

1985

## Population Characteristics and Sulfide Oxidizing Metabolism of the Bivalve *Solemya velum*

Chong Chen  
*University of Rhode Island*

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POPULATION CHARACTERISTICS AND SULFIDE OXIDIZING

METABOLISM OF THE BIVALVE Solemya velum

BY

CHONG CHEN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

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1985

MASTER OF SCIENCE THESIS

OF

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1985

## ABSTRACT

The physiological and biochemical studies of some animals from sulfide-rich habitats, such as deep sea hydrothermal vents, sewage outfall areas, eelgrass beds and mangrove swamps, have shown that sulfide-oxidizing chemoautotrophic bacteria exist endosymbiotically with the animals and provide a major energy source to their host. One of the remaining questions is the degree to which the symbiont supplies the host's need for reduced carbon and energy metabolism.

Solemya velum, a common Atlantic clam from Nova Scotia to Florida, has chemoautotrophic symbionts in the gill tissue (Cavanaugh, 1983). The host animal has a reduced digestive system and lacks some digestive enzymes. The work reported in this thesis was intended to provide information on the population density and growth rate of the species in Ninigret Pond, Rhode Island, and on the energy input, energy output and energy consumption of the animal under the conditions of laboratory measurement. An energy budget based on a number of assumptions was proposed from both field and experimental data.

The growth parameters  $L_{\infty}$  (length at infinity),  $K$  (the rate at which the animal approaches  $L_{\infty}$ ),  $C$  (the intensity of the growth oscillations) and  $WP$  (winter point) of the seasonal von Bertalanffy growth equation were estimated from the length-frequency of Solemya velum using an objective, computer-aided method (Electronic Length Frequency ANalysis, ELEFAN). The results were  $L_{\infty} = 19.6$  mm,  $K = 0.61$ ,  $C = 1.0$  and  $WP = 1.1$  on a yearly basis. A value of total mortality  $Z = 2.63$  was estimated for the adults from a

length-converted catch curve; therefore, the annual survival rate was 7.2% for the population. The highest density of the population was 289 clams/m<sup>2</sup>, and the average density was 120 clams/m<sup>2</sup>. The other population characteristics, such as length vs. width, length vs. dry weight, and wet weight vs. dry weight were also described.

The enzymatic activities of sulfide oxidation were assayed in the gill tissues of Solemya velum. The maximal activities ( $V_{max}$ ) were 4.7 umoles (substrate converted to product) per gram fresh gill per min for thiosulfate transferase, 2.2 umoles/g/min for adenylylsulfate reductase, and 5.0 umoles/g/min for sulfate adenylyl transferase. The presence of sulfide stimulated and enhanced the respiratory rate of the animal. The animal respiration ( $65.2 \pm 11.2$  ul O<sub>2</sub>/g total wet weight (shell included)/hr) accounted 56.8% of the total oxygen consumption ( $115.4 \pm 18.6$  ul O<sub>2</sub>/g/hr), while chemoautotrophic symbionts consumed the rest 43.2% ( $50.2$  ul O<sub>2</sub>/g/hr).

An energy budget of Solemya velum, a chemoautotrophic symbiotic association, has been constructed based the growth rate, oxygen consumption and enzymatic activities of the species. The energy derived from sulfide oxidation may account 29% of the total energy input. The other sources of energy, like feeding and dissolved organic uptake, may provide the rest, 71%. The energy loss due to respiration and excretion may account 85% of the input energy, while only 15% may have been used for body growth. Multiple modes of nutrition are suggested for this protobranch bivalve. An energy flow model is proposed for the species, and may be applicable to some deep-sea hydrothermal vent organisms.

## ACKNOWLEDGMENTS

A man like myself looks back to find that the mental sparkle originated from the early innocence of mind that kept me forever curious and forever open to new ideas, the encouragement and satisfactions during my first two years in an American graduate school, and the constructive and destructive criticisms necessary to make me more thoughtful. I do not simply give my thanks in pages of acknowledgments, because my appreciations to many people, mentioned or not mentioned, are memorized permanently in my heart.

My ambitious father, who had been hoping for a higher education his whole life, had great expectations for his first child. The hopes of my family became an important driving force in my life. There is no way I can fully express my gratitude to my parents and grandparents.

Many people have helped me in one way or another. In particular, I thank Peter Sampou and Jeff Jones for numerous discussions and assistance relating to my research. Steve Kelly, Rick Soderberg and Eric Klos are thanked for teaching me how to use the MERL facilities. I also thank Aimee Keller for her help in using the Statistical Analysis System (SAS), and John Marcus in using the ELEFAN programs. A very special thank is for William Hutchinson for his confidence, moral support and encouragement to me.

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Finally, I would like to thank the Ministry of Education of China and Xiamen University for providing me a two-year fellowship, which has made my education possible.

**This thesis is dedicated to**

**my father, Wenchi Chen,**

**and my mother, Meizheng Chen.**

## PREFACE

This thesis was prepared according to manuscript format. Three manuscripts are presented and each is written in a style appropriate for submission to a particular scientific journal. The first manuscript, about the population characteristics of Solemya velum in Ninigret Pond, Charlestown, Rhode Island, will be submitted to Marine Biology. The second manuscript, on respiration and sulfide-oxidizing enzymatic activities, will be submitted to Biological Bulletin. The third one, on the bioenergetics of the species, will be submitted to Science.

The thesis as a whole is outlined to understand the energetics in a chemoautotrophic symbiotic organism. Two limitations were encountered. First, no information on the proposed energy substrate (i.e. reduced sulfur) in the environment was obtained because of technical difficulty. Thus, the availability of the energy derived from reduced sulfur in that environment is not known. Second, this study provided a good picture of natural population characteristics and nutritional pathways, but in situ metabolism and reproduction are not known. I felt that the present results could stand by themselves with the questions remaining to be answered, serving as an incentive for further study.

TABLE OF CONTENTS

	Page
Thesis Abstract. . . . .	ii
Acknowledgments. . . . .	iv
Preface. . . . .	vi
Table of Contents. . . . .	vii
List of Tables . . . . .	ix
List of Figures. . . . .	.x
I. Population Characteristics of the Bivalve <u>Solemya velum</u> in Ninigret Pond in Charlestown, Rhode Island, USA . . . . .	.1
Abstract. . . . .	.2
Introduction. . . . .	.3
Material and Methods. . . . .	.3
Results . . . . .	10
Discussion and Conclusions. . . . .	22
Literature cited . . . . .	.26
II. The effect of Hydrogen Sulfide on the Metabolism of <u>Solemya velum</u> (Protobranch Bivalvia) and Sulfide oxidation	
Enzymes in the Species . . . . .	29
Abstract. . . . .	30
Introduction. . . . .	31
Material and Methods. . . . .	33
Results . . . . .	36
Discussion. . . . .	45
Literature cited. . . . .	53

### III. Hydrogen Sulfide and the Bioenergetics of a Symbiotic

#### Bivalve Solemya velum (Protobranchia)

Abstract. . . . .	58
Introduction. . . . .	59
Energy input: sulfide-based chemoautotrophics . . . . .	59
Growth of <u>Solemya</u> . . . . .	61
Respiration of <u>Solemya</u> . . . . .	62
Excretion of <u>Solemya</u> . . . . .	63
Energy budget of <u>Solemya</u> . . . . .	63
Energy transfer and efficiency. . . . .	67
Evolutionary perspectives . . . . .	70
References. . . . .	72

<b>Appendix A.</b> The experimental conditions of . . . . .	
the Gilson respirometer. . . . .	76

<b>Appendix B.</b> Oxygen uptake rates . . . . .	
(a). The cumulative oxygen uptakes of <u>Solemya velum</u>	
in the presence and absence of hydrogen sulfide. . . . .	77
(b). Calculating procedures . . . . .	78

<b>Appendix C.</b> Enzymatic activities . . . . .	79
---	----

<b>Appendix D.</b> Bibliography . . . . .	80
---	----

LIST OF TABLES

	Page
I. Population characteristics of the bivalve <u>Solemya velum</u> in Ninigret Pond in Charlestown, Rhode Island, USA.	
Table 1. The density of <u>Solemya velum</u> . . . . .	12
Table 2. The length-frequency data of <u>Solemya velum</u> . . . . .	13
Table 3. The biomass of the population . . . . .	14
Table 4. The growth parameters in some bivalves. . . . .	15
II. The effect of hydrogen sulfide on the metabolism of <u>Solemya velum</u> and sulfide oxidation enzymes.	
Table 1. The effects of hydrogen sulfide on the metabolic rates of <u>Solemya velum</u> . . . . .	39
Table 2. Enzyme activities of sulfide oxidation in marine animals from sulfide-rich habitats. . . . .	40
III. Hydrogen sulfide and the bioenergetics of a symbiotic bivalve <u>Solemya velum</u> .	

LIST OF FIGURES

	Page
I. Population characteristics of the bivalve	
<u>Solemya velum</u> in Ninigret Pond in Charlestown, Rhode Island, USA.	
Figure 1. The reference map of Ninigret Pond. . . . .	5
Figure 2. The water temperature in the sampling site. . . . .	8
Figure 3. The plots of the length-frequency data of <u>Solemya velum</u> , with growth curves super-imposed. . . . .	16
Figure 4. The predicted growth of <u>Solemya velum</u> . . . . .	17
Figure 5. The plot of shell length versus dry tissue weight on log/log basis . . . . .	18
Figure 6. The plot of shell length versus dry shell weight . . . . .	19
Figure 7. The regression of the total wet weight versus the dry tissue weight . . . . .	20
Figure 8. The relationship between two dimensions of the clam. . . . .	21
II. The effect of hydrogen sulfide on the metabolism of <u>Solemya velum</u> and sulfide oxidation enzymes	
Figure 1. The biochemical pathways of sulfide oxidation in chemoautotrophic bacteria . . . . .	41
Figure 2. The cumulative oxygen uptakes in the presence and absence of sulfide . . . . .	42
Figure 3. The reciprocal plot of APS reductase . . . . .	43

Figure 4. The reciprocal plot of sulfate  
adenylyl transferase. . . . . 44

III. Hydrogen sulfide and the bioenergetics of a  
symbiotic bivalve Solemya velum.

Figure 1. A proposed model of energy flow in  
Solemya velum . . . . . 69

Manuscript I

**Population Characteristics of the Bivalve Solemya velum in Ninigret**

**Pond in Charlestown, Rhode Island, USA**

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Suggestion for an abbreviated title: population characteristics of

Solemya velum

**Abstract.** The growth parameters  $L_{\infty}$  (length at infinity),  $K$  (the rate at which the animal approaches  $L_{\infty}$ ),  $C$  (the intensity of the growth oscillations) and  $WP$  (winter point) of the seasonal von Bertalanffy growth equation were estimated from the length-frequency of Solemya velum using an objective, computer-aided method (Electronic Length Frequency Analysis, ELEFAN). The results were  $L_{\infty} = 19.6$  mm,  $K = 0.61$ ,  $C = 1.0$  and  $WP = 1.1$  on a yearly basis. A value of total mortality  $Z = 2.63$  was estimated for the adults from a length-converted catch curve; therefore, the annual survival rate was 7.2% for the population. The highest density of the population was 289 clams/m<sup>2</sup>, and the average density was 120 clams/m<sup>2</sup>. The other population characteristics, such as length vs. width, length vs. dry weight, and wet weight vs. dry weight were also examined.

## **Introduction**

Solemya is a genus of protobranch bivalves belonging to the superfamily Solemyacea. It has been found on the east and west coast of North America, in West Africa, the Mediterranean, Australia and New Zealand (Morse, 1913). This genus of animals has a variety of unusual biological features. The species which have been investigated are all capable of rapid swimming and burrowing (Drew, 1900; Stanley, 1970). As observed originally by Pelseneer in 1891, the gut of Solemya is primitive and reduced or absent (Young, 1939; Reid, 1980; Reid and Bernard, 1980). In the gill tissues of a common Atlantic species Solemya velum, Cavanaugh (1983) has indicated the existence of intracellular bacteria, which are capable of utilizing reduced sulfur energy and fixing carbon.

Even though the biology and nutrition of Solemya have attracted attention for more than one hundred years, none of the studies have been directed to the population characteristics of the bivalves. The present paper is intended to provide preliminary information on variation in such population characteristics as density, size-frequency, growth rate and biomass in the Ninigret Pond of Charlestown, Rhode Island.

## **Material and Methods**

A sample station was established at Hall Point of Ninigret Pond, Charlestown, Rhode Island ( $41^{\circ}22'N$ ,  $71^{\circ}41'W$ ) (Figure 1). The sample area is an eelgrass bed of sandy mud. The mud has a dark color with the smell of hydrogen sulfide. The population was sampled monthly along transects with randomly chosen cores for approximately one year (September 1984 to July 1985). The core was 20.5 cm in diameter and 22

cm deep. The transects (one for each month) started from the shore and extended perpendicularly to a water depth of approximately 1.5 meters, which was about 30 meters from the shore. The whole sample area extended about 50 meters along the shoreline. The animals seemed to be distributed in a patchy pattern; almost no animals were found in the surrounding area 100 or 200 meters away from the sample site in the pond. Transects parallel with the shore were also taken. The cores were rinsed through a 5 mm sieve in field.

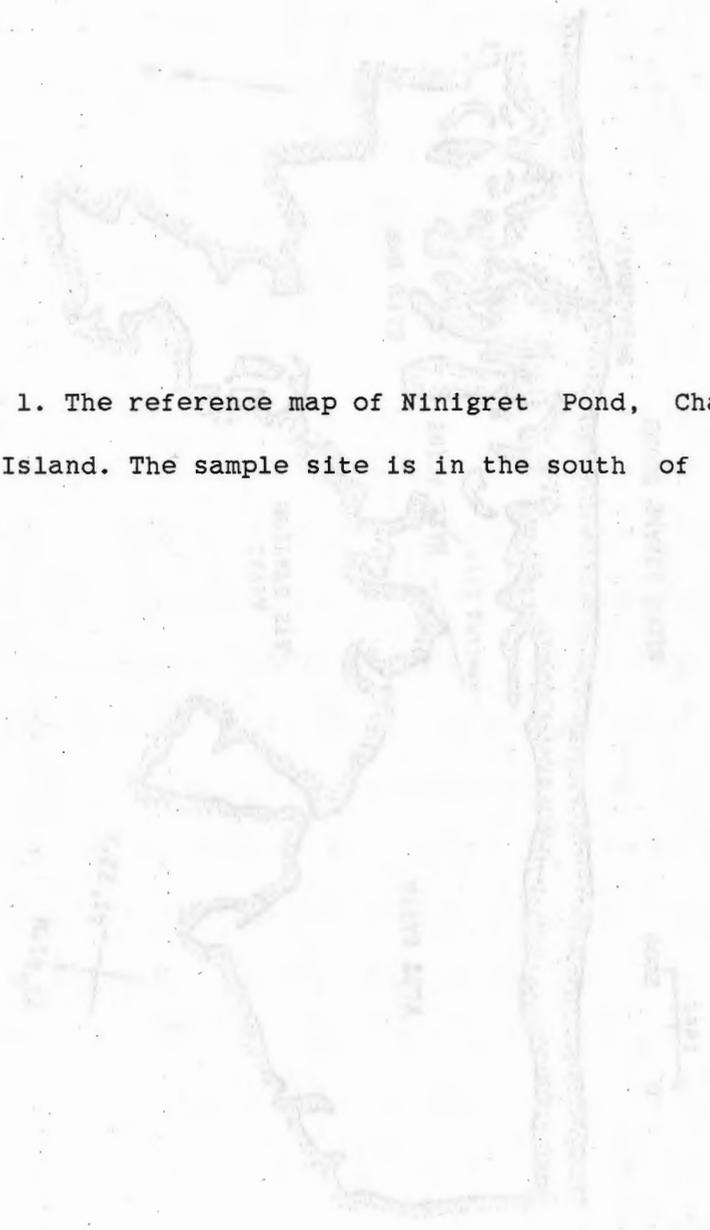
All individuals (>5 mm dia.) in each core were brought back to laboratory alive. They were counted, and measured to the nearest 0.01 mm in shell length and width with calipers. A total of 20 to 100 animals from each month's sample covering the full size range were weighed, dissected and freeze-dried for 48 hours. The dry soft-body and shell weights were measured to the nearest 0.01 mg on an electrical automatic balance.

The computer programs ELEFAN (Electronic Length Frequency Analysis) developed by Pauly and David (1981) was used to analyse the length data to fit the best growth curve of the population. The original programs were written in Radio Shack BASIC II, and have been converted to BASICA in IBM personal computers. The von Bertalanffy growth function (VBGF) was used to describe the growth.

$$L_t = L_{\infty} [1 - e^{-K(t-t_0)}] \quad (1)$$

where  $L_t$  = length at relative age  $t$ ,  $L_{\infty}$  = length at infinity,  $K$  describes the rate at which the animal approaches  $L_{\infty}$ . The parameters  $L_{\infty}$  and  $K$  of the VBGF were estimated on the basis of the length frequency in Table 2.

Figure 1. The reference map of Ninigret Pond, Charlestown, Rhode Island. The sample site is in the south of the Hall Point.



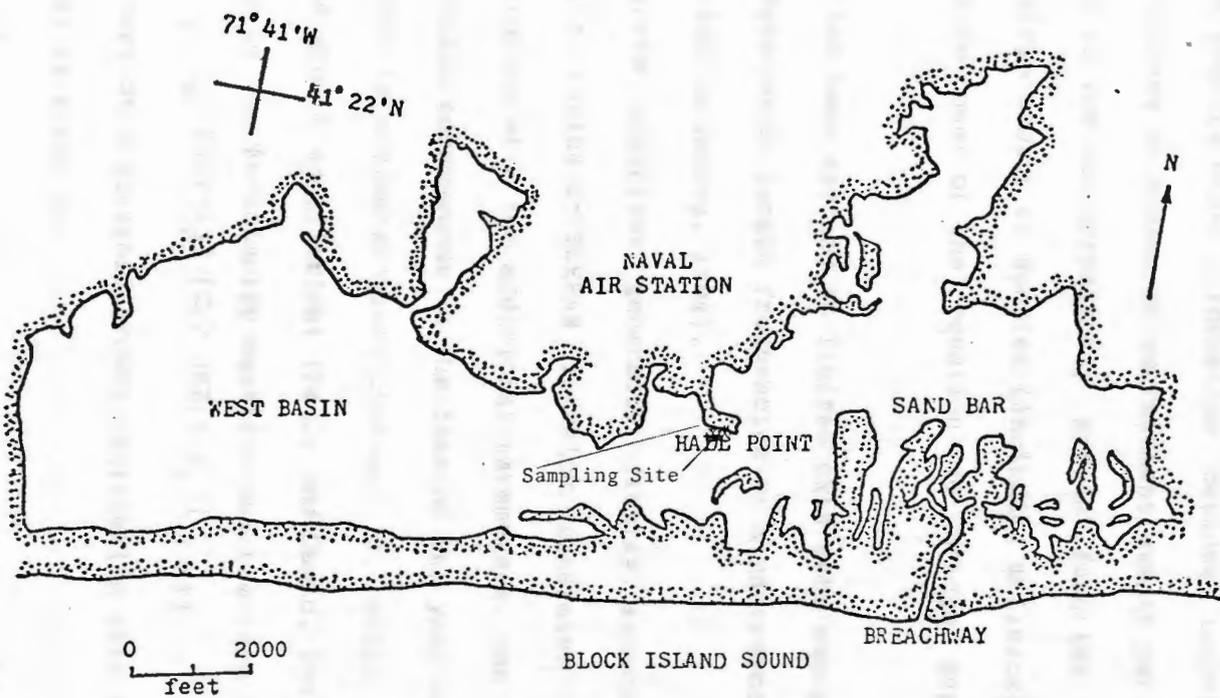


Fig. 1. Reference map of Ninigret Pond, Charlestown, Rhode Island.

Because of the lack of information about age structure, the hypothetical age  $t_0$  is set to zero, i.e.  $t-t_0=t$ . The main features of the method (Pauly and Calumpong, 1984) include :

(1) It does not require other information besides length-frequency data, such as age classes or number of recruitment events per year;

(2) it is based on the von Bertalanffy growth function which has been applied to a large number of species (including molluscs), and it uses the interpolative power of the equation to bridge gaps in the size-frequency;

(3) the method has been applied to limited data sets among a number of species, e.g. seven-month length frequencies of a gastropod Dolabella auricularia (Pauly and Calumpong, 1984).

Polar and temperate organisms generally display seasonal growth patterns. There is a routine in ELEFAN 1 which generates seasonally oscillating growth curves with two additional parameters. One is called Winter Point (WP), which corresponds to the time of the year when growth is slowest. The other is a dimensionless constant C, which expresses the intensity of the growth oscillations (Pauly and David, 1981). Thus, the seasonal version of von Bertalanffy equation has the form

$$L_t = L_{\infty} \left( 1 - e^{-[KD(t-t_0) + (C/2)KD(t-t_s)]} \right) \quad (2)$$

where  $t_s$  sets the start of a sinusoid growth oscillation with regard to  $t=0$ ; the Winter Point is given by

$$WP = t_s + 0.5 \quad (3)$$

D describes the effect of animal size on growth, ranging from 0.3, such as in large tuna, to 1.0, such as in guppies (Pauly, 1979). D was simply set to 1.0 in Solemya velum because of its small size. The value

of C can range from 0 in tropical fishes to 1 in temperate fishes.  $C=1$  is used as trial value in this case. Because the seasonal temperature fluctuation is more than  $10^{\circ}\text{C}$  in Rhode Island (Figure 2), WP is set at 0.6 as a trial value as suggested by Pauly et al. (1983).

The computing procedure traces through a series of length-frequencies sequentially arranged in time with a multitude of growth curves, and then selects a single curve which, by passing through a maximum of peaks, would "explain" these peaks. Six major steps are involved in the procedure (Pauly and David, 1981).

(1) Reconstruct the length-frequency samples. The procedure consists of calculating running average frequencies (over 5 length classes), dividing each length-frequency value by the corresponding running average frequency, then subtracting 1 from the quotient. The positive values are considered as peaks, and the negative ones as troughs.

(2) Calculate the maximum sum of peaks available in the length-frequency samples. This maximum "available sum of peaks" (ASP) is accumulated into a single growth curve.

(3) Analyse the length-frequency samples for a trial value of  $L_{\infty}$  and K. A series of growth curves started from the base of each of the peaks at a starting sample is projected backward and forward in time to fit all samples. The starting sample was iterated by computer.

(4) The points, obtained by each growth curve when passing through peaks (positive points) or the "troughs" separating peaks (negative points), are accumulated.

