PHYSIOLOGICAL INDICES OF STRESS IN *MYTILUS EDULIS*

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(Figs. 1–9)

Multiple regression equations and response surfaces are used to describe the combined effects of body size, food concentration, acclimation temperature and season on physiological integrations such as the scope for growth, growth efficiency and O:N ratio. Maintenance and optimum ration levels are estimated for *Mytilus edulis* adapted to different factor combinations. Response surfaces illustrate the validity and sensitivity of the physiological integrations in quantifying the 'physiological condition' and the degree of stress experienced, under conditions ranging from near-optimal, through sub-lethal to lethal.

INTRODUCTION

In nature, organisms are subjected to a variety of environmental factors acting together and they respond to the total resulting stimulus rather than to single environmental variables. In addition, the animal's response is not in terms of individual physiological rates but rather as a whole organism. Therefore an experimental approach to an animal's response to a complex environment could usefully include (*a*) multivariate experiments and (*b*) integration of individual physiological processes to provide a greater understanding of the 'whole organism' response. In this paper, non-linear multiple regression equations and response surfaces are used to describe and illustrate the combined effects on the common mussel, *Mytilus edulis* L., of body size, ration and season on physiological integrations such as the scope for growth, growth efficiency and the oxygen: nitrogen ratio (reviewed by Bayne, 1975; Bayne, Widdows & Thompson, 1976). The data used to compute these physiological integrations are derived from a series of multivariate experiments described in a preceding paper (Widdows, 1978).

The aims of this research were twofold. Firstly, to provide a greater understanding of the factors affecting the bioenergetics and growth of a dominant suspension feeder in the estuarine environment, and secondly to demonstrate the sensitivity and validity of some physiological stress indices that could be used in experimental and environmental studies to assess the physiological condition of animals subjected to different degrees of environmental stress.

MATERIALS AND METHODS

The combined effects of body size, ration and seasonal acclimation temperature on the physiological responses of *Mytilus edulis* were studied in a series of multivariate experiments and these have been described in a preceding paper (Widdows, 1978). The analysis and interpretation of data derived from these experiments is taken a stage further in this paper, with the integration of individual physiological responses into measures of growth, condition and efficiency. The rates of oxygen consumption and filtration, together with assimilation efficiency, have been converted into calorific equivalents and integrated by means of the basic energy equation of Winberg (1956).

The primary components of the energy budget are described by the following equations, using I.B.P. terminology (Crisp, 1971):

$$C = R + P + F,$$

$$C - F = A = R + P,$$

where C = energy value of food consumed, F = energy value of waste products (faecal and excretory material), A = energy value of assimilated food, R = energy metabolically utilized and P = energy incorporated into the biomass of the organism (growth and gametes).

The energy available for growth or the scope for growth represents the energy balance of an animal under a given set of conditions. It is determined by subtraction of total metabolism (R) from assimilated food (A) after conversion to energy equivalents. The concept of 'scope for growth' (Warren & Davis, 1967; Widdows & Bayne, 1971; Bayne, Thompson & Widdows, 1973; Thompson & Bayne, 1974) provides an index of energy balance without the need to specify the use to which the energy is put (e.g. gametes or somatic growth). Growth is here regarded as the total change in energy content of body materials. Scope for growth may be positive, when surplus energy is available for growth and/or reproduction; it may also be negative, in which case weight is lost due to the utilization of energy reserves in the tissues.

The calculation of scope for growth is as follows:

$$C \text{ (cal day}^{-1}\text{)} = \text{filtration rate (1 day}^{-1}\text{)} \times \text{food concentration (mg l}^{-1}\text{)}$$
$$\times \text{calorific content of food (cal mg}^{-1}\text{)};$$

$$A \text{ (cal day}^{-1}\text{)} = C \times \% \text{ assimilation efficiency};$$

$$R \text{ (cal day}^{-1}\text{)} = \text{metabolic rate (ml O}_2 \text{ consumed day}^{-1}\text{)} \times 4 \cdot 86;$$

$$\text{scope for growth (cal day}^{-1}\text{)} = \text{A-R}.$$

The scope for growth is therefore readily computed from measurements of filtration rate, assimilation efficiency and respiration rate. The calorific value of the food *Phaeodactylum tricornutum*, determined using a Phillipson microbomb calorimeter, was 3·9 cal per mg dry weight. In this paper, ration or the food concentration surrounding and available to the animal is expressed in terms of mg dry algal matter per litre. It can, however, be converted into ingested ration (filtration rate × ration) and also ingested ration as a percentage of body weight, in order to provide comparisons with other published data.

Growth efficiency is the efficiency with which food is converted into body tissues. Gross growth efficiency is $K_1 = (A - R)/C$, where K_1 is the growth per unit of ingested ration. Relationships between growth efficiency and ration provide a useful means of comparing growth data for individual animals in relation to body size, ration, temperature and other factors (Paloheimo & Dickie, 1965, 1966a, b; Thompson & Bayne, 1974).

A third physiological integration used in this study is the oxygen:nitrogen ratio (O:N). This is the ratio of oxygen consumed to nitrogen excreted, in atomic equivalents; it provides an indication of the balance in the animal's tissues between the rates of catabolism of protein, carbohydrate and lipid substrates (Corner & Cowey, 1968; Bayne, 1973a, 1975; Bayne et al. 1976). A low value for O:N indicates that mainly protein is being utilized; whereas a high value indicates catabolism of carbohydrate and/or fat. The theoretical minimum for the O:N ratio is approximately 7 (Mayzaud, 1973), signifying catabolism based entirely upon protein.

These physiological indices of condition and stress were computed from the individual regression equations of oxygen consumption, filtration rate, assimilation efficiency and ammonia excretion against body size (tables 1–4 in Widdows, 1978). Scope for growth and growth efficiency were then described by multiple regression equations, obtained by the method of polynomials, and presented as response surfaces as a function of body size, ration, seasonal cycle and acclimation temperature. A response surface has also been constructed to show the effects of ration and season on the O:N ratio of *Mytilus edulis*, but an adequate equation describing this surface was not obtained. Details of the data handling techniques used in this study have been described in a preceding paper (Widdows, 1978).

