AN ABSTRACT OF THE THESIS OF

Robert Edward Malouf for the degree of Doctor of Philosophy

in Fisheries and Wildlife presented on April 4, 1977

Title: The Effects of Temperature and Food Availability on Energy Partitioning and Growth in the Pacific Oyster *Crassostrea gigas* (Thunberg)

Abstract approved: Redacted for Privacy

Dr. Charles E. Warren

In general terms, the objective of this study was to describe the relationships that exist between food availability, temperature, and the growth of juvenile oysters, *Crassostrea gigas* (Thunberg).

Two types of experiments were conducted to meet this objective. The first were the "open system" experiments conducted in an apparatus that provided controlled flows of untreated seawater at various temperatures to juvenile oysters. As a part of these experiments, concentrations of particulate organic carbon and nitrogen were monitored in the incoming seawater over a period of two years to provide an indication of seasonal fluctuations in naturally occurring food.

Subsequent experiments were conducted in a recirculating seawater system that permitted control of water temperature, water flow rate, and food availability. Animals in the "closed system" experiments were provided with four different concentrations of two

species of algae, Pseudoisochrysis paradoxa (VA-12) and Monochrysis lutheri (Droop) in flowing water at four temperatures, 11°, 15°, 19°, and 23°C. Complete energy budgets were determined for the animals in each of the 16 treatments. Food consumption, oxygen consumption, and growth were measured directly, and the remaining components of the energy budget were obtained by difference.

The open system experiments indicated that growth rate in juvenile Pacific oysters generally increased with increasing temperature up to 15°C. Indications were, however, that if food is plentiful or qualitatively superior, maximum growth might be achieved at temperatures up to 18° - 20°C. These experiments also show that the relationship between temperature and growth rate depends on water flow rate. At very low flow rates, for example, growth rate was inversely related to temperature, but at high flow rates the opposite was true up to a maximum temperature that varied between 15° and 19°C depending on season.

There were significant seasonal fluctuations in the concentration of particulate organics in the incoming seawater, and this variation was reflected in seasonal changes in the growth rate of juvenile oysters held at a constant temperature. Particulate organic carbon for example, varied from a monthly mean of 815 μg/liter in July to 233 μg/liter in April. Oyster growth varied from 0.13 percent per day in November to 0.81 percent per day in August.

Hypothetical curves relating oyster growth to the flow rate of

water at various temperatures are proposed and discussed. These curves, based on the results of the open system experiments, provide a statement of the problem attacked by the closed system experiments.

The closed system experiments reaffirmed the 15°C growth optimum and provided some explanation as to why this occurs. The data show that maximum food consumption occurred at 15°C, and, more importantly, that the energetic difference between food consumed and respiration was greatest at 15°C.

Assimilation efficiency was found to be relatively low in these experiments (22-42 percent of consumption), and it was found to be inversely related to the availability of food.

Factors influencing the absolute values obtained for the components of the energy budget equation are discussed. These factors include food quality, physical factors (temperature, salinity, silt, etc.), water movement, the size and previous handling of the animals, and crowding.

Possible applications of the findings of this study are briefly considered.

The Effects of Temperature and Food Availability on Energy Partitioning and Growth in the Pacific Oyster *Crassostrea gigas* (Thunberg)

by

Robert Edward Malouf

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1977

APPROVED:

Redacted for Privacy

Professor of Fisheries and Wildlife
in charge of major

Redacted for Privacy

Head of the Department of Fisheries and Wildlife

Redacted for Privacy

Dean of Graduate School

Date thesis is presented April 4, 1977

Typed by Velda Mullins for Robert Edward Malouf

ACKNOWLEDGEMENTS

This study was conducted over a period of five years. During that time a number of people provided encouragement and invaluable assistance to me. First of all, I want to thank Professor W.P. Breese, my friend and mentor, for knowing when to leave me alone and when to apply a gentle push. I also owe thanks to Dr. Jerry Davis for his help in the design of this study, and to Dr. Charles Warren for assisting in interpretation of the results and in preparation of the manuscript.

I owe special thanks to Miss Sara Miller for skill and patience above and beyond the call of duty, for hundreds of flows and temperatures checked and adjusted and for thousands of weighings performed. Thanks also to Mr. Tom Breese for enduring the early disasters encountered in perfecting the recirculating system and for several hundred Winkler and dichromate titrations.

Throughout the study, Mr. Dean Satterlee contributed his skill and imagination to the design and refinement of the rather complex systems that the study employed. Technical assistance was provided in the early stages by Mr. Doug Squire and by Mr. Mike Meyers who helped monitor the open system experiments and assisted in construction of the closed system and algae culture facility.

I am grateful to Dr. Larry Small, Mr. Percy Donaghay, and Mr. Mike Demanche of OSU's School of Oceanography for their help

in analysis and interpretation of organic carbon and nitrogen determinations made in both the open and closed system experiments.

I also appreciate the efforts of my graduate committee, Drs. Robert Olson, Richard Caldwell, and Harry Phinney in preparation of this manuscript.

Financial support for this study came partially from a grant, obtained primarily through the efforts of Dr. William McNeil, from Portland General Electric, Pacific Power and Light, and the Eugene Water and Electric Board. Additional support was provided by NOAA through Oregon State University's Sea Grant College Program. I very much appreciate the support of the people and agencies involved.

Finally, I want to thank my family, my wife Judy, and my children Robby and Bethy, for the weekends that went unnoticed, for their patience and understanding when things looked grim, but especially for making all of this worthwhile.

TABLE OF CONTENTS

	Page
I. INTRODUCTION	1
Objectives	1
Energy Budget Equation	5
II MATERIAL AND METHODS	14
Open Systems	14
Apparatus	14
Animals	15
Growth Measurements	15
Monitoring Particulate Organics	18
Experimental Design	20
Closed Systems	20
Apparatus	22
Operation and Monitoring of the Closed System	31
Algae Culture Facility	33
Determination of the Components of the Energy Budget	35
Food Consumption	36
Caloric Conversions	37
Respiration	39
Growth	42
Waste and Efficiency Estimates	46
III RESULTS	47
Open System Experiments	47
Relationships Between Growth and Temperature	47
Seasonal Variation in Oyster Growth Rates	
Relationship Between Water Flow and Oyster	52
Growth	59
Hypothetical Growth Curves	63
Closed System Experiments	67
Experiment I, Energy Budget	68
Experiment II, Energy Budget	70
Estimates of Maintenance Requirements	74

Page

IV. DISCUSSION 89

Hypothetical Growth Curves 89
Factors Influencing Energy Budgets 94
Applications 98

BIBLIOGRAPHY 101

APPENDICES 109

LIST OF TABLES

Table		Page
1	Design of the seven open system experiments.	21
2	Details of the components of the recirculating seawater system used in the closed system experiments.	24
3	Results of five open system experiments showing the effects of temperature on growth and survival of juvenile oysters during different times of the year.	48
4	Results of two factorial experiments showing effects of temperature and water flow rate on growth of shell and meat in juvenile oysters.	49
5	Results of five open system experiments showing seasonal fluctuations in the growth of juvenile oysters at a constant temperature. Means for particulate organic carbon and nitrogen in incoming seawater are also given.	53
6	Energy budget summary from closed system Experiment I.	69
7	Energy budget summary from closed system Experiment II.	71
8	Graphically estimated requirements to maintain zero weight change at four temperatures, given in terms of food availability, food consumption, and assimilation.	80
9	Calculated estimates of requirements to maintain zero weight change at four temperatures, given in terms of food availability, food consumption, and assimilation.	80

LIST OF ILLUSTRATIONS

Figure		Page
1	Schematic drawing of recirculating seawater system used in closed system experiments.	28
2	Recirculating seawater system, details of the oyster holding trays, flow regulation, heating, and algae delivery system.	30
3	Observed relationship between oyster shell growth rate and temperature in six open system experiments.	51
4	Observed relationship between oyster meat growth rate and temperature in five open system experiments.	51
5	Rates of growth of shell from five open system experiments plotted at the mid-point of each experiment. Also shown are mean particulate organic carbon values observed in incoming seawater for each month.	55
6	Rates of growth of shell from the same five experiments shown in Figure 5, but plotted in this case with monthly means of particulate organic nitrogen in incoming seawater.	55
7	Relationship between oyster shell growth rate from five open system experiments and particulate organic carbon concentration in incoming seawater.	58
8	Relationship between oyster shell growth rate from five open system experiments and particulate organic nitrogen concentration in incoming seawater.	58
9	Relationship between oyster meat growth and water flow rate at three temperatures, open system Experiment III.	61

Figure		Page
10	Relationship between oyster meat growth rate and water flow rate at three temperatures, open system Experiment IV	61
11	Hypothetical curves relating oyster growth rate to water flow rate at three temperatures.	65
12	Graphical representation of energy budgets of juvenile oysters held at four temperatures and four ration levels in closed system Experiment II.	73
13	Partial energy budget for juvenile oysters held at 11 C and fed four different ration levels, Experiment II. Shows graphical estimate of maintenance requirement.	76
14	Partial energy budget for juvenile oysters held at 15 C and fed four different ration levels, Experiment II. Shows graphical estimate of maintenance requirement	76
15	Partial energy budget for juvenile oysters held at 19 C and fed at four different ration levels, Experiment II. Shows graphical estimate of maintenance requirement	78
16	Partial energy budget for juvenile oysters held at 23 C and fed four different ration levels, Experiment II. Shows graphical estimate of maintenance requirement.	78
17	Relationship between temperature and estimated assimilation rate required for zero weight change shown with measured assimilation rate at each temperature and at three different ration levels, Experiment II.	83
18	Relationship between food availability as algal cells per oyster per minute and oyster meat growth rate at four temperatures, Experiment II.	87

THE EFFECTS OF TEMPERATURE AND FOOD AVAILABILITY ON ENERGY PARTITIONING AND GROWTH IN THE PACIFIC OYSTER *CRASSOSTREA GIGAS* (THUNBERG)

INTRODUCTION

In general terms, the objective of this study was to describe the relationships that exist between food availability, temperature, and the growth of juvenile Pacific oysters, *Crassostrea gigas* (Thunberg). An energy budget equation was used as the conceptual framework for attacking the problem and for providing insight into the manner in which growth is influenced by variations in temperature and the availability of food.

The growth of commercially important bivalve molluscs, particularly species of the oysters *Ostrea* and *Crassostrea*, and the mussel *Mytilus edulis*, has been studied for hundreds of years. The earliest sutdies, largely unrecorded, were little more than observations. Yonge (1960), for example, quotes a Roman author from the first century B. C. who noted that cultured oysters prefer some freshwater and that they grow best in the early summer.

Spurred by declining production from natural oyster beds, which accompanied the industrial revolution in 19th century England, researchers began a concentrated effort to understand the biology of oysters. These efforts, which emphasized the processes of feeding and growth, have continued, primarily in England, Japan, and the

United States, for the last one hundred years.

Studies of food consumption and growth in bivalves have traditionally relied on three general approaches (Ukeles, 1971). These may be classed very generally as (1) an anatomical approach, (2) an ecological approach, and (3) a physiological approach. In more recent years, a fourth approach based on bioenergetics has been employed with considerable success.

The anatomical approach consisted basically of describing the food gathering and processing structures of the animals and of inferring function from these descriptions. Yonge's (1926) paper is a classic representative of this approach. These studies, which provide detailed and accurate descriptions of the anatomy of bivalve molluscs, and thereby made their own contribution to science, really did not approach the important questions of feeding and growth. On the basis of such studies, one cannot, for example, make any definitive statements concerning how feeding and growth are influenced by environmental factors.

The ecological approach to studies of feeding and growth began in the mid-nineteenth century and continues today. It is characterized by observation of oyster growth under natural or quasi-natural conditions, together with simultaneous analysis of potential food material in the water and in the stomachs of the animals. Based on this approach, Savage (1925) reported unequivocally that the diatom

Nitzschiella was responsible for oyster fattening in one area of the British Isles. However, his conclusions were based on data collected with a 140 micron plankton net. Nannoplankton or soft-bodied organisms would very probably have been excluded from his samples. More recent studies (Sharp, 1973) have shown that particulate organic matter in seawater that is large enough to be retained by a 140 micron plankton net constitutes only a very small fraction of the total particulate matter present. Similarly, Lotsy (1893) "eliminated" the possibility that bivalves utilize dissolved material as food because his data seemed to show that "hardly a trace of such material" could be found in estuarine water in which oysters were known to grow. It has now been established that dissolved organic material constitutes about 90 percent of the total organic material present in seawater (Parsons, 1963; Sharp, 1973).

The analysis of stomach contents employed in conjunction with the above studies tended to reinforce conclusions drawn from plankton sampling data. Studies of feeding by analysis of stomach contents continued for many years (Field, 1909; Savage, 1925). This was done despite the fact that it was early recognized that the method produced a bias in favor of the less digestible or larger diatoms (Lotsy, 1893).

The ecological approach contributed little to our understanding of food consumption and growth in bivalves because of the inability of the researchers employing the technique to adequately determine the

presence or absence of potential food materials in the water. Moreover, there is no a priori reason to assume that the material most abundant during periods of oyster growth must be the food of the animals.

The physiological approach, has taken many forms. These have included studies of enzymes of digestion and the pathways of metabolism (Hammen, 1966), 1969; Hammen, et al, 1966; Hochachka and Mustafa, 1973; Purchon, 1971). Many of these studies have contributed to our understanding of basic metabolic processes in bivalves, but because of their point of view, they leave unanswered a great many questions regarding the quantitative relationships between the growth of these organisms and factors in their environments.

The physiological approach has also included a number of studies that have attempted to answer questions about the food of bivalves by experimenting with artificial diets. These studies, which began many years ago (Mitchell, 1915), have included efforts to feed a variety of materials to oysters and other bivalves. These materials have included starch (Gillespie, et al, 1964; Haven, 1965; Willis, et al, 1976), finely ground macrophytic algae, yeast, extracts of rice and other dissolved materials (Chanley and Normandin, 1967), and corn starch, corn oil, cod liver oil, etc. (Castell and Trider, 1972). Some of these authors reported an increase in the condition (weight per volume) of the animals or an increase in stored glycogen, but

none reported both meat and shell growth among animals fed any of these materials. Because very little is known about the physical acceptability of such diets in terms of particle size or specific gravity, very little can be concluded about the qualitative or quantitative food requirements of bivalves from these studies.

These first three approaches, although having included some fine work, have been relatively nonproductive, if judged solely on their contribution to our understanding of quantitative relationships between environment and the feeding and growth of bivalves.

The use of an energy budget or bioenergetic point of view, pioneered by Ivlev (1945) and Fry (1947), combined with an acceptable artificial diet or at least the ability to control levels of a natural diet, has permitted characterization of important basic principles of feeding and growth for a number of fish species (Warren and Davis, 1967). With these same tools and employing the same approach, similar advances are now being made in our understanding of feeding and growth in bivalves. These studies, although conducted primarily since 1970 (Hughes, 1970; Widdows and Bayne, 1971; Bernard, 1974; Bayne, *et al.*, 1976) and still limited in scope by the lack of an artificial diet, have yielded more quantitative information about bivalve feeding and growth than the work of the previous 70 years.

An energy budget is essentially an effort to list and quantify the energy equivalents of all of the possible fates of consumed energy.

This concept views growth as one of many possible fates of available energy. Determination of the relative magnitudes of these other energy costs to the animal, and consideration of the influence of envirohmental factors on energy partitioning, provide the basis for understanding, not just observing, growth.

A number of different energy budget equations have been proposed. These equations, although generally quite similar, vary in their completeness and in the measurability of their components (Warren, 1971). Perhaps the most useful of the equations was given by Warren and Davis (1967). This equation, in its simplest form, is as follows:

$$Q_c - Q_w = Q_g + Q_r$$

where,

Q_c = the energy content of food consumed by the animal

Q_w = the energy content of waste products (feces, urine, etc.)

Q_g = the energy content of growth

Q_r = energy lost through respiration

Components of the energy budget equation can be further subdivided to consider for example different aspects of growth (muscle, bone, sex products, etc.) or different levels of respiration (active, or standard). This type of subdivision is, in fact, necessary in order to make understanding of growth possible. In practice, the form of the energy budget equation will be determined by the methods used in

measuring the components and on the species of animal being studied.

In one of the more complete studies of energetics of bivalves, Widdows and Bayne (1971) applied the Warren and Davis budget equation without modification to their study of the mussel _M. edulis_. Modifications of the equation, however, may make it more meaningful for application to studies of filter feeding molluscs.

Consider again the basic equation, $Q_c = Q_w + Q_g + Q_r$. A significant fraction of particulate material removed from suspension by the filter of an oyster may never enter the animal's digestive system. Through mechanisms described by Bernard (1974), some of this material may be rejected at the mouth as an amorphous, mucus-bound mass called pseudofeces. Since pseudofeces can have a significant effect on the utilization of material removed from suspension by oysters, it is of interest in considering the animal's energy budget. For purposes of this study, the energy of material actually consumed by the oysters (Q_c) will be determined as the difference between the energy content of material cleared from suspension (Q_{cl}) and the energy content of any pseudofeces formed (Q_p).

Many studies of growth in bivalves have considered only the weight change or energy content of the meat (Trevallion, 1971). Although a few (Hughes, 1970; Dame, 1972) have attempted to estimate the energy content of the shell organics, others (Tenore, et al, 1973) have assumed it to be negligible. At present, there is little evidence

to support or refute this assumption, and until such data are available the growth component (Q_g) should account for the energy content of both the meat (Q_{gm}) and the shell (Q_{gs}). It should further be noted that the meat growth component also includes gains and losses due to gonadal maturation and spawning.

The respiratory component of the equation (Q_r) was partitioned by Warren and Davis (1967) into three subcomponents: Q_a, active metabolism, a variable component dependent on the degree of activity; Q_s, standard metabolism, defined as the metabolic rate of an unfed animal at rest; and Q_d, specific dynamic action, energy utilization and losses owing to food handling. A similar partitioning of respiration in bivalves is also possible. Thompson and Bayne (1972), for example, suggested a useful framework for considering respiration in bivalves. They proposed three levels of metabolism, 1) active metabolism, defined as oxygen consumption associated with short term feeding, an acute response to the presence of food; 2) routine metabolism, defined as oxygen uptake associated with long term feeding, and therefore the most meaningful uptake rate in studies of feeding and growth; and 3) standard metabolism, defined as the oxygen uptake rate associated with prolonged starvation.

The waste component (Q_w) of the budget equation has been further divided by some authors (Warren and Davis, 1967; Harris, 1966) to consider fecal and nitrogenous wastes separately. In fact, Harris

(1966) recommended considering nitrogenous wastes from food and body (endogenous) sources separately. The idea may be conceptually sound, but to my knowledge no such attempt has been made in any study of the energetics of an aquatic organism. Although the production of nitrogenous wastes by bivalves has been the subject of a number of studies (Hammen, et al., 1966; Potts, 1967), the significance of these losses in terms of the energetics and growth of the animals has not been considered.

The energy budget equation that results from the above modifications differs only in detail from that suggested by Warren and Davis (1967), but the consideration of such detail is conceptually, if not operationally, important in understanding the energetics of feeding and growth in bivalves.

The modified equation is as follows:

$$Q_c = Q_{cl} - Q_p = Q_w + Q_g + Q_r$$

where,

Q_c = the energy content of food consumed (ingested)

Q_{cl} = the energy content of all materials removed from suspension by the animal's filtering apparatus

Q_p = the energy content of materials removed from suspension, but not ingested (pseudofeces)

Q_w = the energy content of all waste products (feces, nitrogenous wastes, and any other losses of organic material other than spawning)

Q_g = the energy equivalent of growth and is Q_{gm} (the energy of the meat) + Q_{gs} (the energy content of the organic fraction of the shell)

Q_r = the energy equivalent of respiration measured as oxygen uptake under the conditions of growth

An additional term, not actually a part of the budget equation, is the assimilation term, Q_a. Assimilation approximates in energy terms the amount of material absorbed through the gut wall and is defined as $Q_a = Q_c - Q_w$, or $Q_a = Q_g + Q_r$. The assimilation term is generally used to calculate assimilation efficiency, Q_a/Q_c X 100%. Assimilation efficiencies have been estimated for a wide variety of organisms (Welch, 1968). The significance of these estimates is discussed by Warren (1971).

Consideration of assimilation efficiency is particularly important because the term has been used in studies of bivalve molluscs to calculate, rather than actually to measure, growth rates in molluscs (Widdows and Bayne, 1971; Bayne, Thompson, and Widdows, 1976; Langefoss and Maurer, 1976). The technique, developed by Conover (1966), involves comparing the energy or ash content of the feces with that of the food. From this ratio, percent assimilation is estimated. If food consumption and oxygen consumption are also known, growth can be estimated by the equation,

$$(Q_c \text{ x percent assimilated}) - Q_r = Q_g$$

In studies of bivalve molluscs, the Conover method has a number of possible sources of error. In its application, the method assumes that the energy content of material available to the animals is the same as the energy content of materials that they consume. That is, it assumes that there is no selectivity in feeding. The method also assumes that only the organic component of the food is affected by digestion. These assumptions may not always be valid (Forster and Gabbott, 1971).

Other potential sources of error include the inability to account for the mucus component of the feces of molluscs, and the loss of unassimilated soluble material from the feces prior to sampling. An additional, more serious problem, involves the collection of fecal samples. Studies have shown that bivalves produce two types of feces (Van Weel, 1961; Bayne, 1976). One type, called "intestinal" by Van Weel, is composed largely of undigested, even living, material. Intestinal feces are characteristically produced by animals feeding in high food concentrations. The second type, called "glandular," is more completely digested. The intestinal type is certainly more visible than the glandular type, and could be sampled preferentially. Such sampling bias would lead to underestimation of assimilation efficiency, and of growth, if the Conover method were used.

Various components of the energy budget equation can also be used to calculate other "ecological efficiencies." These include

"gross growth efficiency" which is equal to Q_g/Q_c, and "net growth efficiency" which is defined as $Q_g/Q_c - Q_m$. In these ratios, Q_c and Q_g represent food consumption and growth respectively, and Q_m is the maintenance ration, the ration at which the animal neither gains nor loses weight. The significance of these efficiencies has been considered by a number of authors (Welch, 1968; Warren, 1971) and will not be discussed in detail in the present study.

In this study, oysters were provided with unfiltered seawater at various flow rates and temperatures, and their growth rates were determined. These raw water or "open system" experiments were repeated seasonally, and with concurrent determination of particulate organic carbon and nitrogen in the water, they provide a measure of seasonal fluctuations in oyster growth rates and in potential food material. More importantly, these experiments provide a first approximation of the relationships between temperature, food supply, and oyster growth. The open system studies defined the problem considered more rigorously by the "closed system" energy budget experiments.

The energy budget experiments were carried out in a carefully monitored system that permitted control of both food density and temperature. Food consumption, respiration, and growth were measured directly at four food densities and four temperatures in a factorial design. Data from these experiments were then used to calculate

complete energy budgets for juvenile oysters. These energy budgets offer an explanation for the growth responses observed in the open system experiments.

