ENERGY BALANCE OF A LABORATORY POPULATION OF *OSTREA EDULIS* (L)

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CONTENTS

ABSTRACT

The energy balance components of a laboratory population of juvenile *Ostrea edulis*, fed on *Tetraselmis seucica* at 15°C and 35‰, were investigated. The results show that filtration rate is a power function of body size and is dependent on food concentration. The relationship between food concentration and filtration rate closely approximated a theoretical model proposed for copepods, which was therefore adapted to suit bivalves. Filtration rate showed no selection of particles on the basis of size. However, oysters are probably able to recognise algae on a chemosensory basis and reject undesirable particles. Routine metabolism was found to be proportional to the 1,09 power of body mass. This agrees with expected values of the mass exponent predicted in the literature for animals of this size. No evidence of energy loss as dissolved organic carbon was recorded. A value of 80,32 $\pm$ 9,91% SD for assimilation efficiency was determined using the Conover ratio. A construction of the energy budget using the Winberg equation of change of mass per unit time ($\Delta w/\Delta t$) showed that 1,94 Jh^{-1} was available for somatic growth under optimal conditions.

The acute effect of temperature on the filtration and metabolic rates of oysters acclimated over the range 5 - 25°C

was determined. Results show that both rates were adjusted by lateral translation. The resultant cost of filtration (O_2 consumed/ml cleared) and filtration efficiency (ml cleared/O_2 consumed) showed optimal values at 15°C. Warm acclimated animals therefore showed a maximum scope for growth and percentage daily mass change between 15 and 20°C. These temperatures correspond to the summer temperatures experienced in the environment.

INTRODUCTION

Marine bivalves have been cultured for many years. The voluminous literature that was built up on every aspect of bivalve biology reflects the importance of bivalves, not only as commercial food source, but also because they are an important component of many intertidal marine ecosystems.

As filter feeders considerable attention has been paid to the particle retention ability of these animals. (Jørgensen, 1966; Owen 1966a, 1974b; Purchon, 1968 and Bayne, 1976). The effects of various ecological and physiological parameters on the filtration rate have also been studied (for reviews see Bayne, 1976; Winter, 1977a, b and Newell, 1979). These studies show that filtration rate is a function of body size, particle concentration, physiological state, tidal rhythmicity and temperature. Respiration rates have received as much attention, with studies on the effects of aforementioned factors, as well as pO_2 on the rate of O_2 uptake (Bayne and Livingston, 1977).

More recent studies have attempted to integrate the effect of a particular factor on the physiological reaction as a whole. This type of analysis enables one to look at the

reaction of the whole animal to a change in environmental condition, as opposed to the effect on a single function. Ratios between the volume of water cleared and the amount of oxygen consumed vary considerably with body size (Vahl, 1972, 1973a, b), pO_2 (Bayne *et. al.* 1976b) and temperature (Newell and Kofoed, 1977a, b and Newell *et. al.* 1977). This work has shown that the rate of filtration and respiration are modifiable in response to a change in environmental conditions. With a change in temperature Newell *et.al.* (1977) showed that *Ostrea edulis* regulated its metabolic efficiency over a wide range of acclimated temperatures, thereby increasing its competitive ability in a variety of ecological conditions.

The first part of the present work summarises aspects of energy balance in juvenile *Ostrea edulis* with special reference to the effect of concentration, particle size and mixed algal diets on the filtration rate of *Ostrea edulis*. The second part determined the response of filtration rate and respiratory rate to thermal acclimation in terms of filtration efficiency, scope for growth and predicted daily mass changes. Theoretical models of assimilated ration, respiratory rate and scope for growth were constructed from experimental data.

CHAPTER 1

THE EFFECTS OF BODY SIZE AND FOOD ON THE ENERGY BALANCE OF A LABORATORY POPULATION OF *OSTREA EDULIS*

1.1 DESCRIPTION OF ANIMALS AND HOLDING FACILITIES

1.1.1 INTRODUCTION

Work on the rearing and maintenance of laboratory populations of oysters has been in progress since 1919, but it was only in 1938 that Cole (1938) was able to claim that the rearing and maintenance of oysters on a commercial scale could be predicted. Since these pioneering studies much work has been done on keeping oysters in the laboratory, (Walne, 1956, 1958a, b, 1963, 1970a, b; Calabrese and Davis, 1970; Helm *et. al.* 1973; Langton and McKay 1974; Loosanoff and Murray 1974; Epifanio *et.al* 1975 and Pruder *et. al.* 1977).

What follows is a brief description of the procedures adopted in keeping oysters alive during this program.

1.1.2 METHODS

The main holding facility was a 50 litre tank kept in the Oceanography aquarium at the University of Cape Town. The tank was independent of the water supply in the aquarium, the ambient temperature of which was maintained at 15°C $\pm$ 1°C. Water in the aquarium was obtained from the Sea Point aquarium of the Sea Fisheries Branch on the West Coast of the Cape Peninsula. The sea water was

filtered through Whatmans No 1 filter paper to remove large suspended particles and changed at least once a week to prevent a buildup of excretory products. Water in the tank was circulated using air lifts. These were constructed from 16mm diameter Polyvinylchloride (PVC) piping. The water was allowed to drop into the tank, after lifting, by directing it into a perforated furrow suspended above the oysters.

The animals were obtained from the Fisheries Development Corporation hatchery at Knysna. Batches were flown to Cape Town in moist tissue paper with no apparent ill effects. The oysters were suspended in the tank in PVC dishes made from a section of piping with a diameter of 124mm. The dishes had 0,5mm mesh bottoms. This method is similar to that used by the hatchery in Knysna where it was found that regular shaking prevented the animals from attaching themselves to the mesh. Once a week the oysters were washed in a jet of fresh water to prevent as accumulation of epiphytes.

The animals were maintained on a diet of *Tetraselmis seucica*, added to the tank twice daily. This ration was calculated to supply the oysters with a minimum maintenance ration of 17µg C/animal.day^{-1} (Newell *et. al.*, 1977).

1.1.3 RESULTS

The oysters were maintained in an apparently healthy condition for a period of 14 months. During this period substantial growth was only observed in small oysters below 4mm in length. The larger specimens up to 15mm in length showed no significant increase in size.

1.2 DESCRIPTION OF ALGAE AND CULTURE METHODS

1.2.1 INTRODUCTION

The use of artificially grown phytoplankton as a means of supplying or supplementing the diet of laboratory populations of bivalves is well documented. (Cole, 1939; Walne, 1956, 1963, 1965, 1970a; Helm *et. al.*, 1973; Loosanoff and Murray, 1974; Gabbott *et. al.*, 1975; Winter, 1975; Winter and Langton, 1976 and Helm, 1977).

13 Species of algae were grown as food for the oysters in this study. One of them, *Tetraselmis seucica*, provided the main diet of the oysters while others were used in studying the effect of particle size on filtration rate.

1.2.2 METHODS

All of the species listed in Table 1 were obtained from the Plymouth Laboratory collection in the U K with the exception of *Hynenomonas carterae* and *Pyramimonas sp*, both of which were obtained from the Botany Department of the University of Natal, Pietermaritzburg. Two types of culture, stock and feeding, were maintained under slightly different conditions.

TABLE 1 The taxonomic position and size of the algae (authority is given behind the species in parenthesis)

TAXONOMY		APPROXIMATE SIZE (µm)
Phylum Chlorophyceae		
Order Volvocales		
Family Prasinophyceae		
Pyramimonas amylifera	(Conr.)	15 - 25
Dunaliella primolecta	(Butch)	5 - 12
Tetrasalmis seucica	(Kylin)	6 - 10
Tetrasalmis rubens	(Butch)	3 - 11
Tetrasalmis striata	(Butch)	6 - 8
Phylum Chrysophyta		
Sub-Phylum Chrysophyceae		
Class Haptophyceae		
Hymenomonas carterae	(Braarud)	10 - 15
Cricosphaera carterae	(Braarud)	10 - 15
Isochrysis galbana	(Parke)	4 - 8
Monochrysis lutheri	(Droop)	4 - 6
Sub-Phylum Bacillariophyceae		
Class Centrales		
Chaetoceros calcitrans	(Paulsen)	8 - 10
Phaeodactylum tricornutum	(Bohlin)	8 - 35
Skeletonema costatum	(Cleve)	3 - 5
Thallassiosira pseudonata	(Halse)	10 - 20
Phylum Cryptophyta		
Class Cryptophyceae		
Hemiselmis virescens	(Droop)	5 - 8
Phylum Pyrophyta		
Class Desmophyceae		
Prorocentrum micans	(Ehrenb)	30 - 40

1.2.3 STOCK CULTURES

Stocks were maintained in autoclaved 250ml pyrex conical flasks sealed by a double layer of commercial aluminium cooking foil. The cultures were grown in sea water enriched with a mixture of Erd Schreiber and 'Walnes' culture medium. In addition algae of the order Bacillariophyceae were supplied with sodium metasilicate ($40mg.l^{-1}$) to enhance test growth. A list of components of these ingredients is given in Appendices 1 and 2. Cultures were maintained in the aquarium at 15°C and subjected to a 16 hour light - 8 hour darkness cycle. The light source consisted of 3 Crompton 'cool white' flourescent tubes of 20W and 2800 lux each.

Since it was not necessary to maintain logarithmic growth in the stock cultures they were subcultured once a month.

1.2.4 FEEDING CULTURES

Feeding cultures were maintained in 2,5 litre pyrex flasks or 5 litre pyrex cylindrical flasks (Figure 1). Conditions were essentially similar to those described for stock cultures except for the following differences. Erd Schreiber was omitted from the culture medium and the light source was continious. The most significant difference was that

FIGURE 1 : View of the algal culture stand. The 5 litre culture flasks are on the right hand side. Note the cotton wool filters fitted to the air supply.

the feeding cultures were vigorously aerated, a process that enhances growth by mixing nutrients and allowing the entire volume to be exposed to light instead of just the outer layer. Before entering the culture the air was filtered through 2 cotton wool filters to remove airborne particles and so prevent contamination of the culture. Feeding cultures were maintained in a logarithmic growth condition by subculturing at least once every 10 days. *Tetraselmis seucica* has been shown to remain in a logarithmic growth phase until day 15 (Henning, pers. comm).

1.2.5 MASS-DENSITY RELATIONSHIP

A sample of the cultures was taken between day 8 and 12, ensuring that they were in a logarithmic growth phase. The algal density and particle sizes were determined using a Coulter counter Model T_A II which was fitted with a 70µm aperture tube. Algal densities were expressed as number of cells per litre.

5ml of each species were filtered onto preweighed, 0,45µm Millipore filters. These were then dried to constant mass at 60° for 48 hours and re-weighed on a Mettler (ME 30) microbalance.

1.2.6. RESULTS AND DISCUSSION

In describing the effects of algal densities on various biological functions, a number of different measures have been used. These measures include chlorophyll 'a' values (Kirby-Smith and Barber, 1974) mass (Gabbott and Bayne 1973), packed cell volume (Walne, 1970a), and numbers, using either a haemocytometer (Parsons, *et. al.*, 1961), or a Coulter counter (Vahl, 1973). Since morphological changes in algae can occur with age (Stacey, 1976), it is important to specify the age of culture being used for study purposes. Unfortunately chlorophyll and packed cell volumes were not measured for the algae used in this study, but, a basis for comparison, the mass of 1×10^6 cells in a logarithmic growth phase, is given in Table 2.

The taxonomic position, authority and size of the algae used are given in Table 1. A brief description of the algae is given below.

1.2.7 PRASINOPHYCEAE

Belonging to the phylum Chlorophyta (green plants), these algae are distinguished by their green pigments, the chlorophylls. The cell wall may consist of up to 3 layers, the outer one being mucilaginous or pectose.

TABLE 2 Mass as determined in the logarithmic growth phase of 1×10^6 cells of the algal species used in this study

ALGAE	MASS (mg)
Chaetoceros calcitrans	0,0830
Phaeodactylum tricornutum	0,0572
Monochrysis lutheri	0,0476
Thallassiosira pseudonata	0,0562
Hemiselmis virescens	0,0629
Isochrysis galbana	0,0517
Skeletonema costatum	0,0547
Dunaliella primolecta	0,3919
Tetraselmis striata	0,3340
Tetraselmis seucica	0,4964
Cricosphaera carterae	1,1875
Hymenomonas carterae	1,0372

Pyramimonas however, is unusual in having no cell wall, the outer layer of the cytoplasm forming the cell boundry (Prescott, 1969). All representatives of this family have flagella and the reserve product, when present, is starch (Morris, 1973).

With the exception of *Pyramimonas*, no difficulty was experienced in culturing these algae. Although I was able to keep *Pyramimonas* alive, none of the subcultures reached sufficient density to be used as food and it was therefore discarded. This was unfortunate because Walne (1970a), has shown that algal species that do not have a cell wall are usually good food for bivalve culture. This characteristic may attributed to the relative ease with which they may be assimilated when compared with algae possessing a rigid cell wall.

1.2.8 HYPLOPHYCEAE

Members of the sub-phylum Chrysophyteae are characterised by their golden brown colour. Pigments include chlorophyll 'a' as well as carotenoids such as fucoxanthin (Morris, 1973). A second common feature is the cell wall. Cellulose is absent and the wall is made up of scales which may or may not be calcified (Stacey, 1976). All Haplophyceae are flagellates. The main reserve products are fats and oils (Prescott, 1969), and starch is never present (Morris, 1973).

The chrysophytes are at present the subject of extensive reclassification as can be seen with the genus *Hymenomonas*. The Plymouth Laboratories still refers to the genus as *Cricosphaera* as classified by Braarud (1960). However, Manton and Peterfi (1969), have placed them into the genus *Hymenomonas*.

Cricosphaera and *Hymenomonas* are therfore regarded as strains of the same species for the purpose of this study. The similar results presented in Section 1.4.4 support this assumption.

1.2.9 CENTRALES

Probably the most ubiquitous of all algae are the Bacillariophyceae (Diatoms) of which the Centrales form a part. These algae possesses the same general characteristics of the Phylum Chrysophyta as listed above. In addition they have an isodiametric valve view with radially symmetrical wall ornamentation. The cell wall is silicified, for which reason sodium metasilicate was added to the growth medium.

Although the diatoms may tend to form long chains they are almost all unicellular with a motile state possessing a single flagellum (Morris, 1973). The main reserve products are fats and oils.

1.2.10 CRYPTOPHYCEAE

This is a small group sometimes regarded as being related to the Pyrrophyta (see below). They are mostly naked forms with no rigid cell wall and occur as flagellated, pallenoid or cocoid forms. *Hemiselmis virescens* has a distinctive blueish green colour. Reserve products are stored as starch.

1.2.11 PYRROPHYTA

As with the Cryptophyceae this is a little known group. However, *Procentrum micacans* was not cultured successfully and was discarded.

1.3 MORPHOMETRICS

1.3.1 INTRODUCTION

Linear and mass determination between an intact organism and one of its parts or between two of its parts may be expressed either as a power function of the form,

$$Y = aX^b \qquad (1)$$

or as a linear regression of the form,

$$Y = aX + b \qquad (2)$$

Equation (1) may be expressed in linear form by a logarithmic transformation,

$$\log Y = \log a + b \log X \qquad (3)$$

Y and X are measures of the animal and a and b are constants determined using least squares regression analysis on equations (2) and (3).

1.3.2 METHODS

Oysters were obtained from the Knysna Oster Co. in October 1978 and placed in a sea water aquarium described in Section 1.1. After 1 week the animals were removed and placed individually into water at $\pm$ 80°C until the valves gaped, a process that took up to 30 seconds. Body tissues were carefully separated from their shells under a dissecting microscope and placed into preweighed aluminium dishes.

The latter had been punched out of commercial cooking foil and found to be unaffected by temperatures of up to 500°C. Each was dried to constant mass in an oven at 60°C for 48 hours to determine dry mass of tissue, dry mass of shell and total dry mass, the sum of the two.

Length and width of the shell were determined as the maximum distance from the hinge to the outer edge of the shell and the widest perpendicular to this, respectively. These parameters were measured microscopically using a graduated eyepiece, or in the case of larger specimens, using a pair of Mitutoyo vernier calipers.

Finally the shells and body tissue were ashed in a muffle furnace at 450°C for 12 hours. This temperature was low enough to ensure that carbonates were not burnt off. Ash-free dry masses were calculated by subtracting ash masses from dry masses. All masses were determined using a Mettler Model ME 30 microbalance.

1.3.3 RESULTS AND DISCUSSION

Table 3 gives the co-efficients a and b which best fitted the data on morphological measurements, using either equation (2) or (3). The number of measurements (n) and the co-efficient of determination (r^2) are given.

TABLE 3 Regression analysis for various morphological relationships of *Ostrea edulis*.

	RELATIONSHIP $\frac{Y}{X}$	EQUATION	r^2	n
1.	$\frac{\text{Length}}{\text{Total Dry Mass}}$	$L = 2{,}54TDM^{0{,}37}$	0,70	59
2.	$\frac{\text{Dry Mass Tissue}}{\text{Total Dry Mass}}$	$DMT = 0{,}014TDM + 0{,}0732$	0,91	59
3.	$\frac{\text{Dry Mass Tissue}}{\text{Length}}$	$DMT = 0{,}0028L^{2{,}406}$	0,91	59
4.	$\frac{\text{AFDM Tissue}}{\text{Length}}$	$AFDM = 0{,}0025L^{2{,}388}$	0,90	59
5.	$\frac{\text{AFDM Tissue}}{\text{Total Dry Mass}}$	$AFDM = 0{,}013TDM + 0{,}0618$	0,91	59
6.	$\frac{\text{AFDM Tissue}}{\text{Dry Mass Tissue}}$	$AFDM = 0{,}895DMT - 0{,}0008$	0,99	59

The use of morphological measurements as a means of comparing animals on a spatial basis has been recognised by Wilbur and Jodrey (1952) and Dame (1972). These authors used shell mass: dry body mass ratios as basis of comparison. Moreover Galtsoff (1964) has shown that shells of intertidal oysters are thinner than those of subtidal oysters.

Whereas the data in this study have been primarily used to determine ash-free dry mass from other more easily obtained measurements such as whole mass or length, the entire spectrum has been documented, since their use as a predictive tool in ecological studies is well documented.

1.4 FILTRATION RATE

1.4.1 INTRODUCTION

Filtration rate or clearance rate may be defined as the volume of water filtered free of particles per unit time (Winter, 1969). This is distinguished from the pumping or ventilation rate which represents the volume of water flowing through the gills per unit time. Filtration rate and pumping rate are equal if particle retention efficiency is 100%.

Thus, calculation of filtration rate combined with a knowledge of particle concentration of the water represents the energy intake of the bivalve. Winter (1978), however, points out that this only holds true if no pseudofaeces are produced. To determine filtration rate one in practice measures the pumping rate making the assumption that there is 100% particle retention.

Pumping rates are determined using either 'direct' or 'indirect' methods. The direct method employs some physical attempt to separate the exhalent current and to measure either the velocity or the total water discharged by the animal. The major drawback involves stress caused by manipulating the animal. This probably affects the filtration rate (Winter, 1969).

Using the indirect method the animal is placed in a suspension of known concentration and the decline in particle concentration is monitored. Filtration rate is determined using one of the formulae summarised by Coughlan (1969). Davids (1964), Winter (1969), Owen (1974) and Bayne *et. al.* (1976c) have discussed the disadvantages of the indirect method. The major problem lies in the assumption that particle retention is 100% and that pumping rate is constant. These assumptions may not be true.

Indirect techniques to determine filtration rate can be used with closed or flow-through experimental chambers. The disadvantage in using closed systems lies in the fact that the continuously decreasing particle concentration affects filtration rate. These problems may, however, be overcome by using small animals in large chambers where a decrease in particle concentration does not become limiting (Foster-Smith, 1975a, b). Flow-through determinations allow a more continuous record of filtration rate to be obtained. Under constant concentrations, however, flow rate affects filtration rate (Winter 1975).

It is not within the scope of this study to document the mechanisms involved in particle retention. This subject has been extensively covered by Galtsoff (1964), Dral (1967), Bernard (1974), Owen (1974a), Foster-Smith (1975b), Bayne (1976) and Newell (1979).

1.4.2 THE EFFECT OF BODY SIZE ON THE FILTRATION RATE

1.4.2.1 INTRODUCTION

The effect of body size on biological functions has been the subject of intensive investigation and numerous studies on filtration rates of filter feeding bivalves have been recorded (Winter, 1969; Walne, 1972; McLusky, 1973; Vahl, 1973; Thompson *et. al.*, 1974; Bayne *et. al.*, 1976; Winter, *et. al.*, 1976; Widdows, 1978; Griffiths and King, 1979 and reviews by Bayne, 1976; Winter, 1977a, 1977b and Newell, 1979).

These studies all recognise that filtration rate (Vw) increases with body size and may be described by the power curve :

$$Vw = aM^b$$

where M = ash free dry mass in grams and a and b are constants. A review of the values for a and b obtained by a number of workers is given by Winter (1978). The wide range of b, from 0,29 to 0,82 is probably a result of measuring the filtration rate with different methods, under a variety conditions.

1.4.2.2 METHODS

The oysters used in these experiments were all juveniles and for reasons described above indirect, closed system methods

were used to determine filtration rates. Animals were placed in experimental chambers containing 500ml of fresh sea water filtered through 0,45µm millipore filters. Exposure temperature (T_E) was 15°C and salinity was 35‰. Water inside the chambers was stirred vigorously. The animals were allowed to acclimatize under these conditions for 60 minutes. Experiments were initiated by adding predetermined concentrations of *Tetraselmis suecica* resulting in densities of between 15 and and 25 x 10^6 cells per litre. The depletion of algae was monitored using a Coulter Counter Model Ta II. Concentration was not allowed to drop to less than 50% of the initial value. If filtration rate had not been measured by this stage more algae was added or the results were discarded. Sample time varied from 5 to 15 minutes, depending on the size of the animals.

Filtration rates were calculated using the standard formula described by Coughlan (1969) :

$$Vw(l.hr^{-1}) = \frac{(\log_e N_1 - \log_e N_2) \times V}{T}$$

Where, Vw is filtration rate, N_1 and N_2 are cell concentration at $time_1$ and $time_2$ respectively, T is time elapsed in hours and V is volume of vessel in litres. 3 - 5 such calculations were made to determine a mean rate for each animal.

After each experiment dry mass of body tissue was calculated

by drying at 60°C for 48 hours and ash-free dry mass was determined using equation 6. (Section 1.3).

1.4.2.3 RESULTS AND DISCUSSION

The relationship between body size and filtration rate is shown in Figure 2 and may be described by the power curve :

$$V_w = 34,18M^{0,91}{}_{l.h}{}^{-1} \qquad (r^2 = 0,83)$$

A value of 0,9144 for b as described above is higher than the average value summarised from Winter's (1978) review, but is close to the value of 0,94 obtained by Newell (1977) for *Ostrea edulis*. Most of the values for b obtained by other workers fall between 0,63 and 0,82 (Winter 1978). This implies that the proportionality is situated somewhere between surface area (0,67) and body mass (1,0). Winter (1978) suggests that values below 0,63 probably represent an under-estimation of the filtration rate.

The high value of b presented here shows that over the range of sizes worked with, filtration rate is more proportional to body mass than to surface area. A similar ralationship was found between oxygen consumption and body size (Section 1.5.2.1) which supports findings of other workers that metabolism increases in direct proportion to body mass in small metazoans (Newell, 1979). Because of the relatively high metabolic cost increase over the size range one would expect filtration to increase at a corresponding rate.

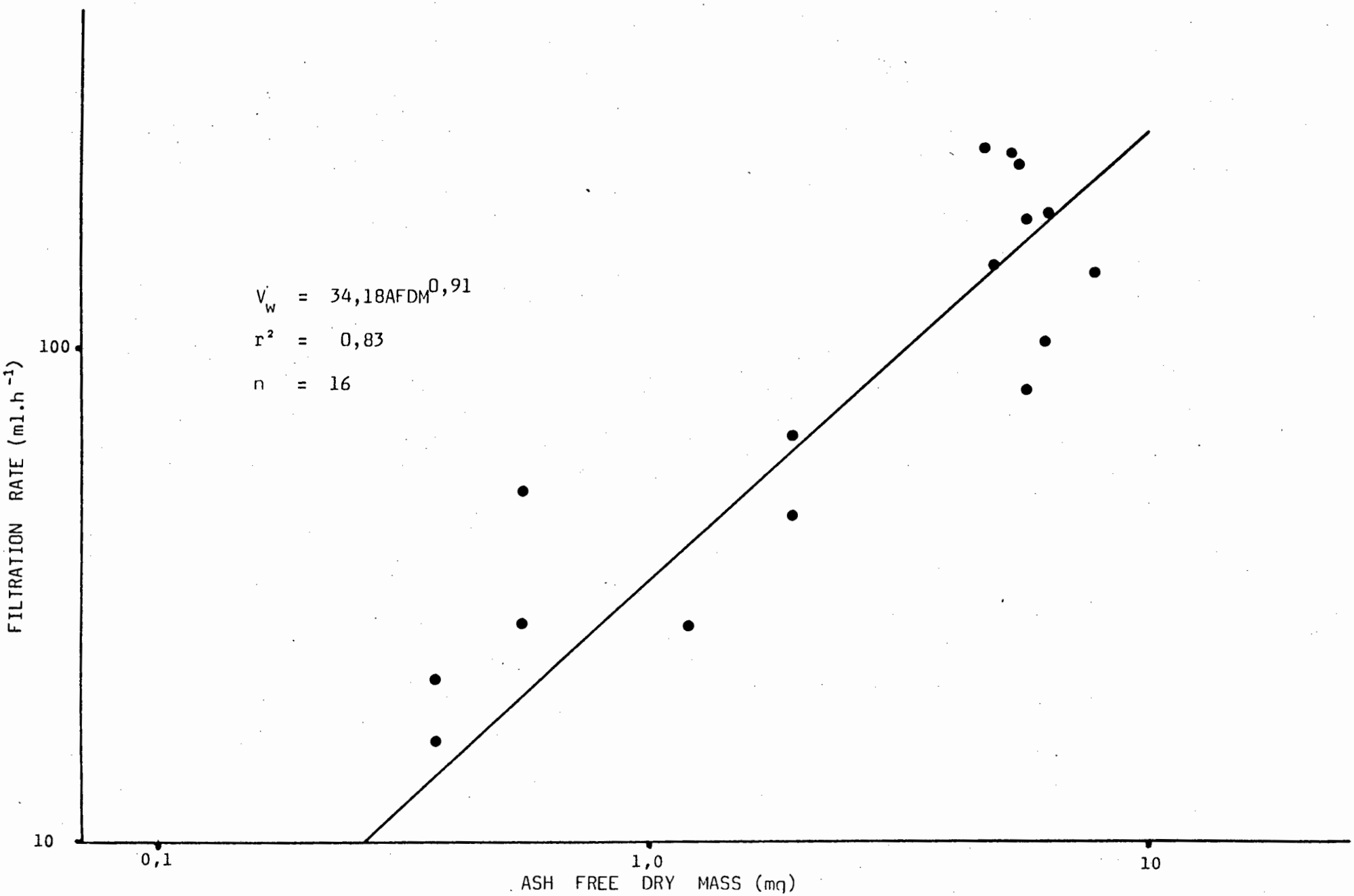

FIGURE 2 : Filtration rate (V_w) of *Ostrea edulis* as a function of ash free dry mass (AFDM)

The relatively high mass exponent of filtration (0,91) in small oysters may be explained by the high metabolic cost in these animals.

1.4.3 THE EFFECT OF PARTICLE CONCENTRATION ON FILTRATION RATE

1.4.3.1 INTRODUCTION

There is a considerable variation of opinion in the literature regarding the effect of phytoplankton concentration on the filtration rate of bivalves (Davids, 1964; Winter, 1969, 1970, 1973, 1977, 1978, 1979; Ali, 1970 and Foster-Smith, 1975a and b). In general terms, however, it is agreed that, at very low concentrations, filtration rate is almost zero and that it increases with increasing concentration until a peak is reached. Thereafter the rate decreases with further increases in particle concentration.

The levels at which these changes in rate take place vary greatly, not only from species to species but also between reports on the same species. The latter probably arises out of differences in experimental technique and animal condition.

1.4.3.2 METHODS

Specimens used in these experiments were obtained from the holding aquarium (see section 1.1). These animals had, by this stage, been maintained for approximately 5 months with no apparent ill effects. This was evident by the fact that mortality was low during this period and that the condition index, relating meat mass to shell mass, remained unchanged over the period in the aquarium.

The experiments were conducted in open 500ml perspex chambers. Fresh sea water was collected from Sea Point or Dalebrook on the West and East Coasts of the Peninsula respectively. The water was filtered through 0,45µm Millipore filters to remove natural particles.

The chamber design was similar to that used in section 1.4.2. Animals were placed on a false bottom and the water was mixed by a magnetic stirrer. Acclimatization continued at 15°C and 35‰ for 1 hour. Experiments were started by adding a predetermined amount of concentrated *Tetraselmis seucica* to the water to bring it to the desired suspension density. Decreasing particle concentration was monitored using a Coulter Counter Model TA II and filtration rate was determined using the equation described in section 1.4.2. A mean of 4 determinations was obtained at each of the densities, 5, 10, 20, 25, 30 and 50 x 10^6 cells per litre.