Multiple regression equations and response surfaces provide a concise and coherent method of describing the large amount of data derived from these multivariate experiments. Each multiple

Table 1. *A summary of data for ingested ration, metabolism and growth at 10 °C in April*

Dry weight (g)	Food concentration (mg l⁻¹)	Filtration rate (l day⁻¹)	Ingested ration (mg day⁻¹)	Calories ingested (cal day⁻¹), C	Assimilation efficiency (%)	Calories assimilated (cal day⁻¹), A	Total metabolism (ml O₂ day⁻¹)	Metabolism (cal day⁻¹), R	Scope for growth (cal day⁻¹), $A-R$	Gross growth efficiency K_1, $(A-R/C)$
0·100	0	—	0	0	—	0	1·63	7·93	− 7·93	—
	0·14	25·65	3·59	14·02	83	11·64	1·63	7·93	+ 3·71	+0·26
	0·28	25·65	7·19	28·04	76	21·31	1·63	7·93	+ 13·38	+0·48
	0·56	25·65	14·38	56·08	52	29·16	1·63	7·93	+ 21·23	+0·38
	1·40	25·65	35·95	140·21	25	35·05	2·64	12·83	+ 22·22	+0·16
0·200	0	—	0	0	—	0	2·57	12·48	− 12·48	—
	0·14	33·60	4·70	18·35	83	15·23	2·57	12·48	+ 2·75	+0·15
	0·28	33·60	9·41	36·69	76	27·88	2·57	12·48	+ 15·40	+0·42
	0·56	33·60	18·82	73·40	53	40·37	2·57	12·48	+ 27·89	+0·38
	1·40	33·60	47·04	183·46	26	47·69	3·72	18·08	+ 29·61	+0·16
0·500	0	—	0	0	—	0	4·68	22·75	− 22·75	—
	0·14	47·28	6·62	25·81	83	21·43	4·68	22·75	− 1·32	−0·05
	0·28	47·28	13·23	51·63	76	39·24	4·68	22·75	+ 16·49	+0·32
	0·56	47·28	26·48	103·26	55	56·79	4·68	22·75	+ 34·04	+0·33
	1·40	47·28	66·19	258·14	31	80·02	5·88	28·58	+ 51·44	+0·19
1·000	0	—	0	0	—	0	7·32	35·58	− 35·58	—
	0·14	61·20	8·57	33·42	83	27·73	7·32	35·58	− 7·85	−0·23
	0·28	61·20	17·14	66·83	76	50·79	7·32	35·58	+ 15·21	+0·23
	0·56	61·20	34·27	133·66	58	77·52	7·32	35·58	+ 41·94	+0·31
	1·40	61·20	85·68	334·15	39	130·32	8·40	40·82	+ 89·49	+0·27
2·000	0	—	0	0	—	0	11·64	56·57	− 56·57	—
	0·14	79·20	11·09	43·24	83	35·89	11·64	56·57	− 20·68	−0·48
	0·28	79·20	22·18	86·48	76	65·73	11·64	56·57	+ 9·16	+0·11
	0·56	79·20	44·35	172·97	62	107·24	11·64	56·57	+ 50·67	+0·29
	1·40	79·20	110·88	432·43	47	203·24	11·88	57·74	+145·50	+0·33

N.B. Additional data for the food concentration 0·14 mg l⁻¹ are based on evidence that *Mytilus edulis* maintains a relatively constant filtration rate and metabolic rate down to at least 1000 cells ml⁻¹ or 0·07 mg l⁻¹ (Thompson & Bayne, 1974). This prevents an erroneous extrapolation between the food levels 0–0·28 mg l⁻¹.

regression equation (*a*) describes the relationship between a dependent variable and two or three independent variables, (*b*) enables the calculation of those response surfaces not presented in this paper, and (*c*) can be used for interpolating between data points.

RESULTS AND DISCUSSION
Scope for growth

The basic components of the energy equation for *Mytilus edulis* were calculated for each experimental condition. A typical example of the conversion of feeding rate, food assimilation and metabolism into calorific equivalents, and the subsequent calculation of scope for growth, is presented in Table 1 (other tables summarizing the data for 5 °C (Feb.), 10 °C (Oct.), 15 °C (June), 20 °C (Aug.) and 25 °C are on file and available from the author). Scope for growth of *Mytilus edulis* in response to body size and ration
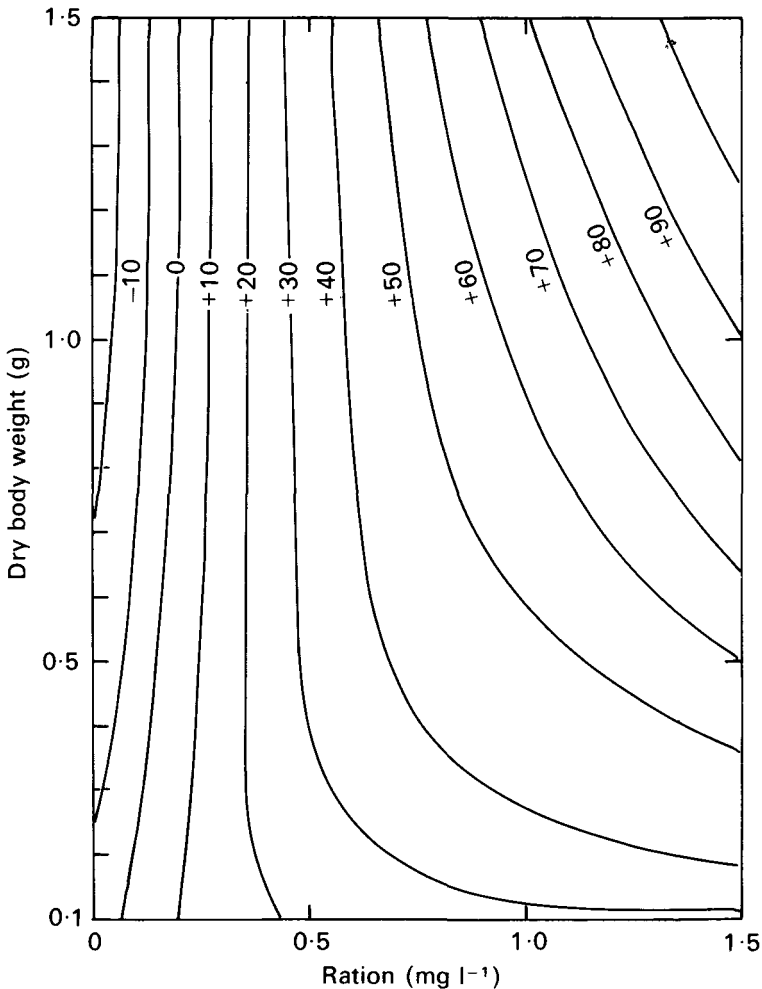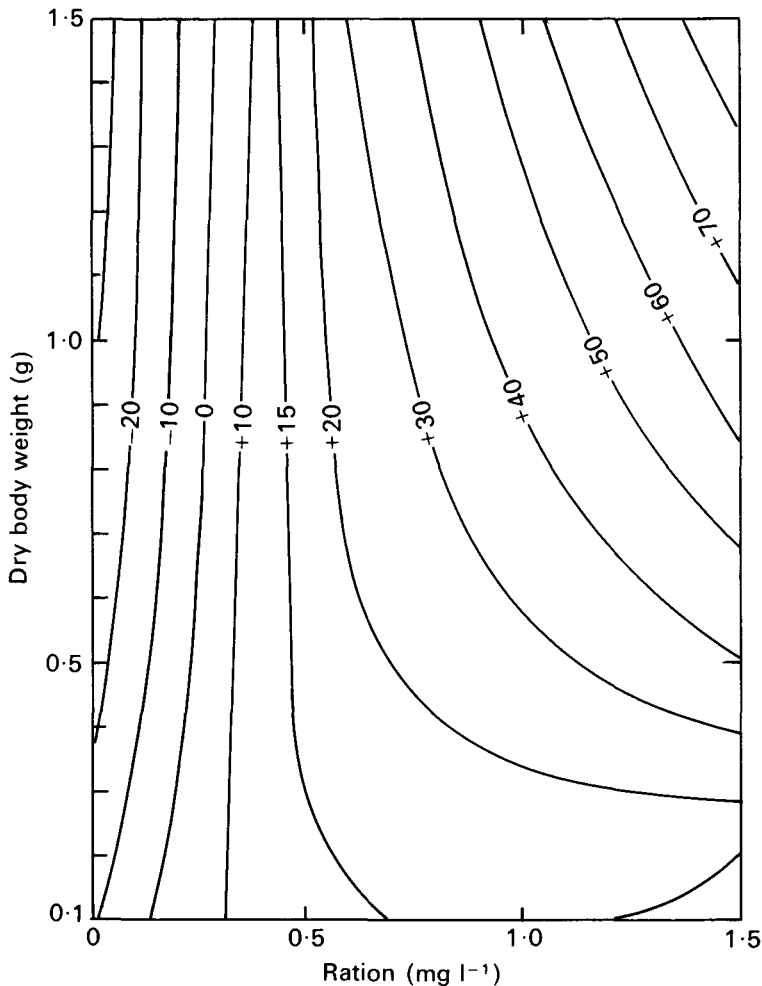