MATERIALS AND METHODS

Open Systems

Apparatus

The open system experiments involved the use of a number of relatively simple systems that maintained a controlled flow of raw Yaquina Bay water at several temperatures. These systems consisted basically of Plexiglas® headboxes heated by Vycor® immersion heaters controlled by Safti-stat ® thermoregulators. The wattage of the immersion heaters was balanced as closely as possible with the heat requirement for a given temperature and flow combination. Temperature fluctuations caused by repeated cycling of the heaters was thereby minimized and regulation to within $\pm$ 0.5° C of the desired temperatures was maintained. Water of the appropriate temperatures was allowed to flow from the headboxes through 5 mm glass tubing, which was bent and inserted through rubber stoppers in such a way that the head and therefore the flow rate could be regulated by rotating the glass bends. These systems generally provided water flows that were within $\pm$ 10 percent of the mean.

The oysters were held on screens in shallow four liter plastic pans. Since the retention time in these pans was relatively long (up to about three hours), the pans were placed in water baths supplied

with excess heated water from the headboxes. The larger volume of the water baths minimized any fluctuation in water temperature.

Water supplied to the headboxes was taken from the seawater system of Oregon State University's Marine Science Center on Yaquina Bay. Other than temperature regulation and the natural settling of large or dense particles that occurred in the Center's seawater system or in the Plexiglas headboxes, the water was untreated.

Animals

Single or "cultchless" juveniles of the Pacific oyster, *C. gigas*, were used in all experiments. The oysters were purchased from commercial oyster hatcheries in Washington and California. The animals were air freighted from the hatcheries and were held for about a week in running seawater prior to the start of an experiment. Since the animals had a size range of at least ± 100 percent of the mean at the time of shipment, they were sorted by size to remove the very large and the very small individuals before random selections were made for the experimental treatments.

Growth Measurements

The growth of oysters held in the open systems was determined by measurements of shell length, dry meat weight, or ash free dry meat weight. The length of the shell, as defined for this study, is the

maximum dimension from the umboe to the ventral margin of the shell in a straight line. Technically this dimension is the height of the animal since it is the dorsoventral axis (Galtsoff, 1964), but since it is also the shell's greatest dimension, it is usually referred as length. Growth in terms of shell length was obtained through periodic measurement of randomly selected animals from each treatment. Large animals were measured to the nearest millimeter with calipers. Smaller animals were measured with a micrometer eyepiece to the nearest one-tenth millimeter.

To obtain meat weights, oysters were shucked by hand onto glass fiber filter papers (Whatman GF/A) or small squares of aluminum foil that had been previously ashed at 450° C and weighed. A random sample of oysters was drawn at the beginning of each experiment to be shucked and weighed. These animals provided a measure of the initial weight of the experimental groups. Growth at each treatment was then expressed as a change (plus or minus) from this initial value. In all cases, oyster meats were dried at 90° C for 20 hours to obtain dry weights. The meats were then ashed at 450° C for 20 hours. The resulting ash was then rehydrated with distilled water, dried at 90° C for 20 hours, and weighed. The weight of the ash was subtracted from the dry meat weight to obtain ash free dry weight.

Preliminary trials showed that hand shucking of oysters less than 10 mm in length was simply not a practical and reliable technique.

Hand shucking of these small animals tends to introduce large errors from shell fragments and from difficulties in recovering all the meat. A technique was therefore developed and tested in which acid (HCl) was used to dissolve the shells of small oysters to permit recovery of the meats and acid insoluble organic shell matrix. The advantages of the method, in addition to the fact that it permits rapid handling of large numbers of animals, include complete recovery of the meats, inclusion of the organic fraction of the shell in measurements of growth, and avoidance of the problem of removing fragments of shell included with the meat after hand shucking. Potential problems with the method include the loss of meat due to action of the acid, incomplete removal of the shell, and incomplete removal of salts ($CaCl_2$) produced by the reaction of HCl with $CaCO_3$ of the shell. Calcium chloride is extremely hygroscopic and is very difficult to weigh accurately in small amounts. It is also somewhat unstable at the 450° C ashing temperature and cannot be corrected for in the ash weight. Trials, data from which are given in Appendix 1, showed that the weight of the meat was unaffected by concentrated HCl, although it was obviously denatured. These trials further showed that the $CaCl_2$ salts could be removed from the meats by a five minute distilled water wash, and, since the ash content of the acid shucked meats was as low or lower than comparable hand shucked meats,

that the shell can be assumed to be completely removed by acid shucking.

Relative growth rates were calculated from initial and final weights or shell length through the use of the exponential equation:

$$k = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W_1 = the initial weight or length

W_2 = the final weight or length

and $t_2 - t_1$ = the duration of the experiment in days

The coefficient k was multiplied by 100 to yield percent change per day.

<u>Monitoring Particulate Organics</u>

Significant seasonal fluctuations in oyster growth rates have been observed by a number of workers at a variety of locations (Quayle, 1969; Dame, 1972; Maurer and Aprill, 1973), and my personal observations led me to expect similar fluctuations in my studies. I further expected such fluctuations in the growth of sexually immature oysters to be related to changes in the quality or quantity of available food. Therefore, as a part of this study, I sought to monitor seasonal fluctuations in materials that might serve as food for juvenile oysters. These data were then to be used as a basis for

selecting artificially controlled levels of food availability in the closed system experiments.

The best evidence currently available indicates that oysters rely primarily on particulate material as their food source, although they can absorb and utilize certain dissolved materials from their environment (Jorgenson, 1976). There is, however, no evidence to indicate that this particulate material must be living. For these reasons, I determined that, of the available analytical techniques, measurement of particulate organic carbon and nitrogen provided the best indicator of available food.

Samples for carbon and nitrogen determination were drawn weekly from the Plexiglas headboxes of the open systems. Generally, a 250 ml sample was drawn and, from this, two 100 ml samples were taken for analysis. The subsamples were gently filtered through Whatman GF/A glass fiber filter papers cut to fit in a 13 mm Millipore Swinnex filter holder. Sampling procedures developed and reported by Donaghay and Small (in press) were followed to insure that cell breakage was minimized. When filtration was completed, the filter papers were rolled into a tight roll with the sample inward and were placed in individual one-half dram vials. The samples were dried at 90° C for 24 hours, capped while hot, and were then frozen for later processing.

Organic carbon and nitrogen were determined with a Carlo-Erba® Carbon, Hydrogen, Nitrogen, Oxygen Analyzer by staff members of Oregon State University's School of Oceanography. The analyses produced data on particulate organic carbon and nitrogen in micrograms per liter as well as the ratio C/N.

Experimental Design

Seven open system experiments were conducted over a two year period. These experiments involved the use of a number of different combinations of temperature and water flow rates and were conducted using animals of different initial sizes. The designs of the seven open system experiments are shown inTable 1. Note that although a variety of water flows, temperatures, and animal sizes were used, the combination of eight milliliters per animal per minute at 15° C with animals having an initial length of 20-25 mm was included in four experiments to provide a measure of seasonal growth variation under the same treatment conditions.

Closed Systems

The open system experiments served to assay, in a relative sense, seasonal changes that can be expected to occur in the rate of oyster growth when temperature, but not food availability, is manipulated. The experiments also provided quantitative information relating

Table 1. Design of the seven open system experiments. In experiments III and IV, water flows rates were used in all possible combinations with the temperatures given. Weights given for experiments III and IV are dry meat weights; weights given for experiments V, VI, and VII are ash free dry meat weights.

Expt. No.	Mid-point of expt (date)	Duration (days)	Flow Rates (ml/oyster/min)	Temps. °C	Osyters' Initial Size	
					Length (mm)	Meat Weight (mg)
I	Feb. 26	54	8	10, 15, 18, 21	25.9	-
II	May 11	84	8	10, 15, 18, 21	25.8	-
III	Nov. 6	57	4, 8, 16, 32	11, 15, 20	21.0	16.7
IV	June 16	63	4, 16, 28, 40	11, 15, 20	23.0	23.1
V	Aug. 24	34	8	11, 15, 19, 23	22.7	12.1
VI	Oct. 21	48	2	11, 15, 19, 23	-	2.78
VII	April 19	56	0.66	11, 15, 19, 23	3.2	0.10

changes in the concentrations of particulate organic carbon and nitrogen to fluctuations observed in oyster growth rate.

Growth, food availability, and temperature relationships for oysters can be adequately defined, however, only under experimental conditions that permit manipulation of both temperature and food density. Such conditions can be maintained most readily in a closed or recirculating system. For this reason, a recirculating seawater system and an algae culture facility were designed and constructed for use in a series of oyster growth experiments.

It was not the objective of this study to develop or refine methodology for operating a marine closed system. The closed system was viewed as a tool, not an end in itself. The design and management of the closed system reflect this philosophy.

Apparatus

There are a great many marine closed systems operating in various parts of the world. There are, however, very few systems that have been used to provide controlled levels of food to filter feeding animals. Most of the recent work has been done by scientists at the University of Delaware (Hartman, et al., 1974; Epifanio, et al., 1975; Epifanio and Mootz, 1976). The design of the closed system used in this study was based on the Delaware system, as well as on other successful systems (Clark and Clark, 1964; Zillioux, 1969;

Spotte, 1970, 1974; Liao and Mayo, 1972). Methods for complete removal of planktonic algae from such systems in order to regulate algal density were not described in the literature and were developed for this study.

The closed system consisted basically of four subsystems: (1) water treatment, (2) temperature control, (3) flow control and animal holding trays, and (4) algal delivery. These subsystems are described briefly in Table 2, and are shown schematically in Figures 1 and 2.

The total capacity of the system was about 1,000 liters, which is relatively large in relation to the biomass (1-10 g dry weight) that it supported (Goldizen, 1970). The water was circulated through the treatment systems at a rate of 10 liters per minute. Seawater was filtered to 0.8 microns, sterilized with an ultra-violet sterilizer, and was added to the system at a rate of 25 percent per day (250 liters per day). This exchange rate is in effect the same as a continuous exchange of about 10 percent of the system's flow rate. Ten percent continuous exchange systems (so called "90 percent reuse" systems) have among the highest water replacement rates reported in the literature (Liao and May, 1972) for closed systems.

Table 2. Details of the components of the recirculating sea water system used in the "closed system" experiments. Components within a subsystem are described in order of their occurrence in the system, but the general arrangement of the components is best seen in the schematic (Figure 1).

Subsystem	Component	Description
(I) Water Treatment	(A) Particle Filters	(1) 116 micron nitex,® monofilament nylon screen
		(2) 5 micron Afco® filter bag
		(3) 3 micron filter cartridge, Pall, Epocel-3®, polypropylene
		(4) 0.8 micron filter cartridge, Pall Ultipor-0.25®
	(B) Foam Column	Design from Zillioux and Lackie (1970); 1.5 m x 7.6 cm diameter plexiglas pipe with 60 mm fritted glass gas dispersion funnel; water flow countercurrent to air bubbles; foam collected in waste container
	(C) Biological Filter	Design from Spotte (1970); 60 cm x 60 cm x 12 cm deep layer of dolomite gravel on perforated fiberglass sheet; perforated plastic dispersion plate on top of gravel
	(D) Carbon Filters	AMF/Cuno® , AP-117 filter cartridges (four) in plastic housings;
	(E) Ultra-violet Sterilizer	Aquanomics® 17 watt unit, rated max flow 8 LPM, one-half of system's total flow passed through the unit with each cycle (5 LPM)

Table 2. Continued.

Subsystem	Component	Description
(II) Temperature Control	(A) Heating	Vycor® immersion heaters in plexiglas headboxes, controlled by mercury relays and H and R Instrument Co. "Red Top" thermo-regulators
	(B) Cooling	Two Blue-M® 3500 BTU/hr compressors with stainless steel coils placed inside glass heat exchangers with 50% glycol solution at 0°C, two-stage cooling, 5 LPM to 14°C, 2.5 LPM of that to 10°C.
(III) Flow Control & Animal holding	(A) Flow Control	Flow meters and valves for flow control to water treatment systems and to control flow to each headbox (Figure 1); flow from headboxes to each of 16 trays (four at each temp) controlled by Fischer-Porter glass valves (10 mm)
	(B) Animal Holding Trays	Four 60 cm x 30 cm x 15 cm deep plexiglas trays subdivided into four compartments containing about four liters of sea water (at a depth of 10 cm); trays were placed in plywood baths for additional temperature control (Figure 2); oysters were placed on 1 mm mesh nylon screens in the trays
(IV) Algae Delivery	(A) Algal Reservoir	48 liter glass carboy partially submerged in a water bath receiving a constant flow of water at 17°C from the closed system (Figure 1); continuously aerated; received 24 hour room light; drained, flushed, refilled every 24 hours

Table 2. Continued

Subsystem	Component	Description
	(B) Algal Pumps	Three Gilson® eight channel peristaltic pumps (one for each feeding rate); Tygon® tubing (1.6mm) from reservoir to pump to holding trays (Figure 2); algae added to incoming water entering holding trays; flow of algae culture (1, 2, and 4 ml/min) insignificant compared to total flow to holding tray (200-400 ml/min)

Figure 1. Schematic drawing of the recirculating sea water system used in the "closed system" experiments. The algal reservoir and water bath are shown, but the algal delivery pumps and tubing have been omitted. Oysters were placed in holding trays details of which are shown in Figure 2.

Figure 1

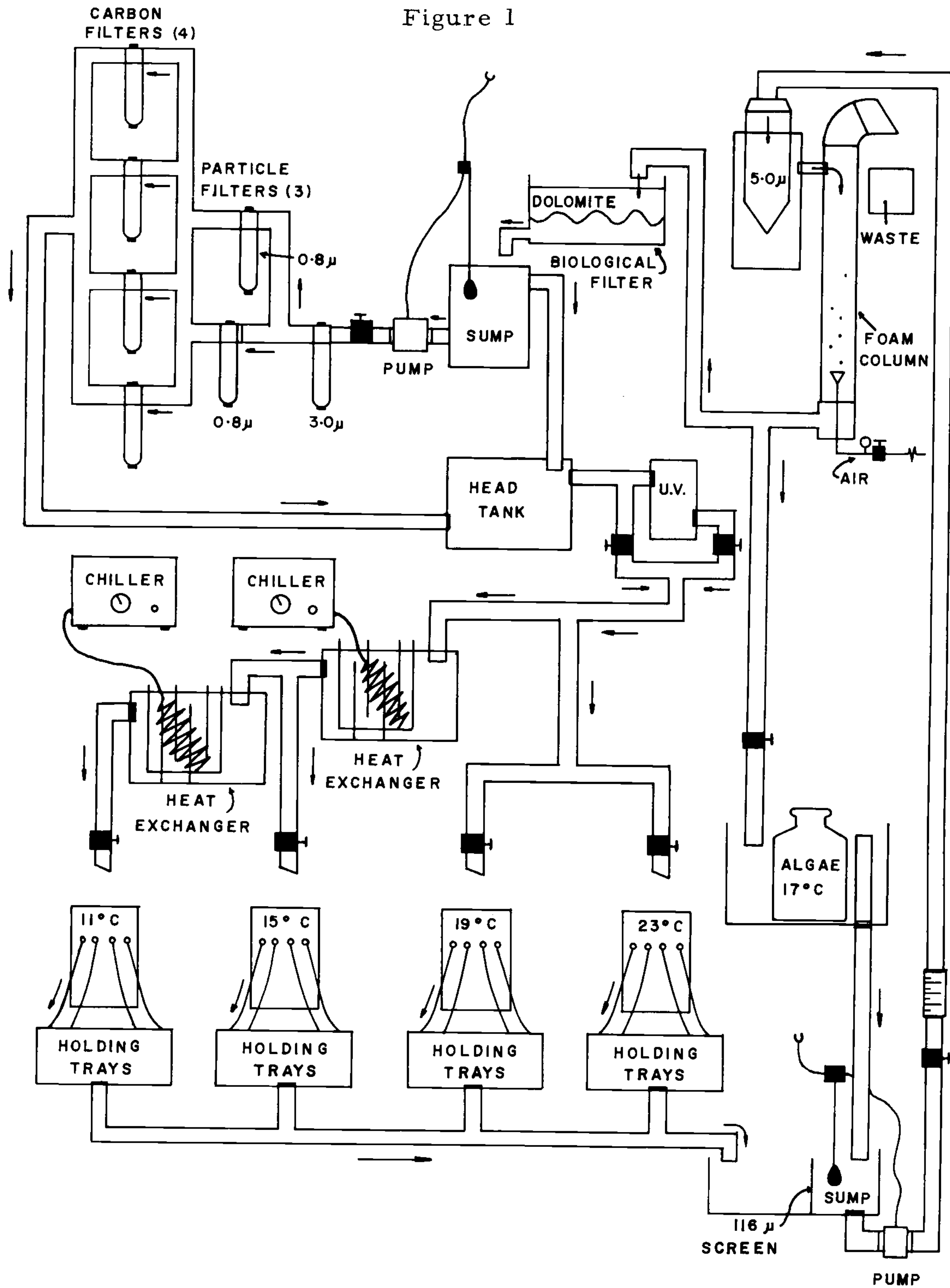


Figure 2. Recirculating sea water system; details of the oyster holding trays, water flow regulation, heating, and algal delivery system. As shown in Figure 1, this system, except for the algal reservoir, was duplicated for each of the four temperatures. Three of the four trays at each temperature received algae from the reservoir. For simplicity, only one tray is shown with its algal feed tube.

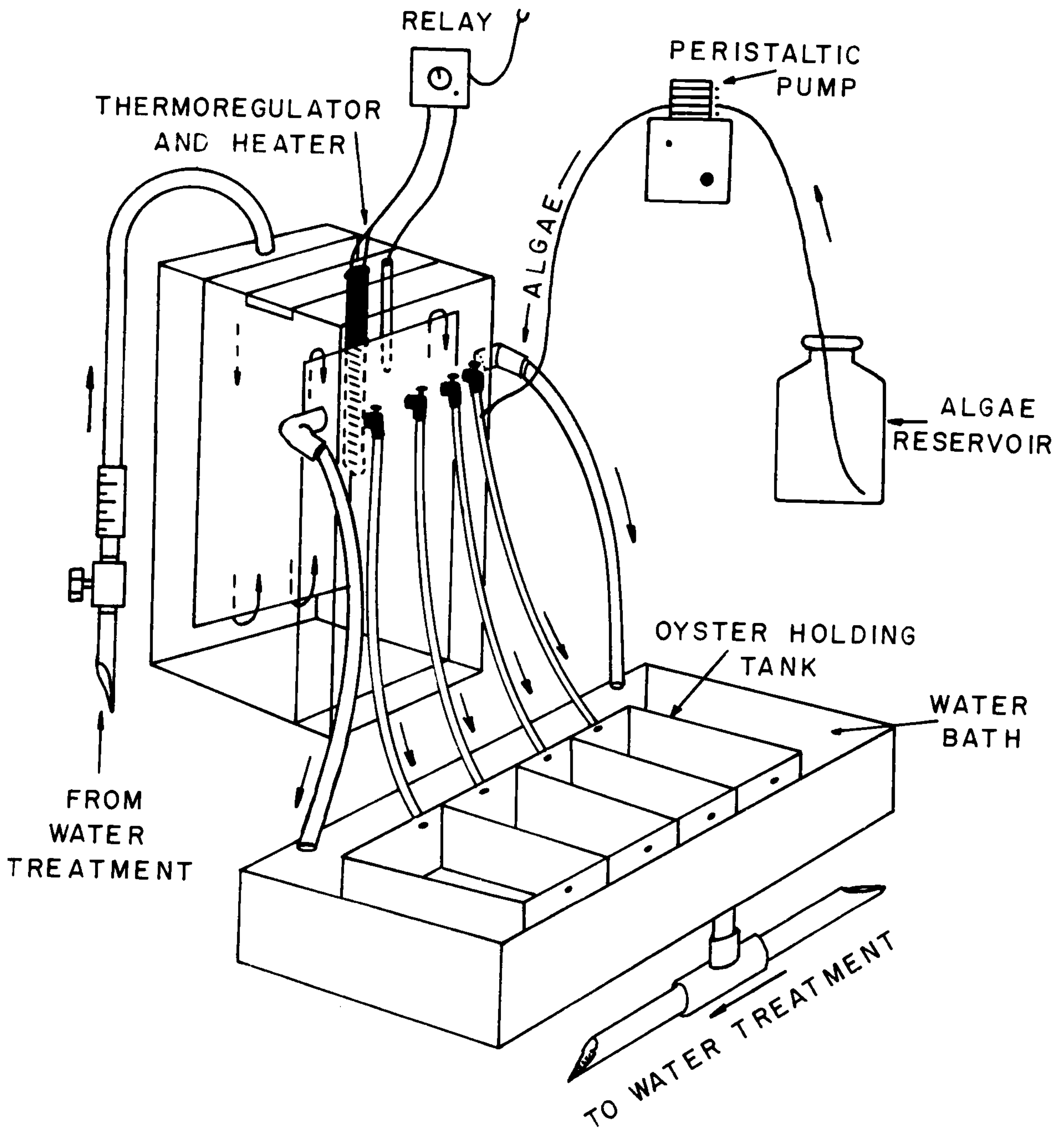


Figure 2

Operation and Monitoring the Closed System

The system was stocked with relatively large oysters and was operated for four months prior to the start of the first experiment. An Orion® model 95-10 ammonia electrode was used, with an Orion® model 701 pH meter according to procedures outlined by Srna, et al. (1973), to monitor the concentration of ammonia in the system during the conditioning period and later during the experiments. The biological filter (Table 2), the use of make-up water in the system, and probably the presence of large numbers of phytoplankters in the system acted to keep ammonia concentrations in the system at a safe level. The highest concentration of ammonia recorded was 3.1 ppm, which occurred three days after the start of the first experiment. After one week, the concentration of ammonia dropped to 0.3 ppm. It generally remained at about 0.5 ppm during the experiments. Oysters do not appear to be adversely affected by concentrations of ammonia below 10 ppm (Epifanio, 1975).

Salinity and pH were monitored weekly during the experiments. The salinity ranged from 29 to 32 ppt. The pH range was from 7.95 to 8.10 throughout all the experiments, but during any one experiment the pH was relatively constant (± 0.1).

Temperatures in the system were checked and recorded twice daily during an experiment. Because of the nature of the system,

temperatures were held very close to those desired. A temperature fluctuation of 0.5° C was considered extreme. In experiment II for example, the means and 95 percent confidence intervals (N=56) for the four temperatures, recorded to the nearest 0.1° C were 11.0°, 15.0°, 19.0°, and 23.0°, ± 0.0° C in each case.

Dissolved oxygen concentrations were determined for each temperature treatment several times during each experiment. The concentration of dissolved oxygen was found to range from 90 to 97 percent of saturation, depending on the temperature.

Rates of water flow and rates of introduction of algae in the closed system were measured and recorded at least once daily during an experiment. These flows generally were within five percent of those desired.