The same animals were used throughout the series of experiments. Only one experiment was performed per day and the animals were returned overnight to the holding aquaria. Finally the dry masses of the animals were obtained by drying at 60°C for 48 hours. Ash-free dry masses were obtained by extrapolation from equation 6 (Section 1.3). Filtration rates were corrected using the equation :

$$V_{ws} = \left(\frac{5,0883}{W_e}\right)^b . V_{we} \qquad \text{(Newell, 1977)}$$

where V_{ws} is the corrected rate for a standard animal of 5,0883mg, V_{we} is the experimentally obtained rate for an animal of mass W_e and b is the mass exponent of filtration obtained in section 1.4.2. The standard animal mass was calculated by taking the geometric mean of all the animals used throughout the study.

1.4.3.3 RESULTS AND DISCUSSION

Theoretical models describing the feeding rates of filter feeding zooplankton have been described by Lehman (1976) and Lam and Frost (1976). The basic assumptions described in the development of these models, however, hold true for filter feeders in general and the model may therefore be applied to bivalves as well. The model predicts that, as optimal foragers, filter feeders will maximise the nett energy intake, i.e. assimilated energy minus energy expenditure in terms of the cost of filtration.

A schematic representation of the model is given in Figure 3. Lam and Frost (1976) described 4 critical regions on the figure. At concentrations below 1 the animal will suffer a nett energy loss. Under these conditions the animal may filter at a minimal rate to maintain its metabolic processes or shut until conditions become more favourable. Beyond 1 filtration rate increases to a maximum between 1 and 3, in this example at 2. Maximal filtration rate is maintained up to 4, beyond which it declines. In accordance with the theory the ingestion rate increases to 2 after which it remains constant. Hence the cost of filtration is reduced, but the nett intake of food remains constant. (Winter, 1969 and Kirby-Smith *et. al.*, 1974).

The results of this study are summarised in Table 4 and depicted graphically in Figure 4. Although the rates show considerable variability it is clear that the model fits the results. An interesting feature of the curve is the fact that maximal filtration rates are reached over a wide range of particle concentrations. This is in contrast to findings of other workers who have reported attainment of maximal rates at low concentrations (Winter, 1970, 1977, 1978; Wayne, 1972; Thompson and Bayne, 1972, 1974 and Widdows *et. al.*, 1979), but agrees with work by Foster-Smith (1975) and Griffiths and King (1979), who reported maximal filtration rates approximately 15 x 10^6 cells/l.

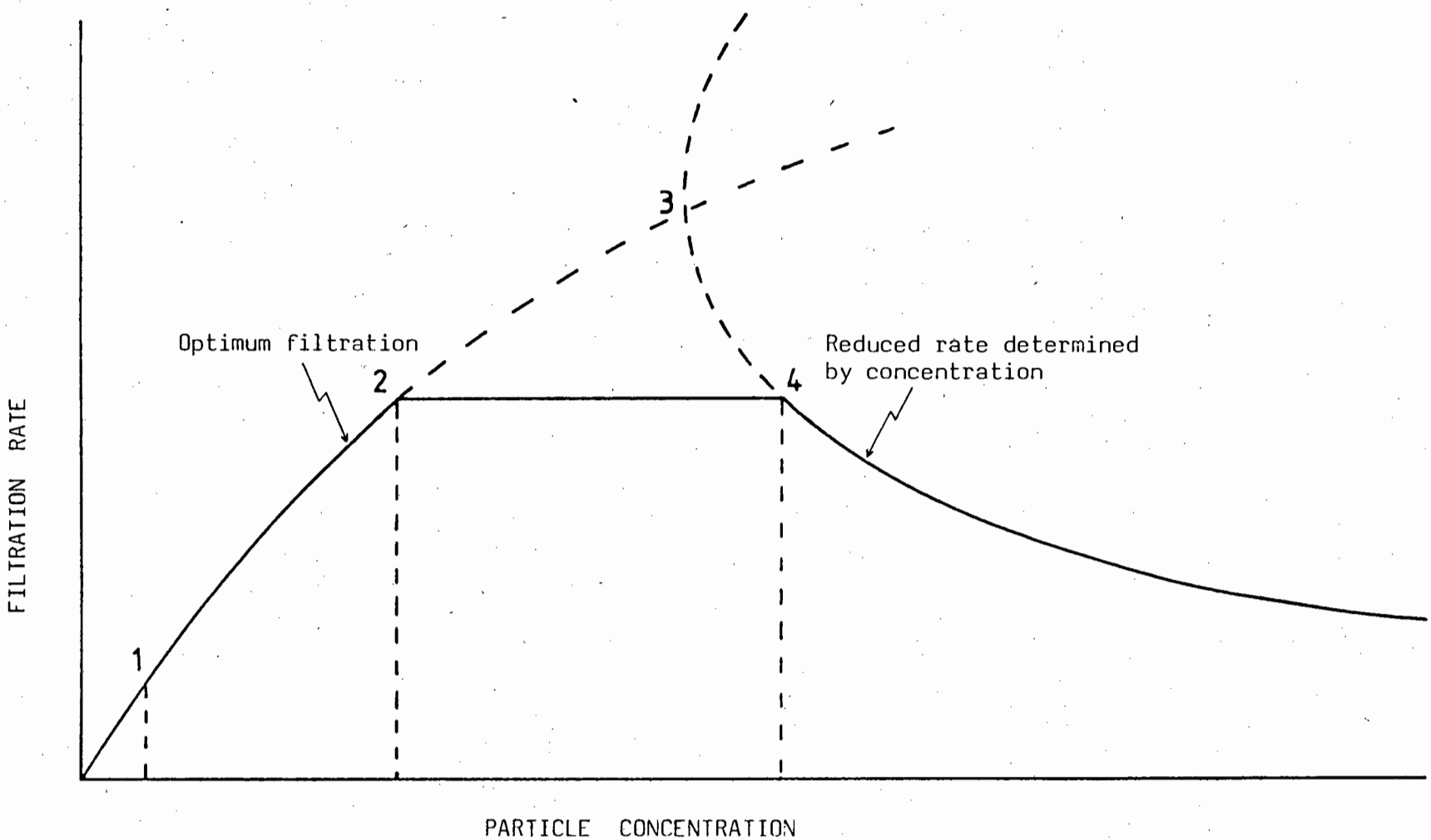

FIGURE 3 : Theoretical model of filtration as a function of concentration proposed by Lam and Frost (1976), For explanation see text.

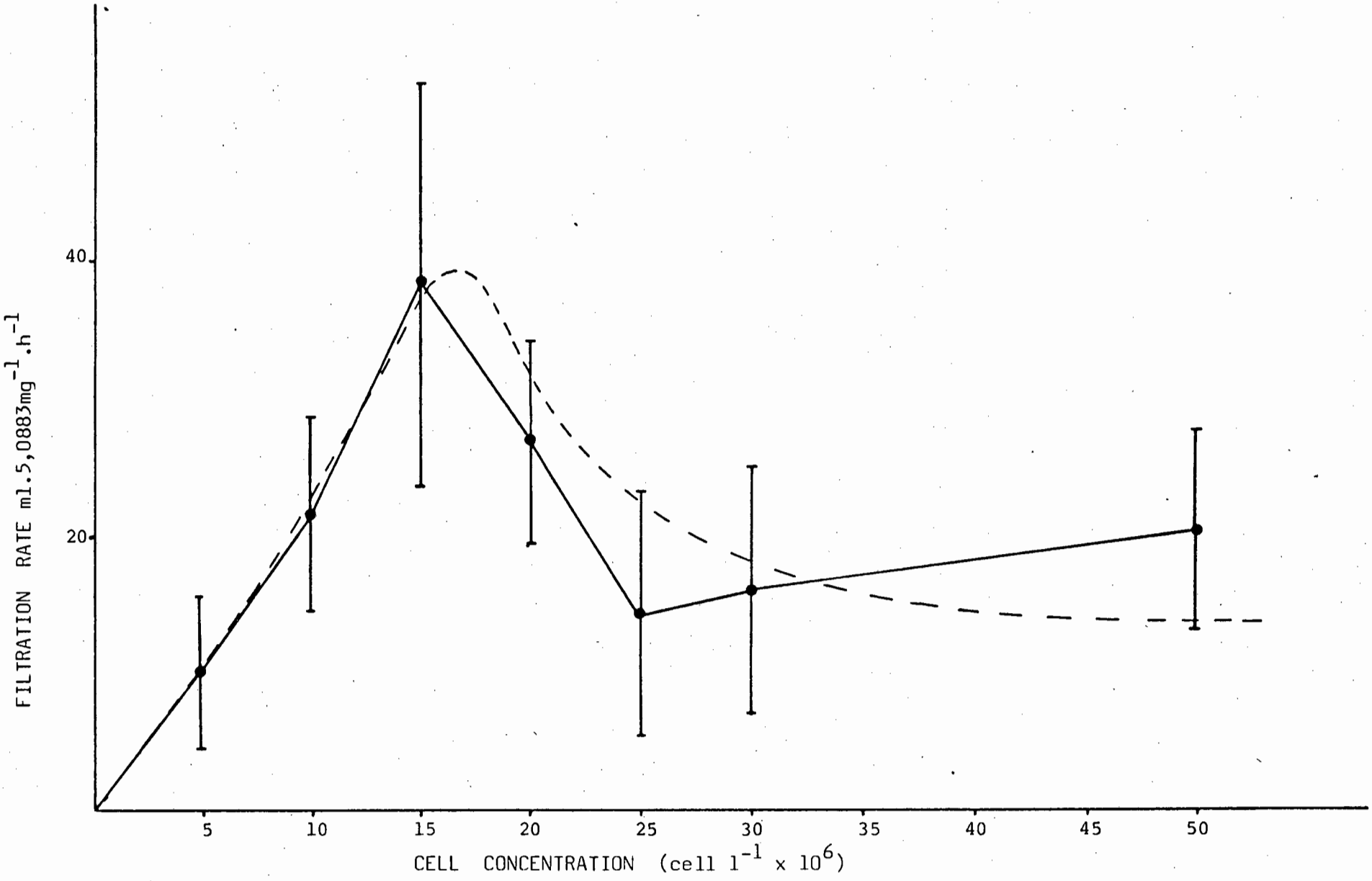

FIGURE 4 : Filtration as a function of cell concentration for *Ostrea edulis* using *Tetraselmis seucica* as food. Dashed line indicates values expected from the model and T-bar = SD. Pseudofaeces were observed at concentrations above 15 x 10^{6}cells/l.

TABLE 4 Summary of the filtration rates obtained at different food concentrations. Each value represents the mean of 4 determinations using different animals. The data are corrected for a standard of 5,0883mg.

CONCENTRATION (cells x 10^6cell.l^{-1})	FILTRATION RATE (ml.5,0883mg^{-1}.h^{-1})	
	$\bar{x}$	s
5	10,48	5,40
10	21,24	7,69
15	38,81	15,29
20	27,03	7,54
25	14,21	0,92
30	16,10	9,18
50	20,78	7,12

Within the framework of the energy optimisation model one would expect filtration rate to increase with increasing ration until it reached an ingestion rate that maintains a packed gut. In this situation there are two possibilities,

i) The filtration rate may decrease maintaining a constant ingestion rate.

ii) The animal may continue filtering at a constant rate and exclude any excess particles as pseudofaeces.

In the present study pseudofaecal production occurred at lower cell concentrations than reported for mussels (Davids, 1964; Winter, 1969 and Schulte, 1975), although Widdows (1978) and Haven and Morales-Amlo (1966) recorded pseudofaeces at low concentrations for mussels and oysters respectively.

For small oysters pseudofaeces-free filtration rates occurred at particle concentrations below approximately 15×10^6 cells/l. When determining pseudofaeces-free cell densities one must take cognisance of the fact that animal size, cell size and observation time may effect the value obtained (Widdows *et. al.*, 1979). In small animals the time taken to reach a fullness equilibrium is probably shorter than for larger animals. This enables observation of pseudofaecal production at relatively low cell densities and high filtration rates

This contrasts Winter (1978), who found that *Mytilus edulis* only produced pseudofaeces at high cell densities. At the same time filtration rate was low (see his Figure 9, concentration c). Ventilation at such low rates however, could become insufficient to meet the oxygen demand. Under these conditions the animal could increase the ostial size. This would allow more water to pass through the gill without affecting the relative ingestion rate because more, smaller particles would pass through the gill. However, Lehman (1976) predicts that an increase in ostial size decreases the nett assimilated ration making this alternative energetically expensive.

The possibility exists that fullness equilibrium will only be reached at higher concentrations in larger animals. This is compounded by the time taken for particles to pass through the gut and the size of the particles (Walne, 1972). These observations would explain high reported values for pseudofaeces-free filtration rates when large animals are used.

A schematic representation of the interaction between filtration, ingestion, pseudofaecal production and increasing cell concentration is proposed in Figure 5. This model is modified from those of Lehman (1976) and Lam and Frost (1976).

At concentrations lower than encountered at A the animal will experience a nett loss of energy because metabolic

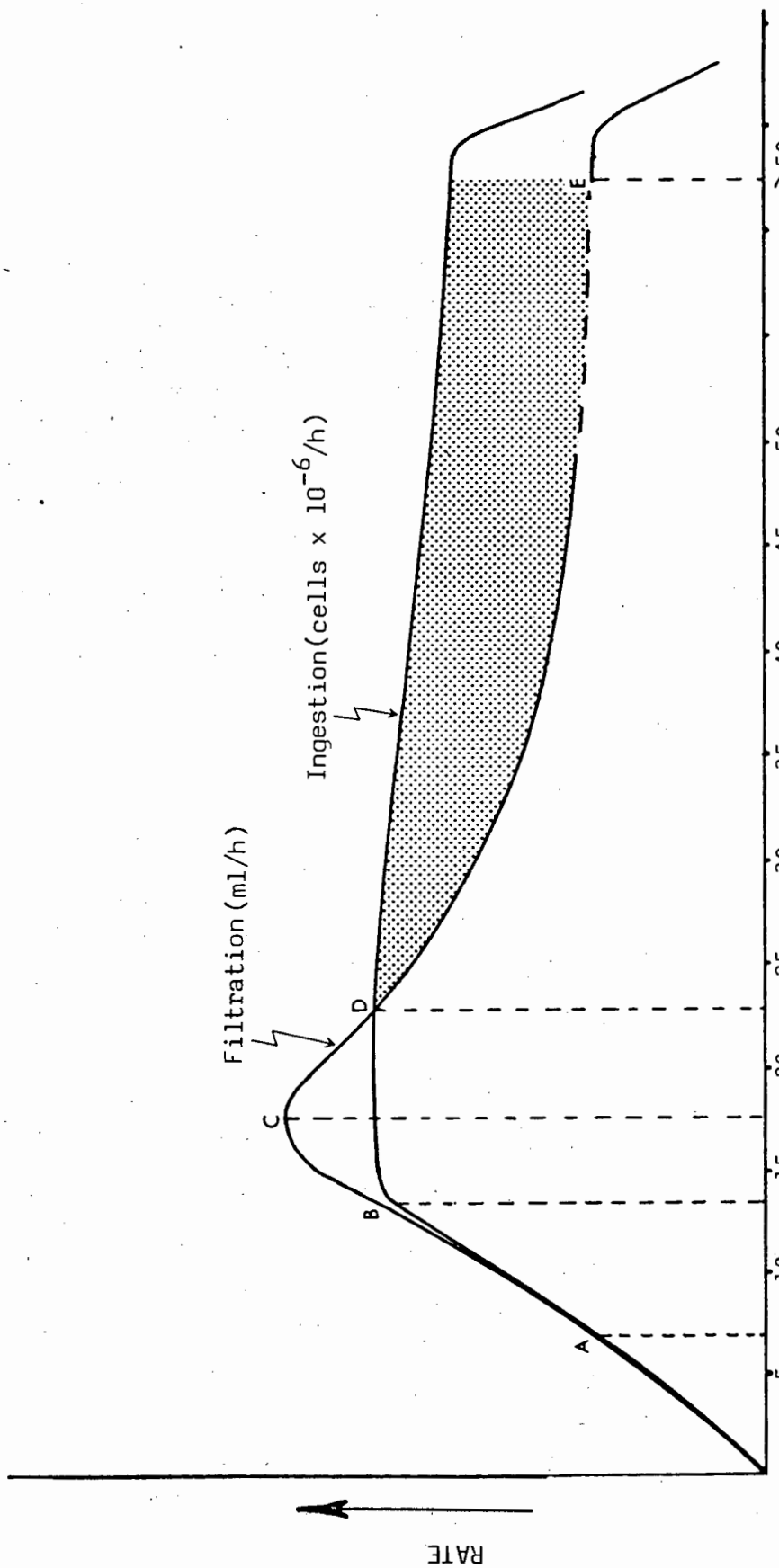

FIGURE 5 : Theoretical model of filtration and ingestion rates of *Ostrea edulis* as a function of cell concentration. (after Lehman, 1976). A, B, C, D and E are explained in the text. Stippled area represents pseudofaecal production.

processes will exceed the intake of energy from filtration (Lam and Frost, 1976). Filtration rate increases almost linearly with increasing cell concentration until a maximum is reached at C. Ingestion rate keeps pace with filtration until a maximum is reached at B. There is a lag between maximum ingestion rate and pseudofaecal production represented by the area between B and D. Over this range ingestion rate is thought to equal the gut passage rate. Beyond C filtration rate declines while ingestion rate stays approximately constant. Excess particulate matter will be disposed of by the production of pseudofaeces (represented by the hatched area). In practice there will be a time lag between the onset of filtration and the production of pseudofaeces, representing the time taken for the gut to reach a fullness equilibrium. In small animals this was short and the production of pseudofaeces was observed at lower densities than previously reported. At some higher concentration E, the mechanics of the gills fail, perhaps due to the production of excess mucus and filtration ceases.

1.4.4 THE EFFECT OF DIFFERENT ALGAL SPECIES ON THE FILTRATION RATE

1.4.4.1 INTRODUCTION

The effects of different algal species on the growth of bivalves have been well documented (Cole, 1936; Davis and Guillard, 1958; Walne, 1963, 1970a and Helm 1977). This work has, however, concentrated on larval bivalves and little has been done on juveniles and adults. The work performed in the present study was designed to supplement the above knowledge working specifically on juvenile *Ostrea edulis* of a maximum length of 12mm.

Growth studies are usually used as a measure of success in this type of experiment. On larvae, this method is particularly successful because of rapid growth rates, significant differences being observed over a matter of days (Walne, 1970a). Available time and size of animals mitigated against recording growth rates in this study.

The present work therefore concentrates on the effect of particle size on the filtration rate. Combined with known data on the success of various algae as food for larval oysters (Walne, 1970c and Helm, 1977), food value for juveniles could be predicted. In addition a number of different algae, were

used to investigate possible selection of particles on a basis other than morphological means.

1.4.4.2 METHOD

The algal species used in the experiments are shown in Table 5, together with the actual size. It will be noted that the size specified by the suppliers often did not correspond to the size found in the present study (cf. Table 1). Four size classes for the algae were constructed :

CLASS	DIAMETER (µm)
1	4,00 - 6,35
2	6,35 - 10,08
3	10,08 - 16,00
4	16,00 - 20,20

The oysters were seperated into two size classes, 0 - 6mm and 6 - 12mm. They were obtained from the main holding aquarium described in Section 1.1. Only one algal type was tested on consecutive days, between which the animals were returned to the main aquarium.

Experiments were conducted in 500ml perspex containers and animals were allowed to acclimatize in filtered sea water, at 15°C, for 1 hour (Section 1.4.3). A known amount of concentrated algae was injected into the chamber to bring

TABLE 5 Algal species and size (diam in µm) used to determine the effect of particle size on filtration rate.

ALGAE	MEAN DIAMETER (µm)	CLASS
Chaetoceros calcitrans	4,0 - 5,04	2
Phaeodactylum tricornutum	4,0 - 5,04	2
Monochrysis lutheri	4,0 - 5,04	2
Thallassiosira pseudonata	4,0 - 5,04	2
Hemiselmis virescens	5,04 - 6,35	2
Isochrysis galbana	5,04 - 6,35	2
Skeletonema costatum	6,35 - 8,00	3
Dunaliella primolecta	6,35 - 8,00	3
Tetraselmis striata	6,35 - 8,00	3
Tetraselmis seucica	6,35 - 8,00	3
Cricosphaera carterae	10,08 - 12,70	4
Hymenomonas carterae	10,08 - 12,70	4

the particle concentration to approximately 20 x 10^6 cells/l. Filtration rate was determined using a Coulter counter as described in Section 1.4.3.

Finally dry mass of tissue and ash-free dry mass of tissue were determined as described in Section 1.4.2.

1.4.4.3 RESULTS AND DISCUSSION

Measurement of a decrease in particle concentration continued until a satisfactory rate could be determined. However, particle concentration was not allowed to decrease below 50% of the initial value. Five independant experiments were run for each algal species using oysters in the 6 - 12mm size class. The filtration rates obtained were corrected for a standard animal of 5,0883mg using the equations described in Section 1.4.3. Data were then pooled to give a mean and standard deviation, and summarised in Tables 6a and b.

The same procedure could not be adopted using small animals. Due to culture difficulties experienced at Knysna the numbers of oysters available for these experiments in the 0 - 6mm range, were very limited. This problem was aggravated by the large numbers needed to measure a significant decrease in algal particles during the experiment. The data obtained

TABLE 6a Filtration rates of large *Ostrea edulis* using various algae.

ALGAE	FILTRATION RATE ($ml.mg^{-1}h^{-1}$)				
Mass (mg)	5,9355	5,3943	5,3763	4,6540	4,5530
Monochrysis lutheri	-	-	30,08	22,97	25,27
Thallassiosira pseudonata	-	62,72	51,23	35,92	85,40
Phaeodactylum tricornutum	-	66,82	41,77	25,74	87,63
Chaetoceros calcitrans	71,55	62,72	51,31	58,67	118,01
Hemiselmis virescens	73,57	57,93	52,23	28,50	64,46
Isochrysis galbana	-	53,94	37,59	52,45	66,43
Dunaliella primolecta	44,80	52,27	50,06	60,98	38,99
Skeletonema costatum	55,26	81,92	114,10	81,61	70,79
Tetraselmis striata	13,56	13,71	40,91	10,42	69,53
Tetraselmis seucica	-	-	43,85	34,99	64,27
Cricosphaera carterae	0,00	0,00	6,41	13,78	0,00
Hymenomonas carterae	6,94	5,94	3,44	7,49	4,68

NB Dashes indicate that data were discarded

TABLE 6b Mean filtration rates, corrected for standard animal mass 5,0883mg AFDM, derived from Table 6a.

ALGAE	FILTRATION RATE ($ml.5{,}0883mg^{-1}.h^{-1}$) $\bar{x}$	s	MASS SPECIFIC FILTRATION RATE ($ml.mg^{-1}h^{-1}$) $\bar{x}$	s
Monochrysis lutheri	129,43	± 55,78	29,08	± 6,65
Thallassiosira pseudonata	252,91	± 120,73	58,82	± 20,84
Phaeodactylum tricornutum	276,76	± 128,29	55,49	± 27,30
Chaetoceros calcitrans	369,67	± 112,35	72,45	± 26,41
Hemiselmis virescens	291,09	± 108,24	55,32	± 16,78
Isochrysis galbana	231,53	± 74,86	52,58	± 11,87
Dunaliella primolecta	231,24	± 56,34	49,37	± 8,25
Skeletonema costatum	416,79	± 119,23	80,74	± 21,58
Tetraselmis striata	153,79	± 111,89	29,63	± 25,50
Tetraselmis seucica	222,02	± 77,97	47,70	± 15,02
Cricosphaera carterae	19,72	± 28,97	4,04	± 6,17
Hymenomonas carterae	29,26	± 9,10	5,70	± 1,65

from two independent experiments for animals of 0,217mg (mean ash free dry mass) are summarised in Table 7. The corrected mass-specific filtration rates for both size classes are plotted in Figure 6.

From Tables 6 and 7 it is clear that the mass specific filtration rates of small animals were higher than those of large animals, following the general principle that body functions are proportional to the 0,75 power of body mass (Hemmingsen, 1960).

Although, in the 6 - 12mm size class of oyster for example, the rates using *Phaeodactylum tricornutum* and *Monochrysis lutheri* are not significantly different ($t = 2,47$; $p < 0,05$; $df = 6$), there is considerable variation in the filtering rate within each algal size class. If the gill was acting purely as a mechanical sieve one would not expect variation in filtration rate of particles of the same size. Because the experiments were repeated five times, the difference is unlikely to be a reflection of different activity levels. Two other alternatives are possible. Firstly, that different shapes of the algae lead to a difference in retention efficiency. Size of algae was determined using a Coulter counter, which measures irregular particles as spherical particles of equivalent volume. *Phaeodactylum tricornutum* for example, is long and slender, whereas *Monochrysis lutheri* is round. It is quite conceivable that the former presents a larger effective

TABLE 7 Filtration rates of *Ostrea edulis* using various algae (Mean oyster mass = 0,217mg AFDM)

ALGAE	FILTRATION RATE ($ml.mg^{-1}h^{-1}$)		FILTRATION RATE ($ml.0,2178mg^{-1}h^{-1}$)		MASS SPECIFIC FILTRATION RATE ($ml.mg^{-1}h^{-1}$)	
Mass (mg)	0,2561	0,1853	$\bar{x}$	s	$\bar{x}$	s
Monochrysis lutheri	38,20	47,88	9,39	± 1,32	43,09	± 6,07
Thallassiosira pseudonata	67,20	106,93	18,89	± 5,74	86,73	± 26,36
Phaeodactylum tricornutum	150,00	162,69	34,19	± 0,91	156,96	± 4,19
Chaetoceros calcitrans	69,44	118,25	20,37	± 7,10	93,53	± 32,59
Hemiselmis virescens	122,79	142,84	28,90	± 2,57	132,69	± 11,68
Osochrysis galbana	70,00	68,13	14,98	± 0,52	68,78	± 2,40
Dunaliella primolecta	28,95	46,23	8,18	± 2,54	37,54	± 11,66
Skeletonema costatum	136,21	153,93	31,57	± 2,09	144,93	± 9,59
Tetraselmis striata	69,45	61,74	14,30	± 1,47	65,66	± 6,75
Tetraselmis seucica	70,91	91,47	17,69	± 2,78	81,20	± 12,75
Cricosphaera carterae	0,00	0,00	0,00		0,00	
Hymenomonas carterae	0,00	0,00	0,00		0,00	

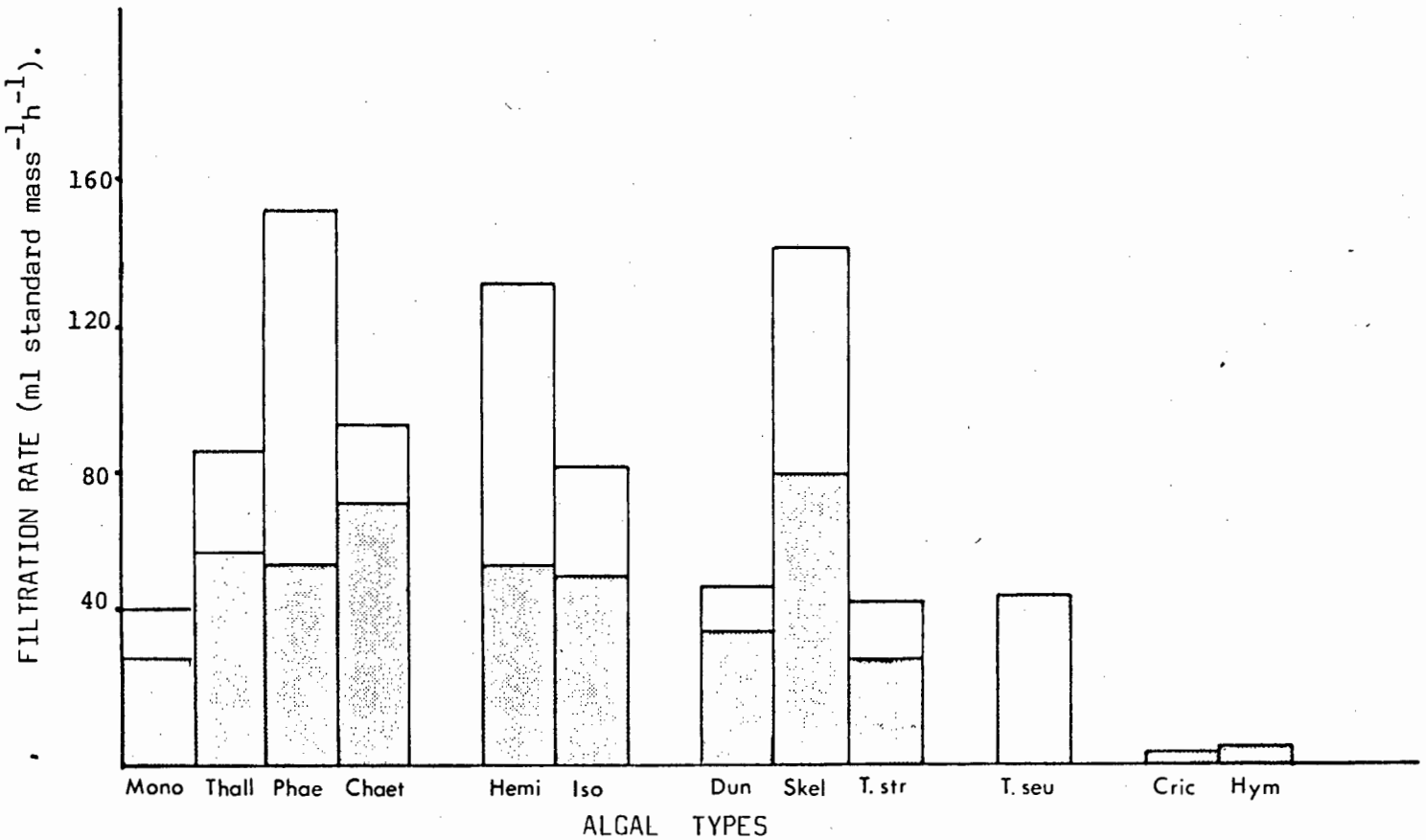

FIGURE 6 : Mass specific filtration rate as a function of particle size (see text) and algal type for *Ostrea edulis*. Stippled areas indicate standard mass of 5,0883mg animal and clear areas indicate 0,2178mg animal.

particle to the gill and is therefore trapped more efficiently. This may account for the high filtration rates of all of the irregular algae, *Thallassiosira pseudonata, Phaeodactylum tricornutum, Skeletonema costatum* and *Hemiselmis virescens.*

The second possibility is that the animals were distinguishing algae using some chemosensory means. Winter (1969) has noted this effect in mussels. A possible reason for this selectivity may be the production of toxic metabolities by the algae. The very low filtration rates obtained when using *Hymenomonas carterae* and *Cricosphaera carterae* were initially attributed to these effects. In an attempt to remove the metabolities the algae were centrifuged at 3000g for 5 minutes and suspended in fresh sea water. This did not improve the filtration rate suggesting that the toxicity remained even after centrifuging.