Fig. 1. Isopleths of scope for growth of *Mytilus edulis* (cal day^{-1}) as a function of available ration and dry body weight; data for animals acclimated to 10 °C in April.

throughout the seasonal cycle at ambient temperature, is described by the following multiple regression equation:

scope for growth

$$
\begin{aligned}
= &-1\cdot87188 - (0\cdot209459)\,T - (0\cdot0534796)\,T^2 + (0\cdot0118615)\,T^3 \\
&- (0\cdot74507 \times 10^{-3})\,T^4 + (0\cdot172659 \times 10^{-4})\,T^5 - (0\cdot132585 \times 10^{-6})\,T^6 \\
&+ (93\cdot4622)\,R - (112\cdot585)\,R^2 + (36\cdot466)\,R^3 - (27\cdot3694)\,W \\
&+ (7\cdot3887)\,W^2 - (1\cdot26195)\,W^3 - (0\cdot347338)\,WT + (82\cdot3995)\,WR \\
&+ (1\cdot10224)\,TR + (0\cdot604698 \times 10^{-2})\,WT^2 - (5\cdot6765)\,WR^2 \\
&- (11\cdot1803)\,RW^2 - (0\cdot0230567)\,RT^2,
\end{aligned}
$$

where scope for growth is expressed in cal day^{-1}, W = body weight (0·1–3·0 g dry weight), T = time in weeks (1–52) and R = ration (from 0 to 1·5 mg l^{-1}). This equation

Fig. 2. Isopleths of scope for growth of *Mytilus edulis* (cal day^{-1}) as a function of available ration and dry body weight; data for animals acclimated to 20 °C in August.

explains 97 % of the total variance in the data (175 data points) and therefore provides a good fit to the experimental results. A series of response surfaces, representing scope for growth as a function of two independent variables, can be generated from this equation. Examples of two such surfaces, where scope for growth is plotted against body weight and ration, are illustrated in Figs. 1 and 2.

The surface in Fig. 1 is for animals acclimated to 10 °C in April (week 14) and shows the effect of ration on the scope for growth with increasing body size. The energy available for growth, measured in terms of calories per day, increases with ration and body weight. In small animals (100 mg dry wt), the maximum growth rate is attained at approximately 0·8 mg dry algal matter per litre and increases in ration above this concentration do not have any further effect on scope for growth. In larger individuals however, the maximum growth rate is not achieved at food concentrations between 0 and 1·5 mg l^{-1}.

An important feature of the response surface is the identification of the maintenance ration or the ration level at which the animal neither gains nor loses weight. This is denoted by the isopleth for zero growth. The area represented by a negative scope for growth indicates the conditions under which the organism utilizes energy reserves stored in its own tissues.

Following Fry (1947), this region of negative scope for growth, in which an organism cannot survive indefinitely, can be referred to as the zone of resistance, and the region of positive scope for growth, the zone of tolerance.

Fig. 2 illustrates the effect of body size and ration on the scope for growth at 20 °C in August (week 35). Although the pattern of response is similar to that recorded in the spring (Fig. 1), the combined effects of higher seasonal temperatures and changes in reproductive condition result in a shift in the maintenance ration to higher food concentrations for animals of all sizes. The overall lower scope for growth is mainly a consequence of the higher metabolic rates recorded in the summer.

The lower maintenance ration or food requirement in winter would be advantageous at a time of year when available food is at a minimum.

The combined effects of seasonal acclimation temperatures between 5 and 25 °C, ration and body weight on the scope for growth of *Mytilus* are described by the following equation:

scope for growth
$$
\begin{aligned}
={}& -18{\cdot}0199 - (49{\cdot}3266)\,W + (15{\cdot}103)\,W^2 - (2{\cdot}93893)\,W^3 \\
& + (11{\cdot}8966)\,C - (2{\cdot}10923)\,C^2 + (0{\cdot}123443)\,C^3 - (0{\cdot}00230472)\,C^4 \\
& + (67{\cdot}9551)\,R - (122{\cdot}588)\,R^2 + (39{\cdot}6555)\,R^3 + (3{\cdot}25855)\,WC \\
& + (76{\cdot}9401)\,WR + (7{\cdot}1392)\,CR - (0{\cdot}264719)\,RC^2 - (0{\cdot}167631)\,WC^2 \\
& - (11{\cdot}433)\,RW^2,
\end{aligned}
$$

where scope for growth is expressed in cal day^{-1}, W = body weight (0·1–3·0 g dry wt), C = acclimation temperature (5–25 °C) and R = ration (from 0 to 1·5 mg l^{-1}).

