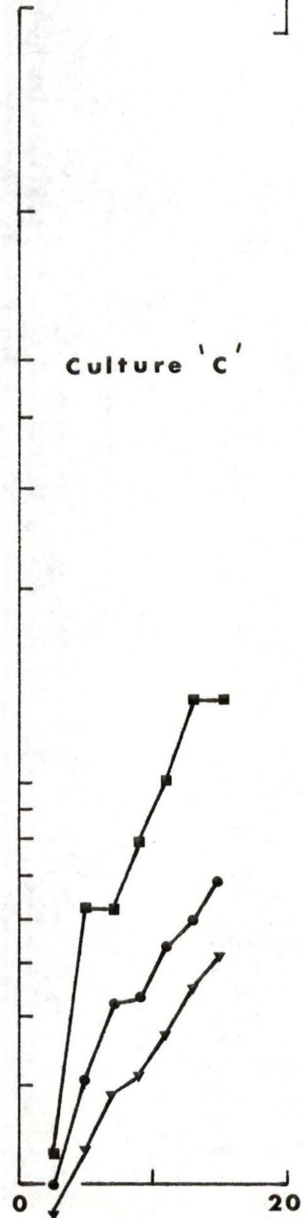
(b) *A. salina* culture 'B'

(c) *A. salina* culture 'C'


RNA / DNA
4.0
2.0
0.0
40
20
0
μg DNA / mg prot.
μg DNA / mg dry wt.
80
60
40
20
0
μg RNA / mg prot.
μg RNA / mg dry wt.
200
100
50
40
30
20
10
9
8
7
6
5
4
3
2
Culture 'A'
weight / individual
μg dry wt.
μg protein
μg DNA × 100
0
20
40
60
age (days)


4.0
RNA/DNA
2.0
0.0
40
20
0
µg DNA/mg prot.
µg DNA/mg dry wt.
100
µg RNA/mg prot.
80
60
µg RNA/mg dry wt.
40
20


200
100
50
40
30
20
10
9
8
7
6
5
4
3
2
Culture 'B'
weight / individual
0
20
age (days)


Culture 'C'
0
20

growth (Figure 4a).

Changes in the amount of DNA per individual were also used to measure growth. In the early stages of each culture, DNA rapidly increased (Figure 4a, 4c). In culture 'A', before the exponential phase DNA changes did not correspond with dry weight changes, and the amount of DNA per individual declined after an initial increase. DNA per individual rapidly increased before and during exponential growth. In culture 'B', the growth curve derived from DNA increase per individual was similar to the growth curves derived from protein and dry weight increases per individual (Figure 4b). In culture 'C', growth measured by the increase in DNA per individual increased rapidly until day 5 and then slowed (Figure 4c). The DNA per individual increased more rapidly than the protein or dry weight per individual.

The indices used to measure growth in the *A. salina* cultures do sometimes change relative to each other. In other words, the protein/dry weight concentration and DNA/dry weight concentration change throughout the culture period. These concentration changes are not necessarily reflected in similar changes in the absolute amounts of protein and DNA per individual.

(ii) Protein Concentration

Protein concentration (protein/dry weight) changed with growth rate (Figure 4, Appendix II). It was highest during exponential growth.

(iii) DNA Concentration

The DNA content can be expressed against dry weight and protein to give information concerning the biochemical growth of an organism (see Chapter IV). DNA/dry weight concentration and DNA/protein concentration usually followed similar patterns. Because DNA was only 1 to 3

per cent of the dry weight or protein of an individual *A. salina* (Figure 4, Appendix II), large changes in the protein/dry weight concentration were required to cause significant disparities between the patterns of the DNA/protein and DNA/dry weight curves.

In all three cultures, the DNA concentration initially increased. The maximum DNA concentrations occurred immediately prior to exponential growth. During and after exponential growth, DNA/protein and DNA/dry weight decreased.

(iv) RNA Concentration and Growth Rate

The amount of RNA per individual was expressed relative to each of the indices used to measure growth (Figure 4, Appendix II). Changes in RNA dry weight concentration and RNA/protein concentration usually coincided. RNA relative to DNA often followed a pattern different from the RNA/protein and RNA/dry weight curves.

In culture 'A', RNA/protein and RNA/dry weight changed with changes in the rate of increase in protein and dry weight. For example, two small peaks in the RNA/dry weight curve (day 13 and day 28) coincided with peaks in the growth curve (dry weight/individual). The RNA concentration reached a near maximum immediately prior to the exponential phase of growth and although it began to decline during exponential growth, it remained high during this phase. After the exponential phase, the RNA/protein and RNA/dry weight gradually declined. The RNA/DNA concentration increased immediately prior to exponential growth but remained essentially constant thereafter, although the growth rate declined. During the period of slow growth in culture 'B', the RNA/protein concentration peaked and then declined until the exponential

phase began. At this time, RNA relative to all three growth indices, increased. The RNA/dry weight and RNA/protein concentrations then decreased, even though exponential growth continued. The RNA/DNA ratio continued to increase. The RNA/dry weight and RNA/protein concentration of culture 'C' increased initially and then declined as the growth rate slowed. With the exception of one sample, the RNA/DNA ratio declined throughout the period of the culture.

There were some patterns of change common to all cultures. RNA relative to dry weight and protein increased most rapidly immediately prior to or during early exponential growth but then began to decline even though exponential growth continued. The RNA/DNA concentration initially decreased in all cultures and often did not coincide with changes in the other two RNA measurements.

The RNA/dry weight value for each *A. salina* sample was used to derive the percent expected dry weight increase per day from the standard *Orchestia platensis* curve (Sutcliffe, 1965). If Sutcliffe's relationship is valid, the expected growth rate should be similar to the observed growth rate for each sample. The observed growth rate, expressed as percent dry weight increase per day, was determined for each *A. salina* sample (Appendix III), using the formula of Winberg (1956):

$$C^1_m = \left[10^{\frac{1}{t}(\log W_n - \log W_{n-1})} - 1 \right] .100$$

where C^1_m = percent dry weight increase per day

t = number of days between samples n and n-1

W_{n-1} = average dry weight of an individual from sample n-1

W_n = average dry weight of an individual from sample n

This formula assumes a constant rate of growth between samples. The growth of the four samples in culture 'A' that underwent "negative growth" was assumed to be zero. The observed (Winberg) and expected (Sutcliffe) growth rates were most comparable during the exponential phase (Table VII). At slower growth rates the relationship between the two values was poor.

Although Sutcliffe's equation did not predict growth accurately, a relationship between the measured RNA concentration of each sample and the observed growth rate of the sample remained a possibility. Therefore, a simple regression of the RNA/dry weight concentration of each sample on its observed growth rate (percent dry weight increase per day) was done (Figure 5a). This line shows the ability of the RNA/dry weight concentration to predict growth rate (i.e. the RNA/dry weight concentration of sample 'n' was paired with the percent dry weight increase per day observed between samples 'n' and 'n + 1'). At the 5 percent interval, the F value shows this relationship is significant (Table VIII). However, the point scatter about the line is large.

Percent growth per day was also determined in terms of protein and DNA increases, using the formula of Winberg (1956) (Appendix III). A saimple regression line of the RNA/protein concentration of each *A. salina* sample on the observed growth rate (percent protein increase per day) was done (Figure 5b). Similarly the RNA/DNA values were regressed on the observed growth rates expressed as percent DNA increase per day (Figure 5c). The F values at the 5 percent interval show these relationships (5b, 5c) are not significant (Table VIII).

TABLE VII. Observed vs. expected percent growth (dry weight increase) per day for *A. salina*.

Culture	Age	Expected !	Observed	Culture	Age	Expected	Observed
A	4	24.3	4.1	B	2	26.0	4.3
	6	22.1	-		4	34.5	8.0
	9	17.4	1.5		5	29.8	14.8
	11	20.4	8.6		6	28.0	4.7
	13	23.1	0		8	25.5	5.7
	15	20.3	0		10	28.4	7.9
	17	18.5	0		11	31.8	0
	21	15.2	1.0		14	30.6	0
	24	16.9	5.8		15	30.8	0
	26	21.2	8.8		*16	30.3	37.6
	28	24.9	0		*19	33.0	27.5
	*31	21.5	7.3		*22	32.8	23.0
	*38	32.8	33.2		*24	32.0	31.8
	*41	33.1	43.3		*27	30.6	27.1
	*44	31.9	7.7		*29	29.5	
	*46	31.4	17.3				
	47	31.3	2.9	C	2½	30.6	15.7
	48	30.6	0		5	34.6	16.4
	49	30.7	29.3		7	33.8	1.2
	50	28.0	9.9		9	32.2	10.2
	52	29.3	0		11	31.5	6.3
	53	29.0	5.5		13	31.5	7.4
	55	27.8	13.5		15	30.2	
	56	28.5	8.9				
	57	29.6	0				
	59	33.5	10.8				
	66	28.5					

! = from % = -5.041 + 21.667 (log RNA) (Sutcliffe, 1965)

* = exponential phase

Figure 5(d) illustrates the ability of the RNA/dry weight concentration to estimate the past growth (i.e. the RNA concentration of sample 'n' was paired with the growth rate observed between samples 'n-1' and 'n'). The F value at the 5 percent interval showed the relationship was significant (Table VIII).

(v) Ribosome efficiency

The use of the term $\frac{\text{protein}}{\text{RNA}}$ x μ is often used to indicate the rate of protein syntheses per unit weight of RNA in bacteria (Rosset et. al., 1966). This value indicates protein synthesis rate per ribosome or ribosome efficiency because RNA is a constant proportion of the total RNA. By replacing μ (doubling per hour) with percent growth per day, an arbitrary value for *A. salina* ribosome efficiency at different growth rates was derived (Table IX). The efficiency was also determined with dry weight instead of protein (Table IX). There is no indication of the efficiency remaining constant at different growth rates.

B. *Euphausia sp.*

Euphausiids from Saanich Inlet were kept at 11°C, one per liter of sea water, in a refrigerator. The test animals were probably *Euphausia pacifica* because this species is the most abundant euphausid in Saanich Inlet (K. Rauchert, personal communication). The animals did not keep well and many died (Appendix VI). Most showed negative growth between molts. Molting rate averaged 6.2 days. No chemical analyses were done and the experiment was terminated after 4 weeks.

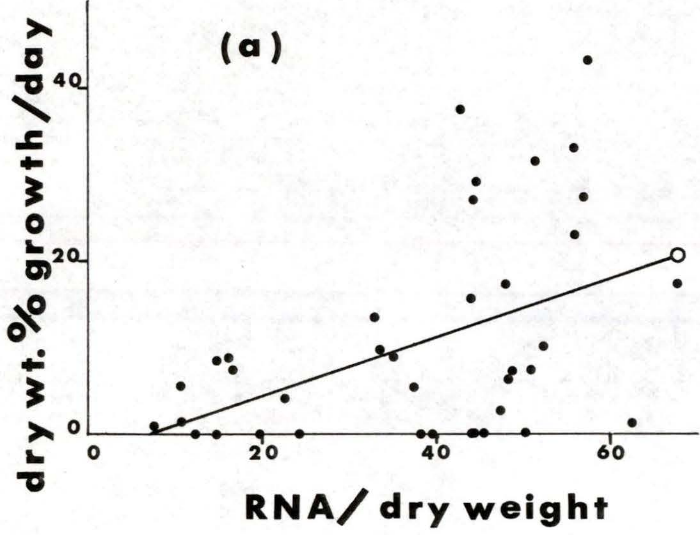
Figure 5. Regression analyses for *A. salina* of:

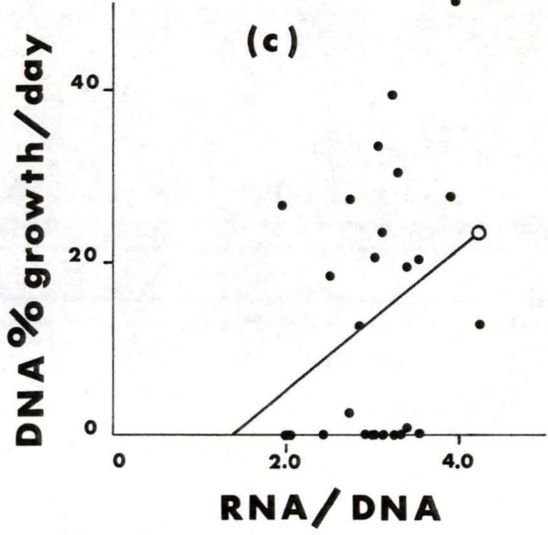
(a) RNA/dry weight concentrations on the percentages of increase in dry weight per day

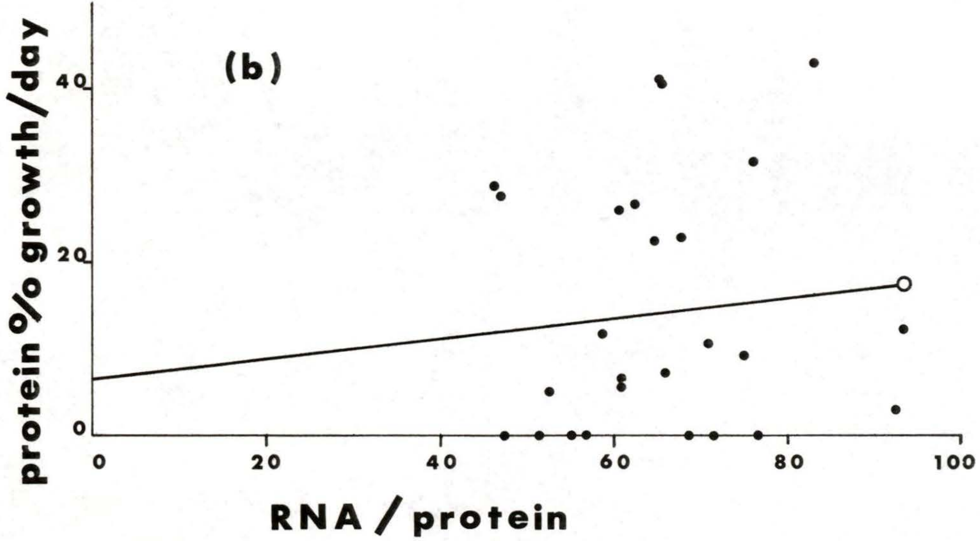
(b) RNA/protein concentrations on the percentages of increase in protein per day

(c) RNA/DNA concentrations on the percentages of increase in DNA per day

(d) RNA/dry weight concentrations on the percentages of increase in dry weight per day.