Bernard (1974) has shown that the ostial size of *Crassostrea gigas* is 2900µm. This would theoretically allow a particle of 55µm in diameter through the gill. It is therefore improbable that *Hymenomonas carterae* and *Cricosphaera carterae* ($\pm$ 11µm) were presenting the upper limit in particle size for successful filtration, unless they were forming aggregates, for which there is no evidence. Unfortunately no other large algae were available for comparison.

The filtration rates presented here are largely of the same order. Slight differences may be explained by either algal

1.4.5 THE EFFECT OF A COMBINATION OF ALGAE ON FILTRATION RATE

1.4.5.1 INTRODUCTION

To supplement the work done in the previous section oysters were offered a diet containing three algal species. The spectrum of particles in the diet covered a range from 2,10µm - 20,2µm. The purpose of the work was to find the lower limit particle retention and secondly to determine whether small oysters selected smaller particles than large animals.

The second consideration is of particular interest in terms of intraspecific co-operation (if different size oysters selected different fractions of the food) or conversely, intraspecific competition for the same food source.

1.4.5.2 METHOD

As before the oysters were obtained from the main aquarium (see section 1.1). Experimental procedure and determination of filtering rates are described in section 1.4.3.
A mixture of *Chaetoceros calcitrans*, *Tetraselmis seucica* and *Skeletonema costatum* provided the necessary range of particle size. With the aid of the Coulter Counter a mixture was made up to consist of equal numbers of particles in each size class.

Size classes of the particles were :

CLASS	PARTICLE SIZE IN µm	
1	2,10 - 4,00	*Chaetoceros calcitrans*
2	4,00 - 6,35	*Chaetoceros calcitrans*
3	6,35 - 10,08	*Tetraselmis seucica*
4	10,08 - 16,00	*Skeletonema costatum*
5	16,00 - 20,20	*Skeletonema costatum*

On the basis of work done in section 1.4.4 these algae were all considered to be well retained by *Ostrea edulis*. Enough of the mixture was added to each chamber to bring the concentration to 20×10^6 cells.l^{-1}.

1.4.5.3 RESULTS AND DISCUSSION

The filtration rate and corrected filtration rate for standard animal of 5,0883gm, are summarised in Table 8. Graphical plots of the filtration rates of the different size animals are shown in Figure 7.

In all 4 size classes of oysters there was a sharp drop in retention efficiency below 6,35µm. This agrees with work on *Mytilus edulis* by Davids (1964) and Dral (1967) but differs from Vahl (1972) who found good retention of particles in the 2 - 5µm range.

TABLE 8 Filtration rates of *Ostrea edulis* using mixed algal diets.

AFDM (mg)	ALGAL SIZE (Class)	FILTRATION RATE ($ml.h^{-1}$)	FILTRATION RATE ($ml.5,0883mg^{-1}.h^{-1}$)
0,1853	1	0,3	6,20
	2	11,0	227,48
	3	9,1	188,19
	4	5,4	111,67
	5	3,1	64,11
0,2561	1	5,8	89,22
	2	6,7	103,07
	3	14,1	216,90
	4	17,7	272,28
	5	10,0	153,83
2,8935	1	0,0	0,0
	2	14,0	23,46
	3	28,0	46,92
	4	29,0	48,59
	5	7,0	11,73
5,9355	1	7,0	6,08
	2	71,0	61,67
	3	97,0	84,25
	4	126,0	109,44
	5	103,9	90,25

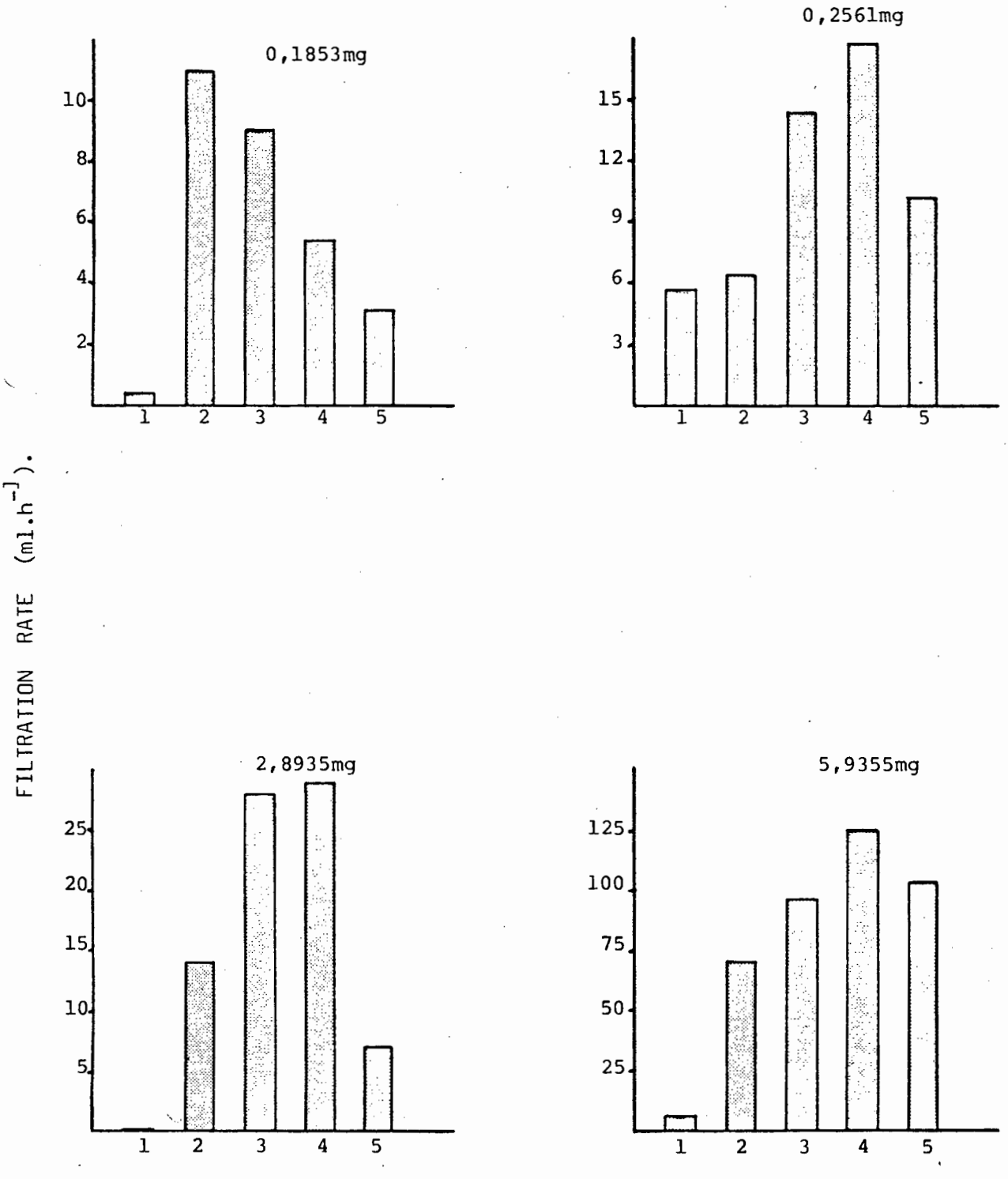

FIGURE 7 : Filtration rate of different sized *Ostrea edulis* using combined algal diets.

Filtration rates increased to a maximum over the 6,35- 16,00µm range. Notably there was an element of skewness; to the right in small animals and to the left in large animals. This suggests a possible difference in either the retention efficiency or particle selection ability of small and large oysters. It is well known that the ostial size in oysters remains unchanged with size of animal (Galtsoff, 1964). A selection of particle sizes purely on the basis of gill morphology therefore, is unlikely. Recent investigation into the ciliary mechanisms of oysters show that the pallial organs do not have a sorting function (Bernard, 1974). He suggests that different types of mucus may be instrumental in sorting particles. Whatever the mechanism, the subject is far from resolved.

Assuming a range of particle sizes was available to the oysters, these results suggest that small and large animals would select different fractions of the particle spectrum. This would alleviate intraspecific competition resulting in an optimisation of food resources.

1.5 ASSIMILATION EFFICIENCY

1.5.1 INTRODUCTION

Assimilation efficiency may be defined as the amount of food assimilated expressed as a percentage of the amount of food ingested (Winter, 1978). It is primarily dependent on the quality and quantity of the ingested material, although a decrease in efficiency with increasing particle concentration has been noted (Winter, 1969; Widdows and Bayne, 1971; Foster-Smith, 1975; Widdows, 1978 and Griffiths and King, 1979). This conflicts with the view of Thompson and Bayne (1972, 1974) and Vahl (1973), who found no evidence of the influence of particle concentration on assimilation efficiency.

Experiménts were designed to measure the assimilation efficiency of *Ostrea edulis* at 15°C and 35‰ under a discontinuous feeding regime using *Tetraselmis seucica* as food.

1.5.2 METHOD

Assimilation efficiency was measured using the method described by Conover (1966). This method was first used to determine the assimilation efficiency of zooplankton, but has been applied to a number of other organisms especially where quantitive collection of faeces is impractical.

Faeces were collected regularly over a period of 12 hours. The frequency of collection ensured that negligable amounts of organic material leached out of the faeces into surrounding sea water. Collection was made on pre-ashed (450°C for 3 hours) GFC filters and washed twice with distilled water to remove salts. Washing with distilled water was found to produce the same results as washing with ammonium formate as described by Conover (1966). Faeces were dried to constant mass at 60°C for 24 hours before being ashed at 450°C for 12 hours. Masses were measured with a Mettler ME 30 microbalance.

The same procedure was followed using 5ml of algal concentrate (*Tetraselmis seucica*) instead of faeces.

Assimilation efficiency was then calculated using the equation :

$$U' = \frac{(F' - E')}{(1 - E')(F')} \times 100$$

Where F' is the ash-free dry mass: dry mass ratio (fraction of organic matter) in the ingested food, and E' is the same ratio in a representative sample faeces. U' is the percentage utilization or assimilation efficiency.

This method requires the basic assumption that the inorganic fraction of the ingested material is unaffected by digestive processes.

1.5.3 RESULTS AND DISCUSSION

It has been shown that the sea urchin *Parechinus angulosus* uses a portion of the ingested inorganic material for shell-building (Buxton, 1977). This makes the Conover ratio unsuitable for determining the assimilation efficiency as it does not fulfil the requirements of the abovementioned assumption. *Ostrea edulis* may also use ingested inorganic material for shell building though no mention of this is made in the literature.

A mean assimilation efficiency of 80,28% $\pm$ 9,03 SD was obtained from six determinations (Table 9). Values of 30,0 - 94,2% for assimilation efficiency in a number of bivalves have been summarised by Winter (1977b). Some of this variation may be due to experimental technique although an inverse relationship exists between assimilation efficiency and concentration above 10 x 10^6cells/l (Widdows and Bayne, 1971; Foster-Smith, 1975b; Winter, 1977b; Widdows, 1978 and Griffiths and King, 1979). Furthermore assimilation may be inversely related to body size, for example, large individuals of *Arctica islandica* show a reduced efficiency (Winter, 1969). This relationship is, however, not evident in *Mytilus edulis* (Vahl, 1973c) or *Modiolus modiolus* (Winter, 1969).

Welch (1968) has shown that the lower the assimilation efficiency, in aquatic consumers, the higher the nett growth

TABLE 9 Assimilation efficiencies for acclimated temperatures of 5, 10, 15, 20 and 25°C

	T_A 5°C	T_A 10°C	T_A 15°C	T_A 20°C	T_A 25°C
	66,33	88,66	90,82	89,66	61,56
	65,44	84,55	84,37	54,31	76,27
	70,32	81,93	72,22	67,32	76,22
	83,21	67,45	83,49	87,00	81,23
		86,57	64,45	79,08	67,32
		86,46	86,32		
n	4	6	6	5	5
$\bar{x}$	71,32	82,60	80,32	75,47	72,62
s	8,21	7,76	9,91	14,68	7,98

Analysis of variance

	ss	d.f	Ms	F
Treatment	491,2	4	122,8	1,22
Error	2110,0	21	100,5	
Total	2602,1	25		

F $(\alpha = 0,05 ; 4,20\ d.f.) = 3,07$

Overall mean = 76,43 $\pm$ 4,88

efficiency. Conversely if nett growth efficiency is low, then assimilation efficiency is high. As will be shown in a later section, the experimental animals were fed a minimum maintenance ration and therefore growth in the holding period was virtually nil. The high assimilation efficiency obtained in this work was therefore to be expected.

1.6.2 METHOD

Oxygen consumption can be measured using open or closed respirometer techniques, the former reguiring a flow-through system. Ingoing and outgoing oxygen are monitored and any difference is attributed to oxygen consumption. This method has the advantage that experiments can be run indefinitely. Closed systems on the other hand, suffer from the drawback that oxygen tension steadily declines within the experimental chamber, the effects of which are well documented (Bayne, 1971a, b; Bayne *et. al.*, 1976, 1977). Combined with the above there is a steady decline in the particle concentration which affects filtration rate and therefore oxygen consumption.

By using large experimental vessels it is possible to reduce the above effects to a minimum. Closed vessel techniques were used in this study.

1.6.2.1 RESPIRATION RATES OF SMALL ANIMALS

Initial attemps to measure consumption of oysters below 4mm. in diameter (0,01mg AFDM), using YSI macroelectrodes, were unsuccessful. Large numbers of animals had to be used to detect a change in oxygen concentration because of the relative insensitivity of these electrodes. This high density resulted in the food becoming a limiting factor (i.e.

it decreased below 50% of the initial value before the termination of the experiment). Walne (1966) experienced similar difficulties using stoppered bottle techniques with oyster larvae. Successive decreases in chamber volume failed to solve this problem because of the relative increase in oxygen probe consumption and differential respirometry failed for similar reasons.

A reasonable measure of success was attained using a PHM 72 MK 2 digital acid base analyser (Radiometer, Copenhagen). This technique was first described by Davenport (1976). The principle is essentially similar to that of YSI electrodes, but the tolerance limits are very much lower. The Radiometer system is able to detect oxygen changes of the order of $10^{-1}\mu l\ O_2$ per individual per hour.

Figure 8 shows the experimental equipment. It consists of a 1ml chamber surrounded by a water jacket. Water of the required experimental temperature is pumped through the jacket to maintain the temperature of the experimental chamber (T_E). The electrode was attached to a continuous chart recorder. Preliminary experiments using a small magnetic stirrer did not improve the circulation produced by the irrigatory current of the oyster.

Animals were acclimatized for 1 hour at 15°C and 35‰ salinity before being transferred to the experimental chamber.

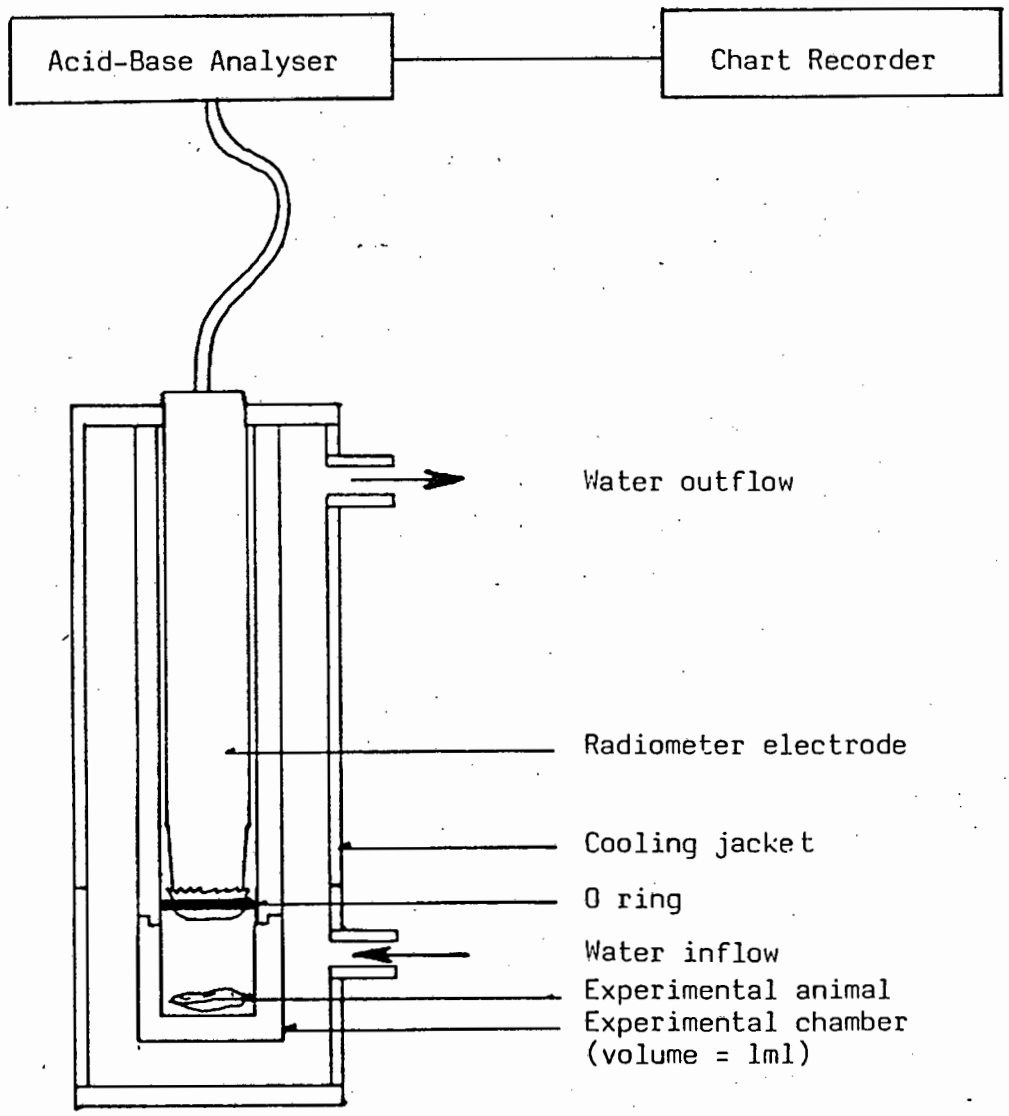

FIGURE 8 : Diagram of equipment used to determine O_2 consumption with the radiometer electrode.

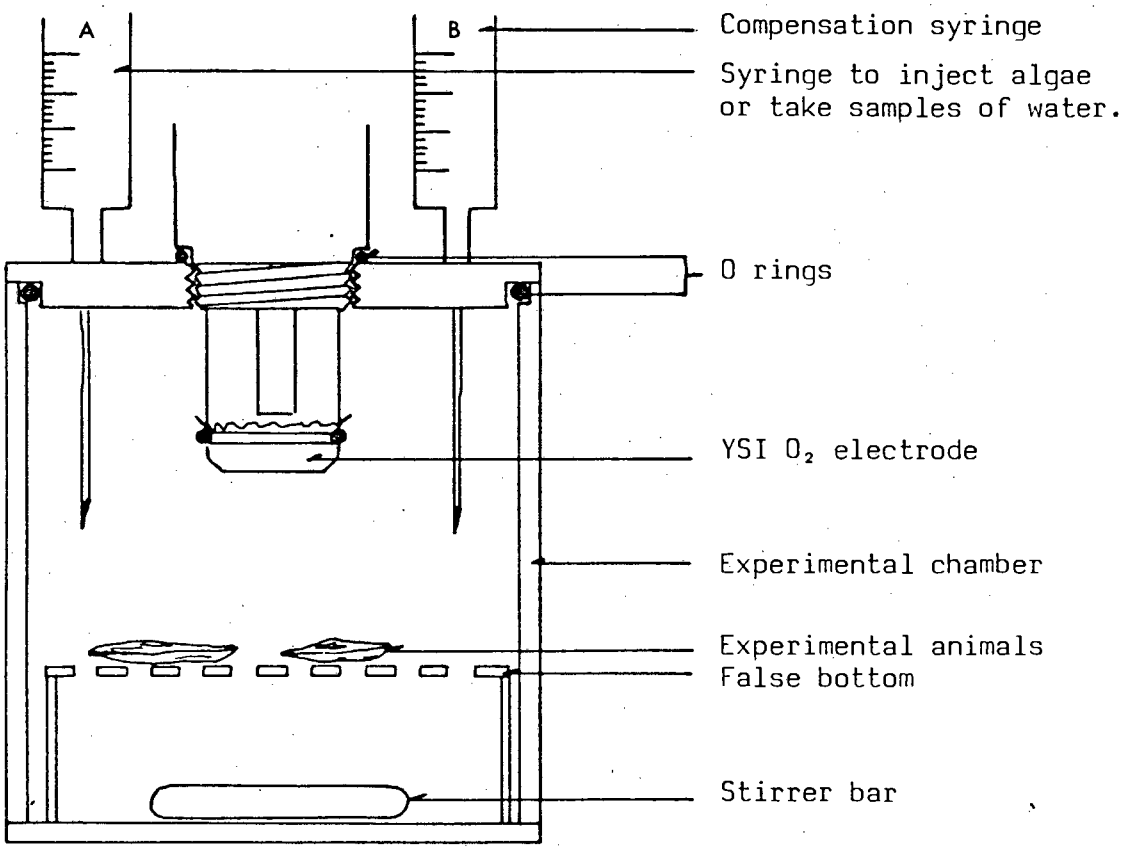

FIGURE 9 : Diagram of the apparatus used to determine O_2 consumption using the YSI electrode.

After completion of the experiments using either electrode, dry mass of tissue was determined by drying at 60°C for 24 hours. AFDM was determined from the following equation :

AFDM = 0,90DMT - 0,0008 (Table 3).

1.6.3 RESULTS AND DISCUSSION

The effect of the body mass on oxygen consumption is usually described by the general allometric equation :

$$V_{O_2} = aM^b$$

where V_{O_2} = respiratory rate, M = mass and a and b are constants. The value of the mass exponent b has been well studied for a broad spectrum of animals (Hemmingsen, 1960 and Zeuthen, 1953), including bivalves (Bayne *et. al.*, 1976). Corrected for temperature and activity rate, the value of b is usually 0,75 $\pm$ 0,015 (Newell, 1979). This fundamental relationship is attributed to the fact that metabolism is related to suface area and not volume (Newell, 1979).

The results obtained in this study are summarised in Figures 10 and 11. For small oysters the relationship between size and respiration was best described by a linear relationship :

$$V_{O_2} = 1,990\ AFDM - 0,086 \qquad (r = 0,98)$$

while for large animals the relationship was described by the power function :

$$V_{O_2} = 0,301\ AFDM^{1,69} \qquad (r = 0,71)$$

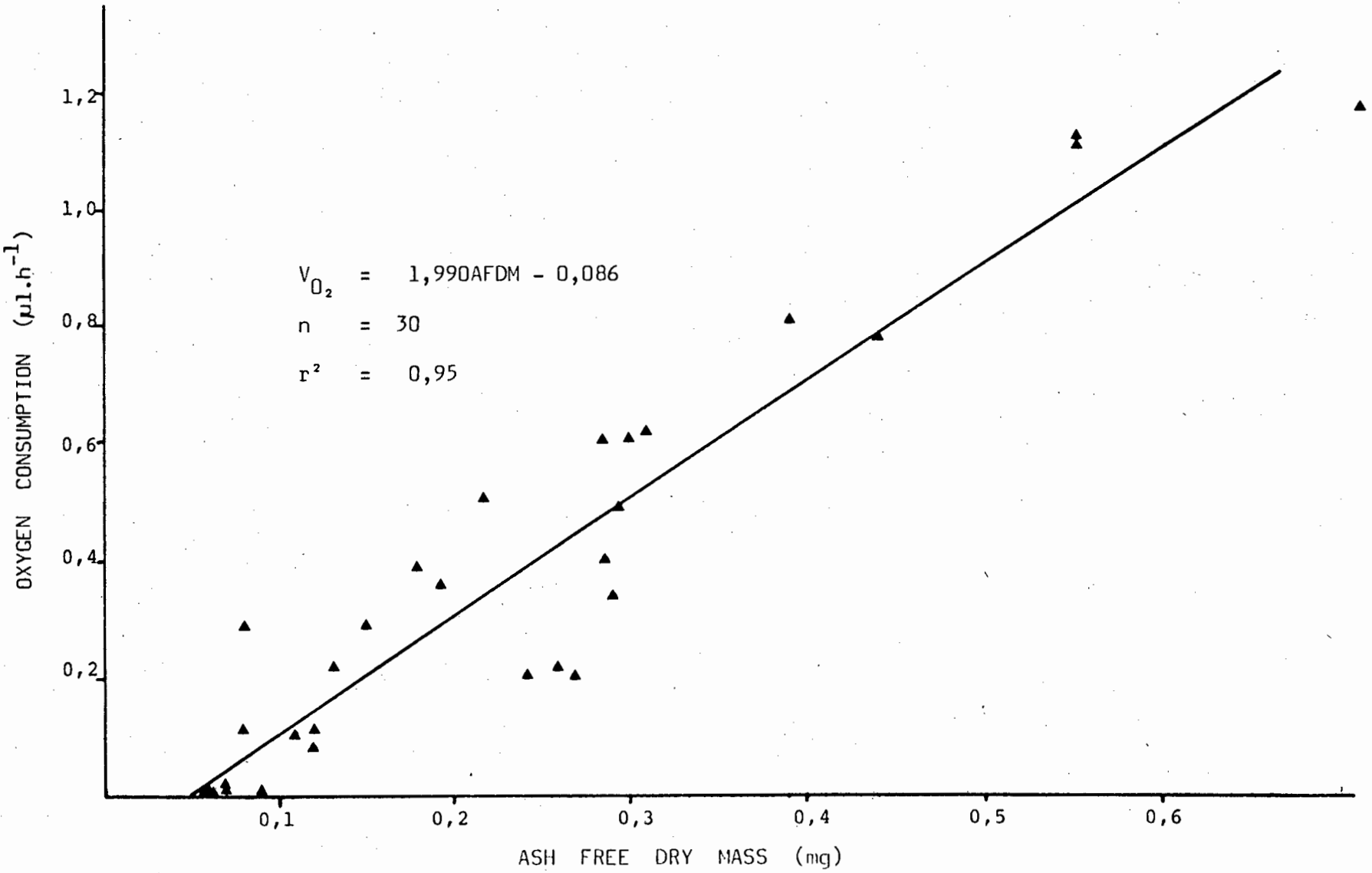

FIGURE 10 : Oxygen consumption (V_{O_2}) of juvenile *Ostrea edulis* as a function of ash free dry mass determined using Radiometer techniques.

