Feeding and metabolism in the
mussel *Mytilus edulis* L.

by

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Three levels of metabolism have been identified in the mussel *Hytilus edulis* L. Standard metabolism is attained after prolonged starvation. On feeding, there is an initially elevated metabolic rate, termed active metabolism, which is characterised by increases in oxygen uptake, ventilation rate and filtration rate. After three days feeding, oxygen consumption decreases to a value intermediate between standard and active levels; this is defined as routine metabolism.

Integrated measurements of ingested ration, assimilated ration and metabolic rate provide an estimate of energy balance which is a useful index descriptive and predictive of the effects of sublethal stress. Growth efficiency increases hyperbolically with increasing ration to reach a maximum after which efficiency decreases as ration is further raised. The optimum ration for efficient growth is an increasing function of mussel weight.

Gametogenesis in *Hytilus edulis* occurs in winter when food is scarce. Energy for gonad maturation is obtained from reserves built up during summer when food is abundant. The mantle and digestive gland are particularly important storage sites. The digestive gland also regulates the flow of assimilated material to other tissues. During prolonged stress the digestive tubules become considerably degenerate but the tissue possesses the capacity to recover after such periods of stress.
Lipid, carbohydrate and protein are synthesised in summer and metabolic rate is low. During winter, gametogenesis results in an inflated metabolic rate and turnover of metabolic pools is rapid as reserves are utilised. Sublethal stress also leads to catabolism of energy reserves but it is not possible to define the degree of stress in terms of biochemical parameters such as the carbohydrate : protein ratio because they tend to be regulated to similar values which are independent of the severity of the stress experienced.
The mussel *Mytilus edulis* L. (Mollusca Lamellibranchia), a filter feeding marine bivalve mollusc, is a very common littoral and shallow sublittoral species which is abundant on many British shores. Mussel populations often occupy a central position in the shallow water benthic community, forming an important link in the trophodynamic structure of the marine ecosystem (Lewis, 1964; Newell, 1971). They may also be fished commercially, so the species is of some economic importance. For these reasons the biology of the genus *Mytilus* has been more extensively studied than that of any other bivalve, with the exception of the oysters *Ostrea* and *Crassostrea*.

The species *Mytilus edulis* is particularly well known. Its morphology has been described in detail (Field, 1921; White, 1937). Many aspects of its ecology have been defined, for instance the zonation of the species (Lewis, 1964), its population structure and growth (Baird, 1966) and its growth efficiency (Jørgensen, 1952). The reproductive cycle has been established (Chipperfield, 1953; Lubet, 1955) and the biology of the larval stages described (Bayne, 1963a,b) including settlement and metamorphosis (Bayne, 1965, 1966, 1971). There is some information concerning seasonal changes in biochemical composition (Daniel, 1921, 1922; Williams, 1969). Some aspects of the physiology of *M. edulis* have also been investigated, particularly filtration (Tammes and Dral, 1955; Jørgensen, 1966), gill structure related to function (Dral, 1968; Moore, 1971), and respiration (Bruce, 1926; Kruger, 1960; Read, 1962). The importance
of nitrogen metabolism has recently been realised (Hammes, 1968).

Not only is there extensive information available on the biology of *Mytilus edulis*, but the species also has several features which contribute to its suitability for experimental work. Mussels of all sizes are available throughout the year. They are easy to maintain in the laboratory with little mortality, they feed readily on suitable algae and are relatively insensitive to disturbance during physiological experiments. Various procedures may be carried out using implanted electrodes or pressure transducers e.g. to measure heart perfusion and pressure in the pericardial cavity. A considerable advantage in using *M. edulis* for biochemical analysis in relation to the reproductive cycle lies in the ease with which the mantle (germinal tissue) may be separated from non-mantle (somatic tissue). Finally, gravid mussels, both male and female, may be induced to spawn in the laboratory if suitably stimulated (Bayne, 1963a) and larvae subsequently reared through metamorphosis to maturity.

Many marine invertebrates exhibit seasonal changes in metabolism (at least in temperate waters) by which metabolic reserves are synthesised during periods of food availability and utilised when food is scarce (Giese, 1959, 1966; Barnes et al. 1963; Ansell et al., 1964). Such changes are often associated with a seasonal pattern of reproduction, as in *Mytilus edulis* (Chipperfield, 1953). The mussel builds up reserves in summer when food is abundant and catabolises them in winter when gametogenesis is proceeding (Daniel, 1921, 1922; Williams, 1969).

Much of the work described in this investigation concerns these metabolic changes resulting from seasonal variation and sublethal stress factors applied in the laboratory. Stress may be defined as a disturbance of the physiological steady state of an organism or
system, leading by readjustment of rate functions to the establishment of a new steady state which may or may not be identical with the original steady state. It is implied in this definition that differences between steady state conditions are measurable. The use of radi nuclide tracers is particularly important in this context because such methods yield information about the dynamics of a system. Tracer studies in bivalve physiology are few (Allen, 1962, 1970) and have yet to realise their full potential.

It must be emphasised that stress is not necessarily confined to laboratory conditions. In the natural environment conditions are constantly changing, although such changes are likely to be more gradual than in the laboratory. Adjustments of the steady states of physiological functions will therefore occur in the field. The importance of laboratory studies of stress lies in the precision with which conditions may be controlled and the identification, by physiological monitoring, of the range of responses shown. This leads to the derivation of physiological indices which may be both descriptive and predictive of stress. Stress prediction may be regarded as particularly useful in commercial cultivation practice. The development of empirical growth models for fish (Beamish and Dickie, 1967; Warren and Davis, 1967) has stimulated interest in their application to marine invertebrates, and it is probable that this approach will prove very useful in future studies of stress physiology.