Animals used in the closed system experiments were clutchless spat purchased from the same hatcheries that supplied animals for the open system experiments. The animals were handled as previously described for the open system experiments.

During an experiment, the oysters were removed from the system and were rinsed with fresh water every 48 hours. At the same time the holding trays were siphoned out and cleaned. When the animals were returned to the holding trays, they were mixed and their positions within a treatment were randomly rearranged to insure

that they were not always in the same position relative to the inflow of food organisms.

Algae Culture Facility

The use of a closed system for studies of feeding and growth in oysters necessitated the use of a supplemental food for the animals. Our understanding of the nutritional requirements of oysters at this time is such that the only material known with confidence to serve as food for oysters is living phytoplankton. Therefore, an algae culture facility was designed and constructed to provide food for the closed system experiments.

Two flagellate species, Monochrysis lutheri Droop from the University of Indiana algae collection, and Pseudoisochrysis paradoxa (VA 12), from Dr. John Dupuy of the Virginia Institute of Marine Science, were chosen for use in these experiments. These species are relatively easy to culture, stay in suspension well after they are introduced, and are known to be at least adequate food for oysters.

The culture system used to produce algae was, with slight modification, the same type of system used successfully in Oregon State University's Pilot Oyster Hatchery (Breese and Malouf, 1975). The system involved a series of cultures increasing sequentially in volume from 250 ml to three liters and finally to 50 liters. Erlenmeyer flasks were used for the two smaller culture volumes. The 50 liter

culture vessels were fabricated from Plexiglas sheets and were 30 cm x 30 cm x 75 cm high. All the cultures were lighted continuously with fluorescent bulbs, one-fourth of which were Sylvania Gro-Lux® bulbs, three-fourths being Sylvania Life-Line® bulbs. The two larger culture volumes were continuously aerated with compressed air filtered to 0.25 microns. The 250 ml flasks were not aerated but were agitated by hand twice daily.

Seawater used in the 250 ml cultures was autoclaved. Water for the two larger culture types was filtered to five microns and disinfected at least 24 hours with 5-10 ppm chlorine (from sodium hypochlorite). The water was then passed at a rate of 1 liter/min sequentially through a three micron filter (Pall, Ultipor®), a 5 cm x 3.5 cm column of activated charcoal containing approximately eight liters of grannular carbon (Calgon, filtrasorb 400®), an ultra-violet sterilizer (Aquanomics, 17 watt) and then into the culture vessel. Nutrient media in all cases was based on the Matthiessen and Toner (1966) formulation. In addition, 0.2 grams per liter of sodium bicarbonate was added to the larger cultures as a carbon source.

Only the 50 liter cultures were harvested for feeding. These cultures reached a maximum density of about 25 million cells per ml with *P. paradoxa* and about 12 million cells per ml with *M. lutheri*. Both species, however, were harvested only in their log growth phase, or at about 8-10 million cells per ml and 4-5 million cells per ml

respectively. With both species, the desired culture density was reached in 4-5 days after innoculation.

The growth of the larger cultures was monitored daily with a Coulter Counter Model ZBI®. A Coulter P-64® size distribution analyzer was used with the counter to permit monitoring of the cell volume distributions in the cultures. This instrument, used with an X-Y plotter, was calibrated with 2-10 micron diameter polyvinyl-toluene beads (Particle Information Services, Inc., Grants Pass, OR) and produced size frequency histograms that were read in cubic microns.

The organic carbon and nitrogen content of the algal cells was determined with the Carlo-Erba® CHNO analyzer as previously described. Samples from cultures of both algal species were counted and their size distribution in cubic microns per cell was plotted. Subsamples were then analyzed for organic carbon and nitrogen.

Determination of the Components of the Energy Budget

The energy budget equation used in this study was:

$Q_c = Q_{cl} - Q_p = Q_w + Q_g + Q_r$, that is, in energy terms: food consumed = food cleared-pseudofeces = waste + growth + respiration.

Food Consumption (Q_c). No pseudofeces production was observed under any of the treatment conditions in any of the closed system experiments. Therefore, Q_p must be assumed to equal zero for those experiments, and Q_c is equal to Q_{cl}. The quantity of algae cleared from suspension by the oysters per unit of time was determined as a measure of their food consumption rate. Samples were taken from the inflow and outflow of the individual Plexiglas subcompartments of the oyster holding trays and were counted on the Coulter Counter to determine the algal cell densities. When no animals were present in the trays, the inflow and outflow algal concentrations differed by a mean of only 1.6 percent. When animals were present, the difference between the inflow and outflow concentrations was taken to represent algal cells cleared by the animals. The animals cleared up to 60 percent of the algal cells, although under poorer conditions clearing rates occasionally dropped to as low as 5 percent.

Rates of food consumption were determined several times during each experiment. Because of the schedule used in replenishing the algal reservoir, these determinations were generally made during the evening after the system had equilibrated. Counts were made occassionally at other times during the day and night. There was no evidence from any of the counts that the oysters were any more or less active in their feeding at any particular time. In one food consumption trial, counts were made of the inflow and outflow concentrations at

four hour intervals for 18 hours. These data, which are given in Appendix 2, further indicate that, although the behavior of individual oysters undoubtedly varied, the feeding of the population was relatively constant. These data also show that equilibration of the inflow and outflow concentrations was completed and that feeding was normal within about four hours after the interruption for refilling the algal reservoir. All the rates of food consumption reported in this study were determined at least six hours after feeding had resumed in the system.

Cyclic patterns of feeding behavior in bivalves have been reported by some workers (Rao, 1954), but most studies have failed to demonstrate any correlation between feeding activity and circadial, lunar, or tidal rhythms by animals held in artificial systems (Winter, 1973). In their studies with the oyster Ostrea lurida, Langton and Gabbott (1974) reported that there were cyclic changes in the pH, length, and amylase content of the animals' crystalline style. They found that these changes were correlated with the tides and that they disappeared if the animals were held under constant laboratory conditions for two weeks.

Caloric Conversions. Food consumption data, first collected in terms of algal cells consumed per unit of time, were converted to caloric terms in the following manner. Wet oxidation of samples

containing a known number of algal cells were carried out following procedures outlined by Strickland and Parsons (1972), Standard Methods (1975), and Maciolek (1962). The method consists basically of the addition of a known quantity of strongly acid potassium dichromate to a sample of known weight (or in this case, a known number of cells). The sample was then heated at about 100° C for three hours. Finally, the sample was cooled and the remaining dichromate was determined by titration. The quantity of dichromate reduced in oxidizing the sample and therefore the sample's chemical oxygen demand (COD) could be calculated. Oxygen demand was converted to calories through the use of an oxycalorific coefficient of 3.42 calories per mg of oxygen (Maciolek, 1962).

Chemical oxygen demand determinations with dichromate have been used by a number of authors to estimate caloric values in bioenergetics studies. The method was applied to studies of molluscan growth by Russel-Hunter, *et al.* (1958) and by Langefoss and Mauer (1976). Caloric values obtained in this way tend to be somewhat lower than those obtained by bomb calorimetry. Dichromate oxidation, although very efficient with carbohydrates, is only about 90% complete with proteins (Maciolek, 1962). In addition, bomb calorimetry assumes the oxidation of nitrogenous compounds to N_2, while dichromate oxidation does not. Caloric determinations using the two methods

may not be comparable if the material oxidized is highly proteinaceous (Maciolek, 1962).

The results of the caloric determinations made on samples of *P. paradoxa* and *M. lutheri* are given in Appendix 3. From these data, I determined conversion factors of 9.22 cal/mg of carbon for *P. paradoxa* and 9.65 cal/mg of carbon for *M. lutheri*. Platt and Irwin (1973) suggested a general conversion factor of 11.4 cal/mg C for natural phytoplankton samples. However, they used bomb calorimetry to establish the caloric values of their samples. Moreover, their data show a direct relationship between the calories per carbon in their samples and the samples' C/N ratios. In other words, samples containing more nitrogen (protein) relative to carbon had a lower caloric value per unit of carbon. The cultured algae that I used in these experiments had relatively low C/N ratios (about 6.0). Platt and Irwin's samples had a mean C/N ratio of about 9.7. Considering these factors, caloric values used in this study are in reasonable agreement with those reported by Platt and Irwin (1973).

Respiration (Q_r). Respiration was measured as oxygen consumption. The method used was designed to minimize disturbance to the animals and to duplicate as much as possible the conditions under which growth was measured. To accomplish this, small (about 90 ml) jars were fitted with silicone rubber stoppers through which two holes

had been bored to permit insertion of glass tubing into the jar. One of the glass tubes, the inflow, extended nearly to the bottom of the jar, while the other, the outflow, was flush with the inner surface of the stopper. The inflow glass tubing was connected to Tygon tubing and received a flow of water diverted from the inflow to the appropriate animal holding tray. In this manner, animals were placed in small containers that could be closed for determination of oxygen depletion, but were still exposed to the same water source (including food) in which they were growing. The jars were placed in the water baths that surrounded the animal holding trays as previously described, so that a stable temperature was maintained.

Animals were placed in the jars, and a flow of water, about 15 ml per minute, was maintained through them for at least 18 hours. In Experiment I, three animals were placed in each jar, while 20 of the smaller animals were used in Experiment II. After the acclimation period, the inflow and outflow tubes to each jar were closed with tubing clamps. This was done in a manner such that disturbance to the animals was minimized. The time was noted as each jar was closed, and the jars remained sealed for two to four hours. The time used was the same for any one experiment, but was varied with the size of the animals in an effort to prevent the dissolved oxygen concentration in the jars from dropping below 50 percent.

After the required time, the contents of each jar were siphoned into a 65 ml glass stoppered reagent bottle using the jar's inflow tube (which extended to the bottom of the jar) as a siphon. The dissolved oxygen content of the water was then determined using Winkler titrations as described by Strickland and Parsons (1972). The oxygen consumption of the animals was determined by comparing the reduced oxygen content of the jars containing animals with the oxygen content of the two jars per treatment containing no animals, but which were otherwise handled identically. The volume of each jar, corrected for the displacement of each group of oysters, was also determined. These data, together with the ash free dry weights of the animals, permitted calculation of oxygen consumption in terms of ml of oxygen per mg of animal tissue per hour.

Conversion of the animals' weight to caloric values will be described in the next section. Conversion of the oxygen consumption data to caloric values involved the use of an oxycalorific coefficient of 3.42 calories per mg of oxygen (Brody, 1945; Warren, 1971). A correction of this coefficient for animals respiring nitrogenous substrates and execreting ammonia has been suggested (Brafield and Solomon, 1972). This correction would reduce the coefficient by about six percent to 3.20. I have insufficient information concerning the protein content of the algae used as food in these experiments to justify use of the lower value.

Conditions in the jars used for oxygen uptake measurement were identical to those under which growth rates were determined only at the start of each oxygen consumption trial. Obviously, feeding by the oysters in a closed container must have altered the food concentration in the jars as the trial progressed. It is unlikely that the food was ever entirely removed, although it is mathematically possible considering the rate of filtration by the animals and the algae content of the jars. Exactly how this declining food density affected the animals' oxygen uptake is not known. However, Thompson and Bayne (1972) measured food and oxygen consumption by the mussel *Mytilus edulis* in a flow-through system, and found that a characteristic rate of oxygen consumption was stimulated by the presence of particulate food but that this rate was maintained by the animals for at least an hour after the food was removed entirely.

Growth (Q_g). Determinations of growth were made as previously described for the open system experiments. Animals used in Experiment I were relatively large, and were hand shucked. Animals used in Experiment II were very small (3-4 mm) and were acid shucked.

Caloric determinations were made on meats (Experiments I and II) and shell organics (Experiment II only) by means of dichromate wet oxidation as previously described for estimating the

caloric content of the algae (Appendix 4).

There are a number of points regarding the conversion of oyster growth to its caloric equivalent that deserve some consideration at this point. As previously mentioned, dichromate wet oxidation does yield lower caloric values for proteinaceous materials than those obtained by bomb calorimetry. This, again, is because of the resistance to oxidation of such material and because bomb calorimetry, unlike dichromate oxidation, includes the oxidation of organic nitrogen to N_2 (Maciolek, 1962). Consequently, as is apparent from Appendix 4, the caloric values obtained for this study are lower than those generally reported for mollusc tissue. Representative values from the literature include 4700 cal/g (Carefoot, 1970), 5400 cal/g (Russel-Hunter, et al., 1968), 5066 cal/g (Dame, 1972), 5033 cal/g (Bernard, 1974), and 5097 cal/g (Hughes, 1970). The mean of these five values is 5059 cal/g, which is 19 percent higher than the value that I obtained. It should be noted that caloric values for oysters used in Experiment II include the shell matrix, which is primarily protein (Galtsoff, 1964), and that the meats of young oysters have a high protein content (about 70 percent of the ash free dry weight, not including the proteinaceous shell matrix) (Holland and Spencer, 1973).

A second point that should be considered here is that the

caloric value per unit weight of the meat of these very small oysters does not change significantly with starvation. As demonstrated by Holland and Spencer (1973), the composition of these animals, which are low in lipids to begin with, is not greatly altered by starvation. Multiplying the relative lipid, carbohydrate, and protein components of starved and unstarved animals by accepted caloric equivalents (Maciolek, 1962) shows that a 28 percent weight loss resulted in an estimated 1.5 percent loss of caloric content per unit of weight. In a similar study with the bivalve Donax vittatus, Ansell and Sivadas (1974) reported a 34 percent drop in the dry weight and total caloric content per animal, but no change in the caloric content per gram dry weight after two months of starvation.

Based on the evidence provided by the studies described above, and my inability to demonstrate a difference in the caloric content per unit weight of meats from fed and unfed animals in this study, a single conversion term was used for all treatment groups.

Additionally, I believed that it was important to use the conversion factor that I obtained for oyster meats by dichromate oxidation, even though that value is somewhat lower than the literature values, because the caloric equivalent of food consumption was obtained by dichromate oxidation.

Average relative growth rates were calculated from the mean initial and the mean final caloric content per animal using the

equation given below (Warren, 1971):

$$\text{(1)} \qquad \text{Growth Rate} = \frac{W_2 - W_1}{(0.5)(W_1 + W_2)(t_2 - t_1)} \times 1,000$$

where, W_1 = the initial mean caloric content per oyster

W_2 = the final mean caloric content per oyster

and $(t_2 - t_1)$ = the duration of the experiment in hours

The equation expresses the change in caloric content per animal relative to the mean caloric content per animal during the experiment. The growth rates so calculated were expressed as calories per kilocalorie per hour.

The average relative growth rate equation given above was used to calculate growth rates in the closed system experiments, because the rate of food consumption in caloric terms was also expressed relative to the mean caloric content of the animals at each treatment during each experiment. As a matter of interest, these growth rates were also calculated using the exponential equation:

$$\text{(2)} \qquad k = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \times 1,000$$

Growth rates so calculated were found to differ from those obtained from equation (1) by a mean of only 2.4 percent.

Waste (Q_w) and Efficiency Estimates. The caloric equivalent of waste material was estimated from the previously determined values for food consumption, oxygen consumption, and growth from the equation,

$$Q_w = Q_c - (Q_r + Q_g)$$

Assimilation efficiency was estimated from food consumption, respiration and growth using the equation

$$\text{Assimilation Efficiency} = \frac{Q_r + Q_g}{Q_c} \times 100$$

RESULTS

Open System Experiments

The results of the seven open system experiments are shown in Tables 3 and 4. Details of experimental design for these experiments were given previously in Table 1.

There are three main points that I would like to develop from these data. These points, which will be discussed in more detail in the following section are: (1) the observed lack of consistent growth enhancement by temperatures in excess of 15° C, (2) the significant seasonal fluctuation in oyster growth under conditions of constant temperature and water flow rate, and (3) relationships between water flow rate and oyster growth. From these last relationships, hypothetical curves relating growth and food availability at different temperatures are presented.

Relationships Between Growth and Temperature

Despite extreme seasonal fluctuations in absolute values, the general relationship between growth and temperature is apparent in Figures 3 and 4. These figures show mean growth for all flows at a given temperature and are based on meat growth and increase in shell length respectively. The data show little or no increase in

Table 3. Results of five "open system" experiments conducted to determine the effects of temperature on the growth of shell and meat in juvenile Pacific oysters during different times of the year. Water flow rate and temperature were controlled, but the seawater was otherwise untreated.

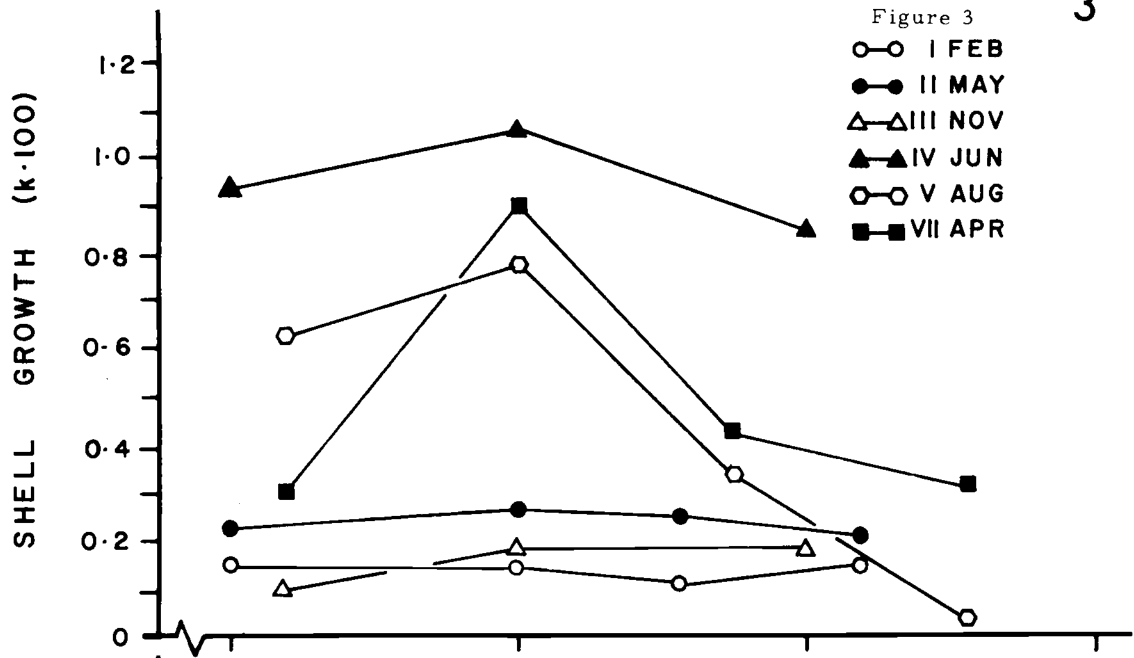
Expt. No.	Date Started - Ended	Water flow rate (ml/oyster/min)	Initial size Length (mm)	Initial size Weight (mg)	Temp. ^{o}C	Shell growth (k x 100)	Meat growth (k x 100)	Percent mortality
I	Jan 23-Mar 18	8	25.8	-	10	0.16	-	9.3
"	"	"	"	-	15	0.16	-	35.3
"	"	"	"	-	18	0.12	-	38.0
"	"	"	"	-	21	0.14	-	48.7
II	Mar 30 - Jun 22	8	25.8	-	10	0.23	-	13.0
"	"	"	"	-	15	0.26	-	14.0
"	"	"	"	-	18	0.26	-	14.0
"	"	"	"	-	21	0.19	-	14.0
V	Aug 7 - Sep 9	8	22.7	12.1	11	0.63	+1.99	-
"	"	"	"	"	15	0.81	+1.72	-
"	"	"	"	"	19	0.34	+1.28	-
"	"	"	"	"	23	0.00	-0.12	-
VI	Sep 28 - Nov 14	2	-	2.78	11	-	+0.16	2.0
"	"	"	-	"	15	-	+0.07	7.0
"	"	"	-	"	19	-	-0.73	17.0
"	"	"	-	"	23	-	-1.72	29.0
VII	Mar 23 - May 17	0.66	3.17	0.102	11	0.32	+1.28	-
"	"	"	"	"	15	0.92	+2.54	-
"	"	"	"	"	19	0.41	+0.90	-
"	"	"	"	"	23	0.32	+0.71	-

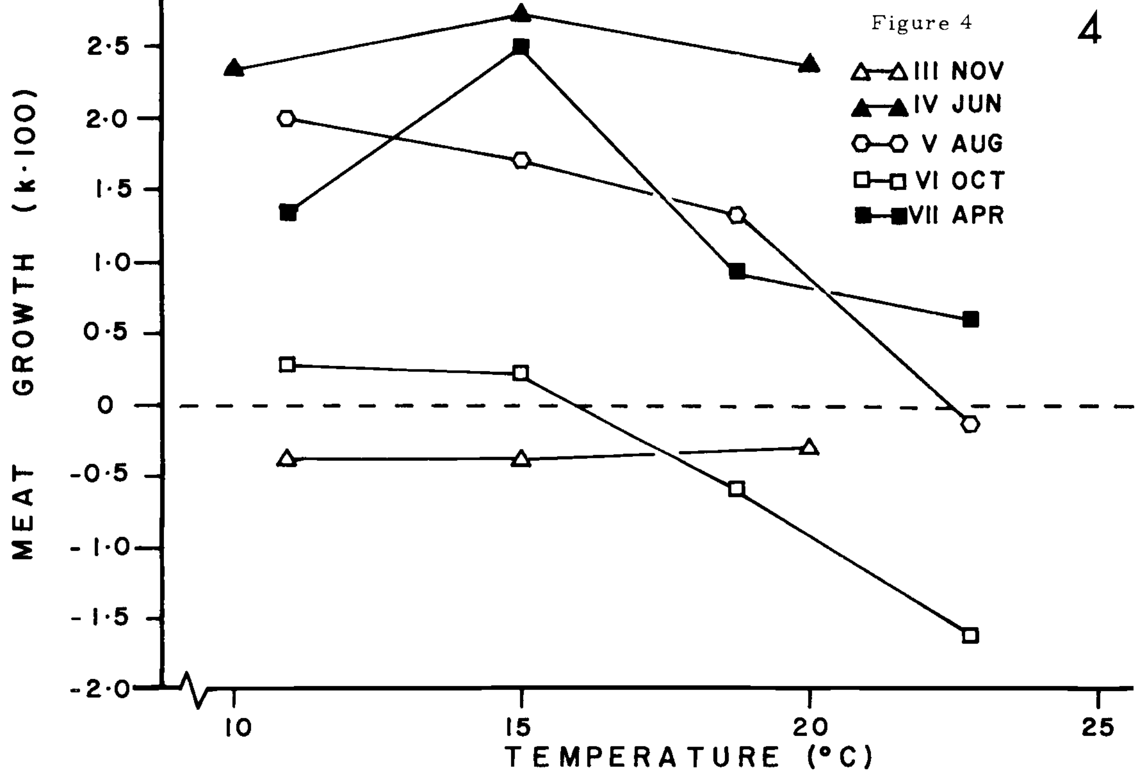
Table 4. The results of two factorial experiments conducted to determine the effects of water flow rate and temperature on shell and meat growth in juvenile Pacific oysters in unfiltered seawater without supplemental feeding.