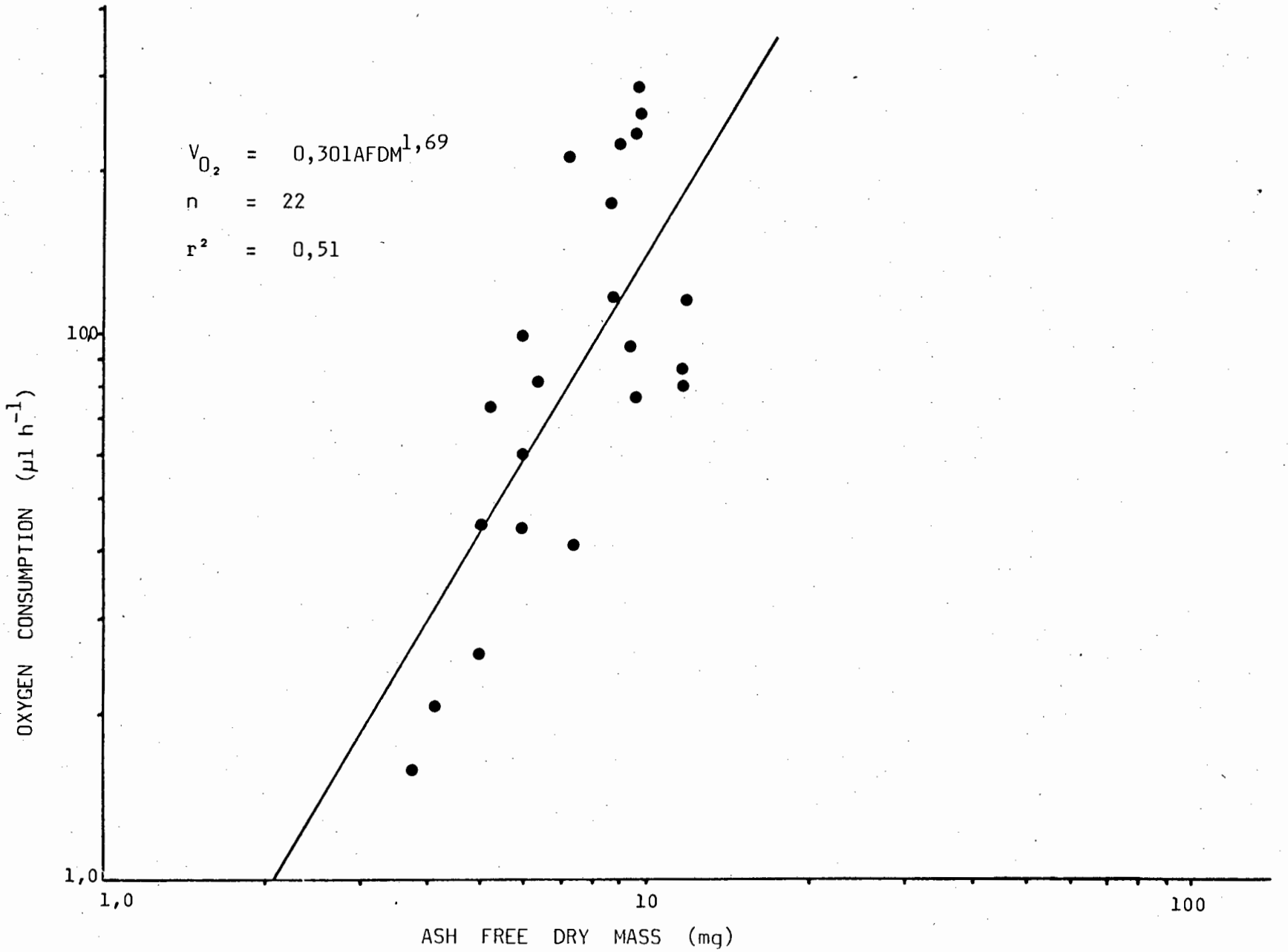

FIGURE 11 : Oxygen consumption (V_{O_2}) of *Ostrea edulis* as a function of ash free dry mass determined with YSI Oxygen electrodes.

These values are both significantly different from those summarised by Hemmingsen (1960) (Table 10). Combining the two sets of data, however, produced a power curve :

$$V_{O_2} = 1,13\ AFDM^{1,09} \qquad (r = 0,94)$$

The data are summarised in Figure 12.

Hemmingsen (1960) recognised 3 phases of respiration over the range of animals examined. Phase 1 described unicellular forms with a b-value of 0,75. Phase 3 describes a similar value for large metazoans. Phase 2, however, which connected 1 and 3 showed a much steeper slope of the order of 1,0 i.e. V_{O_2} was directly proportional to body mass.

It is felt that the anomalous values for b, obtained for small and large animals individually, are a result of the relatively narrow size range examined in each case. Combining the two sets of data produced a better result with a b-value of 1,09. This value is close to the expected result of 1,0 predicted by Hemmingsen (1960) for animals between $10^{-6,5}$ and $10^{-2,5}$g (i.e. phase 2).

To explain the mass exponent b, Zeuthen (1953) suggested that respiration was related to surface area at the cellular level. This would result in a $^2/_3$ proportionality to mass. Hemmingsen (1960) modified this by adding that due to vascularisation and complex respiratory surfaces the

TABLE 10 Statistical testing of b against general value of Hemmingsen (1960)

SOURCE	MASS EXPONENT (b) $\bar{x}$	s	t(0,05)	n	
Small oysters	1,99	0,13			
			15,08	21	Significant
Hemmingsen (1960)	0,75	0,015			
			7,04	21	Significant
Larger oysters	1,69	0,36			

($p \geqslant 2{,}08$; for df 20)

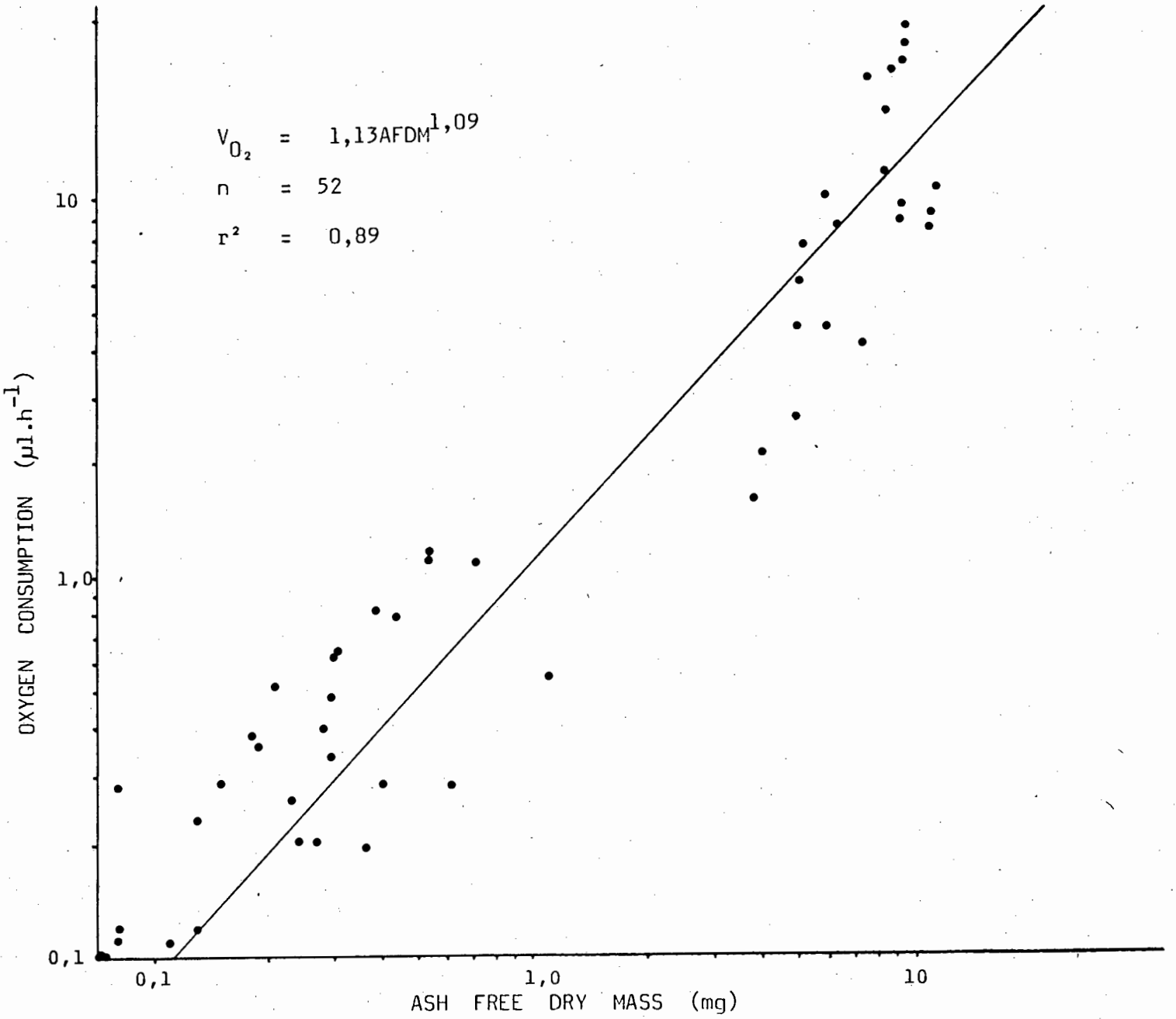

FIGURE 12 : Oxygen consumption as a function of body mass for *Ostrea edulis*.

value would be higher, i.e. 0,75 proportionality to mass. The relative high value of b obtained in this study can be attributed to the small body mass of the experimental animals and the complexity of the lamellibranch gill with its high degree of vascularisation.

1.7 RELEASE OF DISSOLVED ORGANIC CARBON

1.7.1 INTRODUCTION

In the balanced energy equation of Ricker (1968),

$$P = C - R + F + U$$

where P = production, C = consumption, R = respiration, F = faecal production and U = excretion. In marine organisms excretion is difficult to qualify because waste products dissolve in the surrounding sea water. Although it is often discarded as being insignificant (Kofoed, 1975 and Newell and Kofoed, 1977), Field, (1972) shows that dissolved organic carbon from excretion and faecal production form a major component of the energy balance of *Strongylocentrotus droebachiensis*. A Beckman Total Organic Carbon Analyser (Model 915A) was used to measure dissolved organic carbon (DOC) in the sea water surrounding feeding oysters.

1.7.2 METHOD

Oysters were obtained from the main holding aquarium. Three to five animals were placed in 500ml of sea water filtered through 0,45µm Millipore filters. The experimental chambers are shown in Figure 9. Water was circulated by a magnetic stirrer and animals were acclimatized at 15°C and 35‰ for 1 hour. To start an experiment *Tetraselmis seucica* was

added to the chamber to bring the particle concentration to 15×10^6 cells/l. 5ml samples were taken every 30 minutes for a period of 8 hours. These were drawn through 0,45µm Sartorius filters to remove suspended matter and frozen to -30°C.

Samples were injected into the Carbon Analyser within 5 minutes of thawing, using an automatic syringe. Levels of total and inorganic carbon were recorded automatically and concentrations were determined from a standard curve.

1.7.3 RESULTS AND DISCUSSION

Four experiments, using different sample times and experimental animals, were conducted. The results of one of these are summarised in Table 11. Results in all four experiments were similar in that no change in carbon levels was detected.

On the basis of these results the following conclusions were drawn :

a) Amounts of dissolved organic carbon, from either excretory or faecal production, were negligable in small oysters.

b) Carbon production by the above processes could be obscured by carbon assimilation. Hammen, (1969), found that oysters used dissolved carbon for shell growth.

TABLE 11 Analysis of Dissolved Organic Carbon production in *Ostrea edulis*.

STANDARDS					SAMPLES			
TOTAL C		INORG. C		TIME	TOTAL C		INORG. C	
Y	X	Y	X	(min)	$\hat{Y}$	X	$\hat{Y}$	X
50	92,8	50	92,0	0	26,1	45,1	29,1	47,2
30	45,3	30	39,4	30	27,1	47,1	29,3	47,6
10	18,3	10	19,3	60	28,7	50,0	27,4	43,9
				120	24,7	42,6	29,7	48,5
				240	25,0	43,2	29,6	48,3
				480	25,0	43,2	29,0	47,2
Y int =	2,15		5,61					
Slope =	0,53		0,50					
r^2 =	0,98		0,93					

Y = standard solutions (ppm)

X = peak height on chart recorder

$\hat{Y}$ = extrapolated concentration in ppm

The results presented above indicate therefore, that dissolved organic carbon does not form a significant loss in terms of the energy budget of juvenile *Ostrea edulis*.

1.8 CONCLUSIONS

The results presented above, detail the components of energy balance for juvenile specimens of *Ostrea edulis* maintained at 15°C and fed *Tetraselmis seucica*. Routine metabolism was established under these conditions and the data presented are presumed to be a function of this metabolic state.

There is considerable evidence in the literature that filtration rate is a function of body size and this is confirmed for *Ostrea edulis*. A mass exponent for filtration rate of 0,91 was derived. Filtration rate was also found to be dependent on the concentration of food presented to the animal. Results show that the optimal filtration rates occur at concentrations of approximately 15 x 10^6 cells l^{-1}. The relationship between filtration rate and food concentration was found to closely approximate the theoretical model proposed by Lehman and Frost (1976). This model was modified and applied to this species. No conclusive evidence was found to suggest that *Ostrea edulis* selects particles on the basis of size, but the possibility that they may distinguish algae using chemosensory mechanisms cannot be discounted.

The mass exponent of routine oxygen consumption was determined at 1,09. This value, although considerably higher than the standard value of 0,75 obtained for Metazoa, fitted

the phase 2 respiratory level proposed by Hemmingsen (1960). Therefore, over the range of sizes worked with, metabolism was directly proportional to body size. If the mass exponent of filtration in small oysters approximated the general value of 0,75 energy gain by juvenile oysters would be insufficient to meet metabolic demands with an increase in mass. This situation has been compensated for by the relatively high value of 0,91 for the mass exponent of filtration. Within the range of sizes worked with the smallest animals would have a slight advantage over the larger animals in terms of energy gain of filtration relative to energy loss of metabolism. This allows a maximum competitive ability during the early stages of growth.

Measurable amounts of dissolved organic carbon were not detected in this study. Widdows and Bayne (1971) report that during protein catabolism there is no calorific loss because nitrogen is given off as ammonia to the surrounding sea water. Physiologically useful energy is therefore equal to assimilated energy. Hammen (1968) has measured leakage of amino acids into the mantle cavity, but notes that it is only significant when the animal is stressed. Under aquaculture conditions where the animal is not exposed to intertidal stress, this factor becomes minimal and for the purposes of this study excretory losses were ignored.

Using the data described above it is possible to construct an overall energy budget for juvenile *Ostrea edulis* of standard

mass, 5,0883 mg. The rates used were obtained under optimal conditions. The energy equation of Winberg (1956) provides a method for determining the expected change in mass (Δw) with time (Δt) :

$$\Delta w/\Delta t = Ab - R$$

Ab is the energy equivalent of the proportion of food consumed, C, that is not rejected as faeces. The possible energy loss of excretion is disregarded. R is the energy loss of metabolism represented by respiration. $\Delta w/\Delta t$ is determined by subtracting the metabolic energy expenditure from the energy of assimilated ration. The derivation of $\Delta w/\Delta t$ is shown in Table 12.

The calculated value of $1,94 J.h^{-1}$ for $\Delta w/\Delta t$ gives the energy available to the oyster for growth. No differentiation between somatic growth (P_g) and reproductive effort (P_r) is made in the equation. Oysters of this size, however, are immature, hence the entire amount is available for somatic growth.

The energy budget calculated above was determined under optimal conditions, although a number of factors mitigate against the achievement of optimal levels of filtration and respiration. These include concentration of food and food type as well as oxygen tension and temperature. The effects of temperature on biological rate functions are dealt with in Chapter 2.

TABLE 12 Data used to determine $\Delta w/\Delta t$ for juvenile *Ostrea edulis* at 15 x 10^6 cells $.l^{-1}$ and 15°C.

CONSTANTS

Energy content of 1 x 10^6 cells *Tetraselmis seucica* = 1,54J (Widdows and Bayne, 1971)

Oxycalorific equivalent of 1ml O_2 = 19,85J (Thompson and Bayne, 1974)

Assimilation efficiency = 80,32 $\pm$ 9,91 (Table 9)

Ingested ration	=	filtration rate x food concentration
	=	111,85 x 15 x 10^6
	=	1,68 x 10^6 cells.h^{-1}
	=	2,58J.h^{-1}
Assimilated ration	=	assimilation efficiency x ingested ration
	=	0,80 x 2,58
	=	2,07 J.h^{-1}
Respiratory rate	=	6,73ul.h^{-1}
	=	0,13J.h^{-1}
$\Delta w/\Delta t$	=	Assimilated ration - Respiratory rate
	=	2,07 - 0,13
	=	1,94J.h^{-1}

CHAPTER 2

THE INFLUENCE OF TEMPERATURE ON THE ENERGY BALANCE OF A LABORATORY POPULATION OF *OSTREA EDULIS*

2.1 INTRODUCTION

Growth may be defined as the change of body mass (Δw) with time (Δt), represented by the energy gain (assimilated ration, Ab) minus energy loss (metabolism, R). This relationship is summarised by the general energy equation of Winberg (1956) :

$$\frac{\Delta w}{\Delta t} = Ab - R$$

$\Delta w/\Delta t$ has been called 'scope for growth' (Warren and Davis, 1967) and may be positive when a surplus of energy is available for growth (Pg) and reproduction (Pr), or negative if the metabolic costs exceed the assimilated ration. The relationship, however, does not distinguish between Pr and Pg and may therefore be regarded as an overall index of energy balance (Bayne, 1976).

Recent work on the relationship between scope for growth and temperature (Widdows and Bayne, 1971; Newell and Kofoed, 1977a, b; Newell *et. al.* 1977) suggests that the ability of an animal to adjust components of their energy balance by acclimation may enhance their ability to survive or conversely, limit its existence in a particular ecological niche.

Regulation of biological rate functions in response to a change in temperature, by a process of acclimation, is widely recognised (for reviews see Kinne, 1963a, b, 1970 and Newell, 1979). Crisp and Ritz (1967) define acclimation

as 'any non-genetic adjustment by an organism as a direct response to a change in a single factor in its environment'. The term acclimitization is used to describe acute adjustment to a change in the environment.

In the time course of non-genetic adjustment, Kinne (1964) has distinguished 3 phases :

i) An immediate or acute response in which a change in rate is observed within minutes of exposure to temperature change. This response is a shock response characterised by over- and undershoots.

ii) Stabilization : This begins within hours of the change in temperature and leads to a progressive constancy of performance, gradually approaching a new steady level.

iii) New steady state : This results hours or days after the change depending on the time course taken for the rate to stabilize.

The attainment of a new steady state, however, may or may not occur, and may adopt various incomplete stages. These stages or patterns of acclimation are summarised by Precht *et. al.* (1975) and Prosser (1973).

This work summarises the acute response to temperature change of filtration and respiratory rates for *Ostrea edulis* acclimated for 30 days over a temperature range of 5 - 25°C. Scope for

growth, assimilated ration and the predicted change in mass with food concentration are also discussed.

2.2 MATERIALS AND METHODS

Oysters used in these experiments were obtained from Knysna (see section 1.1) and were all between 8 - 12mm in length. They separated into 5 groups, one of which was placed in the main holding facility at 15°C, while the others were placed in 4 separate 37,5l aquaria. These aquaria were maintained at 5, 10, 20 and 25°C respectively.

Fresh water in a cooling tank was maintained at 3°C using a Haake (Model EK 51) dip cooler. This water was circulated through 2 simple coiled-tube heat exchangers in the 5 and 10°C sea water tanks. Heating against the coiling coils with Lopis aquarium heaters maintained the desired temperatures. The 20°C and 25°C tanks were heated against the ambient temperature which was below 20°C for the duration of the experiment. Circulation in the tanks was maintained using a similar air-lift system to that described in Section 1.1 .

Fresh sea water was obtained every week and filtered through Watmans No. 1 filters before being placed in the aquaria. The animals were kept free of epiphytes by spraying them with fresh water once a week. During the acclimation period they were fed a surplus of *Tetraselmis seucica*. This ration was

sufficient for the animals to achieve a routine state of metabolism. The acclimation period of 30 days was considered long enough for achievement of a new steady state (c.f. Winter, 1971, who noted complete acclimation of *Mytilus edulis* after 14 days).

2.2.1 FILTRATION RATE

Sixteen animals were removed from each acclimation regime, four animals in each of four experimental cahmbers were run together with 1 control over an exposure temperature range of 5 to 30°C in the sequence 5, 20, 15, 30, 10, 25. Shuffling the exposure temperatures in this way precluded any possible secondary acclimation effects. The same groups were used throughout the range of exposure temperatures while only one experiment was performed each day with any 1 group. After each experiment the animals were returned to their respective acclimation regimes.

The procedure followed in determining the filtration rate is outlined in section 1.4 .

2.2.2 RESPIRATORY RATES

The same animals used to measure filtration rates were used to determine oxygen consumption over the range of temperatures described above. YSI oxygen electrodes were used

throughout as described in section 1.5.2.1.

Consumption rates were corrected for a standard animal of 5,0883mg using the following equation :

$$V_{O_2 s} = \left(\frac{5,0883}{W_e}\right)^{1.09} . V_{O_2 e}$$

where $V_{O_2 s}$ and $V_{O_2 e}$ are the oxygen consumption rates for the standard and experimental animals respectively, W_e is the mass of the experimental animal and 1,09 is the mass exponent of respiration listed in section 1.6.2.1.

Finally dry mass was calculated by drying the tissues at 60°C for 48 hours.

2.2.3 ASSIMILATION EFFICIENCY

The animals remaining in each acclimation reqime were used to measure tha assimilation efficiency at each acclimation temperature. The oysters were fed continuously using a drip method which maintained the concentration in each tank at approximately 15 x 10^6cells.l^{-1}. Efficiencies were calculated using Conover ratios described in section 1.5.

2.3 RESULTS

2.3.1 THE EFFECT OF TEMPERATURE ON THE FILTRATION RATE

Filtration rate at each exposure temperature was a mean of four determinations. Sample intervals varied between 10 and 30 minutes depending on the clearance rate and at no stage was the concentration of cells allowed to drop below 50% of the initial value. Corrected rates from four chambers were pooled to determine a mean and standard deviation at each exposure temperature. Each rate was therefore a mean of 16 determinations. The results are summarised in Tables 13 to 17.

The acute rate:temperature curves for filtration are shown in Figure 13a. The cold acclimated animals (T_A 5 and T_A 10) show slow rates of filtration. In both cases peak filtration is reached at temperatures higher than the acclimated temperature. T_A 15 on the other hand shows a peak filtration at acclimated temperature, while T_A 20 and T_A 25 shows a maximum filtration rates 5° above T_A. These results show evidence of a lateral translation of the rate:temperature curve (Type II-Precht 1958). The very high rate of filtration at 25°C for T_A 20 may reflect an active rate as opposed to routine rates at the other temperatures. The acclimated rate:temperature curve for filtration is shown in Figure 13b.

TABLE 13 Summary of mean V_{O_2} (ul.5,0883mg^{-1}.h^{-1}). V_w (ml.5,0883mg^{-1}.h^{-1}), filtration efficiency (V_w/V_{O_2}) and cost of filtration (V_{O_2}/V_w) in oysters acclimated to 5°C and measured at exposure temperatures (T_E) of 5°C to 30°C.

	5°C		10°C		15°C		20°C		25°C		30°C	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Mean V_{O_2} ul.5,0883mg^{-1}h^{-1}	7,95	5,58	10,42	4,0	33,07	4,38	5,63	4,04	20,13	3,11	-	-
Mean V_w ml.5,0883mg^{-1}h^{-1}	32,37	12,69	46,28	11,57	65,27	13,39	36,83	23,70	30,06	8,28	31,61	12,03
V_{O_2}/V_w	0,2455		0,2251		0,5067		0,1527		0,6696		-	
V_w/V_{O_2}	4,0730		4,4429		1,9737		6,5473		1,4935		-	

TABLE 14 Summary of mean V_{O_2}, V_W, V_W/V_{O_2} and V_{O_2}/V_W in oysters acclimated to 10°C and measured at T_E of 5°C to 30°C.

	5°C		10°C		15°C		20°C		25°C		30°C	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Mean V_{O_2} ul.5,0883mg^{-1}h^{-1}	3,63	2,19	4,65	2,5	7,40	2,42	9,69	1,52	12,27	1,69	14,88	2,95
Mean V_W ml.5,0883mg^{-1}h^{-1}	8,21	5,22	66,11	14,18	83,15	16,51	41,31	14,78	100,38	5,08	19,39	7,43
V_{O_2}/V_W	0,4420		0,0703		0,0890		0,2345		0,0100		0,7674	
V_W/V_{O_2}	2,2620		14,2224		11,2336		4,2645		8,1816		1,3031	

TABLE 15 Summary of V_{O_2}, V_w, V_w/V_{O_2} and V_{O_2}/V_w in oysters acclimated to 15°C and measured at T_E of 5°C to 30°C.

	5°C		10°C		15°C		20°C		25°C		30°C	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Mean V_{O_2} ul1.5,0883mg^{-1}h^{-1}	2,21	1,78	3,86	1,55	6,21	2,51	8,53	3,97	13,77	3,85	14,29	1,96
Mean V_w ml1.5,0883mg^{-1}h^{-1}	0,00	0,00	93,2	4,5	162,25	74,36	145,38	41,27	118,64	30,85	10,61	6,52
V_{O_2}/V_w	0,0000		0,0349		0,0383		0,0587		0,1161		1,3474	
V_w/V_{O_2}	0,0000		24,52		26,1091		17,0402		8,6133		0,7422	

TABLE 16 Summary of mean V_{O_2}, V_W, V_W/V_{O_2} and V_{O_2}/V_W in oysters acclimated to 20°C and measured at T_E of 5°C to 30°C.

	5°C		10°C		15°C		20°C		25°C		30°C	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Mean V_{O_2} ul1.5,0883mg^{-1}h^{-1}	1,31	1,11	3,37	5,12	5,34	3,36	7,27	2,37	24,57	5,30	21,54	5,19
Mean V_W ml1.5,0883mg^{-1}h^{-1}	4,53	6,40	0,00	0,00	48,94	6,39	114,57	45,92	350,92	42,23	55,24	17,45
V_{O_2}/V_W	0,2889		0,0000		0,1091		0,0503		0,0700		0,3906	
V_W/V_{O_2}	3,4615		0,0000		9,1619		19,8965		14,2848		2,5602	

TABLE 17 Summary of mean V_{O_2}, V_w, V_w/V_{O_2} and V_{O_2}/V_w in oysters acclimated to 25°C and measured at T_E of 5°C to 30°C.

	5°C		10°C		15°C		20°C		25°C		30°C	
	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s	$\bar{x}$	± s
Mean V_{O_2} ul1.5,0883mg^{-1}h^{-1}	17,98	16,19	23,84	7,50	22,16	11,41	20,22	11,74	22,31	22,15	63,76	35,80
Mean V_w ml1.5,0883mg^{-1}h^{-1}	8,71	12,81	27,51	18,93	53,78	12,26	105,13	40,04	135,00	60,63	184,33	99,51
V_{O_2}/V_w	2,0632		0,8665		0,4120		0,1923		0,1652		0,3459	
V_w/V_{O_2}	0,4847		1,1541		2,4273		5,2005		6,0522		2,8910	

2.3.2 THE EFFECT OF TEMPERATURE ON THE RESPIRATORY RATE

As with the filtration rate, respiratory rate was calculated as the mean of 16 determinations, converted to standard mass. The results are summarised in Tables 13 and 17.

The acute rate:temperature curves for routine oxygen consumption are shown in Figure 14a. Rates for T_A 5°C show an increase to a maximum at 15°C with a Q_{10} of 4,19. T_A 10, 15 and 20, however, show a lower increase in rate with a Q_{10} of approximately 2,00 over the T_E 5 - T_E 20 range. T_A 25 appears to be relatively independent of temperature, moreover the location of the curve has shifted dramatically resulting in an increased metabolic cost over the entire temperature range. The curves show evidence of lateral translation in T_A 5, 10, 15 and 20, but this effect is small from T_A 10 to T_A 20. T_A 25 may be a combination of translation and rotation (Type IV - Precht, 1958).

The acclimated rate:temperature curve for routine oxygen consumption is shown in Figure 14b. In effect translation of the rate:temperature curves maintains a low metabolism at T_A throughout the range, but metabolic costs increase dramatically between 20°C and 25°C.

2.3.3 THE EFFECT OF TEMPERATURE ON FILTRATION EFFICIENCY AND THE COST OF FILTRATION

The ratio of the volume of water cleared per volume of oxygen consumed is termed the irrigation or filtration efficiency (V_w/V_{O_2}) (Bayne, 1976; Newell *et. al.*, 1977). Values of this ratio obtained in this study are shown in Tables 13 to 17, and plotted graphically in Figure 15a. The results show that at T_A 5 and T_A 25 the V_w/V_{O_2} is relatively low over the entire range of exposure temperatures. For T_A 10, 15 and 20, however, V_w/V_{O_2} increases rapidly, peaking at the exposure temperature corresponding to the temperature of acclimation.

The acclimation rate:temperature curve for filtration efficiency is shown in Figure 15b. It is clear from the figure that the filtration efficiency increases from a low level at T_A 5 to a maximum at T_A 15, and then decreases to a low level at T_A 25. This may be explained by the fact that through T_A 10 to T_A 20 oxygen consumption is constant. Filtration efficiency therefore is dependent on filtration rate, which increases to a maximum at T_A 15 before decreasing again. The low value of V_w/V_{O_2} for T_A 25 may be explained by the high of oxygen consumption at 25°C.