The equation explains 98 % of the total variance in the data (125 data points). Two surfaces have been plotted (Figs. 3 and 4) using this equation, to illustrate the responses of large (1 g dry weight) and small (0·2 g) animals to increasing seasonal acclimation

temperatures and ration levels. The scope for growth of a large animal increases at higher food concentrations and decreases with increasing temperatures above 17 °C (Fig. 3).

Between 5 and 20 °C there is complete temperature acclimation of filtration rate and metabolism by *Mytilus edulis* (Widdows, 1973; Bayne *et al.* 1973), and as a result the scope for growth is relatively independent of temperature over this range.
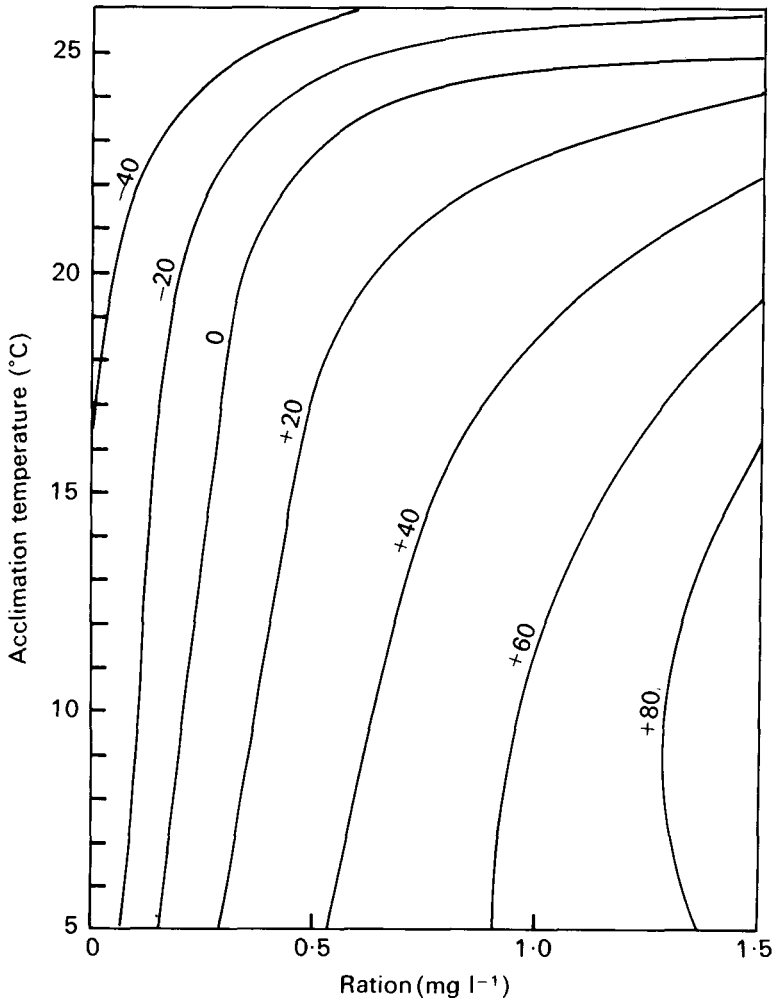

Fig. 3. Isopleths of scope for growth of *Mytilus edulis* (cal day⁻¹) as a function of available ration and acclimation temperature; data for an animal of 1 g dry weight.

However, above 20 °C there is a breakdown in mechanisms of temperature adaptation, resulting in an increase in the metabolic rate and a decline in filtration rate (Widdows, 1973, 1976). This is reflected in a reduced scope for growth, so that above 25 °C there is no energy available for growth and the animal has to utilise its energy reserves in order to survive.

This physiologically determined response provides a greater understanding of the

processes limiting growth at high temperatures and corresponds to both field observa-
tions and the maximum limit of thermal tolerance. Wells & Gray (1960) have stated
that the southern limit of distribution for *Mytilus edulis* coincided with a mean summer
sea-water temperature of 26·7 °C. More recently, thermal tolerance studies by Read &
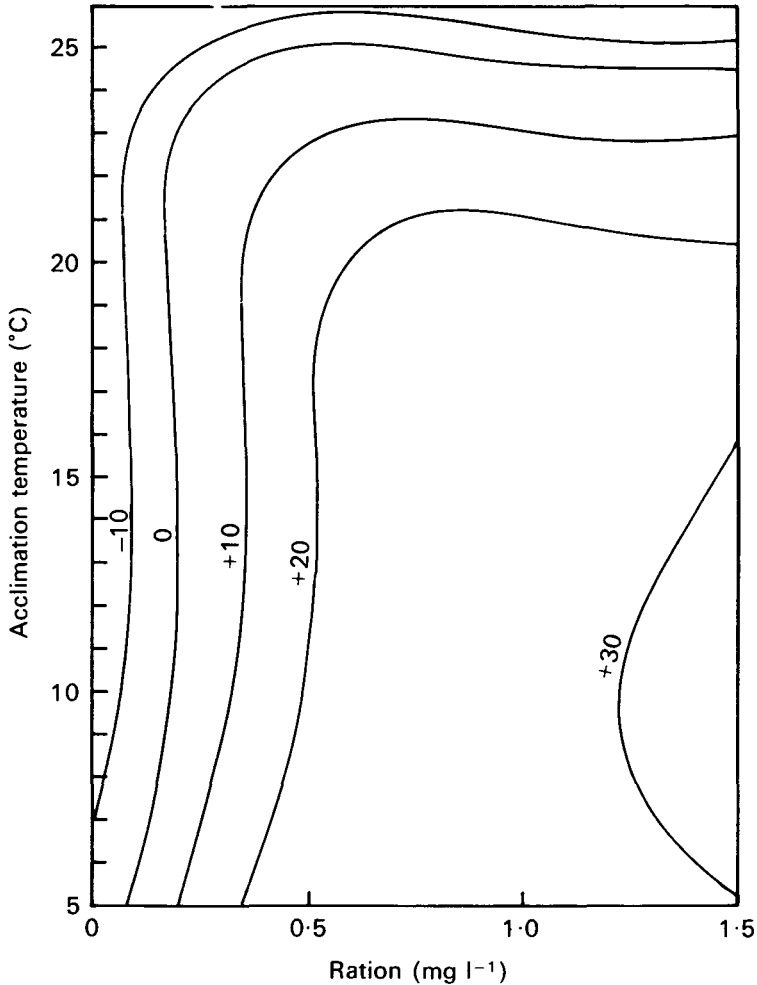Cumming (1967), Wallis (1975) and Bayne, Widdows & Worrall (1977) have recorded

Fig. 4. Isopleths of scope for growth of *Mytilus edulis* (cal day^{-1}) as a function of available
ration and acclimation temperature; data for an animal of 0·2 g dry weight.

upper lethal temperatures for *Mytilus edulis* between 27 and 28·2 °C. Evidence suggests
that the upper lethal limits could be reduced to lower temperatures, closer to 25 °C, by
increasing the time course from weeks to months in median mortality-time experiments.
For example, scope for growth is negative during starvation, but death would not occur
in a mortality study until up to 6 months of starvation (Thompson, Ratcliffe & Bayne,
1974).