	Temp °C	Experiment III: Oct 11-Dec 15 Initial size: L = 21.0 mm, Wt. = 16.7mg				Experiment IV: May 15-Jul 17 Initial size: L = 23.0 mm, wt. =23.1 mg			
		Water flow (ml/oyster/min)				Water flow (ml/oyster/min)			
		4	8	16	32	4	16	28	40
Meat growth (k x 100)	11	-0.72	-0.57	-0.21	+0.02	+1.06	+2.26	+2.79	+3.07
	15	-1.12	-0.40	-0.01	+0.10	+1.16	+2.55	+3.27	+3.79
	20	-0.95	-0.77	+0.11	+0.17	+0.75	+1.93	+2.97	+3.51
Shell growth (k x 100)	11	0.03	0.06	0.13	0.13	0.52	1.04	1.03	1.09
	15	0.04	0.13	0.22	0.30	0.60	1.05	1.33	1.23
	20	0.05	0.08	0.14	0.40	0.45	0.64	1.11	1.24

Figure 3. The observed relationship between shell growth rate and temperature in six "open system" experiments. Growth rates shown for Experiments III and IV are means for the four flow rates used.

Figure 4. The observed relationship between meat growth rate and temperature in five "open system" experiments.


3
Figure 3
I FEB
II MAY
III NOV
IV JUN
V AUG
VII APR
SHELL GROWTH (k·100)
1·2
1·0
0·8
0·6
0·4
0·2
0


4
Figure 4
III NOV
IV JUN
V AUG
VI OCT
VII APR
MEAT GROWTH (k·100)
2·5
2·0
1·5
1·0
0·5
0
-0·5
-1·0
-1·5
-2·0
10
15
20
25
TEMPERATURE (°C)

growth rate at temperatures exceeding 15° C, and in fact, a negative relationship between temperature and meat growth resulted in two experiments, numbers V and VI.

It should be noted that, contrary to the impression given by considering the means for all flow rates at a given temperature (Figs. 3 and 4), maximum growth was obtained at 20° C in two of the seven experiments (III and IV). However, this occurred only at the highest flow rates, and was only slightly greater than growth at 15° C (Table 4).

Seasonal Variation in Oyster Growth Rates

Five of the seven open system experiments (Table 5) were conducted using oysters of essentially the same initial size, 21-25 mm shell length. Four of these five experiments included among their treatments the same combination of flow and temperature, 8 ml/min/oyster at 15° C. The remaining experiment (IV) included, at 15° C, water flow rates that bracketed 8 ml/min/oyster, so that growth at that flow could be estimated graphically. Also given in Table 5 are the mean concentrations of particulate organic carbon and nitrogen recorded in the incoming bay water during each of the experiments. The remaining data concerning particulate organic carbon and nitrogen are presented in Appendix 5, and are shown graphically along the oyster growth rates in Figures 5 and 6.

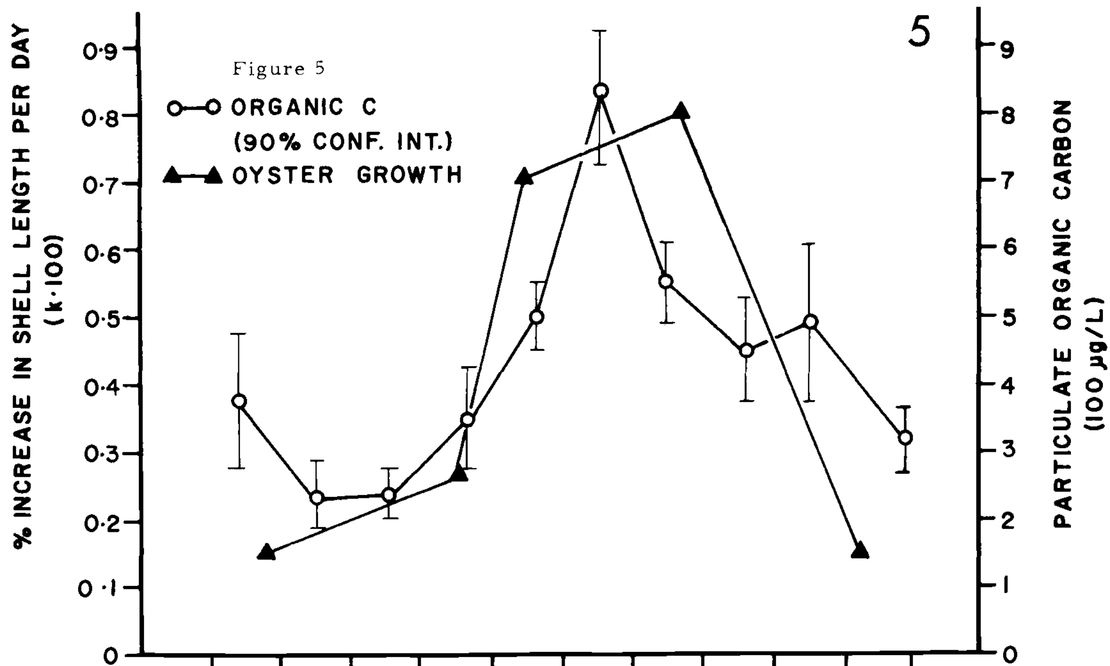
Table 5. Results from five experiments conducted to assess seasonal fluctuations in the growth of juvenile Pacific oysters under constant conditions of temperature (15°C) and water flow rate (8 ml/oyster/min). Also shown are the mean values for particulate organic carbon and nitrogen concentration recorded from the incoming seawater in the interval of each experiment. Carbon and nitrogen data were taken over a period of two years.

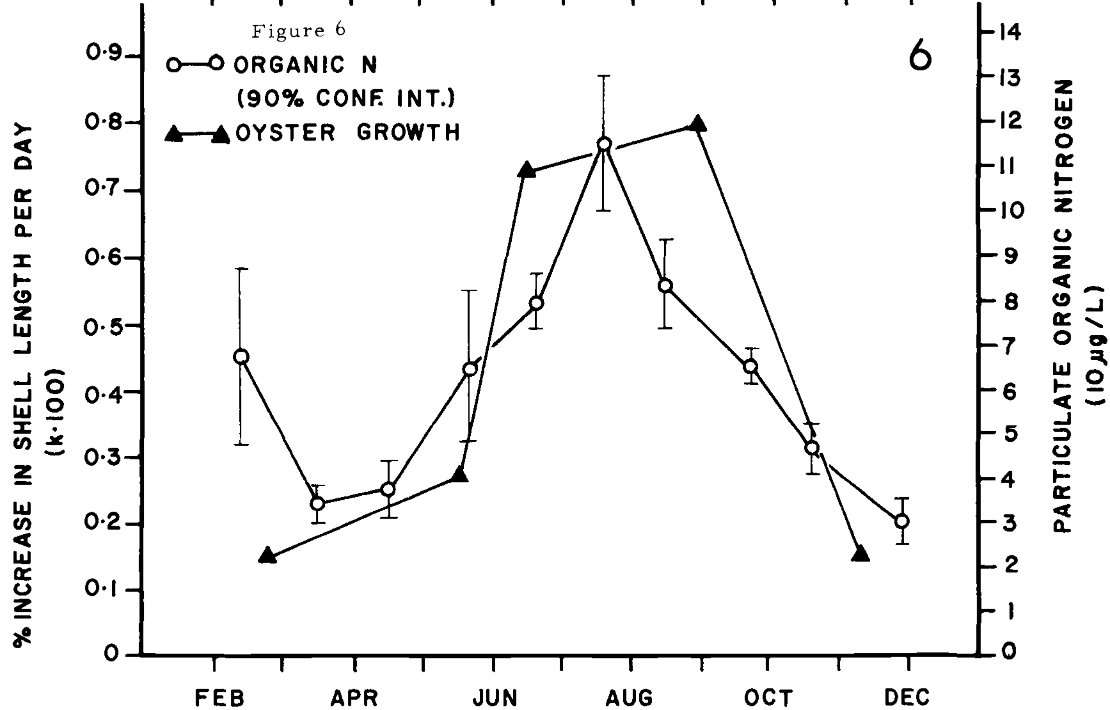
Expt. No.	Mid-point of expt. (date)	Duration (days)	Initial length (mm)	Final length (mm)	Shell growth rate (k x 100)	Particulate organic carbon (μg/L)	Particulate organic nitrogen (μg/L)
I	Feb 26	54	25.0	27.3	0.16	311.0	51.5
II	May 11	84	25.0	31.3	0.26	361.5	59.1
III	Nov 6	57	21.0	22.6	0.13	415.4	44.7
IV [1]	Jun 16	63	23.0	38.4	0.75	555.5	85.5
V	Aug 24	34	22.7	29.9	0.81	499.8	76.8

[1] Growth rate at 8 ml/oyster/min in Expt. IV was estimated graphically based on growth at 4 ml/oyster/min and 16 ml/oyster/min.

Figure 5. Rates of growth of shell from five "open system" experiments plotted at the mid-point of each experiment. Also shown are mean particulate organic carbon values observed for each month in the incoming seawater. Carbon values were collected over a two year period.

Figure 6. Rates of growth of shell from the same five experiments shown in Figure 5, but plotted in this case with monthly means of particulate organic nitrogen.


5
Figure 5
ORGANIC C
(90% CONF. INT.)
OYSTER GROWTH
% INCREASE IN SHELL LENGTH PER DAY
(k·100)
PARTICULATE ORGANIC CARBON
(100 µg/L)
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
9
8
7
6
5
4
3
2
1
0


6
Figure 6
ORGANIC N
(90% CONF. INT.)
OYSTER GROWTH
% INCREASE IN SHELL LENGTH PER DAY
(k·100)
PARTICULATE ORGANIC NITROGEN
(10µg/L)
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
FEB
APR
JUN
AUG
OCT
DEC

The extreme seasonal influence on the growth of oysters is apparent in Figures 5 and 6. Since these experiments were all conducted with young oysters that were not undergoing seasonal gonadal changes, and since temperature and flow rate were held constant, it is likely that the observed growth fluctuations are related to changes in the quality or quantity of available food. This hypothesis is supported by the close relationship between seasonal changes in the levels of particulate organics in the water and changes in oyster growth rate (Figs. 5 and 6). This relationship is shown more clearly in Figures 7 and 8 in which oyster growth rate is graphed with particulate organic carbon and nitrogen respectively.

Particulate organic nitrogen appears from these data to be a better indicator of available food than particulate organic carbon. Particulate carbon levels increased slightly with the onset of Fall rains (Fig. 5); particulate nitrogen did not. The increased particulate carbon during the Fall was not accompanied by increased oyster growth.

Although the particulate organic carbon and nitrogen data reported here may relate to the quantity of food available to my experimental animals, they were not intended to represent, in more than a relative sense, conditions in Yaquina Bay. Nevertheless, data from other sources (Karentz, 1975) indicate that phytoplankton densities do show extreme seasonal fluctuations in the bay.

Figure 7. The relationship between oyster shell growth rate from five open system experiments and the mean particulate organic carbon concentration observed in incoming seawater in the interval of, but not necessarily concurrent with, each experiment.

Figure 8. The relationship between oyster shell growth rate from five open system experiments and the mean particulate organic nitrogen concentration observed as in Figure 7.

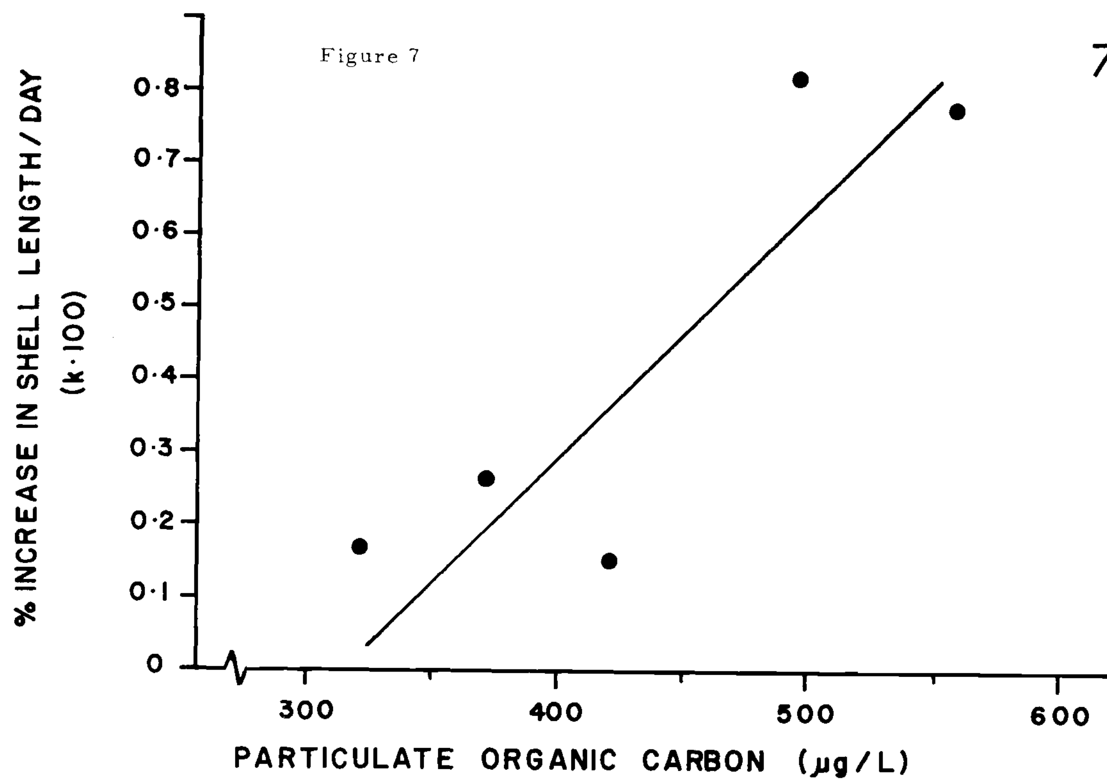


Figure 7
7
% INCREASE IN SHELL LENGTH / DAY
(k·100)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
300
400
500
600
PARTICULATE ORGANIC CARBON (µg/L)

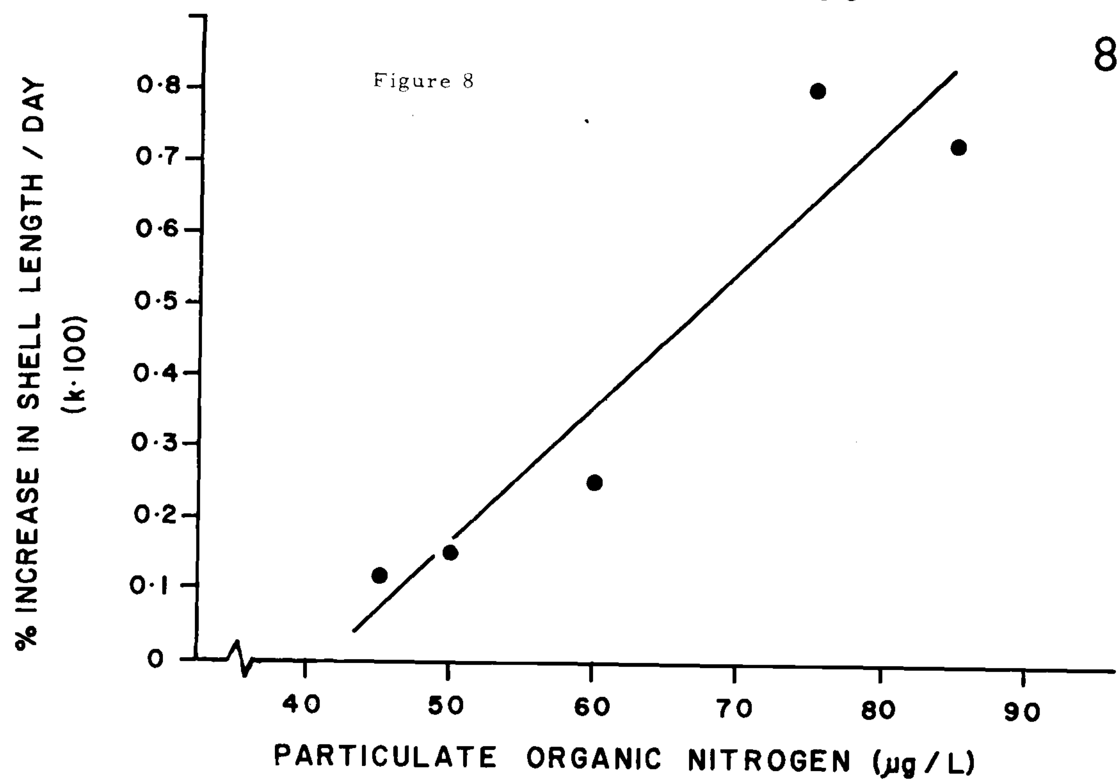


Figure 8
8
% INCREASE IN SHELL LENGTH / DAY
(k·100)
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
40
50
60
70
80
90
PARTICULATE ORGANIC NITROGEN (µg / L)

Phytoplankton populations are very low between November and March, and reach a peak in May or June. Particulate organics, observed in the present study, were also lowest between November and March but appeared to peak later in the summer (July-August) than did the phytoplankton populations.

Relationships Between Water Flow and Oyster Growth

Experiments III and IV of this study were of a factorial design, and included three temperatures at four flow rates. From these experiments, a generalized relationship between water flow and oyster growth at different temperatures can be considered.

The influence of water flow rate on oyster growth at three temperatures is shown in Figures 9 and 10, based on data from Experiments III and IV respectively (Table 4). Note in particular that, although the absolute values obtained for growth are vastly different in the two experiments, the general shapes of the curves are similar. Increases in water flow resulted in increases in oyster growth at all temperatures. But, the response of the animals in terms of growth was less pronounced at low temperatures (11° C) compared to growth at the higher temperatures (20° C). That is, the slope of the curve relating growth to flow is steeper at 20° C than at 11° C. In addition, the point at which further increases in flow do not yield increases in growth is reached at a lower flow rate in cold water than in warmer

Figure 9. Observed relationship between oyster meat growth rate and water flow rate at three temperatures from open system Experiment III.

Figure 10. Observed relationship between oyster meat growth rate and water flow rate at three temperatures from open system Experiment IV.

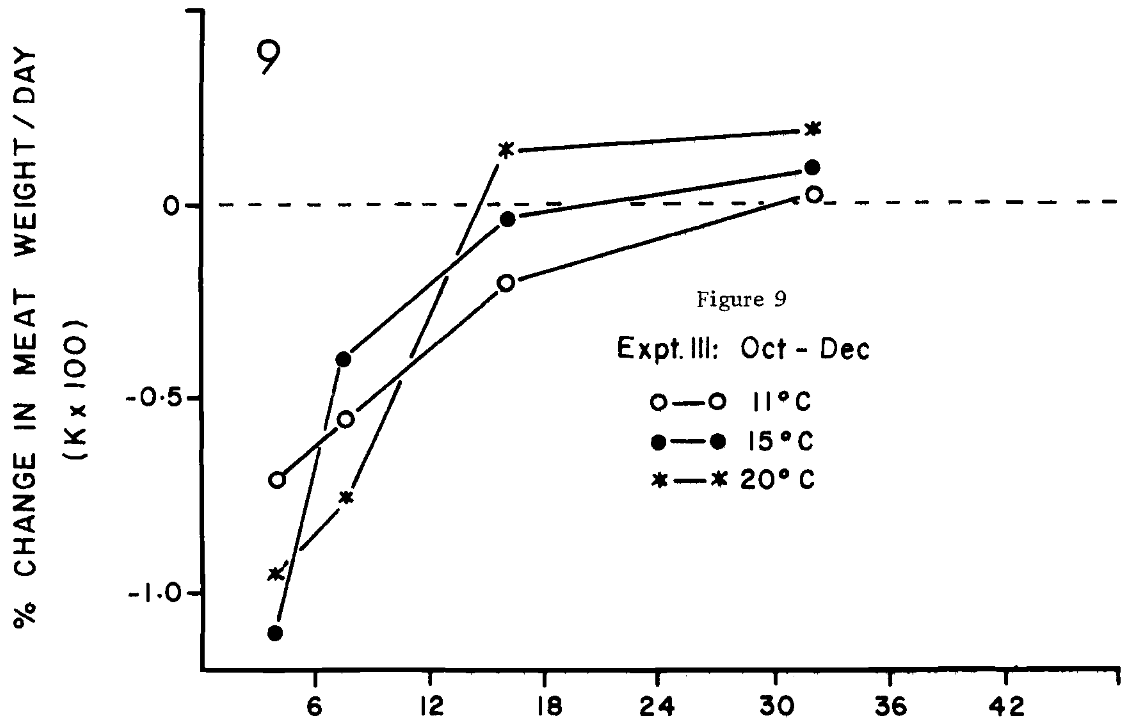


Figure 9

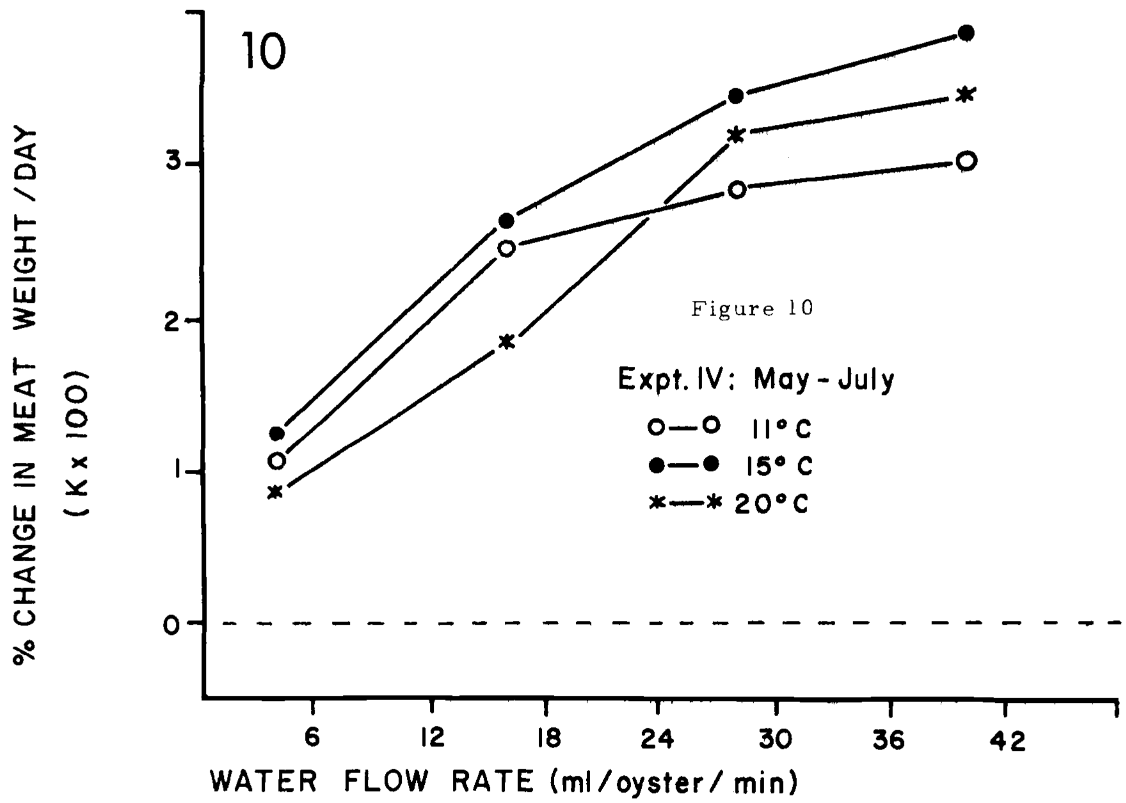


Figure 10

water.