The ratio of volume of oxygen consumed per volume of water cleared is shown in Figure 16a. This ratio represents the

cost of filtration values for which are summarised in Tables 13 to 17. Figure 16a shows that the relative cost of filtration is highest at the two extremes of acclimated temperature, T_A 5 and T_A 25. This effect is particularly noticable for T_A 25°C because of the relatively higher oxygen consumption over the entire range of exposure temperatures. For warm acclimated animals V_{O_2}/V_W increased dramatically beyond 25°C, this reflects the sharp decline in filtration rate shown in Figure 13.

The acclimated rate:temperature curve for cost of filtration is shown in Figure 16b. A minimum value 0,03 was recorded for T_A 15°C. This reflects the maximum V_W (Figure 13) and low V_{O_2} (Figure 14) for T_A 15°C.

2.3.4 ASSIMILATION EFFICIENCY

Assimilation efficiencies calculated for the range of acclimation temperatures are summarised in Table 9. Analysis of variance showed no significant differences between the means at the 5% level (F_s = 1,22 ; $p > 0,05$).
A mean value for assimilation over the entire range of acclimation temperatures was therefore calculated as 76,43 $\pm$ 4,88% SD.

2.3.5 SCOPE FOR GROWTH

The index of energy balance was calculated by subtracting energy of respiration from the assimilated ration. These date were calculated in energy equivalents using the following conversion factors :

a) Energy content of 10^6 cells of *Tetraselmis seucica* = 1,54J (converted from Widdows and Bayne, 1971).

b) An oxycalorific equivalent of 19,85J.$ml^{-1}O_2$ (Griffiths and King, 1979).

Ingested ration is calculated as the product of filtration rate and food concentration (15 x 10^6 cells.l^{-1} throughout). Assimilated ration is calculated as the product of assimilation efficiency and ingested ration.

The data are summarised in Table 18. It is noticable that scope for growth has a positive index throughout, indicating a surplus amount of energy available for growth (P). A graphical summary of the data on scope for growth is shown in Figure 17. The ingestion rate increases to a maximum at 15°C after which it declines, reflecting the decrease in the filtration rate shown in Figure 13. Because assimilation efficiency remains unchanged over the range of acclimation temperatures, the assimilated ration follows the same pattern. Scope for growth is indicated by the hatched area which represents the energy gain of assimilation minus the energy expenditure of metabolism.

TABLE 18 Data used for the calculation of the scope for growth of *Ostrea edulis* of standard mass (5,0883mg). Cell concentration = 15 x 10^6cell.l^{-1}. Conversion factors are given in the text.

Acclimation temperature °C	Respiratory rate $\bar{x}$ ± s		J.h^{-1}	Filtration rate $\bar{x}$ ± s		Ingestion ration x10^6cells.h^{-1}	J.h^{-1}	Assimilated ration J.h^{-1}	Scope for growth J.h^{-1}	Growth efficiency K_1
5	7,95	5,6	0,16	32,37	12,67	0,49	0,76	0,53	0,37	+ 0,49
10	4,65	2,5	0,09	66,11	14,18	0,99	1,53	1,25	1,16	+ 0,76
15	6,21	2,5	0,12	162,25	74,30	2,43	3,74	3,00	2,88	+ 0,77
20	7,26	2,4	0,14	144,57	45,90	2,17	3,33	2,52	2,38	+ 0,71
25	22,30	22,0	0,44	135,00	60,62	2,03	3,12	2,26	1,82	+ =,58

NOTE : Respiration rate (µl O_2.5,0883mg^{-1}.h^{-1}) and filtration rate (ml.5,0883mg^{-1}.h^{-1}) were obtained from Tables 13 - 17.

2.3.6 DAILY PERCENTAGE MASS CHANGE

Using the values of clearance and respiratory rates documented above it is possible to calculate the predicted daily percentage mass change for different concentrations of food, over the range of acclimation temperatures. These were calculated in carbon equivalents (as opposed to Joules) to facilitate comparison with other work (e.g. Newell, 1977).

The following assumptions were made :

a) that the carbon content of an oyster consisted of 50% of the dry mass. The standard animal of 5,0833mg therefore consisted of 2,450mg C. (Newell *et. al.*, 1977).

b) that the RQ was 1,0 and the carbon equivalent of 1ul O_2/µg C (Newell *et. al.*, 1977).

Values of the mean V_{O_2} and V_W over the range of acclimation temperatures were obtained from Tables 13 to 17. The mean assimilation efficiency of 76,43% was obtained from Table 10. Daily percentage mass loss of respiration, at zero ration, and the carbon required to maintain body mass are shown in Table 19. The calculations are shown in Appendix 3. The carbon content of *Tetraselmis seucica* was

TABLE 19 Predicted daily percentage mass change and carbon required to maintain the body mass per day for juvenile *Ostrea edulis*.

ACCLIMATION TEMPERATURES	5	10	15	20	25
Mean V_{O_2} $\mu l.5,0883 mg^{-1}.h^{-1}$	7,9	4,6	6,2	7,2	22,3
Mean V_w $ml.5,0883 mg^{-1}.h^{-1}$	32,4	66,1	162,3	144,8	135,0
Carbon equivalent of V_{O_2} (μg C.24h)	101,52	59,28	79,68	92,64	286,8
% Daily mass loss at 0 ration	-3,99	-2,33	-3,13	-3,65	-1,29
Carbon req. to maintain body mass (μg C.$24h^{-1}$)	132,82	77,56	104,25	121,20	375,24

determined using the Beckman Carbon Analyser. A mean of 5 determinations gave the carbon content of 1×10^6 cells. The predicted daily percentage mass change with food concentration for different acclimation temperatures is shown in Table 20. Calculations are shown in Appendix 4. The daily mass changes for each T_A against food concentrations are plotted in Figure 18.

2.3.7 REGRESSION ANALYSIS OF ASSIMILATED RATION AND RESPIRATORY RATE TO DETERMINE SCOPE FOR GROWTH

Stepwise multiple linear regression analysis (Allen, 1973) was used to obtain polynomial expressions for assimilated ration and respiratory rate. The programme involved the progressive introduction or subtraction of independent variables and transformations, (Appendix 5) in a curvilinear model of the form :

$$Y = a + b_1X_1 + b_2X_2^2 \qquad + b_nX_n^i$$

where Y is the dependent variable and X_n the independent variables. The resultant equation combined the least number of independent variables with the best coefficient of determination.

Four data points were plotted for each acclimated temperature (T_A 5 - T_A 25) at each exposure temperature (T_E 5 - T_E 30). Regression equations are summarised in Table 21.

TABLE 20 Predicted mass change per day at different food concentrations, for T_A 5 to 25°C.

RATION LEVEL ($\mu g\ C.l^{-1}$)	ACCLIMATION TEMPERATURE 5	10	15	20	25
13,50	-3,68	-1,69	-1,56	-2,24	-9,27
27,00	-3,37	-1,05	+0,01	-0,84	-7,97
40,50	-3,05	-0,14	+1,58	+0,56	-6,66
54,00	-2,74	+0,23	+3,16	+1,97	-5,35
81,00	-2,11	+1,51	+6,30	+4,78	-2,73

TABLE 21 Regression equations used to produce the models described in section 2.4 .

1. FIGURE 19 : (r^2 = 0,050)

$$\text{Assimilation Ration} = 0,29 + 0,34 \times 10^{-4} T_A T_E 3 - 0,91 \times 10^{-6} T_A T_E 4 - 0,19 \times 10^{-6} T_A T_E$$
$$0,21 \times 10^{-5} T_E 4$$

2 FIGURE 22 : (r^2 = 0,54)

$$\text{Respiration Rate} = 0,49 + 0,57 \times 10^{-7} T_A T_E + 0,18 \times 10^{-5} T_A 4 - 0,035 T_A - 0,5 \times 10^{-7} T_E 4$$

3. FIGURE 26 : (r^2 = 0,64)

$$\text{Assimilation Ration} = -8,07 - 0,63 \times 10^{-5} T_A T_E 4 + 0,38 \times 10^{-3} T_A T_E 3 - 0,0076 T_A T_E 2 + 0,0024 T_A 2 T_E$$
$$- 0,047 T_A 2 + 0,406 T_E + 1,132 T_A$$

4. FIGURE 28 : (r^2 = 0,77)

$$\text{Respiratory Rate} = 0,043 - 0,38 \times 10^{-6} T_A T_E 4 + 0,23 \times 10^{-4} T_A T_E 3 - 0,45 \times 10^{-3} T_A T_E 2$$
$$+ 0,12 \times 10^{-3} T_A 2 T_E + 0,024 T_E - 0,0055 T_A 2$$

These regressions were entered into a Graphics Display Package (GDP)(Woodhouse Enterprises, Computer Centre, University of Cape Town). This programme subtracted respiratory rate from assimilated ration to produce scope for growth. From the GDP the data was previewed via a GDP Calcomp Interface to produce two dimensional and three dimensional plots of the dependent variables (Figures 19 to 25).

2.3.8 DISCUSSION

Filtration rates shows a thermal optimum between 15°C and 25°C (Figure 13). Within each acclimated regime maximal rates of filtration occurred at or above the temperature of acclimation, an effect recorded for large *Ostrea edulis* (Newell *et. al.*, 1977). Lateral translations of the acute rate:temperature curves recorded here, were not as pronounced as those recorded for *Ostrea edulis* (Newell *et. al.*, 1977) or *Crepidula fornicata* (Newell and Kofoed, 1977). Warm acclimated animals showed higher filtration rates than cold acclimated ones, with a maximum at T_A 15°C (Figure 13B). This ensures a large intake of food during the warm summer months, for somatic and reproductive growth. Similar results were observed in the winkle *Littorina littorea* (Newell and Pye, 1971a), the slipper limpet *Crepidula fornicata* (Newell and Kofoed, 1977) and large *Ostrea edulis* (Newell *et. al.*, 1977).

The acute rate:temperature curves for oxygen consumption in juvenile *Ostrea edulis* showed evidence of lateral translation,

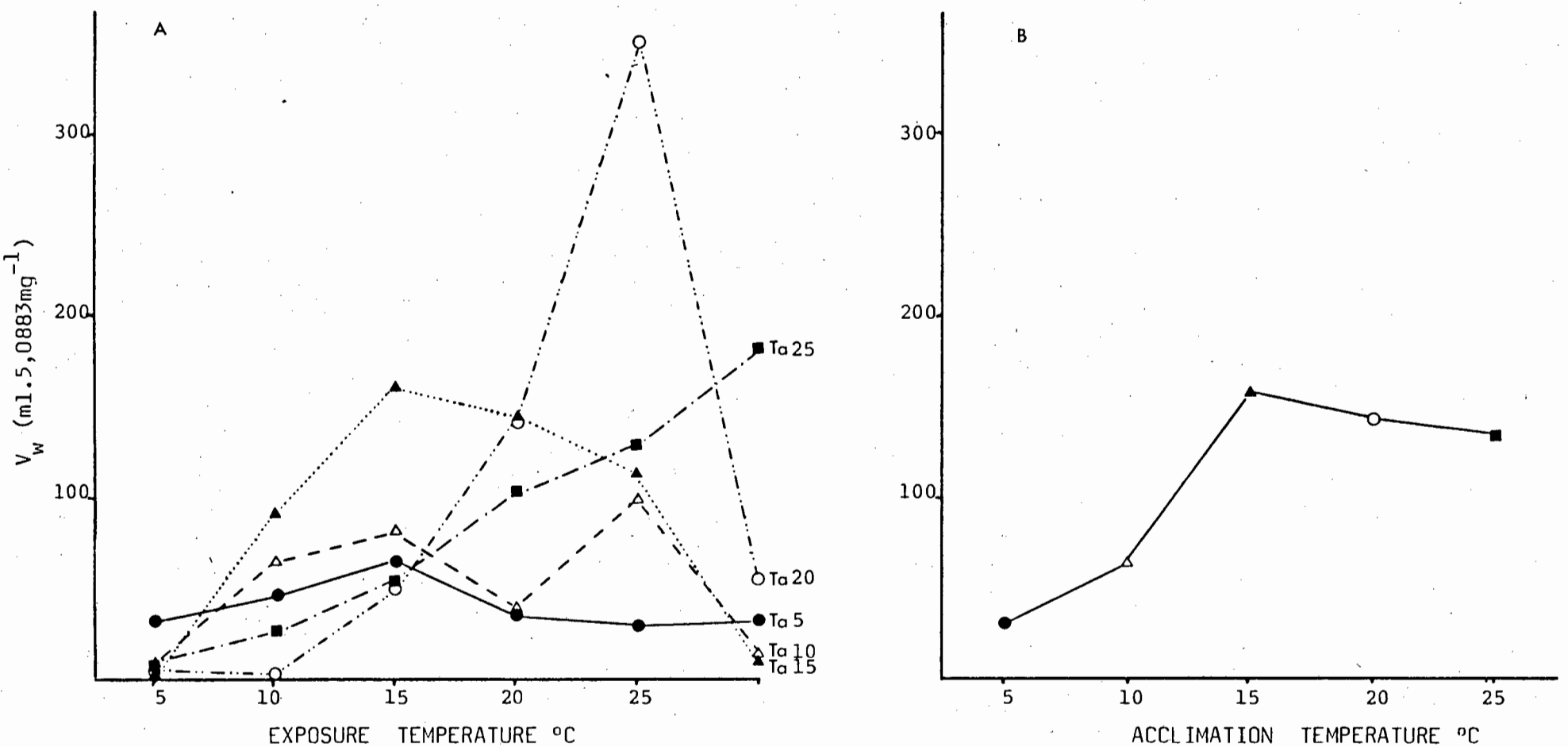

FIGURE 13 : A. The acute rate:temperature curves for clearance of *Tetraselmis seucica* by *Ostrea edulis* following acclimation to temperatures from 5 - 25°C.

B. The acclimated rate:temperature curve for clearance of *Tetraselmis seucica* by *Ostrea edulis*.

maintaining a low V_{O_2} over most of the acclimated regime (Figure 14). This result differed from Newell *et. al.*, (1977), who found no evidence of lateral translation of the acute RT curves for respiration in adult *Ostrea edulis*. Q_{10} values for T_A 10 to T_A 20, between T_E 10 and T_E 20, are 2,30; 2,08 and 2,18 respectively. These values agree with those for routine V_{O_2} in other bivalves (Widdows, 1972, 1973b; Bayne *et. al.*, 1973 and Bayne, 1976). The relative independence of T_A 15°C to increasing exposure temperatures agrees with Read (1962a), who showed that Q_{10} values in *Modiolus demissus* declined as the upper limit of thermal tolerance was reached. Read explains this as a means of conserving energy. The high levels of respiration of juvenile oysters at T_A 25 are difficult to explain in these terms as the animals are definitely not conserving energy. These rates probably represent respiration under thermal stress.

Compensatory responses of both filtration and respiration enable *Ostrea edulis* to optimise filtration efficiency following warm acclimation (Figure 15). However, above T_A 20°C increasing metabolic costs and decreasing filtration rates result in a poor filtration efficeincy. The high value of 24,7 for V_w/V_{O_2} in juvenile *Ostrea edulis* is considerably higher than the value obtained for adult *Ostrea edulis* (Newell *et. al.* 1977), but agrees with values obtained for other bivalves (Jorgensen, 1966 and Vahl 1973a, b).

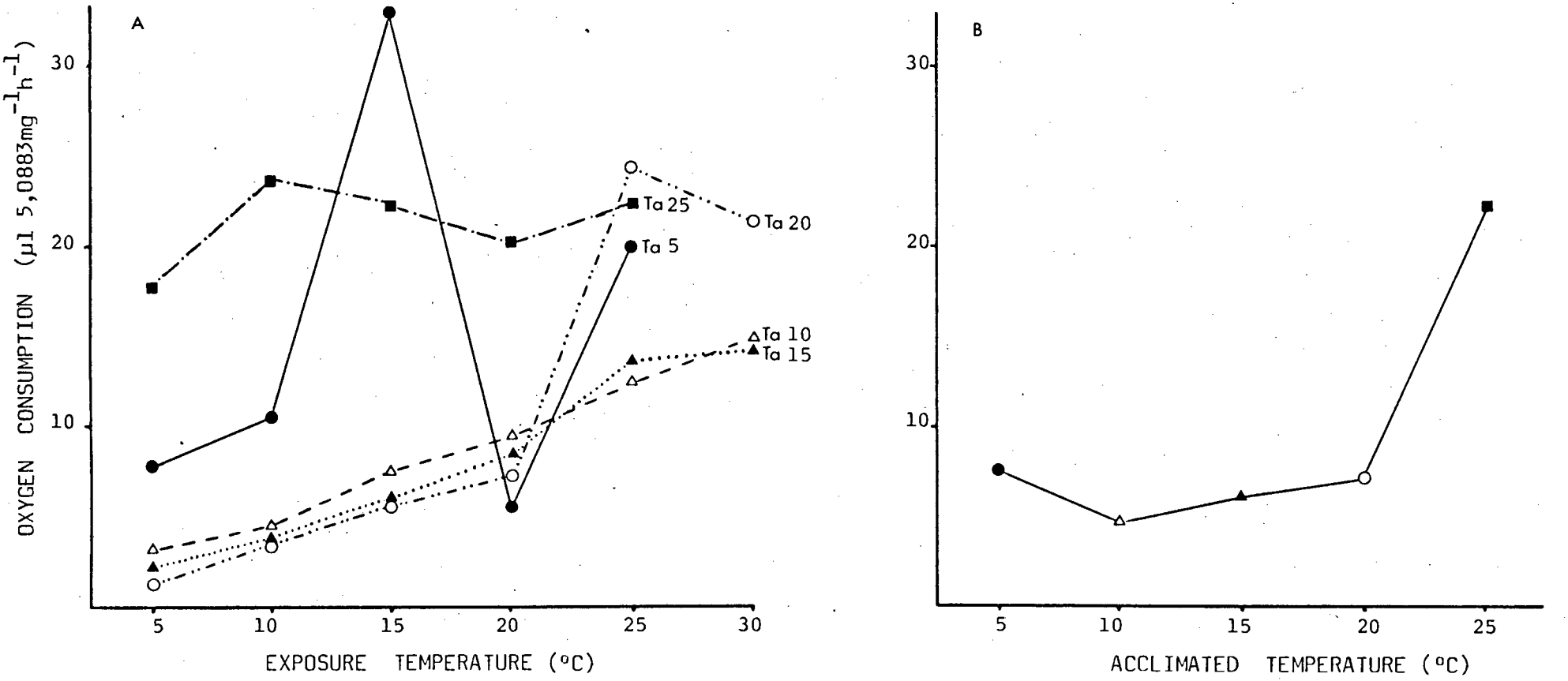

FIGURE 14 : A. The acute rate:temperature curves for Oxygen consumption of *Ostrea edulis* fed *Tetraselmis seucica*. Acclimation was between 5 and 25°C.

B. The acclimated rate:temperature curve for Oxygen consumption of *Ostrea edulis*.

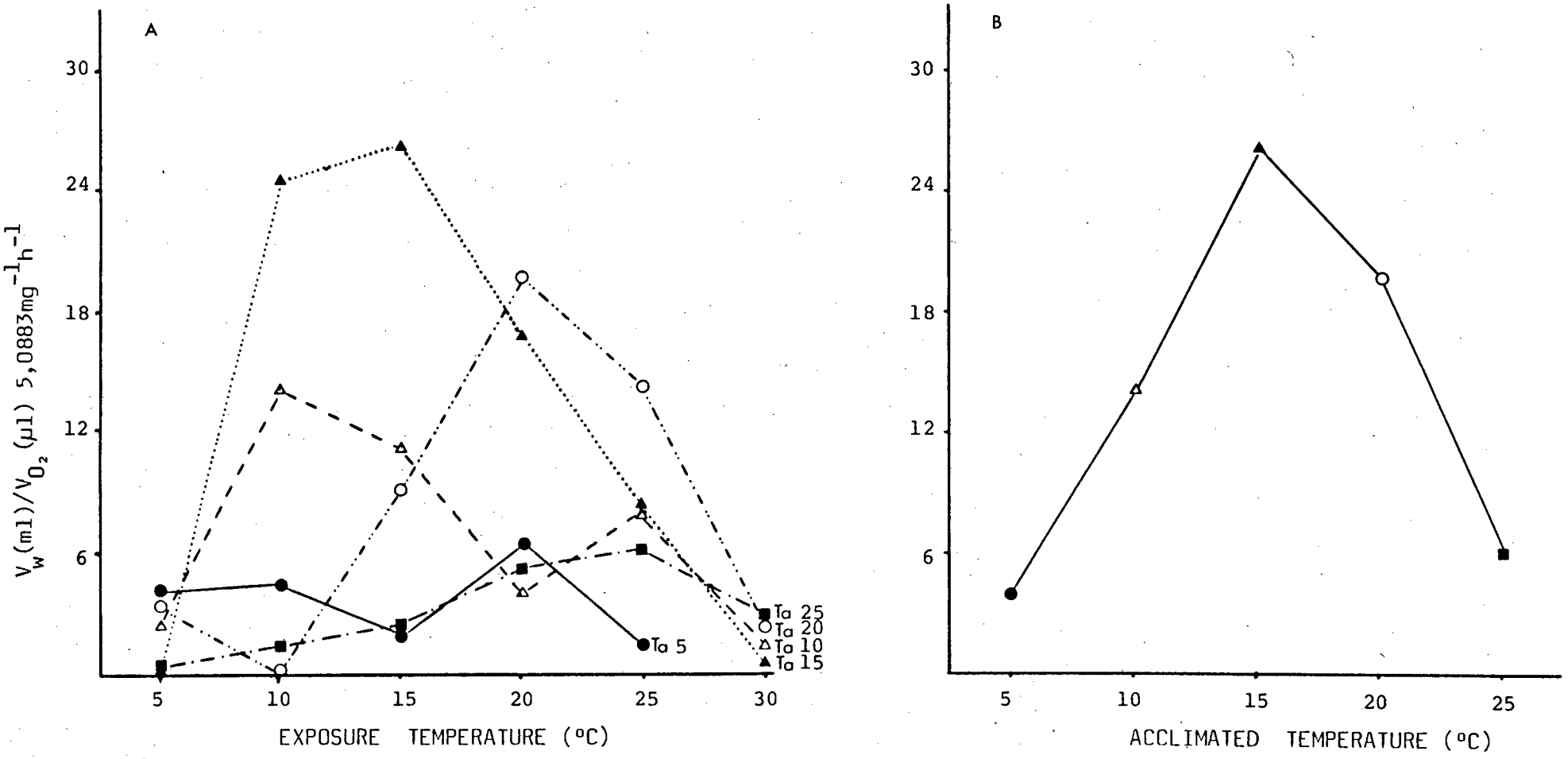

FIGURE 15 : A. Filtration efficiency (V_w/V_{O_2}) of *Ostrea edulis* at different exposure temperatures following acclimation over the range 5 to 25°C.

B. The acute rate:temperature curve of V_w/V_{O_2}.

The cost of filtration (V_{O_2}/V_W) for juvenile *Ostrea edulis* is shown in Figure 16. Following warm acclimation V_{O_2}/V_W is suppressed. This is due to increased filtration and a decline in the metabolic rate at these temperatures. A similar pattern was found in adult *Ostrea edulis* (Newell *et. al.*, 1977). In adults, however, suppression of V_{O_2}/V_W is achieved by an increase in V_W without a corresponding decrease in V_{O_2}. In juveniles V_{O_2}/V_W increases above 20°C due to higher V_{O_2} and a decline in V_W.

These results indicate that the competitive ability of juvenile *Ostrea edulis* is best following warm acclimation to temperatures between 15 and 20°C. At these temperatures maximum filtration efficiency and minimum cost of filtration are achieved.

Scope for growth, as the assimilated energy remaining after metabolic costs have been taken into account, is positive over the entire range of the acclimated temperatures (Figure 17). Warm acclimated animals show a relatively large scope for growth with a maximum at 15°C. These results agree with Widdows (1971), who found that scope for growth of *Mytilus edulis* was relatively insensitive to temperature between 10 and 20°C. At either extreme metabolic compensation breaks down and scope for growth decreases. Bayne *et. al.*, (1973), show that for any given temperature scope for growth increases with ration. Small animals may further

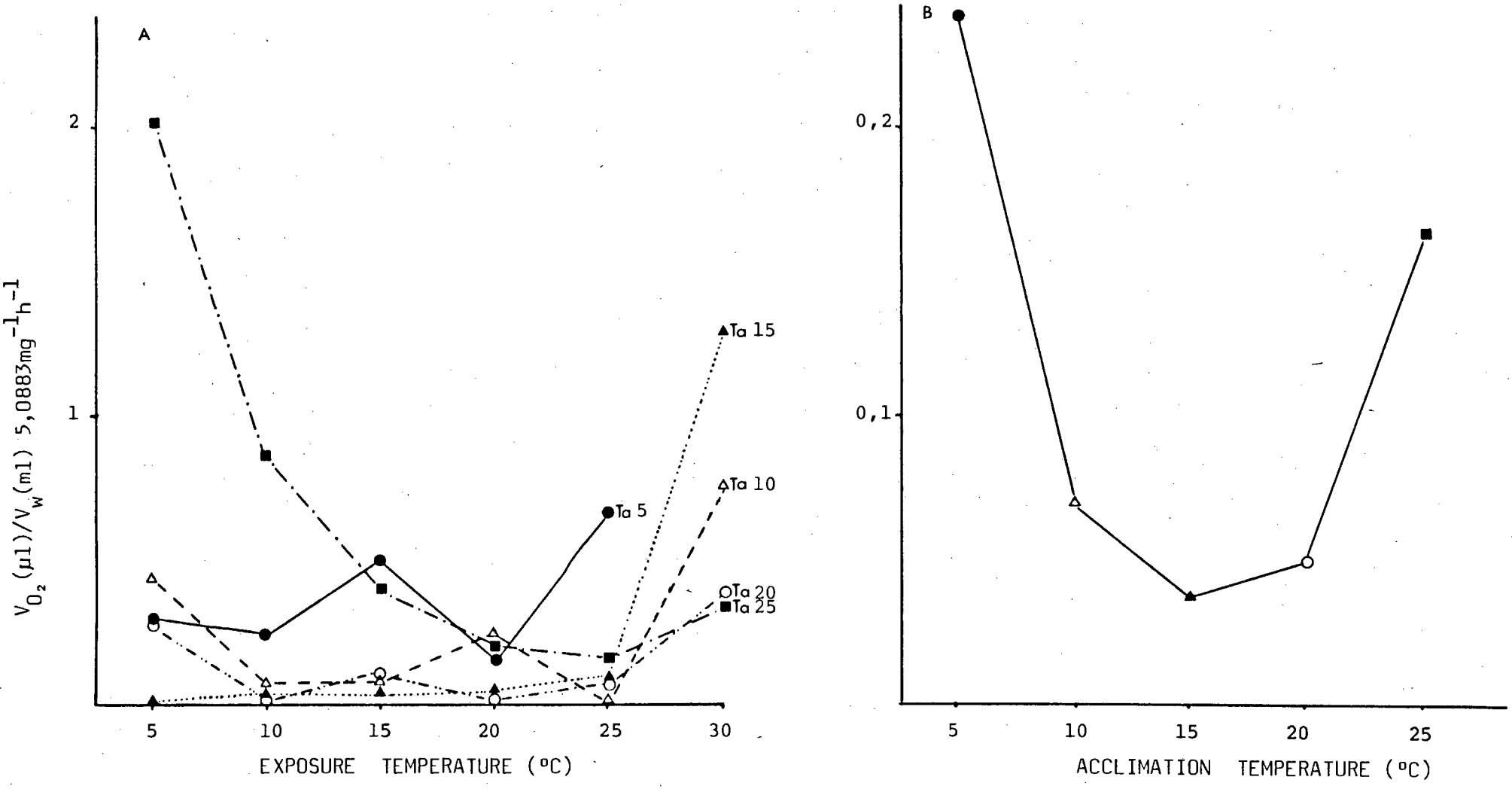

FIGURE 16 : A. The cost of filtration (V_{O_2}/V_w) of *Ostrea edulis* at different exposure temperatures for acclimated temperatures from 5 to 25°C.