The configuration of the response surface indicates that at high temperatures above 25 °C there is little interaction between ration and temperature, and as a result the upper thermal limit of growth is not significantly affected by increasing the ration level.

The response surface for a 200 mg animal (Fig. 4) shows a similar relationship between scope for growth, ration and temperature, with a marked decline in the energy available for growth at 25 °C, irrespective of ration level. In addition, the isopleths indicate that ration levels above approximately 0·5 mg l^{-1} have relatively little effect on the growth rate.

Gross growth efficiency

The gross growth efficiency or K_1 is the scope for growth per unit of ingested ration. Such a simple index not only indicates the areas or conditions under which the growth of the animal is most efficient but also provides a measure of the most economical use of food. Multiple regression equations have been calculated to describe the effects of body size and ration on the growth efficiency of *Mytilus edulis* at each seasonal acclimation temperature. These equations are presented in Table 2.

Table 2. *Equations describing the gross growth efficiency of* M. edulis *at five acclimation temperatures*

5 °C $K_1 = 0·251777 - (0·0426504)\,W + (0·0804841)\,W^2 - (0·0206052)\,W^3$
$- (0·652635)\,R + (0·361218)\,R^2 + (1·16116)\,R^3 + (0·492353)\,WR$
$- (0·109989)\,RW^2 - (0·211919)\,WR^2$

10 °C $K_1 = 0·241504 - (0·0410542)\,W + (0·0911514)\,W^2 - (0·0238976)\,W^3$
$- (0·786425)\,R + (0·282753)\,R^2 + (1·36804)\,R^3 + (0·747506)\,WR$
$- (0·12951)\,RW^2$

15 °C $K_1 = 0·231171 - (0·167763)\,W + (0·211136)\,W^2 - (0·0515543)\,W^3$
$- (0·76773)\,R + (0·245494)\,R^2 + (1·53133)\,R^3 + (0·79856)\,WR$
$- (0·130526)\,RW^2$

20 °C $K_1 = 0·190186 - (0·0362725)\,W - (0·800444)\,R - (1·15194)\,R^2$
$+ (1·05096)\,WR - (0·201827)\,RW^2 + (2·46706)\,WR^2 + (2·97287)\,WR^3$

25 °C $K_1 = -0·0508724 - (0·217712)\,W + (0·170826)\,W^2 - (0·0401042)\,W^3$
$- (0·272261)\,R + (1·99067)\,R^2 + (3·62257)\,R^3 + (2·34843)\,WR$
$- (1·50684)\,RW^2 - (0·904301)\,WR^2 + (0·306157)\,RW^3$

$K_1 =$ gross growth efficiency, $W =$ dry weight (0·1–2 g) and $R =$ logarithm (to base 10) of ration (mg l^{-1}) All equations explain at least 96 % of the total variance.

A response surface of growth efficiency as a function of body size and ration can be generated from each of the regression equations in Table 2. The response surfaces for animals acclimated to temperatures between 5 and 20 °C all show similar configurations. Two examples have been chosen to illustrate the effects of ration, body size and acclimation temperature on the growth efficiency of *Mytilus edulis*, namely 10 °C (Fig. 5) and 20 °C (Fig. 6). The zero isopleth is again equivalent to the maintenance ration and the limit of growth, the area of negative growth efficiencies represents the zone of resistance, and the region of positive growth efficiencies corresponds to the zone of tolerance.

The relationship between growth efficiency and ration is usually represented by a simple two-dimensional K-line, which can be divided into an ascending phase and a descending phase. Paloheimo & Dickie (1965, 1966a, b) analysed the growth of fishes in terms of growth efficiency and K-lines, but only demonstrated a decline in K_1 with

increasing ration (descending phase) because the ration levels considered were supra-optimal. However, more recent growth studies on fish (Brett, Shelbourn & Shoop, 1969; Kerr, 1971 a, b) and on bivalves (Thompson & Bayne, 1974; Winter, 1974) have shown an ascending phase over lower food concentrations; that is, growth efficiency is an increasing function of ration until an inflection to a negative slope is reached at the
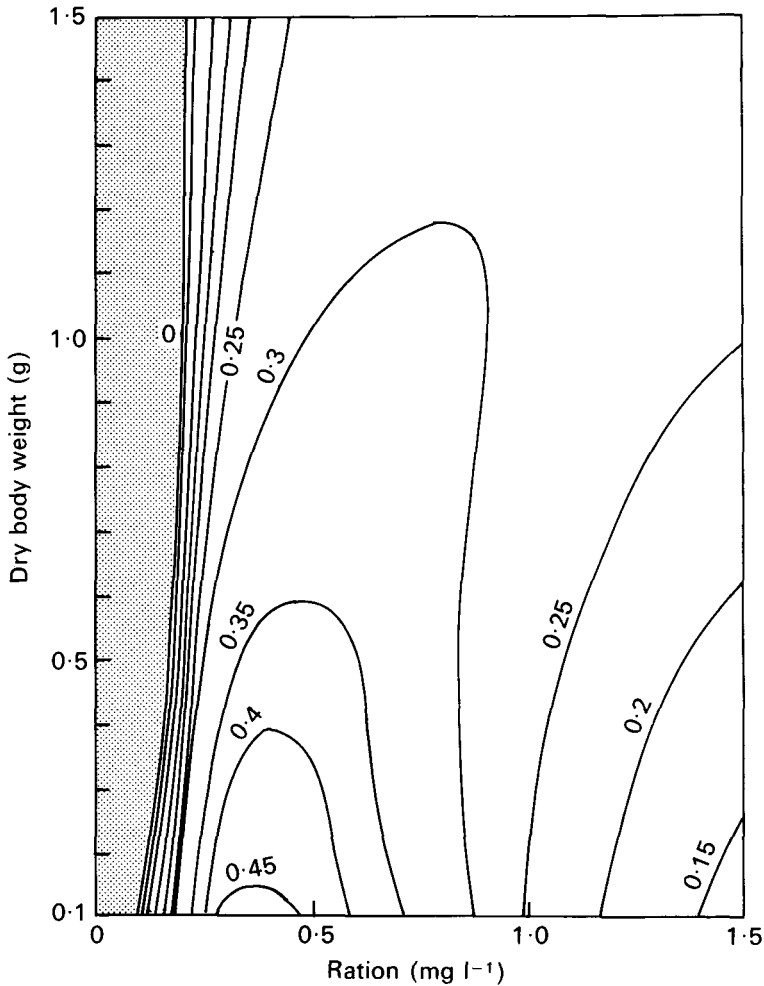

Fig. 5. Isopleths of growth efficiency (K_1) of *Mytilus edulis* as a function of available ration and dry body weight; data for animals acclimated to 10 °C in April. Stippled region represents the area of negative growth.

optimum ration level. (The term optimum is used in this paper to refer to the experimental condition which results in a maximum growth efficiency for a particular body size.)