Laboratory studies of physio-ecological problems are often criticised on the grounds that they have little relevance to the field situation, because conditions in the laboratory are essentially constant whereas those in the natural environment are continuously changing. This is a near-vitalistic argument which cannot be supported. It must be emphasised that laboratory conditions are not designed
to mimic the environment. Experiments carried out under controlled conditions are essential to any understanding of function in Nature in terms of physiology and metabolism. Certainly, the application of laboratory data to the field situation must be done intelligently and with care, but there is no reason to suppose that the physiological and metabolic responses shown by an animal in the natural environment should radically alter merely because that animal is brought into a laboratory. It is now being realised that laboratory studies and fieldwork need not be mutually exclusive; indeed, both are necessary for an understanding of the complex mechanisms which operate to maintain steady states in a wide variety of marine organisms under changing conditions in the sea.

The first of the four sections in this study deals with the physiology of feeding in *Mytilus edulis* and shows that the feeding process is a stimulus-response situation characterised by a high metabolic level, termed active metabolism. This represents an interesting aspect of the subject, since most previous studies of bivalve filtration (Jørgensen, 1966; Winter, 1969; Ali, 1970) comprise isolated measurements of filtration rate separately related to temperature, size and other variables.

In section II measurements of filtration rate, metabolic rate and assimilation efficiency are integrated into an empirical model defining the relationships between growth, food and metabolism. Derived values for growth efficiency provide an acute index of stress under any given set of conditions at any given time.

The third section describes the utilisation of metabolic reserves during environmental stress. The gonad serves as a major energy reservoir and there is a complex relationship between the catabolism of these reserves during unfavourable conditions and the maintenance
of mature gametes with their subsequent resorption after prolonged stress.

Finally, section IV is concerned with radionuclide tracer studies which provide an indication of the dynamic processes involved in the synthesis and utilisation of reserves in various tissues and tissue fractions. This information complements the quantitative biochemical analyses and the data as a whole provides a comprehensive picture of metabolism in terms of seasonal changes and stress effects.
SECTION I

Active metabolism associated with feeding
INTRODUCTION

It is well known that fish have a capacity to raise or lower their metabolic level between certain limits, which vary according to acclimation conditions. Fry (1957) introduced the concept of a "scope for activity" between standard and active metabolism, an idea further developed by Brett (1964). Scope for activity is usually associated with animals moving in search of food, but may also be applied meaningfully to a near-sessile animal (such as Mytilus edulis) in order to distinguish between a non-feeding (standard) metabolism and a feeding (active) metabolism. This distinction, however, is likely to prove more complex than a simple "activity cost" of the feeding behaviour itself. Thus Saunders (1963) has shown that the respiration of starved Atlantic cod (Gadus morhua L.) increases immediately after feeding and remains high for some days.

This section describes physiological responses to a variety of feeding stimuli in the common mussel, in order to distinguish between an active metabolism, related to feeding in the short term, a routine metabolism, associated with long-term feeding, and a standard metabolism, attained after prolonged starvation. It is important that these three levels of metabolism have been identified with some precision for a benthic bivalve.
Mussels were collected from a population at Hesacham, Norfolk. They were cleaned of any epibiotic growth and kept in specimen tanks in the aquarium. One group of mussels was starved, the inflowing water being passed through a candle filter retaining 98% of all particles larger than 2 μm diameter. A second group was fed with the flagellate Tetraselmis suecica (Kylin) Butch., which was grown in monoculture and continuously dosed into the tank to give a food concentration of 5,000 to 10,000 cells ml⁻¹ of water. Animals were allowed at least two weeks to acclimate before use in experiments.

Filtration rate, ventilation rate and oxygen consumption were measured in the flow system illustrated in fig. 1. Inflow water passed through a candle filter A (porosity 2 μm) before entering a constant head device B. It then flowed via a temperature equilibration coil K to flask C, which contained the animal, and finally to a second flask D in which was mounted an oxygen sensor E coupled to an amplifier F and pen recorder G. Water in flask D was continuously agitated by means of an immersible magnetic stirrer H. Rate of water flow was controlled at 35 ± 2 ml min⁻¹ (2.1 ± 0.12 l h⁻¹) by a tap I. When required, food was continuously added to the constant head B. Samples of inflow water were taken from tap J. Flasks C and D and the coil K were kept in a water bath to minimise temperature fluctuations.

Respiration was monitored with a polarographic oxygen electrode (Beckman model 777) coupled to a pen recorder (Rikadenki Kogyo model B-34). Oxygen content of the inflowing water was measured before an animal was placed in the system. Each mussel was allowed at least one hour to equilibrate in the system before food was added. In no case was feeding commenced until a steady reading was obtained for oxygen.
Fig. 1 Apparatus used for simultaneous measurement of oxygen uptake and filtration rate. For explanation see text.
content of the outflowing water, i.e. until the animal was respiring at a steady "routine" rate. Variations in the oxygen content of the inflowing water were less than 1% during the course of any one experiment, and the total error in each final estimate of oxygen consumption was less than 10%.

Filtration rate was measured at intervals by means of an electronic particle counter (Coulter Counter model A). Inflow samples of water were taken at J, outflow samples at 1, and the number of particles of 6 μm diameter and greater counted in each sample. Filtration rate, defined as the volume of water cleared of particles in unit time, was calculated as the product of particle retention efficiency and water flow rate. Values were obtained of both filtration rate and oxygen consumption before feeding with Tetraselmis, during feeding and after feeding.

The same technique was used to determine ventilation rate, i.e. rate of water flow over the gill, but in this case it was necessary to use particles large enough to guarantee complete retention by the animal, so that filtration rate and ventilation rate were equal. The diatom Phaeodactylum tricornutum Bohlin fulfilled this condition and was used as food in two experiments when ventilation rate measurements were obtained. Actively feeding animals, however, often closely approached 100% retention efficiency with Tetraselmis also, although the cell size of this species is smaller than Phaeodactylum. In such cases filtration rate was the same as ventilation rate.

Activated charcoal was used for filtration rate measurements when inert material was required. Particles were suspended in seawater and the suspensions filtered through a 45 μm diameter mesh before use. Some settlement of these particles occurred in the apparatus, and appropriate corrections were applied to filtration rate calculations.
Non-particulate food extracts were prepared from *Tetraselmis* in the following way. Approximately 250 ml of culture were centrifuged to separate cells from medium. The supernatant culture medium was then filtered (0.45 μm Millipore) before use. The cells were resuspended in water and broken up with an ultrasonic disintegrator. Five minutes of this treatment ruptured all the cell membranes. The homogenate was then added to 250 ml of fresh seawater and filtered (0.45 μm Millipore). All debris was thus removed and a particle-free, soluble cell extract obtained. Total carbohydrate in the filtered supernatant was determined as glucose equivalents by the phenol-sulphuric acid method (Dubois et al., 1956).