B. The acclimated rate:temperature curve for V_{O_2}/V_w.

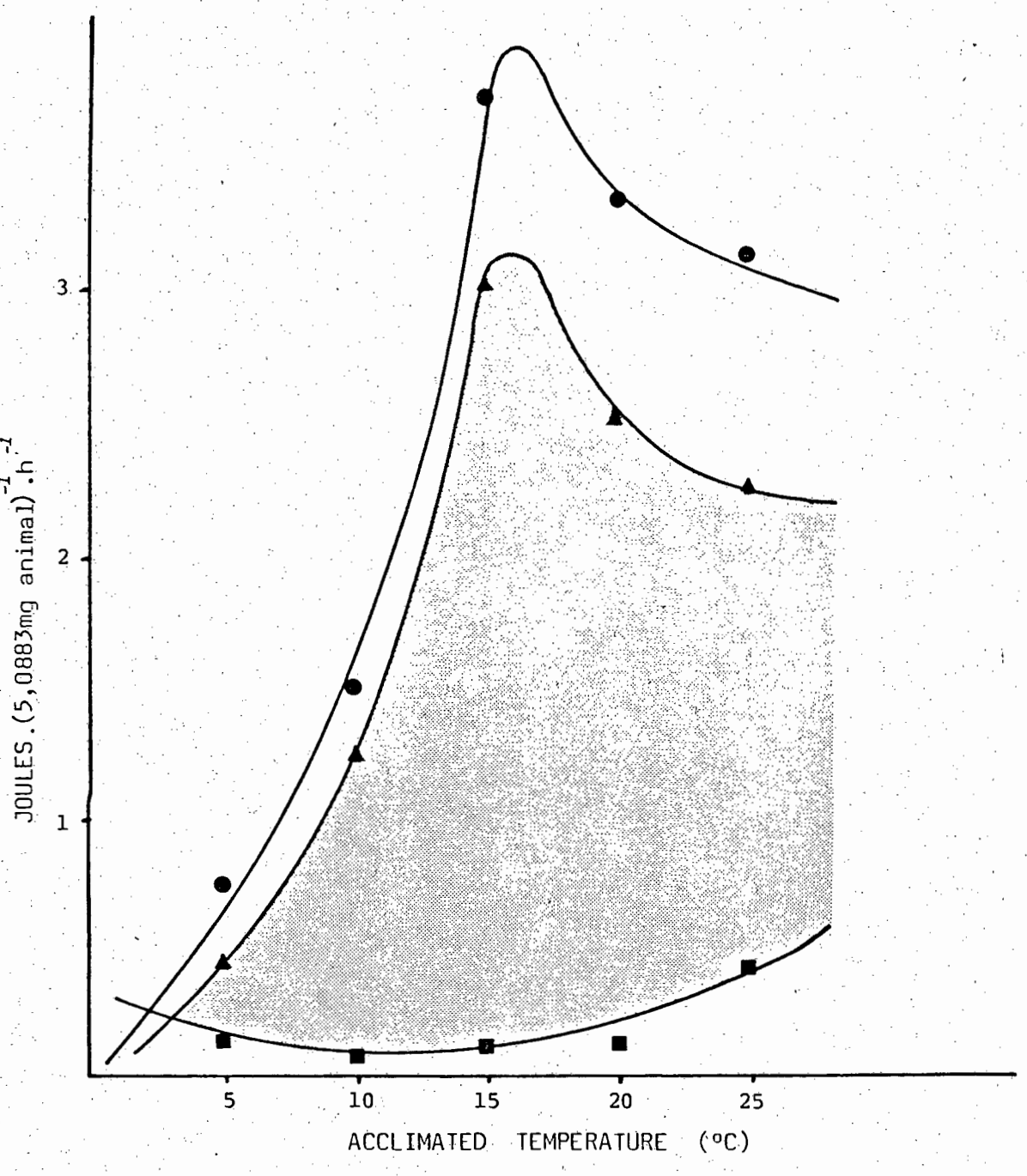

FIGURE 17 : Ingested ration (●), Assimilated ration (▲), Oxygen consumption (■) and Scope for growth (stippled) expressed as a function of Acclimated temperature.

show a greater potential for growth than large ones (Thompson and Bayne, 1974).

These results show that variation in scope for growth with temperature only becomes critical when ration levels are outside optimum values of approximately 15 x 10^6 cells/l. At low ration levels negative scope for growth results because of a low filtraion efficiency brought about by high metabolic costs. Conversely at high rations, low filtration and assimilation rates combined with high metabolic costs, contribute to a negative scope for growth.

An apparent contradiction exists between the positive scope for growth (Figure 17) and the negative daily percentage mass change (Figure 18) at T_A 5 and T_A 25°C. This situation could possibly arise from the many assumptions made to predict the daily percentage mass change. The graph nevertheless, does serve as an indication of the relative mass changes that may be expected at different food concentrations. Growth potential decreases at acclimated temperatures above and below 15°C and at ration levels above 27µg C (10 x 10^6 cells *Tetraselmis seucica*/l), juvenile *Ostrea edulis* maintain positive growth between T_A 10 and T_A 20°C.

Theoretical modelling of assimilated ration, respiratory rates and scope for growth (Figures 19 to 25) showed that optimal assimilated ration occurred at T_A 21,84 and

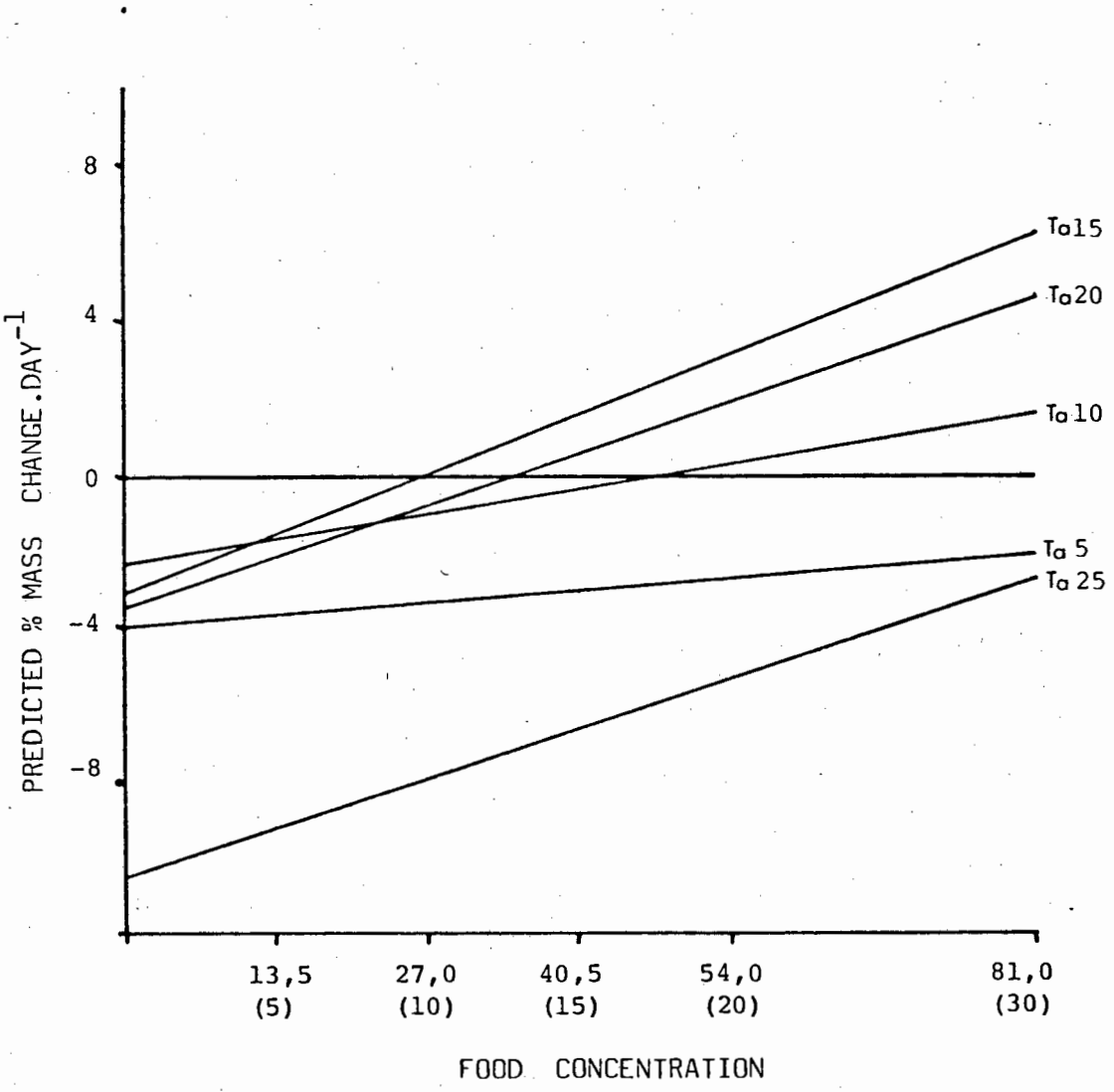

FIGURE 18 : Predicted daily % mass change for *Ostrea edulis* in different concentrations of food ($\mu gC.l^{-1}$). Cells x 10^6/litre are given in parentheses.

T_E 24,73°C (Figure 19). Minimal rates of respiration exist between T_A 10 and T_A 20°C (Figure 22) and scope for growth achieved a maximum at T_A 19,74 and T_E 23,42°C (Figure 24).

The model predicts that assimilated ration will be relatively independent of temperature at T_A 5°C but becomes increasingly dependent on exposure temperature as the acclimated temperature increases above 10°C (Figure 19). The response to exposure temperature is similar for each T_A (Figure 21). Rate increases from a low level at T_E 5 °C to a maximum between T_E 15 and 25°C and decreases beyond T_E 25°C. The rapid decrease in rate after T_E 25°C reflects a breakdown in biological function as lethal temperatures are approached.

A trough of optimal respiration occurs between T_A 10 and T_A 20°C at exposure temperatures from T_E 5 to T_E 22°C (Figure 22). Above T_A 20°C respiration increases with exposure temperature to a maximum at T_E 30°C. In warm acclimated animals a dramatic rise in respiration occurs above T_E 22°C. This probably reflects the respiratory rate under temperature stress, following which metabolic processes would breakdown.

Scope for growth was obtained by subtracting metabolic costs of respiration from the energy gain of assimilated ration (Figure 24). Maximum growth would be obtained between

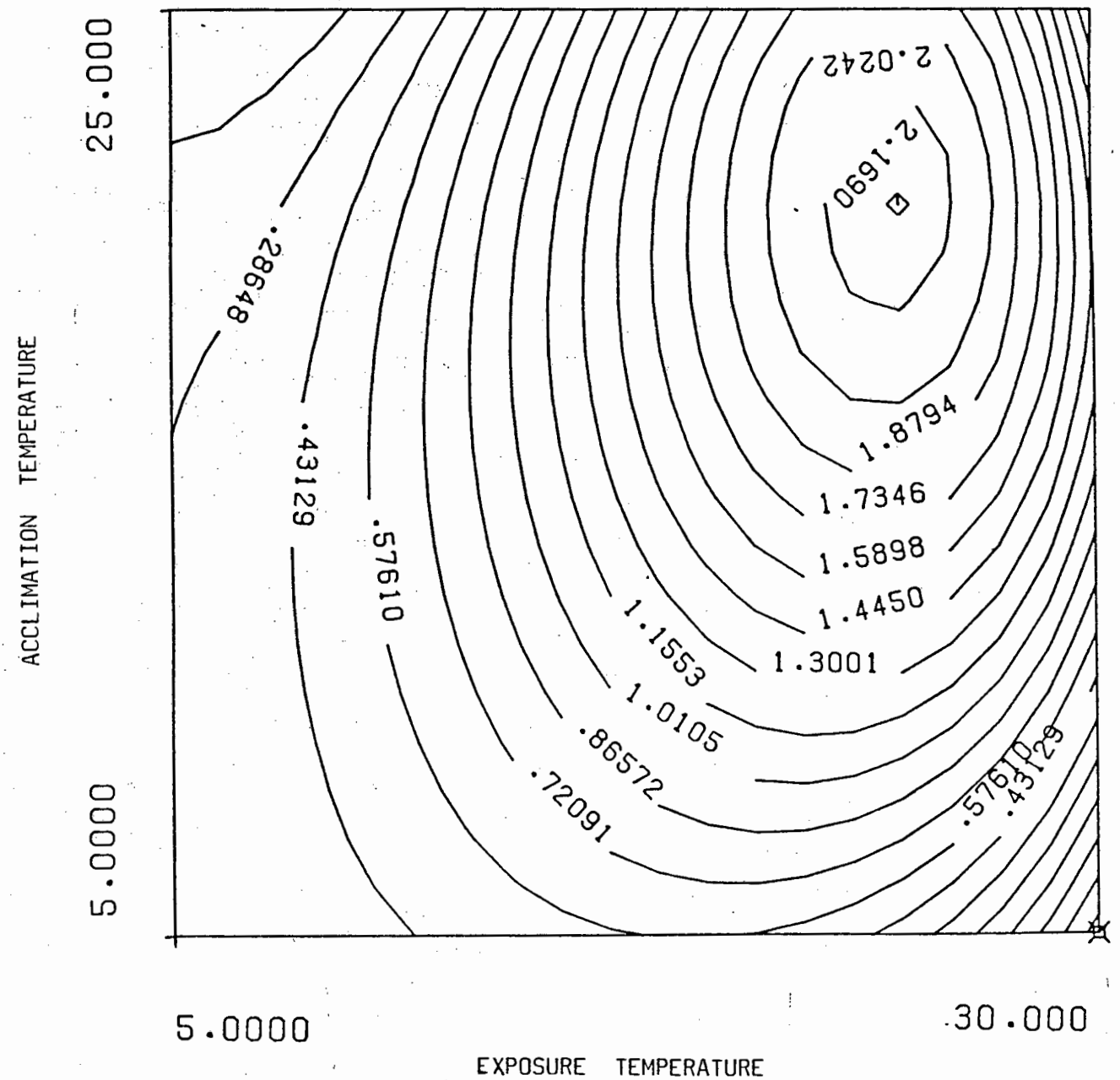

FIGURE 19 : Contour plot of Assimilated ration ($J.h^{-1}$) for juvenile *Ostrea edulis* acclimated between 5 and 25°C and exposed to temperatures ranging from 5 to 30°C.

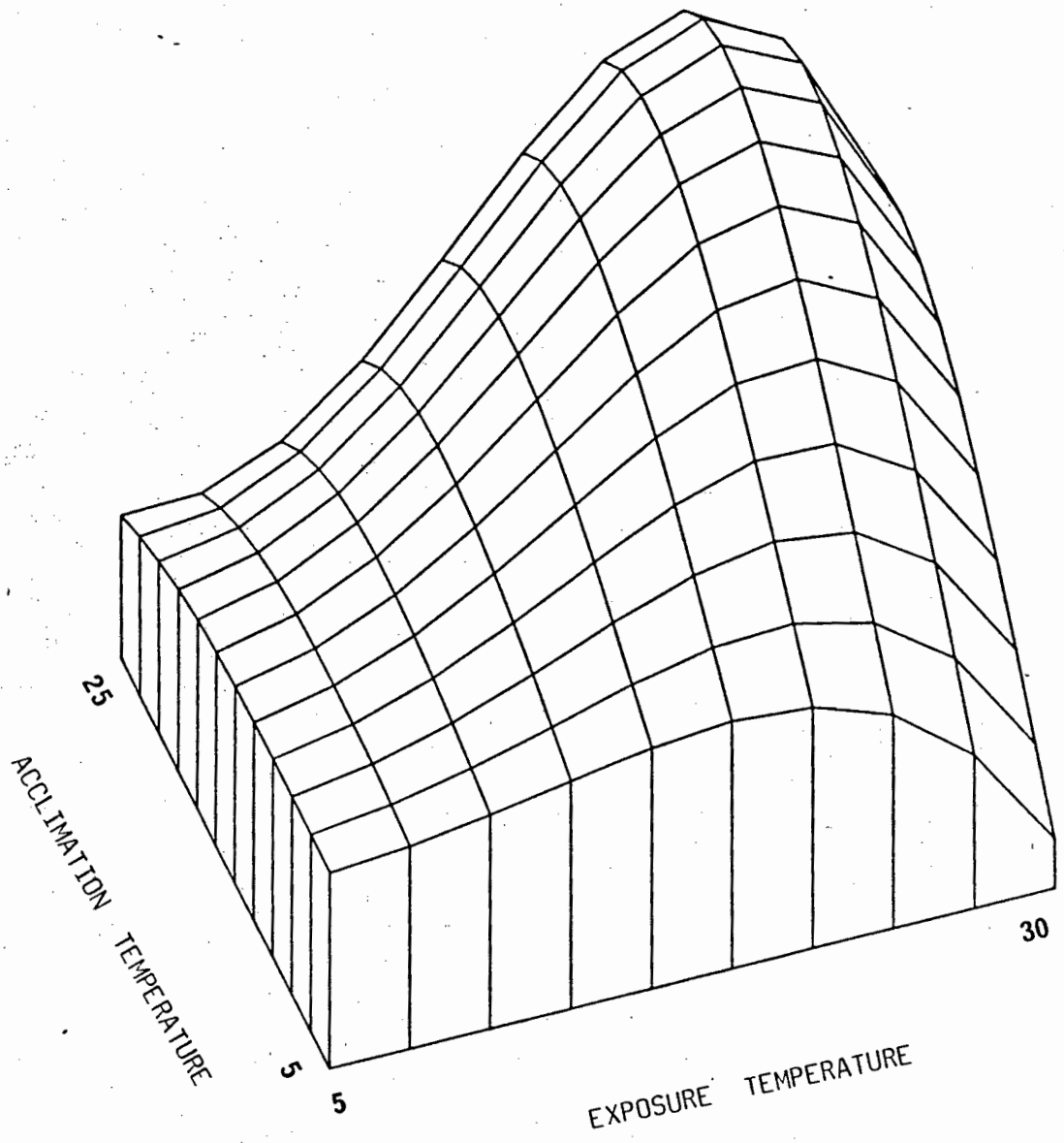

FIGURE 20 : Response surface diagram relating T_A, T_E and Assimilated ration.

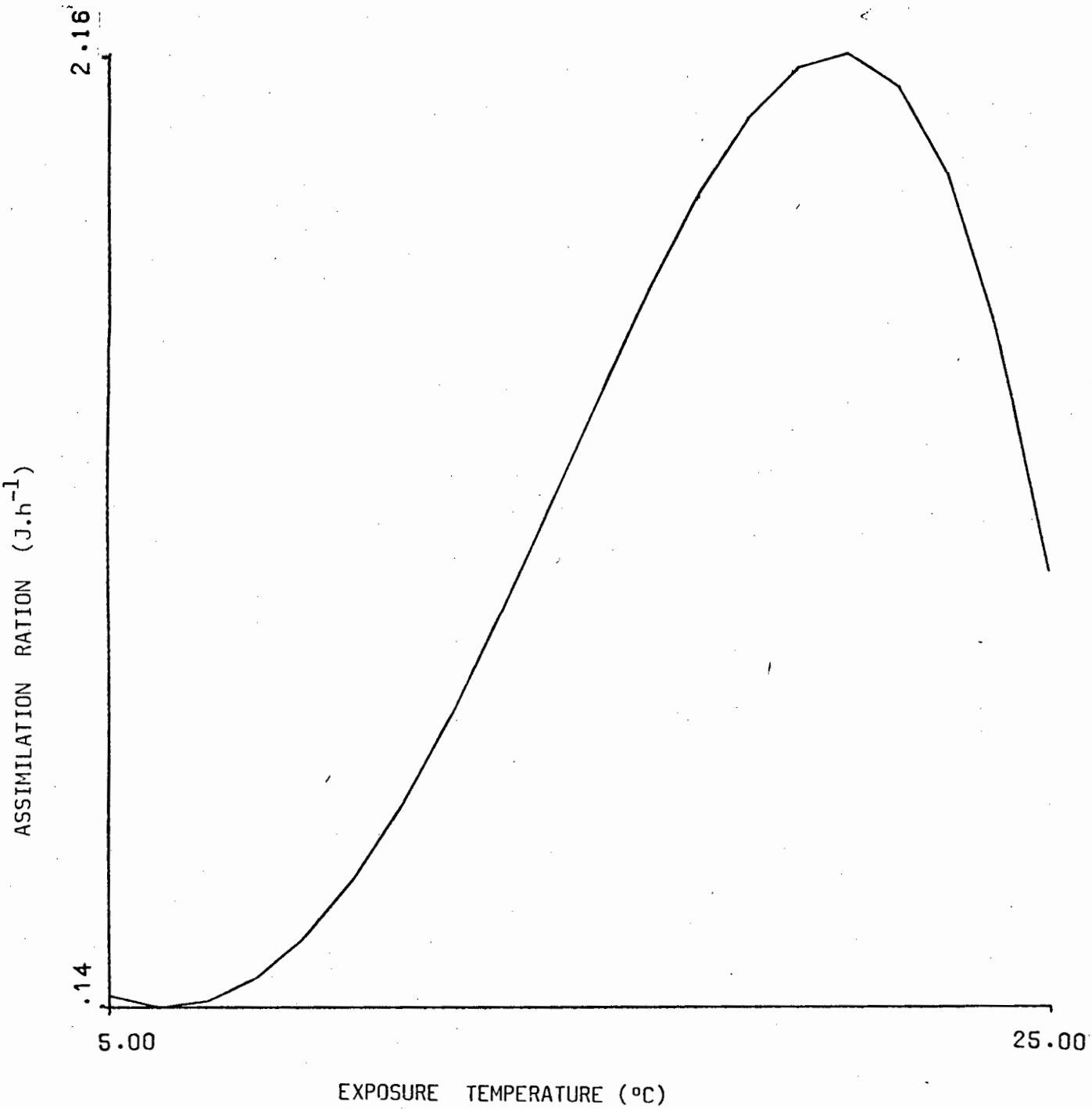

FIGURE 21 : Assimilated ration at T_A21,84 over exposure temperatures from 5 to 30°C.

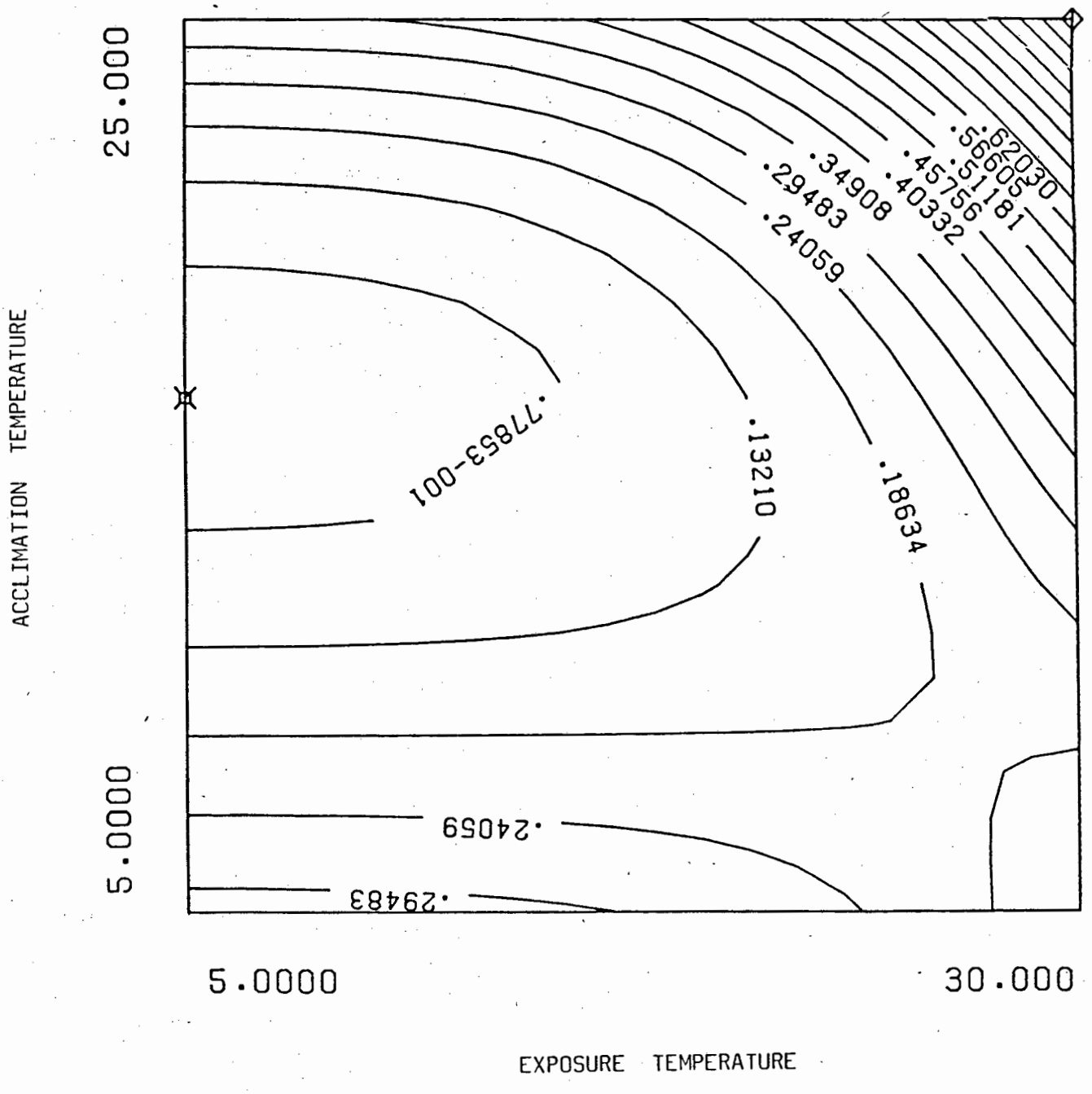

FIGURE 22 : Contour plot of Respiratory Rate ($J.h^{-1}$) in juvenile *Ostrea edulis* acclimated from 5 to 25°C and exposed to temperatures from 5 to 30°C.

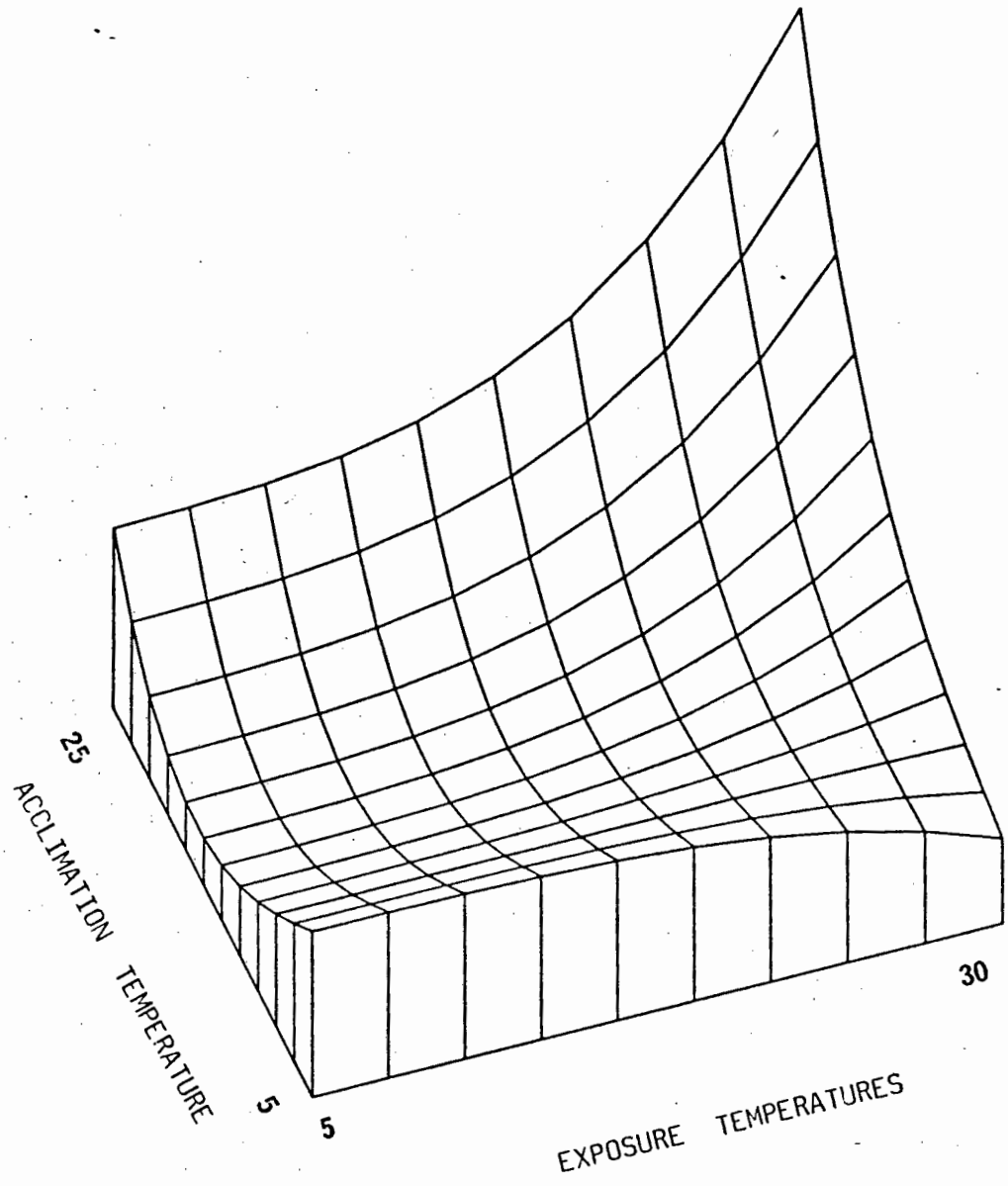

FIGURE 23 : Response surface diagram relating T_A, T_E and Respiratory rate.