In this study, growth efficiency shows the same relationship with ration but is presented in the form of surfaces (Figs. 5, 6.)

At very low ration levels *Mytilus edulis* has negative growth efficiencies or K_1 values,

but small increases in ration and therefore food consumption, result in greatly improved growth efficiencies. When the energy assimilated is equal to the total energy metabolized, K_1 is zero and the animal is at the maintenance ration. Further increases in food concentration leads to greater growth efficiencies until an inflexion is reached, above which growth efficiency declines.
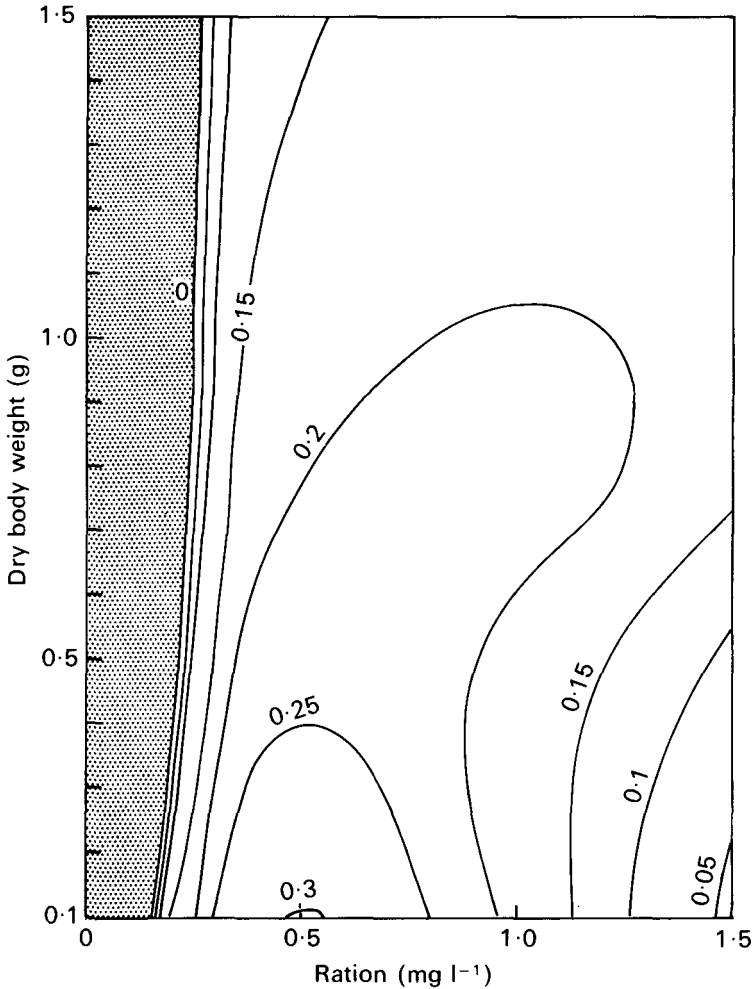

Fig. 6. Isopleths of growth efficiency (K_1) of *Mytilus edulis* as a function of available ration and dry body weight; data for animals acclimated to 20 °C in August. Stippled region represents the area of negative growth.

Growth efficiency of *Mytilus edulis* is also dependent on body size. The response surfaces of K_1 against body size and ration (Figs. 5, 6) show a similar relationship to that previously recorded by Thompson & Bayne (1974). The effect of increasing body size is to reduce the maximum value for K_1 and increase the maintenance ration: A ridge in the response surface (Figs. 5, 6) represents the maximum growth efficiency (K_{max}) at the optimum ration. The optimum ration also increases as a function of body weight,

reflecting the greater energy input required to offset the total metabolism of a larger animal. In small mussels (100–200 mg), maximum growth efficiencies are attained at food concentrations between 0·3 and 0·5 mg l^{-1} (Figs. 5, 6) depending upon acclimation temperature, whereas the K_{max} for animals greater than 1·5 g occurs at ration levels in excess of 1·5 mg l^{-1}.
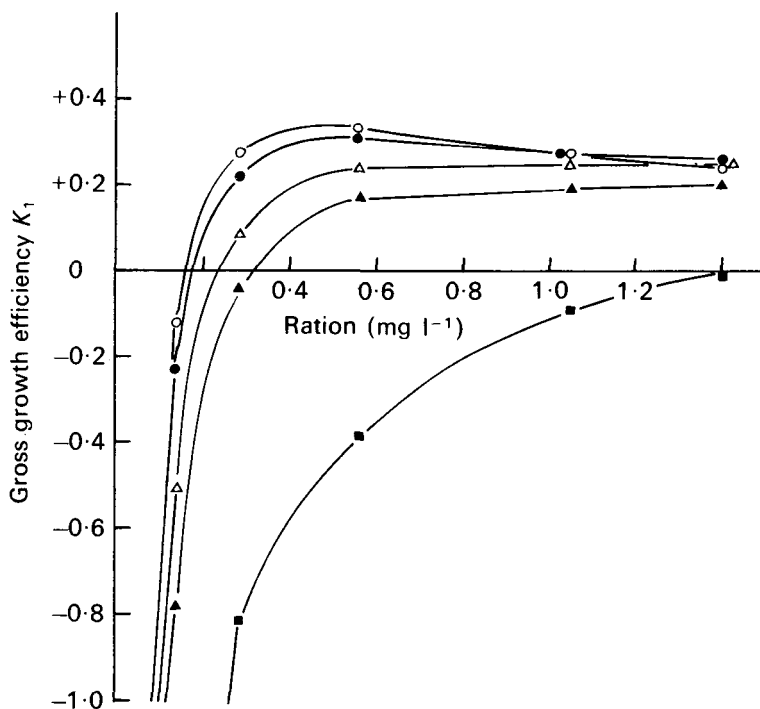

Fig. 7. Effect of acclimation temperature on the relationship between growth efficiency (K_1) of *Mytilus edulis* (1 g dry tissue weight) and available ration. ○, 5 °C; ●, 10 °C; △, 15 °C; ▲, 20 °C; ■, 25 °C.