Heart preparations were made as follows. A groove was filed in each shell valve, 2 or 3 mm measured laterally from the hinge, and parallel to it. A small hole was then bored in the centre of each groove, and a pair of electrodes inserted dorsally. The electrodes were directed posteriorly to lie alongside the heart, and sealed in position with paraffin wax. Resistance across the electrodes was monitored by an impedance pneumograph coupled to a Physiograph (type DHP 4A, L&H Instrument Co.), which recorded heart rate and frequency. The measurements were made in a slightly modified version of the apparatus illustrated in fig. 1. Comparisons of heart amplitude of different preparations was made possible by an internal standard in the impedance pneumograph.

Assimilation efficiency was determined for animals fed with various concentrations of *Tetraselmis*. Samples of faeces were taken at intervals from the specimen tanks and washed three times with 0.9% ammonium formate solution (isotonic with seawater). The material was dried to constant weight at 60°C under a 100 torr vacuum, and combusted to constant weight at 430°C. Assimilation efficiency was calculated
from the ratio ash-free dry weight to dry weight of faeces, as described
by Conover (1966).

The flow system used in these experiments is in many ways more
satisfactory than the static or closed systems of earlier workers
(reviewed by Jørgensen, 1966). In the closed system, the decrease
in oxygen tension or food concentration is measured for a period of
time, and the respiration or filtration rate calculated from the rate
of decrease. Unfortunately, when oxygen tension falls to 70% of full
saturation, compensatory physiological mechanisms begin to operate
to combat respiratory stress (Bayne, 1971a, 1971b). Oxygen uptake
measurements are then not typical of values obtained under normal
conditions. Furthermore, accumulation of excreted and other toxic
metabolites inhibits normal physiological behaviour. Closed systems,
therefore, are useful only for short-term measurements and have a
limited application.

In the flow system, however, the animal is allowed to equilibrate
in water which is at nearly maximum oxygen saturation throughout an
experiment. The inflowing oxygen and food concentrations can be kept
constant, and it is then only necessary to monitor their values in
the outflow. In the case of oxygen measurements, this necessitates
using a sensitive method for detecting quite small differences between
inflow and outflow content. The polarographic electrode enables this
to be done. A considerable advantage of the flow system is that it
allows lengthy experiments to be carried out, greatly facilitating
studies on respiratory and feeding physiology of filter-feeders.
RESULTS

Responses to feeding were examined in two groups of animals, one group starved before use, the other fed. Both groups increased filtration rate and oxygen uptake when fed in the flow system, but the responses of starved animals were more discrete. All results to be described were obtained with starved animals, unless otherwise indicated.

Filtration rate and respiration rate

Figure 2 shows that respiration rate increased immediately after food was added, whether the stimulus was in the form of algal culture, inert particulate matter or non-particulate algal extract. Filtration rate similarly increased if the food was particulate. An interesting feature, repeatedly found, was the absence of any filtration activity before addition of food. During the prefeeding period the particle concentration never exceeded 300 per ml and was often less than 200 per ml, so there was a threshold concentration below which animals would not filter at all. When particulate matter was presented in excess of this threshold concentration, filtration rate rapidly increased to a relatively steady value which was maintained throughout feeding. Oxygen consumption also rose from a pre-feeding level (here termed "standard metabolism") to a higher value during feeding ("active metabolism").

Although the initial respiratory response to feeding did not vary with the nature of the stimulus, this was not the case for the response to removal of the stimulus. If the stimulus was non-particulate, metabolism immediately returned to the pre-feeding value, whereas removal of particulate food resulted in maintained active metabolism for at least a further hour. Filtration rate
Fig. 2 The effects of four separate food stimuli on oxygen uptake (dashed line) and filtration rate (circles, continuous line).

A. *Tetraselmis* culture  B. *Phaeodactylum* culture
C. Activated charcoal  D. *Sonicated* *Tetraselmis* extract.

Feeding period indicated by arrows.
Table I  Oxygen uptake and filtration rate of starved animals used in activity experiments.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Particle used</th>
<th>Oxygen uptake (ml h(^{-1}))</th>
<th>Filtration rate (ml min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>routine</td>
<td>active</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.20</td>
<td>0.41</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.10</td>
<td>0.31</td>
</tr>
<tr>
<td>3</td>
<td>Tetraselmis</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.21</td>
<td>0.42</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.21</td>
<td>0.31</td>
</tr>
<tr>
<td>6</td>
<td>Non-particulate extract</td>
<td>0.25</td>
<td>0.70</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>0.19</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>0.31</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>10</td>
<td>Phaeodactylum</td>
<td>0.10</td>
<td>0.25</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>0.11</td>
<td>0.22</td>
</tr>
<tr>
<td>12</td>
<td>Glucose 10mM</td>
<td>0.23</td>
<td>0.55</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>0.22</td>
<td>0.44</td>
</tr>
<tr>
<td>14</td>
<td>Activated charcoal</td>
<td>0.19</td>
<td>0.38</td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>0.16</td>
<td>0.33</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>0.14</td>
<td>0.19</td>
</tr>
</tbody>
</table>
returned to zero under these conditions. Filtration and respiration rate data for a number of preparations are given in table I.

Figure 3 summarises the ways in which various food stimuli affected respiration, which is expressed as a percentage of the standard value. These data amplify those already described for the preparations in fig. 2 and show that non-particulate culture supernatant and 10mM glucose (one experiment only) elicited similar responses to those of culture extract. Information from these experiments is listed in table II. Of a total of 31 observations on starved animals after various treatments, 27 responded to stimulation and 4 showed no response. Of a total of 26 experiments with fed animals, 23 responded and 3 did not.