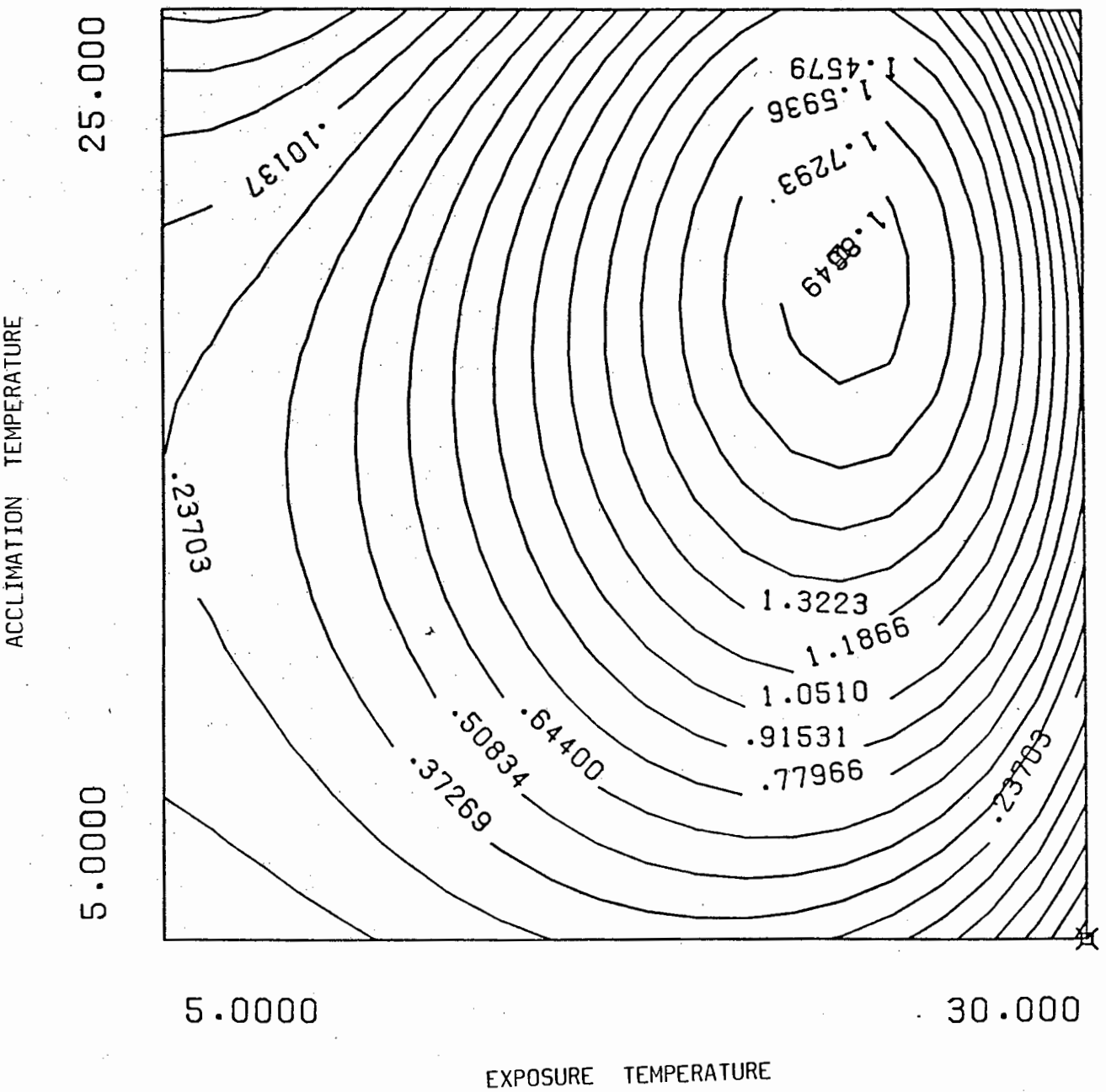

FIGURE 24 : Contour diagram of Scope for Growth ($J.h^{-1}$) in juvenile *Ostrea edulis*. The plot was obtained by subtracting Respiratory rate (Figure 22) from Assimilated ration (Figure 19).

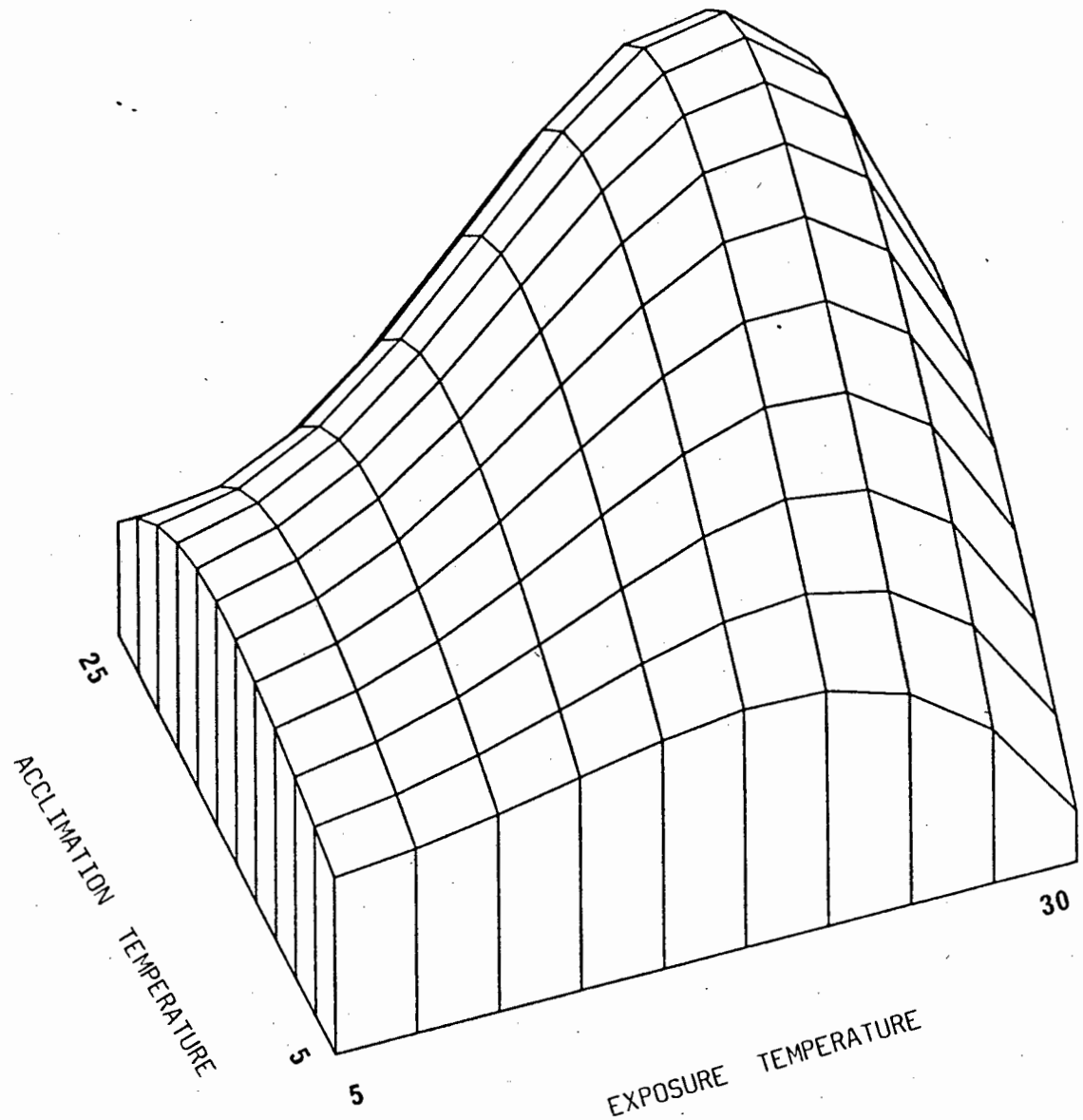

FIGURE 25 : Response Surface diagram relating T_A, T_E and Scope for growth in juvenile *Ostrea edulis*.

T_A 15 and 22°C at exposure temperatures from 20 to 27°C. Optimal scope for growth was reached at exposure temperatures above the acclimation temperature, providing the most growth during the summer breeding season.

Although respiratory rates are lowest at cold temperatures, assimilated ration at these temperatures is not sufficient to support good growth. This reflects the dormant state of oysters during winter months when growth is minimal. It follows that growth could be enhanced if the water temperature was artificially increased in winter, for example, by utilising power station effluent.

The standardised residuals between observed and calculated values of the dependant variables showed that most of the variation, between data and the model, occurred at T_A 5 and T_A 25°C. The variation was probably due to thermal stress at extreme temperatures. Re-entering the data, omitting T_A 5 and T_A 25°C, produced figures 26 to 31. This improved the coefficient of determination and predictive accuracy of each model. For practical purposes therefore, predictions of scope for growth, assimilated ration and respiratory rate at temperatures normally encountered in the environment, should be made with figures 26 to 31.

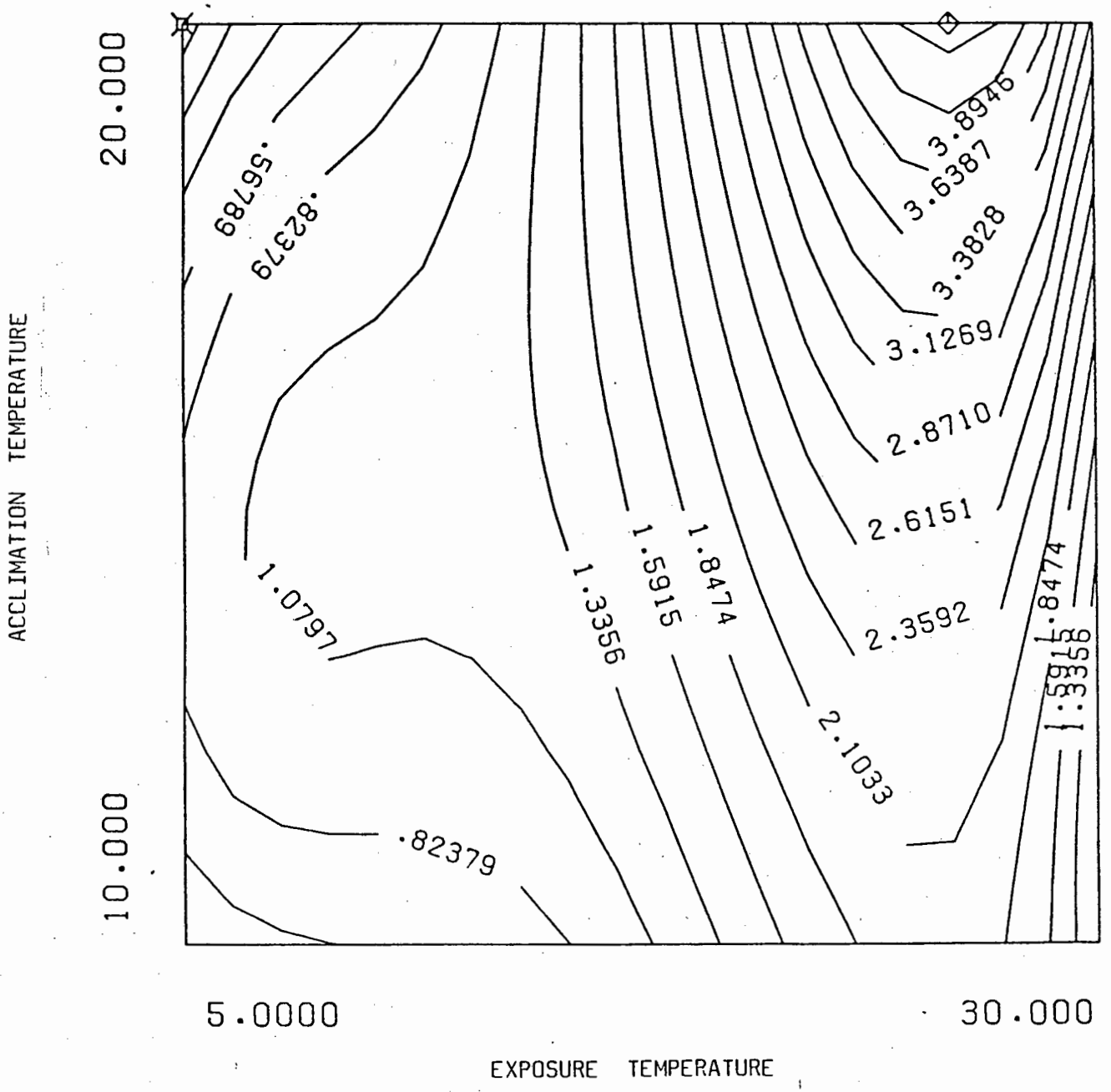

FIGURE 26 : Contour plot of Assimilated ration ($J.h^{-1}$) for juvenile *Ostrea edulis* acclimated between 10 and 20°C and exposed to temperatures ranging from 5 to 30°C.

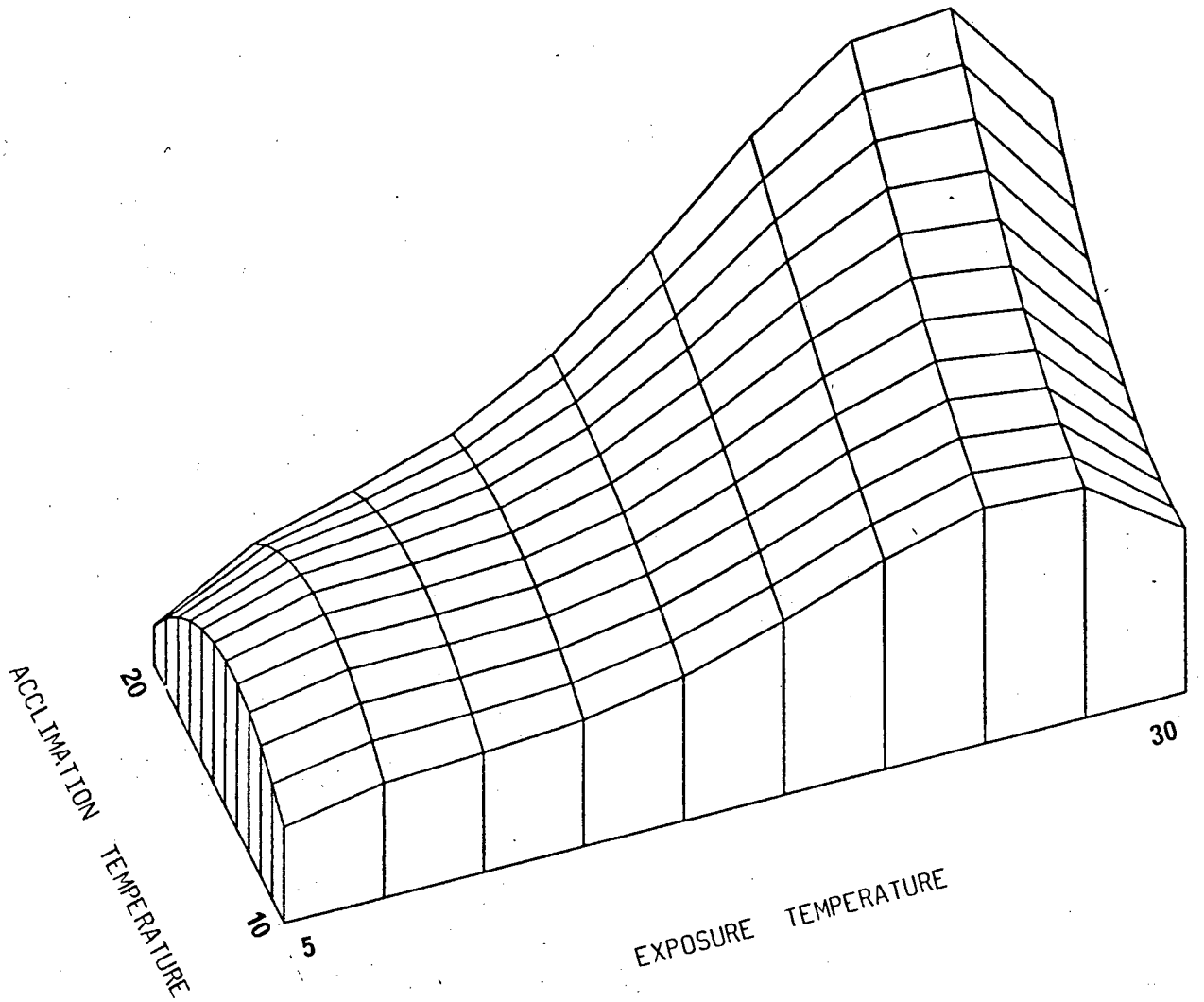

FIGURE 27 : Response surface diagram relating T_A, T_E and Assimilated ration

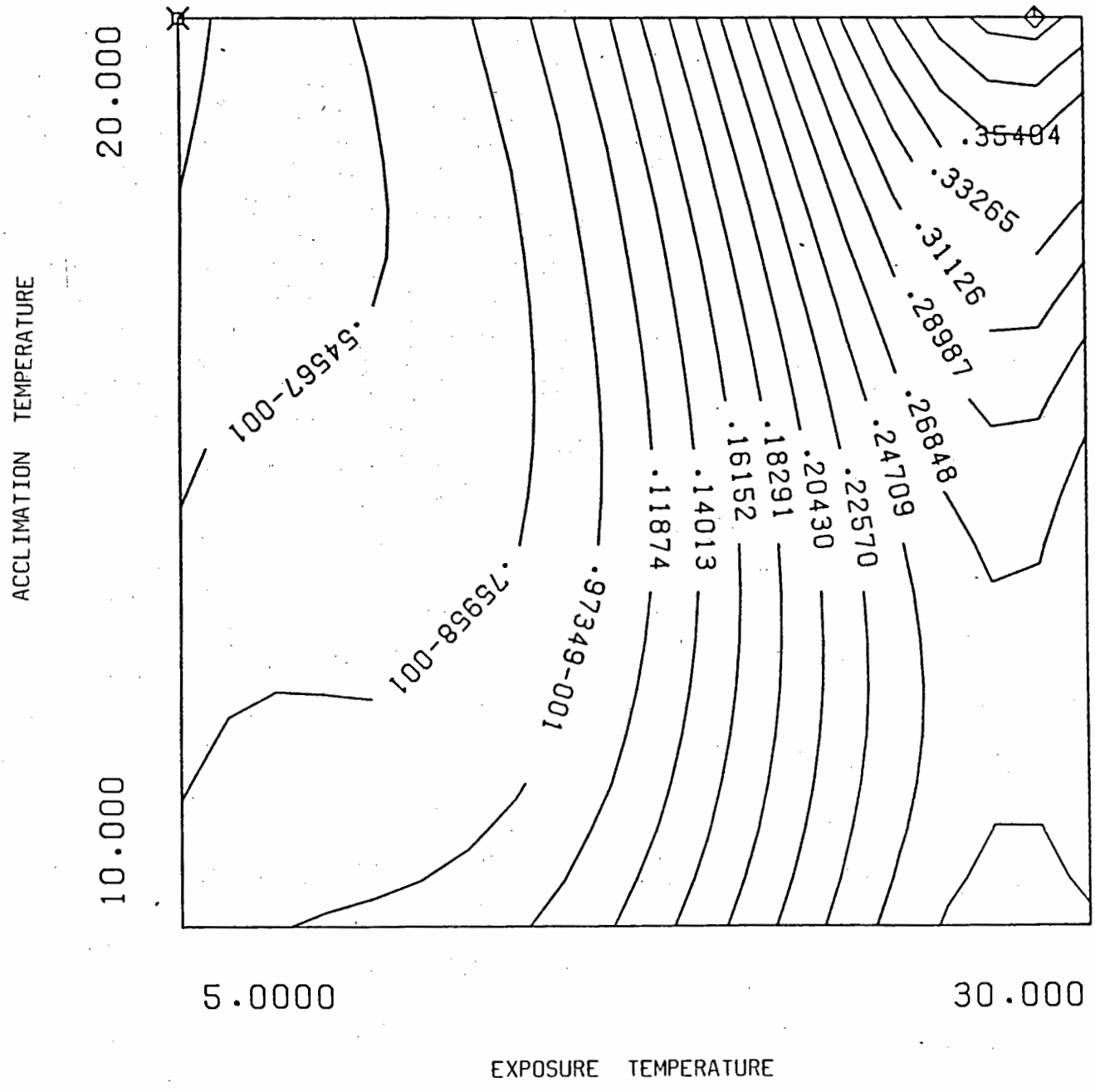

FIGURE 28 : Contour plot of Respiratory Rate ($J.h^{-1}$) in juvenile *Ostrea edulis* acclimated from 10 to 20°C and exposed to temperatures from 5 to 30°C.

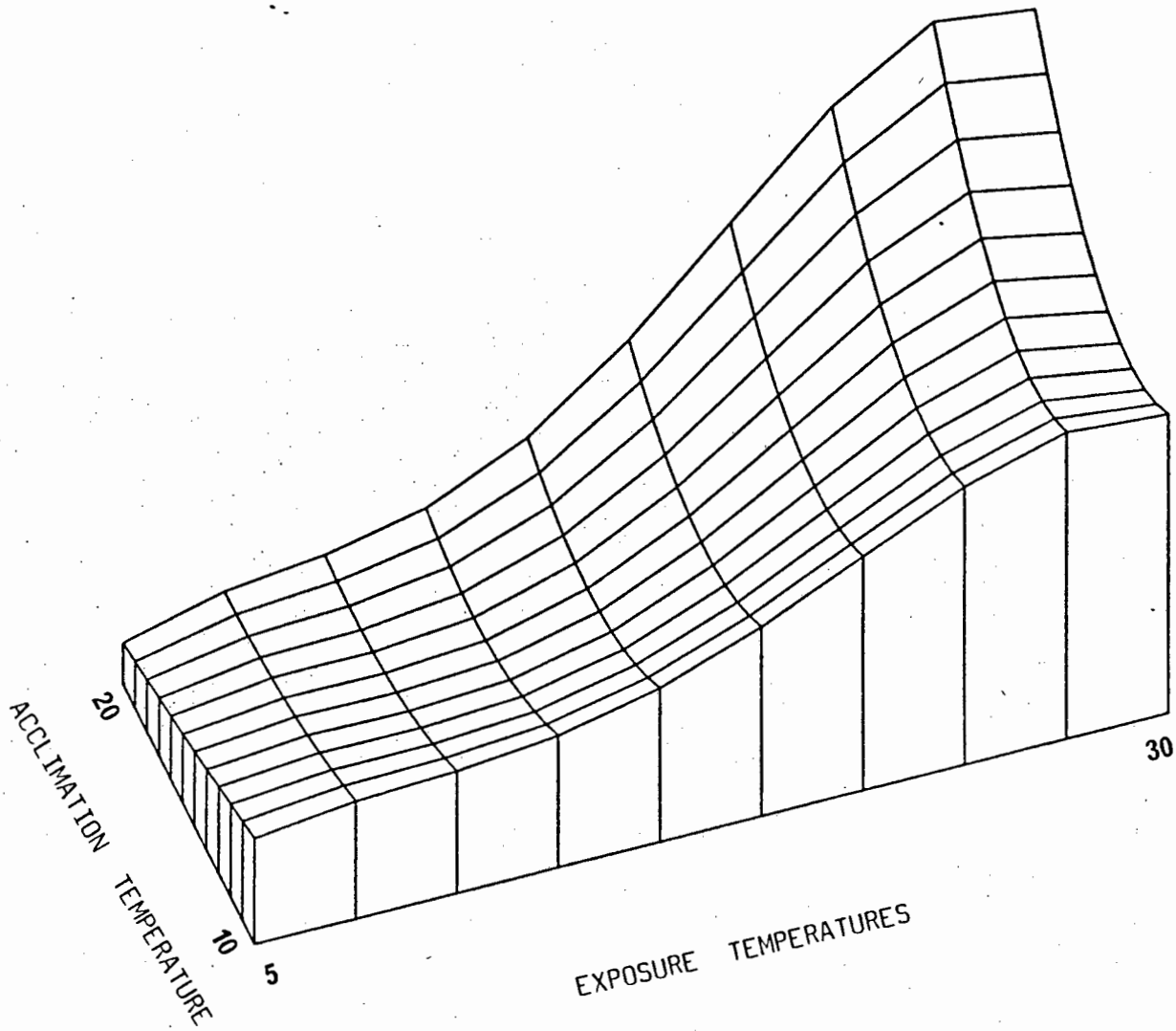

FIGURE 29 : Responses surface diagram relating T_A, T_E and Respiratory rate.

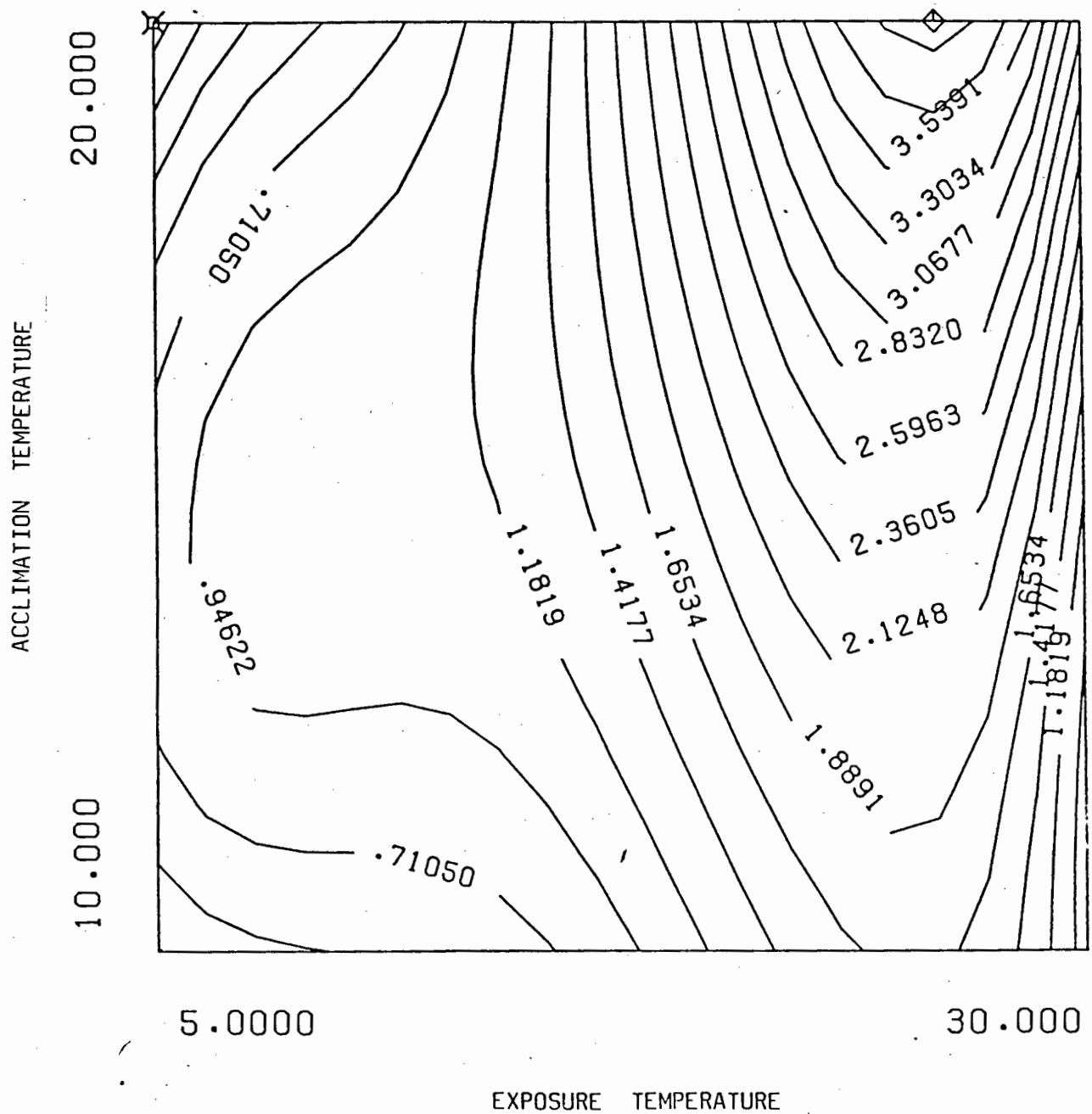

FIGURE 30 : Contour diagram of Scope for Growth ($J.h^{-1}$) in juvenile *Ostrea edulis*. The plot was obtained by subtracting Respiratory rate (Figure 28) from Assimilated ration (Figure 26).