At low absolute ration levels the small animal is most efficient in converting food into body tissue, although the ration is larger in relation to body weight. Conversely, large animals grow more efficiently at higher absolute ration levels, which are relatively low in comparison to body weight.

The maximum values for gross growth efficiency that are cited in the literature are variable and dependent on several factors, such as species, body size, diet, feeding regime, temperature and salinity. The maximum growth efficiencies for small mussels, recorded in this study, are comparable with those for small fishes in growth studies by Pandian (1967) and Warren & Davis (1967), namely 44% and 37% respectively. The growth efficiencies for large animals, although relatively high, are below those estimated for *Mytilus edulis* by Jørgensen (1952).

The effects of acclimation temperature on the positioning of the K lines for 1 g animals are shown in Fig. 7. An increase in the rate of oxygen consumption, which is associated with an increase in the seasonal ambient temperature and gametogenic

activity, results in reduced growth efficiency, as well as a shift in the maintenance and optimum ration to higher food concentrations. For example, the maximum growth efficiency for a 1 g animal at 10 °C (Figs. 5, 7) is 0·3 (30 %), and declines to 0·2 (20 %) at 20 °C (Figs. 6, 7) in the summer. In addition, the maintenance ration increases from 0·2 to 0·25 mg organic matter per litre and the optimum ration from 0·7 to 1·1 mg l^{-1} with an increase in temperature from 10 to 20 °C. Brett *et al.* (1969) in their study of young sockeye salmon (*Oncorhynchus nerka*) also noted a progressively higher maintenance ration and optimum ration with increasing temperature.
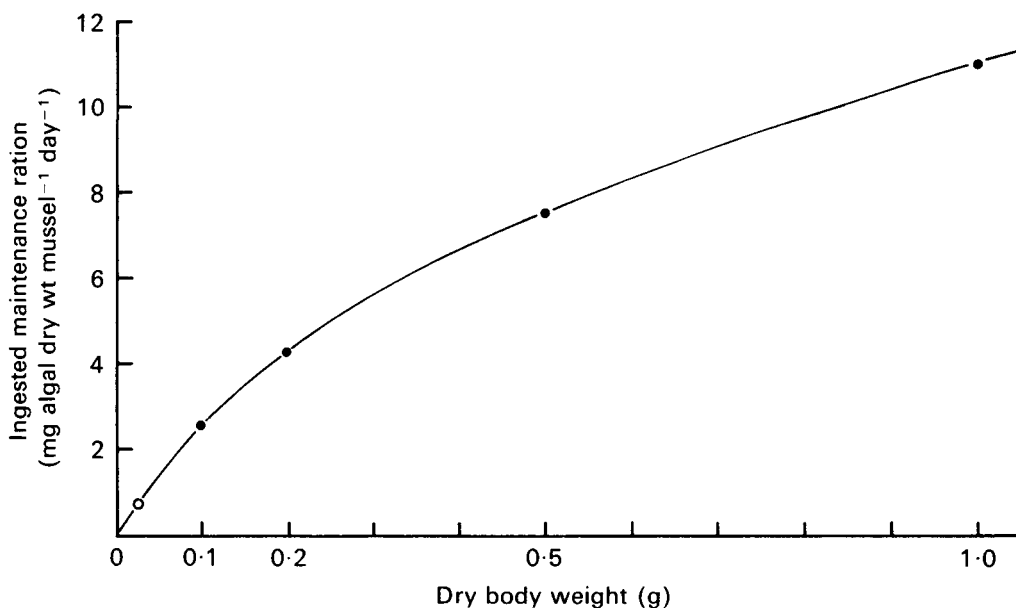

Fig. 8. The relationship between ingested maintenance ration and dry tissue weight of *Mytilus edulis*. ●, From present study; ○, from Winter & Langton (1976).

At the high temperature of 25 °C the growth efficiency of *Mytilus edulis* remains negative over the complete surface as there is no energy available for growth. However, the surface does indicate that smaller individuals have a higher efficiency, albeit slightly negative, than the larger animals. This suggests that the smaller mussels are less stressed and more tolerant of high temperatures, a feature subsequently confirmed in recent thermal tolerance studies (Bayne, Widdows & Worrall, 1977).

At a given ration level, growth efficiency is inversely proportional to the metabolic rate or maintenance cost. Therefore a reduction in the maintenance cost in the autumn and early winter, during a period of gametogenic quiescence, represents the main contributor to the enhanced growth efficiency. This is perhaps ecologically significant because it enables body reserves to be built up rapidly after the termination of spawning in September and before the onset of winter. In addition, it results in a more efficient utilization of food during the winter when ration levels are reduced.

The results of a recent growth study on *Mytilus edulis* by Winter & Langton (1976)

complement the findings from this study. The results are comparable even though the experimental approaches were different. Winter & Langton determined growth and growth efficiency directly on small animals (approx. 30 mg) fed at different ration levels, whereas in this study growth has been estimated from physiological measurements on mussels ranging from 0·1 to 2·0 g. The agreement between the two studies is illustrated in Fig. 8 where the 'ingested maintenance' ration is plotted against body weight.

The oxygen:nitrogen ratio

The O:N ratio reflects the relative utilization of protein in energy metabolism. A high rate of protein catabolism relative to lipid and carbohydrate catabolism results in a low O:N ratio which is generally indicative of a stressed condition. The calculated O:N ratios of *Mytilus edulis* in response to ration and seasonal acclimation temperatures are presented in Table 3.

Table 3. *O:N as a function of ration level and acclimation temperature*

Temperature (°C)	Food concentration (mg l^{-1})	O:N (for a 1 g animal)
5° (Feb.)	0·0	25·2
	0·28	21·1
	0·56	39·5
	1·40	55·0
10° (Apr.)	0·0	14·7
	0·28	16·5
	0·56	46·5
	1·40	44·2
15° (June)	0·0	20·6
	0·28	28·5
	0·56	20·1
	1·40	23·2
20° (Aug.)	0·0	22·5
	0·28	22·0
	0·56	34·3
	1·40	57·7
25°	0·0	20·1
	0·28	26·9
	0·56	22·9
	1·40	29·5
10° (Nov.)	0·0	25·9
	0·28	28·5
	0·56	32·7
	1·40	39·5

A response surface has been constructed to illustrate the effects of ration and season on the O:N ratio of a 1 g *Mytilus edulis* (Fig. 9). Ration is an important factor determining the O:N ratio. Starvation and very low ration levels result in low O:N values of approximately 20, but these values generally increase to 40 or 50 at high food concentrations (1·5 mg l^{-1}). The decline in O:N was a consequence of the small reduction in metabolic rate and/or an increase in the rate of ammonia excretion. Similar effects of

starvation on the O:N ratio have been recorded for *Mytilus edulis* by Bayne (1973 *a*, *b*) and for *Donax vittatus* by Ansell & Sivadas (1973). Furthermore, Gabbott & Bayne (1973) have demonstrated that a significant fraction of the body protein is lost from *Mytilus edulis* during starvation. An O:N ratio of approximately 25–30 appears to be closely associated with the maintenance ration throughout the year, with the exception of late spring when the ratio is extremely low at all ration levels.