The basis for the above observations may lie in the effect of temperature on the filtering rates of the animals. Although water flow does have an independent effect on the filtering rate of molluscs (Walne, 1972), the dominant effect of temperature on the filtering rates of most molluscs is well known (Bayne, 1976). If the oysters held at 11°C had reduced filtering rates relative to animals in warmer water, they would be unable to take full advantage of high water flow rates. Their food consumption and therefore their growth rate would become limited by their filtering rate, not water flow rate, at a lower flow rate in cold water than in warm water.

It is clear that meaningful definition of relationships between temperature and growth for oysters is impossible in a system where food and water flow cannot be controlled independently. In the previously described open system experiments, food availability and water flow rate were assumed to be equivalent. This may in fact be essentially valid within a range of flow rates, but the width of this range varies constantly with temperature and food density. We know that the food content of the water varied greatly between experiments, and perhaps within experiments. The mean particulate organic nitrogen concentration during Experiment III for example was 44.7 micrograms per liter; during Experiment IV, the nitrogen averaged 85.5 micrograms per liter.

Obviously a given flow rate provided different food availability in the two experiments, and no universally applicable statements about either water flow or food requirements can be made based on these experiments.

Despite the inadequacies of the open system experiments, they do provide an important indication of the form of the relationships that we may expect to find in experiments involving controlled feeding at different temperatures. A generalized form of those relationships is shown in Figure 11. The curves in Figure 11 are hypothetical, but their form is based on experimental data (Figs. 9 and 10) and on similar relationships that have been clearly demonstrated for other animals (Brett, 1969).

The curves have four areas of interest (shown in Fig. 11), which will be considered in greater detail. The first of these (1 on Fig. 11), growth at very low levels of food availability, shows an inverse relationship between temperature and growth. This relationship implies increased metabolic costs, reduced food consumption, reduced assimilation efficiency, or a combination of effects at increased temperatures. The second area, where the growth curves cross the zero-growth or maintenance line, indicates that greater quantities of food must be available to the animals at higher temperatures for maintenance alone. The basis for this increased maintenance requirement in terms of food availability may be, as in area

Figure 11. Hypothetical curves relating oyster growth rate to water flow rate at three temperatures. Four areas of particular biological interest are circled with dashed lines and are discussed in the text.

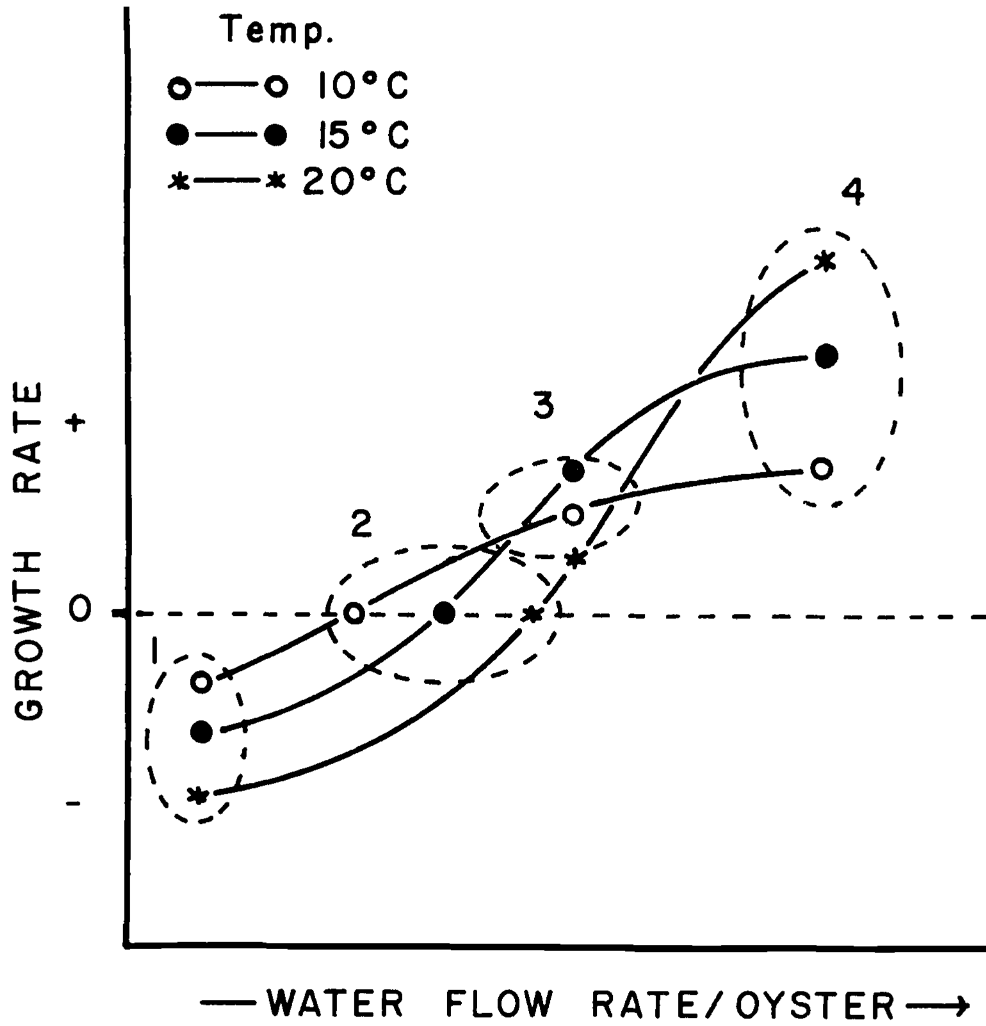


Figure 11

one, in increased metabolic costs, reduced consumption, reduced assimilation, or any combination of these factors. The third area, where the growth curves intersect each other, is an area of food availability where temperature effects are the least obvious. Where the growth curves for 20° and 10° intersect, growth would appear, for example, to be relatively independent of temperature. This is also an area where slight changes in food availability can cause reversal of the observed relationships between temperature and growth rates. The fourth and last area, an area of high food availability, shows a positive relationship between temperature and growth rate up to some maximum temperature.

These hypothetical curves, then, provide a definition of the problems attacked by the closed system experiments. The results of controlled feeding experiments and the energy budgets developed from them can be used to determine, first of all, if such curves do result when food availability is varied at different temperatures. Secondly, the energy budget can provide explanations as to why the curves take this form, and why the four areas described above occur. Specifically, the energy budget will show if higher maintenance costs, reduced food consumption, reduced assimilation, or some other factors are involved in shifting the growth curves to the right with increasing temperature in Figure 11.

Closed System Experiments

The results of two closed system experiments are given in this section. As previously discussed, the experiments were very similar in design, but differed in two important respects. Experiment I was conducted using relatively large animals (about 3 mg ash free dry weight) that were fed a single species of algae, *Pseudoisochrysis paradoxa*. Experiment II used much smaller animals (about 0.1 mg ash free dry weight) that were fed two species of algae, *P. paradoxa* and *Monochrysis lutheri*, on alternate days. Because the animals used in Experiment I were large and the carrying capacity of the closed system was limited, fewer animals were used in that experiment than in Experiment II. The significance of these design differences will be made clear as the results of the two experiments are considered.

Although all the data were eventually converted to caloric terms, some of the data are also given in their unconverted form. These data, such as food consumption in algal cells per animal per unit of time or growth in terms of increase in shell length, are in themselves of limited value and can be misleading. They are presented here only because they may permit the reader to consider aspects of the energy budgets in terms perhaps more familiar than calories.

Experiment I. Parts A, B, and C of Appendix 6 present food consumption, oxygen consumption, and growth data respectively. These data are summarized in the form of an energy budget in Table 6.

Problems created by the use of relatively few larger animals are apparent in these data. The most significant problem is that, since the animals were large, their rate of growth was slow, and differences in growth between treatments are not significant. In addition, the use of fewer animals allowed behavioral differences of individual animals to have a more important influence on food and oxygen consumption data.

Despite these difficulties, there are some trends apparent in the data that warrant consideration. Note, for example, that weight loss was greatest at the higher temperatures, particularly at 23° C (Table 6). The data also generally show an inverse relationship between food consumption and assimilation efficiency, but there appears to be no clearly definable relationship between temperature and assimilation efficiency. Respiration was highest at 23° C (Appendix 6B), but this increased metabolic activity was not accompanied by increased food consumption. A final point is that, although none of the mortalities were particularly high for this type of experiment, mortality was clearly greater at 23° C than at 11° C.

Table 6. Energy budget summary from closed system Experiment I. Data used are given in Appendix 6.

Temp. °C	Tray No.	Food available (cal/kcal/hr)	(Q_c) Food consumed (cal/kcal/hr)	(Q_r) Oxygen consumed (cal/kcal/hr)	(Q_g) Growth rate (cal/kcal/hr)	(Q_r+Q_g) Assimilation (cal/kcal/hr)	Assimilation efficiency (percent)	Mortality (percent)
11	1	33.26	2.75	1.07	+0.22	1.29	47	13
	2	17.21	3.10	0.82	+0.29	1.11	36	14
	3	8.70	1.87	0.94	+0.30	1.24	66	11
	4	unfed	--	0.85	+0.01	--	--	15
15	5	35.75	6.67	0.93	+0.26	1.19	18	21
	6	17.24	3.54	0.87	+0.23	1.10	31	26
	7	9.73	2.32	1.58	+0.11	1.69	73	15
	8	unfed	--	0.59	-0.13	--	--	23
19	9	46.61	5.50	1.17	-0.08	1.09	20	22
	10	23.89	4.93	1.47	+0.12	1.59	32	27
	11	11.65	2.17	0.99	-0.08	0.91	42	22
	12	unfed	--	1.22	-0.19	--	--	20
23	13	37.70	4.51	1.89	+0.16	2.05	45	22
	14	29.42	4.98	1.26	-0.37	0.89	18	29
	15	16.02	2.99	1.96	-0.43	1.53	51	31
	16	unfed	--	0.85	-0.05	--	--	30

Experiment II. The results of closed system Experiment II are given in Appendix 7, parts A through E. The energy budgets derived from those data are presented in Table 7 and are shown graphically in Figure 12.

Growth during Experiment II was sufficient to permit calculation of the animals' energy budgets. The response of individual oysters, in terms of growth, to a particular treatment was highly variable. This variability is evidenced by the width of the confidence intervals for final weight given in Appendix 7E. But the consistency of the growth trends, both in shell growth and meat growth, lends support to the conclusions.

An interesting feature of the food consumption (Appendix 7) is the obvious difference in the rate at which the two algal species were consumed during the experiment. In every treatment, but particularly at high temperatures, the animals consumed more of the P. paradoxa than of the M. lutheri. At 23° C, for example, the oysters cleared 6.4 percent of the P. paradoxa, but only 2.7 percent of the M. lutheri available to them. In a study of feeding by Mytilus edulis provided with a number of different species of phytoplankton, Davids (1964) found that different algal species elicited very different feeding responses from the animals. The results of his work led Davids to conclude that the mussels "did not like Chlorella as a

Table 7. Energy budget summary from closed system Experiment II. Data used are given in Appendix 7. Oysters used had an initial length of 3.17 mm and an initial ash free dry weight of 0.102 mg.

Temp. °C	Tray No.	Food available (cal/kcal/hr)	(Q_c) Food consumed (cal/kcal/hr)	(Q_r) Oxygen consumed (cal/kcal/hr)	(Q_g) Growth rate (cal/kcal/hr)	Qw Waste (cal/kcal/hr)	Assimilation efficiency (percent)
11	1	240.7	17.35	3.47	+0.39	13.49	22.3
	2	132.2	12.60	2.85	+0.35	9.40	25.4
	3	82.4	7.29	2.14	-0.04	5.19	28.8
	4	unfed	--	0.61	-0.04	--	--
15	5	193.7	20.67	4.09	+0.65	15.93	22.9
	6	116.7	14.50	3.60	+0.47	10.43	28.1
	7	65.0	12.50	3.69	+0.36	8.45	32.4
	8	unfed	--	1.84	-0.20	--	--
19	9	223.5	13.43	4.54	+0.51	8.38	37.6
	10	144.7	15.31	3.84	+0.36	11.11	27.4
	11	85.6	9.27	3.51	-0.04	5.80	37.4
	12	unfed	--	1.84	-0.14	--	--
23	13	394.4	11.01	3.48	-0.05	7.58	31.2
	14	213.9	12.66	3.42	-0.31	9.55	24.6
	15	117.7	6.96	3.34	-0.36	4.00	42.8
	16	unfed	--	0.55	-0.56	--	--

Figure 12. Graphical representation of the energy budgets of juvenile oysters held at four temperatures and four ration levels in "closed system" Experiment II. Q_c = food consumption rate; Q_w = rate of waste production; $Q_g + Q_r$ = assimilation rate; and Q_r = respiration rate.

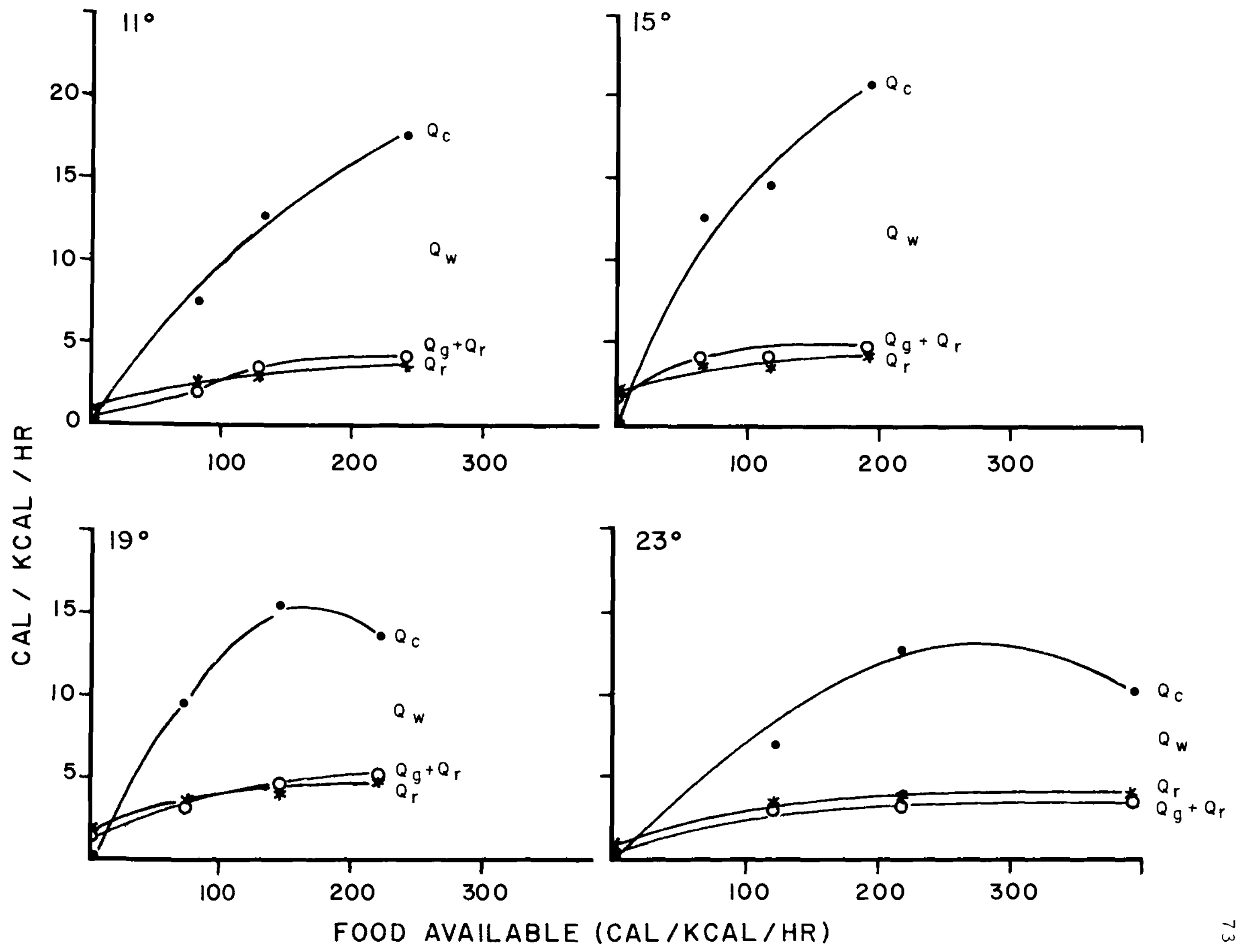


Figure 12

food".

The complete energy budgets for each temperature (Fig. 12) demonstrate the magnitude of the animals' food consumption (Q_c) relative to the other components of the energy budget. Clearly, the most significant fate of consumed food in this experiment was waste (Q_w), which amounted to from 60 to 78 percent of consumption. Conversely, assimilation efficiencies were quite low, 22 to 43 percent. Thompson and Bayne (1972) reported assimilation efficiencies for *M. edulis* as high as 89 percent. However, they used the Conover (1966) method, which as previously discussed, makes some highly tenuous assumptions in its application to bivalve molluscs. The authors also noted an inverse relationship between food availability and assimilation efficiency. This inverse trend is supported in studies by Winter (1970), Thompson and Bayne (1974), and Foster-Smith (1975). In the present study, I also observed an inverse relationship between food availability and assimilation efficiency (Table 7), although the relationship is less clear at the higher temperatures. Microscopic examination of feces produced by oysters in Experiment II showed that the fecal strands contained a large number of intact algal cells, some of which were still motile. The indications are, then, that the assimilation efficiencies obtained from Experiment II are reasonably accurate, and that the assimilation efficiencies that I obtained are low because food density was high.

Food consumption data from Experiment II indicate that the oysters' feeding was inhibited by the combination of high food density and high temperature (Fig. 12). Similar results were obtained at 23° C in Experiment I (Table 6). The feeding inhibition was particularly pronounced when M. lutheri was used as food.

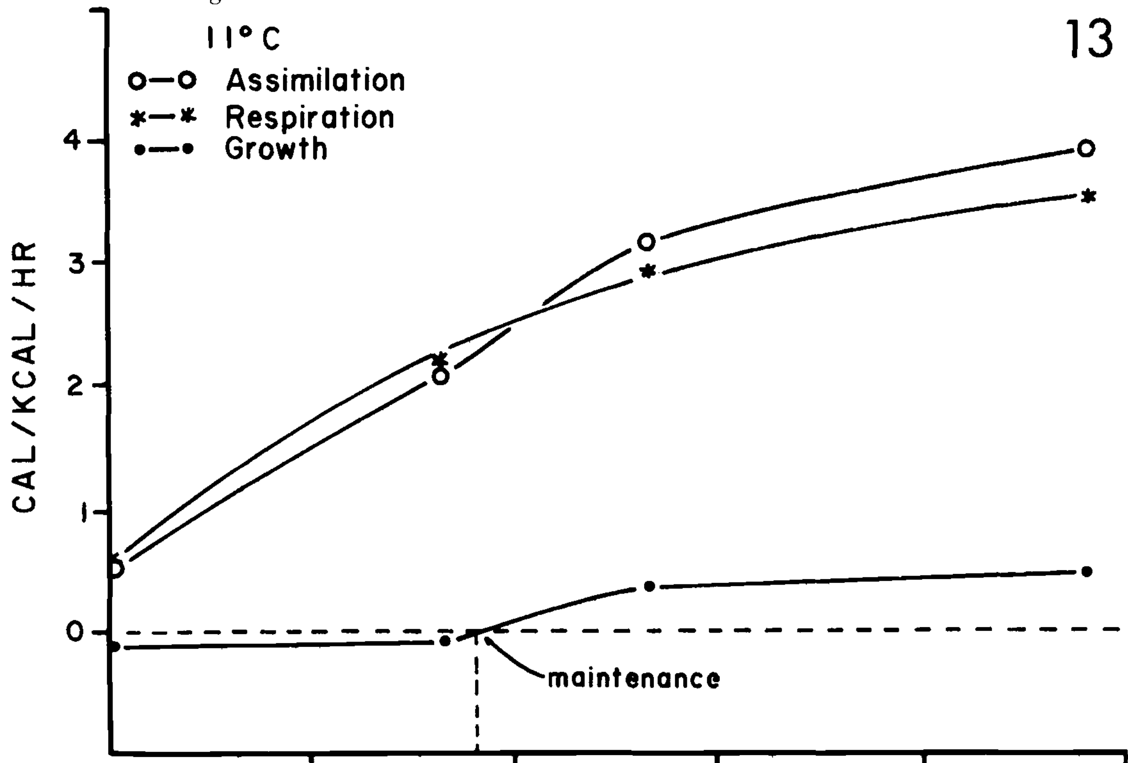
Partial energy budgets, in which food consumption has been omitted to permit use of a larger scale can be used to graphically estimate maintenance requirements at each of the temperatures (Figs.13 through 16). Maintenance requirement may be defined as the energy that must be available, consumed, or assimilated in order for respiration to be exactly equal to assimilation. Since assimilation equals the sum of respiration and growth, growth must equal zero at the maintenance requirement. Maintenance requirements in terms of availability are assumed to be met at the points where the growth curves (Figs. 13 and 14) just cross the zero growth line. Note that the growth curves tend to be sigmoidal. The change in growth rate occasioned by declining food availability is reduced once growth becomes negative. This feature is particularly evident in the 11° C and 19° C growth curves.

Estimates of the maintenance requirement can also be obtained by using the data (Table 7) to estimate the respiration rate (Q_r) at zero growth. Approximated assimilation efficiencies, again at zero growth, can be used to calculate an estimate of maintenance rates of

Figure 13. A partial energy budget (Q_c and Q_w omitted) for juvenile oysters held at 11° C and fed four different ration levels. Graphical estimate of food availability necessary to maintain zero weight change is shown.

Figure 14. A partial energy budget as in Figure 13, but for oysters held at 15° C.