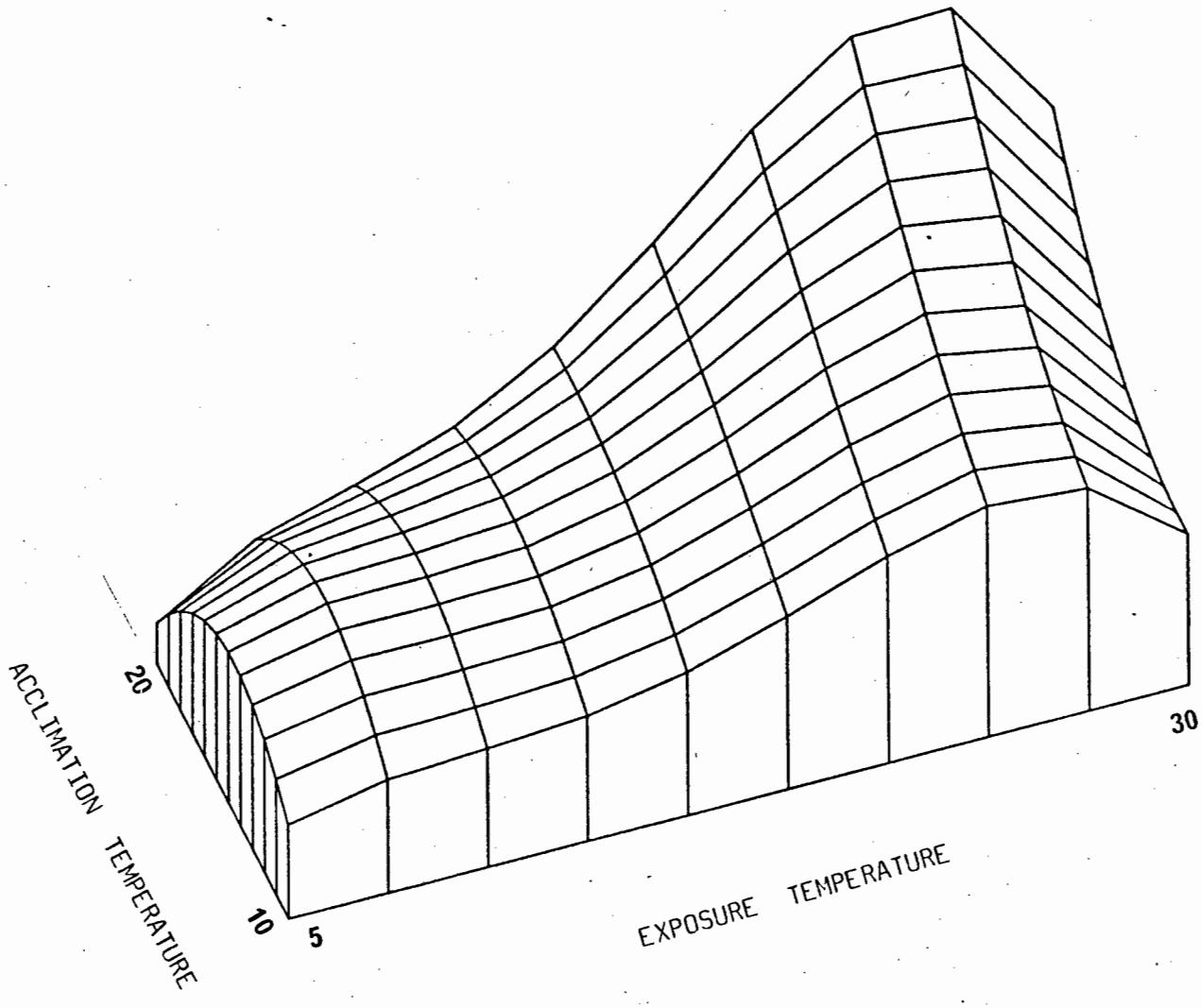

FIGURE 31 : Response Surface diagram relating T_A, T_E and Scope for growth in juvenile *Ostrea edulis*.

CHAPTER 3

GENERAL CONCLUSIONS

This study presents an investigation into the energy balance of juvenile *Ostrea edulis*, made up as energy gain of ingested ration and energy loss of metabolism, release of dissolved organic carbon and faecal production. A study of the effects of body size, food concentration, food type, and temperature on biological rate functions provided information on the optimal conditions of growth. These studies could possibly be extended to adults, to describe reproductive growth, although it must be stressed that rate functions of adults may differ considerably from those of juvenile oysters.

Results presented in Chapter 1 showed that routine metabolism in juvenile oysters was directly dependent on body size with a mass exponent of 1,09. With growth, the cost of filtration (V_{O_2}/V_w) would become proportionately greater were it not for the high mass exponent of filtration (0.91) described for juvenile oysters. The competitive ability of post-larval oysters is therefore comparable to that of juveniles of 5,08mg.

Filtration rate also showed a marked dependence on particle concentration. With increasing concentration, filtration increased to a maximum at 15 x 10^6 cells/l followed by a gradual decline to a basal rate above 25 x 10^6 cells/l. Pseudofaecal production was observed at values as low as 15 x 10^6 cells/l. These results show that maximal ingestion can be

obtained at relatively low levels of food concentration and that increasing the available food above 15 x 10^6cells/l will not improve growth.

Filtration rate was not significantly affected by a particle range of 4,00 - 20.00μm, although evidence exists to suggest that juvenile oysters are able to select particles on a basis other than size. This finding is important to further work on micro-encapsulated diets for oysters. Efforts will have to be concentrated on the palatibility of the microcapsules to oysters rather than the retention efficiency of various sized particles.

The effects of temperature on biological functions are well known and these were confirmed for juvenile oysters. Although assimilation efficiency was not effected by acclimation the filtration rate, oxygen consumption, filtration efficiency, cost of filtration, assimilated ration and hence growth, achieved optimal values following warm acclimation. Temperatures between 15 and 20°C provide the best conditions for growth. In addition it was found that growth would improve if the exposure temperatures increased within 5°C of the acclimation temperature. This would ensure optimum growth in warm summer conditions.

From the findings we can conclude that if the mariculture

programme pioneered at Knysna is to be continued, the possibility of maintaining the temperature of the culture tanks should be investigated. The use of power station coolant could provide a means of achieving this situation.

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REFERENCES

Allen, J. (1973). *STEPREG 1.* Stepwise linear regression analysis. University of Wisconsin, Madison.

Ali, R.M. (1970). The influence of suspension density and temperature on the filtration rate of *Hiatella arctica*. *Mar. Biol. 6*, 291 - 302.

Bayne, B.L. (1971a). Oxygen consumption by three species of lamellibranch molluscs in declining ambient oxygen tension. *Comp. Biochem. Physiol., 40A*, 955-970.

Bayne, B.L. (1971b). Ventilation, the heart beat and oxygen uptake by *Mytilus edulis* L. in declining oxygen tension. *Comp. Biochem. Physiol., 40A*, 1065 - 1085.

Bayne, B.L. (1976). *Marine mussels : their ecology and physiology*, 1 - 494. Cambridge Univ. Press.

Bayne, B.L., Bayne, C.J., Carefoot, T.C. and Thompson, R.J. (1976b). The physiological ecology of *Mytilus californicans* Conrad 2. Adaptations to low oxygen tension and air exposure. *Oecologia* (Berl.), *22*, 229 - 250.

Bayne, B.L., Thompson, R.J. and Widdows, J. (1973). Some effects of temperature and food on the rate of oxygen consumption by *Mytilus edulis* L. In: *Effects of temperature on ectothermic organisms* (ed. W. Wieser), 181 - 193. Springer - Verlag, Berlin.

Bayne, B.L. Thompson, R.J. and Widdows, J. (1976c). Physiology 1. In : *Marine mussels : Their ecology and physiology*, 121 - 206. Cambridge Univ. Press.

Bayne, B.L. and Livingston, D.R. (1977). Responses of *Mytilus edulis* L. to low oxygen tension: Acclimation of the Rate of Oxygen Consumption. *J. comp. Physiol. B.*, *114*, 129 - 142.

Bernard, F.R. (1974). Particle sorting and labial palp function in pacific oyster *Crassostrea gigas* (Thunberg 1795). *Biol. Bull. 146*(1), 1 - 9.

Braarud, T. (1960). On the coccolithophorid genus *Cricosphaera* n. gen. *Nytt. Mag. Bot 8*, 211 - 212.

Buxton, C.D. (1977). The feeding ecology of *Parechinus angulosus* unpubl. BSc hons. thesis, U.C.T.

Calabrese, A and Davis, H.C. (1970). Tolerances and requirements of embryos and larvae of bivalve Molluscs. *Helgol. wiss. Meersunters.*, *20*, 553-574.

Cole, H.A. (1936). Experiments in the breeding of oysters (*Ostrea edulis*) in tanks, with special reference to the food of the food of the larvae and spat. *Fish. Invest. ser. II. XV* (4), 1 - 28.

Conover, R.J. (1966). Assimilation of organic matter by zooplankton. *Limnol. Oceanogr. II*, 338 - 354.

Coughlan, L. (1969). The estimation of filtering rate from the clearance of suspensions. *Mar. Biol.*, 35, 79 - 93.

Crisp, D.J. and Ritz, D.A. (1967). Temperature acclimation in barnacles *J. exp. mar. Biol. Ecol.*, *1*, 236 - 256.

Dame, R.F. (1972). The ecological energies of growth, respiration and assimilation in the inter-tidal Ameri- oyster, *Crassostrea virginica*. *Mar. Biol.*, *17*, 243 -250.

Davenport, J. (1976). A technique for the measurement of O_2 consumption in small aquatic organisms. *Lab. prac.*, *25*, 693 - 695.

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) *Neth. J. Sea Res.*, 2(2), 233 - 249.

Davis, H.C. and Guillard, R.R. (1958) Relative value of ten genera of micro-organisms as food for oyster and clam larvae. *Fish. Bull. U.S. Fish. Wild. Ser.*, 58, 293 - 304.

Dral, A.D.G. (1967). The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel (*Mytilus edulis*). *Neth. J. Sea. Res.* 3(3), 391 - 422.

Epifanio, E CH., Mootz logan. C. and Turk, C.L. (1975). Culture of six species of Bivalves in a recirculating seawater system. *10th Eur. Symp. Mar. Biol.* Ostend Belgium.1, 97 - 108.

Field, J.G. (1972). Some observations on the release of Dissolved Organic Carbon by the sea urchin *Strongylocentrotus droebachiensis*. *Limnol. Oceanogr.*, 17.(5), 759 - 761.

Foster-Smith, R.L. (1975a). The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule*(L.) and *Venerupis pullastra* (Montagu). *J. exp. mar. Biol. Ecol.*, *17*, 1 - 22.

Foster- Smith, R.L. (1975b). The effect of concentration of suspension and inert material on the assimilation of algae by three bivalves. *J. mar. biol. Ass. U.K.*, 55, 411 - 418.

Fry, F.E.J. (1957). The aquatic respiration of fish. In : *The physiology of fishes* (ed. M.E. Brown), vol 1, 1 - 63. Academic Press, New York.

Gabbott, P.A. and Bayne, B.L. (1973). Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L. *J. mar. biol. Ass. U.K.*, 53, 269 - 286.

Gabbott, P.A., Jones, D.A. and Nichols, D.H. (1975). Studies on the design and acceptability of microencapsulated diets for marine particle feeders. II Bivalve molluscs. *10th Eur. Sym. Mar. Biol.* Ostend Belgium. *1*, 127 - 141.

Galtsoff, P.S. (1964). The American oyster. *Bull. US Fish. Wild Serv.*, *64*, 1 - 480.

Griffiths, C.L. and King, J.A. (1979). Some relationships between size, food availability and energy balance in the ribbed mussel *Aulacomya ater*. *Mar. Biol.*, *51*, 141 - 149.

Hammen, C.S. (1969). Metabolism of the oyster, *Crassostrea virginica*. *Am. Zool.*, *9*, 309 - 318.

Haven, D.S. and Morales - alamo, R. (1966). Aspects of biodeposition by oysters and other invertebrate filter feeders. *Limnol. Oceanogr.*, *11*, 487 - 498.

Helm, M.M. (1977). Mixed algal feeding of *Ostrea edulis* larvae with *Isochrysis galbana* and *Tetraselmis seucica*. *J. mar. biol. Ass. U.K.*, *57*, 1019 - 1029.

Helm, M.M., Holland, D.L. and Stephenson, R.R. (1973). The effect of supplimentary algal feeding of a hatchery breeding stock of *Ostrea edulis* L. on Larval vigour. *J. mar. biol. Ass. U.K.*, *53*, 673 - 684.

Hemmingsen, A.M. (1960). Energy metabolism as related to body size and respiratory surfaces and its evolution. *Rep. Steno. Mem Hosp. Nord. Ins.*, *4*, 7 - 58.

Jørgensen, C.B. (1966). *Biology of suspension feeding*. Pergamon Press, Oxford. 1 - 357.

Kinne, O. (1963a). The effects of temperature and salinity on marine and brackish water animals. 1. Temperature *Oceanogr. Mar. Biol. Ann. Rev., 1,* 301 - 340.

Kinne, O. (1963b). Adaptation, a primary mechanism of evolution. In: *Phylogeny and Evolution of Crustacea.* H.B. Whittington and W.D.I. Rolfe (Eds). Museum of Comparative Zoology, Cambridge, Mass., 27 - 50. (Spec. Publ.)

Kinne, O. (1964b). Non-genetic adaptation to temperature and salinity. *Helgol. wiss. Meeresunters.,9,* 433 - 458.

Kinne, O. (1970c). Temperature - invertebrates. In: *Marine Ecology* (ed. O. Kinne). *1*(1), 407 - 514. Wiley - Interscience, N.Y.

Kirby-Smith, W.W. and Barber, R.J. (1974). Suspension feeding aquaculture systems : effects of phytoplankton concentration and temperature on growth of the bay scallop. *Aquaculture, 3,* 135 - 145.

Kofoed, L.H. (1975). The feeding biology of *Hydrobia ventrosa* (Montagu) 1. The assimilation of different components of the food. *J. exp. mar. Biol. Ecol., 19,* 233 - 241.

Lam, R.K. and Frost, B.W. (1976). Model of copepod filtering response to changes in size and food concentration. *Limnol. Oceanogr.*, *21*(4), 490 - 500.

Langton, R.W. and McKay, G.U. (1974). The effect of continuous versus discontinuous feeding on the growth of hatchery reared spat of *Crassostrea gigas*. *J. cons. int. Explor. Mer.*, 35(3), 361 - 363.

Lehman, J.T. (1976). The filter feeder as an optimal forager and the predicted shapes of feeding curves *Limnol. Oceanogr.*, *21*(4), 501 - 516.

Loosanoff, V.L. and Murray Jr. T. (1974). Maintaining adult bivalves for long periods on artificially grown phytoplankton. *Veliger*, *16*(1), 93 - 94.

Manton, I. and Peterfi, L.S. (1969). Observations on the fine structure of coccolith, scale and the protoplast of the freshwater coccolithophorid, *Hymenomonas roseola* Stein, with supplimentary observations on the protoplast of *Cricosphaera carterae*. *Proc. R. Soc. B.*, *172*, 1 - 15.

McLusky, D.S. (1973). The effect of temperature on the oxygen consumption and filtration rate of *Chlamys* (*Aquipecten*) *operularis* (L.) (Bivalvia) *Ophelia*, *10*, 141 - 154.

Morris, I. (1973). *An introduction to the Algae.* Hutchinson Univ. Press. London.

Newell, R.C. (1979). *Biology of Intertidal Animals.* 3rd Ed. Mar. Ecol. Surv. Faversham, Kent, U.K. 1 - 781.

Newell, R.C. (1980). The maintenance of energy balance in marine invertebrates exposed to changes in environmental temperature. In: *Animals and Environmental fitness.* Proc 1st. congr. Eur. soc comp. phys. bioch. Pergamon (in press).

Newell, R.C., Johnson, L.G. and Kofoed, L.H. (1977). Adjustment of the components of energy balance in response to temperature change in *Ostrea edulis.* *Oecologia* (Berl.) *30*, 97 - 100.

Newell, R.C. and Kofoed, L.H. (1977a). The energetics of suspension feeding in the gastropod *Crepidula fornicata* L. *J. mar. biol. Ass. U.K., 51*, 161 - 180.

Newell, R.C. and Kofoed, L.H. (1977b). Adjustment of the components of energy balance in the gastropod *Crepidula fornicata* in response to thermal acclimation. *Mar. Biol., 44*, 275 - 286.

Newell, R.C. and Pye, V.I. (1971a). Quantitative aspects of the relationship between metabolism and temperature in *Littorina littorea* (L.). *Comp. Biochem. Physiol.*, *38 B*, 635 - 650.

Owen, G. (1966a) Feeding. In : *Physiology of Mollusca* (ed. K.M. Wilbur and C.M. Yonge). *2*, 1 - 51. Academic Press N.Y. and London.

Owen, G. (1974a). Studies on the gill of *Mytilus edulis*: the eu-latero-frontal cirri. *Proc. Roy. soc. Lond. Ser. B*, *187*, 83 - 91

Owen, G. (1974b). Feeding and digestion of the bivalvia. *Adv. Comp. Physiol. Biochem.*, 5,1 - 35.

Parsons, T.R. Stephens, K. and Strickland, J.O.H. (1961). On the chemical composition of 11 species of marine phytoplankters. *J. Fish. Res. Bd. Can.*, *18*(b), 1001 - 1015.

Precht, H. (1958). Concepts of the temperature adaptations of unchanging reaction systems of cold-blooded animals. In : *Physiological adaptation* (ed. C.L. Prosser), 50 - 78. Ronald Press Co. N.Y.

Precht, H., Christopherson, J., Hensel, H. and Larcher, W (1973). *Temperature and Life*. 1 - 761. Springer verlag Berlin, Heidelberg. N.Y.

Prescott, G.W. (1969). *The Algae : a review*. Riverside studies in biology. Nelson.

Prosser, C.L. (1973). *Comparitive animal physiology* 3rd ed. 1 - 966. Saunders Philadelphia.

Pruder, G.W., Bolton, E.T. and Faunce, S.F. (1977). System configuration and performance in bivalve molluscan mariculture. *Delaware sea grant technical report, Del S.G.* 1 - 77.

Purchon, R.O. (1968). *The biology of the Mollusca*. Pergamon Press Oxford.

Read, K.R.H. (1962a). Respiration of the bivalve molluscs *Mytilus edulis* L. and *Brachidontes demissus plicatulus* Lamarck as a function of size and temperature. *Comp. Biochem. Physiol.*, 7, 89 - 101.

Ricker, W.E. (1968). Methods for assessment of fish production in fresh waters. *IBP Handbook No. 3*, 1 - 313. Blackwell, Oxford.

Schulte, E.H. (1975). Influence of algal concentration and temperature on the filtration rate of *Mytilus edulis*. *Mar. Biol. 30*, 331 - 341.

Stacey, V.J. (1976). An ultrastructural study of the various strains of *Hymenomonas carterae*. Unpubl. MSc thesis. Univ. of Witwatersrand, Johannesburg, South Africa.

Thompson, R.J. and Bayne, B.L. (1972). Active metabolism associated with feeding in the mussel *Mytilus edulis* *J. exp. mar. Biol. Ecol., 8*, 191 - 212.

Thompson, R.J. and Bayne, B.L. (1974). Some relationships between growth, metabolism and food in the mussel, *Mytilus edulis*. *Mar. Biol., 27*, 317 - 326.

Vahl, O. (1972). Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* (L) (Bivalvia). *Ophelia, 10*, 67 - 74.

Vahl, O. (1973a). Porosity of the gill, oxygen consumption and pumping rate in *Cardium edule* (L.). (Bivalvia). *Ophelia, 10*, 109 - 118.

Vahl, O. (1973b). Pumping and oxygen consumption rates of *Mytilus edulis* L. of different sizes. *Ophelia, 14*, 45 - 52.

Walne, P.R. (1956). Experimental rearing of the larvae of *Ostrea edulis* L. in the laboratory. *Fish, Inv. Min. Agri. food. fish. Lond., Ser. 11, 20,* 1 - 23.

Walne, P.R. (1958a). The importance of bacteria in laboratory experiments on rearing the larvae of *Ostrea edulis* (L.). *J. mar. biol. Ass. U.K., 37,* 415 - 425.

Walne, P.R. (1958b). Growth of oysters (*Ostrea edulis* L.) *J. mar. biol. Ass. U.K., 38,* 591 - 602.

Walne, P.R. (1963a). The culture of marine molluscs and crustacae. In : *The better use of the worlds fauna for food* (ed. J.D. Ovington). 147 - 175. Institute of Biology, London.

Walne, P.R. (1963b). Observations on the food value of seven species of algae to the larvae of *Ostrea edulis* 1. Feeding experiments. *J. mar. biol. Ass. U.K., 43,* 767 - 784.

Walne, P.R. (1965). Observation on the influence of food supply and temperature on the feeding and growth of the larvae of *Ostrea edulis* L. *Fish. Inv. Min. Agri. Food. Fish. Lond., Ser. 11, 24,* 1 - 45.

Walne, P.R. (1966). Experiments in the large scale culture of the larvae of *Ostrea edulis* L. *Fish. Inv. Min. Agri. Food. Fish. Lond., Ser. 11, 25,* 1 - 53.

Walne, P.R. (1970a). Present problems in the culture of the larvae of *Ostrea edulis. Helgol. wiss. meersunters., 20,* 514 - 525.

Walne P.R. (1970b). The seasonal variation of meat and glycogen content of seven populations of oysters *Ostrea edulis* L. and a review of the literature. *Fish. Inv. Min. Agri. Food. Fish. Lond., Ser. 11, 26,* 35pp.

Walne, P.R. (1970c). Studies on the food of 19 genera of algae to juvenile bivalves of the genera *Ostrea, Crassostrea, Mercenaria* and *Mytilus. Fish. Inv. Min. Agri. Food. Fish, Lond., Ser. 11,* 26(5), 62pp.

Walne, P.R. (1972). The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves. *J. mar. biol. Ass. U.K., 52,* 345 - 374.

Warren, C.E. and Davis, G.E. (1967). Laboratory studies on the feeding bioenergetics and growth of fish. In: *The biological basis of freshwater fish production* (ed. S.O. Gerking), 175 - 214. Blackwell Scientific Publ. Oxford.

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

Widdows, J. (1973a). Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis*. *Mar. Biol.*, *20*, 269 - 276.

Widdows, J. (1973b). The effects of temperature on the metabolism and activity of *Mytilus edulis* L. *Neth. J. sea. Res.*, *7*, 387 - 398.

Widdows, J. (1978a). Combined effects of body size, food concentration and season on the physiology of *Mytilus edulis*. *J. mar. biol. Ass. U.K.* *58*, 109 - 124.

Widdows, J. and Bayne, B.L. (1971). Temperature acclimation of *Mytilus edulis* with reference to its energy budget. *J. mar. biol. Ass. U.K.*, *51*, 827 - 843.

Widdows, J., Fieth, P. and Worrail, C.M. (1979). Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. *Mar. Bio.*, *50*, 195 - 207.

Wilbur, K.M. and Jodrey, L.H. (1952). Studies on shell formation. 1. Measurement of the rate of shell formation using Ca^{45}. *Biol. Bull.*(Woods Hole), *103*, 269 - 276.

Winberg, G.G. (1956). Rate of metabolism and food requirements of fishes (Translated from Russian by *Fish. Res. Bd. Can. Translation series, 194*, 1960).

Winter, J.E. (1969). Influence of food concentration and other factors on filtration rate and food utilization in *Arctica islandica* and *Modiolus modiolus*. *Mar. Biol.*, *4*(2), 87 - 135.

Winter, J.E. (1970). Filter feeding and food utilization in *Arctic islandica* L. and *Modiolus modiolus* L. at different food concentrations. In: *Marine food chains* (ed. J.H. Steele), 196 - 206. Univ. Calf. Press Berkley California.

Winter, J.E. (1973). The filtration rate of *Mytilus edulis* and its dependance on algal concentration, measured by a continuous automatic recording apparatus. *Mar. Biol.*, *22*, 317 - 328.

Winter, J.E. (1975). Feeding experiments with *Mytilus edulis* L. at small laboratory scale. 11. The influence of suspended silt in addition to algal suspensions on growth. *10th. Eur. Sym. Mar. Biol. Ostend. Belgium. 1*, 583 - 600.

Winter, J.E. (1977a). Suspension feeding in lamellibranchiate bivalves with particular reference to aquaculture. *Medio-Ambiente*, 3(1), 48 - 69.

Winter, J.E. (1977b). A critical review on some aspects of filter-feeding in lamellibranchiate bivalves. *Haliotis*, 7.

Winter, J. E. (1978). A review on the knowledge of suspension feeding in lammelibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture*, 13, 1 - 33.

Winter, J.E. and Langton, R.W. (1976). Feeding experiments with *Mytilus edulis* L. at small laboratory scale. 1. The influence of the total amount of food ingested and food concentration on growth. *10th. Eur. Sym. Mar. Biol. Ostend Belgium.*, 1, 565 - 581.

Zeuthen, E. (1953). Oxygen uptake as related to body size in organisms. *Quart. Rev. Biol.*, 28, 1 - 12.

APPENDIX 1 : Preparation of Erd Scheiber culture medium (as prepared at the Plymouth Laboratory)

1 litre filtered sea water

0,2g $NaNO_3$

*50ml soil extract

0,02g $Na_2HPO_4.12H_2O$

1. Filter sea water through No 1 Whatman paper.
2. Reduce sea water to 95% with glass distilled water and then autoclave it at 15 lb pressure to sterilize (about 30 minutes fo 2 litre volume).
3. Autoclave *soil extract at 15 lb pressure for 35 minutes using a separate small flask for soil extract for each large flask to medium to be made up.
4. Autoclave the solution of salts (made up together in glass distilled water so that 1ml of solution gives the required amount of salts for 1 litre of culture solution).
5. Add required amount of solution of salts to cold soil extract and then add the soil extract + salts to the cold sea water. Allow this culture solution to reach the temperature of the cultures to be subcultured before using.

*Soil Extract

1Kg finely sieved garden soil to 1 l tap water; autoclave for 1 hour at 15 lb pressure. If there is time, allow soil to settle and use clear liquid, but if needed quickly centrifuge to clear.

APPENDIX 2 : Preparation of Walne's culture medium (Walne, 1970a).

1.

$FeCl_3 6H_2O$	2,60g
$MCnl_2 4H_2O$	0,72g
H_3BO_3	67,20g
E D T A (Na salt)	90,00g
$NaH_2PO_4H_2O$	40,00g
$NaNO_3$	200,00g
Trace metal solution	2,00ml
Distilled water	to 2 litres

1ml is added to each litre of sea water

2. The trace metal solution has the following composition :

$ZnCl_2$	2,1 g
$CoCl_2 6H_2O$	2,0 g
$(NH_2)_6Mo.O_{24}4H_2O$	0,9 g
$CuSO_4 5H_2O$	2,0 g
Distilled water	to 100 ml

It is necessary to acidify this solution with HCl to obtain a clear liquid.

3. Vitamin : stock solution

B_{12}	10mg
B_1 (Thiamine)	200mg
Distilled water	to 200ml

10ml are added to each 100 litres of sea water.

APPENDIX 3 : Calculations used to determine the percentage daily mass loss at zero ration and the daily maintainance, in carbon equivalents for T_A 5°C.

NOTES :

1. Mean V_{O_2} ($\mu l.5,0883 mg^{-1}.h^{-1}$)
2. Mean V_w ($ml.5,0883 mg^{-1}.h^{-1}$)
3. Assimilation efficiency = 76,43%
4. $1 \mu l^{-1}$ oxygen = 0,536µg C
5. Carbon equivalent of a standard animal of 5,0883 mg = 2,54mg

Carbon equivalent of V_{O_2} = 7,90 x 0,536 µg

($\mu gC.h^{-1}$) = 4,23

Carbon equivalent of V_{O_2} = 4,23 x 24

($\mu gC.24h^{-1}$) = 101,52

% Daily mass loss $= \left(\frac{101,52}{1\ 000 \times 2,54}\right) \times 100$

= - 3,99%

Carbon rewuired to maintain body mass = 101,52 x 100/76,43

($\mu gC.24h^{-1}$) = 132,82

APPENDIX 4 : Calculations used to determine the predicted percentage daily mass loss for juvenile *Ostrea edulis*, in carbon equivalents, e.g. T_A 5°C.

NOTES :

1. Carbon equivalent of a standard animal of 5,0883mg = 2,54mg.
2. Assimilation efficiency = 76,43%.
3. Clearance is assumed to be 100%
4. Mean V_w ($ml.5,0883mg^{-1}.h^{-1}$) = 32,40 (APP. 3)
5. Carbon equivalent of V_{O_2} ($\mu gC.24h^{-1}$) = 101,52 (APP. 3)

V_w per day ($ml.5,0883mg^{-1}.h^{-1}$) = 32,40 x 24

= 777,6

At each particle concentration :

e.g. $13,50\mu gC.l^{-1}$

ugC cleared per day (at $13,50\mu gC.l^{-1}$) = $777,6 \times \frac{13,50}{1\ 000}$

= 10,49

% Daily mass change = $\frac{(10,49 \times 0,7643)-101,52}{2540} \times \frac{100}{1}$

= -3,68

APPENDIX 5 : Independant variables and their transformations used in the stepwise regression analysis described in section 2.3.7.

IND. VARIABLE	ACCLIMATION TEMP. (T_A)	EXPOSURE TEMP. (T_E)
TRANSFORMED VARIABLES	$T_A2 = (T_A)^2$	$T_E2 = (T_E)^2$
	$T_A3 = (T_A)^3$	$T_E3 = (T_E)^3$
	$T_A4 = (T_A)^4$	$T_E4 = (T_E)^4$
	$T_A\ T_E$	
	T_A2T_E	
	T_A3T_E	
	T_A4T_E	
	$T_A\ T_E4$	
	$T_A\ T_E3$	
	$T_A\ T_E2$	