The marked seasonal cycle in the O:N ratio appears to reflect the reproductive condition of the species. For most of the year the O:N ratio of animals fed above the main-
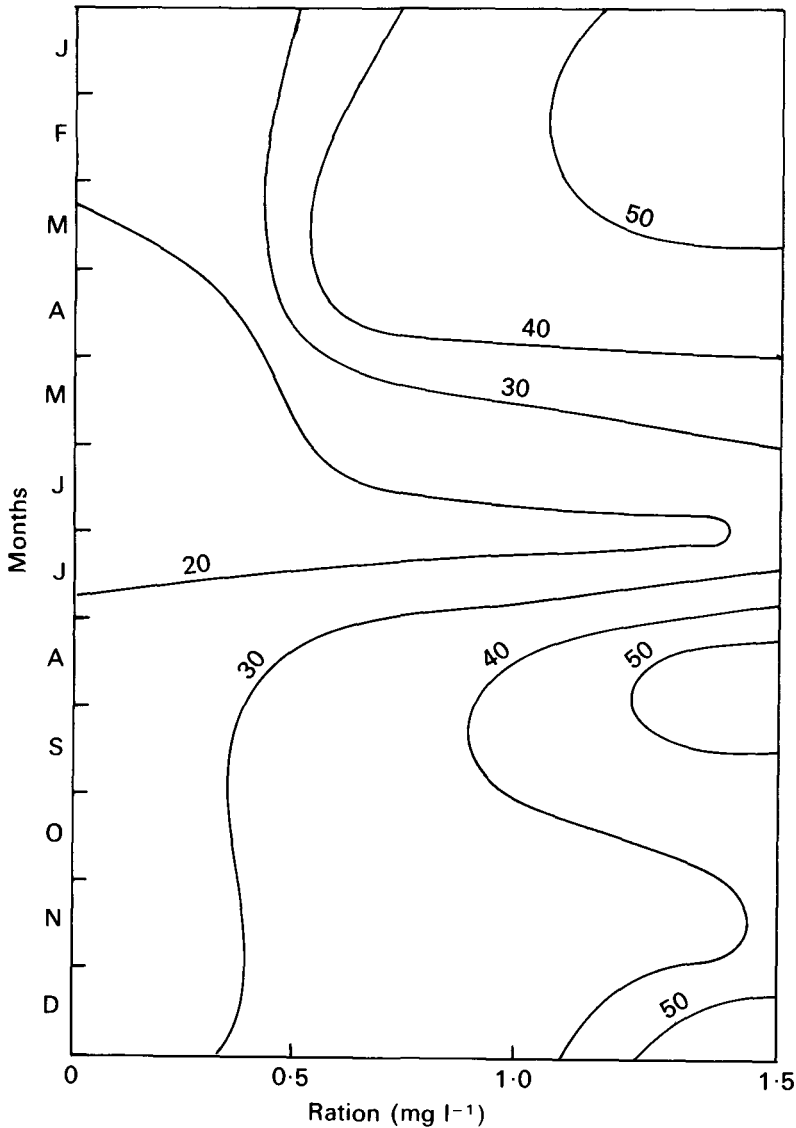

Fig. 9. Isopleths of O:N ratio of *Mytilus edulis* (1 g dry weight) as a function of season and available ration.

tenance ration are above 30. However, in the late spring (Fig. 9) the O:N ratios are low (< 30). This appears to be associated with the immediate post-spawning period when the mantle is thin, and probably undergoing tissue breakdown, regeneration and re-organization. It is during this time of year that *Mytilus edulis* have also been shown to be less tolerant of thermal stress (Bayne *et al.* 1977). Therefore, although O:N values do not convey a direct 'disadvantage' to an organism, as do scope for growth and growth efficiency, there is evidence that it is correlated with a stressed condition and can perhaps be regarded as a symptom of stress (Bayne, 1973*a*, *b*, 1975).

General conclusions

The physiological data derived from these multivariate experiments and presented in the form of response surfaces have not only provided confirmation of many conclusions drawn from earlier studies, but also a summary of the physiological responses of *Mytilus edulis* to a range of factor combinations and a better understanding of the degree to which factors interact. Furthermore, the study has demonstrated how physiological integrations (Bayne *et al.* 1976) can be applied to the problem of assessing the physiological condition of organisms subjected to different environmental conditions.

In this study it has been possible to determine how body size, ration and seasonal temperature affect the scope for growth and efficiency of food conversion by *Mytilus edulis*. The estimation of maintenance and optimum ration levels under these conditions are important in contributing to an improved understanding of the food requirements of natural populations as well as providing fundamental information for purposes of aquaculture.

The multiple regression equation describing scope for growth as a function of body size, ration and season also provides a simple, empirically based growth model for *M. edulis*. Ration has been shown to be a major factor determining growth and it has helped demonstrate the general lack of information in the literature concerning the quality and quantity of utilizable food available to suspension feeders in the estuarine and near-shore environments. This is an important area of research, which is at present receiving attention.

It is generally recognized in environmental biology that there is an urgent need to establish methods and criteria for the measurement of sub-lethal effects of environmental changes on marine organisms. Recently, several physiological integrations such as scope for growth, growth efficiency and the O:N ratio, have been suggested as possible indices of physiological condition and stress (Widdows & Bayne, 1971; Bayne, Thompson & Widdows, 1973; Bayne, 1973*a*, *b*; Bayne, 1975; Bayne *et al.* 1976), where stress is defined as 'a measurable alteration of a physiological (or behavioural, biochemical or cytological) steady-state which is induced by an environmental change and which renders the individual (or the population or the community) more vulnerable to further environmental change' (Bayne, 1975).

In this study, response surface techniques have been used to illustrate the validity and sensitivity of some physiological stress indices under a variety of conditions ranging from near-optimal, through sub-lethal to lethal. These stress indices, derived from physiological integrations of basic rate functions, provide a method of quantifying the

'physiological condition' and the degree of stress experienced by organisms under different field as well as laboratory situations.

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