### WATER TEMPERATURE DURING SAMPLING

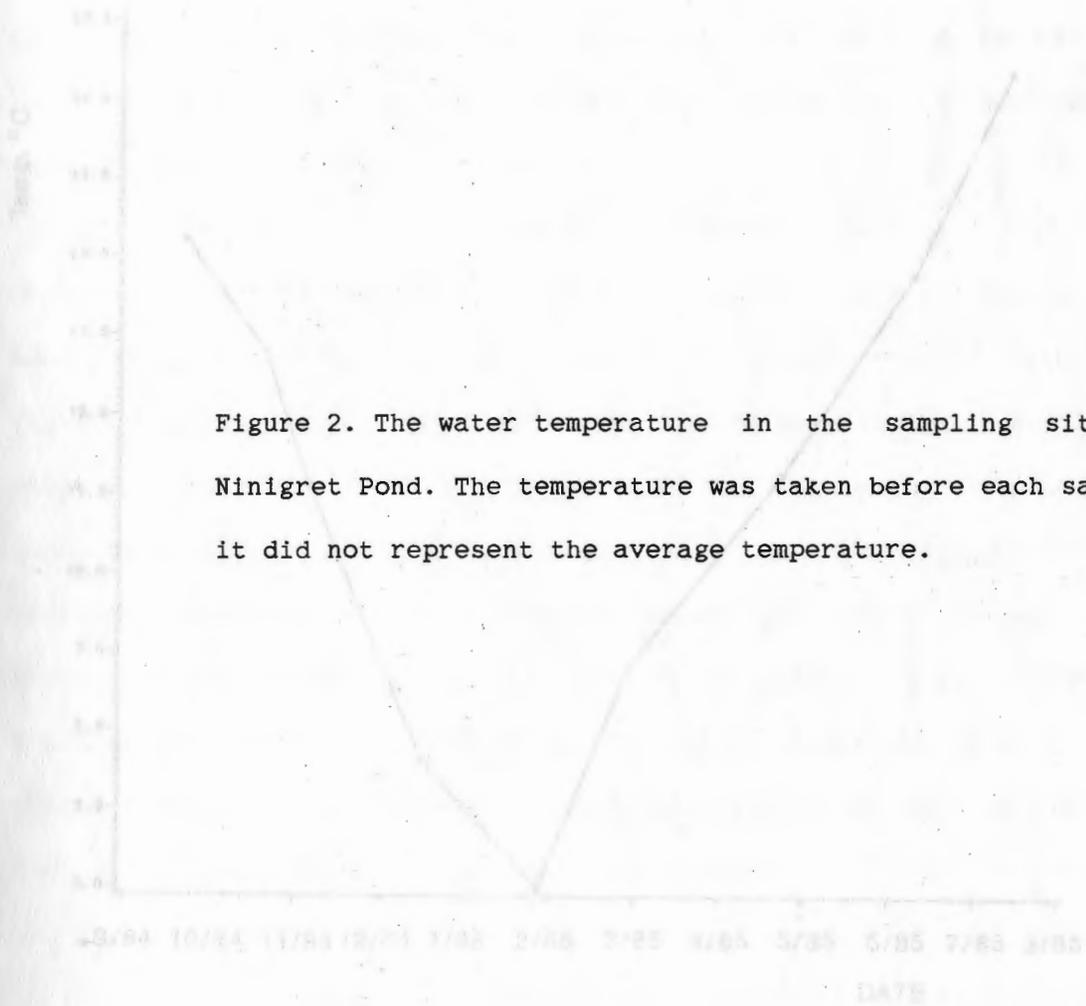
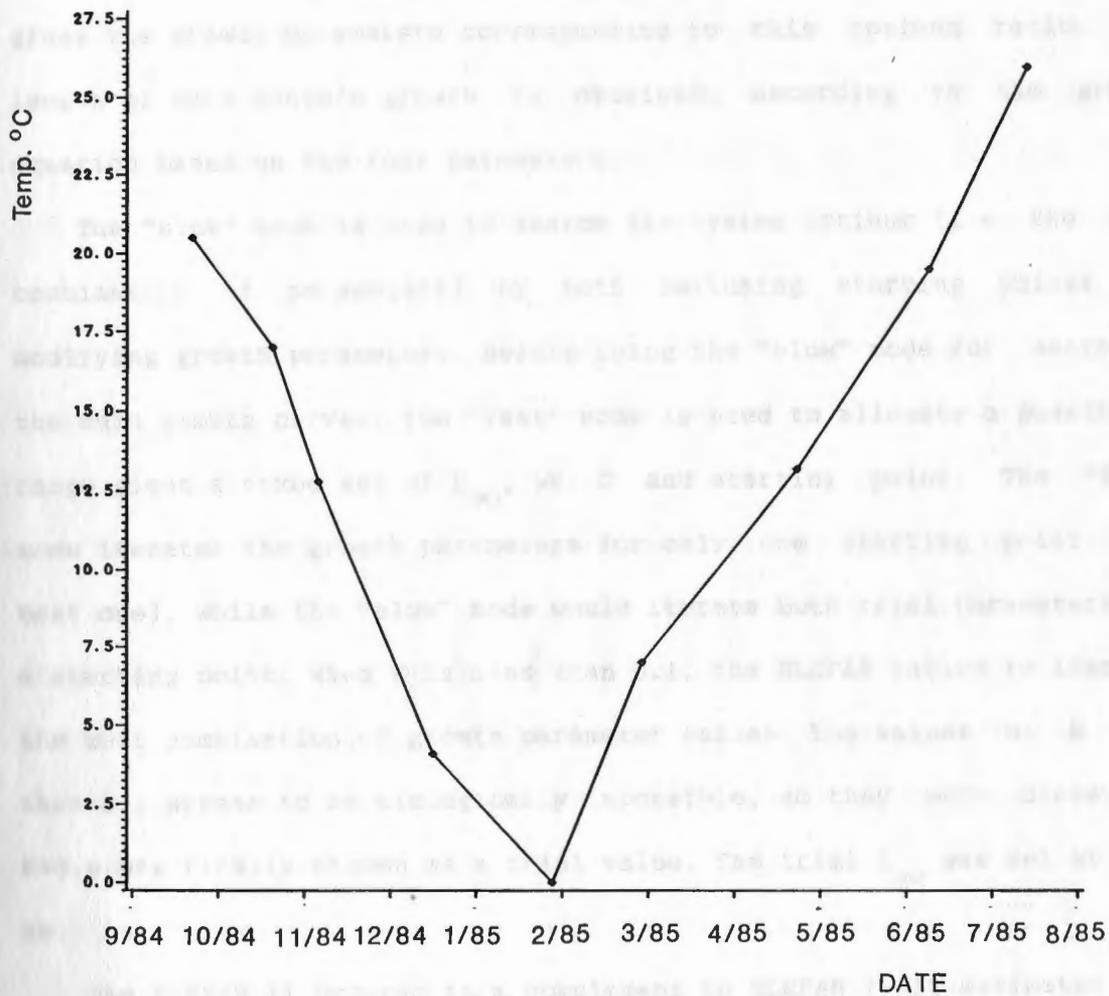


Figure 2. The water temperature in the sampling site of Ninigret Pond. The temperature was taken before each sample; it did not represent the average temperature.

### WATER TEMPERATURE DURING SAMPLING



(5) The curve which passes through most peaks and avoids most "troughs" is selected. The largest "explained sum of peaks" (ESP) is accumulated.

(6) The trial values of growth parameters ( $L_{\infty}$ , K, WP, C) are increased or decreased until the ratio ESP/ASP reaches a maximum, and gives the growth parameters corresponding to this optimum ratio. The length of each month's growth is obtained, according to the growth equation based on the four parameters.

The "slow" mode is used to search the system optimum (i.e. the best combination of parameters) by both switching starting points and modifying growth parameters. Before using the "slow" mode for searching the main growth curves, the "fast" mode is used to allocate a possible K range given a fixed set of  $L_{\infty}$ , WP, C, and starting point. The "fast" mode iterates the growth parameters for only one starting point (the best one), while the "slow" mode would iterate both trial parameters and a starting point. When K is less than 0.1, the ELEFAN failed to identify the best combination of growth parameter values. The values of K less than 0.1 appear to be biologically impossible, so they were discarded. K=0.6 was finally chosen as a trial value. The trial  $L_{\infty}$  was set at 19.5 mm.

The ELEFAN II program is a complement to ELEFAN I. It estimates the total mortality (Pauly et al., 1982). The total mortality (Z) of the population was estimated by the means of length-converted catch curve, which based on the length-frequency samples and estimated parameters  $L_{\infty}$  and K. The total mortality (Z) is defined by

$$N_t = N_0 e^{-Zt} \quad (4)$$

where  $N_0$  and  $N_t$  are the numbers of specimens at the beginning and the end of a time period  $t$ . Assuming a steady state in which growth, mortality and recruitment are constant,  $Z$  can be estimated from the slope of the regression

$$\ln [N_i / t_i] = a - Zt_i' \quad (5)$$

where  $N_i$  is the number of specimens in a given class (i),  $t_i$  is the time needed to grow through the length class (i),  $a = \ln (N_0/t_i)$ , and  $t_i'$  is the relative age corresponding to the midrange of the length class (i). The values of  $t_i'$  and  $t_i$  are obtained from

$$t_i' = -(1/K) \ln [1 - (L/L_\infty)] \quad (6)$$

and, 
$$t_i = (1/K) \ln [(L_\infty - L_1)/(L_\infty - L_2)] \quad (7)$$

where  $L_1$  and  $L_2$  correspond to the lower and upper size limits of a class, respectively.

The length and weight were converted to logarithms and a linear regression was calculated. The regressions between length and width, wet weight and dry weight, length and dry shell weight were made for better defining the population. The resulting equation relating shell length to dry tissue weight were used with size frequency and density data to estimate monthly biomass.

## Results

At the Ninigret Pond station, Solemya velum was the only bivalve which was relatively abundant. Polychaetes and flatworms were predominant among the infauna. Almost no crustaceans were found in the infauna (field observation). The highest density was recorded with 289 clams/m<sup>2</sup> in December 1984 (Table 1). The average densities from five transects were available. They were 105 (Oct.84), 116 (Nov.), 129

(Dec.), 96 (Jan.85), and 147 (Aug.85), respectively. In the area of sampling, a zero density was found only once.

Figure 3 shows a plot of the length-frequency samples in Table 2, with growth curves super-imposed. The growth parameter values estimated by ELEFAN are  $L_{\infty} = 19.6$  mm,  $K = 0.6$ ,  $WP = 1.1$ , and  $C = 1.0$ . The  $L_{\infty}$  value (19.6 mm) is very close to the maximum length, 18.5 mm, in the entire sampling. Thus the von Bertalanfy equation (2) describing the growth of Solemya velum was as follows:

$$L_t = 19.6 [ 1 - e^{-(0.9 t - 0.6 t_0 - 0.2)} ] \quad (8)$$

where the unit of  $L_t$  is mm,  $t$  is the age in years after animal has spawned. The length was plotted against the age in Figure 4.

The estimated total mortality ( $Z$ ) is 2.63.  $Z$  is defined as the coefficient of the instantaneous total mortality, which describes the rate at which the numbers in the population are decreasing (Equation (4)). This  $Z$  value suggested that the annual survival rate (i.e.  $(N_t/N_0) = e^{-Zt}$ ,  $t=1$  yr.) of Solemya velum was 7.2% in Ninigret Pond.

Figure 5 shows the relationship between shell length and dry tissue weight on a log/log basis. The equation is a straight line ( $r^2=0.88$ ):

$$\log_{10} W = -2.143 + 3.003 \log_{10} L \quad (9)$$

The biomass for five months available is shown in Table 3. The highest value recorded was 17.3 g total wet wt/m<sup>2</sup>; the average biomass was approximately 6.7 g/m<sup>2</sup>. The dry shell weight (SW, mg) and shell length (L, mm) are also fit into a linear regression on log/log basis ( $r^2=0.89$ ) (Figure 6).

$$\log_{10} SW = -1.873 + 2.993 \log_{10} L \quad (10)$$

Table 1. The density of Solemya velum at Hall Point, Ninigret Pond, Charlestown, R.I. for 5 months.

Time Day/Mo/Yr	No. of samples	Density (#/m <sup>2</sup> )			
		Highest	Lowest	Average	s.d.
21/10/84	17	217	19	105	63
06/11/84	9	202	58	116	47
16/12/84	17	289	0	129	78
29/01/85	11	202	29	96	67
09/08/85	8	223	32	147	71

Note: (1). The densities were sampled for five months by the same coring device (20.5 cm diameter, 22 cm depth).

(2). The cores were taken along transects (one for each month) either perpendicular to or parallel with the shoreline.

(3). The s.d. represents a standard deviation of each density sample.

Table 2. Length-frequency data of Solemya velum collected in Ninigret Pond, Rhode Island.

class limits		midlength	sample #1	2	3	4	5	6	7	8	9	10	11	12
lower	upper	(in mm)	Jan29	Feb27	Mar19	Apr10	Jun9	Jun26	Jul20	Aug	Sept28	Oct21	Nov9	Dec16
5.00	5.99	5.50	0	0	0	0	0	0	5	--	0	0	0	4
6.00	6.99	6.50	3	0	0	0	1	2	22	--	1	4	3	1
7.00	7.99	7.50	14	5	0	3	1	0	60	--	5	5	3	3
8.00	8.99	8.50	12	6	2	6	4	2	42	--	3	14	14	3
9.00	9.99	9.50	14	16	9	11	11	7	28	--	9	23	16	11
10.00	10.99	10.50	24	10	19	11	21	16	12	--	15	23	7	15
11.00	11.99	11.50	23	11	22	16	20	22	6	--	9	31	18	17
12.00	12.99	12.50	25	7	17	12	15	17	3	--	8	22	14	15
13.00	13.99	13.50	14	7	5	6	2	11	5	--	2	8	4	4
14.00	14.99	14.50	15	3	3	4	2	5	2	--	4	8	4	1
15.00	15.99	15.50	6	1	2	2	1	4	0	--	2	1	3	1
16.00	16.99	16.50	0	0	0	0	0	0	0	--	0	0	0	0
17.00	17.99	17.50	0	1	0	0	0	0	0	--	0	0	0	0
18.00	18.99	18.50	1	0	0	0	0	0	0	--	0	0	0	0
total			150	67	79	71	78	86	185	--	59	139	86	75

Note: 1). The frequency is represented by the number of animals in each sample.

2). -- means that data was not available.

Table 3. The biomass of the population of Solemya velum.

Species: S. velum in Ninigret Pond, Rhode Island. Authority:

MILLER & MILLER (1986)

Sample Time (Day/Mo/Yr)	Biomass (g total wet wt/m <sup>2</sup> )		
	Highest	Lowest	Average
21/10/84	13.0	1.1	6.3
06/11/84	12.1	3.5	7.0
16/12/84	17.3	0	7.7
29/01/85	12.1	1.7	5.8
09/08/85	20.0	2.0	9.5

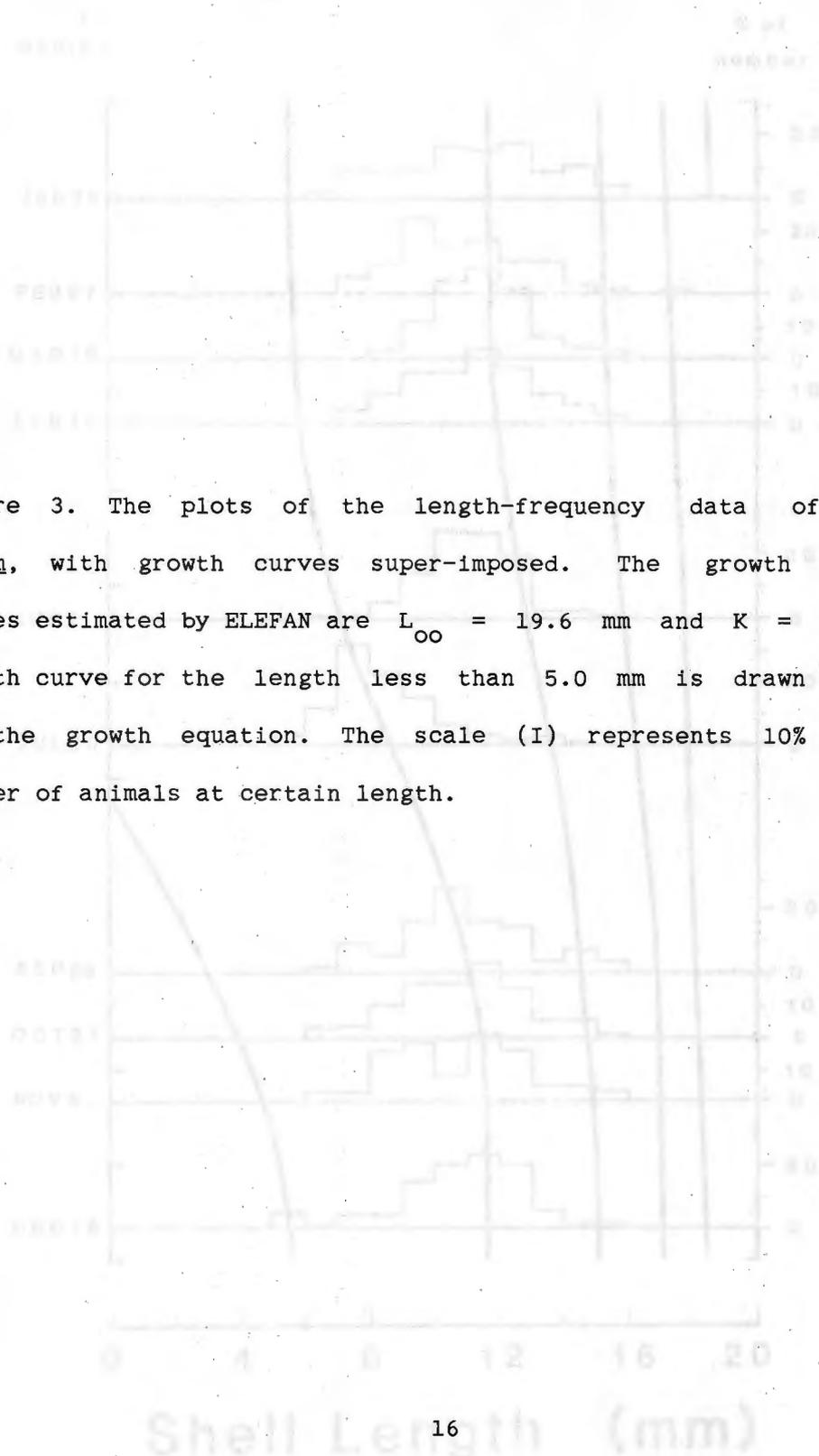
Note: the biomass was estimated from the densities in Table 1.

Lengths were converted to wet weights by the equation (11).

Table 4. The growth parameters in some bivalves.

Species & location	$L_{\infty}$	K	Authority
<u>Mytilus edulis</u> (mussels)			
Danish Wadden Sea	77.6	0.561	Thiesen (1968)
Conway, North Wales	72.7	0.343	Thiesen (1968)
Greenland	77.5	0.022	Thiesen (1973)
Morecambe Bay, England	62.5	0.810	Dare (1975)
<u>Mya arenaria</u> (clam)			
Quonchontaug Pond, R.I.	93.2	0.118	Appeldoorn (1980)
<u>Solemya velum</u> (clam)			
Ninigret Pond, R.I.	19.6	0.610	Author (1985)

Figure 3. The plots of the length-frequency data of Solemya velum, with growth curves super-imposed. The growth parameter values estimated by ELEFAN are  $L_{\infty} = 19.6$  mm and  $K = 0.61$ . The growth curve for the length less than 5.0 mm is drawn according to the growth equation. The scale (I) represents 10% of the number of animals at certain length.



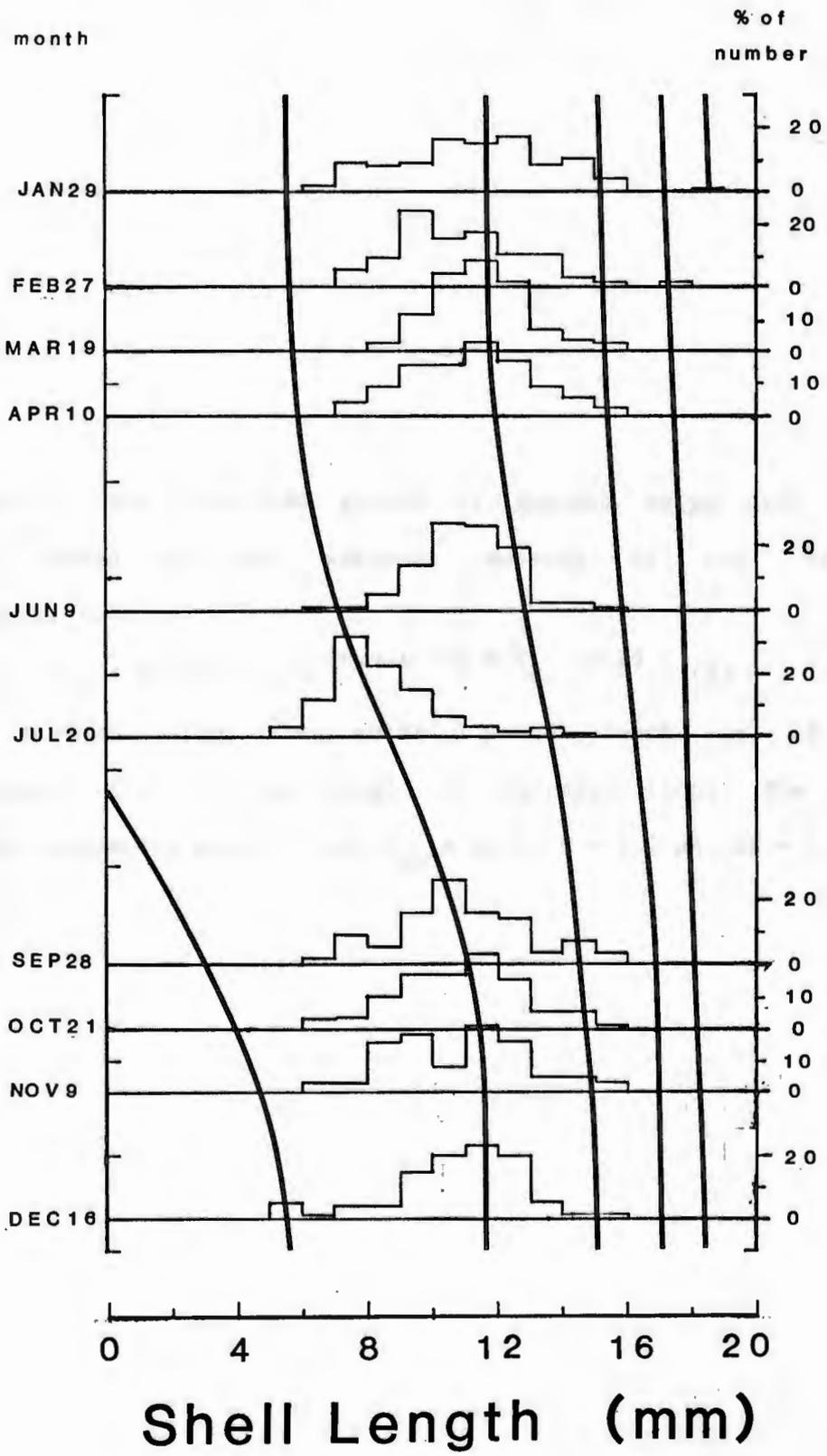
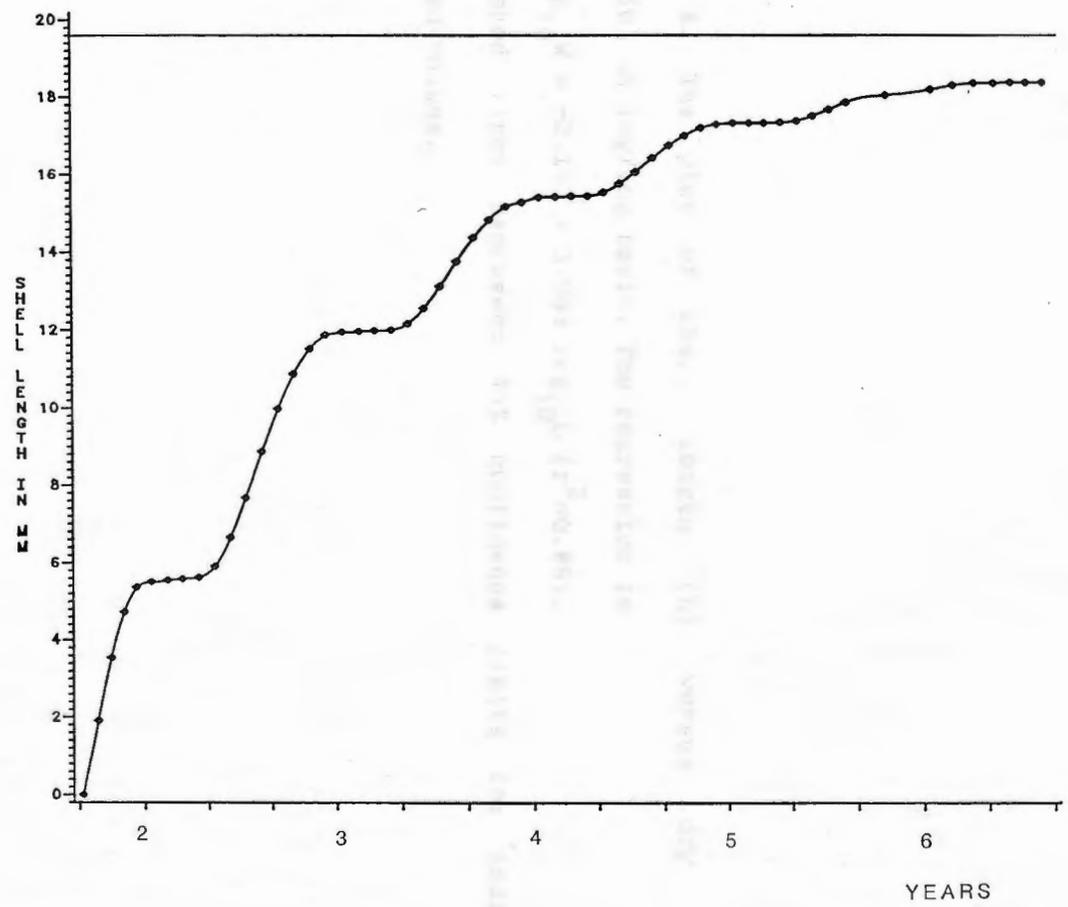


Figure 4. The predicted growth of Solemya velum over its life span, based on the seasonal version of von Bertalanffy equation, i.e.

$$L_t = 19.6 [ 1 - e^{-(0.9 t - 0.6 t_0 - 0.2)} ] \quad (8)$$

The maximum size the animal would reach is 18.5. The horizontal line is the length at infinity ( $L_{\infty}$ ). The estimated growth parameters are  $K = 0.6$ ,  $L_{\infty} = 19.6$ ,  $C = 1.0$  and  $WP = 1.2$ .