(a)
dry wt. % growth/day
RNA/ dry weight
0
20
40
60


(c)
DNA % growth/day
RNA/ DNA
0
20
40
2.0
4.0


(b)
protein % growth/day
RNA / protein
0
20
40
60
80
100

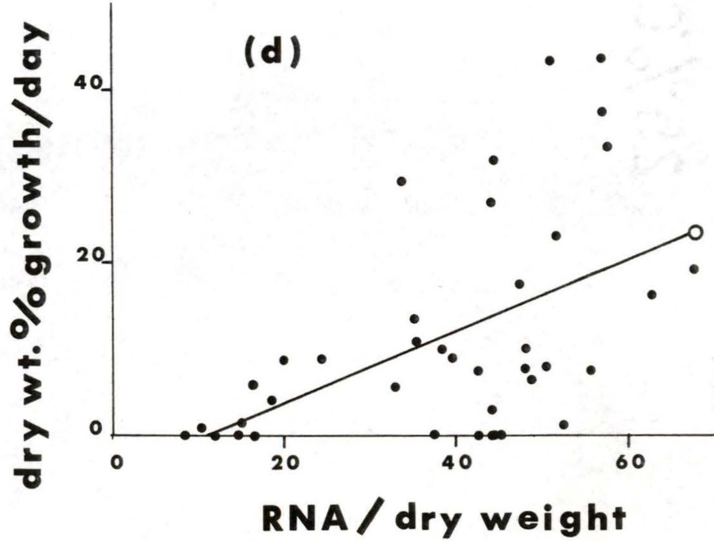

(d)
dry wt. % growth/day
RNA / dry weight
0
20
40
60

TABLE VIII. Statistical significance of the relationships between RNA concentration and growth rate.

Equation form	No. of data points	F value
Fig. 5a	38	9.93
5b	27	0.26
5c	27	2.94
5d	39	13.25

C. *Euchaeta japonica*

(i) Growth

Any attempt to correlate RNA concentration and growth rate requires that both be known. The growth rate of *E. japonica* cannot be determined from laboratory experiments because it has not been successfully cultured. Estimation of generation time from generation times of other copepods would be approximate at best. Field data must be used.

There are 12 stages, 6 naupliar and 6 copepodite in a calanoid life cycle. Time between molts can be determined by following the relative abundance of each stage over a period of time (Mullin and Brooks, 1967). More eggs are produced in the spring, per female *E. japonica* than at other times of the year (Lewis and Ramnarine, 1969). A peak in relative abundance should be observed for each successive stage until, a generation time period later, there is a peak in the number of adults. A similar approach is applicable to the copepodite stage 3 to stage 6 period. Therefore, the change in the relative frequency of each stage with time was plotted

TABLE IX. *A. salina* ribosome efficiency

Culture	Sample Age	$\frac{\text{dry wt}}{\text{RNA}}$ x growth rate	$\frac{\text{protein}}{\text{RNA}}$ x growth rate
A	4	180	
	6	-	
	9	137	
	11	577	
	13	0	
	15	0	
	17	0	
	21	117	
	24	558	
	26	543	
	28	0	
	31	436	516
	38	598	627
	41	753	108
	44	151	202
	46	360	90
	47	61	0
	48	0	0
	49	659	0
	50	294	0
	52	0	0
	53	148	523
	55	410	568
	56	253	0
	57	0	96
B	11	0	0
	14	0	0
	15	0	0
	16	884	621
	19	485	416
	22	412	335
	24	620	426
	27	615	429
C	2½	356	998
	5	243	129
	7	19	30
	9	196	121
	11	130	149
	13	152	109

(Figure 6). The absolute numbers of each stage caught are shown in Appendix IV.

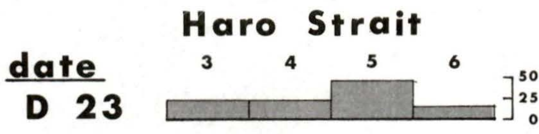
There were three discernable broods in Haro Strait. Stage 3 developed to stage 6 in about 10 weeks, from mid-February to May 1. From May 7 to June 5, a period of 4 weeks, stage 4 developed to stage 6 and from June 5 to June 26, 3 weeks, stage 3 developed to stage 5. In Saanich Inlet stage 3 molted to stage 4 and some stage 4 molted to stage 5 in the period from June 9 to June 23. The intermolt periods were derived from Haro Strait only (Table X).

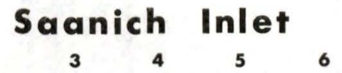
TABLE X. Intermolt periods of Haro Strait *E. japonica*.

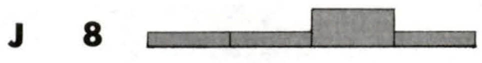
Stage changes	Time Taken	Period
3 - 4	10 days	(1) Mid-Feb. to May 1
	7 days	(3) June 5 to June 26
4 - 5	37 days	(1)
	9 days	(2) May 7 to June 5
	14 days	(3)
5 - 6	27 days	(1)
	20 days	(2)

The times are approximate because samples were taken weekly. Agreement is good except for the 37 day period spent as stage 4 in March. There was a month between samples involving this 37 day period and changes in population structure might have occurred during this time. This figure was therefore excluded from calculations. The average time spent as stage 3 was 8.5 days, stage 4 was 11.5 days and stage 5 was 23.5 days.

Figure 6. The relative frequencies of each *E. japonica* copepodite stage over the collection period.


Haro Strait
date
3
4
5
6
50
25
0
D 23

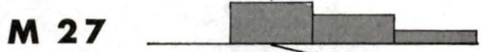

Saanich Inlet
3
4
5
6

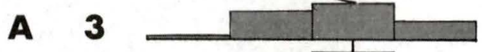

J 8

F 16

26

M 7
50
25
0


M 27

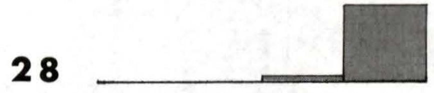

A 3

10

17

14

M 1


28

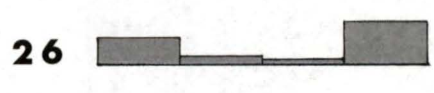
7

16

12

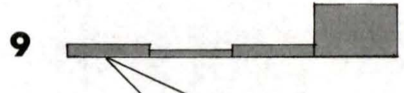
22

29


26

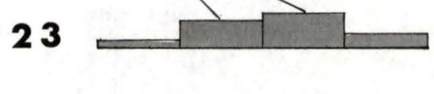
J 5

12


9

19

16
5 predominated


23

As well as the intermolt period, the individual dry weight of each stage is needed to determine growth rate. It was found the dry weight per individual changes with time (Table XI a). DNA per individual also changes (Table XI b). The mean dry weight per individual for each Haro Strait stage was .07 mg for stage 3, .23 mg for stage 4, .92 mg for stage 5 and 1.62 mg for stage 6. The dry weight value for stage 6 is based on the mean dry weight of both sexes and a ratio of 4 females to 1 male. Because females are heavier than males (Table XI a), the proportion of males to females is important in determining the average adult weight. The average ratio found in Haro Strait was 4 females to each male. Conover (1965) noted 6 females to each male for *Calanus hyperboreus*. Using the Winberg growth formula (1956), dry weight increases per day of 15.03 percent for stage 3, 12.81 percent for stage 4 and 2.52 percent for stage 5 were obtained.

There were at least two differences between Saanich Inlet and Haro Strait populations. First, stage 5 was usually the most numerous stage in Haro Strait samples while stage 6 was usually most numerous in Saanich Inlet samples (Figure 6). Second, Haro Strait individuals were sometimes as much as twice the dry weight of Saanich Inlet individuals of the same stage (Table XIa).

(ii) Haro Strait

RNA relative to protein and dry weight fluctuated in each stage (Figure 7. Appendix V). Changes in the RNA concentration usually coincided in all stages, especially the RNA/protein concentrations.

TABLE XI (a). Dry weight (mg) per individual *E. japonica*

	Haro Strait					Saanich Inlet				
Date	stg 3	4	5	6	6	stg 3	4	5	6	6
Dec. 17			.74	1.49						
23	.05	.22	.58	1.32	1.08					
Jan. 8	.06	.19	.88	1.24	1.16					
Feb. 16	.09	.17	.64							
May 11									1.22	
16			.75	1.73	1.14					
26						.10				
29		.26	1.07	1.74	0.84					
June 5			1.36	1.92	1.19					
9						.10	.17	.66	1.12	.56
12		.29	1.24	2.25	1.16					
19		.26	1.25	2.26	1.27					
23							.30	.86	1.30	
26			.71							

TABLE XI (b). DNA (μg) per individual *E. japonica*

	Haro Strait					Saanich Inlet				
Date	stg 3	4	5	6f	6m	stg 3	4	5	6f	6m
Dec. 23	2.1	3.8	5.6	9.4	9.5					
Jan. 8	3.1	3.4	6.3	8.7	10.5					
Feb. 16		3.0	6.3							
May 11									8.0	
16			7.9	9.6	10.0					
26		4.4	8.3	10.2	10.0	2.9				
June 5			11.9	11.1	11.0					
9						1.9	3.0	5.8	7.1	5.5
12		4.3	8.2	9.8	10.1					
19		4.5	8.1	10.1	11.0					
23							4.4	6.3	7.4	
26			7.0							

Figure 7. RNA concentration changes in Haro Strait *E. japonica* from December 1968 to June 1969:

(a) RNA/protein concentrations

(b) RNA/dry weight concentrations

Figure 8. RNA/dry weight concentrations vs. the percentages of increase in dry weight per day for *E. japonica*.

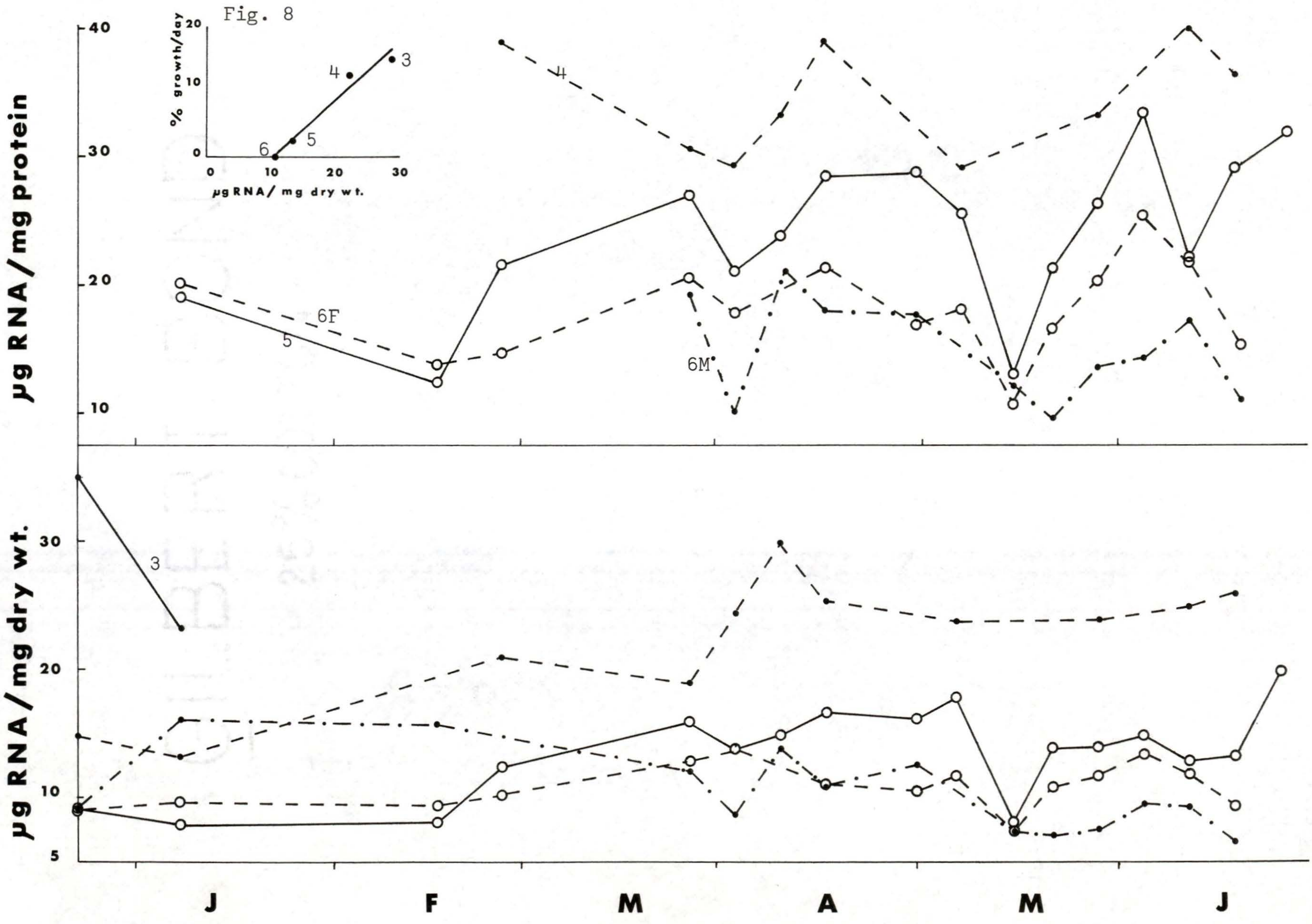


Fig. 8
% growth/day
20
10
0
0
10
20
30
µg RNA/ mg dry wt.
3
4
5
6
µg RNA / mg protein
40
30
20
10
6F
5
4
6M
µg RNA / mg dry wt.
30
20
10
5
3
J
F
M
A
M
J

For example, on April 3 all stages showed a decrease in RNA/protein concentration but stage 4 and adult females did not decrease in RNA/dry weight concentration. Stage 4 RNA concentration was low in December and January. After February 16, stage 5 had higher RNA concentration than either of the adult sexes. Stage 6F and 6M sometimes differed (December to February) and other times (April to mid-May) were similar in RNA concentration. The general pattern appeared to be that the RNA concentration was highest in stage 3, lower in stage 4, lower still in stage 5 and lowest in the adults. This correlates with the patterns of observed growth rates. The relationship between the mean RNA/dry weight concentration of each stage and the observed growth rate for each stage is shown in Figure 8. The RNA/DNA concentration of all stages fluctuated over wide ranges (Figure 11a, Appendix V). The RNA content differences between stages were not as evident in the RNA/DNA ratios as they were in the other RNA measurements. With the exception of adult males, RNA/DNA tended to be highest in the oldest stages and lowest in the youngest stages.

The *Orchestia platensis* derived equation (Sutcliffe, 1965) and the *Artemia salina* derived equation (section A) were used with the mean RNA/dry weight concentration of each stage, to predict the percent dry weight growth per day of each stage. These values were compared to the observed values (Table XII).

TABLE XII. RNA concentration and growth rate of *E. japonica* copepodites.

Stage	µg RNA / mg dry weight	Observed C^1m	Sutcliffe* C^1m	*A. salina*** C^1m
3	29.0	15.0	26.6	7.8
4	22.3	12.8	24.2	5.5
5	13.5	2.5	19.4	2.4
6	10.7	0.0	17.3	1.4

* $C^1m = -5.051 + 21.667 (\log RNA)$

** $C^1m = -2.33 + .35 (RNA)$

The DNA/protein and DNA/dry weight curves (Figure 9, Figure 10) usually coincide in trend although the range of the fluctuation is often markedly different (e.g. March to mid-May, stage 4). Concentrations were highest in stage 3, lower in stage 4, and still lower in stage 5. The DNA concentration in adult males was often higher than that of stage 5 but never as high as stage 4. Adult females had the lowest concentration of all stages with the exception of the April 17 sample.