**Heart frequency and amplitude**

The frequency and amplitude of heart beat were not affected by feeding (fig. 4). Results from five heart preparations are summarised in table III. Oxygen uptake and filtration rate increased typically in each case, but there was no indication of any change in perfusion index (the product of heart frequency and amplitude). Responses from one preparation are illustrated in fig. 5.

**Withdrawal effectiveness**

The efficiency of oxygen withdrawal, or withdrawal effectiveness, was determined for animals actively metabolising during feeding. The calculation was made using measurements of ventilation rate, oxygen content of the water and the rate of oxygen uptake. The results are tabulated in table IV. For experiments in which ventilation rate was calculated from the rates of removal of Tetraselmis and activated charcoal, percentage effectiveness was estimated at approximately 4%, being similar to estimates based on experiments with Phaeodactylum.
Fig. 3 Summary of the effects of various food stimuli on oxygen uptake. Feeding period indicated by arrows.
Table II  Synopsis of experiments, starved animals.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total observations</th>
<th>&quot;on&quot; only</th>
<th>&quot;on&quot;/&quot;off&quot;</th>
<th>no response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetraselmis culture</td>
<td>9</td>
<td>7</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Sonicated Tetraselmis</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Culture supernatant</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Soluble culture extract</td>
<td>7</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Glucose 10mM</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Activated charcoal</td>
<td>7</td>
<td>5</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Phagodactylum culture</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>
Fig. 4 Recordings from heart preparations A. before feeding with *Tetraselmis* culture B. during feeding and C. after cessation of feeding.
Table III.  Data from heart preparations.  Standard deviations indicated where appropriate.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Oxygen uptake (ml h⁻¹)</th>
<th>Filtration rate (ml min⁻¹)</th>
<th>Heart frequency (beats min⁻¹) and amplitude (arbitrary units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-feeding</td>
<td>Feeding</td>
<td>Non-feeding</td>
</tr>
<tr>
<td>1</td>
<td>0.25</td>
<td>0.31</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.30</td>
<td>0.0</td>
</tr>
<tr>
<td>3*</td>
<td>0.16</td>
<td>0.24</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>0.29</td>
<td>0.67</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>0.40</td>
<td>0.92</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*10.5°C
Fig. 5  The effect of a feeding stimulus (Tetraselmis culture) on oxygen uptake (dashed line), filtration rate (circles, continuous line), heart frequency (dotted line) and heart amplitude (dashed/dotted line). Feeding period indicated by arrows. Data from a single preparation.
### Table IV

**Effectiveness of oxygen removal.**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Type of particle used</th>
<th>% effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Phaeodactylum</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>Phaeodactylum</td>
<td>3.7</td>
</tr>
<tr>
<td>3</td>
<td>Tetraselmis</td>
<td>4.2</td>
</tr>
<tr>
<td>4</td>
<td>Tetraselmis</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>Tetraselmis</td>
<td>2.5</td>
</tr>
<tr>
<td>6</td>
<td>Activated charcoal</td>
<td>5.7</td>
</tr>
</tbody>
</table>

### Table V

**Statistical information relating to data in fig. 6.**

\[ y = \text{oxygen uptake (\mu l h^{-1})}, \quad x = \text{dry weight (mg)}. \]

<table>
<thead>
<tr>
<th>Condition</th>
<th>Equation</th>
<th>Number of observations</th>
<th>Correlation coefficient</th>
<th>Standard error of coefficient for slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starved standard</td>
<td>[ y=3.64x^{0.616} ]</td>
<td>28</td>
<td>+0.78</td>
<td>±0.097</td>
</tr>
<tr>
<td>Starved active</td>
<td>[ y=2.22x^{0.797} ]</td>
<td>14</td>
<td>+0.92</td>
<td>±0.099</td>
</tr>
</tbody>
</table>
The relationship between respiration and dry flesh weight is shown in fig. 6 with appropriate statistical data in table V. The weight exponent for standard metabolic rate was 0.62, which approximated to surface proportional metabolism. In the case of active respiration of starved animals, however, the weight exponent was 0.50, i.e. intermediate between surface proportional and weight proportional metabolism.

The reasons for this difference between the weight exponents are not clear. There is no general agreement regarding the significance of the weight exponent of metabolism, and little data defining the conditions under which it may be altered (von Bertalanffy, 1964; Pacheco and Dickie, 1966). In a discussion of this problem von Bertalanffy (1964) points out that in some cases the weight exponent is greater for fed than for starved animals. Other observations (also see Bayne, 1971) indicate that in Mytilus metabolism is surface proportional in winter but weight proportional in summer, confirming that the exponent value is variable. In the case of the starved animals in these experiments the "scope for activity" of smaller individuals is in some way reduced disproportionately to larger individuals, possibly due to stress.

Standard metabolism

Figure 7 indicates a linear relationship between oxygen uptake and filtration rate. A single preparation is used for illustration, but the same relationship was found in several animals. The intercept on the ordinate represents oxygen uptake at zero filtration rate, and has a value 0.34 ml oxygen per hour. For an animal starved for several weeks this must closely approximate true standard metabolism. The value for "routine" metabolism of a starved
Fig. 6 The relationship between oxygen uptake and dry flesh weight (logarithmic scales). 95% confidence limits shown.
Fig. 7 Filtration rate related to oxygen consumption.
animal of the same weight (1.8 gram) is 0.36 ml per hour (fig. 6). This provides evidence that respiration of the starved group prior to feeding experiments represents true standard metabolism at 15°C, which has significance in view of the difficulty of determining with certainty conditions for standard metabolism in lamellibranchs and other filter feeders.

**Routine metabolism**

Figure 8 represents a combination of data from two experiments in which five animals of uniform length were first starved for two weeks and then continuously fed for a similar period. Respiration measurements were made at intervals, and the dry weight of each animal determined at the end of the experiment. All oxygen uptake data was then expressed in terms of a gram "standard" mussel, and confirmed the initial active metabolism associated with feeding which was characteristic of the short-term experiments. When feeding was continued for several days, however, active metabolism was reduced to a level here termed "routine".