# GROWTH CURVE OF *Solemya velum*



17a

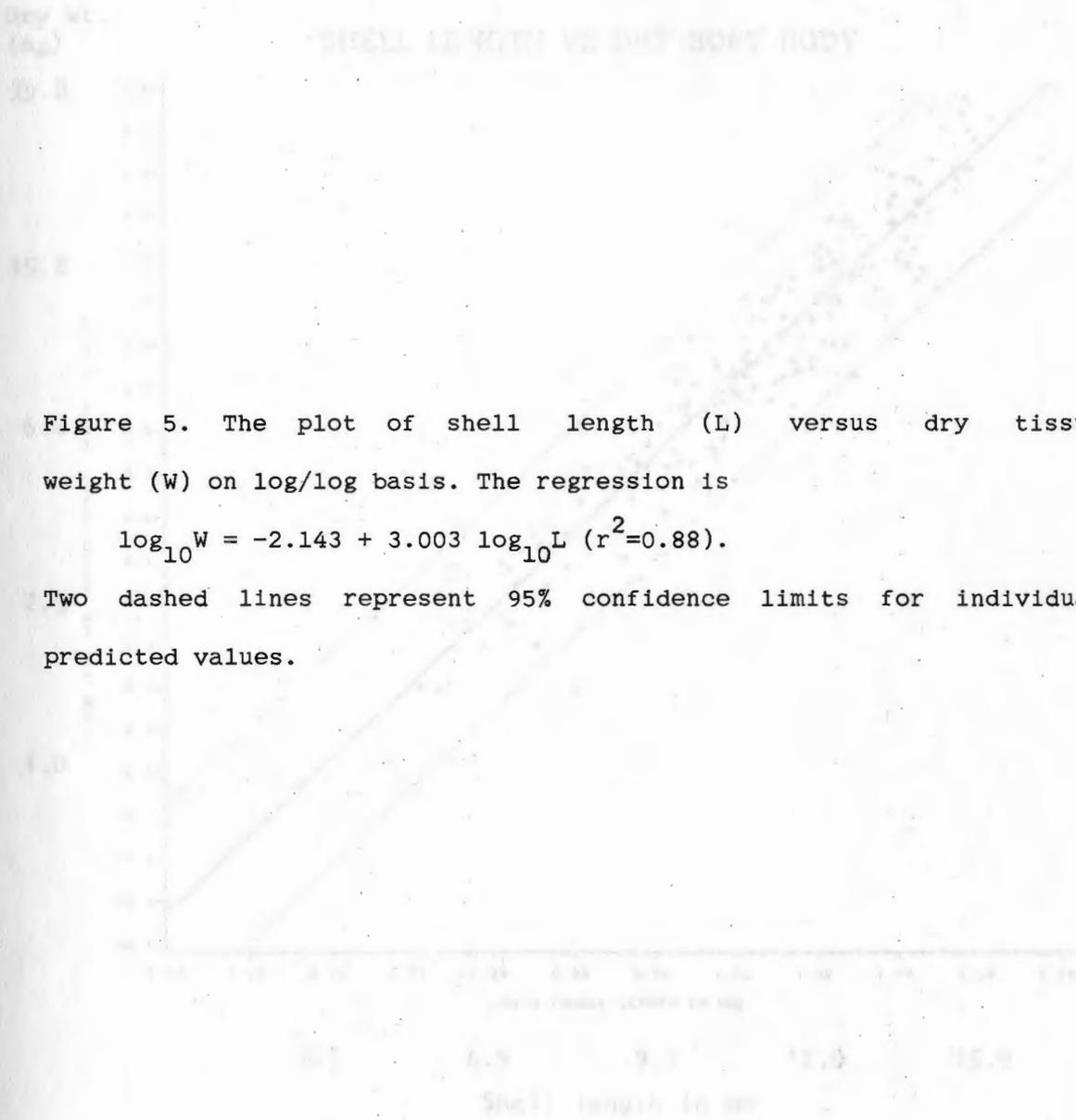


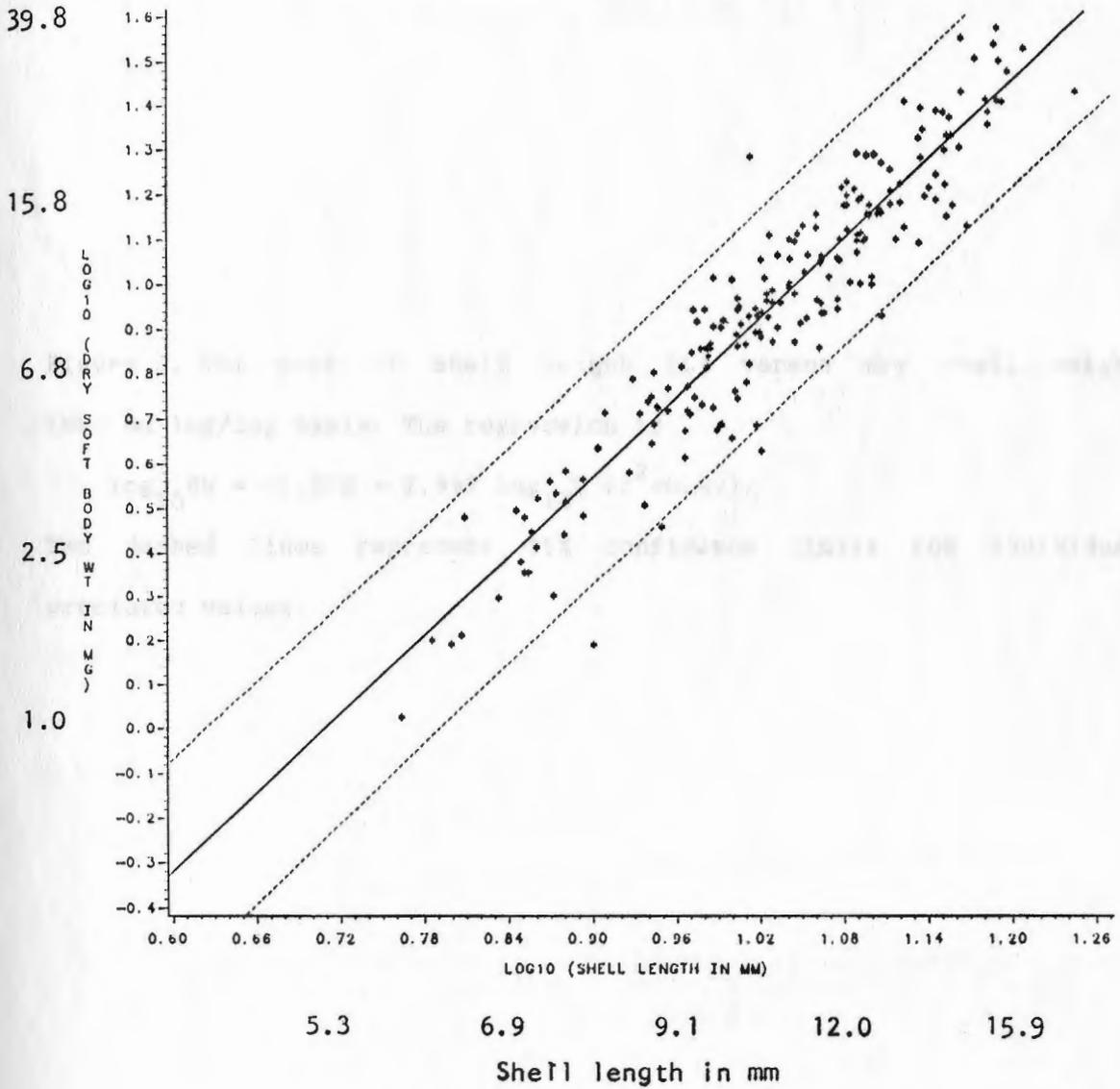
Figure 5. The plot of shell length (L) versus dry tissue weight (W) on log/log basis. The regression is

$$\log_{10} W = -2.143 + 3.003 \log_{10} L \quad (r^2=0.88).$$

Two dashed lines represent 95% confidence limits for individual predicted values.

Dry wt.  
(mg)

### SHELL LENGTH VS DRY SOFT BODY



Dry wt.  
(mg)

### DRY SHELL WEIGHT VS. LENGTH

70.4

39.7

Figure 6. The plot of shell length (L) versus dry shell weight (SW) on log/log basis. The regression is

$$\log_{10} SW = -1.873 + 2.993 \log_{10} L \quad (r^2=0.89).$$

Two dashed lines represent 95% confidence limits for individual predicted values.

5.0

2.5

5.0

6.3

7.9

10.0

12.6

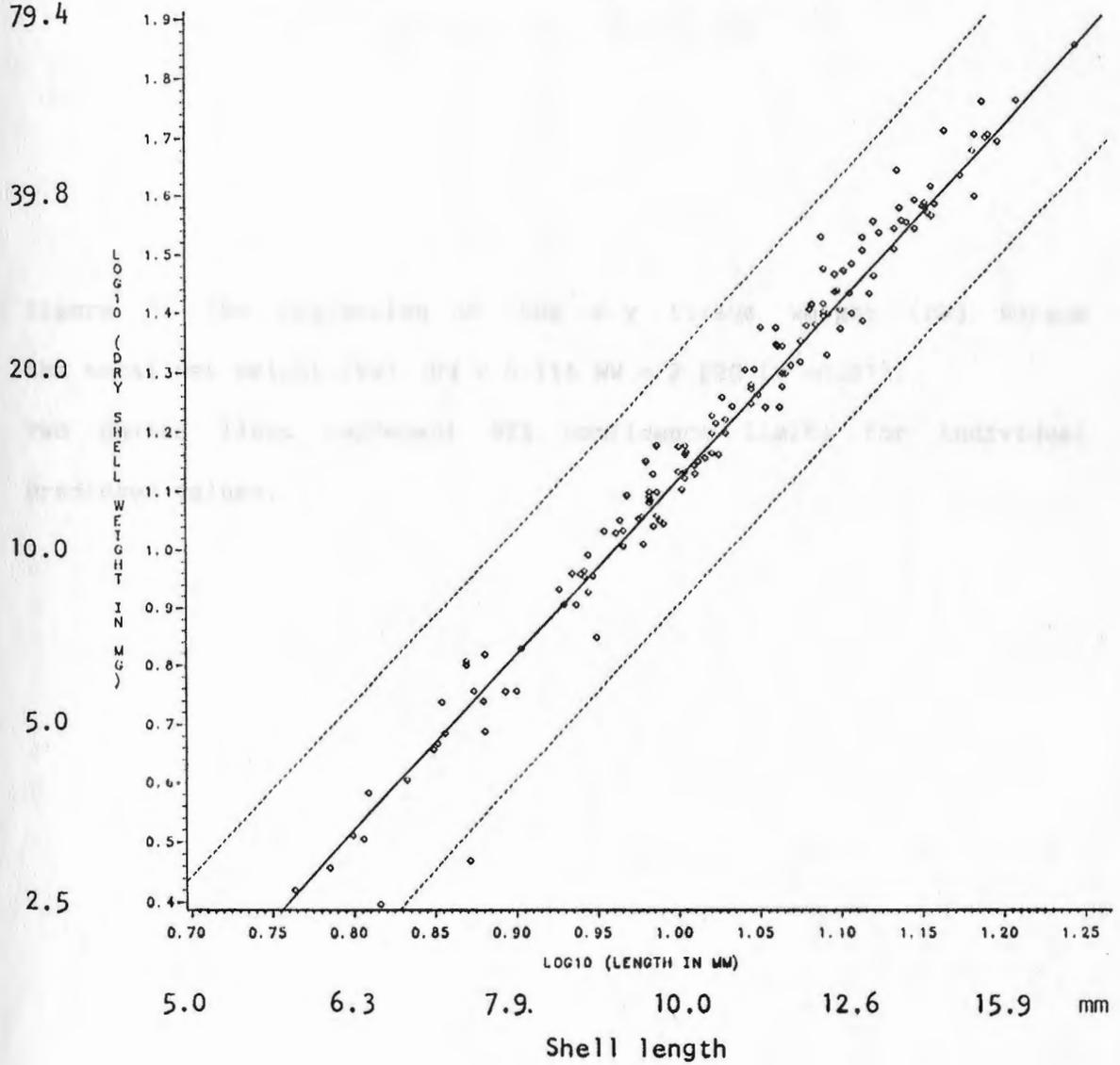
16.3

20.0

shell length

Dry wt.  
(mg)

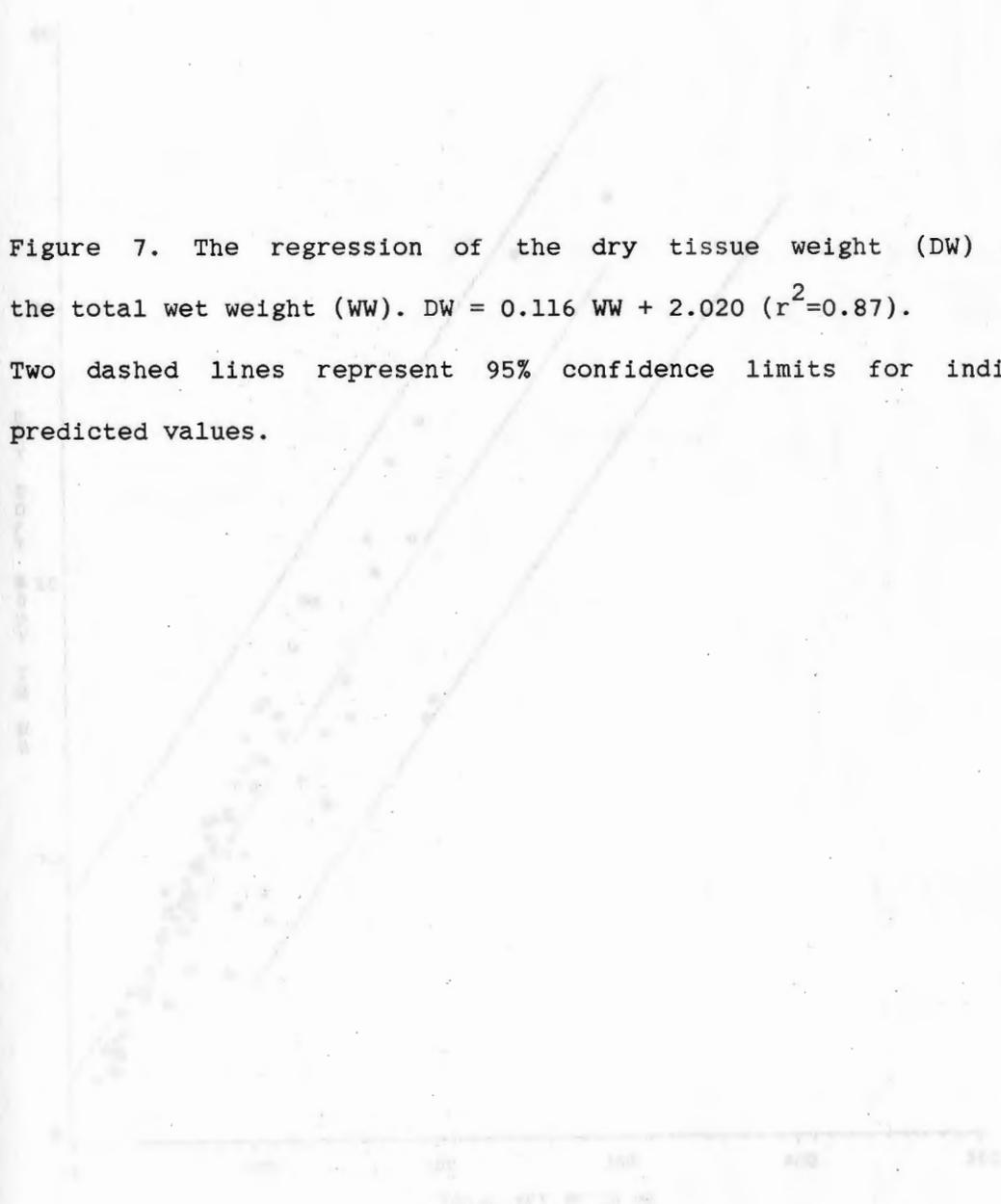
### DRY SHELL WEIGHT VS. LENGTH



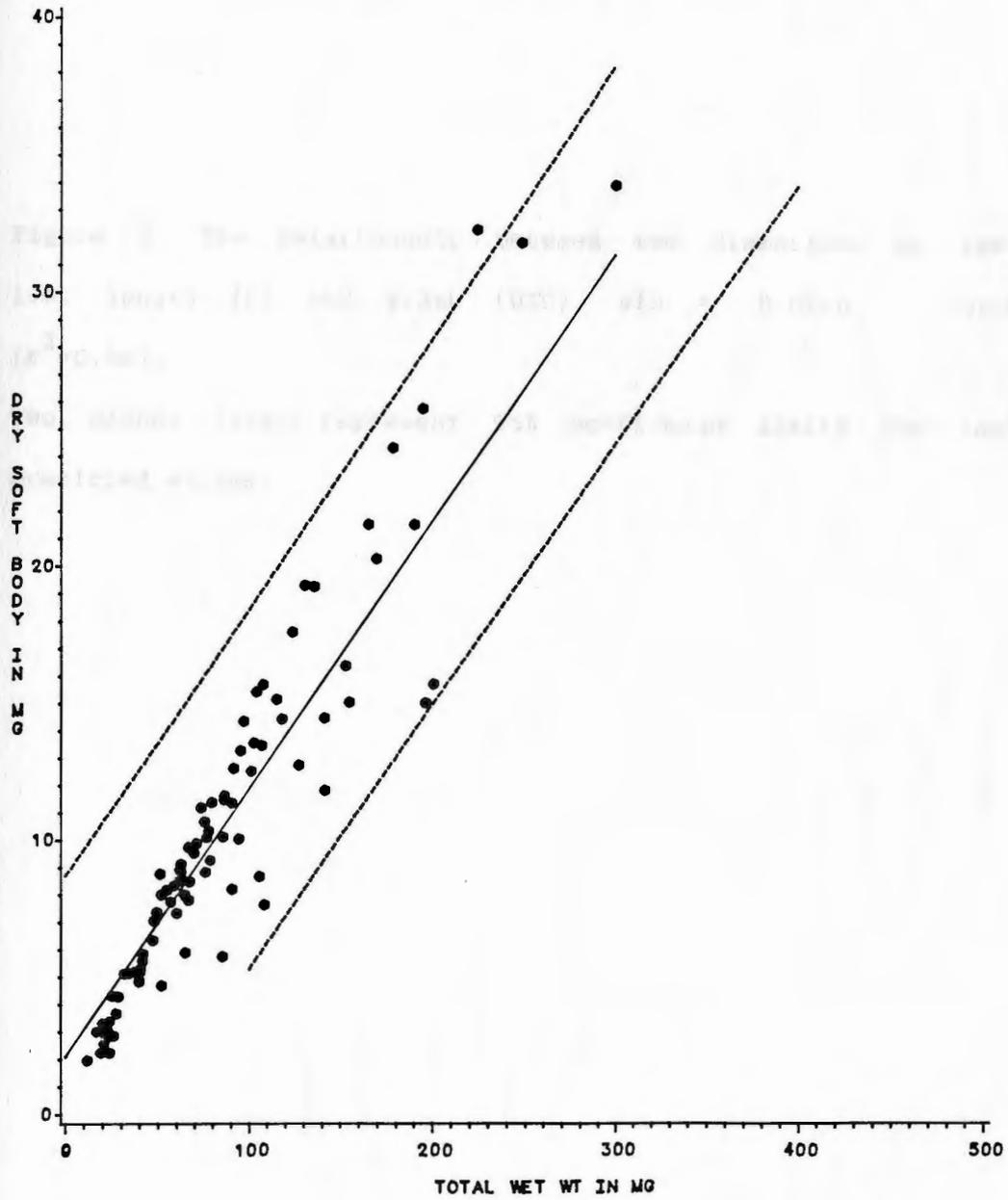
DRY TISSUE WEIGHT VS. TOTAL WET WT

Figure 7. The regression of the dry tissue weight (DW) versus the total wet weight (WW).  $DW = 0.116 WW + 2.020$  ( $r^2=0.87$ ).

Two dashed lines represent 95% confidence limits for individual predicted values.