Large changes in protein concentration occurred over short periods of time (Figure 11 b). For example, stage 6F on April 3 was 76.7 percent protein and on April 17 was 56.3 percent protein. The direction of change was usually the same for all stages. After April 3, stage 4 had the highest protein concentration of all stages.

(iii) Saanich Inlet

Most of the general patterns of Haro Strait *E. japonica* are more clearly seen in the Saanich Inlet samples (Figures 12, 13, Appendix VI).

Figure 9. DNA/protein concentration changes in Haro Strait *E. japonica* from December, 1968 to June, 1969.

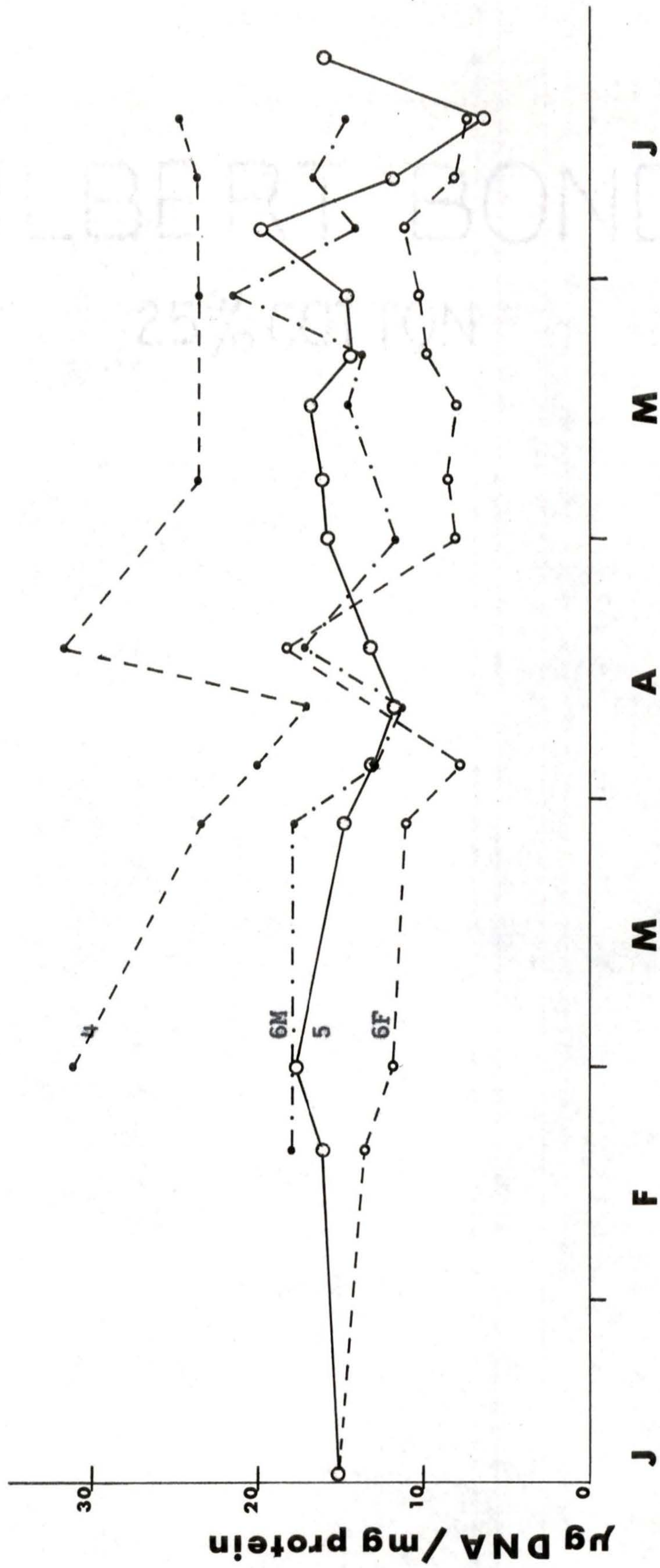

µg DNA / mg protein
0
10
20
30
J
F
M
A
M
J
4
6M
5
6F

Figure 10. DNA/dry weight concentration changes in Haro Strait *E. japonica* from December, 1968 to June, 1969.

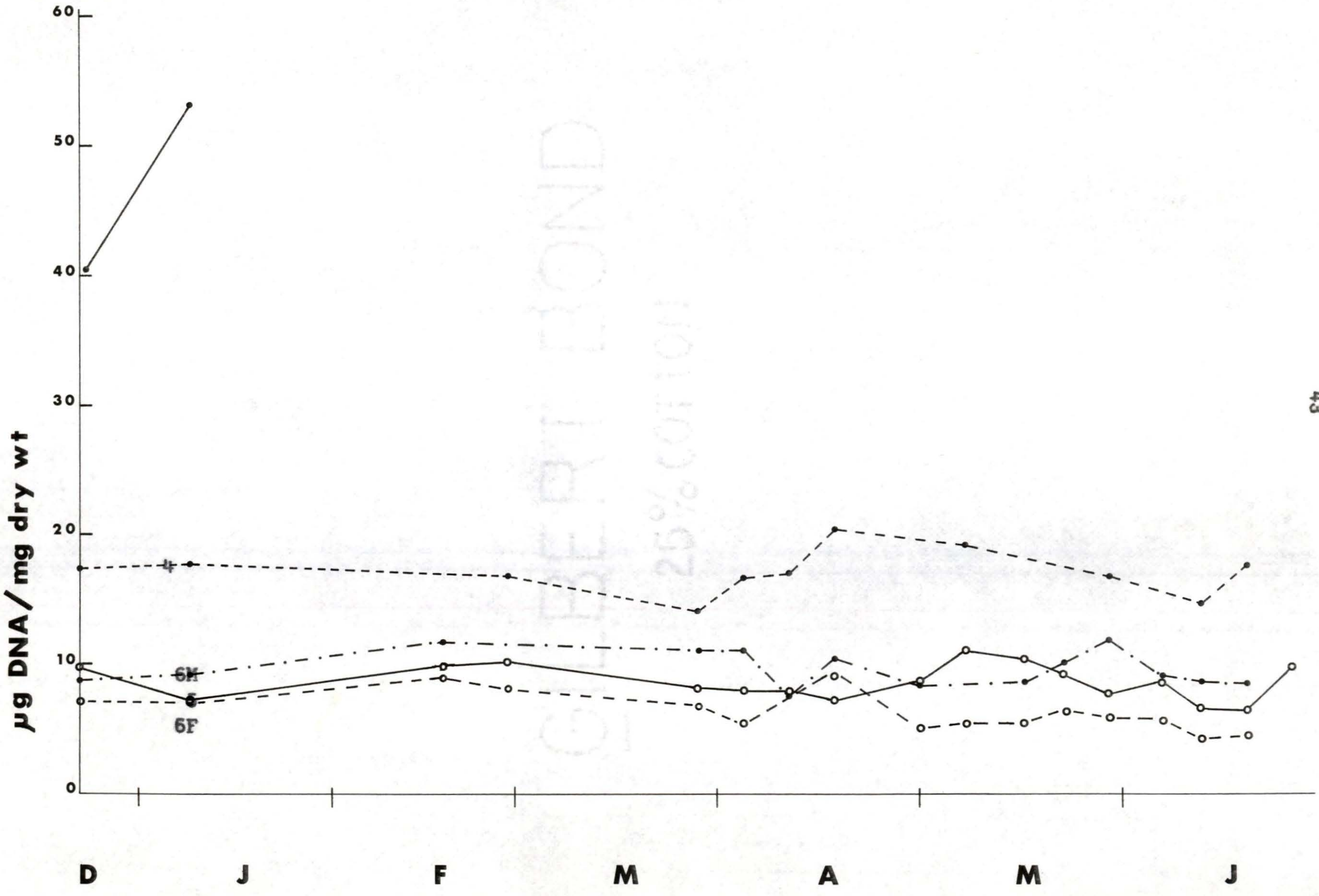
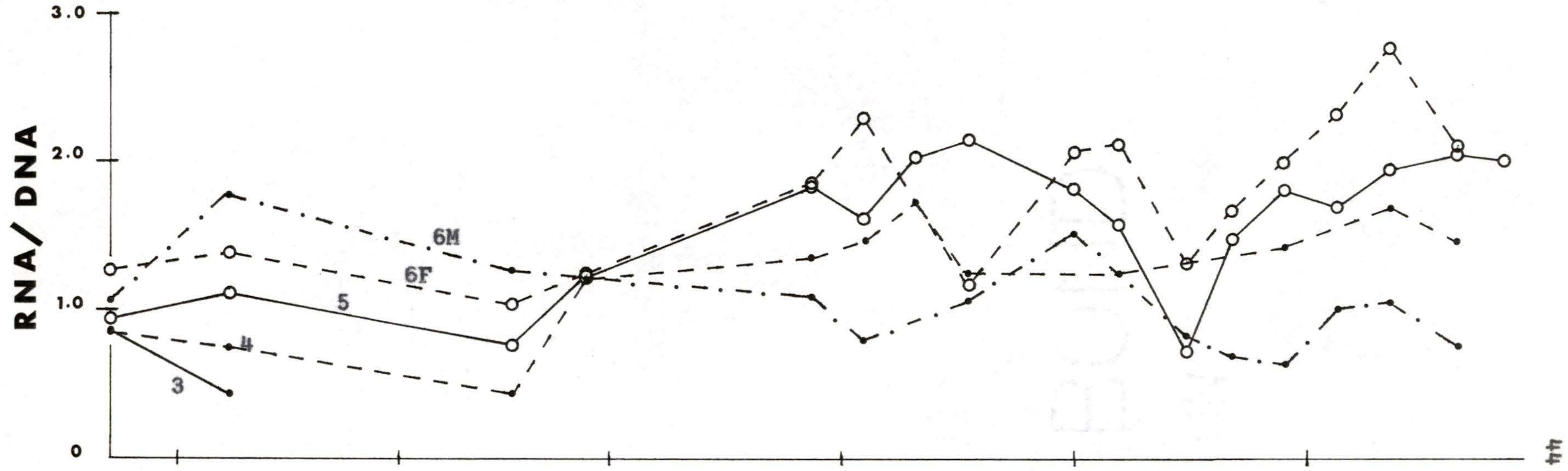
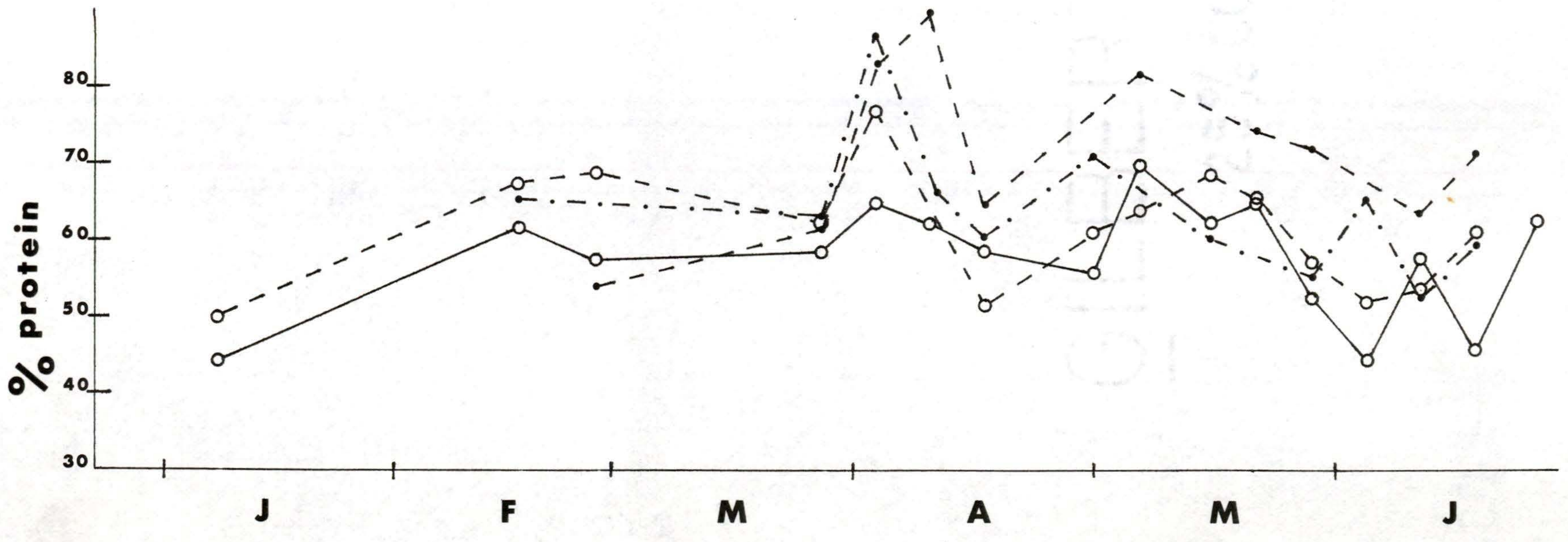

µg DNA / mg dry wt
60
50
40
30
20
10
0
4
6M
6F
D
J
F
M
A
M
J

Figure 11. Changes in Haro Strait *E. japonica* from December, 1968 to June, 1969 in:

(a) RNA/DNA ratios

(b) percent protein


RNA/ DNA
3.0
2.0
1.0
0
6M
6F
5
4
3


% protein
80
70
60
50
40
30
J
F
M
A
M
J

For example, in Saanich Inlet, the RNA/protein concentration of each stage was distinct from that of the other stages except for the May 12 adult male sample (Figure 12c). Sometimes (May 26 - June 9) the RNA concentration of all stages would simultaneously increase. Both RNA and DNA concentrations of each stage were more uniform in the Saanich Inlet samples than the Haro Strait samples. Stage 3 DNA concentration was much higher than the stage 4 concentration (Figure 13). The protein concentration (Figure 13c) fluctuated considerably. The maximum protein concentrations in the Saanich Inlet samples were lower than the maximum protein concentrations in the corresponding stages of the Haro Strait samples. The adults tended to have the highest protein concentrations in the Saanich Inlet *E. japonica*.