**Assimilation efficiency**

In order to determine whether faeces were subject to breakdown by micro-organisms in the experimental tanks, values for assimilation efficiency of a single faecal sample were determined at intervals up to one week. The results (table VI) indicated that microbial attack on the organic matter in faeces was negligible. Reliable estimates of assimilation efficiency could therefore be obtained by sampling faeces regularly.

Results from assimilation efficiency experiments are presented in fig. 9. At low food concentrations (1000 cells ml⁻¹ or less) efficiency was 89%, but as the cell concentration increased the assimilation value decreased approximately linearly to reach zero.
Fig. 8 The effect of a feeding stimulus (Tetraselmis culture) on the oxygen uptake of animals previously starved. Mean ± S.D. Data from two experiments.
Table VI  The effect of prolonged exposure to seawater on estimation of assimilation efficiency. Samples taken at intervals from a quantity of faeces isolated at time zero.

<table>
<thead>
<tr>
<th>Duration of exposure</th>
<th>Estimate of assimilation efficiency by Conover's (1966) method.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 hour</td>
<td>0.93</td>
</tr>
<tr>
<td>36 hours</td>
<td>0.90</td>
</tr>
<tr>
<td>60 hours</td>
<td>0.88</td>
</tr>
<tr>
<td>1 week</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Table VII  Energy cost of ventilation.

<table>
<thead>
<tr>
<th>Ventilation rate ($1 \text{ h}^{-1}$)</th>
<th>Calculated cost of ventilation ($\text{cal h}^{-1} \times 10^{-3}$)</th>
<th>Calculated cost of ventilation expressed as percentage of total oxygen uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.48</td>
<td>0.08</td>
</tr>
<tr>
<td>1.0</td>
<td>1.92</td>
<td>0.32</td>
</tr>
<tr>
<td>1.5</td>
<td>4.31</td>
<td>0.73</td>
</tr>
<tr>
<td>2.0</td>
<td>7.66</td>
<td>1.30</td>
</tr>
</tbody>
</table>
Fig. 9  The relationship between assimilation efficiency and food particle concentration.
at a concentration of 25,000 cells ml$^{-1}$. The appearance of faeces varied according to the amount of food available. Below 1,000 cells ml$^{-1}$ the faeces appeared as a single brown ribbon, whereas at 25,000 cells ml$^{-1}$ the ribbon was bright green. At intermediate concentrations it was possible to distinguish two components in the faeces, one brown and apparently digested, the other green and packed with *Tetraselmis* cells. In some instances the cells survived passage through the gut, and could be seen swimming from the faecal strip. This implies that when food was abundant, variable proportions of ingested material could pass through the gut without apparent digestion, presumably by-passing the digestive gland. Similar observations were reported by van Weel (1961).

**DISCUSSION**

During feeding, metabolism in *Hytilus* is raised above the non-feeding, or standard condition, to a level here termed "active metabolism". Filtration rate increases also, but there is no change in the relative perfusion index (the product of heart frequency and amplitude). Ventilation rate measurements during active metabolism indicate that the withdrawal efficiency for oxygen, or effectiveness, is typically 4% and does not exceed 6%. These estimates do not exceed normal routine values and are possibly less than normal (Kazelhoff, 1935; Bayne, 1967). The observed increase in oxygen consumption is therefore explicable only in terms of a similar increase in ventilation rate, making more oxygen available to the animal in unit time. Increased ventilation rate, with no parallel increase in perfusion rate, increases the
ventilation: relative perfusion ratio with consequent reduction of the effectiveness of oxygen withdrawal to the low values observed (table IV; fig. 10). This is in accordance with the relationship between percentage effectiveness and the ventilation: relative perfusion ratio recorded by Bayne (1971b). During routine activity, the ventilation: relative perfusion ratio is lower than during active metabolism, predicting a higher effectiveness; values calculated from the present experiments and other data, (Bayne, personal communication), indicate a ventilation: perfusion ratio of 4 for starved animals (representing low routine, or standard metabolism), with a predicted effectiveness of 12-13%. Dral (1968), however, has reported that under conditions of low food availability Mytilus may employ less than half the total surface of the gill in moving water through the mantle cavity. In this situation the area available for gas exchange will diminish, effectiveness will be lowered and oxygen uptake reduced.

It appears, therefore, that a basic relationship between ventilation and perfusion may be used by Mytilus in different ways in response to different situations. During hypoxia, the ventilation: relative perfusion ratio is reduced and effectiveness increased (Bayne, 1971b). In response to a feeding stimulus, ventilation: perfusion ratio increases and the resulting reduction in effectiveness is compensated by the increased availability of oxygen. When no food is available, ventilation is low and gas exchange is reduced, possibly by a decrease in the area of contact between gill and water. Thus the animal is able to vary the parameters of gas exchange in accordance with its oxygen requirement.

Assuming a decrease in effectiveness from 8% to 4% during feeding, ventilation must rise to 3 or 4 times the non-feeding
Fig. 10  Percentage effectiveness of oxygen uptake, related to ventilation : perfusion ratio.
Dashed lines indicate ventilation : perfusion ratio at 4% and 8% effectiveness.
Figure modified from Bayne (1971b).
value to produce the observed 70% increase in oxygen consumption. Fry (1957) quotes precisely the same values for the rainbow trout, a fourfold ventilation increase producing a 70% rise in metabolism. Similar increases in ventilation have been described for other fish, usually in response to hypoxia or hypercapnia. The trout, for instance, may exhibit active ventilation five times the resting value (Hughes, 1963). There is, of course, a point when further ventilation increases are profitless, since the cost of the pump becomes excessive. The value for the trout seems to be a typical optimum for fish according to the theoretical considerations of Hughes and Shelton (1962), although these authors indicate that their model is semi-quantitative only. Active fish under these conditions utilise approximately three times the standard oxygen requirement, rather more than does the mussel. Fry (1957) also points out that active oxygen uptake is restricted to a few multiples of standard rate, and considers that a fourfold increase is a maximum. Although these ventilation increases in fish are responses to respiratory stress and not to feeding as in *Mytilus*, it seems reasonable to draw attention to the similarities between the two in quantitative terms. The cost of respiration in water is high, and it seems that the causative physical and biological factors impose similar limitations on ventilation in two groups of aquatic organisms with quite different ventilation mechanisms.