# DRY TISSUE WEIGHT VS. TOTAL WET WT

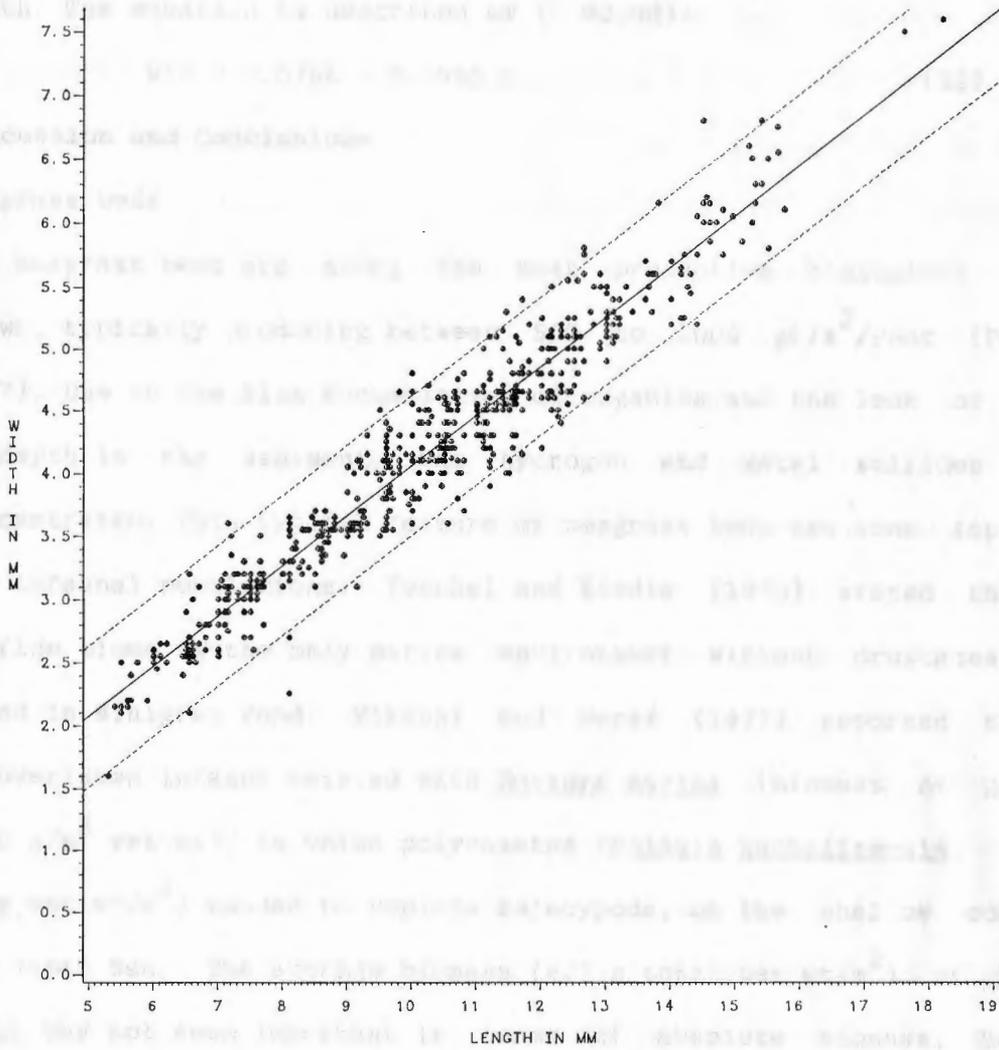


SHELL LENGTH VS. WIDTH

Figure 8. The relationship between two dimensions of the clam, i.e. length (L) and width (WID).  $WID = 0.0766 + 0.3985 L$  ( $r^2=0.90$ ).

Two dashed lines represent 95% confidence limits for individual predicted values.

### SHELL LENGTH VS. WIDTH



The total wet weight (WW, mg, including shells) is regressed with the dry tissue weight (DW, mg) by a straight line in Figure 7 ( $r^2=0.87$ ).

$$DW = 0.133 WW - 0.801 \quad (11)$$

Figure 8 shows the relationship between two dimensions, length and width. The equation is described as ( $r^2=0.90$ ):

$$Wid = 0.0766 + 0.3985 L \quad (12)$$

## Discussion and Conclusions

### Seagrass beds

Seagrass beds are among the most productive biological systems known, typically producing between 500 to 1000  $gC/m^2/year$  (Fenchel, 1977). Due to the high accumulation of organics and the lack of oxygen at depth in the sediment, the hydrogen and metal sulfides become concentrated. This typical feature of seagrass beds has some impact on the infaunal populations. Fenchel and Riedle (1970) stated that the sulfide biome is the only marine environment without crustaceans, as found in Ninigret Pond. Kikuchi and Peres (1977) reported that an impoverished infauna existed with Zostera marina (biomass of Zostera,  $3500 g/m^2$  wet wt), in which polychaetes (Spisula sachalinensis, biomass  $88 g$  wet wt/ $m^2$ ) tended to replace pelecypods, on the shallow coast of the Japan Sea. The average biomass ( $6.7 g$  total wet wt/ $m^2$ ) of Solemya velum may not seem important in terms of absolute biomass, but its average density ( $120/m^2$ ) is relatively high. Because of its unique nutrition based on sulfide oxidation (Cavanaugh, 1983; Felbeck, 1983; Chen, 1985b) and the active locomotion behavior (Morse, 1931; Stanley, 1971), the ecological role of the animal in system energetics may be of special interest.

## Growth equation

The von Bertalanffy equation has been widely used in fisheries (Gulland, 1983). Considering the physiological basis of the equation, it should be generally applicable to the animal kingdom, even though it may not fully describe a particular growth relation. The determination of growth through length-frequency analysis was based on the assumption that there was little year to year variation in growing conditions. The growth curves obtained estimate 'average' growth and in this sense they represent an integration of growth rates over slightly varying conditions.

Pauly and Calumpong (1984) have obtained useful information on the life history of Aplysiid gastropods from field data by utilizing equations pertaining to fish. Thiesen (1973) suggested that the von Bertalanffy equation may only be valid for Mytilus (mussels) about one-third of their maximum size, and for small mussels the Gomperts equation, using the logarithm of length, may provide a better fit. The ELEFAN programs have been used to estimate the growth curve of a gastropod (Dolabella auricularia) from two locations (Pauly and Calumpong, 1984). Two different values of parameters  $W_{\infty}$  (weight at infinity) and  $K$  were defined ( $W_{\infty} = 512$  g,  $K = 0.8$ ;  $W_{\infty} = 475$  g,  $K = 1.0$ ). They also obtained  $t_0$ , recruitment pattern and total mortality ( $Z=3.66$ ). Table 3 lists the  $L_{\infty}$  and  $K$  values in some bivalves.

Biologically, the parameter  $L_{\infty}$  in the equation is the maximum size that the animal would reach if no catch, predation or disease occurred;  $K$  describes the rate at which the animal approaches this limiting size. The length of Solemya velum was given from 1/2 to 1 inch (12.5 mm to 25

mm) in a number of seashell books (Abbott, 1954). Solemya population in Ninigret Pond had the maximum length of 18.5 mm which was less than the  $L_{\infty}$  value of 19.6 mm. The animals in Woods Hole, MA. are usually less than 20 mm (personal communication, collecting people in Marine Biological Laboratory, Woods Hole). The K (0.61) value indicates that Solemya velum is a relatively fast growing species. Generally, an animal of small size grows relatively fast. The hypothetical age  $t_0$  can not be derived from the field data because of the lack of information on size of animals less than 5 mm.

The seasonal growth oscillation was shown in the growth curve, which was generated from the growth equation (8) over a hypothetical life span (Figure 4). The animal grows slowly from December to April. In the first year class, the growth rate was 2.3 mm/month from August to November; and about 1 mm/month from December to April; then 1.1 mm/month from May until November. Entering the third year, the growth was much slower, approximately, 0.2 mm/month in the fast growth season.

#### Recruitment and life span

The estimated growth curve suggests that recruitment individuals enter the population around August. Most of the animals disappear from the population after they reach greater than 12 to 14 mm (Table 2 and Figure 3). Thus, the percentage of various sizes of animals indicates an average life span of two years (Figure 3). It would take six years or less for an individual (if it survived) to reach maximum size, which was recorded at 18.5 mm (Figure 4). Because of the lack of information on reproduction cycle and life history, I can not definitely indicate

either recruitment pattern or life span of the species. But the model does give a clue for further research on the species.

#### Mortality

The high total mortality ( $Z=2.63$ ) may be attributed to density effects, predation or environmental effects. The value of  $Z$  is also an estimate of natural mortality, since there is no commercial fishing on these small clams. The annual survival rate was estimated to be 7.2%. This survival rate applies to all age classes together; it may be lower for early age class individuals and higher for later age class individuals.

#### Chemoautotrophics

The sulfide-based chemoautotrophics is believed to be the major energy source for Solemya (Felbeck et al, 1983). This may provide the species a survival advantage over other bivalves in a sulfide-rich environment. Does this nutrition also change the growth mode of the animal? It would be of interest to conduct a comparative study of the growth pattern among comparable bivalves.

In summary, some of the methods developed from fish population dynamics have been applied to invertebrates, for example, the gastropod Dolabella auricularia (Pauly and Calumpong, 1984) and the protobranchia Solemya velum. These methods provide certain information about population characteristics of the animals.

## Literature cited

- Abott, R. T.: American Seashells, 541pp. New York: von Nostrand Reinhold  
1954
- Appeldoorn, R. S.: The growth and life-history strategy of the  
soft-shell clam, Mya arenaria L.. 115pp. Ph.D., University of  
Rhode Island, Kingston, Rhode Island (1980)
- Cavanaugh, C. M.: Symbiotic chemoautotrophic bacteria in marine  
invertebrates from sulphide-rich habitats. Nature, Lond. 302,  
58-61 (1983)
- Dare, P. J.: Settlement, growth and production of the mussel Mytilus  
edulis L. in Morecambe Bay. Fishery investigations. Ministry of  
Agriculture, Fisheries and Food, London, Series II, pp34 (1975)
- Drew, G. A.: Locomotion in Solenomya and its relatives. Science, N.Y.  
11, 171-172 (1900)
- Felbeck, H.: Sulfide oxidation and carbon fixation by the gutless clam  
Solemya reidi: an animal-bacteria symbiosis. J. Comp. Physiol.  
152(B), 3-11 (1983)
- Fenchel, T.: Aspects of the decomposition of seagrasses. In: Seagrass  
ecosystems, a scientific perspective, pp.123-145. ed. by C. P.  
McRoy and C. Hefferich. New York: Marcel Dekker 1977
- Fenchel, T. M. and R. J. Riedl: The sulfide system: a new biotic  
community underneath the oxidized layer of marine sand bottoms.  
Marine Biology 7, 255-268 (1970)
- Gulland, J. A.: Fish stock assessment, pp83-95. Paris: The Food and  
Agriculture Organization, the United Nations 1983

- Kikuchi, T. and Peres, J. M.: Consumer ecology of seagrass beds. In: Seagrass ecosystems, a scientific perspective, ppl47-185. ed. by C.P.McRoy and C.Helfferich. New York: Marcel Dekker (1977)
- Morse, E. D.: Observations on living Solenomya (velum and borealis). Biol. Bull mar. biol. lab., Woods Hole 25: 261-281 (1913)
- Pauly, D.: Gill size and temperature as governing factors in fish growth: a generalization on von Bertalanffy's growth formula. Ber. Inst. Meereskunde, Kiel, No.63, 156pp (1979)
- Pauly, D. and D. David: ELEFAN 1, a BASIC program for the objective extraction of growth parameters from the length-frequency data. Meeresforsch, Hamburg 28: 205-211 (1981)
- Pauly, D., D. David and J. Ingles: ELEFAN 1: user's instruction and program listing (Rev. 2). pp.1-28. Published by the International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines 1982a
- Pauly, D., D. David and J. Ingles: ELEFAN II: user's instruction and program listing. 67pp. ICLARM, Manila, Philippines 1982b
- Pauly, D. and H. Calumpong: Growth, reproduction and mortality of the sea hare Dolabella auricularia (Gastropoda: Aplysiidae) in the Central Visayas, Philippines. Mar. Biol. 79: 289-293 (1984)
- Pelseneer, P.: Contribution 'a 1' e'tude des lamellibranches. Arch. Biol. 11: 147-312 (1891)
- Reid, R. G. B.: Aspects of the biology of a gutless species of Solemya (Bivalvia: Protobranchia). Can. J. Zool. 58: 386-393 (1980)
- Reid, R. G. B. and F. R. Bernard: Gutless bivalves. Science, N.Y. 208: 609-610 (1980)

- Stanley, S. W.: Adaptations for burrowing in soft substrata. In:  
Relation of shell form to life habitats of the bivalves  
(Mollusca), pp.45-85. The Geological Society of America 1970
- Thiesen, B. F.: Growth and mortality of culture mussels in the Danish  
Wadden sea. Meddelelser fra Danmarks Fiskeri-og Havundersogelser,  
N. S., 6: 47-78 (1968)
- Thiesen, B. F.: The growth of Mytilus edulis L. (Bivalvia) from Disko  
and Thule district, Greenland. Ophelia 12: 59-77 (1973)

Manuscript II

**THE EFFECT OF HYDROGEN SULFIDE ON THE METABOLISM OF SOLEMYA VELUM  
(PROTOBRANCH BIVALVIA) AND SULFIDE OXIDATION ENZYMES IN GILL TISSUE**

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Index words: chemoautotrophy, sulfide, respiration, enzymes,

Solemya velum, symbiosis, gill

Suggestion for abbreviated title: respiration and sulfide oxidation

of Solemya velum

**Abstract.** The presence of sulfide stimulated and enhanced the respiratory rate of Solemya velum from Ninigret Pond, Charlestown, Rhode Island. The oxygen consumption of whole animals with and without 0.5 mM sulfide indicated that the respiration ( $65.2 \pm 11.2$  ul  $O_2/g$  total wet weight (shell included)/hr) accounted 56.8% of the total oxygen consumption ( $115.4 \pm 18.6$  ul  $O_2/g/hr$ ), while chemoautotrophic symbionts consumed the rest 43.2% ( $50.2$  ul  $O_2/g/hr$ ). The enzymatic activities of sulfide oxidation were assayed in the gill tissues of the animal. The maximal activities ( $V_{max}$ ) were 4.7 umoles (substrate converted to product) per gram fresh gill per min for thiosulfate transferase, 2.2 umoles/g/min for adenylylsulfate reductase, and 5.0 umoles/g/min for sulfate adenylyl transferase. Approximately 5% of  $V_{max}$  was suggested to be a reasonable activity of sulfide oxidation under intracellular conditions.

## Introduction

Living systems consisting of prokaryotic endosymbionts with metabolism based on sulfur have been discovered among a number of marine bivalves (Cavanaugh et al., 1981; Felbeck, 1981; Felbeck et al., 1981; Felbeck and Somero, 1982; Cavanaugh, 1983). Schematically, this type of metabolism consists of the following pathways: sulfide oxidation, carbon dioxide fixation, nitrate reduction, and organic translocation and oxidation (Felbeck et al., 1983). Three diagnostic enzymes involved in sulfide oxidation are characteristic in many marine symbiotic animals from sulfide-rich habitats (Felbeck et al., 1981; Fisher and Hand, 1984). All were originally reported from sulfur-oxidizing bacteria (Kelly, 1982). They are thiosulfate sulfurtransferase (EC 2.8.8.1), APS reductase (adenylylsulfate reductase, EC 2.7.1.19), and sulfate adenylyl transferase (EC 2.7.7.4).

The chemoautotrophic nutrition based on sulfide oxidation has been found common in diverse habitats with a rich source of sulfide. In the vicinity of deep-sea hydrothermal vents, the environmental factors affecting animal metabolism are more or less unique and homogeneous. These include high pressure, continuous darkness, stable temperature, and constant supply of reduced sulfur. The organisms in those habitats may have unique metabolic activities, such as respiration rate, sulfur enzyme activities, etc. On the other hand, the shallow water environments show many diverse and changing features, e.g. normal pressure, seasonal photoperiod, fluctuating temperature, and the availability of various kinds of organic foods, etc. Many aspects of

physiological ecology in shallow environments have been comparatively well studied, for example, the seasonal variation associated with temperature effects on metabolism of marine and freshwater bivalves (e.g. Shumway, 1983).

There are two kinds of factors which modify metabolism. One is related to the physiological or genetic constitution of the animal; the other is related to an ever changing environment (Hoar, 1983). One important factor of the former kind is body size; the second category includes the effect of substrate level, in this case, sulfide.

A shallow water bivalve, Solemya velum, was chosen to study the effect of sulfide on metabolism, and the activities of the sulfide-oxidizing enzymes. The percentage of oxygen consumption due to chemoautotrophics will be estimated. By making a few physiologically reasonable assumptions, the capacity of the organism to utilize reduced sulfur as an energy source will be estimated. The potential flux of sulfur through the species will be calculated. A comparison of energy metabolism in various vent animals, Solemya and Mercenaria was based on the work of Hand and Felbeck (1983).

## **MATERIAL AND METHODS**

Animals were collected January to March 1985 for oxygen consumption studies, and April to June for the enzymatic essays, from Hall Point in Ninigret Pond, Charlestown, Rhode Island. In most cases, the animals were measured within two days. The water temperature was 0 °C (Jan.), 7 °C (Mar.), 10 °C (Apr.) and 15 °C (Jun.), respectively in the field. The animals were kept in sea water from their habitats in a cold box, in which temperature gradually went up to 15-20 °C in 24 hours. Salinity was approximately 28-32 parts per thousand. Six to eight animals were used in each experiment. The length of the animals ranged from 10 to 15 mm.

A Gilson differential respirometer was used to measure the oxygen uptake rate in the presence or absence of hydrogen sulfide. The respirometer is a constant pressure system. The change in volume due to respiration is compensated by measured movement of a plunger in the enclosed space (Gilson, 1963). Three experiments were conducted at 15 °C and one at 20 °C (See Appendix A for detailed experimental conditions). Hydrogen sulfide was made from sodium sulfide and diluted in filtered sea water. Crystal form of sodium sulfide was weighed by analytical balance. The seawater was purged with nitrogen gas to get rid of oxygen before the addition of sulfide. The solution was added to the vessels from side-arms. The initial concentration of sulfide in vessels was brought to 0.1, 0.5 and 0.8 mM respectively for three experiments. The initial sulfide was not known in one experiment. The three treatments were animal plus sulfide, animal only and sulfide only. Each treatment had three or four replicates. Four separate experiments were conducted.

The possibility that sulfide oxidizing bacteria may exist outside of the animal soft-body or on the shells was not tested, but Cavanaugh (1983) has shown that the abundant existence of intracellular symbionts, which were sulfur-based chemoautotrophs.

Biochemical pathway of sulfide oxidation and their catalytic enzymes are presented in Figure 1. Three enzymatic assays were performed using fresh gill tissue which was homogenized in the buffer of appropriate pH (described below) with a Wheaton ground glass homogenizer. The concentration of the extract of fresh gill tissue was 50 mg/ml. The crude homogenates were centrifuged at 5000 g for 15 minutes at 0°C and the supernatant fractions were used for the assays (Fisher and Hand, 1984).

Sulfate adenylyl transferase was assayed according to the method of Felbeck (1981). The homogenizing medium consisted of 0.1 M triethanolamine-HCl buffer (pH 7.3). The assay medium consisted of 0.1 M tris-HCl buffer (pH 7.3), 2.5 umoles magnesium acetate, 25 umoles glucose, 25 umoles pyrophosphate, 0.05 umoles  $\text{NADP}^+$ , 0.05 umoles adenosine phosphosulfate (APS), 2.5 units hexokinase, 1.25 units glucose-6-phosphate dehydrogenase, and 1 umole  $\text{P}^1, \text{P}^5$ -di(adenosine-5') pentaphosphate in a total volume of 3 mls. The substrate (APS) concentration varied from 0.05 to 0.5 mM.

The method described by Peck et al. (1965) was used for the assay of APS reductase. Gill tissue was homogenized in 0.3 M tris-HCl buffer (pH 8.0). The 3.0 ml reaction mixture contained 300 umoles tris-HCl buffer (pH 8.0), 10 umoles AMP, 4 umoles sodium ferricyanide, and various amounts of sodium sulfite. The substrate, sodium sulfite, varied from

1.0 to 12.0 umoles. The reduction of the ferricyanide ion was monitored at 410 nm on a spectrophotometer. The molar absorptivity of oxidized ferricyanide in distilled water was empirically determined to be  $877 \text{ M}^{-1} \text{ cm}^{-1}$  at 410 nm. The blank rate of the reaction was corrected by the control, which contained all components except the enzyme extract.

Thiosulfate sulfurtransferase, which participates in the oxidation of thiosulfate to sulfite, was assayed following the method of Smith and Lascelles (1966). In the original method, the 3 ml reaction mixture consisted of 300 umoles tris-HCl buffer (pH 8.7), 150 umoles  $\text{Na}_2\text{S}_2\text{O}_3$ , 200 umoles half-neutralized sodium cyanide (NaCN:HCl=2.5:1 molar ratio), 0.5 umole 2, 6-dichlorophenol-indophenol (DCIP dye), and 0.25 mg phenazine methosulfate (PMS). The content of the DCIP was modified to 0.005 umoles instead of 0.5 umole in the reaction mixture, to avoid a high background rate of chemical reaction. Gill tissues were homogenized in 0.3 M tris-HCl buffer (pH 8.7). The reduction of DCIP dye was followed at 600 nm on a spectrophotometer. The background rate of the reduction of DCIP was corrected by the control, which had all chemicals except the enzyme extract.

## RESULTS

### Respiration

The weight specific metabolic rates of Solemya velum varied from 0.031 to 0.311 ul O<sub>2</sub>/mg total wet wt/hr. The total wet weight of the animals, including shell, ranged from 105 to 278 mg. The log-log regression of the weight specific respiration rates and weights was a negative linear correlation. Following Sutcliff's notation (1984),

$$\log R_s = 0.93 - 0.38 \log W \quad (r^2=0.86) \quad (1)$$

where  $R_s$  = weight-specific metabolic rate,  $W$  = weight.

Animals showed a higher respiratory rate in the presence of hydrogen sulfide. Appendix B indicates the calculating procedure and lists the results obtained from four experiments using the Gilson respirometer, in which twenty eight animals were examined. The highest respiratory rate, which was observed when animals were exposed to 0.8 mM H<sub>2</sub>S at 20°C, was  $215.96 \pm 51.4$  ul O<sub>2</sub>/g/hr. Because many other factors like body size, season, nutritional condition and sex, etc, would affect the metabolic rates (Newell, 1975), the results from different experiments were not comparable in this study. The variation of the respiratory rates in the absence of H<sub>2</sub>S were high, especially in experiments 1 and 2. The reason for this high variation may be that the bivalves often remained closed for the entire measurement, a major difficulty in working with bivalves. But for all the experiments, animals always maintained a high metabolic rate in the presence of sulfide. Therefore, they all showed a stimulation and enhancement of animal-mediated sulfide oxidation.

The comparisons were done within the same experiment. Experiment 4 will be used for the comparison because of low standard deviations among

the replicates. The low variation in this experiment may have been due to the fact that field and laboratory temperature were not as different as in the other three experiment. Figure 2 shows the weight-specific cumulative oxygen consumptions (ul/g) of three experimental treatments, i.e. animal with sulfide, animal only and sulfide only, from the experiment 4 with the Gilson respirometer. The mean and standard deviation of each treatment were calculated from four replicates.

In Table 1, the variation among four replicates and the differences between the paired variables, i.e. respiratory rates in the presence and absence of sulfide over the whole time period, were calculated; and a t-test was run to see whether there was a significant difference between the two treatments. A t-statistic and a probability value for the hypothesis that the mean differences was equal to zero were listed in Table 1. The confidence level of the t-test was set to 0.05, therefore the differences between two treatments were all significant.