(iv) Geographical distribution

The six areas considered in the regional study are shown in Figure 1. Differences in RNA concentration between areas were greater in the younger stages than in the adults (Figure 14 a, b, c, Appendix V). Regional differences were present. For example, except in the adults, Georgia Strait RNA concentrations were higher than those of Haro Strait. However, no area was consistently (i.e. for all stages) higher in RNA concentration than another area. For example, RNA concentration was higher in (b) Jervis Inlet than in Bute Inlet (station 8) in stage 3 and 4 but not in stage 5. There was no consistent difference between the Inlets (areas 1, 2, 3, 4) and Straits (areas 5 and 6). Wider ranges in RNA concentration were present in stages 3 and 4 than in the older stages. Although some regional differences in RNA concentration occurred, a pattern of variation between stages was also present. The RNA concentration in stages 3 and 4 was high. In stage 5 RNA concentration was lower and it was lower still

Figure 12. RNA concentration changes in Saanich Inlet *E. japonica* from March to June, 1969 in:

(a) RNA/dry weight concentrations

(b) RNA/DNA ratios

(c) RNA/protein concentrations

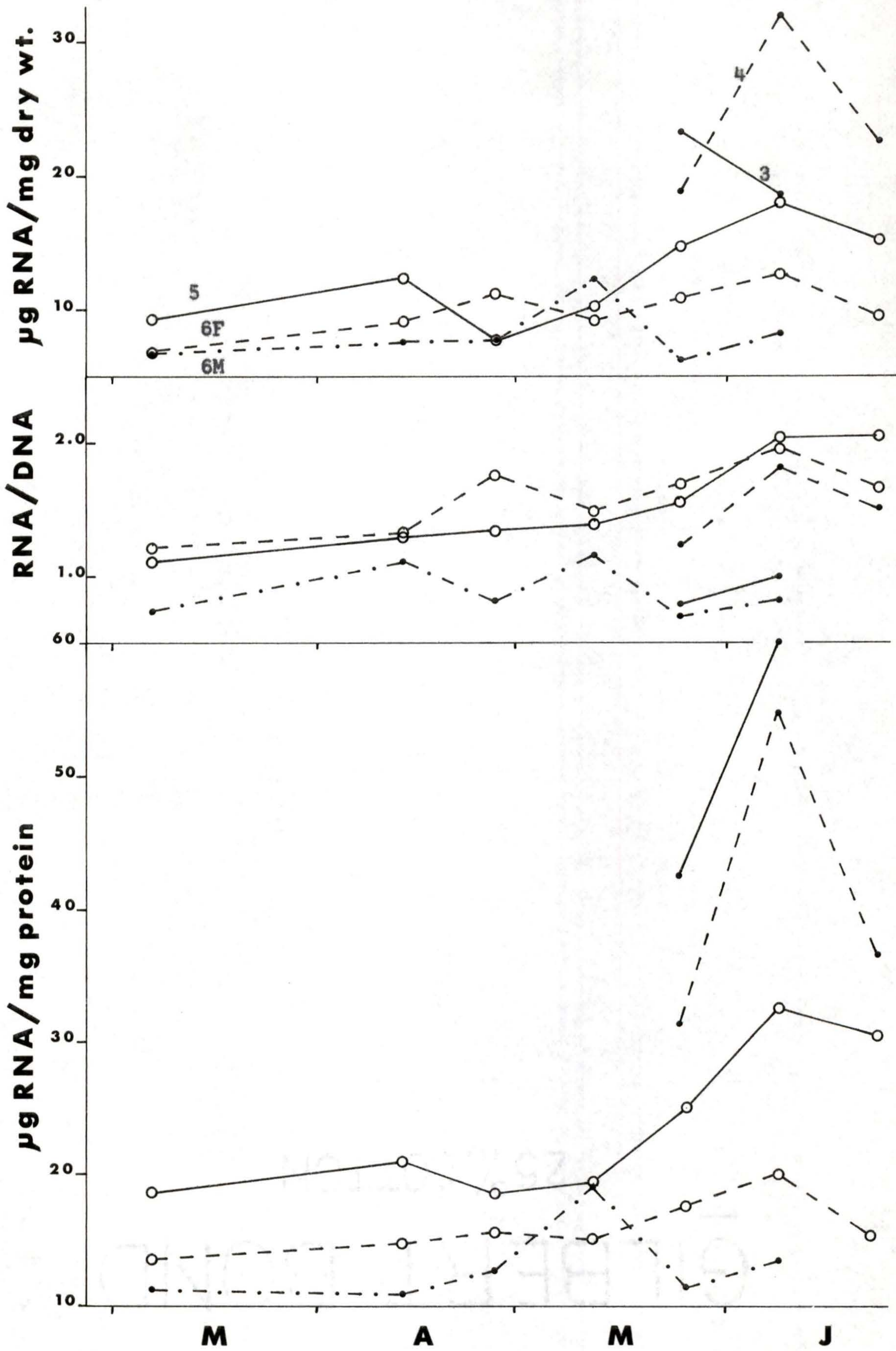
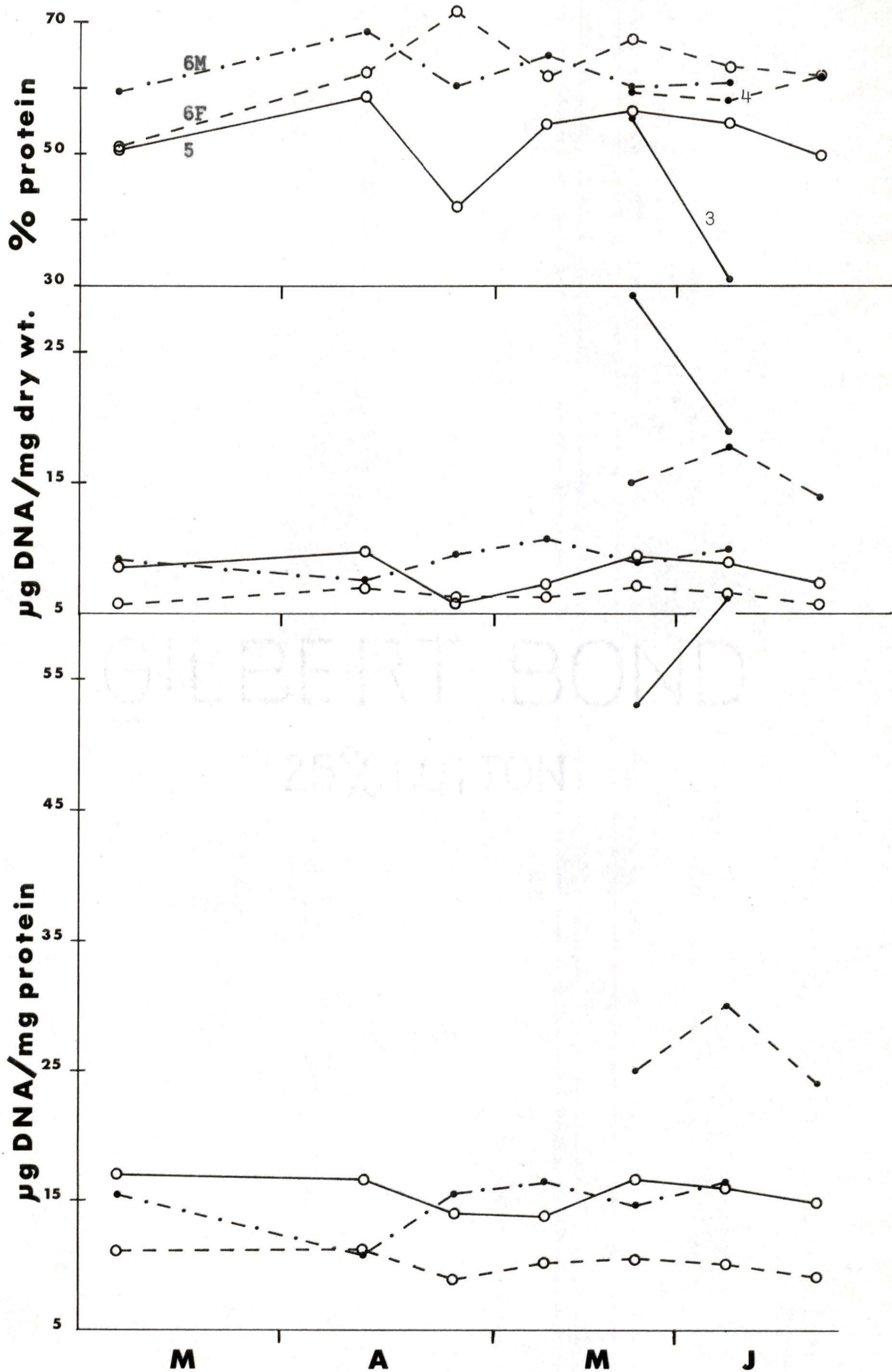

µg RNA/mg dry wt.
30
20
10
4
3
5
6F
6M
RNA/DNA
2.0
1.0
µg RNA/mg protein
60
50
40
30
20
10
M
A
M
J

Figure 13. Changes in Saanich Inlet *E. japonica* from March to June, 1969 in:

(a) percent protein

(b) DNA/dry weight concentrations

(c) DNA/protein concentrations


% protein
70
50
30
6M
6F
5
3
4
μg DNA/mg dry wt.
25
15
5
μg DNA/mg protein
55
45
35
25
15
5
M
A
M
J

in the adults. This general pattern is similar to that of Haro Strait and Saanich Inlet, except in this study the RNA concentration was slightly higher in stage 4 than in stage 3.

Regional differences in DNA concentration were smallest in the adults. Geographical variation in DNA concentration was less than RNA concentration but some regional patterns were evident. The Georgia Strait sample had a higher DNA concentration than the Haro Strait sample for all stages except adult males. The pattern due to stage differences in DNA concentrations was similar to the general pattern of Haro Strait and Saanich Inlet samples.

Protein concentration varied regionally (Figure 14f). Some trends due to stage was present as the adults were generally higher than the younger stages. However, there were regional differences consistent for all stages. For example, the protein concentration of each stage of the Bute Inlet sample was lower than the corresponding stage of the (b) Jervis Inlet sample (Table XIII). Further data are shown in Appendix V.

TABLE XIII. Example of regional differences in protein concentration

Stage	% Protein	
	(b) Jervis	Bute (station 8)
3	61	50
4	63	42
5	51	45
6F	71	60
6M	68	69

Figure 14. Geographical variation for *E. japonica* in March, 1969 of:

(a) RNA/protein concentrations

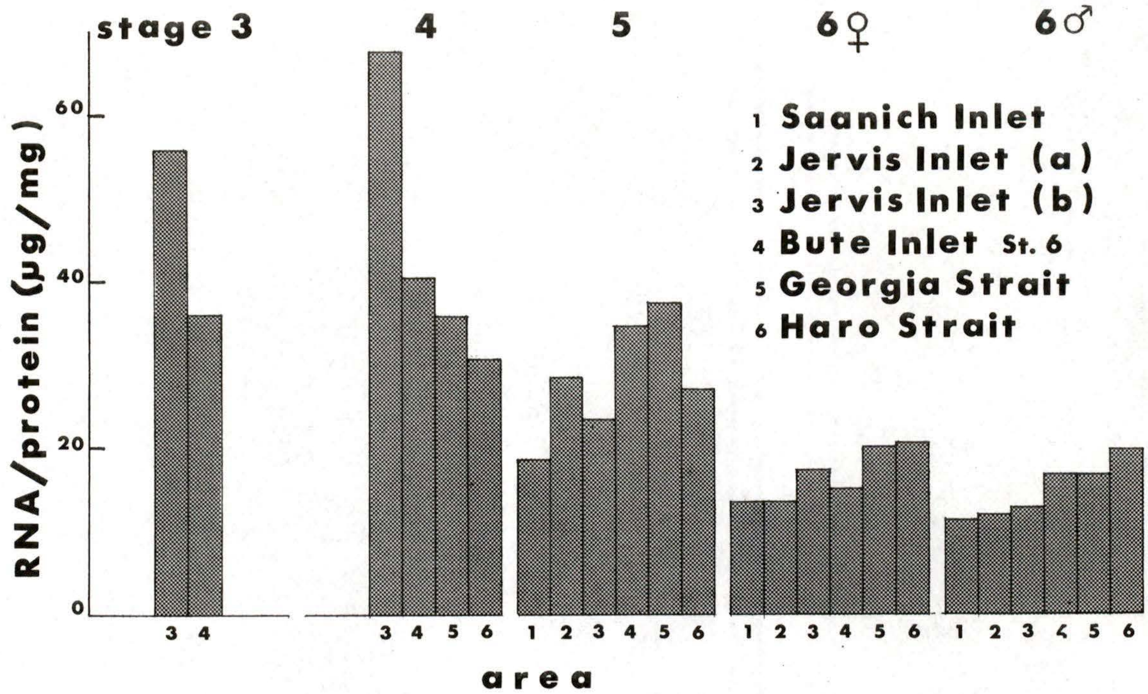
(b) RNA/dry weight concentrations

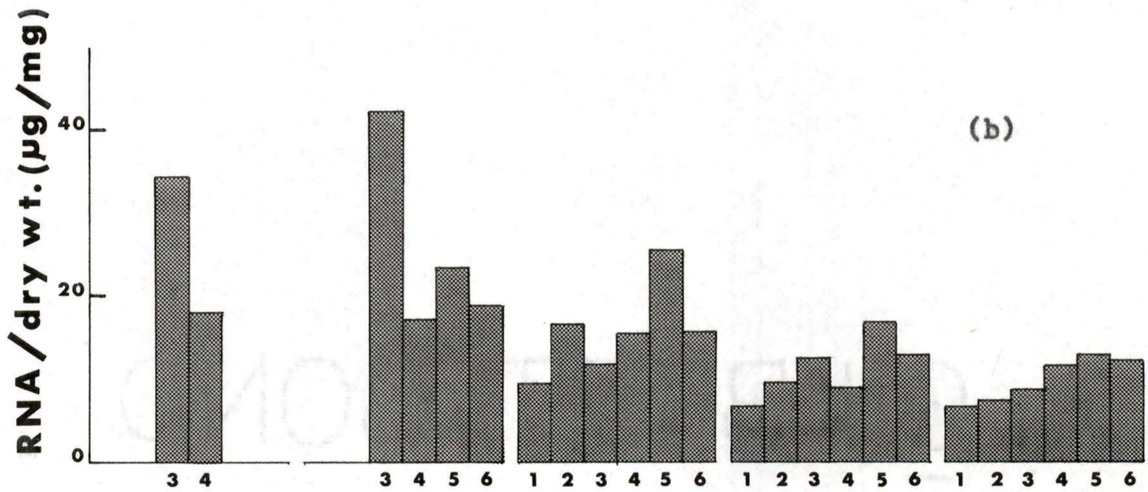
(c) RNA/DNA ratios

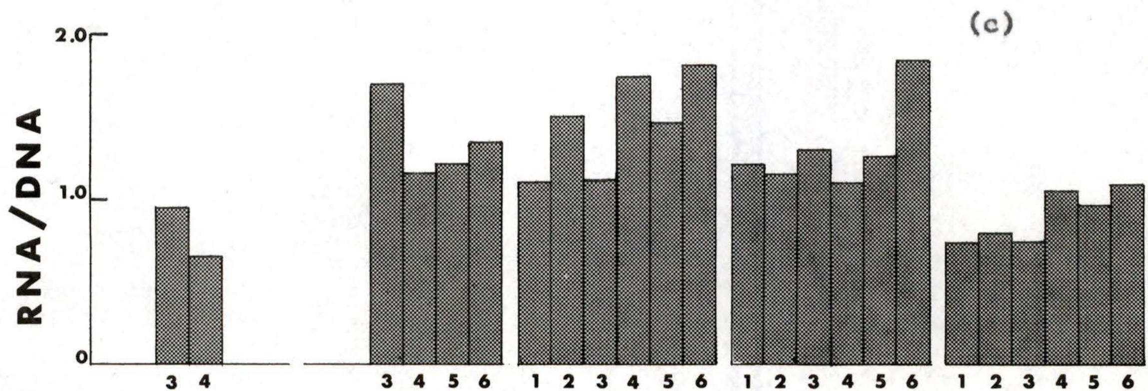
(d) DNA/protein concentrations

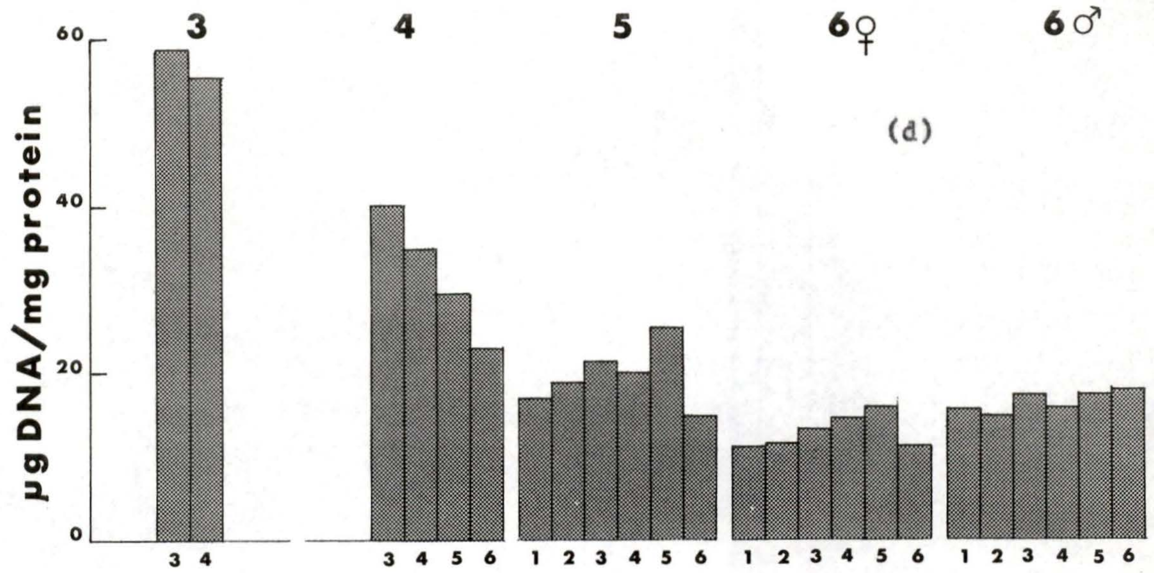
(e) DNA/dry weight concentrations

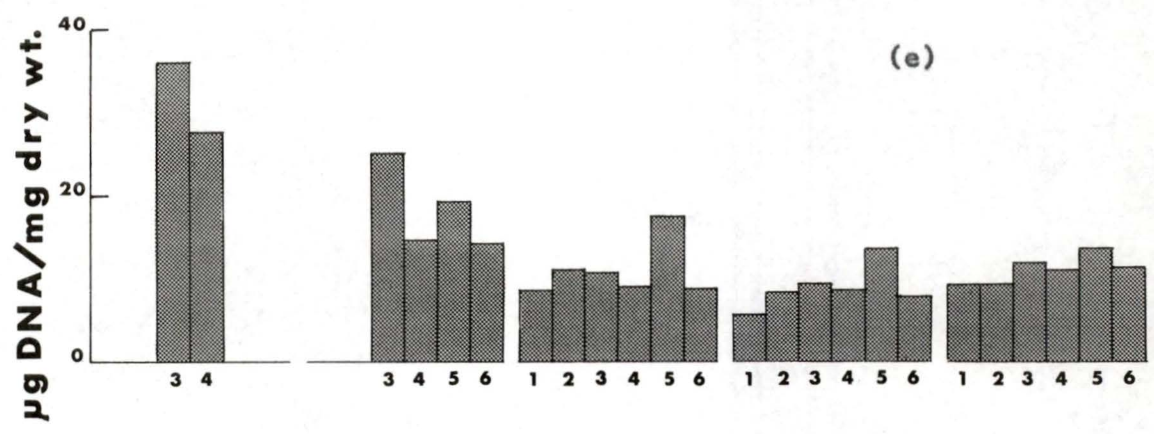
(f) percent protein

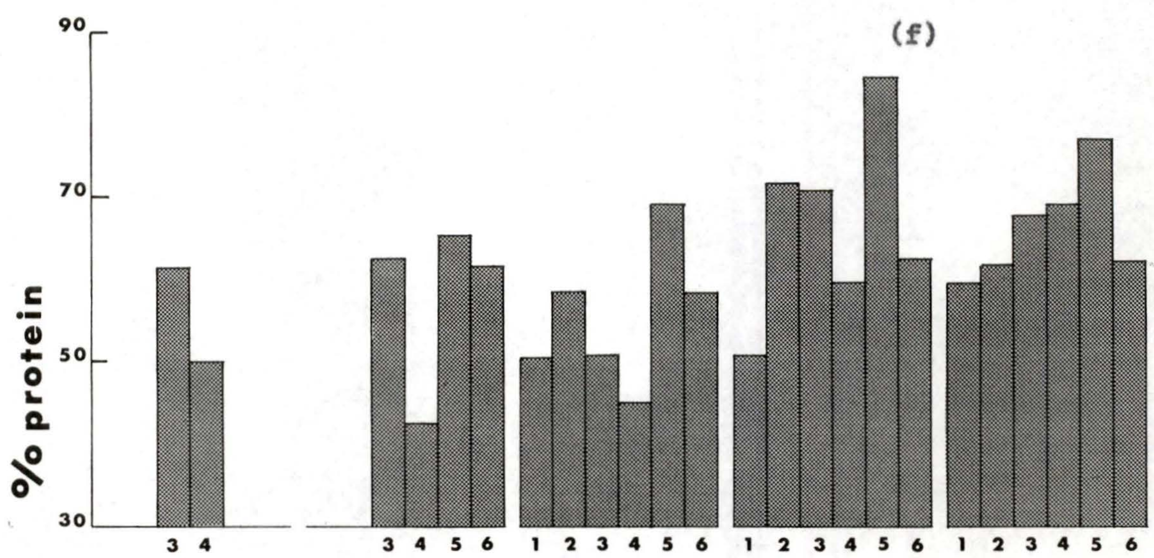

stage 3
4
5
6♀
6♂
1 Saanich Inlet
2 Jervis Inlet (a)
3 Jervis Inlet (b)
4 Bute Inlet St. 6
5 Georgia Strait
6 Haro Strait
RNA/protein (µg/mg)
60
40
20
0
3 4
3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
area


RNA/dry wt. (µg/mg)
(b)
40
20
0
3 4
3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6


(c)
RNA/DNA
2.0
1.0
0
3 4
3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6


3
4
5
6♀
6♂
(d)
µg DNA/mg protein
60
40
20
0
3 4
3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6


(e)
µg DNA/mg dry wt.
40
20
0
3 4
3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6


(f)
% protein
90
70
50
30
3 4
3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6
1 2 3 4 5 6

The differences are not of uniform magnitude. The Georgia Strait sample had the highest protein concentrations. No differences corresponding to Straits and Inlets were found.

(v) Biomass

Station 6 was at the mouth of Bute Inlet and station 8 was in the central area of the Inlet (Figure 1). For both stations, numbers of each stage per m^3 were calculated. Wet-weight per individual was determinted from a prosome length-wet weight curve for copepods (Fulton, 1968). The product of wet weight per individual and number per m^3 is the biomass of each stage per m^3 (Table XIV). Wet weight per individual was converted to dry weight per individual by use of a wet weight-dry weight curve for copepods (Fulton, 1968). The dry weight per individual values were similar to the lowest values encountered in Saanich Inlet and Haro Strait *E. japonica*.

The biomass of *E. japonica* at the mouth of the inlet was approximately twice that of station 8 (Table XIV). The average (day and night) biomass for the 40 m plus the 250 m samples was 27.05 mg for station 6 and 9.38 mg for station 8. The day values for the 40 m plus 175 m plus 250 m samples were 39.18 mg (station 6) and 22.20 mg (station 8). The total zooplankton biomass for the two stations was similar (Golberg, personal communication).

TABLE XIV. Biomass (mg wet weight/m^3) of *Euchaeta japonica* for station 6 and station 8 (Bute Inlet)

Depth	Day or Night	Stn.6	Stn.8	Stn.6	Stn.8	Stn.6	Stn.8	Stn.6	Stn.8	Stn.6	Stn.8	TOTAL Stn.6	TOTAL Stn.8
40 m.	D	.01	.08	.04	0	0	.05	0	0	0	0	.05	.13
40 m.	N	.84	.44	2.97	.62	2.24	3.14	.45	.47	0	0	6.50	4.67
175 m.	D	1.90	.21	4.46	.60	1.49	12.33	..90	1.80	0	0	8.75	14.94
175 m.	N	-	1.10	-	3.13	-	1.17	-	1.88	-	.42	-	7.70
250 m.	D	9.50	1.77	7.16	2.34	6.27	2.55	6.61	.47	.84	0	30.38	7.13
250 m.	N	10.99	0.63	3.27	2.67	3.36	2.62	2.15	.90	.39	0	17.16	6.82
400 m.	D	11.80	-	10.10	-	3.74	-	4.05	-	1.61		31.30	
400 m.	N	15.39	-	5.05	-	2.99	-	3.15	-	0		26.58	

CHAPTER IV

Discussion

A. Growth and RNA Concentration

In these experiments, two basic methods have been used to measure growth. The first is the increase in mass as measured by dry weight and protein increments. The second is the increase in cell numbers as measured by DNA per individual. The RNA content can be expressed in reference to any of these growth measurements. Each method has disadvantages.

Protein synthesis is dependent on RNA. For bacteria, the rate of protein synthesis is directly related to the RNA concentration (Neidhardt and Fraenkel, 1961). Dry weight growth is related to the RNA concentration as long as the protein to dry weight ratio does not change. Protein concentration is not uniform at all times in *A. salina* (Figure 4). It is highest during exponential growth, indicating that almost all growth is due to new protein synthesis. No lipid storage appears to take place. The amount of protein per cell, as indicated by the DNA/protein concentration, also increases during the period of exponential growth. Before and after exponential growth, the protein concentration is lower. These changes in the protein/dry weight values cause changes in the RNA/dry weight concentration which do not reflect growth rate changes. In *E. japonica* large changes in protein concentration occurred within short periods of time (Figure 11). This is, no doubt, partly due to individual variation. For example, adult female *Paraeuchaeta norvegica* from a single sample in the Gulf of Maine ranged in protein content from approximately 37-60 percent (Conover and Corner, 1968). However, it seems unlikely

that individual variation is the only factor involved because protein concentration changes often coincided in all stage (Figure 11). Periodic changes in lipid content are common to most zooplankton species (Corner and Cowey, 1968). Lipid values as high as 67 percent have been observed in *E. japonica* (Rauchert, personal communication). Under certain conditions (e.g. low food availability, egg production) lipid reserves are at least partially used. At normal respiration rates the large lipid store of CV *Calanus finmarchicus* would last 2 to 3 weeks (Marshal and Orr, 1960). If *E. japonica* were similar, a 3 to 4 day period without food would cause a reduction of lipid reserves and protein would increase relative to dry weight. The growth rate might or might not change but the RNA/dry weight concentration would change. Conversely, a period of lipid increase would cause a decrease in RNA/dry weight concentration, although the protein growth rate might remain unchanged. Some copepods use body protein as an energy source during part of the year (Martin, 1968). This also could cause the RNA/dry weight concentration to change and probably also affect the RNA/protein concentration.

Three *E. japonica* samples were analyzed for protein by the Kjeldahl method as well as the Lowry method. The Kjeldahl values were lower (58, 48 and 60 percent) than the Lowry values (68, 58 and 63 percent). The total nitrogen (Kjeldahl) cannot be greater than the protein nitrogen. For the Lowry method, tyrosine and tryptophan residues in the protein are more chromogenic than other amino acids (Geise, 1967). Therefore, the discrepancy between the two techniques would be at least partially accounted for if the concentration of tyrosine and tryptophan was higher in *E. japonica* than in the standard casein solution.

The second basic method used for growth measurement was the increase in cell numbers. The amount of DNA/cell and the total DNA/individual provide an estimate of the cell number (Church and Robertson, 1966). The total DNA/individual will give a relative value for cell numbers if the DNA/cell does not change. For practical purposes, DNA/cell will not change unless DNA/nucleus changes, because extranuclear DNA constitutes only about 3 percent of total cellular DNA (Cook, 1969). However, this possibility cannot be discounted because DNA/nucleus is not an unchanging entity. For example, polyteny occurs in two forms of *Pseudocalanus*, possibly the same species (Woods, 1969). The two forms have the same chromosome number (n = 16) and differ only in size. The large form has conspicuously larger chromosomes (containing seven times more DNA) than the small form. In hemimetabalous Diptera, growth by cell size and polyteny occurs in the larval stages (Church and Robertson, 1966). In ten different species of unicellular algae, the amount of DNA is linearly related to cell size (Holm-Hansen, 1968). On the other hand changes in DNA/protein and DNA/dry weight do not necessarily indicate polyteny but can also be indicative of changing amounts of protein/nucleus (i.e. change in cell size).

The DNA concentration of *A. salina* increased during the early stage of each culture, until day 5 or 6, regardless of dry weight growth. It also increased prior to exponential growth. These increases probably indicate that cell division was proceeding faster than protein synthesis and dry weight growth (i.e. average cell size was decreasing). During and after exponential growth, the DNA concentration decreased, probably indicating protein synthesis and dry weight growth were proceeding faster than cell division. In *E. japonica*, the DNA concentration is different for each stage. This could be due to the different growth rates of the

various stages. Alternately, it could be intrinsic to the stage, regardless of the growth rate. The range of observed values is slightly greater than in *A. salina* (6.3 - 32.3 µg/mg dry weight for *A. salina* vs. 4.3 - 40.5 µg/mg dry weight for *E. japonica*). The high concentration in the young stages probably indicates that growth proceeds by increase in cell size as well as cell number. If growth was by cell size only, the amount of DNA/individual would be the same for all stages. This is not the case (Table XI b). Adult males have higher DNA concentrations than adult females because of spermatogenesis. In both *A. salina* and *E. japonica*, the DNA concentration declines as the growth rate slows. In *A. salina* the concentration rises before the period of most rapid growth. If *E. japonica* behaves similarly, then the most rapid growth must be before or during stage 3 because the highest DNA concentrations were found in this stage.