The changes that occur on removal of the feeding stimulus are interesting. If a purely chemical stimulus is withdrawn, metabolism immediately returns to the standard value, whereas on removal of a particulate stimulus active metabolism continues. This implies that the presence of particulate matter in the gut leads to a continued high oxygen requirement. There is no data
regarding the utilisation of the additional oxygen obtained during active metabolism, but a significant proportion must represent the cost of the respiratory pump. An unsuccessful attempt to calculate this cost is detailed below. Work done, however, increases disproportionately to the volume moved in nearly all pump systems, so that considerable energy is probably expended in ventilating during feeding. That part of the additional oxygen consumed during active metabolism which does not represent the cost of increased ventilation may be considered as the physiological cost of feeding, which may include the specific dynamic action (Brody, 1946).

Collier et al. (1953) maintain that the American oyster Crassostrea virginica increases ventilation in response to dissolved carbohydrate, but this is disputed by Galtsoff (1964). The controversy is centred around the question whether dissolved organic matter, especially carbohydrate, contributes significantly to the nutrition of lamellibranchs. This emphasis seems misplaced. Dissolved material certainly can stimulate ventilation, but this does not necessarily imply that such matter is significant nutritionally. It appears more likely that a sensory system is present which can respond to chemical stimuli. There is no need to postulate any nutritional value of the dissolved material itself. The concentration of extracellular carbohydrate in the Tetraselmis culture used in these experiments was 3.3 mg l\(^{-1}\), similar to that in natural seawater (Jørgensen, 1966).

The mussel possesses two known pallial sense organs, the osphradium and the abdominal organ, which could be responsible for testing the inhalent current (White, 1937). There may also be receptors on the mantle edge. It is generally assumed that the mollusc osphradium is chemosensory, but the only direct electro-
physiological evidence is that of Bailey and Laverack (1966), who have shown that the osphradium of the gastropod *Buccinum undatum* L. responds to a range of chemical stimuli, including "mussel extract" and certain organic acids found in mussels, common prey of the whelk. There was no response to suspended particulate matter. Unfortunately no similar information appears to exist for the bivalve osphradium or other receptors, but it seems reasonable that a suspension feeder should be adapted to respond to both chemical and mechanical stimuli.

There is considerable variation in assimilation efficiencies reported for aquatic invertebrate consumers. Welch (1966) gives examples from the literature from 14 to 88%. Little is known regarding the effects of food concentration on assimilation efficiency for particular species, with the exception of some zooplankton, especially copepods (Corner and Cowey, 1968), which are the subject of controversy. One view is that zooplankton assimilate ingested algae efficiently even when food is present in large quantities, whereas the opposite view is that under such conditions more food is ingested than is utilised, relatively little being assimilated. The data for *Mytilus* indicates that the latter occurs in the mussel, also a filter feeder, but Winter (1969) claims that in the closely related species *Modiolus modiolus* L. assimilation efficiency is independent of food availability. His data, however, are for protein assimilation, and may not be comparable with the values given here for assimilation of organic matter as a whole.

The mussel filters continuously if food is present, unless the particle concentration reaches 25,000 cells ml⁻¹, when pseudofaeces are produced. Assimilation efficiency decreases
regularly with increasing food concentration. Presumably the digestive tubules become full of algae, and as feeding continues a proportion of the undigested material by-passes the digestive gland. The higher the food concentration, the greater the undigested portion, and the lower the assimilation efficiency. This explanation would also account for the presence of two distinct components in the faeces of animals having abundant food supply.

*Mytilus* appears, therefore, to be adapted to a discontinuous food supply. When little or no food is accessible, no filtration occurs, but the presence of algae (or detritus) above threshold concentration stimulates ventilation and filtration so that more material is brought to the gills and maximum benefit is obtained from food which may only be available for a limited period. The energy cost of this activity is met from the increased oxygen made available for uptake. On removal of a food stimulus, filtration decreases, but the physiological cost of feeding continues to inflate the animal's oxygen requirement. It is not known whether this extra oxygen is obtained by an increase in the effectiveness of oxygen uptake, or by maintained high ventilation.

Normally, particle concentrations in the sea approximate to the minimum values used in these laboratory experiments, i.e. below 1,000 cells ml⁻¹, so that typically assimilation efficiency may be high in the field. During phytoplankton blooms, however, cell concentration may increase considerably, with correspondingly lower assimilation efficiency for *Mytilus*.

It is possible to identify three distinct levels of metabolism in *Mytilus*—standard, active and routine. Working definitions of standard and active levels have already been given, (see introduction), but further work must be done before the term
"routine metabolism" can acutely be defined. Neither is it yet certain by which mechanism active metabolism is reduced to a routine level when feeding is prolonged. Clearly, the classical concept of standard metabolism cannot be applied to poikilotherms; for example, Beamish and Mooijerjii (1964) have demonstrated that standard metabolism of the goldfish Carassius auratus L. is temperature dependent. Nor is it possible, in the case of a filter feeder like Mytilus, to define standard metabolism in terms of minimum activity, since this is within the limits of the term "routine metabolism". Standard metabolism is here defined as the minimum metabolic rate reached by a starved animal at any given temperature.

ESTIMATION OF WORK DONE IN VENTILATION

Figure 11A is a diagrammatic representation of a transverse section through the mantle cavity, showing the 8 gill lamellae. Water is pumped from the inhalent cavity, through the spaces between adjacent gill filaments, into the exhalent cavity. For the purposes of the present treatment, the gill is regarded as a continuous sheet penetrated by a number of uniform water channels, each perfectly cylindrical.

Total work done is estimated in two parts:

a) Work done in driving water through the gills. This involves a knowledge of the dimensions of the gill lamellae, filaments and water channels. Published data has been used to derive many of these dimensions.

b) Work done in bringing water from the inhalent
Fig. 11 Anatomy of the mantle cavity and gills.
For explanation see text.
to the gills, and taking water from the gills to the exhalent siphon.