The biochemical pathway of sulfide oxidation in the chemoautotrophic symbionts is similar to that in free chemoautotrophic bacteria, such as Thiobacillus and Thiomicrospira (see Figure 1; Felbeck et al., 1983; Kuenen and Beudeker, 1982). The experimental measurements of enzymatic activity are presented in Appendix C. The enzyme, thiosulfate sulfurtransferase, catalyses the oxidation from thiosulfate ( $S_2O_3^{2-}$ ) to sulfite ( $SO_3^{2-}$ ). This enzyme may not be as reliable an indicator of sulfide metabolism as the other two, because the assay method did not give linear reaction rates. A similar problem was found in Lucina floridana (Fisher and Hand, 1984). The activity of the enzyme was observed in six trials. The  $V_{max}$  of 4.73 umoles /g/min (substrate

converted to product) was estimated from the linear portion of the reciprocal plot based on three trials, and is presented with less confidence than APS reductase and ATP sulfurylase.

APS reductase catalyses the production of adenosine phosphosulfate (APS) from AMP and sulfite. A double reciprocal plot gives a  $V_{max}$  of 2.19 umoles/g/min and a  $K_m$  value of 2.42 mM for sulfite (Figure 3,  $r^2=0.965$ ).

Sulfate adenylyl transferase phosphorylates adenosine phosphosulfate (APS) to form ATP and sulfate. Figure 4 shows the double reciprocal plot ( $r^2=0.919$ ).  $V_{max}$  and  $K_m$  values are 5.02 umole/g/min and 0.463 mM (APS), respectively. A comparison of enzymatic activities in a number of well studied species is presented in Table 2.

Table 1. The effects of hydrogen sulfide on the metabolic rates of Solemya velum in Gilson respirometer. The experimental conditions and the original data are listed in the Appendixes A and B respectively.

Exp. no.	Temperature (°C)		H <sub>2</sub> S (mM)	Metabolic rate * (ul O <sub>2</sub> /g/hr)		Prob>t***
	lab	field		H <sub>2</sub> S	no H <sub>2</sub> S	
1	15	0	0.1	108.7	38.0	0.0001
2	15	4	**	41.8	33.7	****
3	20	5	0.8	216.0	170.3	0.0455
4	15	8	0.5	115.4	65.2	0.0007

Note: \*the rates were averaged over two or three hours.

The unit is ul O<sub>2</sub> per gram (shell-included total wet weight) per hour.

\*\*failed to estimate the H<sub>2</sub>S concentration.

\*\*\*the t-test of the difference between two means from two treatments.

\*\*\*\*two numbers are not sufficient to run the test.

Table 2. Enzyme activities\* of sulfide oxidation in marine animals from sulfide-rich habitats.

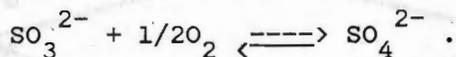
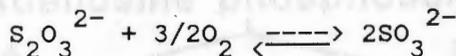
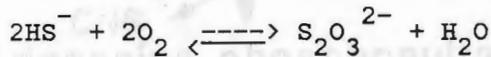
Habitat & species	Thiosulfate transferase	APS reductase	+ ATP sulfurylase	Reference
Hydrothermal vent				
<u>Riftia pachyptila</u>	7.6	7.6	74.0	Felbeck et al., 1982
Sewage outfall				
<u>Solemya reidi</u>	0.7	4.1	77.0	same.
Seagrass Beds				Fisher & Hand, 1984.
<u>Lucina floridana</u>	++	2.1	2.9	
<u>Solemya velum</u>	4.7	2.2	5.0	This paper.

Note: \*Enzyme activities are expressed as umoles of substrate converted to product per min per gram fresh weight of tissue, at a measurement temperature of 20-25 °C.

+i.e. sulfate adenylyl transferase.

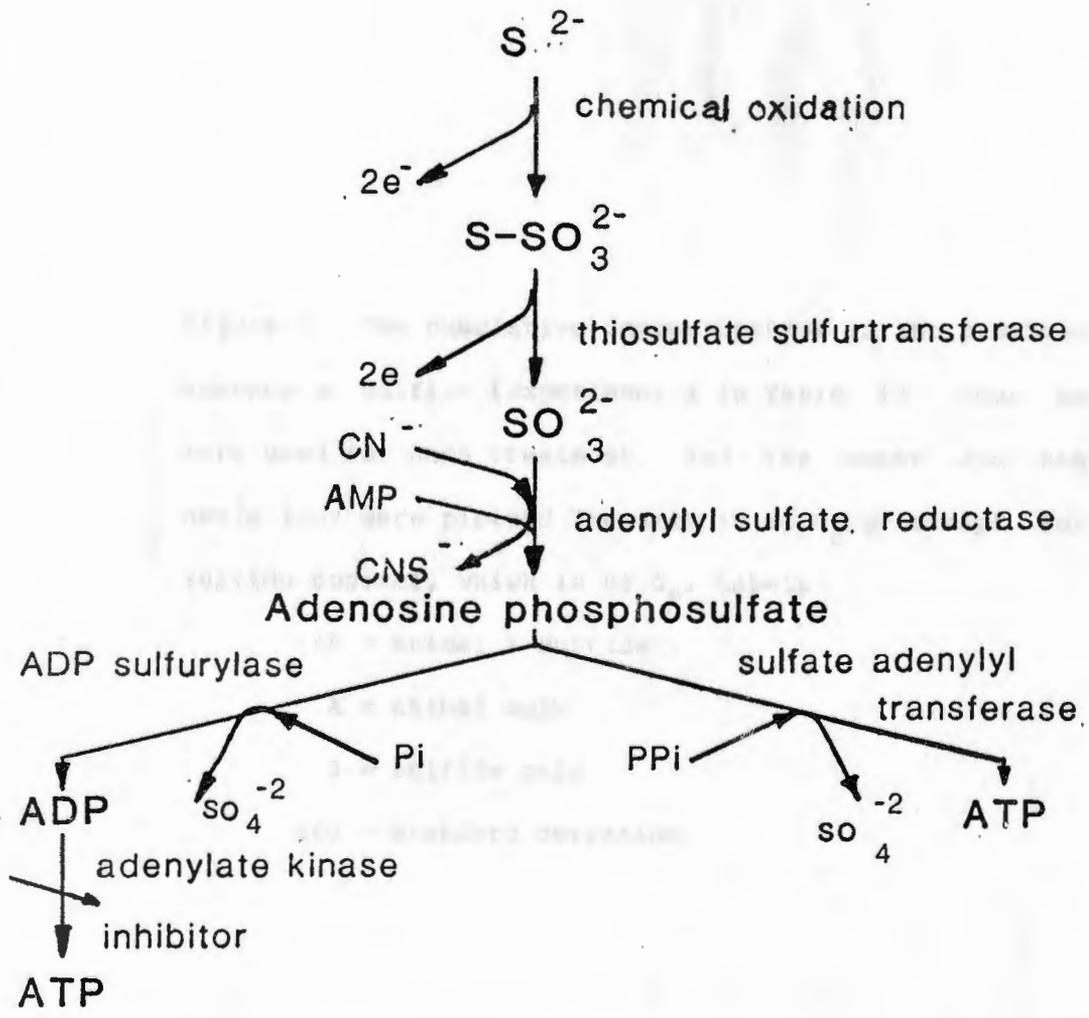
++the enzyme activity was detected, but quantitative measurement was not reported.

Figure 1. The biochemical pathways of sulfide oxidation in chemoautotrophic bacteria (based on Peck, 1960 and 1968). Only diagnostic enzymes are indicated. The enzymatic pathways may be expressed as following reactions:



Note: The first two reactions possibly take place by inorganic ways or by biological ways as assumed here.

See the text for more detailed discussion.



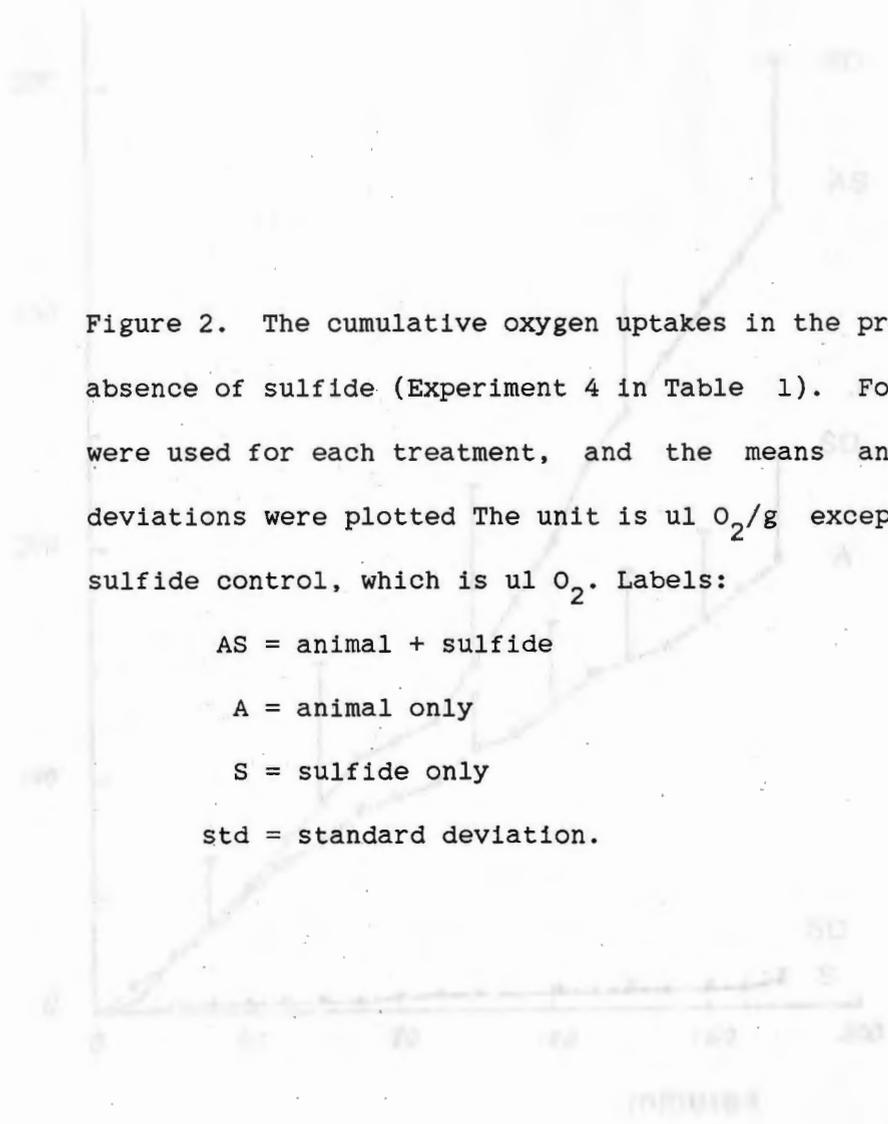


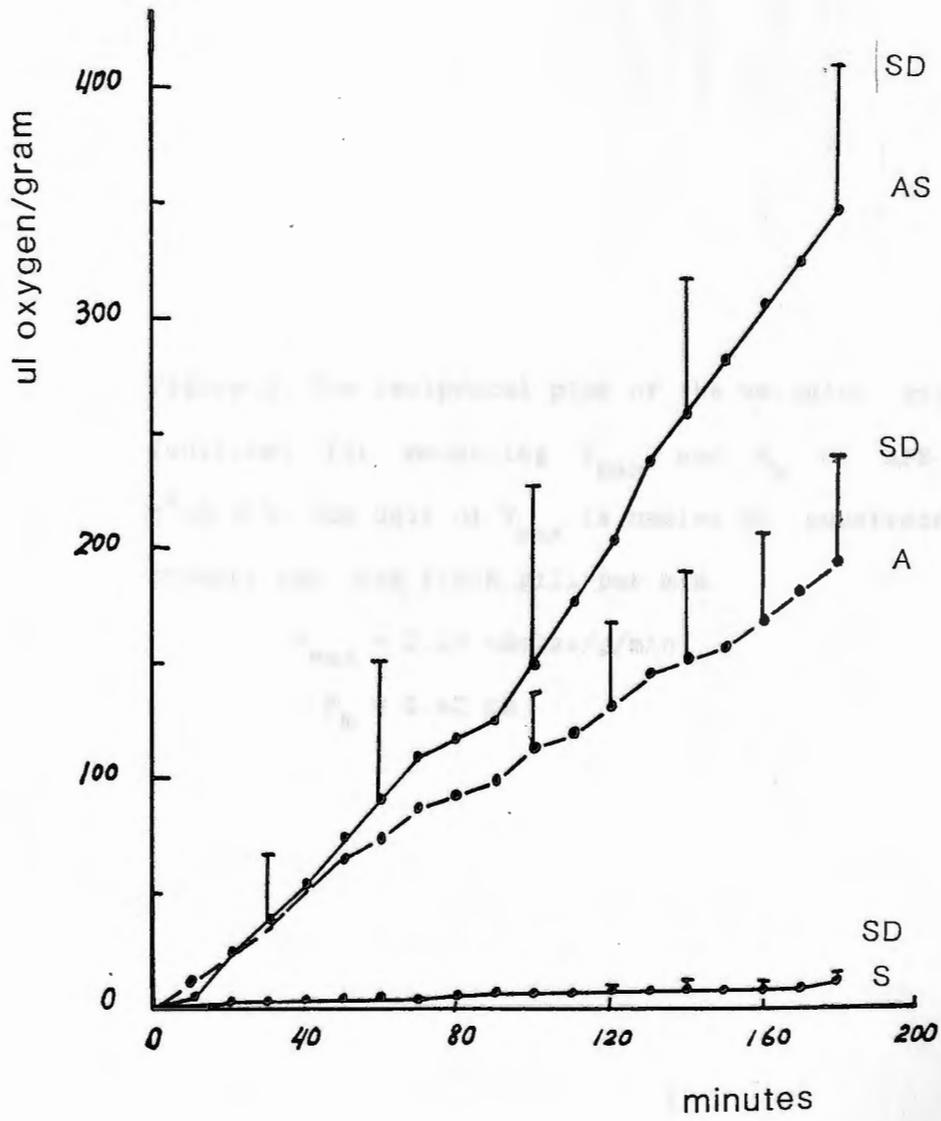
Figure 2. The cumulative oxygen uptakes in the presence and absence of sulfide (Experiment 4 in Table 1). Four animals were used for each treatment, and the means and standard deviations were plotted. The unit is  $\mu\text{l O}_2/\text{g}$  except for the sulfide control, which is  $\mu\text{l O}_2$ . Labels:

AS = animal + sulfide

A = animal only

S = sulfide only

std = standard deviation.



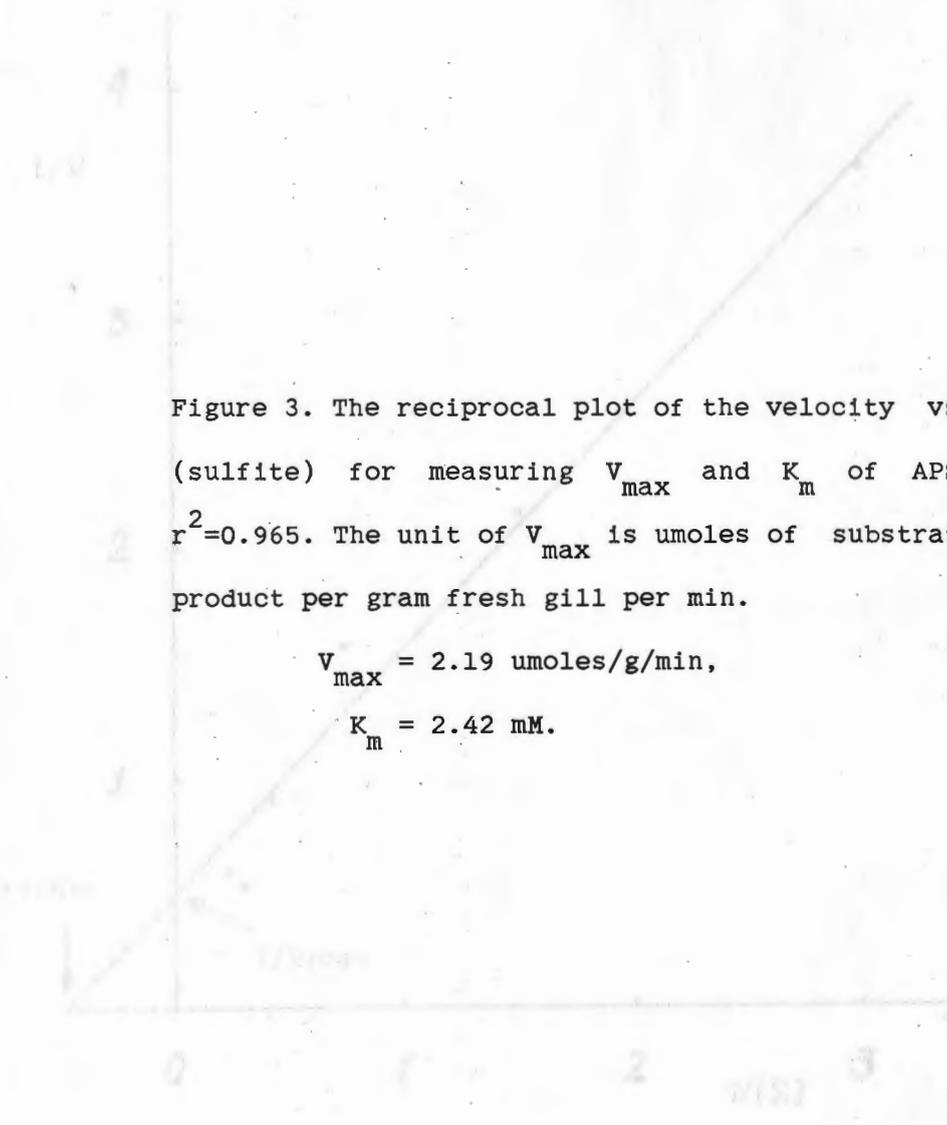
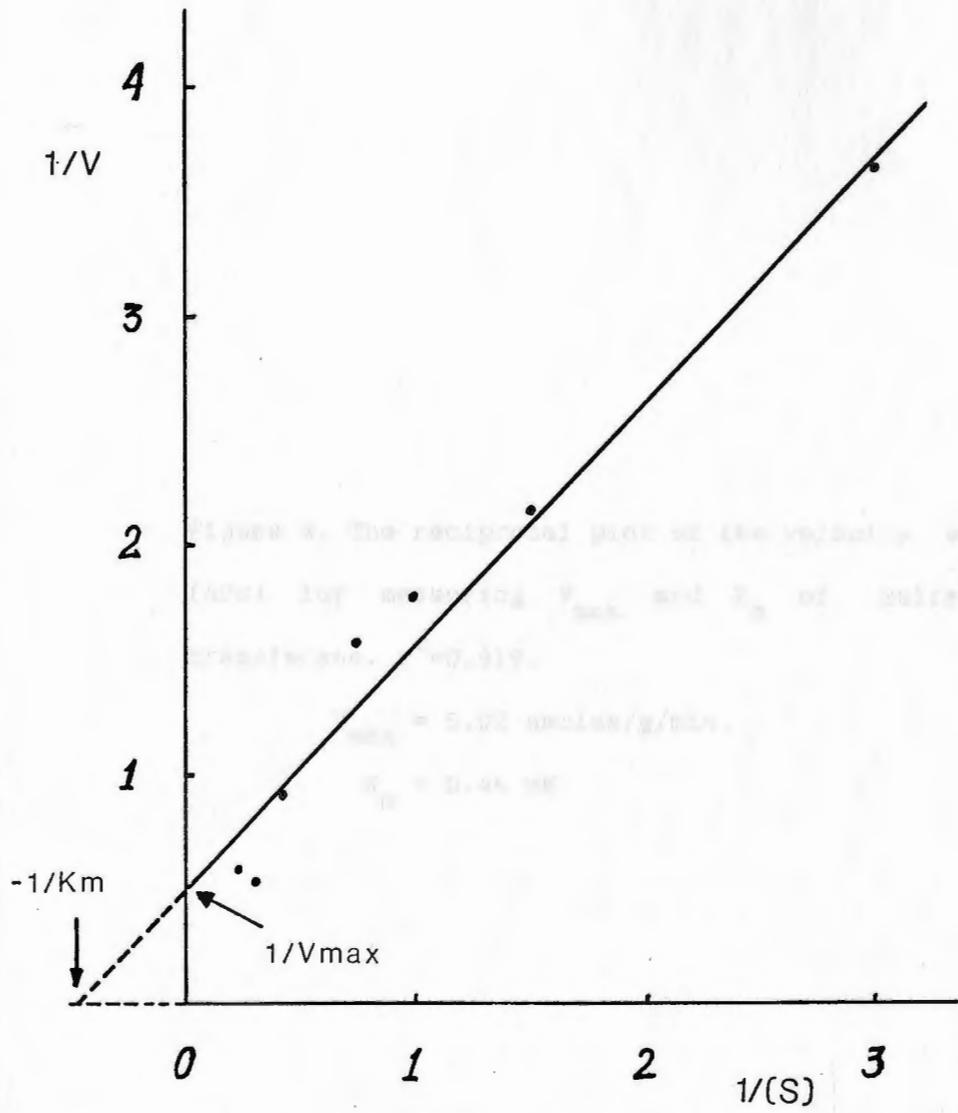


Figure 3. The reciprocal plot of the velocity vs. substrate (sulfite) for measuring  $V_{\max}$  and  $K_m$  of APS reductase.  $r^2=0.965$ . The unit of  $V_{\max}$  is umoles of substrate converted product per gram fresh gill per min.

$$V_{\max} = 2.19 \text{ umoles/g/min,}$$

$$K_m = 2.42 \text{ mM.}$$



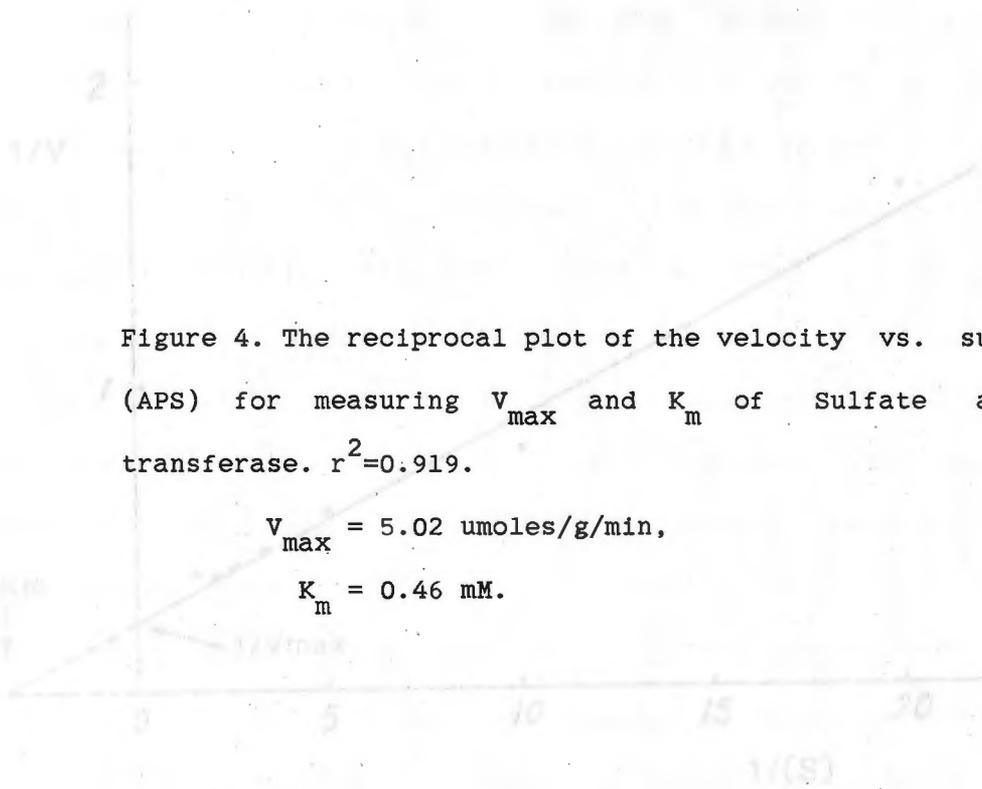


Figure 4. The reciprocal plot of the velocity vs. substrate (APS) for measuring  $V_{\max}$  and  $K_m$  of Sulfate adenylyl transferase.  $r^2=0.919$ .