Prior to the exponential phase in *A. salina* culture 'A', the rate of increase in dry weight per individual was irregular. Apparently, individuals of two of the samples underwent negative growth (i.e. were smaller than individuals in the previous sample). This could be due, in part, to sampling error. During slow growth, if samples were not representative of the population, a fluctuating growth line would likely result. At high growth rates, deviations due to the same sampling error would not be as obvious. Alternatively, the fluctuations could be indicative of change in the population. Although RNA concentration cannot be used to predict growth rate, Figure 4 shows that fluctuations in the growth curves usually coincide with fluctuations in the RNA concentration. The RNA/dry weight concentrations of the two samples that appeared to undergo negative growth were lower than those found in previous samples. This might indi-

cate that fluctuations in the growth curves are representative of the population or it might mean that some members of the population grow faster than others and the samples are not representative of the population. Euphausiids sometimes show negative growth. The apparent negative growth in *A. salina* cannot be solely assigned to sampling errors.

Mason (1963) stated than an endogenous nutrition prevails for *A. salina* during the first four days after hatching (approximately six days on my age scale), and food level has no effect on growth rate. This conclusion is not borne out by my data because visible size differences did occur between the early nauplii of my three cultures. This is to be expected because the nauplii begin to feed voraciously approximately 36 hours after hatching (Reeve, 1963). Before the yolk is exhausted high concentrations of RNA and DNA build up (Figure 4). At the initiation of feeding, if conditions are poor (e.g. culture 'A'), there is a rapid decline in nucleic acid concentration. If, at the initiation of feeding, conditions are optimum, the nucleic acid concentration remains high or even increases (e.g. Sutcliffe, 1965). If feeding conditions at this time are intermediate (e.g. culture 'C' and 'D') nucleic acid concentration slowly declines. These patterns are illustrated by RNA and DNA expressed relative to dry weight and protein. The RNA/DNA value does not follow the pattern of the two other curves. Because RNA is representative of protein synthesis capacity and DNA of cell number, the RNA/DNA gives a relative value for the protein synthesis capacity/cell (Lang et. al., 1965). Changes in *E. japonica* and *A. salina* cell size result in changing the number of cells per unit of dry weight. Therefore,

changes in DNA concentration are often independent of changes in protein and dry weight. This explains why the RNA/DNA ratio does not always follow the same pattern as the other two RNA concentration measurements do.

The *Orchestia platensis* standard curve (Sutcliffe, 1965) does not accurately predict *A. salina* growth rates. The best correlation between observed and predicted growth rates is during exponential growth. All other observed growth rates were lower than those predicted from the standard curve. Pease (1968) suggested that the RNA growth relationship only predicts growth of zooplankton species in the exponential phase were used in Sutcliffe's (1965) *A. salina* experiment. Although the RNA growth rate (dry weight increase per day) regression lines are significant, the point scatter is large (Figure 5a, 5d). It is unlikely that accuracy in predicting future growth or determining past growth can be obtain from the equations of these two curves. The relationships between RNA concentration and the rate of protein increase and RNA concentration and the rate of DNA increase are not significant. The poor relationship between RNA/protein concentration and growth measured by protein increases is unexplainable. In theory this relationship should be the most significant. Perhaps this illustrates that the valid relationship of the RNA/dry weight vs. growth rate equations is coincidental even though they are statistically valid.

The value of both *A. salina* derived growth equations (by dry weight increases per day) in predicting the growth of *E. japonica* copepodites is shown in Table XII. Neither equation accurately predicts the growth rate for all stages. The possibility that the observed growth values for

E. japonica copepodites were erroneous was considered. Growth values for *Acartia clausi* and *Calanus hyperboreus* in the Black Sea (Petipa, 1967) were similar but slightly higher to those of *E. japonica*. Stage 3 values were 24.3% and 21.7%; stage 4 values were 13.7% and 21.5%; stage 5 values were 8.3% and 5.9%; and stage 6 female values were 1.2% and 3.2% (due to egg production). Petipa discusses five calanoid life forms. The fourth life form includes copepodite stages 1 to 4 and is characterized by intensive feeding and rapid growth. The fifth life form includes stages 5 and 6 and is characterized by an abrupt slowing of growth. This same pattern is seen in *E. japonica* observed growth rates. It is probable the observed values for *E. japonica* are not seriously in error and are not the cause of the discrepancies between the observed growth rates and the two sets of predicted rates.

There is little point scatter about the RNA/dry weight vs. observed growth rate (dry weight increase per day) line for *E. japonica* (Figure 8). Unfortunately only four points are available. The slope of the line is assumed to be representative of the RNA-growth relationship for *E. japonica*. It is different from the slope of the RNA/dry weight vs. percent dry weight increase per day line of *A. salina* (Figure 5a). Therefore, even if the RNA-growth relationships of these two crustaceans are useful in predicting their respective growth rates, the relationships are specific for each of them. Slopes of RNA-growth lines in some bacteria are species specific (Rosset et. al., 1966). If this is true for crustaceans, any value the RNA-growth relationship might have (even if it was valid for individual species) would be largely lost.

The adult males of *E. japonica* do not feed (Campbell, 1934), and

presumably do not grow. Their RNA concentration probably is representative of the amount required for body maintenance. The adult female RNA concentration is slightly higher than the male because of egg production and possibly, digestive enzyme synthesis. Presumably any RNA above the concentration required for maintenance is potentially available for growth. By subtracting the RNA concentration of adult males from the RNA concentrations observed in other stages, the amount of RNA available for protein synthesis for growth can be determined. If it is assumed all the RNA in an organism is being used to its fullest extent, the relative amount of newly synthesized material that goes into growth can be determined. For stage 3 the value is 64.1%, for stage 4 it is 53.4% and for stage 5 it is 23.0%. Adult females use 2.8% of their newly synthesized material for egg production. This verifies that the life form division (Petipa, 1967) is the same in *E. japonica* as it is in *A. clausi* and *C. hyperboreus*.

The low *E. japonica* stage 4 RNA/dry weight and RNA/protein concentrations in December and January possibly indicate a slow growth rate. Winter is a period of low food availability in temperate waters (Raymont, 1963). At all other times during the study, the RNA/dry weight and RNA/protein concentrations of each stage were usually distinct from the RNA concentrations of the other stages. This probably means food availability is good and allows for maximum or near maximum growth. Fluctuations in RNA concentration usually coincide in all *E. japonica* stages. The range of RNA changes is greater in the younger stages than in the older stages. Because young stages are growing rapidly, they are susceptible to growth restricting factors. Similar factors have a lesser effect on the growth

rates of stages 5 and 6. Food availability cannot be the only environmental factor causing RNA concentration changes because the RNA concentration of adult males, which do not feed (Campbell, 1934), also changes.

Low food level may affect crustaceans in two ways. It may delay development or it may reduce the final size attained (Mason, 1963). Biochemically, these are not inseparable processes because protein synthesis is curtailed in both. In delaying development, all processes concerned with growth are slowed. In reducing the final size, molting proceeds at regular intervals regardless of food concentration, but the final size of the animal is smaller than it would have been if food availability had been high. The *Euphausiid* intermolt period depends on temperature and is not affected by the amount of food eaten (Lasker, 1966). Molting containues at the expense of body tissues if food is not available. *E. japonica* stages in Haro Strait are larger than the comparable stages in Saanich Inlet (Table II). Therefore assuming there is more food in Haro Strait, food level probably effects the final size attained in this species. However, molting is probably not completely independent of food supply in calanoids (Conover, 1965), as it is in Euphausiids. *E. japonica* seems to present an intermediate position between the two methods suggested by Mason (1963).

Further work is necessary to determine the reason for stage 6 predominating in Saanich Inlet while stage 5 predominates in Haro Strait.

B. Geographical Distribution of RNA, DNA, protein and dry weight in *Euchaeta japonica*

Some regional trends were evident for RNA and DNA concentrations but none were consistent for all stages. This made interpretation of

the cause of the changes difficult. Geographical variation in protein concentration was present. This might be a reflection of the food availability of the different areas. Protein concentration changes rapidly in *E. japonica* of Haro Strait and Saanich Inlet (Figures 11 and 13). Probably the *E. japonica* of these other areas are similar and the regional differences are not permanent. In all areas the young stages appear to be slightly lower in protein concentration than the adults. The younger *E. japonica* are the fastest growing. The fastest growing *A. salina* have the highest protein concentration. This illustrates a major difference in the growth processes of these two organisms. In *A. salina* the major growth process is protein synthesis and little or no lipid is stored during rapid growth. In *E. japonica,* the protein concentration increases as growth rate slows, indicating lipid storage is important in the growth process of this species.

Table XIV shows there were differences in *E. japonica* biomass between the Bute Inlet stations. It is reasonable to expect different numbers of *E. japonica* in different areas. Seasonal abundance of food may be responsible for the number of eggs produced and not for their viability (Lewis and Romnarine, 1969). Possibly, the abundance of food is primarily responsible for the numbers of *E. japonica* that are produced and only slightly affects their growth rate, except under extreme conditions such as in wintertime. This might explain the usually small regional variations in RNA concentration, within each stage. However, these differences cannot be entirely discounted, especially in the younger stages (3 and 4) and probably indicate that growth rates vary regionally. Perhaps a potential growth rate is a characteristic of the stage and the environment

either provides suitably for this growth rate or restricts it.

E. japonica contributed a significant amount to the total biomass of the Bute Inlet stations. The dry weights of the individuals of the samples were as low as any encountered in Haro Strait or Saanich Inlet and it is felt these are minimum biomass values. Copepodites of *E. japonica* are mainly carnivorous, although they have been found to feed on large members of the phytoplankton (Lewis and Ramnarine, 1969). The Bute Inlet biomass of *E. japonica* would exert considerably predatory pressure on the zooplankton.

C. The Feasability of Secondary Productivity Determination from RNA Concentration

For all growth rates, the bacterial cell ribosomes work at a constant, near maximum rate (Neidhardt, 1964). This conclusion was based on three major factors. First, there is a correlation between rRNA content and protein synthesis rate during balanced growth. Second, rRNA is inhibited when the rate of protein synthesis decreased. Third, there is a lack of ribosome efficiency increase during accelerated growth rate. Not all the literature substantiates these observations. Ribosome efficiency does change with growth rate for some bacteria (Rosset et. al., 1966). However, the rate of efficiency change in uniformly related to the growth rate change and therefore only the slope of the RNA concentration-growth rate line changes. It has also been shown that the slope of this line varies for different bacterial species (Rosset et. al., 1966). During development of the amphibian *Xenopus laevis*, rRNA synthesis begins at gastrulation but new ribosomes are not used until the early swimming stage has been reached (Brown and Littna, 1964).

The function of the rRNA in the ribosome is not known, although it has been assigned a structural role (Ingram, 1965). Only mRNA breaks down rapidly. Ninety-five percent of the total RNA is stable (Spiegleman, 1965). A decrease in growth rate results in a decline in the rate of synthesis of new RNA. The difference between the amount of RNA present in a cell before the decrease in growth rate, and the amount needed for growth after the decrease, is excess RNA. Most of this is stable. Even mRNA does not break down under amino-acid starvation conditions but is held by the unfinished peptide chains (Fan et. al., 1964). Most of the excess rRNA is probably absorbed by the daughter cells of each new generation. This might only take a few minutes in bacteria because growth is rapid (i.e. it is often measured in generations/hour). The time required for an individual *A. salina* to reduce its RNA concent after a decrease in growth rate might be as long as a week. However the times required for the bacteria culture and the individual *A. salina* to reduce their RNA contents might be comparable if expressed as a percentage of their respective generation times.

A culture of *A. salina* can easily filter 94 to 99% of its food from solution in 24 hours (Mason, 1963). When this happens, a rapid decrease in growth rate must occur due to starvation. This rapid decrease in growth rate cannot be immediately reflected by the RNA concentration because 95% of it is stable. *E. japonica* growth rates probably are not subject to such rapid changes because of their lipid stores.

A rapidly increasing growth rate will possibly be reflected in the RNA concentration but RNA concentration can also decrease during rapid

growth in *A. salina*. The RNA-growth relationship is probably most valid under steady growth conditions.

Crustaceans are not like bacteria. A rapidly growing bacterial cell may be 50 percent ribosomes by dry weight (Neidhardt and Fraenkel, 1961). The highest value I found for total RNA content was 3.4% of the dry weight. There are numerous crustacean characteristics, not related to growth, which change the protein synthesis rate or protein content of the organism and thus its dry weight. For some copepods, proteins for metabolism are required in different amounts at different times during the year (Conover, 1962). Other factors, previously discussed, cause changes in apparent RNA concentration independently of growth. The growth rates associated with a given RNA concentration range tremendously. The measurement of RNA and DNA is always of interest in growth studies but RNA concentration appears to be of little value as a method of determining secondary productivity.

CHAPTER V

SUMMARY

1. The concentration of RNA, DNA and protein and the dry weight of three *Artemia salina* cultures was measured to investigate the usefulness of the RNA-growth relationship in estimating growth or productivity in other species. Similar analyses were done on copepodite stages 3 to 6 of *Euchaeta japonica*, collected periodically over 7 months in Haro Strait and over 4 months in Saanich Inlet, B. C.

2. The Schmidt-Thannhauser (1945) technique as modified by Munro and Fleck (1966) was adopted for the extraction and measurement of RNA. DNA was determined by the indole method of Ceriotti (1952) and protein was determined by the method of Lowry et. al. (1951). The technique of Wannemacher et. al. (1965) for the separation and determination of RNA and DNA was unsatisfactory.

3. In *A. salina*, RNA, DNA, and protein relative to dry weight, all changed with growth rate. RNA relative to dry weight and protein, increased rapidly immediately prior to or at the beginning of exponential growth but declined as exponential growth continued. The RNA/DNA ratio often did not coincide with changes in the other two RNA measurements. Protein concentration was highest during exponential growth. The maximum DNA concentration occurred immediately prior to exponential growth. During and after exponential growth, DNA concentration decreased.

4. Regression analyses of RNA/dry weight concentration on percent dry weight increase per day values showed the relationship between the two was significant for *A. salina*. However, the point scatter about the line was large. Regression analyses of RNA/protein and RNA/DNA values

on percent protein and percent DNA increase per day respectively, were not significant.

5. The efficiency of *A. salina* ribosomes was not uniform but varied over wide ranges.

6. Intermolt periods and the mean dry weight per individual were used to determine growth rates of *E. japonica* copepodites 3 to 6.

7. The slope of the line of RNA/dry weight concentration vs. percent dry weight increase per day for *E. japonica* copepodites was different from corresponding line in *A. salina*.