Figure 13



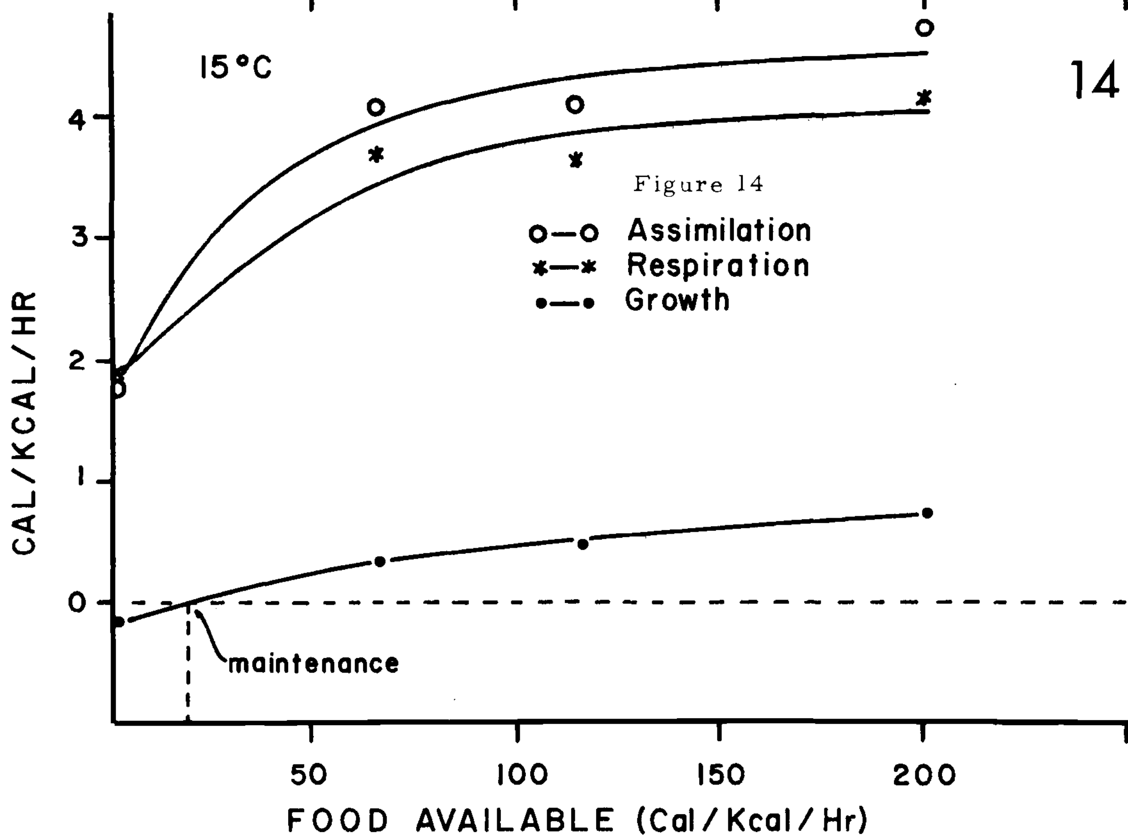


Figure 15. A partial energy budget (Q_c and Q_w omitted for juvenile oysters held at 19° C and fed four different ration levels. Graphical estimate of food availability necessary to maintain zero weight change is shown.

Figure 16. A partial energy budget as in Figure 15, but for juvenile oysters held at 23° C.

Figure 15

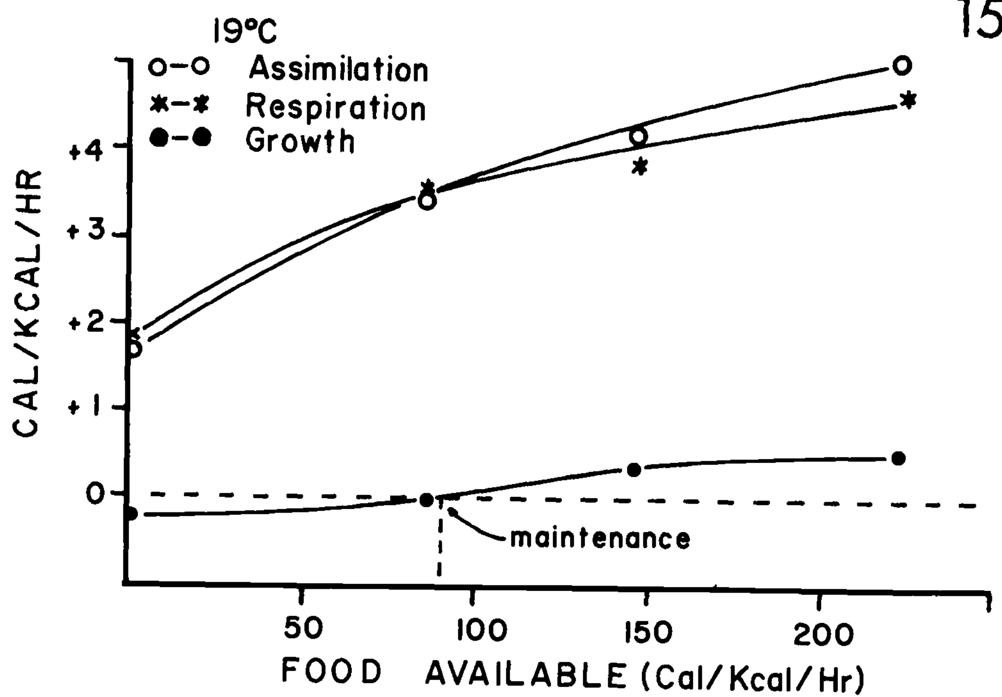
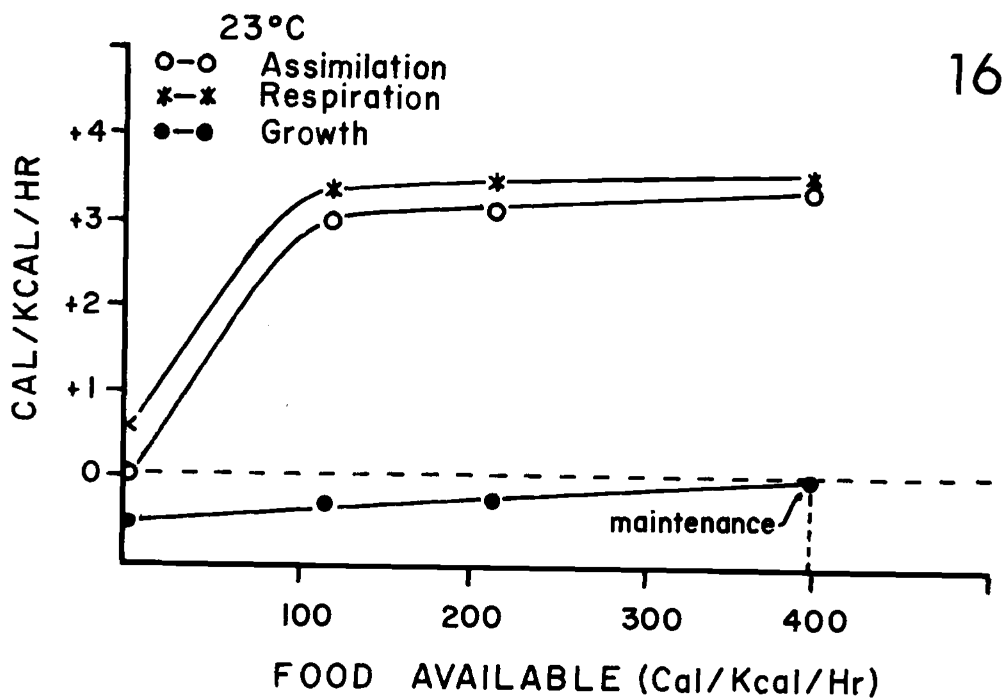


Figure 16



food consumption:

$$\text{Assimilation Efficiency} = \frac{\text{Respiration } (Q_r) + \text{Growth } (Q_g)}{\text{Food Consumption } (Q_c)}$$

Since at the maintenance point $Q_g = 0$,

$$\text{Maintenance } Q_c = \frac{Q_r}{\text{Assimilation Efficiency}}$$

Finally, an estimate of food consumption rate, as a percentage of food availability, at the zero growth point can be used to calculate maintenance availability requirements:

$$\text{Maintenance Availability} = \frac{\text{Maintenance Food Consumption } (Q_c)}{\text{Percentage of available food consumed at zero growth}}$$

The results of these calculations for each of the temperatures are given along with graphically determined values in Tables 8 and 9. Note that the graphically determined estimates in Table 8 differ from the calculated values (Table 9) only for 15° C. Since the 15° C growth curve has only a single point below the zero growth line, and since it is very likely that the 15° C growth curve should flatten out somewhat below that line (such as the curves for the other temperatures in Figs. 13 and 14), it is difficult to obtain an accurate estimate for 15° C from Figure 13, and it is probable that the graphically obtained value is an underestimate. The calculated estimate is, therefore, considered the more accurate for 15° C.

Calculated maintenance requirements, in terms of assimilation,

Table 8. Graphically estimated requirements to maintain zero weight change at four temperatures. Requirements presented in terms of food availability, food consumption, and assimilation.

Temp. °C	Maintenance requirement (cal/kcal/hr)			Estimated assimilation efficiency at zero weight change (percent)
	Availability	Consumption	Assimilation	
11	92	8.4	2.3	27
15	24	6.4	2.4	38
19	93	10.6	3.4	32
23	390	12.0	3.5	29

Table 9. Calculated estimates of requirement to maintain zero weight change at four temperatures. Requirements presented in terms of food availability, food consumption, and assimilation. Calculations are based on estimated respiration at zero weight change, and on estimates of percent consumed (of available) and percent assimilated (of consumed).

Temp. C	Maintenance requirement (cal/kcal/hr)			Estimated consumption at zero growth as percent of available	Estimated assimilation efficiency at zero growth (percent)
	Availability	Consumption	Assimilation		
11	91	8.2	2.3	9	28
15	40	7.9	2.6	20	33
19	94	10.3	3.4	11	33
23	390	11.7	3.5	3	30

are shown graphically in Figure 17 with actually measured assimilation rates for each of the temperature and ration combinations used in Experiment II. These plots show that maintenance requirements increase with increasing temperature, but that assimilation tends to reach a maximum between 15° and 19° C, and then declines with further temperature increases. The distance between the two curves is therefore greatest at 15° C.

At the lowest food availability, the highest temperature at which any positive growth can be expected is about 18° C (Fig. 17). An approximate doubling of food availability increased the maximum temperature for growth to about 22° C, but further increases in ration did not increase the maximum temperature at which positive growth could occur.

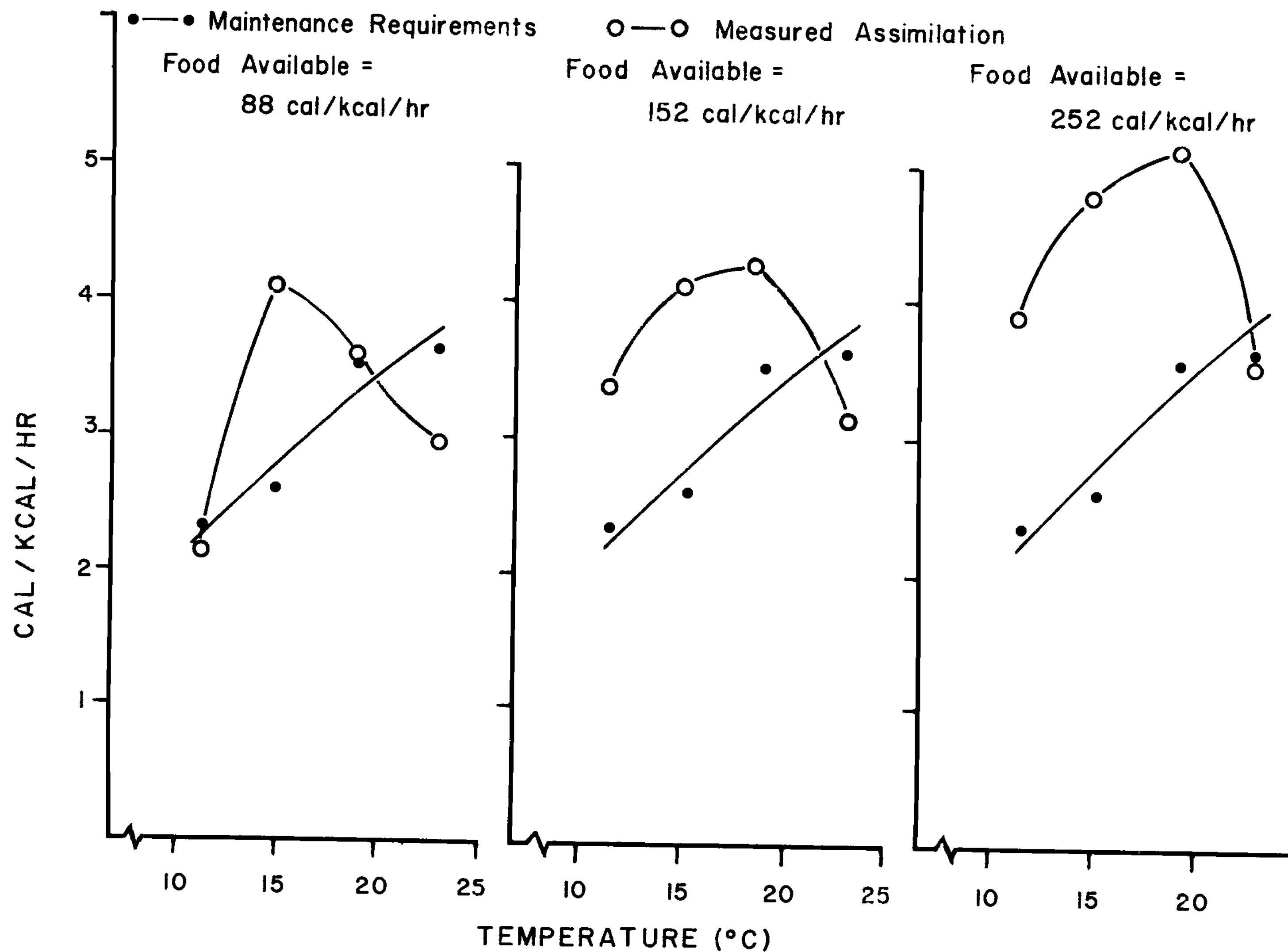
Maintenance assimilation increases with increasing temperature, but at temperatures over 15° C food consumption does not increase (Table 7). Consequently, the maintenance requirement in terms of food availability increases disproportionately to increases in respiration.

Note that approximately a three fold increase in food availability is required to maintain zero weight change with each increase in four degrees Celsius over 15° C.

Despite a lower respiration rate at 11° C compared to 15° C (Table 7), food consumption and availability requirements are higher

Figure 17. The relationship between temperature and the estimated assimilation rate required for zero weight change shown with the measured assimilation rate at each temperature. This relationship is shown for each of three different levels of food availability. Data from closed system Experiment II.

Figure 17



at 11° C than at 15° C. This is a reflection of the reduced food consumption, as a percent of availability (Appendix 7c), at 11° C compared to 15° C. Reduced assimilation efficiency at 11° C (Table 7) also contributes to the high maintenance requirement in terms of availability and consumption at 11° C.

Maintenance requirements have been estimated from zero weight change by a number of authors (Brett, et al., Thompson and Bayne, 1974). Although such estimates are useful in providing a comparison between the effects of temperature on maintenance costs and the effects of temperature on assimilation rate (Fig. 17), they should be viewed with caution.

Recall that maintenance was defined for purposes of the present study as the energy that must be available, consumed, or assimilated under a given set of environmental conditions such that the weight of the animals neither increases nor decreases (Brett, et al., 1969). However, maintenance in the strictest sense should be defined as the sum of all metabolic costs. Clearly, metabolic costs are not independent of food consumption rate (Table 7), and no single estimate of maintenance can be strictly valid for all levels of food availability (Warren, pers. comm.).

The absolute difference between the temperature-dependent maintenance curves and the temperature-dependent assimilation curves in Figure 17 is not "scope for growth" as defined by Warren

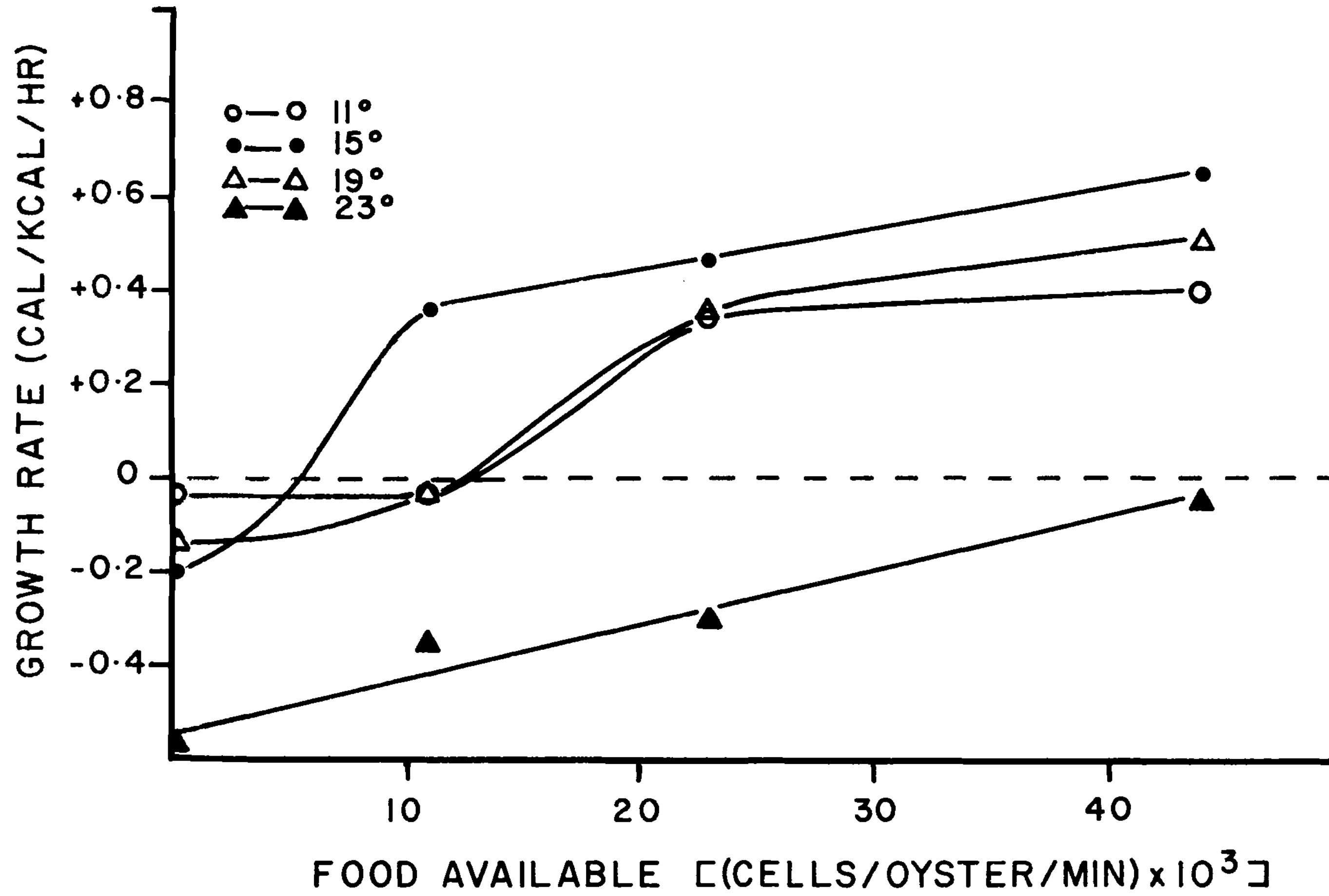
and Davis (1967) and Warren (1971). Scope for growth is best estimated as the difference between the energy content of food consumed and the energy content of all of the remaining components of the energy budget equation other than growth (Q_w and Q_r) under a given set of environmental conditions. An accurate measure of Q_w is difficult to obtain for bivalves and was not attempted in the present study. Therefore, calculation of scope for growth using estimated Q_w's (an estimate that involves the use of growth data, would be no more useful than the growth curves given in Figures 13-16.

When oyster growth rate was graphed against water flow rate in open system experiments at a number of different temperatures, a family of curves resulted (Figs. 9 and 10). These curves served as the basis for defining problems attacked by the more controlled closed system experiments. In order to provide a comparison between the form of those open system curves and similar data from Experiment II, Figure 18 is presented.

Note from Figure 18 that the growth curves assume a characteristic sigmoidal shape and that they are generally displaced to the right with increasing temperatures over 15° C. As in Figure 9, Figure 18 shows that the 11° C curve crosses the zero growth line at a point to the right of the 15° C curve. That is, below 15° C the curves may also be displaced to the right if growth is plotted against food concentration. As pointed out previously, this is because food

Figure 18. The observed relationship between food availability in terms of algal cells per oyster per minute and meat growth rate at four temperatures. Algal species used were *Pseudoisochrysis paradoxa* and *Monochrysis lutheri*. Data from closed system Experiment II.

Figure 18



food consumption, and assimilation efficiency are both reduced at 11° C compared to 15° C (Table 7) such that animals at the lower temperature apparently are not able to take full advantage of increases in food concentration (or flow rate) by increasing food consumption and growth.

DISCUSSION

Observations of the growth of oysters in untreated seawater at various temperatures and flow rates provided a statement of the objectives of this study, and permitted development of a first approximation of the kinds of relationships under consideration. Seasonal changes in the concentration of particulate organic matter in the seawater drastically affected the absolute values of growth rate observed but did not alter the general form of the relationships. Closed system studies, in which both temperature and food availability could be controlled, verified the nature of previously described relationships and provided some explanation of the form of those relationships. With this background, there are some additional features of the temperature x food density x growth relationships that warrant further consideration.

Recall that, after presenting the results of experiments conducted in the open systems, I suggested generalized curves relating oyster growth rate to water flow rate at various temperatures (Fig. 11). These hypothetical curves were based in part on observations and in part on similar relationships known to exist in other animals (Brett, 1969). I further suggested that, for purposes of discussion, the curves could logically be divided into four areas. Alternative biological explanations for each of the areas were suggested, but the

relative merits of these alternatives could not be considered solely on the basis of data obtained from the open system experiments. Now, with the additional information provided by closed system studies, we can reconsider the hypothetical curves and discuss their implications.

The first area of the curve, the area of low food availability, is characterized by an inverse relationship between temperature and growth and by the relative insensitivity of growth rate to changes in food density. My data indicate that the inverse relationship between temperature and growth rate is due to the combined effects of reduced food consumption and increased maintenance requirements at high temperatures. I found no evidence to indicate that assimilation efficiency was adversely affected by high temperatures.

Bayne (1976) found a similar increase in metabolic costs and decrease in scope for growth at high temperatures in his studies of feeding and growth in Mytilus edulis. His earlier studies (Widdows and Bayne, 1971) indicate that the reported decline in the scope for growth of the animals was in part due to an inverse relationship between temperature and assimilation efficiency. Winter (1969), however, reported that temperature had no effect on the assimilation efficiencies of the bivalves Artica islandica and Modiolus modiolus. I found no evidence in my data to support the idea that assimilation efficiency is adversely affected by increased temperatures.