$$V_{\max} = 5.02 \text{ umoles/g/min,}$$

$$K_m = 0.46 \text{ mM.}$$

Discussion

Biological factors involved in the evolution

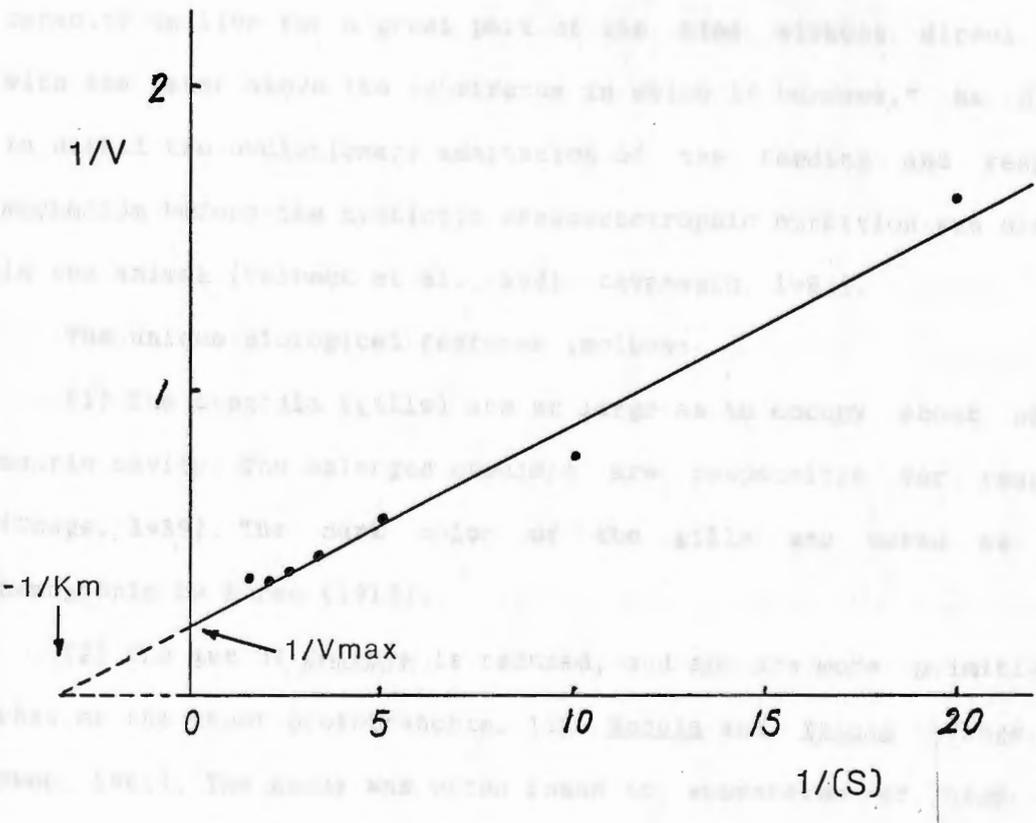
All members of the Phrynosoma are specialized for surviving in the desert. They are characterized by their ability to store fat in their tails and to absorb water from the soil. They are also able to tolerate high temperatures and to survive in the desert for long periods of time.

The tail is a great part of the body which is used for protection. It is also used for storage of fat. The tail is also used for defense. It is used to store fat and to absorb water from the soil. It is also used to tolerate high temperatures and to survive in the desert for long periods of time.

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## Discussion

### Biological features related to metabolism

All species of the Protobranchia are specialized for burrowing into and moving through a soft substratum (Yonge, 1939). Solemya is especially adapted for the life under the surface, and its shell is modified for its habitat, e.g. it has an elongated shell with smooth surface. As Yonge (1939) pointed out, "this genus appears unique in its capacity to live for a great part of the time without direct contact with the water above the substratum in which it burrows." He discussed in detail the evolutionary adaptation of the feeding and respiratory mechanism before the symbiotic chemoautotrophic nutrition was discovered in the animal (Felbeck et al., 1981; Cavanaugh, 1983).

The unique biological features include:

(1) The ctenidia (gills) are so large as to occupy about half the mantle cavity. The enlarged ctenidia are responsible for respiration (Yonge, 1939). The dark color of the gills was noted as due to hemoglobin by Morse (1913).

(2) The gut of Solemya is reduced, and appears more primitive than that of the other protobranchia, like Nucula and Yoldia (Yonge, 1939; Owen, 1961). The genus was often found in substratum of high organic content, e.g. sewage outfalls and eelgrass beds (Stempell, 1899; Reid, 1980; Reid and Bernard, 1980).

(3). The Solemyidae probably represents one of the first lines along which the early Lamellibranchia evolved. They are a very old group appearing in the Devonian, and of the six genera only Solemya persists. It is surprising that any species of a group, which evolved so early,

and along such specialized lines, have survived.

Based on the experimental results, some of physiological and biochemical functions of Solemya velum will be discussed, and correlated with the above unique biological features. Symbiosis may be responsible for the unusual features in the genus.

#### Respiratory response to hydrogen sulfide

The relation between animal size and metabolic rates is a complex allometric adaptation (Hoar, 1983). It is well known from the studies of Zeuthen (1953) and Hemmingsen (1960) that the slope of a common regression line relating log metabolism to body weight of protozoans through to the larger poikilotherm metazoans is approximately -0.25. This slope varies from animal to animal, for example, in Mercenaria sp., the slope was -0.34 (Loveland and Chu, 1969); and the slope of a freshwater limpet was -0.27 (Berg et al., 1962). The slope of Solemya was -0.38 (Eq.(1)). The negative slope shows that younger or smaller animals have a higher rate of oxygen consumption on a weight-specific basis than older or larger ones. The relationship may be explained by the differences in the rate of enzyme production with increased size, variation in the cell surface to volume relationship (as proposed by von Bertalanffy, 1957), and in larger or old animals a greater production of tissues that have low metabolic rates (Prosser, 1975).

In another protobranch bivalve Nucula turgida, Wilson and Davis (1984) found the absolute rate of oxygen consumption in the range of temperature between 5-35°C was greater in summer-conditioned animals than in winter-conditioned ones. The oxygen consumption of winter

conditioned animal (Nucula) at 5°C was about 67% of that at 15°C. The animals in this study may be considered as winter conditioned because they were sampled at field temperatures of 0-7°C. Therefore, their respiration at situ temperature may be approximately 60% of the measurements at 15°C. Even so, the measured temperature impact on each experimental animal was not estimated.

The presence of hydrogen sulfide stimulated and enhanced the metabolic rates of Solemya velum (Table 1). Hydrogen sulfide, in general, is a toxin to many organisms. It blocks the electron transfer system in the respiratory chain. Normally, Mollusca would simply close their shells and slow down metabolism (Hammen, 1979). The possibility that there may have been sulfide-oxidizing bacteria on the outside of the animal soft-body or shells has not been excluded in this particular research, but it does not seem likely that these were abundant enough to have influenced the results. The outsides of the shells of Solemya were quite smooth and clean, and were further washed in clean sea water before measurements were begun. Cavanaugh (1983) observed rod-shaped intracellular symbionts (with characteristics of prokaryotic bacteria) in the gill tissue of Solemya velum; and that the presence of sulfide enhanced the carbon dioxide fixation in the gill tissues by 10 times. Neither mantle nor foot has shown the same effect. It seems reasonable to believe that the difference of the respiratory rates in the presence and absence of H<sub>2</sub>S is due to symbiotic chemoautotrophics in the gill tissues of the animal.

The respiratory rates were different among the four experiments. Reasons could include differences in season, size, sex, etc. Experiment

3 was conducted at 20 °C, in which the respiration both with or without sulfide were two or three times higher than the other three (at 15 °C). In experiment 2, low respiration was noted for both treatments, with and without sulfide. The respiration with sulfide was lower than that without sulfide for the first 90 minutes, and then the respiration with sulfide exceeded the other treatment for the last 30 minutes. This single experiment does not give a conclusive evidence of sulfide metabolism, but it has suggested that sulfide stimulated the respiration of the animals.

The impacts of temperature and sulfide on metabolism were also reported in Solemya reidi (McMahon and Reid, 1984). The weight specific oxygen uptake rate of Solemya reidi was reported to be inhibited below 6°C and above 18 °C; and was normal at 1.0 mM hydrogen sulfide, elevated at 0.5 mM, and nil above 2.5 mM.

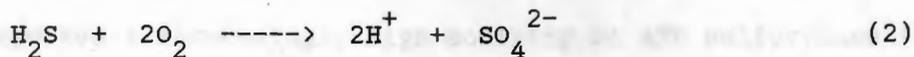
A comparison of sulfide-enhanced respiration was made within Experiment 4 (Table 1). The statistical tests show significant differences at a 0.05 confidence level between treatments in this experiment (t-test in Table 1). The animal respiration without sulfide ( $65.2 \pm 11.21$  ul O<sub>2</sub>/g/h) accounts for 56.8% of total oxygen consumption with sulfide  $115.4 \pm 18.6$  O<sub>2</sub>/g/h). Thus, the chemoautotrophic symbionts consumed 43.2% of the total oxygen, i.e. 50.2 ul O<sub>2</sub>/g/hr.

$$50.2 \text{ ul O}_2/\text{g/hr} = 2.24 \text{ umole O}_2/\text{g/hr}.$$

The free energy for the reduction from CO<sub>2</sub> to glucose is 118 KCal/mole, the energy from oxidation of H<sub>2</sub>S to SO<sub>4</sub><sup>2-</sup> is 160 KCal/mole. Thus, the energy ratio (Carbon incorporated/Reductant oxidized) is 0.738 (Kuenen and Beudeker, 1982). For comparison, if the energy coupling

efficiency between sulfide oxidation and CO<sub>2</sub> fixation were 100%, the oxidation of every mole of sulfide would fix 1.36 mole CO<sub>2</sub>.

According to the overall equation,



the oxidation of each mole of sulfide needs two moles of oxygen. Therefore (at 100% efficiency), the consumption of 2.24 umole O<sub>2</sub>/g/hr, would fix:

$$(2.24/2) * 1.36 = 1.52 \text{ umoles CO}_2/\text{g total wet wt/hr.}$$

Cavanaugh (1983) reported 4.5 umoles/g gill tissue/hr carbon fixation rate in Solemya velum in the presence of 0.2 mM sodium sulfide. In average, gill tissue weighed about 25% of total wet weight (including shell) (Chen, 1985a). Therefore, Cavanaugh's result is equivalent to 1.1 umoles CO<sub>2</sub>/g total wet wt/hr. It should be noted that Cavanaugh used a different method (the uptake of labeled carbon dioxide) and the gill tissues for her estimations, while the whole live organisms were used in this research. Of course, the assumption of 100% energy coupling efficiency is not realistic. A 20% efficiency was used for the energy coupling in thiobacilli (chemoautotrophic free bacteria) by Kuenen and Beudeker (1982). Thus, the CO<sub>2</sub> fixation rate would be 0.30 umoles/g total wet wt/hr at a 20% efficiency, which is about one order magnitude lower than Cavanaugh's estimate (1.1 umoles CO<sub>2</sub>/g total wet wt/hr). This may be due to the fact that Cavanaugh conducted her experiment in vitro.

## Enzymatic activities in sulfide oxidation

The enzymatic activities of sulfide oxidation in Solemya velum were in the range of values reported by other workers (Table 3). Felbeck et al (1981) reported a surprisingly high activity of ATP sulfurylase (77.0 umole/g fresh tissue/min) in Solemya reidi. The results of this study were very close to those from another bivalve, Lucina floridana (Fisher and Hand, 1984; see Table 3). Generally, the deep-sea vent animals, such as Riftia pachyptila (Pogonophora) and Calyptogena pacifica (Mollusca), have higher enzymatic activities. The hydrothermal vents provide a high, constant supply of hydrogen sulfide (Edmond et al, 1982), which serves as a major, if not the only, source of energy for the living systems. In shallow water, the percentage of energy expenditure derived from reduced sulfur is not clear, but may not be so large. Multi-nutritional modes may exist in shallow water species, such as Solemya velum, as Yonge (1939) argued almost fifty years ago but without evidence.

In the pathway of sulfide oxidation (Figure 1), the number of enzymes involved, other than three diagnostic enzymes, is not clear. It is not known whether there is any enzyme catalyzing the first step from  $S^2$  to  $S_2O_3^{2-}$ . A study of sulfide oxidation in seawater suggested that the initial oxidation was simply a chemical reaction forming thiosulfate (Almgren and Hagstrom, 1974). Biological oxidation was subsequently responsible for the further oxidation of thiosulfate to sulfate (Tuttle and Jannasch, 1973). Thus, the above calculation and comparison are not intended to provide a true picture of this energetics, but rather to explore one possibility in an effort to find the actual pathway.

Adenosine phosphosulfate (APS) is a central intermediate in the

pathway (Kelly, 1982). It may be reasonable to assume that APS reductase is a key enzyme which controls the rate of the entire reaction. Also, the activity of APS reductase is the lowest among three enzymes in this study. For APS reductase,

$$V_{\max} = 2.2 \text{ umoles/g gill/min} = 0.029 \text{ mole H}_2\text{S/animal/yr,}$$

given that the average size of experimental animals has 0.025 g fresh gill tissue in the total wet weight of 0.1 g (including shell). The oxygen consumed in the sulfide oxidation is ( $O_{\text{sulfide}}$ ),

$$50.2 \text{ ul O}_2/\text{g total wet wt/hr} = 0.0014 \text{ mole O}_2/\text{animal/yr,}$$

assuming a normal size animal weighs 0.1 g and in which the rate remains constant over the year. Two moles of oxygen are needed to oxidize one mole of sulfide. In other words, the oxygen consumption based on Gilson respirometer could only account for

$$0.5 \times 0.0028 = 0.0007 \text{ mole H}_2\text{S per animal per year. Obviously, } V_{\max}$$

does not represent the real physiological activity because the substrate concentration in the animal is usually close to or lower than  $K_m$ , in which  $1/2 V_{\max}$  is shown. And,

$$0.0007/0.029 = 0.024 = 2.4\%,$$

which suggests approximately 2.4%  $V_{\max}$  would represent the limit of physiological activity.

## Comparison of energy metabolism

As Hand and Somero (1983) concluded: "In general, the enzymatic activities found in the tissue of the vent animals were qualitatively and quantitatively similar to those of phylogenetically related shallow-living marine species, suggesting that the types of energy pathways and the potential flux rates through these pathways were similar in both groups." The important energy pathways include glycolysis, the citric acid cycle, and the electron transport system.

Many studies show that the activities of enzymes of energy metabolism correlate well with rates of oxygen consumption, even in organisms having widely different metabolic capacities, such as deep sea animals (Childress and Somero, 1979; Siebenaller and Somero, 1982).

The activity of Cytochrome C oxidase, which was found in the foot of Solemya, indicates a high aerobic capacity (Hand and Somero, 1983). Phosphofructokinase and pyruvate kinase are indicators of total glycolytic flux potential. The high activities of malate dehydrogenase may be indicative of high activities for the type of anaerobic scheme found in many invertebrates.

Based on the above discussion, we may conclude that the unique character of Solemya is the pathway of sulfide oxidation, which generates energy for organic synthesis, while its energy metabolism remains similar to other bivalves, such as Mercenaria (Hand and Somero, 1983).

## Literature cited

- Almgren, T. and I. Hagstrom. 1974. The oxidation rate of sulfide in seawater. Water research 8: 395-400.
- Bertalanffy, L. von. 1957. Quantitative laws in metabolism and growth. Q. Rev. Biol. 32: 217-231.
- Berg, K., P.M.Jonasson and K.W.Ockelman. 1962. Respiration of freshwater invertebrates. Hydrobiologia 19: 1-39.
- Cavanaugh, C.M., S.Gardiner, M.K.Jones, H.W.Jannasch, and J.B.Waterberry. 1981. Prokaryotic cells in the hydrothermal vent tube worm Riftia pachyptila Jones: possible chemoautotrophic symbionts. Science 213: 340-342.
- Cavanaugh, C.M. 1983. Symbiont chemoautotrophic bacteria in marine invertebrates from sulfide-rich habitats. Nature 302: 58-61.
- Childress, J.J. and G.N.Somero. 1979. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Mar. Biol. 52: 273-283.
- Edmond, J.M., K.L.von Damm, R.E.McDuff, and C.I.Measures. 1982. Chemistry of hot springs on the East Pacific Rise and their effluent dispersal. Nature 297: 187-191.
- Felbeck, H. 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, Riftia pachyptila Jones (Vestimentifera). Science 213: 336-338.
- Felbeck, H. 1983. Sulfide oxidation and carbon fixation by the gutless clam Solemya reidi: an animal-bacteria symbiosis. J. Comp. Physiol. 152 (B): 3-11.
- Felbeck, H., J.J.Childress and G.N.Somero. 1981. Calvin-Benson cycle and

- sulphide oxidation enzymes in animals from sulfide-rich habitats.  
Nature 293: 291-293.
- Felbeck, H. and G.N.Somero. 1982. Primary production in deep-sea hydrothermal vent organisms: role of sulfide-oxidizing bacteria. Trends Biochem. Sci. 7: 201-204.
- Felbeck, H., J.J.Childress, and G.N.Somero. 1983. Sulfide-based symbioses between sulfide-oxidizing bacteria and bivalves. Pp.339-354 in The Mollusca, Vol.2., P.W.Hochachka, ed. Academic Press, New York.
- Fisher, M.R. and S.C.Hand. 1984. Chemoautotrophic symbionts in the bivalve Lucina floridana from seagrass beds. Biol. Bull. 167:455-459.
- Gilson, W.E. 1963. Differential respirometer of simplified and improved design. Science 141: 531-532.
- Hammen, C.S. 1979. Metabolic rates of marine bivalve molluscs determined by calorimetry. Comp. Bioch. Physiol. 62(A): 955-959.
- Hand, S.C. and G.N.Somero. 1983. Energy metabolism pathways of hydrothermal vent animals: adaptations to a food-rich and sulfide-rich deep-sea environment. Biol. Bull. 165:167-181.
- Hemmingsen, A.M. 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Report of the Steno Memorial Hospital and Nordisk Insulin Laboratorium 9(2):7-110.
- Hoar, N.S. 1983. Oxygen availability: metabolic and respiratory responses. Pp.548-572 in: General and comparative physiology, Chapter 14. 3rd edition. Prentice-Hall, New Jersey.
- Kelly, D.P. 1982. Biochemistry of the chemolithotrophic oxidation of

- inorganic sulfur. Phil. Trans. R. Soc. London B 298:499-528.
- Kuenen, J.G. and R.F.Beudeker. 1982. Microbiology of Thiobacilli and other sulfur-oxidizing autotrophs, mixotrophs and heterotrophs. Phil. Trans. R. Soc. Lond. B 298:473-497.
- Loveland, R.E. and D.S.K.Chu. 1969. Respiratory movements and pumping in Mercenaria. Comp. Biochem. Physiol. 29:173-184.
- McMahon, R.F. and R.G.B.Reid. 1984. Respiratory responses of the gutless bivalve, Solemya velum, to temperature, hypoxia,  $\text{HS}^-$ , and dissolved organic matter. (Abstract). Am. Zool. 24(3):136(A).
- Morse, E.D. 1913. Observations on living Solenomya (velum and borealis). Biol. Bull. 25: 261-281.
- Newell, R.C. 1975. Factors controlling metabolic capacity adaptation in marine invertebrates. Pp.111-129 in Physiological ecology of estuarine organism, F.J.Vernberg ed. University of South Carolina Press, Columbia, S.C..
- Owen, G. 1961. A note on the habitats and nutrition of Solemya parkinsoni (protobranchia, bivalve). Quart. J. Micro. Sci. 102: 15-21.
- Peck, H.D. 1960. Adenosine 5'-phosphosulfate as an intermediate in the oxidation of thiosulfate by Thiobacillus thioparus. Proc. Natn. Acad. Sci. U.S.A. 46: 1053-1057.
- Peck, H.D., T.E.Deacon, and J.T.Davison. 1965. Studies on adenosine 5'-phosphosulfate reductase from Desulfovibrio desulfuricans and Thiobacillus thioparus. I. The assay and purification. Biochem. Biophys. Acta 96:429-432.
- Prosser, C.L.,1973. Oxygen: respiration and metabolism. Pp.165-211 in

- Comparative animal physiology, vol.1, Chapter 5, C.L.Prosser ed.  
W.B.Saunders Co., Philadelphia.
- Reid, R.G.B. 1980. Aspects of the biology of a gutless species of Solemya (Bivalvia: Protobranchia). Can. J. Zool. 58: 386-393.
- Reid, R.G.B. and F.R.Bernard. 1980. Gutless bivalves. Science 208: 609-610.
- Shumway, S.E. 1983. Factors affecting oxygen consumption in the coot clam Mulinia lateralis (Say). Ophelia 22(2): 143-171.
- Siebenaller, J.F., and G.N.Somero. 1982. The maintenance of different enzyme activity levels in congenetic fishes living at different depths. Physiol. Zool. 55:171-179.
- Smith, A. J., and J. Lascelles. 1966. Thiosulphate metabolism and rhodanese in Chromatium sp. strain D. J. Gen. Microbiol. 42: 357-370.
- Stempell, W. 1899. Zur anatomie von Solemya togata Poli. Zoo. Jb. Abt. 13(2): 89-170.
- Tuttle, J.H. and H.W.Jannasch. 1973. Sulphide and thiosulphate-oxidizing bacteria in anoxic marine basins. Mar. Biol. 20: 64-71.
- Wilson, J.G. and J.P.Davis. 1984. The effects of environmental variables on the oxygen consumption of the protobranch bivalve Nucula turgida (Leckenby and Marshall). J. Moll. Stud. 50:73-77.
- Yonge, C.M. 1939. The protobranchiate mollusca; a functional interpretation of their structure and evolution. Phil. Trans. R. Soc. Lond. B 230: 79-147.
- Zeuthen, E. 1953. Oxygen uptake as related to body size in organisms. Q. Rev. Biol. 28: 1-12.

Manuscript III

**Hydrogen Sulfide and the Bioenergetics of a Symbiotic Bivalve**

**Solemya velum (Protobranchia)**

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**Abstract.** An energy budget of Solemya velum, a chemoautotrophic symbiotic association, has been constructed based on the growth rate, oxygen consumption, ammonia excretion and enzymatic activities of the species. The energy derived from sulfide oxidation may account for 29% of the total energy input. The other sources of energy, like feeding and dissolved organic uptake, may provide the rest, 71%. The energy loss due to respiration and excretion may account 85% of the input energy, while only 15% may have been used for body growth. Multiple modes of nutrition are suggested for this protobranch bivalve containing a reduced gut. An energy flow model is proposed for the species, and may be applicable to some deep-sea hydrothermal vent organisms. Many unusual biological features in Solemya suggest that the symbiotic chemoautotrophy may have provided the genus a significant survival advantage.