8. Variation in the RNA concentration of *E. japonica* copepodites 3 and 4 with time or with region was sometimes large. Similar variation was small in stage 5 and adults.

9. In *E. japonica* the DNA concentration appeared to be mainly a characteristic of the stage. It was highest in the youngest stages and lowest in the oldest stages with the exception of adult males which had high DNA contents because of spermatogenesis.

10. RNA and DNA concentration were more uniform with time in *E. japonica* of Saanich Inlet than of Haro Strait.

11. The relationship between RNA concentration and growth rate derived from *Orchestia platensis* by Sutcliffe (1965) did not accurately predict growth rates for *A. salina* or *E. japonica*. Also, the relationship between RNA concentration and growth rate derived from my *A. salina* cultures did not accurately predict *E. japonica* growth rates.

12. RNA concentration-growth rate relationships are of little value in predicting or determining secondary production because wide ranges of growth rates were found associated with a single RNA concentration.

REFERENCES CITED

Brown, D. D., and E. Littna. 1964. RNA synthesis during the development of *Xenopus laevis*, the South African clawed toad. J. Mol. Biol., 8: 669-687.

Campbell, M. H. 1934. The life history and post embryonic development of the copepods, *Calanus tonsus* Brady and *Euchaeta japonia* Marukawa. J. Biol. Can., 1: 1-65.

Ceriotti, G. 1952. A microchemical determination of Desoxyribonucleic acid. J. Biol. Chem., 198: 297-303.

Church, R. B. and F. W. Robertson. 1966. A biochemical study of the growth of *Drosophila melanogaster*. J. Exp. Zool., 162: 337-352.

Conover, R. J. 1965. Notes on the molting cycle, development of sexual characters and sex ratio in *Calanus hyperboreus*. Crustaceana, 8: 308-320.

-----1968. Zooplankton - Life in a nutritionally dilute environment. Am. Zoologist, 8: 107-118.

-----1962. Metabolism and growth in *Calanus hyperboreus* in relation to its life cycle. Rapp. Proc.-Verb. Cons. Intern. Explor. Mer, 153: 190-197.

----- and E.D.S. Corner. 1968. Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. J. Mar. Biol. Ass. U.K., 48: 49-75.

Cook, J. R. 1969. Photo-inhibition of cell division and growth in Euglenoid flagellates. J. Cell. Physiol., 71: 177-184.

Corner, E.D.S., and C. B. Cowey. 1968. Biochemical studies on the production of marine zooplankton. Biol. Rev., 43: 393-426.

Cushing, D. H. 1959. On the nature of production in the sea. Min. Agr. Fish. Food, U.K., Fish Invest. Ser. II., 22: 1-40.

Davis, C. C. 1949. The pelagic copepoda of the northeastern Pacific Ocean. Univ. Wash. Publ. Biol, 14: 1-118.

Dische, Z., A. Devi, and G. Zelmenis. 1961. Influence of age and cateract formation on the ribonucleic acid of the lens. Am. J. Opthalmol., 51: 993-1003.

Fan, D. P., A. Higa, and C. Levinthal. 1964. Messenger RNA decay and protection. J. Mol. Biol., 8: 210-222.

Fulton, J. MS, 1968. A laboratory manual for the identification of British Columbia marine zooplankton. Fish. Res. Bd. Can., Tech. Rept. 55. 141 p.

Gaarder, T., and H. H. Gran. 1927. Investigations of the production of plankton in the Oslo Fjord. Rapp. Proc.-Verb. Cons. Int. Explor. Mer. 42: 1-48.

Giese, A. D. 1967. Some methods for study of the biochemical constitution of marine invertebrates. p. 159-186. *In* H. Barnes (ed.), Oceanog. Mar. Biol. Amm. Rev. V.5, George Allen and Unwin Ltd., London.

Holm-Hansen, O. 1969. Algae: Amounts of DNA and organic carbon in single cells. Science, 163: 87-88.

Hutchison, W. D., and H. N. Munro. 1961. The determination of nucleic acids in biological materials. A review. Analyst, 86: 768-813.

Ingram, V. M. 1965. The synthesis of macromolecules, p. 125-172. *In* J. A. Moore (ed.), Ideas in modern biology. The Natural History Press, Garden City.

Kjeldgaard, N. O. 1961. The kinetics of ribonucleic acid and protein formation in *Salmonella typhimurium* during the transition between different states of balanced growth. Biochem. Biophys. Acta, 49: 64-76.

Lang, C. A., H. Y. Lau, and D. J. Jefferson. 1965. Protein and nucleic acid changes during growth and aging in the mosquito. Biochem. J., 95: 372-377.

Lasker, R. 1966. Feeding, growth, respiration, and carbon utilization of a Euphausiid Crustacean. J. Fish. Res. Bd. Can., 23: 1291-1317.

----- and G. H. Theilacker. 1965. Maintenance of Euphausiid shrimps in the laboratory. Limnol. Oceanog., 10: 287-288.

Lewis, A. G., and A. Ramnarine. 1969. Some chemical factors affecting the early developmental stages of *Euchaeta japonica* (Crustacean: Copepoda: Calanoida) in the laboratory. J. Fish. Res. Bd., Can., 26: 1347-1362.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Martin, J. H. 1968. Phytoplankton-zooplankton relationships in Narragansett Bay. III. Seasonal changes in zooplankton excretion rates in relation to phytoplankton abundance. Limnol. Oceanog., 13: 63-71.

Marshall, S. M., and A. P. Orr. 1960. Feeding and nutrition. p. 227-258. *In* T. H. Waterman (ed.), The physiology of crustacea. V.1, Academic Press, London.

Mason, D. T. 1963. The growth response of *Artemia salina* (L.) to various feeding regimes. Crustaceana, 5: 138-150.

McAllister, C. D. 1969. Aspects of estimating zooplankton production from phytoplankton production. J. Fish. Res. Bd. Can., 26: 199-220.

Mullin, M. M., and E. R. Brooks. 1967. Laboratory culture, growth rate, and feeding behaviour of a planktonic marine copepod. Limnol. Oceanog., 12: 657-666.

Munro, H. N., and A. Fleck. 1966. Recent developments in the measurement of nucleic acids in biological materials. A supplementary review. Analyst, 91: 78-88.

Neidhardt, F. C. 1964. The regulation of RNA synthesis in bacteria, p. 145-181. *In* J. N. Davidson and W. F. Cohn (eds.), Progress in Nucleic acid research. V. 3, Academic Press, New York.

----- and D. G. Fraenkel. 1961. Metabolic regulation of RNA synthesis in bacteria. Cold Spr. Harb. Symp. Quant. Biol., 26: 63-74.

----- and B. Magasanik. 1960. Studies on the role of ribonucleic acid in the growth of bacteria. Biochem. Biophys. Acta, 42: 99-116.

Parsons, T. R., and R. J. Le Brasseur. 1968. A discussion of some critical indices of primary and secondary production for large-scale ocean surveys. Calif. Mar. Res. Comm., CalCOFI Rept., 12: 54-63.

Pease, A. K. 1968. The use of estimates of ribonucleic acid to predict growth rates of zooplanktonic organisms. M.Sc. Thesis. Univ. of British Columbia. 107 p.

Raymont, J. E. G. 1963. Plankton and productivity in the oceans. The MacMillan Company, New York. 660 p.

Reeve, M. R. 1963. Growth efficiency of *Artemia* under laboratory conditions. Biol. Bull. Mar. Biol. Lab., Woods Hole, 125: 133-145.

Riley, G. A. 1963. Theory of food-chain relations in the ocean. p. 438-463. *In* M. N. Hill (ed.), The sea. V. 2, John Riley and Sons, New York.

Rosset, R., J. Julien, and R. Monier. 1966. Ribonucleic acid composition of bacteria as a function of growth rate. J. Mol. Biol., 18: 308-320.

Schmidt, G., and S. J. Thannhauser. 1945. A method for the determination of deoxyribonucleic acid, ribonucleic acid, and phosphoproteins in animal tissues. J. Biol. Chem., 161: 83-89.

Schneider, W. C. 1957. Determination of nucleic acids in tissues by pentase analysis. p. 680-684. *In* S. P. Colwick and N. O. Kaplan (eds.), Methods in enzymology. V. 3, Academic Press, New York.

Spiegel, M. R. 1961. Theory and problems of statistics. Schaum Publishing Co., New York. 359 p.

Spiegelman, S. 1965. Gene action, p. 17-78. *In* J. A. Moore (ed.), Ideas in modern biology. The Natural History Press, Garden City.

Steemann Nielsen, E. 1952. Use of radio-active carbon (C^{14}) for measuring organic production in the sea. J. Cons. Int. Explor. Mer, 18: 117-140.

-----1963. Productivity, definition and measurement. p. 129-164. *In* M. N. Hill (ed.), The sea. V. 2, John Riley and Sons, New York.

Sutcliffe, W. H. Jr. 1965. Growth estimates from ribonucleic acid content in some small organisms. Limnol. Oceanog. 10: (Suppl.) R253-R258.

Sverdrup, H. V. 1953. On conditions for the vernal blooming of phytoplankton. J. Cons. Int. Explor. Mer., 18: 287-295.

Vickers, D. H., and N. Mitlin. 1965. Changes in nucleic acid content of the boll weevil *Anthonomus grandis* Bodeman during its development. Physiol. Zool, 39: 70-76.

Wannemacher, R. W. Jr., W. L. Banks, Jr., and W. H. Wunner. 1965. Use of a single tissue extract to determine cellular protein and nucleic acid concentrations and rate of amino acid incorporation. Analyt. Biochem., 11: 320-326.

Winberg, G. G. 1960. Rate of metabolism and food requirements of fishes. Nauchnge Trudy Belorussk-ovo Gosudartvennovo Universiteta imeni V.I. Lenina, Minsk, 342 p. Fish. Res. Bd. Can. Translation Ser. No. 194.

Woods, S. M. 1969. Polyteny and size variation in the Copepod *Pseudo-calanus* from two semi-landlocked fiords on Baffin Inland. F. Fish. Res. Bd. Can., 26: 543-556.

Appendix I. Length range in some *A. salina* samples

Culture	Sample number	Sample size	$\bar{x}$ length mm.	σ mm.
A	15	31	3.48	0.81
	21	30	4.37	0.68
	27	29	5.63	1.00
B	2	30	0.76	0.10
	7	30	1.50	0.10
C	2	30	.94	0.00
	5	30	1.40	0.28

Appendix II. RNA, DNA and protein concentrations of *A. salina* samples

Culture	Sample	RNA / DNA	µgRNA / mg dry wt	µgRNA / mg prot	µgDNA / mg drywt	µgDNA / mg prot	protein / drywt.
A	1	2.70	22.7		8.4		
	2	1.24	18.5		14.9		
	3	1.12	10.9		9.7		
	4	1.31	14.9		11.4		
	5	1.86	19.9		10.7		
	6	1.74	14.8		8.5		
	7	1.56	12.2		7.9		
	8	1.40	8.6		6.1		
	9	1.65	10.4		6.3		
	10	1.68	16.2		9.7		
	11	1.80	24.2		13.5		
	12	1.75	16.7		9.6		
	13	2.74	55.7	83.0	20.4	30.4	70.7
	14	3.21	57.3	65.2	17.8	20.3	87.9
	15	3.11	50.9	60.9	16.4	19.6	83.6
	16	3.29	48.0	58.6	14.1	17.2	81.9
	17	3.02	47.4	60.9	15.7	20.1	78.1
	18	3.53	44.1	55.1	12.5	15.6	80.0
	19	2.91	44.5	47.4	15.3	17.9	85.2
	20	3.01	33.6	56.9	11.2	20.0	55.8
	21	3.26	38.4	51.6	11.8	16.6	71.1
	22	3.33	37.4	51.6	11.2	18.1	62.1
	23	3.10	32.9	47.0	10.6	17.1	61.9
	24	3.07	35.2	46.1	11.5	16.5	69.7
	25	2.79	39.5	48.0	14.1	17.2	82.4
	26	3.24	35.2	45.2	10.9	14.0	77.7
B	1	4.05		88.2		22.4	
	2	3.25		93.2		28.6	
	3	2.91		99.1		34.0	
	4	2.80		86.9		31.1	
	5	2.70		80.7		29.9	
	6	2.57		79.8		31.1	
	7	2.41	50.3	76.3	20.8	31.6	65.9
	8	2.00	44.2	71.3	22.1	35.7	62.0
	9	2.05	45.2	68.7	22.0	33.4	65.9
	10	1.92	42.6	65.5	22.1	34.1	65.0
	11	3.40	56.9	76.0	18.7	25.0	74.9
	12	3.52	55.9	67.9	15.9	19.3	82.4
	13	3.40	51.3	62.5	15.2	18.5	82.1
	14	3.90	44.1	60.6	11.3	15.5	72.8
	15	3.43	39.2	54.9	11.4	16.0	71.4

Appendix II, cont'd.

Culture	Sample	RNA / DNA	µgRNA / mg drywt	µgRNA / mg prot	µgDNA / mg drywt	µgDNA / mg prot	protein / drywt.
C	1	3.95	44.0	64.8	11.2	16.5	67.8
	2	3.43	67.7	93.2	19.7	27.0	72.7
	3	4.21	62.3	92.5	14.8	22.0	67.3
	4	2.83	52.2	75.0	18.4	26.4	69.6
	5	2.51	48.4	70.9	19.3	28.3	68.4
	6	2.03	48.7	66.0	23.8	32.3	73.8
	7	2.09	42.4	57.6	20.4	27.8	73.6

Appendix III. Observed growth rates (percent/day) of *A. salina* samples

Culture	Age (Days)	Sample number	dry wt. increase	protein increase	DNA increase
A	4	1			
	6	2	4.1		sporadic fluctuations with periods of negative growth
	9	3	-		
	11	4	1.5		
	13	5	8.6		
	15	6	0		
	17	7	-		
	21	8	1.7		
	24	9	1.0		
	26	10	5.7		
	28	11	8.8		
	31	12	0		
	38	13	7.3		
	41	14	33.2	43.0	27.3
	44	15	43.3	41.0	39.4
	46	16	7.7	6.6	0
	47	17	17.3	11.8	30.2
	48	18	2.9	5.5	0
	49	19	0	0	0
	50	20	3.5	0	0
	52	21	9.9	0	0
	53	22	2.2	0	0
	55	23	0	0	0
	56	24	13.5	27.8	23.2
	57	25	8.9	28.7	33.5
	66	26	5.6	5.0	2.7
B	2	1			
	4	2	4.3		
	5	3	8.0		
	6	4	14.8		
	8	5	4.7		
	10	6	5.7		
	11	7	7.9		
	14	8	0		
	15	9	0	0	0
	16	10	0	0	0
	19	11	37.6	40.6	26.7
	22	12	43.9	31.5	20.8
	24	13	23.0	22.8	20.2
	27	14	31.8	26.6	19.4
	29	15	27.1	26.0	27.9

Appendix III, cont'd.