The calculation has been made for a 4.5 cm length mussel at various ventilation rates. The example given below assumes a ventilation rate of 1 l h⁻¹.

Conversion factors used:

\[
1 \text{ calorie} = 4 \times 10^7 \text{ ergs} \\
1 \text{ mg oxygen} = 3.5 \text{ calories}
\]

Work done in driving water through the gills

Total area of gill presented to inflowing water = 41 cm² (Hughes, 1969).

According to Dral (1967), surface area of ostia

\[
= 0.67 \times \text{total surface area of gill} \\
= 0.67 \times 41 \text{ cm}^2 \\
= 27.3 \text{ cm}^2
\]

Volume of gill estimated as follows (Bayne, personal communication). Gills excised from 10 animals (mean length 4.5 cm) and weighed. Weight of each gill then divided by 1.07 (density of gill; Lange, 1966).

Mean volume of gill = \(0.224 \text{ cm}^3\)

Length of water channel

\[
= \frac{\text{volume of gill}}{\text{area of gill}} \\
= \frac{0.224}{41} \text{ cm} \\
= 5.5 \times 10^{-3} \text{ cm}
\]

(i.e. \(l = 55 \mu\text{m}\); Galtsoff (1964) gives a value 80 \(\mu\text{m}\) for the American oyster).
Radius of channel = 0.5 \times \text{inter-filament space}
\quad = 0.5 \times 6 \mu\text{m (Drel, 1967)}.
\quad a = 0.3 \times 10^{-3} \text{cm}

Cross-sectional area of channel = \pi a^2
\quad = 2.8 \times 10^{-7} \text{cm}^2

Number of channels in gill
\quad = \frac{\text{total area of channels}}{\text{area of single channel}}
\quad = \frac{27.3}{2.8 \times 10^{-7}}
\quad = 9.7 \times 10^7
\quad n = 9.7 \times 10^7 \text{channels}

For a gill "sieve" of the above dimensions, and at a flow rate 1 \text{ l h}^{-1}, the appropriate Reynolds number is less than 10. This is well below the value 2,000 at which laminar flow gives way to turbulence. Poiseuille's equation can therefore be applied to this situation.

Ventilation rate = 1 \text{ l h}^{-1}
\quad = 0.278 \text{ ml sec}^{-1}

Coefficient of viscosity = 0.01 \text{ poise} = \eta

Length of channel \text{l} = 5.5 \times 10^{-3} \text{ cm}

Cross-sectional area \text{a} = 3 \times 10^{-4} \text{ cm}

Number of channels \text{n} = 9.7 \times 10^7

\text{P}_1 - \text{P}_2 = \text{pressure drop across gills}

\text{Work done} = V(\text{P}_1 - \text{P}_2)
\quad = \frac{\delta V^2 \eta}{\pi a^4}
For a gill with n channels this becomes

\[ V(P_1 - P_2) = \frac{8V_1^2 n}{11a^4 n} \]

\[ = \frac{8 \times 0.278^2 \times 0.01 \times 5.5 \times 10^{-3}}{3.14 \times 9.7 \times 10^{-7} \times 81 \times 10^{-16}} \]

\[ = 13.8 \text{ ergs sec}^{-1} \]

Energy used = \(1.24 \times 10^{-3} \text{ cal h}^{-1}\)

Work done in moving water to and from gills

Assume that inhalent and exhalent channels are cylinders, each 4.5 cm long and of 0.22 cm radius (measured radius of exhalent siphon).

At 1 l h\(^{-1}\) flow rate,

\[ \text{work done} = \frac{8 \times 0.278^2 \times 0.01 \times 4.5}{3.14 \times 1 \times 0.22^4} \]

\[ = 3.78 \text{ ergs sec}^{-1} \]

Energy used = \(0.34 \times 10^{-3} \text{ cal h}^{-1}\)

This is for one cylinder only. For two cylinders energy used

\[ = 2 \times 0.34 \times 10^{-3} \]

\[ = 0.68 \times 10^{-3} \text{ cal h}^{-1} \]

Total work done

Total work = work done in moving water from inhalent siphon to gill + work done in pumping water through the gill + work done in moving water from gill to exhalent siphon.

Total work = 3.78 + 13.8 + 3.78

\[ = 21.4 \text{ ergs sec}^{-1} \]

Total energy used = \((1.24 + 0.68) \times 10^{-3}\)
Standard metabolism for 4.5 cm animal

\[ = 0.17 \text{ mg oxygen h}^{-1} \]

\[ = 0.59 \text{ cal h}^{-1} \]

Energy used by respiratory pump

\[ = \frac{1.92 \times 10^{-3} \times 10^2}{0.59} \% \text{ of energy cost of respiration} \]

Cost of pump = 0.32\% of cost of respiration

The relationship between cost of pump and ventilation rate is shown in figure 12.

Pressure difference across the gills

Dral (1967) gives a value 0.5 cm water for the pressure difference across the gills. This can be compared with a value derived from the present calculation.

Assuming a ventilation rate of 1.5 l h\(^{-1}\),

\[ \text{total work done} = 48.05 \text{ ergs sec}^{-1} \]

\[ \text{Now work done} = (V_1 - V_2)V \]

\[ = V (h \rho g) \]

where \( h \) = height of water column

\( \rho \) = density of water

\( g \) = acceleration due to gravity

\( h = \) work done

\[ = \frac{V \rho g}{981 \times 0.42} \]

\[ = 0.12 \text{ cm water} \]

Thus Dral's figure is approximately four times the value
Fig. 12 The relationship between ventilation rate and the calculated cost of the respiratory pump, expressed as a percentage of oxygen uptake. Logarithmic scales.
Conclusions

If the above treatment is accurate, about 1% of the oxygen obtained by a mussel is used by the respiratory pump under normal conditions (table VII). It is difficult to give credence to such a low figure for aquatic respiration. Hughes (1963) considers 1% to be typical for terrestrial mammals, and estimates that the minimum cost of aquatic respiration in fish is likely to be 10% of the total oxygen obtained, and under certain conditions this must be exceeded. Similar values probably hold for other aquatic organisms such as bivalves.