## Introduction

Host-symbiont interactions in molluscs may confer nutritional advantages on the host (Felbeck et al., 1983). The benefits reaped by the symbiont bacteria are still poorly understood at the biochemical level, although symbionts live in a microenvironment where abundant reduced sulfur energy is available. From an evolutionary point of view, does the symbiosis in Molluscs give the host a better survivorship? The integration of studies on Solemya may provide a useful clue.

This manuscript focuses on an energy budget indicating the quantitative importance of the translocated products from the symbionts to the metabolic requirements of the mollusc. Studies on photosynthetic symbiotic coral reefs will serve as a framework in the discussion (Falkowski et al., 1984).

The major components of sulfide-based chemoautotrophic symbiotic systems consist of 1) a sulfide energy conversion system which produces ATP and NADPH, 2) carbon and nitrogen reduction systems which produces organics, 3) an organic translocating system, and 4) an animal metabolism system which produces biomass (Felbeck et al., 1983).

### Energy input: sulfide-based chemoautotrophics

Three diagnostic enzymes in sulfide oxidation in Solemya are thiosulfate sulfurtransferase (EC 2.8.8.1), APS reductase (adenylylsulfate reductase, EC 2.7.1.19) and sulfate adenylyl transferase (EC 2.7.7.4). Their activities in Solemya velum are 4.7, 2.2 and 5.0 umoles (substrate converted to product) per gram fresh gill per min, respectively (Chen, 1985b). Adenosine phosphosulfate (APS) is a

central intermediate in the pathway of sulfide oxidation in chemoautotrophic bacteria (Kelly, 1982). Kelly (1982) has reviewed the most likely alternative mechanisms of energy generation from thiosulfate among the thiobacilli. The same scheme can be probably applied equally to sulfide oxidation (Kelly, 1982).

The study of sulfide oxidation in seawater suggests that the initial oxidation (i.e. from  $\text{HS}^-$  to  $\text{S}_2\text{O}_3^{2-}$ ) is simply a chemical reaction forming thiosulfate (Almgren and Hagstrom, 1974). Biological oxidation is subsequently responsible for the further oxidation of thiosulfate to sulfate (Tuttle and Jannasch, 1973). The principal energetic pathway of Solemya is assumed similar to the pathway of thiobacilli as indicated by the presence of the same enzymes. The APS-dependent phosphorylation is considered to be a control mechanism in the sulfide energy-yielding pathway of Solemya. Thus, APS reductase is considered as a control enzyme for the whole pathway.

The enzymatic activity of APS reductase was 2.2 umoles/g fresh gill/min (i.e.  $V_{\text{max}}$ ) (Chen, 1985b). It had the lowest activity among the three enzymes measured. Therefore, the estimation of energy flow based on APS reductase is conservative. One average size animal weighs about 0.1 gram (wet weight including shell), of which 25% is the weight of fresh gill (Chen, 1985a). Thus, on the yearly basis, the maximum activity of sulfide oxidation would be 0.029 mole/animal/year.

The growth yield, i.e. grams dry mass fixed per mole of thiosulfate, is 6.7 in aerobic thiobacilli (Kelly, 1982). The overall mean carbon content is 47% of dry biomass averaged from five species of Thiomicrospira (Kelly, 1982). The  $\text{CO}_2$  fixed to give that yield would be

0.262 mole (Kelly, 1982). Assuming that Solemya had similar conversion factors, for one average size animal (0.1 g total wet wt), the symbionts could oxidize 0.029 mole reduced sulfur per year, and fix

$$(0.029 \text{ mol H}_2\text{S/yr}) \times (0.262 \text{ CO}_2/\text{H}_2\text{S}) = 0.008 \text{ mole CO}_2 \text{ fixed/yr,}$$

and  $(0.008 \text{ mol C/yr}) \times (12) \times (1/47\%) = 0.2 \text{ g dry biomass/animal/yr,}$  which represents a theoretical productivity based on enzymatic activity. The actual growth may be only a fraction of this estimate (See following section). It should be noted the growth yield is based on experimental measurements in free bacteria, therefore the energy transformation efficiency has been taken into consideration, even though it may be different from Solemya.

#### Growth of Solemya

The simplified growth equation of Solemya velum is

$$L_t = 19.6 (1 - e^{-0.61t}) \quad (1)$$

where  $L_t$  is the length of animal at age  $t$ ,  $t$  is relative age in years (Chen, 1985a). Based on the equation, the yearly growth increment of a second-year animal is  $L_2 - L_1 = 13.77 - 8.86 = 4.91 \text{ mm/year}$ . Converting length to dry soft-body weight by the equation (Chen, 1985a),

$$\log W = -2.143 + 3.003 \log L,$$

thus the yearly increment of dry soft-body weight will be 0.014 gram/year based on the growth model of Solemya.

## Respiration of Solemya

The oxygen consumption, which was measured in Gilson respirometer, included metabolic and chemoautotrophic portions when sulfide was present (Chen, 1985b). In experiment 4, the respiration, 65  $\mu\text{l O}_2$  per gram total wet weight (including shells) per hour, accounts 57% of the total rate (115.4  $\mu\text{l O}_2/\text{g}$ ); and chemoautotrophic consumption accounts the remaining 43%, i.e. 50  $\mu\text{l O}_2/\text{g/hr}$ , of the total rate. On yearly basis, one average size animal (0.1 g) could consume

$$(65 \times 10^{-6} / 32 \text{ mol O}_2/\text{g/hr})(0.1 \text{ g/animal})(24 \times 365 \text{ d/yr}) = 0.0018 \text{ mol}$$

oxygen for respiration; and

$$(50 \times 10^{-6} / 32)(0.1)(24 \times 365) = 0.0014 \text{ mol O}_2$$

for chemoautotrophic metabolism.

The average effect of temperature on respiration was considered. One year was divided into a winter averaged temperature of 5 °C and a summer averaged temperature of 20 °C. Wilson and Davis (1984) measured the oxygen consumption of the protobranch bivalve Nucula turgida under both summer and winter conditions in relation to temperatures (5-35 °C). For a winter conditioned animal, they found that the consumption at 5 °C was 67% of that at 15 °C; while in a summer conditioned animal, the consumption at 20 °C is 180% of that at 15 °C. The respiration of Solemya was measured at 15 °C. Therefore, an approximate temperature factor over the year could be  $(0.67+1.80)/2=1.24$ . Here, I simply use 1 for the temperature factor for a conservative estimation in the energy budget of Solemya velum.

### Excretion of Solemya

The ammonia excretion of Solemya velum has been measured by Hammen (1968). He found a range of excretion rate of 7.212 to 9.541  $\mu\text{mol NH}_3/\text{g}$  total wet wt/day. The mean value is approximately 8.4  $\mu\text{mol NH}_3/\text{g/day}$ .

According to Gnaiger and Bitterlich (1984), the C/N ratio of protein metabolism is

$$\text{C/N} = 0.529/0.173 = 3.06.$$

Therefore, the ammonia excretion of the animal is equivalent to

$$(8.4 \times 10^{-6} \text{ mol NH}_3/\text{g/d})(3.06 \text{ C/N})(0.1 \text{ g/animal})(365 \text{ d/yr}) = \\ 0.00094 \text{ mol C/animal/yr.}$$

### Energy budget of Solemya

The bioenergetic balance equation of a symbiotic association may be expressed as follows:

$$E_{\text{input}} = E_{\text{sulfide}} + E_{\text{others}} = E_{\text{growth}} + E_{\text{reprod}} + E_{\text{loss}} \quad (2)$$

where  $E_{\text{input}}$  = the total energy input to the animal;

$E_{\text{sulfide}}$  = the energy derived from sulfide oxidation;

$E_{\text{others}}$  = the energy derived from the other sources than sulfide, such as particulate or dissolved organics;

$E_{\text{growth}}$  = the energy used for body growth;

$E_{\text{reprod}}$  = the energy used for reproduction, which is not known and for the purpose of this calculation is assumed to be zero;

$E_{\text{loss}}$  = the energy lost by respiration ( $E_{\text{resp}}$ ) and excretion ( $E_{\text{excr}}$ ).

Dry biomass is converted into energy equivalents by the substrate

specific molar enthalpy of combustion based on carbon ( $H_C$ , KJ/mol C) in bomb calorimetry (Gnaiger, 1983). Here, KJ=1000 Joules. Therefore,

$$E_{\text{growth}} = (W_2 - W_1)(40\%)(1/12)(H_C), \quad (3)$$

where  $(W_2 - W_1)$  is the yearly increment of dry soft-body weight in grams; the carbon content of the animal was assumed to be 40% of dry soft-body mass; the carbon content in grams was converted to moles by carbon molecular weight, 12;  $H_C$  is the carbon molar enthalpy of combustion. To satisfy the energy conservation law of thermodynamics in Eq (2), oxygen uptake was converted to the enthalpy of combustion equivalent of the catabolic organic mass, with consistently derived oxygen enthalpic equivalents ( $H_O$ , KJ/mol  $O_2$ ) (Gnaiger, 1983),

$$E_{\text{resp}} = H_O \times N_O, \quad (4)$$

where  $N_O$  is oxygen consumption by the animal in moles. The average values of substrate specific oxygen and carbon enthalpic equivalents  $H_O$  and  $H_C$  were used (Gnaiger, 1983):

$$H_O = 480 \text{ KJ/mol } O_2$$

$$H_C = 542 \text{ KJ/mol C}.$$

Hammen (1979) has examined the ratio of heat production to oxygen consumption  $Q_H/Q_O$  (KJ/mol  $O_2$ ) in four marine bivalves. The ratio for Mytilus edulis was 635 KJ/mol  $O_2$ , and the ratio for Mya arenaria was 462 KJ/mol  $O_2$ . He concluded that the ratio was analogous and numerically equivalent to oxyenthalpic equivalents ( $H_O$ ) for combustion of food substrates.

Based on the discussion of growth and respiration, the energy components of a single average size animal on yearly basis are as follows:

$$E_{\text{growth}} = (0.014 \text{ g})(40\%)(1/12)(542 \text{ KJ/mol C}) = 0.25 \text{ KJ};$$

$$E_{\text{resp}} = (0.0018 \text{ mole O}_2)(480 \text{ KJ/mol O}_2) = 0.86 \text{ KJ}.$$

The carbon enthalpic equivalent for protein (Gnaiger and Bitterlich, 1984) is

$$H_C (\text{protein}) = 543 \text{ KJ/mol C}.$$

Thus, the energy content of the excretion equals to

$$E_{\text{excr}} = 0.00094 \text{ mol C/animal/yr} (543 \text{ KJ/mol C}) = 0.51 \text{ KJ}.$$

The energy loss is

$$E_{\text{loss}} = E_{\text{resp}} + E_{\text{excr}} = 0.86 + 0.51 = 1.37 \text{ KJ}.$$

Considering the overall reaction in energy generation:



the ratio of  $\text{H}_2\text{S}/\text{O}_2$  is 2. Based on the oxygen consumption by the chemoautotrophic sulfide oxidation,

$$E_{\text{sulfide}} = (0.0014/2 \text{ mol H}_2\text{S})(672 \text{ KJ/mol H}_2\text{S}) = 0.47 \text{ KJ}.$$

In summary, the energy equation (Eq.2) on yearly basis is,

$$E_{\text{input}} = E_{\text{sulfide}} + E_{\text{others}} = E_{\text{growth}} + E_{\text{loss}}$$

$$\text{i.e. } E_{\text{input}} = 0.47 \text{ KJ} + E_{\text{others}} = 0.25 \text{ KJ} + 1.37 \text{ KJ} = 1.62 \text{ KJ}.$$

Therefore, by difference,

$$E_{\text{others}} = 1.15 \text{ KJ} \quad (5)$$

Another way to estimate  $E_{\text{sulfide}}$  was based on the  $V_{\text{max}}$  of APS reductase. As discussed before,  $V_{\text{max}} = 2.2 \text{ umoles/g fresh gill/min} = 0.03 \text{ mole/animal/yr}$ . The energy released from the complete oxidation of thiosulfate to sulfate is 392 KJ/mol (expressed as thiosulfate-S). As discussed in the section of energy input, the biological oxidation is assumed to start from thiosulfate. Therefore,

$$E'_{\text{sulfide}} = (0.03 \text{ mole})(392 \text{ KJ/mol}) = 11.76 \text{ KJ} \quad (6)$$

on yearly basis. Obviously,  $E'_{\text{sulfide}}$  (11.76 KJ/animal/yr) is much greater than  $E_{\text{sulfide}}$  (0.47 KJ/animal/yr) estimated from respiration.

It is well known that metabolic enzymes usually do not perform at maximal velocity ( $V_{\text{max}}$ ). Enzymes may perform at the level of  $K_m$  or lower.  $K_m$  is the substrate concentration at which the velocity is half maximal. Under intracellular conditions, what catalytic velocity is reasonable in the overall pathway of sulfide oxidation ?

The energy input calculated from the oxygen consumption seems to be reasonable with the comparison to the other two terms, growth and loss. Many workers have shown that the activities of enzymes of metabolism correlate well with rates of oxygen consumption, even in organisms having widely different metabolic capacities, e.g. deep-sea animals (Childress and Somero, 1979; Siebenaller and Somero, 1982). It is reasonable to assume that the energy input based on oxygen consumption represents a physiological capacity. Thus,

$$E_{\text{sulfide}}/E'_{\text{sulfide}} = (0.47 \text{ KJ})/(11.75 \text{ KJ}) = 0.04.$$

The ratio 0.04 suggests that the APS reductase might only perform at approximate 5% of the maximal velocity.

## Energy transfer and efficiency

The energy transfer efficiencies can be derived from the energy equation (4).

$$E_{\text{sulfide}}/E_{\text{input}} = 0.47/1.62 = 0.29$$

$$E_{\text{sulfide}}/E_{\text{other}} = 0.47/1.15 = 0.41$$

$$E_{\text{growth}}/E_{\text{input}} = 0.25/1.65 = 0.15$$

$$E_{\text{loss}}/E_{\text{input}} = 1.37/1.62 = 0.85$$

The calculation indicates the chemoautotrophy may have provided 29% of the host's need. The energy derived from sulfide may be equal to 41% of the other energy sources, such as feeding and dissolved organic uptake. A total of 85% of the energy input may have been used for respiration and excretion, while only 15% may have been used for growth. A conceptual model of energy flow in this chemoautotrophic symbiotic system is presented in Figure 1. The above discussion on the energy budget of Solemya velum may not be always true, but it does indicate that multiple modes of nutrition may exist in the species. More experimental efforts and theoretical calculations are needed in order to reconstruct the detailed features of the model. It may be interesting to compare those efficiencies to the energy flow model in Figure 1.

The measurement of stable carbon isotope ratio ( $^{13}\text{C}/^{12}\text{C}$ ) indicates that the chemoautotrophic nutrition is more important than the uptake of dissolved organic matter in Solemya reidi (Felbeck, 1983). The  $\text{CO}_2$  fixation mechanism in Solemya reidi appears to involve an initial trapping of  $\text{CO}_2$  into 4-carbon intermediate (Felbeck, 1983). The principal forms of reduced carbon and nitrogen, that are translocated from the symbionts to the host, are unknown.

Two approaches can be used in the future to study the energy transfer in Solemya. The whole animal can be incubated in seawater containing  $^{14}\text{C}$ -bicarbonate, then labeled organic compounds can be separated and identified in the host to describe the nature of carbon transfer.

The other approach is to analyse the nitrogen budget in the animal, like the studies in the symbiotic association of coral reefs (Falkowski et al, 1984). The carbon translocation rate was extremely high (90%) in a coral symbiotic association (Davies, 1984), but the overall transfer efficiency from primary producer to primary consumer approximately equalled the rate in actively foraging herbivores (Slobodkin, 1960). When balanced growth of carbon and nitrogen was assumed, it was found that much of the organic material translocated by zooxanthellae was deficient in nitrogen (Muscatine et al, 1984). The coral animal had a need to supplement its phototrophic carbon diet with nitrogen rich material--presumably from zooplankton or dissolved organic nitrogen compounds. By regulating the nitrogen nutrition of zooxanthellae, the animal host may control the symbiont's growth (Falkowski et al, 1984). It is possible that the nutritional interaction between symbionts and the animal (Solemya) would also regulate the growth of both.

Figure 1. A proposed model of energy flow in Solemya velum, a chemoautotrophic simbiotic association. The following energy components may be applied to the model.

$$E_{\text{input}} = 1.62 \text{ KJ/animal/year,}$$

$$E_{\text{sulfide}} = 0.47 \text{ KJ/animal/year,}$$

$$E_{\text{other}} = 1.15 \text{ KJ/animal/year,}$$

$$E_{\text{growth}} = 0.25 \text{ KJ/animal/year,}$$

$$\text{and } E_{\text{loss}} = 1.37 \text{ KJ/animal/year.}$$

The energy transfer efficiencies:

$$E_{\text{sulfide}}/E_{\text{input}} = 0.29,$$

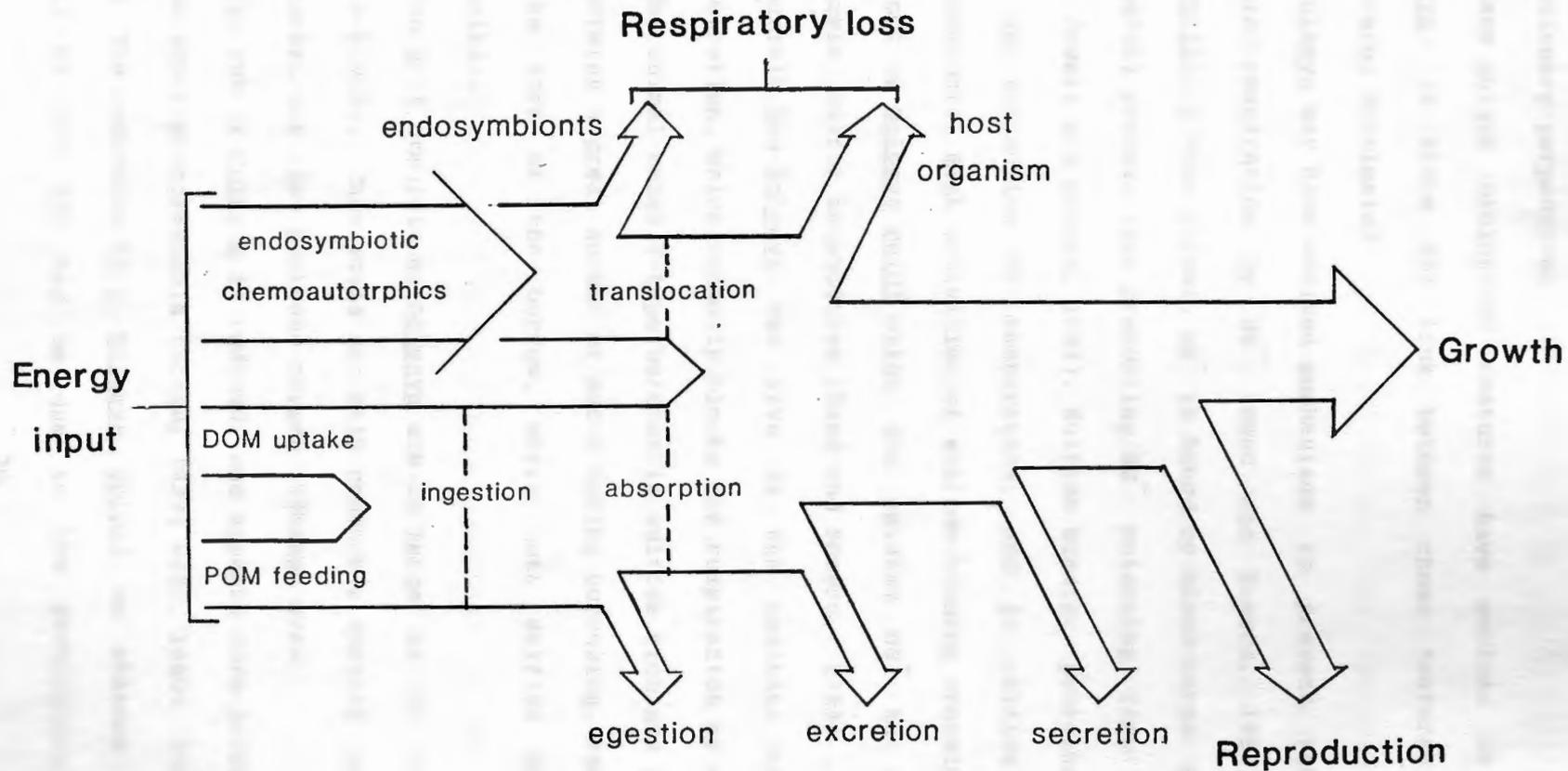
$$E_{\text{others}}/E_{\text{input}} = 0.71,$$

$$E_{\text{growth}}/E_{\text{input}} = 0.15,$$

$$E_{\text{loss}}/E_{\text{input}} = 0.85.$$

See the text for explanations.

69a



## Evolutionary perspectives

Many unique biological features have evolved in the genus of Solemya. Is there any link between those features and recently discovered symbiosis?

Solemya may have evolved mechanisms to prevent the poisoning of aerobic respiration by  $\text{HS}^-$  (Hand and Somero, 1983). In Rifitia pachyptila, a vent animal,  $\text{HS}^-$  is bound by blood-borne sulfide binding (transfer) protein thus preventing  $\text{HS}^-$  poisoning (Arp and Childress, 1983; Powell and Somero, 1983). Sulfide binding proteins may function both in protection of respiration, and in sulfide transport to endosymbionts. High activities of sulfide binding proteins were found in the foot of Solemya reidi which can oxidize  $\text{HS}^-$  to less toxic, or non-toxic, sulfur metabolites (Hand and Somero, 1983). This evidence may explain how Solemya can live in the habitats of high sulfide concentration, which normally blocks the respiration of organisms.

The animal makes Y-type burrows in sulfide rich mud (Stanley, 1970). It excretes a great amount of mucus during burrowing. The animal stays at the fork of the burrow, where both sulfide and oxygen are accessible.

The gill ctenidia of Solemya are so large as to occupy half the mantle cavity. This would not only provide a spatial microhabitat for symbionts, but also increase oxygen exchange area.

The gut of Solemya is reduced, and appears more primitive than that of the other protobranchia (Yonge, 1939; Reid, 1980; Reid and Benard, 1980). The reduction (e.g. Solemya velum) or absence (e.g. Solemya reidi) of the gut may be due to the development of symbiotic

sulfide-based nutrition. The controversy on the nutritional modes of Solemya remains today. Yonge found "the most minute particles" in the gut (1939). Felbeck (1983) found Solemya reidi takes up the dissolved organic matter in water, although it is less important than sulfide energy source. Kuzenetsov et al. (1983) claimed, "Solemya velum is a seston phage-filtrator feeding on the organic matter of the water entering the burrow ... The only species with the completely reduced intestine, Solemya reidi began to feed on the molecular organic matter dissolved in water."