Culture	Age (Days)	Sample number	dry wt. increase	protein increase	DNA increase
C	2½	1			
	5	2	19.2	22.3	50.4
	7	3	16.4	12.1	0.8
	9	4	1.2	2.8	12.9
	11	5	10.0	9.1	12.5
	13	6	6.5	10.6	18.3
	15	7	7.4	7.2	0

Appendix IV. Numbers of *E. japonica* caught per sample

Location	Date	Stage - numbers of each caught					
		3	4	5	6F	6M	Total
Haro St.	Dec.23/68	12	12	25	6	2	57
	Jan. 8/69	12	12	29	11	1	65
	Feb.16	16	14	36	11	2	79
	26	16	24	27	12	1	70
	Mar.27	0	12	8	2	2	24
	Apr. 3	5	46	58	21	9	139
	10	0	12	17	2	1	32
	17	0	14	27	7	4	52
	May 1	0	1	10	8	3	22
	7	1	10	28	9	1	48
	16	2	6	41	23	6	78
	22	1	9	53	22	12	97
	29	4	17	48	9	8	86
	Jun. 5	2	2	9	2	5	20
	12	4	20	57	21	25	127
	19	3	11	7	5	3	29
Saanich Inlet	Mar. 7	1	2	11	14	0	28
	Apr.14	0	3	6	77	26	112
	28	0	0	3	26	5	34
	May 12	4	1	4	45	3	57
	26	28	10	6	34	12	90
	Jun. 9	28	6	24	95	18	181
	23	9	28	36	15	0	88

Appendix V (a). RNA, DNA and Protein Concentration for Haro Strait *E. japonica* samples.

Location	Date	RNA/DNA					μg RNA/mg Dry Weight					μg RNA/mg protein				
		3	4	5	6F*	6M*	3	4	5	6F	6M	3	4	5	6F	6M
Haro St.	Dec. 23	0.86	0.85	0.94	1.27	1.07	34.9	14.8	9.2	9.0	9.4					
	Jan. 8	0.44	0.74	1.11	1.38	1.77	23.1	13.2	7.8	9.6	16.0					
	Feb. 16		0.44	0.76	1.03	1.27			8.0	9.3	15.2			12.4	13.8	23.3
	26		1.22	1.20	1.23			20.9	12.4	10.1			38.8	21.6	14.7	
	Mar. 27		1.34	1.82	1.84	1.09		18.9	15.8	12.8	12.1		30.6	27.0	20.6	19.4
	Apr. 3		1.46	1.62	2.29	0.78		24.3	13.7	13.7	8.7		29.3	21.2	17.9	10.1
	10		1.72	2.02				29.8	14.8		13.9		33.3	23.9		21.1
	17		1.24	2.14	1.16	1.05		25.2	16.6	11.0	10.9		39.0	28.5	21.4	18.0
	May 1			1.81	2.06	1.52			16.1	10.5	12.6			28.8	16.9	17.7
	7		1.23	1.57	2.11			23.8	17.8	11.6			29.2	25.6	18.1	
	16			0.71	1.31	0.83			8.1	7.3	7.3			13.1	10.7	12.2
	22			1.47	1.66	0.69			13.8	10.8	7.1			21.3	16.6	9.6
	29		1.42	1.80	1.98	0.63		23.9	13.9	11.6	7.5		33.3	26.4	20.4	13.5
	June 5			1.69	2.32	1.02			14.8	13.3	9.4			33.5	25.5	14.4
	12		1.68	1.94	2.75	1.06		24.9	12.8	11.7	9.2		40.0	22.2	21.8	17.6
	19		1.47	2.04	2.09	0.76		25.9	13.3	9.3	6.6		36.4	29.1	15.3	11.2
	26			2.00					19.8					31.9		

* F - female

* M - male

Appendix V (a) - continued

Location	Date	µg DNA/mg dry weight					µg DNA/mg protein					protein/dry weight %				
		3	4	5	6F*	6M*	3	4	5	6F	6M	3	4	5	6F	6M
Haro St.	Dec. 23	40.5	17.4	9.8	7.1	8.8										
	Jan. 8	53.1	17.7	7.2	7.0	9.1			15.1	15.1				44.1**	49.9**	
	Feb. 16			9.9	9.0	11.7			16.2	13.5	18.0			61.4	67.2	65.0
	26		16.8	10.2	8.1			31.2	17.8	11.8			53.8	57.2	68.4	
	Mar. 27		14.1	8.7	6.9	11.1		22.9	14.8	11.1	17.9		61.6	58.3	62.0	62.1
	Apr. 3		16.7	8.5	5.4	11.2		20.1	13.1	7.9	13.0		82.9	64.7	76.7	85.6
	10		17.1	8.5		7.5		17.1	11.9		11.4		89.3	62.0		66.0
	17		20.5	7.8	9.5	10.4		31.7	13.3	18.4	17.2		64.7	58.4	51.3	60.5
	May 1			8.8	5.1	8.3			15.8	8.2	11.7			55.7	60.9	71.0
	7		19.3	11.2	5.5			23.7	16.2	8.6			81.6	69.6	63.8	
	16			10.5	5.5	8.8			16.9	8.1	14.6			62.1	68.1	60.0
	22			9.3	6.5	10.2			14.4	9.9	13.8			64.8	65.1	74.4
	29		16.8	7.7	5.9	11.9		23.5	14.7	10.3	21.5		71.7	52.7	57.0	55.4
	June 5			8.7	5.8	9.2			19.8	11.1	14.2			44.2	52.0	65.3
	12		14.8	6.6	4.3	8.7		23.8	11.8	8.1	16.6		63.4	57.8	53.5	52.7
	19		17.6	6.5	4.5	8.7		24.8	6.5	7.3	14.6		71.2	45.8	61.0	59.2
	26			9.9					16.0					62.2		

* F - female

* M - male

** Kjeldahl determinations

Appendix V (b). RNA, DNA, and protein concentration for Saanich Inlet *E. japonica* samples.

Location	Date	RNA/DNA					μg RNA/mg dry weight					μg RNA/mg protein				
		3	4	5	6F*	6M*	3	4	5	6F	6M	3	4	5	6F	6M
Saanich Inlet	Mar. 7			1.10	1.21	0.73			9.3	6.8	6.7			18.6	13.5	11.3
	Apr. 14			1.28	1.31	1.10			12.3	9.0	7.5			21.0	14.7	10.9
	28			1.34	1.75	0.81			7.8	11.1	7.6			18.5	15.6	12.7
	May 12			1.38	1.48	1.15			10.1	9.1	12.3			19.3	15.0	19.0
	26	0.79	1.23	1.55	1.68	0.70	23.2	18.7	14.6	11.7	6.2	42.0	31.4	25.8	17.5	10.4
	June 9	0.98	1.80	2.03	1.95	0.82	18.5	31.8	17.9	12.5	8.2	60.0	54.7	32.6	19.9	13.5
	23		1.52	2.05	1.66			22.5	15.1	9.4			36.6	30.5	15.3	

Location	Date	μg DNA/mg dry weight					μg DNA/mg protein					% protein/dry weight				
		3	4	5	6F	6M	3	4	5	6F	6M	3	4	5	6F	6M
Saanich Inlet	Mar. 7			8.5	5.7	9.2			17.0	11.1	15.5			50.4	50.8	59.4
	Apr. 14			9.7	6.9	7.5			14.3	11.2	10.7			58.5	62.3	68.3
	28			5.9	6.3	9.5			14.0	8.9	15.7			41.9	71.3	60.3
	May 12			7.2	6.2	10.6			13.8	10.2	16.5			51.7	61.7	64.7
	26	29.2	15.0	9.4	7.0	8.8	53.0	25.1	16.7	10.5	14.7	55.2	59.7	66.4	67.1	60.0
	June 9	18.8	17.5	8.7	6.3	10.0	61.1	30.1	16.0	10.1	16.5	30.9	58.0	54.7	63.0	60.6
	23		13.8	7.3	5.7			24.1	14.8	9.2			61.5	49.5	61.7	

* F - female

* M - male

Appendix V (c). Geographical Distribution of RNA, DNA and protein for *E. japonica* in March, 1969.

Location	Date	RNA/DNA					µg RNA/mg dry weight					µg RNA/mg protein				
		3	4	5	6F*	6M*	3	4	5	6F	6M	3	4	5	6F	6M
Saanich In.	Mar. 7			1.10	1.21	0.73			9.3	6.8	6.7			18.6	13.5	11.3
(a)Jervis In.	Mar. 11			1.50	1.15	0.79			16.6	9.5	7.3			28.4	13.3	11.9
(b)Jervis In.	Mar. 12	0.95	1.69	1.11	1.30	0.74	34.2	42.2	11.8	12.2	8.7	55.7	67.5	23.3	17.2	12.8
Bute Inlet	Mar. 14	0.65	1.16	1.74	1.10	1.05	18.0	17.1	15.6	8.9	11.5	36.0	40.4	34.7	15.0	16.7
Georgia St.	Mar. 15		1.21	1.46	1.26	0.96		23.4	25.6	16.9	12.9		35.9	37.1	20.0	16.8
Haro St.	Mar. 27		1.34	1.82	1.84	1.09		18.9	15.8	12.8	12.1		30.6	27.0	20.6	19.4

Location	Date	µg DNA/mg dry weight					µg DNA/mg protein					% protein/dry weight				
		3	4	5	6F	6M	3	4	5	6F	6M	3	4	5	6F	6M
Saanich In.	Mar. 7			8.5	5.7	9.2			17.0	11.1	15.5			50.4	50.8	59.4
(a)Jervis In.	Mar. 11			11.0	8.2	9.2			18.9	11.5	14.9			58.5	71.5	61.7
(b)Jervis In.	Mar. 12	36.0	25.0	10.7	9.3	11.7	58.6	40.0	21.1	13.2	17.2	61.4	62.5	50.7	70.8	67.9
Bute In.	Mar. 14	27.7	14.7	9.0	8.7	10.9	55.3	34.9	20.0	14.6	15.8	50.0	42.3	45.0	59.5	69.0
Georgia St.	Mar. 15		19.3	17.5	13.5	13.4		29.6	25.4	15.9	17.4		65.2	69.0	84.4	77.1
Haro St.	Mar. 27		14.1	8.7	6.9	11.1		22.9	14.8	11.1	17.9		61.6	58.3	62.0	62.1

* F - female

* M - male

Appendix VI. Molting and Growth Records of Saanich Inlet *Euphausiids*

Number represents uropod length of molt. If no measurement was available, the molt is indicated by 'M'.

Date	*Euphausiid* 1	2	3	4	5	6	7	8	9	10
Aug.20	M				M	M				
21										M
22			M			died	M			
23										
24				3.15				3.06	3.42	1.42
25		3.19								
26	3.17				3.13					
27							died			
28										
29			3.14							1.42
30				3.00				died		
31		3.23							3.34	
Sept.1	3.34				2.98					
2										
3										1.47
4										
5		3.04								
6										
7			3.12		2.91				3.19	
8	3.12									
9										
10										1.53
11										
12				M						
13		2.94								

Appendix VI, cont'd.

Date	*Euphausiid*									
	11	12	13	14	15	16	17	18	19	20
Aug.20			M							
21						died				died
22	M							M		
23		3.27								
24				2.08						
25							2.98		3.29	
26			3.12							
27										
28	2.38									
29				M	3.18			M		
30										
31		3.27								
Sept.1									died	
2	2.34									
3										
4			M	M	3.15					
5										
6										
7	2.34		2.87							
8										
9		3.17		1.87						
10				died	3.04					
11										
12								died		
13	2.30		2.86				2.61			

Appendix VI, cont'd.

Date	*Euphausiid*					
	21	22	23	24	25	26
Aug.20						
21						
22	M		M			died
23						
24		3.08		2.93		
25						
26						
27			died	died	3.16	
28						
29						
30	3.24	2.98				
31						
Sept.1						
2					3.10	
3						
4						
5		2.95				
6						
7						
8						
9	3.15					
10						
11						
12						
13		2.95				

VITA

Surname: DAGG　　Given Names: MICHAEL JOHN

Place of Birth: Vancouver, B.C.　　Date of Birth: April 7, 1945

Educational Institutions Attended, with Dates of Entering and Leaving:

Prince of Wales College,	P.E.I.	1962-1964	
Mount Allison University,	New Brunswick	1964-1966	B.Sc.
University of Victoria,	Victoria, B.C.	1966-1969 (May 1970)	M.Sc.
University of Washington,	Seattle, Wash.	1969-	

Experience:

Teaching Assistant	Biological Oceanography	1967-1969

THE UNIVERSITY OF VICTORIA LIBRARY

MANUSCRIPT THESIS

AUTHORITY TO DISTRIBUTE

AUTHOR: This dissertation may be lent or microfilm copies made available:

(a) Without restriction

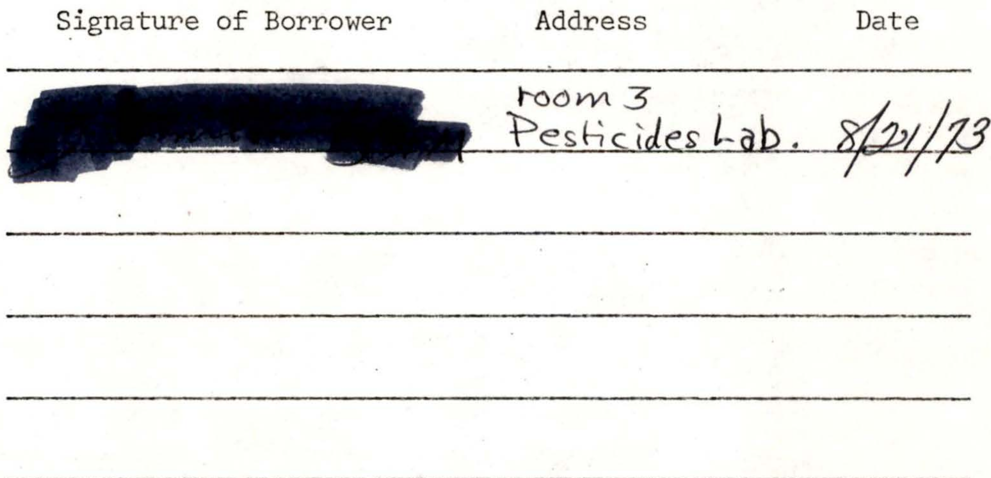
(b) With the restriction that, for a period of five years (until) the written approval of the following is required:

(1) The Chairman, School of Graduate Studies ______

(2) The Author ______

(3) Both the Chairman, School of Graduate Studies, and the Author ______

BORROWERS: The borrower undertakes, by signing below, to give proper credit for any use made of the dissertation, and to obtain the consent of the author if it is proposed to make extensive quotations, or to reproduce the dissertation in whole or in part.



Signature of Borrower	Address	Date
	room 3 Pesticides Lab.	8/21/73