Assimilation declined with increasing temperatures (over 15° C) in my experiments because food consumption declined.

The relative insensitivity of growth to changes in food density at very low food densities (and very high food densities) gives the curves their characteristic sigmoidal form. At very low food densities, below some threshold level, it has been suggested that oysters are inactive and do not pump (Collier, 1959). If this were true, there would be little or no difference between the metabolic activity, as measured by oxygen consumption, of unfed animals and animals fed at some very low density. As a consequence, then, the weight change of animals fed over a range of low food densities would be similar, and growth rate would be essentially unrelated to food density.

My data indicate that, although oxygen consumption is depressed in those animals that were not fed at all, the animals fed even the lowest ration showed considerable metabolic activity. There is no evidence in my data that would support the idea that animals fed at the lowest rations were inactive. Thompson and Bayne (1974) found that oxygen consumption increased very rapidly with increases in ration at low rations, and that it reached an asymptote at which it remained over a broad range of high food densities.

My data do show, however, that metabolic activity (oxygen consumption) declines with declining food availability but that

assimilation efficiency generally is inversely related to food density. The negative effect of declining food density is apparently offset by increased assimilation efficiency and declining metabolic costs so that the net effect is to stabilize weight change despite decreased ration.

The second area of interest is the area of the curves where growth is essentially zero. As I have previously indicated, this is the point at which respiration and assimilation are equal. The hypothetical curves (Fig. 11) suggested that this point of zero change in weight is displaced to the right with increasing temperatures. Data from the closed system experiments show that zero growth does in fact occur at increasing ration levels as temperatures increase over 15° C. The data further show that the increasing maintenance requirement in terms of food availability at high temperatures is due to decreasing assimilation coupled with increasing respiration rates.

Perhaps of more interest is the fact that the 11° C curve shows a higher maintenance requirement in terms of food availability than the 15° C curve (Fig. 18). It must be remembered that we are considering the relationship between food availability and growth, not food consumption and growth. The data show that food consumption and assimilation efficiency are as low or lower at 11° C than at 15° C. The net effect is that a greater ration, in terms of food available, is required for zero weight change at 11° C than at 15° C

despite the fact that respiration is higher at 15° C than at 11° C. This relationship was observed in the open system experiments as well (Fig. 9). It appears to have a sound biological basis, however, since a curve relating maintenance requirements in terms of food availability to temperature would be concave with its minimum at about 15° C (Table 9). Although additional temperatures below 11° C should be tested to provide confirmation, existing data indicate that the sigmoidal curves relating food availability to growth are displaced to the left as temperatures increase up to 15° C, above which further temperature increases displace them to the right.

The third area, an area of intermediate food density, is the area in which growth rate is most affected by food density and the growth curves for different temperatures intersect. At these ration levels, food consumption is directly related to food density, and assimilation (not assimilation efficiency) increases more rapidly than respiration. The net effect is a more or less linear increase in growth rate with increases in ration. At intermediate temperatures, the oysters, because of their higher maximum clearing rates, appear to respond more dramatically to increases in ration by increased growth than they do at the high or very low temperatures.

The fourth area, the area of very high ration levels, is where growth again becomes relatively independent of ration. Further increases in ration either have little or no affect on growth rate or

they have a negative effect. At high ration levels, growth rate is relatively independent of ration level because the food handling ability of the animal, which is of course limited, becomes saturated. Therefore, despite very high food consumption rates, assimilation efficiency declines, assimilation levels off, and growth rate may remain unaffected by relatively large changes in food availability.

Evidence that growth rate may decline at very high ration levels is provided by the closed system experiments, which showed a slightly negative effect of ration on food consumption at the higher temperatures. Evidence from other studies relating high food densities to reduced feeding in filter feeders is in the literature (Loosanoff and Engle, 1947; Rice and Smith, 1958).

There is evidence from the present study and from others that the absolute values obtained for growth rate under a given set of conditions are influenced by a number of factors other than temperature and food availability. It is not my objective to review all these factors. Nevertheless, because some of them are sufficiently important to influence the general applicability of the conclusions of this study, I will briefly consider them here.

In the present study, for reasons that have been considered, food availability and food consumption were expressed in terms of calories. The caloric value of food materials provides a common denominator for studies of bioenergetics, but consideration of only

the caloric content of the food may obscure important qualitative differences among different food materials. Evidence was previously presented to show that oysters are likely to respond differently in terms of their food consumption and growth to different species of algae, despite the fact that the algae may provide exactly the same caloric value to the animals.

Food availability in this as in most studies was expressed in terms of the quantity of food available per animal per unit of time. It has, however, been shown (Walne, 1972) that the movement of water has an effect on the food consumption rate and presumably on the growth rate of oysters. The interactions of water flow rate, food density, and food availability and their effects on the animals' energy budget are not known.

Factors related to the density of the animals, such as the build up of waste products or the "shadowing" of one animal by another, probably have effects on growth that are independent of or interactive with food density and temperature.

Physical and environmental factors such as the dissolved oxygen concentration, silt, salinity, etc., have been shown by a number of authors to affect various components of the energy budget, particularly food and oxygen consumption (Newell, 1970). The effects of these factors on the animals used in a study such as this must be assumed to be minimal as long as those factors are maintained in

an acceptable range. Their precise effect on the energy budget is nonetheless unknown.

Previous handling or holding conditions for the experimental animals are also known to influence their food consumption (Davids, 1964) and oxygen consumption (Thompson and Bayne, 1972). Clearly, prior treatment can be expected to have some influence on the absolute values, if not the general relationships, obtained in an energy budget study of this nature.

Components of the energy budget are known from a number of studies (reviewed by Bayne, 1976) to be related directly to weight on an absolute basis and inversely to weight on a relative basis. That is, oxygen consumption, for example, is greater in a large animal, but oxygen consumption per unit of body weight declines with increasing body weight. Weight specific oxygen consumption can be related to weight by the equation:

$$Q_{o_2} = a \times W^{b-1} \quad \text{where,}$$

Q_{o_2} = oxygen uptake, usually as ml 0_2/g dry weight/hr.

a = the intercept of a fitted line and is a constant under constant conditions.

b-1 = the slope of a fitted line.

The biological significance of specific values of a and b have been considered by a number of authors (reviewed by Newell, 1970). It

is sufficient for purposes of this study to note that a value of 0.75 is usually found for poikilotherms and that this value is roughly intermediate between a surface proportional ($b=0.67$) and a weight proportional ($b=1.0$) value. Dame (1972) in studies with the Eastern oyster, C. virginica, found that the value of a was related to temperature by the equation $a = (69.7 + 12.6\ T\ C)^{-3}$.

Basically, only two different sizes of oysters were used in the present study, since determination of the relationship between weight specific respiration and body weight was not the objective of the study. It should be noted, however, that there are clear differences in the weight specific oxygen consumption, food consumption, and growth rates between the larger oysters (3.5 mg) used in Experiment I (Table 11) and the small oysters (0.2 mg) used in Experiment II (Table 12). Further, if the equation $Q_{o_2} = a \times W^{b-1}$, using Dame's (1972) values for a and b, are applied to oysters at 11° C in the two experiments of this study, values of 1.01 cal/kcal/hr for Experiment I and 2.32 cal/kcal/hr for Experiment II result. Mean values obtained experimentally are 0.94 cal/kcal/hr and 2.82 cal/kcal/hr respectively. This result suggests that the values obtained in the present study agree reasonably well with those predicted on the basis of studies intended to describe the effect of changing body weight and temperature on the weight specific respiration rate of oysters.

Applications

This study was initiated by interest in the possibility of using the heated effluent from coastal nuclear power plants for culturing oysters. Since ambient temperatures on this coast are generally below the 15° C optimum for C. gigas, it appears that such effluents could be put to beneficial use for this purpose. However, natural food supplies for the oysters would have to be supplemented between the months of November and March in order to take full advantage of the increased temperature.

More recently, interest in constructing such plants on the Oregon coast has, for a number of reasons, diminished. However, the possibility that small, "cultchless" oysters might be cultured intensively on a commercial scale still exists. These animals, unlike most other types of oyster seed, are now readily available, and they can be shipped anywhere in the world from existing commerical oyster hatcheries. However, these very small individual animals (about 3 mm in length) are very vulnerable to siltation and to predators and cannot be placed directly into the field without special handling. There may be advantages, therefore, in maintaining the seed under carefully controlled conditions and perhaps in providing them with supplemental food to obtain growth prior to placing them in the field.

Maintaining the seed under controlled conditions will require the kind of information generated by this study. Information concerning the effects of variations in temperature on food consumption, food conversion, and growth by such animals is basic to intensive culture. In addition, data that give some indication as to how natural food supplies fluctuate seasonally would be important in determining the need for supplemental feeding and would help determine the optimum time for field planting.

Obviously, the efficient operation of an artificial culture system would require specific information concerning a great number of factors, most of which are outside the scope of this study. However, there can be little doubt that understanding the energy budget of an animal would be critical to the biological, to say nothing of the commercial, success of an intensive culture facility. It should be equally clear that there are a number of important questions that are suggested, but not answered, by this study.

One question that is particularly intriguing concerns the effects of food quality on the oysters' energy budget. This question, to which I briefly alluded previously, has never been dealt with for any bivalve. Excellent growth was obtained in open systems that received relatively little food in caloric terms. Such a result implies that the natural food was somehow superior to cultured algae in a qualitative sense. How the natural food differs from cultured algae

and exactly how the energy budget of the animals is affected by differences in food quality are questions that should be considered in future studies.

Another question that is of interest concerns the relationship between the animals' size and their energy budget. I discussed previously some of the ways in which body size affect weight specific rates among components of the energy budget. From an applied point of view, however, a great deal more information would be required before decisions could be made that would optimize productivity in an intensive oyster culture system containing oysters of a number of different sizes.

BIBLIOGRAPHY

American Public Health Association et al., 1975. Standard methods for examination of water and wastewater. 14th ed. American Public Health Association, Inc. New York. 1193 pp.

Ansell, A. D. and P. Sivadas. 1973. Some effects of temperature and starvation on the bivalve Donax vittatus (da Costa) in experimental laboratory populations. Journal of Experimental Marine Biology and Ecology 13:229-262.

Bayne, B. L., R. J. Thompson, and J. Widdows. 1976. Physiology. In: Marine mussels, their ecology and physiology, ed. by B. L. Bayne, Cambridge University Press, Cambridge. p. 121-291

Bernard, F. R. 1974a. Particle sorting and labial palp function in the Pacific oyster Crassostrea gigas (Thunberg, 1795). Biological Bulletin 146(1):1-10.

Bernard, F. R. 1974b. Annual biodeposition and gross energy budget of mature Pacific oysters, Crassostrea gigas. Journal of the Fisheries Research Board of Canada 31(2):185-190.

Brafield, A. E. and D. J. Solomon. 1972. Oxy-calorific coefficients for animals respiring nitrogenous substrates. Comparative Biochemistry and Physiology 43A: 837-841.

Breese, W. P. and R. E. Malouf. 1975. Hatchery manual for the Pacific oyster. Corvallis, Oregon State University Sea Grant College Program publication no. ORESU-H-75-002. 22 pp.

Brett, J. R., J. E. Shelbourn, and C. T. Shoop. 1969. Growth rate and body composition of fingerling Sockeye salmon, Oncorhynchus nerka, in relation to temperature and ration size. Journal of the Fisheries Research Board of Canada 26(9): 2363-2394.

Brody, S. 1945. Bioenergetics and growth. Reinhold Publishing Corp., New York. 1023 pp.

Carefoot, T. H. 1970. A comparison of absorption and utilization of food energy in two species of tropical Aplysia. Journal of Experimental Marine Biology and Ecology 5: 57-62.

Castell, J. D. and D. J. Trider. 1974. Preliminary feeding trials using artificial diets to study the nutritional requirements of oysters (Crassostrea virginica). Journal of the Fisheries Research Board of Canada 31(1):95-99.

Chanley, P. and R. F. Normandin. 1967. Use of artificial foods for larvae of the hard clam, Mercenaria mercenaria (L). Proceedings of the National Shellfisheries Association 57: 31-37.

Clark, J. R. and R. L. Clark. 1964. Sea-water systems for experimental aquariums, a collection of papers. U.S. Fish and Wildlife Service, Bureau of Sport Fisheries and Wildlife, Research Report no. 63.

Collier, A. 1959. Some observations on the respiration of the American oyster Crassostrea virginica (Gmelin). Institute of Marine Science 6: 92-108.

Conover, R. J. 1966. Assimilation of organic matter by zooplankton. Limnology and Oceanography 11: 338-354.

Dame, R. F. 1972. The ecological energies of growth respiration and assimilation in the intertidal American oyster Crassostrea virginica. Marine Biology 17(3): 243-250.

Davids, C. 1964. The influence of suspensions of micro-organisms of different concentrations on the pumping and retention of food by the mussel Mytilus edulis (L.). Netherlands Journal of Sea Research 2: 233-249.

Epifanio, C. E. and C. A. Mootz. 1975. Growth of oysters in a recirculating system. Proceedings of the National Shellfisheries Association 65: 32-37.

Epifanio, C. E. Mootz Logan, and C. Turk. 1975. Culture of six species of bivalves in a recirculating seawater system. 10th European Symposium on Marine Biology, Ostend, Belgium 1: 97-108.

Epifanio, C. E. and R. F. Srna. 1975. Toxicity of ammonia, nitrite ion, nitrate ion, and orthophosphate to Mercenaria mercenaria and Crassostrea virginica. Marine Biology 33: 241-246.

Field, I. A. 1909. The food value of sea mussels. Bulletin of the U.S. Bureau of Fisheries no. 24: 85-128.

Forster, J.R.M. and P. A. Gabbott. 1971. The assimilation of nutrients from compounded diets by the prawns Palaemon serratus and Pandalus platyceros. Journal of the Marine Biological Association of the United Kingdom 51: 943-961.

Forster-Smith, R. L. 1975. The effect of concentration of suspension on the filtration rates and pseudofaecal production for Mytilus edulis (L.), Cerastoderma edule (L.) and Venerupis pullastra (Montagu). Journal of Experimental Marine Biology and Ecology 17: 1-22.

Fry, F.E.J. 1947. Effects of the environment on animal activity. University of Toronto Studies Biological Series 55. Ontario Fisheries Research Laboratory Publication no. 68. 62 pp.

Galtsoff, P.S. 1964. The American oyster. Bulletin of the U.S. Fish and Wildlife Service 64. 480 pp.

Gillespie, L., R. M. Ingle, and W. K. Havens. 1964. Glucose nutrition and longevity in oysters. Quarterly Journal of the Florida Academy of Science 27(4): 279-288.

Goldizen, V. C. 1970. Management of closed marine aquariums. Helgolander Wissenschaftliche Meeresuntersuchungen 20: 637-641.

Hammen, C. S. 1966. Carbon dioxide fixation in marine invertebrates. V Rate and pathway in the oyster. Comparative Biochemistry and Physiology 17:289-296.

_______________ 1969. Metabolism in the oyster, Crassostrea virginica. American Zoologist 9: 309-318.

Hammen, C. S., H. F. Miller, and W. H. Greer. 1966. Nitrogen excretion of Crassostrea virginca. Comparative Biochemistry and Physiology 17: 1199-1200.

Harris, L. E. 1966. Biological energy interrelationships and Glossary of energy terms. National Research Council of National Academy of Science Publication no. 1411. 35 pp.

Hartman, M., C. E. Epifanio, G. Pruder, and R. Srna. 1974. Farming the artificial sea: growth of clams in a recirculating seawater system. Proceedings of the Gulf and Caribbean Fisheries Institute 26: 59-74.

Haven, D. S. 1964. Supplemental feeding of oysters with starch. Chesapeake Science 6: 43-51.

Hochachka, P. W. and T. Mustafa. 1973. Enzymes in facultative anaerobiosis of molluscs. I. Malic enzyme of oyster adductor muscle. Comparative Biochemistry and Physiology 45B: 625-637.

Holland, D. L. and B. E. Spencer. 1973. Biochemical changes in fed and starved oysters, Ostrea edulis L., during larval development, metamorphosis, and early spat growth. Journal of the Marine Biological Association of the United Kingdom 53: 287-298.

Hughes, R. N. 1970. An energy budget for a tidal-flat population of the bivalve Scrobicularia plana (da Costa). Journal of Animal Ecology 39: 357-387.

Ivlev, V. S. 1945. The biological productivity of waters. (Translated from Russian.) Journal of the Fisheries Research Board of Canada 23:1707-1759.

Jorgensen, C. B. 1976. August Putter, August Krogh, and modern ideas on the use of dissolved organic matter in aquatic environments. Biological Reviews 51(3): 291-328.

Karentz, D. 1975. The distribution of planktonic diatoms in Yaquina estuary, Oregon. Masters Thesis. Corvallis, Oregon State University, 137 numb. leaves.

Langefoss, C. M. and D. Maurer. 1975. Energy partitioning in the American oyster, Crassostrea virginica (Gmelin). Journal of the National Shellfisheries Association 65: -20-25.

Langton, R. W. and Gabbott, P. A. 1974. The tidal rhythm of extracellular digestion and the response to feeding in Ostrea edulis L. Marine Biology 24: 181-187.

Liao, P. B. and R. D. Mayo. 1972. Salmonid hatchery reuse systems. Aquaculture 1: 317-335.

Loosanoff, V. L. and J. B. Engle. 1947. Effect of different concentrations of microorganisms on the feeding of oysters (Ostrea virginica). U.S. Fish and Wildlife Service Fishery Bulletin 51: 37-57.

Lotsy, J. P. 1895. The food of the oyster, clam, and ribbed mussel. Report of the U.S. Commissioner of Fisheries 893(19): 375-386.

Maciolek, J. A. 1962. Limnological organic analysis by quantitative dichromate oxidation. Report of the Bureau of Sport Fisheries and Wildlife 60, 61 pp.

Matthiessen, G. C. and R. C. Toner. 1966. Possible methods of improving the shellfish industry of Martha's Vineyard, Duke's County, Massachusetts. Edgartown, A publication by the Marine Research Foundation Inc. Part I, Sec. I, p. 5-6.

Maurer, D. and G. Aprill. 1973. Feasibility study of raft culture of oysters in the Delaware Bay area. Lewes, Report submitted to the Delaware River Basin Commission. 180 pp.

Mitchell, P. H. 1916. Nutrition of oysters: glycogen formation and storage. Bulletin of the U.S. Bureau of Fisheries 35: 151-161.

Newell, R. C. 1970. Biology of intertidal animals. Logos Press, London. 555 pp.

Parsons, T. R. 1963. Suspended organic matter in sea water. Progress in Oceanography 1: 203-239.

Platt, T. and B. Irwin. 1973. Caloric content of phytoplankton. Limnology and Oceanography 18(2):306-310.

Potts, W.T.W. 1967. Excretion in the molluscs. Biological Reviews 42: 1-41.

Purchon, R. D. 1971. Digestion in filter feeding bivalves - a new concept. Proceedings of the Malacological Society of London 39:253-262.

Quayle, D. B. 1969. Pacific oyster culture in British Columbia. Bulletin of the Fisheries Research Board of Canada 169. 193 pp.

Rao, K. P. 1954. Tidal rhythmicity of rate of water propulsion in Mytilus and its modificibility by transplantation. Biological Bulletin. Marine Biological Laboratory, Woods Hole, Massachusetts 106: 353-359.

Rice, T. R. and R. J. Smith. 1958. Filtering rates of the hard clam (Venus mercenaria) determined with radioactive phytoplankton. U.S. Fish and Wildlife Service Fishery Bulletin 58(129): 78-82.

Russel-Hunter, W. D., R. T. Meadows, M. L. Apley, and A. J. Burky. 1968. On the use of a "wet oxidation" method for estimates of total organic carbon in mollusc growth studies. Proceedings of the Malacological Society of London 38 (1): 1-11.

Savage, R. E. 1925. The food of the oyster. Ministry of Agriculture and Fisheries, London, Series II, 8(1). 53 pp.

Sharp, J. H. 1973. Size classes of organic carbon in seawater. Limnology and Oceanography 18(3):441-447.

Spotte, S. H. 1970. Fish and invertebrate culture. John Wiley and Sons, Inc., New York. 145 pp.

________________ 1974. Aquarium techniques: Closed system marine aquariums. In: Experimental marine biology, ed. by R. N. Mariscal. Academic Press, New York. p. 2-19.

Srna, R. C., C. Epifanio, G. Pruder, M. Hartman, and A. Stubbs. 1973. The use of ion specific electrodes for chemical monitoring of marine systems. I. The ammonia electrode as a sensitive water quality indicator probe for recirculating mariculture systems. University of Delaware, Lewes, Sea Grant Publication no. DEL-SG 14-73. 20 pp.

Strickland, J.D.H. and T. R. Parsons. 1972. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin 167. 310 pp.

Tenore, K. R., J. C. Goldman, and D. P. Clarner. 1973. The food chain dynamics of the oyster, clam, and mussel in an aquaculture food chain. Journal of Experimental Marine Biology and Ecology 12:157-165.

Thompson, R. J. and B. L. Bayne. 1972. Active metabolism associated with feeding in the mussel Mytilus edulis L. Journal of Experimental Marine Biology and Ecology 8: 191-212.

________________ 1974. Some relationships between growth, metabolism and food in the mussel Mytilus edulis. Marine Biology 27: 317-326.

Trevallion, A. 1971. Studies on Tellina tenius Da Costa. III. Aspects of general biologyand energy flow. Journal of Experimental Marine Biology and Ecology 7: 95-122.

Ukeles, R. 1971. Nutritional requirements in shellfish culture. In: Artificial propagation of commercially valuable shellfish, ed. by K. S. Price and D. L. Maurer. University of Delaware Press, Newark. pp. 43-64.

Van Weel, P. B. 1961. The comparative physiology of digestion in molluscs. American Zoologist 1: 245-252.

Walne, P. R. 1972. The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. Journal of the Marine Biological Association of the United Kingdom 52: 345-374.

Warren, C. E. and G. E. Davis. 1967. Laboratory studies on the feeding, bioenergetics and growth of fishes. In: The biological basis of freshwater fish production, ed. by S. D. Gerking, Oxford, Blackwell Scientific Publications. 495 pp.

Warren, C. E. 1971. Biology and water pollution control. W. B. Saunders Company, Philadelphia. 434 pp.

Welch, H. E. 1968. Relationships between assimilation efficiencies and growth efficiencies for aquatic consumers. Ecology 49 (4): 755-759.

Widdows, J. and B. L. Bayne. 1971. Temperature acclimation of Mytilus edulis with reference to its energy budget. Journal of the Marine Biological Association of the United Kingdom 51: 827-843.