Solemyidae (family) are a very old group appearing in the Devonian, and of the six genera only Solemya persists. The species of Solemya are widely distributed (Morse, 1913). They are found on the east and west coast of North America, in West Africa, the Mediterranean, the Canaries, Australia and New Zealand (Morse, 1913). It is surprising that any species of a group which evolved so early along such specialized lines have survived (Yonge, 1936). The symbiotic chemoautotrophy might have provided this genus with a significant survival advantage.

In summary, Solemya velum may have multiple nutritional modes. In the course of evolution, the species developed the nutrition of symbiotic chemoautotrophy. Thus, the original feeding nutrition became less useful, but might not have been abandoned. The species may also directly take up dissolved organic matter from water. The survival of the genus may indicate that the multiple nutritional modes have given the genus survival advantage, when the other genera in Solemyacea family went extinct.

## References

- Almgren, T. and I. Hagstrom. 1974. The oxidation rate of sulfide in seawater. *Water Research* 8: 395-400.
- Arp, A. and J.J. Childress, 1983. Sulfide binding by the blood of the hydrothermal vent tube worm Riftia pachyptila. *Science* 219: 295-297.
- Chen, C. 1985a. Population characteristics of the bivalve Solemya velum in Ninigret Pond, Charlestown, Rhode Island, USA. M.S. thesis, University of Rhode Island, in prep.
- Chen, C. 1985b. The effect of hydrogen sulfide on the metabolism of Solemya velum (Protobranch bivalvia) and sulfide oxidation enzymes in the species. M.S. thesis, University of Rhode Island, in prep.
- Childress, J.J. and G.N. Somero, 1979. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. *Marine Biology* 52: 273-283.
- Davies, P. S. 1984. The role of zooxanthellae in the nutritional energy requirements of Pocillopora eydouxi. *Coral Reefs* 2: 181-186.
- Falkowski, P.G., Z. Dubinsky, L. Muscatine and J. W. Porter, 1984. Light and bioenergetics of a symbiotic coral. *BioScience* 34(11):705-709.
- Felbeck, H., 1983. Sulfide oxidation and carbon fixation by the gutless clam Solemya reidi: an animal-bacteria symbiosis. *J. Comp. Physiol.* 152(B): 3-11.
- Felbeck, H., J. J. Childress and G. N. Somero, 1983. Sulfide-based symbioses between sulfide-oxidizing bacteria and bivalves. In: K.W. Wilbur (editor-in-chief) *The mollusca*, vol.2, Environmental

- biochemistry and physiology. pp.339-354. Academic Press, Inc. New York.
- Gnaiger, E., 1983. Calculation of energetics and biochemical equivalents of respiratory oxygen consumption. In: E. Gnaiger and H. Forstner (eds.) Polarographic oxygen sensors: aquatic and physiological applications. pp.337-345. Springer-Verlag Berlin Heidelberg, New York.
- Gnaiger, E. and G. Bitterlich. 1984. Proximate biochemical composition and caloric content calculated from elemental carbon, hydrogen, nitrogen analysis: a stoichiometric concept. *Oecologia (Berl)* 62(3): 289-298.
- Hammen, C.S. 1968. Aminotransferase activities and amino acid excretion of bivalve molluscs and brachiopods. *Comp. Bioch. Physiol.* 26A: 697-705.
- Hammen, C.S. 1979. Metabolic rates of marine bivalve molluscs determined by calorimetry. *Comp. Bioch. Physiol.* 62 (A): 955-959.
- Hand, S.C. and G.N.Somero, 1983. Energy metabolism pathways of hydrothermal vent animals: adaptations to a food-rich and sulfide-rich deep-sea environment. *Biol. Bull.* 165:167-181.
- Kelly, D.P., 1982. Biochemistry of the chemolithotrophic oxidation of inorganic sulfur. *Phil. Trans. R. Soc. Lond.* 298(B):499-528.
- Kuzenetsov, A.P., J. Hampson, H. Sanders and C. Jenner, 1983. On the structure of gill-palp system in Solemya velum, character of feeding of the Solemyidae and their status in the system of protobranchia (Bivalvia). *Zoologicheskii Zhurnal* 62(6): 830-838.
- Morse, E.D., 1913. Observations on living Solenomya (velum and borealis)

Biol. Bull. 25:261-281.

- Muscantine, L., P. Falkowski, J. Porter and Z. Dubinsky. 1984. Fate of photosynthetically-fixed carbon in light- and shade-adapted colonies of the symbiotic coral Stylophora pistillata. Proc. R. Soc. Lond. B 222: 181-202.
- Powell, M.A. and G.N.Somero, 1983. Blood components prevent sulfide poisoning of respiration of the hydrothermal vent tube worm Raftia pachyptila. Science 219:297-299.
- Reid, R.G.B., 1980. Aspects of the biology of a gutless species of Solemya (Bivalvia: Protobranchia). Can. J. Zool. 58:386-393.
- Reid, R.G.B. and F.R.Bernard, 1980. Gutless bivalves. Science 208: 609-610.
- Siebenaller, J.F. and G.N.Somero, 1982. The maintenance of different enzyme activity levels in congenetic fishes living at different depths. Physiol. Zool. 55: 171-179.
- Slobodkin, L. B. 1960. Ecological energy relationships at the population level. Am. Nat. 95: 213-236.
- Stanley, S.W., 1970. Adaptations for burrowing in soft substrata. In: S.W.Stanley, Relation of shell form to life habitats of the bivalvia (Mollusca). pp.45-85. The Geological Society of America, Inc. USA.
- Tuttle, J. H. and H. W. Jannasch. 1973. Sulphide and thiosulfate-oxidizing bacteria in anoxic marine basins. Marine Biology 20: 64-71.
- Wilson, J.G. and J.P.Davis, 1984. The effects of environmental variables on the oxygen consumption of the protobranch bivalve Nucula turgida

(Leckenby and Marshall). J. Moll. Stud. 50: 73-77.

Yonge, C.M., 1939. The protobranchiate mollusca; a functional interpretation of their structure and evolution. Phil. Trans. R. Soc. Lond. B 230: 79-147.

Appendix 2. The environmental conditions of the Wilson  
Experiment.

Date	Temp °C	Humidity %	Time (hours)	pH
10/10/50	15	80	1	8.2
11/10/50	15	80	1	8.2
12/10/50	15	80	3	8.2
13/10/50	15	80	3	8.2

Note: -Using an electrode for pH measurement.

Appendix A. The experimental conditions of the Gilson respirometer.

Date	pH	Temp °C		Replicates	Time (hours)		H <sub>2</sub> S (mM)
		Field	Lab		(A only)	(B only)	
30Jan85	0	15	15	3	2	2	0.1
07Feb85	4	15	15	3	2	2	--
14Feb85	5	20	20	4	3	3	0.8
21Mar85	8	15	15	4	3	3	0.5

Note: --failed to estimate the H<sub>2</sub>S concentration.

**Appendix B. The oxygen uptakes.**

(a). The cumulative oxygen uptakes of Solemya velum in the presence and absence of hydrogen sulfide. The experiments were conducted in a Gilson respirometer.

Date	Min	(A + S) ul O <sub>2</sub>			(A only) ul O <sub>2</sub>			(S only) ul O <sub>2</sub>					
<u>Animal Wt (g)</u>		<u>.21</u>	<u>.21</u>	<u>.20</u>	<u>.21</u>	<u>.17</u>	<u>.14</u>						
Jan30	30	20	19	16	13	1	2	3	6	4			
	60	33	31	25	24	0	5	5	6	5			
	90	43	40	30	35	0	3	5	6	4			
	120	55	51	37	43	0	3	5	6	4			
<u>Animal Wt (g)</u>		<u>.32</u>	<u>.27</u>		<u>.28</u>	<u>.27</u>	<u>.36</u>						
Feb07	30	5	3		4	5	29	1	2	3			
	60	11	3		5	6	42	4	3	4			
	90	17	4		5	7	52	1	4	6			
	120	24	10		5	8	59	1	4	6			
	150	37	25		6	10	70	1	4	6			
<u>Animal Wt (g)</u>		<u>.16</u>	<u>.10</u>	<u>.11</u>	<u>.16</u>	<u>.11</u>	<u>.16</u>	<u>.12</u>	<u>.13</u>				
Feb14	30	9	8	8	25	20	11	14	11	1	0	1	2
	60	24	23	22	49	39	24	33	21	1	2	2	3
	90	42	42	34	62	59	42	45	33	2	3	3	4
	120	58	61	47	74	63	54	54	49	3	5	5	5
	150	77	74	55	81	68	58	56	58	3	5	5	7
	180	94	85	63	89	72	63	58	63	4	6	8	8
<u>Animal Wt (g)</u>		<u>.17</u>	<u>.13</u>	<u>.32</u>	<u>.22</u>	<u>.17</u>	<u>.16</u>	<u>.15</u>	<u>.23</u>				
Mar21	30	1	4	11	18	8	5	5	9	0	3	5	7
	60	7	10	20	40	17	10	12	14	1	5	8	9
	90	14	10	31	53	22	13	16	20	2	5	9	9
	120	31	22	51	64	31	17	21	25	3	6	12	11
	150	48	36	67	76	35	20	26	30	5	7	14	13
	180	62	45	85	86	42	28	30	39	6	9	15	15

(b). Calculating Procedures.

1. Notations: (A + S) = animal in the presence of sulfide;

(A only) = animal in the absence of sulfide;

(S only) = sulfide without animal (chemical blank).

2. The mean blank (S only) accounting for chemical oxidation was subtracted from the  $O_2$  uptake of each animal (A + S). The resulting value (O) contains both animal respiration and symbiotic chemoautotrophics.

$$O_{(A+S)} - O_{(S \text{ only})} = O_{\text{both}} \text{ (ul } O_2) \quad (1)$$

3. The respiratory rate of animals was calculated by dividing the uptakes by time interval.

$$O_{\text{both}} / \text{time (hrs.)} = R'_{\text{both}} \text{ (ul/h)} \quad (2)$$

$$\text{or } O_{(A \text{ only})} / \text{time (hrs.)} = R'_A \quad (3)$$

4. The weight-specific respiratory rate was then obtained by dividing the respiratory rate by each animal's weight.

$$R' / \text{Wt (g)} = R \text{ (ul/g/h)} \quad (4)$$

5. Finally, the oxygen consumption due to symbiotic chemoautotrophics was determined by

$$R_{\text{both}} - R_A = R_{\text{chemoautotrophics}} \quad (5)$$

**Appendix C. The Measurements of Enzymatic Activities.**

<u>Enzyme</u>	<u>Substrate</u>	<u>Substrate Conc.</u>	<u>Velocity</u>
APS reductase	$\text{Na}_2\text{SO}_3$	(mM)	( $\mu\text{mol/g gill/min}$ )
		1.0	0.0020
		2.0	0.0034
		3.0	0.0041
		4.0	0.0046
		7.0	0.0080
		10.0	0.0144
		12.0	0.0121
Sulfate adenylyl transferase	Adenosin phosphosulfate	0.05	0.485
		0.10	0.152
		0.20	1.968
		0.30	2.161
		0.40	2.392
		0.50	2.585
Thiosulfate sulfur-transferase	$\text{Na}_2\text{S}_2\text{O}_3$	8.30	0.264
		16.70	0.584
		33.30	0.804

Note: [s] = substrate concentration;

[V] = reaction velocity (Figures 3&4 in m.s. II).

#### Appendix D. Bibliography

- Abbott, R.T. 1954. American seashells. 541pp. von Nostrand Reinhold, New York.
- Almgren, T. and I. Hagstrom. 1974. The oxidation rate of sulfide in seawater. Water research 8: 395-400.
- Appeldoorn, R.S. 1980. The growth and life-history strategy of the soft-shell clam, Mya arenaria L.. 115pp. Thesis, University of Rhode Island.
- Arp, A. and J.J.Childress. 1983. Sulfide binding by the blood of the hydrothermal vent tube worm Riftia pachyptila. Science 219: 295-297.
- Bertalanffy, L. von. 1957. Quantitative laws in metabolism and growth. Q. Rev. Biol. 32: 217-231.
- Berg, K., P.M.Jonasson and K.W.Ockelman. 1962. Respiration of freshwater invertebrates. Hydrobiologia 19: 1-39.
- Cavanaugh, C.M., S.Gardiner, M.K.Jones, H.W.Jannasch, and J.B.Waterberry. 1981. Prokaryotic cells in the hydrothermal vent tube worm Riftia pachyptila Jones: possible chemoautotrophic symbionts. Science 213: 340-342.
- Cavanaugh, C.M. 1983. Symbiont chemoautotrophic bacteria in marine invertebrates from sulfide-rich habitats. Nature 302: 58-61.
- Chen, C. 1985a. Population characteristics of the bivalve Solemya velum in Ninigret Pond, Charlestown, Rhode Island, USA. Thesis, University of Rhode Island.
- Chen, C. 1985b. The effect of hydrogen sulfide on the metabolism of

- Solemya velum (Protobranch bivalvia) and sulfide oxidation enzymes in the species. Thesis, University of Rhode Island.
- Childress, J.J. and G.N.Somero. 1979. Depth-related enzymic activities in muscle, brain and heart of deep-living pelagic marine teleosts. Mar. Biol. 52: 273-283.
- Dare, P.J. 1975. Settlement, growth and production of the mussel Mytilus edulis L. in Morecambe Bay. Fishery investigations. pp34. Ministry of Agriculture, Fisheries and Food, London, Series II.
- Davies, P.S. 1984. The role of zooxanthellae in the nutritional energy requires of Pocillopora eydouxi. Coral Reefs 2: 181-186.
- Drew, G.A. 1900. Locomotion in Solenomya and its relatives. Science 11: 171-172.
- Edmond, J.M., K.L.von Damm, R.E.McDuff, and C.I.Measures. 1982. Chemistry of hot springs on the East Pacific Rise and their effluent dispersal. Nature 297: 187-191.
- Falkowski, P.G., Z. Dubinsky, L.Muscatine and J.W.Porter. 1984. Light and bioenergetics of a symbiotic coral. BioScience 34(11):705-709.
- Felbeck, H. 1981. Chemoautotrophic potential of the hydrothermal vent tube worm, Riftia pachyptila Jones (Vestimentifera). Science 213: 336-338.
- Felbeck, H. 1983. Sulfide oxidation and carbon fixation by the gutless clam Solemya reidi: an animal-bacteria symbiosis. J. Comp. Physiol. 152 (B): 3-11.
- Felbeck, H., J.J.Childress and G.N.Somero. 1981. Calvin-Benson cycle and sulphide oxidation enzymes in animals from sulfide-rich habitats. Nature 293: 291-293.

- Felbeck, H. and G.N.Somero. 1982. Primary production in deep-sea hydrothermal vent organisms: role of sulfide-oxidizing bacteria. Trends Biochem. Sci. 7: 201-204.
- Felbeck, H., J.J.Childress, and G.N.Somero. 1983. Sulfide-based symbioses between sulfide-oxidizing bacteria and bivalves. Pp.339-354 in The Mollusca, Vol.2., P.W.Hochachka, ed. Academic Press, New York.
- Fenchel, T. 1977. Aspects of the decomposition of seagrasses. In: C.P.McRoy and C. Hefferich (eds.) Seagrass ecosystems, a scientific perspective. pp.123-145. Marcel Dekker, New York.
- Fenchel, T.M. and R.J.Riedl. 1970. The sulfide system: a new biotic community underneath the oxidized layer of marine sand bottoms. Marine Biology 7:255-268.
- Fisher, M.R. and S.C.Hand. 1984. Chemoautotrophic symbionts in the bivalve Lucina floridana from seagrass beds. Biol. Bull. 167:455-459.
- Gilson, W.E. 1963. Differential respirometer of simplified and improved design. Science 141: 531-532.
- Gnaiger, E. 1983. Calculation of energetics and biochemical equivalents of respiratory oxygen consumption. In: E.Gnaiger and H.Forstner (eds) Polarographic oxygen sensors: aquatic and physiological applications. pp.337-345. Springer-Verlag Berlin Heidelberg, New York.
- Gnaiger, E. and G. Bitterlich. 1984. Proximate biochemical composition and caloric content calculated from elemental carbon, hydrogen, nitrogen analysis: a stoichiometric concept. Oecologia (Berl)

- 62(3): 289-298.
- Gulland, J.L. 1983. Fish stock assessment. pp83-95. The Food and Agriculture Organization, the United Nations, Paris.
- Hammen, C.S. 1968. Aminotransferase activities and amino acid excretion of bivalve molluscs and brachiopods. Comp. Bioch. Physiol. 26A: 697-705.
- Hammen, C.S. 1979. Metabolic rates of marine bivalve molluscs determined by calorimetry. Comp. Bioch. Physiol. 62(A): 955-959.
- Hand, S.C. and G.N.Somero. 1983. Energy metabolism pathways of hydrothermal vent animals: adaptations to a food-rich and sulfide-rich deep-sea environment. Biol. Bull. 165:167-181.
- Hemmingsen, A.M. 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. Report of the Steno Memorial Hospital and Nordisk Insulin Laboratorium 9(2):7-110.
- Hoar, N.S. 1983. Oxygen availability: metabolic and respiratory responses. Pp.548-572 in: General and comparative physiology, Chapter 14. 3rd edition. Prentice-Hall, New Jersey.
- Kelly, D.P. 1982. Biochemistry of the chemolithotrophic oxidation of inorganic sulfur. Phil. Trans. R. Soc. London B 298:499-528.
- Kikuchi, T. and J.M.Peres. 1977. Consumer ecology of seagrass beds. In: C.P.McRoy and C.Helfferich (eds.) Seagrass ecosystems, a scientific perspective. ppl47-185. Marcel Dekker, New York.
- Kuenen, J.G. and R.F.Beudeker. 1982. Microbiology of Thiobacilli and other sulfur-oxidizing autotrophs, mixotrophs and heterotrophs. Phil. Trans. R. Soc. Lond. B 298:473-497.
- Kuzenetsov, A.P., J.Hampson, H.Sanders and C.Jenner. 1983. On the

- structure of gill-palp system in Solemya velum, character of feeding of the Solemyidae and their status in the system of protobranchia (Bivalvia). Zoologicheskii Zhurnal 62(6):830-838.
- Loveland, R.E. and D.S.K.Chu. 1969. Respiratory movements and pumping in Mercenaria. Comp. Biochem. Physiol. 29:173-184.
- McMahon, R.F. and R.G.B.Reid. 1984. Respiratory responses of the gutless bivalve, Solemya velum, to temperature, hypoxia,  $HS^-$ , and dissolved organic matter. (Abstract). Am. Zool. 24(3):136(A).
- Morse, E.D. 1913. Observations on living Solenomya (velum and borealis). Biol. Bull. 25: 261-281.
- Muscatine, L., P.Falkowski, J.Porter and Z.Dubinsky. 1984. Fate of photosynthetically-fixed carbon in light- and shade-adapted colonies of the symbiotic coral Stylophora pistillata. Proc. R. Soc. Lond. B. 222, 181-202.
- Newell, R.C. 1975. Factors controlling metabolic capacity adaptation in marine invertebrates. Pp.111-129 in Physiological ecology of esturine organism, F.J.Vernberg ed. University of South Carolina Press, Columbia, S.C..
- Owen, G. 1961. A note on the habitats and nutrition of Solemya parkinsoni (protobranchia, bivalve). Quart. J. Micro. Sci. 102: 15-21.
- Pauly, D. 1979. Gill size and temperature as governing factors in fish growth: a generalization on von Bertalanffy's growth formula. Ber. Inst. Meereskunde, Kiel, No.63, 156pp.
- Pauly, D. and D.David. 1981. ELEFAN 1, a BASIC program for the objective extraction of growth parameters from the length-frequency data.

- Meeresforsch, Hamburg 28: 205-211.
- Pauly, D., D.David and J.Ingles. 1982. ELEFAN 1: user's instruction and program listing (Rev. 2). pp.1-28. Published by the International Center for Living Aquatic Resources Management (ICLARM), Manila, Philippines.
- Pauly, D., D.David and J.Ingles. 1982b. ELEFAN II: user's instruction and program listing. 67pp. CLARM, Manila, Philippines.
- Pauly, D. and H.Calumpong. 1984. Growth, reproduction and mortality of and the sea hare Dolabella auricularia (Gastropoda: Aplysiidae) in the Central Visayas, Philippines. Mar. Biol. 79: 289-293.
- Peck, H.D. 1960. Adenosine 5'-phosphosulfate as an intermediate in the oxidation of thiosulfate by Thiobacillus thioparus. Proc. Natn. Acad. Sci. U.S.A. 46: 1053-1057.
- Peck, H.D., T.E.Deacon, and J.T.Davison. 1965. Studies on adenosine 5'-phosphosulfate r[[uctase from Desulfovibrio desulfuricans and Thiobacillus thioparus I. The assay and purification. Biochem. Biophys. Acta 96:429-432.
- Pelseneer, P. 1891. Contribution 'a l' e'tude des lamellibranches. Arch. Biol. 11: 147-312.
- Powell, M.A. and G.N.Somero. 1983. Blood components prevent sulfide poisoning of respiration of the hydrothermal vent tube worm Riftia pachyptila. Science 219:297-299.
- Prosser, C.L.,1973. Oxygen: respiration and metabolism. Pp.165-211 in Comparative animal physiology, vol.1, Chapter 5, C.L.Prosser ed. W.B.Saunders Co., Philadelphia.
- Reid, R.G.B. 1980. Aspects of the biology of a gutless species of

- Solemya (Bivalvia: Protobranchia). Can. J. Zool. 58: 386-393.
- Reid, R.G.B. and F.R. Bernard. 1980. Gutless bivalves. Science 208: 609-610.
- Shumway, S.E. 1983. Factors affecting oxygen consumption in the coot clam Mulinia lateralis (Say). Ophelia 22(2): 143-171.
- Siebenaller, J.F., and G.N. Somero. 1982. The maintenance of different enzyme activity levels in congenetic fishes living at different depths. Physiol. Zool. 55:171-179.
- Slobodkin, L.B. 1960. Ecological energy relationships at the population level. Am. Nat. 95:213-236.
- Smith, A. J., and J. Lascelles. 1966. Thiosulphate metabolism and rhodanese in Chromatium sp. strain D. J. Gen. Microbiol. 42: 357-370.
- Stanley, S.W. 1970. Adaptations for burrowing in soft substrata. Relation of shell form to life habitats of the bivalvia (Mollusca). pp.45-85. The Geological Society of America.
- Stempell, W. 1899. Zur anatomie von Solemya togata Poli. Zoo. Jb. Abt. 13(2): 89-170.
- Thiesen, B.F. 1968. Growth and mortality of culture mussels in the Danish Wadden sea. Meddelelser fra Danmarks Fiskeri-og Havundersogelser, N. S. 6:47-78.
- Thiesen, B.F. 1973. The growth of Mytilus edulis L. (Bivalvia) from Disko and Thule district, Greenland. Ophelia 12:59-77.
- Tuttle, J.H. and H.W. Jannasch. 1973. Sulphide and thiosulphate-oxidizing bacteria in anoxic marine basins. Mar. Biol. 20: 64-71.
- Wilson, J.G. and J.P. Davis. 1984. The effects of environmental variables

on the oxygen consumption of the protobranch bivalve Nucula turgida  
(Leckenby and Marshall). J. Moll. Stud. 50:73-77.

Yonge, C.M. 1939. The protobranchiate mollusca; a functional  
interpretation of their structure and evolution. Phil. Trans. R.  
Soc. Lond. B 230: 79-147.

Zeuthen, E. 1953. Oxygen uptake as related to body size in organisms.  
Q. Rev. Biol. 28: 1-12.