Willis, S. A., W. K. Havens, and R. M. Ingle. 1976. Quality improvement of oysters, Crassostrea virginica (Gmelin), using artificial foods. Florida Marine Research Publications no. 20 of The Florida Department of Natural Resources, St. Petersburg. 16 pp.

Winter, J. E. 1970. Filter feeding and food utilisation in Arctic islandica L. and Modiolus modiolus L. at different food concentrations. In: Marine food chains, ed. by J. H. Steele, Berkeley, University of California Press. pp. 196-206.

____________ 1973. Filtration rate of Mytilus edulis and its dependence on algal concentration, measured by a continuous automatic recording apparatus. Marine Biology 22: 317-328.

Yonge, C. M. 1926. Structure and physiology of organs of feeding and digestion in Ostrea edulis. Journal of the Marine Biological Association of the United Kingdom 14: 295-386.

____________ 1960. Oysters. Collins Press, London. 209 pp.

Zillioux, E. J. and N. F. Lackie. 1970. Advances in the continuous culture of planktonic copepods. Helgolander Wissenschaftliche Meeresuntersuchungen 20: 325-332.

APPENDICES

Appendix 1. The results of a series of prelininary trials to test the validity of the acid shucking technique.

Part A. Test of effects of acid (conc. HCl) on the weight of oyster meats. Hand shucked three juvenile oysters (about three inches in length), homogenized the meats, and then divided the homogenate into 10 aliquots. Dried all ten on tared glass fiber papers, cooled and weighed. Treated five with acid, and five with distilled water for 1/2 hour. Redried the samples, cooled, and reweighed.

	Dry weight before treatment	Dry weight after treatment	Ash free dry weight after treatment	% Ash
A. Acid treated	8.067 mg	8.070 mg	6.525 mg	19.1
B. Water treated	7.187 mg	7.331 mg	5.774 mg	21.2

Ratio of the weights:

Before treatment = $\frac{A}{B}$ = 1.12; After treatment = $\frac{A}{B}$ = 1.10

Part B. Test of effects of acid treatment on the weight of oyster meats. Methods as described above for Part A.

	Dry weight before treatment	Dry weight after treatment	Ash free dry weight after treatment	% Ash
A. Acid treated	7.720 mg	8.050 mg	6.122 mg	24.0
B. Water treated	7.951 mg	8.192 mg	6.140 mg	25.0

Ratio of the weights:

Before treatment = $\frac{A}{B}$ = 0.97; After treatment = $\frac{A}{B}$ = 0.98

Appendix 1--continued.

Part C. Test of washing procedures for acid shucking technique. Randomly selected 25 oysters and divided them into five groups of five. Dried at 90° C for 24 hrs.; ashed at 450° C for 20 hrs. Treatments of the five groups were as shown below.

	Mean length (mm)	Dry weight (mg)	Ash free dry weight (mg)	% Ash
Hand shucked	14.2	3.99	3.27	18.0
Acid shucked-no wash	16.6	13.19	7.10	46.2
Acid shucked-dip wash	14.7	7.74	5.75	25.8
Acid shucked- 1 min.wash	15.5	6.37	4.69	26.3
Acid shucked- 5 min. wash	14.9	4.44	3.80	14.5

Data above show that a five minute wash is sufficient to remove the $CaCl_2$ salts produced by the action of HCl on the oysters' shell. Weights of acid shucked animals includes the shell matrix.

Part D. Test to determine the relative contribution of meat and shell matrix to the organic content of juvenile oysters. Oysters were shucked with conc. HCl and were washed for five minutes in distilled water. Samples were dried for 24 hrs. at 90° C, and ashed for 20 hrs. at 450° C, re-hydrated with distilled water, dried 24 hrs. at 90° C, cooled, and weighed.

Run No.	Sample size	Shell length (mm)	Ash free dry meat weight (mg)	Ash free dry matrix weight (mg)	Meat as % of total	Matrix as % of total
1	5	15.6	3.44	1.20	74.1	25.9
2	4	16.5	4.57	0.97	79.0	21.0
3	5	9.0	0.67	0.23	74.9	25.1
4	6	8.5	2.36	0.62	79.1	20.9
5	10	8.9	0.89	0.19	82.4	17.6
$\bar{X}$	30				77.9	22.1

Appendix 2a. Density of algal cells in the inflow and outflow of three trays containing 180 oysters each (Expt. II). <u>Pseudoisochrysis paradoxa,</u> from a common reservoir (appendix 2b), was used in each case. Trays were covered to exclude light after the 1930 count. Counts show no particular pattern of activity or inactivity. Routine counts during the experiments were made at about 2000 hr.

Time	Elapsed time (hrs)	Inflow (cells/ml)	Outflow (cells/ml)	Change in cell density (cells/ml)	Apparent consumption (cells/oyster/min)
			Tray No. 1 (11° C)		
1330	0	44, 034	43,630	404	468
1530	2	44, 165	43, 498	667	796
1730	4	50, 869	47, 912	2957	2875
1930	6	55, 563	53, 736	1827	1929
2130	8	56, 302	53, 648	2653	2904
2330	10	47, 415	44, 593	2852	3169
0130	12	43, 146	39, 759	3387	3575
0330	14	37, 441	39, 510	1931	2360
0530	16	38, 953	36, 535	2418	2552
			Tray No. 5 (15° C)		
1330	0	49, 349	56, 119	3230	3589
1530	2	52, 021	48, 927	3094	3351
1730	4	55 185	51, 887	3298	3664
1930	6	57 300	50, 920	6380	5317
2130	8	59, 389	53, 320	6069	6642
2330	10	56, 241	50, 773	5468	6076
0130	12	54, 811	48, 753	6058	6395
0330	14	53, 313	46, 087	6560	6378
0530	16	48, 943	42, 048	6895	6512
			Tray No. 6 (15° C)		
1330	0	21, 955	20, 773	1192	1291
1530	2	22, 030	20, 273	1757	2001
1730	4	24, 693	22, 101	2592	2837
1930	6	25, 860	23, 347	2513	2750
2130	8	25, 930	23, 540	2390	2589
2330	10	23, 876	20, 940	2936	3181
0130	12	21, 162	19, 159	2003	2226
0330	14	20, 319	17, 559	2760	2990
0530	16	17, 665	15, 807	1858	2064

Appendix 2b. Changes in the condition of algae, *Pseudoisochrysis paradoxa*, held in the algae feed reservoir of the closed system during Experiment II. The algae was held at 17° C in constant room light while in the reservoir.

Elapsed time (hrs)	Cells/ ml (X 10^6)	Nitrogen/ cell (X 10^{-6} µg)	Carbon/ cell (X 10^{-6} µg)	C/N Ratio	Mean cell vol. (um³)	Carbon/ um³ (X 10^{-6} µg)	Nitrogen/ um³ (X 10^{-6} µg)	pH
0	2.9	0.99	6.10	6.15	22.0	0.28	0.045	8.49
4	3.1	0.92	5.43	5.89	19.0	0.29	0.048	8.46
8	3.4	0.83	4.54	5.53	14.8	0.31	0.056	8.38
12	3.4	0.93	4.60	4.95	13.7	0.34	0.068	8.33
16	3.1	0.83	4.66	5.48	13.3	0.35	0.064	8.31
20	2.5	0.81	4.90	6.03	13.1	0.37	0.062	8.24
Net change		-18.2%	-19.7%		-40.5%	+32.1%	+37.8%	

Appendix 3

Part A. Determination of the caloric content of cells of _Pseudoisochrysis paradoxa._ Caloric determinations made using dichromate wet oxidation; cell counts and cell volumes made using Coulter Counter (ZBI); organic carbon and nitrogen determination made with Carlo-Erba carbon, hydrogen, nitrogen analyzer. Interval shown is the 95% confidence interval of the mean.

Run No.	Carbon/ sample (mg)	Cells/ sample (X 10^6)	Calories/ sample	Calories/ mg carbon
1	0.62	94	5.90	9.52
2	"	"	5.97	9.63
3	"	"	5.83	9.40
4	"	"	5.97	9.63
5	"	"	5.97	9.63
6	0.90	126	7.27	8.08
7	"	"	7.54	8.38
8	"	"	7.60	8.44
9	"	"	7.34	8.16
10	0.67	115	6.56	9.79
11	"	"	6.50	9.70
12	"	"	6.56	9.79
13	"	"	6.36	9.49
14	"	"	6.36	9.49
$\bar{X}$				9.22 ± 0.34

Part B. Determination of the caloric content of cells of _Monochryis lutheri._ Methods as described above and in the text.

Run No.	Carbon/ sample (mg)	Cells/ sample (X 10^6)	Calories/ sample	Calories/ mg carbon
1	0.84	90	7.83	9.32
2	"	"	8.10	9.64
3	"	"	8.43	10.04
4	"	"	7.90	9.41
5	"	"	7.43	8.85
6	0.55	53	5.36	9.75
7	"	"	5.30	9.64
8	"	"	5.50	10.00
9	"	"	5.50	10.00
10	"	"	5.43	9.87
$\bar{X}$				9.65 ± 0.23

Appendix 4. Determination of the caloric content of oyster meat and organic shell matrix. Caloric values were determined by dichromate wet oxidation on samples of known dry weight and ash content. Interval shown is the 95% confidence interval of the mean.

Type of sample	Ash free dry weight (mg)	Calories	Cal/g
Initial group, Expt. I			
(meat only)	2.69	10.90	4052
"	2.76	12.22	4428
"	2.44	9.83	4029
"	2.89	11.69	4045
"	2.64	11.29	4277
Final group, Expt. I			
(tray 1, meat only)	5.096	19.72	3870
"	0.836	3.72	4450
"	2.851	10.18	3571
"	1.542	6.75	4377
"	0.966	4.26	4410
"	3.804	15.68	4122
"	3.810	16.35	4292
"	2.469	8.67	3524
"	1.465	5.26	3592
Final group, Expt. 1			
(tray 13, meat only)	3.471	13.68	3941
"	2.593	11.53	4447
"	3.270	14.75	4511
Initial group, Expt. II			
(meat and matrix)	0.587	2.06	3509
"	0.334	1.20	3593
"	0.368	1.66	4511
"	0.339	1.46	4307
Initial group, Expt. II			
(meat and matrix)	0.674	3.00	4451
"	0.726	2.73	3760
"	0.977	3.67	3756
"	0.747	3.13	4190
"	0.565	2.33	4124
Final group, Expt. II			
(open system ambient)	2.653	11.35	4278
"	3.448	13.90	4031
"	3.470	13.50	3891
Mean			4081 ± 119

Appendix 5. Mean and 90 percent confidence intervals for particulate organic carbon and nitrogen entering the oyster holding trays of the open system experiments. The water was pumped from Yaquina Bay through the seawater system of Oregon State University's Marine Science Center. The water was not filtered but the system included a settling tank that removed the large and dense particulate matter. Each sample consisted of 100 ml of water filtered through a glass fiber filter disc as described in the text. Carbon and nitrogen were determined with A Carlo-Erba CHNO analyzer. Intervals shown are the 90 percent confidence intervals.

Month	No. of samples	Carbon (μg/L)	Nitrogen (μg/L)	C/N ratio
Jan	0	-	-	-
Feb	6	382.1± 96.7	68.4± 21.3	5.59
Mar	6	239.8± 49.2	34.4± 5.5	6.97
Apr	10	233.3± 22.0	36.2± 4.9	6.45
May	6	335.3± 81.0	63.5± 19.5	5.28
Jun	14	496.2± 48.9	80.6± 7.1	6.15
Jul	10	815.5± 119.4	115.8± 16.2	7.04
Aug	15	543.3± 62.8	84.1± 10.3	6.46
Sep	5	449.7± 76.4	62.5± 8.5	7.19
Oct	10	469.6± 105.9	45.8± 6.5	10.25
Nov	6	303.0± 42.6	28.9± 4.8	10.48
Dec	4	411.7± 103.2	62.7± 6.4	6.57

Appendix 6a. Results of five food consumption determinations made on each treatment group in closed system Experiment I. Food consisted of living *Pseudoisochrysis paradoxa* cells.

Temp. °C	Tray No.	Food available		Food consumed		Percent consumed	
		Cells/oyster/min	Cal/kcal/hr	Cells/oyster/min	Cal/kcal/hr	of food available	of body weight/day
11	1	149,581	33.26	12,348	2.75	8.3	6.6
	2	81,279	17.21	14,645	3.10	18.0	7.5
	3	41,226	8.70	8,862	1.87	21.5	5.5
	4	unfed	--	--	--	--	--
15	5	165,877	35.75	30,848	6.67	18.6	16.0
	6	78,253	17.24	16,070	3.54	20.5	8.5
	7	40,876	9.73	9,750	2.32	23.9	5.6
	8	unfed	--	--	--	--	--
19	9	176,040	46.61	20,778	5.50	11.8	13.2
	10	100,684	23.89	20,796	4.93	20.7	11.8
	11	44,015	11.65	8,196	2.17	18.6	5.2
	12	unfed	--	--	--	--	--
23	13	163,275	37.70	19,527	4.51	12.0	10.8
	14	95,712	29.42	16,275	4.98	16.9	11.9
	15	50,497	16.02	9,433	2.99	18.7	7.2
	16	unfed	--	--	--	--	--

Appendix 6b. Means of oxygen consumption determinations made during Experiment I. Intervals given are 90 percent confidence intervals.

Temp. °C	Tray No.	Oxygen uptake Run A (ml/g/hr)	Oxygen uptake Run B (ml/g/hr)	Mean oxygen uptake (ca /kcal/hr	Mean for all determinations at each temp.
11	1	0.66± 0.16	1.14± 0.50	1.07	0.92
	2	0.67± 0.16	0.70± 0.21	0.82	
	3	0.85± 0.25	0.73± 0.11	0.94	
	4	0.51± 0.28	0.88± 0.52	0.85	
15	5	0.71± 0.31	0.85± 0.29	0.93	0.99
	6	0.83± 0.37	0.62± 0.14	0.87	
	7	1.89± 1.11	0.76± 0.32	1.58	
	8	0.47± 0.23	0.51± 0.17	0.59	
19	9	0.82± 0.34	1.14± 0.64	1.17	1.21
	10	0.41± 0.29	1.05± 0.45	1.47	
	11	1.35 ± 0.41	0.63± 0.39	0.99	
	12	1.40± 0.60	0.65+ 0.17	1.22	
23	13	1.28± 0.35	1.87± 0.53	1.89	1.49
	14	1.08± 0.25	1.01± 0.40	1.26	
	15	2.24± 0.81	1.04± 0.23	1.96	
	16	0.90± 0.18	0.53± 0.25	0.85	

Appendix 6c. Growth results from closed system Experiment I. Growth rate calculation is based on comparison of termination weights with weights of randomly selected initial sample. Initial ash free dry weight = 2.78 mg (11.40 cal.). 90 percent confidence intervals are shown.

Temp. °C	Tray No.	Final ash free dry weight (mg)	Final cal. per oyster	Percent change	Growth rate cal/kcal/hr
11	1	3.58 ± 0.88	14.68	+29	+0.22
	2	3.90 ± 1.38	15.99	+40	+0.29
	3	3.92 ± 1.33	16.07	+41	+0.30
	4	2.83 ± 0.47	11.60	+ 1	+0.01
15	5	3.77 ± 1.31	15.46	+36	+0.26
	6	3.64 ± 0.76	14.92	+31	+0.23
	7	3.15 ± 1.19	12.92	+13	+0.11
	8	2.40 ± 0.95	9.84	-14	-0.13
19	9	2.55 ± 0.69	10.46	- 8	-0.08
	10	3.18 + 0.85	13.04	+14	+0.12
	11	2.55 ± 0.65	10.46	- 8	-0.08
	12	2.24 ± 0.49	9.18	-19	-0.19
23	13	3.33 ± 1.40	13.65	+20	+0.16
	14	1.81 ± 0.43	7.42	-35	-0.37
	15	1.61 ± 0.67	6.60	-40	-0.43
	16	2.64 ± 0.63	10.82	- 5	-0.05

Appendix 7a. Experiment II - Consumption of *Pseudoisochrysis paradoxa* by juvenile oysters.

Temp. °C	Tray No.	Food available: Cells/ml	Food available: Cells/oyster/min	Food available: Cal/kcal/hr	Food consumed: Cells/oyster/min	Food consumed: Cal/kcal/hr	Food consumed: Percent of available	Food consumed: Percent of body wt./day
11	1	53,082	42,798	226.0	3809	19.39	8.6	46.5
	2	27,780	22,832	123.1	2443	12.99	10.6	31.2
	3	11,989	11,048	70.0	1160	8.09	11.6	19.4
	4	unfed	--	--	--	--	--	--
15	5	55,131	46,227	181.0	5501	21.73	12.0	52.2
	6	27,178	23,054	107.8	3412	16.29	15.1	39.1
	7	13,129	11,054	57.4	2642	13.94	24.3	33.5
	8	unfed	--	--	--	--	--	--
19	9	56,077	47,459	215.8	4034	18.63	8.6	44.7
	10	28,494	22,395	130.2	3628	20.05	15.4	48.1
	11	13,639	10,170	79.8	1078	7.67	9.6	18.4
	12	unfed	--	--	--	--	--	--
23	13	53,222	42,848	317.9	1971	14.12	4.4	33.9
	14	29,669	23,892	206.0	1983	16.55	8.0	39.7
	15	16,639	10,920	117.9	950	8.12	6.9	19.5
	16	unfed	--	--	--	--	--	--

Appendix 7b. Closed system Experiment II - Consumption of *Monochrysis lutheri*.

Temp. °C	Tray No.	Food available: Cells/ml	Food available: Cells/oyster/min	Food available: Cal/kcal/hr	Food consumed: Cells/oyster/min	Food consumed: Cal/kcal/hr	Food consumed: Percent of available	Food consumed: Percent of body wt./day
11	1	40,664	31,549	280.7	1609	13.63	4.9	32.7
	2	20,138	17,260	147.6	1347	11.94	8.1	28.7
	3	10,378	8,152	102.2	481	5.55	5.4	13.3
	4	unfed	--	--	--	--	--	--
15	5	40,275	33,000	218.2	1716	18.82	8.6	45.2
	6	20,344	19,270	133.8	1426	11.35	8.5	27.2
	7	10,172	7,184	73.8	1135	9.99	13.5	24.0
	8	unfed	--	--	--	--	--	--
19	9	39,863	34,813	248.9	557	4.29	1.7	10.3
	10	20,756	15,524	152.0	978	9.00	5.9	21.6
	11	9,760	7,803	94.8	1038	12.19	12.9	29.3
	12	unfed	--	--	--	--	--	--
23	13	41,100	29,936	403.5	464	5.54	1.4	13.3
	14	19,725	14,582	227.7	420	5.85	2.6	14.0
	15	10,790	7,609	122.9	350	4.99	4.1	12.0
	16	unfed	--	--	--	--	--	--

Appendix 7c. Closed system Experiment II - Food consumption, weighted means for the 840 hours that they were fed *Pseudoisochrysis paradoxa*, and 480 hours that they were fed *Monochrysis lutheri* during the 56 day experiment.

Temp. °C	Tray No.	Food available (cal/kcal/hr)	Food consumed (cal/kcal/hr)	Percent consumed	
				Of available	Of body wt/day
11	1	240.7	17.35	7.21	41.5
	2	132.2	12.60	9.53	30.3
	3	82.4	7.29	8.85	17.2
	4	unfed	--	--	--
15	5	193.7	20.67	10.67	49.7
	6	116.7	14.50	12.43	34.8
	7	65.0	12.50	19.22	30.1
	8	unfed	--	--	--
19	9	223.5	13.43	6.01	32.2
	10	144.7	15.31	10.58	38.5
	11	85.6	9.27	10.83	22.4
	12	unfed	--	--	--
23	13	349.4	11.01	3.15	26.4
	14	213.9	12.66	5.92	30.4
	15	117.7	6.96	5.91	16.8
	16	unfed	--	--	--

Appendix 7d. Oxygen consumption determinations made during Experiment II. Intervals shown are 90 percent confidence intervals.

Temp. °C	Tray No.	Oxygen uptake Run A (ml/g/hr)	Oxygen uptake Run B (ml/g/hr)	Mean oxygen uptake (cal/kcal/hr)	Mean uptake for each temp. (cal/kcal/hr)
11	1	3.09± 0.46	2.72± 0.12	3.47	2.27
	2	2.56± 0.39	2.22± 0.24	2.85	
	3	1.53± 0.33	2.15± 0.67	2.14	
	4	0.76± 0.47	0.28± 0.30	0.61	
15	5	3.86± 0.26	2.97± 0.17	4.09	3.31
	6	3.87± 0.56	2.16± 0.14	3.60	
	7	4.19± 0.48	2.00± 0.43	3.69	
	8	1.34± 0.32	1.74± 0.77	1.84	
19	9	13.18± 0.78*	3.80± 0.39	4.54	3.43
	10	6.03± 0.66	3.22± 0.46	3.84	
	11	3.93± 0.47	1.94± 0.31	3.51	
	12	1.70± 0.76	1.38± 0.39	1.84	
23	13	12.08± 1.99*	2.92± 0.48	3.48	2.70
	14	2.30± 0.18	2.86± 0.29	3.42	
	15	3.14± 1.11	2.44± 0.74	3.34	
	16	0.43± 0.17	0.47± 0.53	0.55	

* Values omitted from calculation of means; caused by acute response to an increase in temperature, and are not representative.

Appendix 7e. Results of growth determinations, closed system Experiment II. Initial ash free dry weight was 102 micrograms; initial caloric content per animal was 0.418 cal.; and the initial shell length was 3.17 mm. 90 percent confidence intervals are shown.

Temp. °C	Tray No.	Final shell length (mm)	Percent increase in shell per day (k x 100)	Final ash free dry weight (micrograms)	Change in mean weight (micrograms)	Final calories per animal	Relative growth rate (cal/kcal/hr
11	1	4.59	0.67	175 ± 35	+73	0.718	+0.39
	2	3.85	0.35	164 ± 27	+62	0.672	+0.35
	3	3.29	0.06	97 ± 7	- 5	0.398	-0.04
	4	3.18	0.01	97 ± 17	- 5	0.398	-0.04
15	5	4.82	0.76	256 ± 45	+154	1.050	+0.65
	6	4.31	0.56	194 ± 70	+92	0.795	+0.47
	7	3.87	0.36	166 ± 30	+64	0.681	+0.36
	8	3.30	0.07	78 ± 15	-24	0.321	-0.20
19	9	4.36	0.57	204 ± 30	+102	0.836	+0.51
	10	3.83	0.34	166 ± 39	+64	0.681	+0.36
	11	3.24	0.04	97 ± 20	- 5	0.398	-0.04
	12	3.21	0.02	85 ± 18	-17	0.349	-0.14
23	13	3.28	0.06	95 ± 22	- 7	0.390	-0.05
	14	3.37	0.11	67 ± 5	-35	0.275	-0.31
	15	3.31	0.08	63 ± 8	-39	0.258	-0.36
	16	3.20	0.02	47 ± 6	-55	0.193	-0.56