In all probability the treatment is inappropriate. There are several possible reasons:

1. Poiseuille's equation is applicable in terms of flow rate and tube dimensions because the Reynolds number is very low. The gill channel, however, cannot be treated as a cylinder because of its irregular shape and the presence of cilia (fig. 11B). Poiseuille's treatment applies only to laminar flow in non-obstructed pipes. Masses of cilia clearly interfere with the flow through gill channels.

2. The presence of particles in the water affects the value of η, the coefficient of viscosity.

3. Channels are further blocked by food particles when the animal is filtering.

4. All pipes and cylinders have a "loss constant" which depends on the geometry of the entrance and exit. Some pressure is inevitably lost.

The probable functioning of the gill system is illustrated...
in fig. 110. Water is first passed over the laterofrontal cilia, which act as a filter. The lateral cilia then move the water through the channel, thus acting as a pump. Most of the work is probably done by the laterals in moving water along, creating a suction which brings water across the filter. The pressure across A - Y, which corresponds both to Dral's measurement and to that derived here, may be considerably lower than the pressure A - B across the pump proper. It is a value for this pressure A - B which is required to make an accurate estimate of the work done by the pump.

There are thus two ways in which the work done in ventilation may be measured; first, by determining the pressure across the pump proper, and calculating the work done directly; secondly, by constructing a scale model of the gill in a flow system and determining appropriate Reynolds numbers. Neither method is a practical proposition at present.

Although the values in fig. 12 are unlikely to be accurate in absolute terms, they do illustrate an important aspect of the system. The work done by a pump is usually a power function, often a square or greater, particularly if there is turbulence. Hence the cost of the pump increases disproportionately to the oxygen obtained.
SECTION II

Relations between growth, rations and metabolism
INTRODUCTION

Physiological studies on feeding naturally pose questions regarding relationships between growth, food and metabolism. Empirical models of metabolic phenomena may contribute significantly to an understanding of the mechanisms underlying growth as a biological production system. Such models have proved particularly useful in studies of fish population dynamics (Faloheimo and Dickie, 1965, 1966a, b; Ursin, 1967). The work described here extends these concepts to a benthic filter feeder, Mytilus edulis, in an attempt to derive mathematical relationships between food availability, metabolism, body weight and growth efficiency, and also to interpret such relationships in terms of the biology of the mussel.

There are two methods of approaching this problem, both of which have enjoyed support from fisheries biologists. The first is that of Pütter (1920), greatly expanded by von Bertalanffy (1964) and applied to fish growth dynamics by Ursin (1967). This model defines growth as the algebraic sum of anabolic and catabolic processes, thus

\[ \frac{dw}{dt} = Hw^m - Dw^n \]

where \( H \) and \( D \) are coefficients of the rates of anabolism and catabolism, \( m \) and \( n \) the corresponding exponents, and \( w \) is body weight.

The second approach makes use of the basic energy equation as expressed by Winberg (1956). That is,

\[ \frac{\Delta w}{\Delta t} = R - T \]
where $\Delta H$ is the energy equivalent of growth for any period of time $\Delta t$, $R$ is assimilated ration and $T$ the total metabolic energy expenditure. This concept has been explored in detail by Palcheimo and Dickie (1965, 1966a,b), who found it applicable to much existing data concerning growth and metabolism of fishes. Use of the Winberg equation avoids the necessity of dealing with somewhat abstract concepts of anabolism and catabolism, and lends itself readily to laboratory investigations, such as those described here for Mytilus.

**Materials and Methods**

Measurements were made of oxygen uptake, filtration rate and assimilation efficiency in relation to food availability and body weight. Experimental procedures have already been described (section I). Temperature was maintained at $15^\circ C \pm 1^\circ C$ throughout, and all data obtained during the winter season (October - March).

**Results and Discussion**

**Conversion Factors**

Ingested energy, assimilated energy and energy expended in metabolism are expressed in terms of calories. The conversion factors used are as follows:

- Energy content of Tetraselmis cells = 5.6 cal g dry weight$^{-1}$
  
  (Widdows and Bayne, 1971)

- Weight of $10^6$ Tetraselmis cells = 0.066 mg
  
  (Widdows and Bayne, 1971)
Total metabolism

Oxygen consumption is used as a measure of total metabolism $T$. The relationship between oxygen uptake and dry flesh weight (fig.6) has already been discussed for both standard and active metabolism, and describes a power function:

$$T = a \cdot w^b$$

where $a$ is a constant which defines metabolic expenditure per unit time, and $b$ an exponent defining the rate of change of metabolism with body weight $w$. This is a fundamental law of physiology (e.g. Prosser and Brown, 1961; von Bertalanffy, 1964). Its application to the consideration of fish metabolism is comprehensively described by Paloheimo and Dickie (1966a), who refer to the relationship between the logarithms of metabolic rate and tissue weight as the "T-line". They conclude that temperature, food ration and other factors regulate metabolic level '$a$', but that the weight exponent '$b$' of the T-line is more stable. The conditions under which $b$ may change are not clear. With regard to the present study of growth, food and metabolism of Mytilus, it is of particular interest that greater '$a$' values in fish result from increased food availability.

It is therefore important to determine whether metabolic level in Mytilus is dependent on food concentration. Active metabolism experiments (section 1) have shown that there is a threshold food concentration at which the mussel begins to feed, and that during the feeding period metabolism increases from a standard (starved) to an active value, before decreasing to a routine level on prolonged feeding. There is no evidence that either active or routine oxygen uptake varies with food availability,
a view shared by Widdows (personal communication). In order to confirm this, an experiment was carried out to obtain acute measurements of oxygen uptake for animals maintained at two food levels.

Animals of uniform length were selected from mussels brought into the laboratory in November, and divided into two groups. One group was maintained at a food concentration of 1000-2000 *Tetraselmis* cells ml$^{-1}$, the other at 7000-10000 cells ml$^{-1}$. The duration of the feeding period was three weeks, after which the oxygen uptake of the animals in each group was measured. The dry weight of each mussel was then determined, and oxygen uptake expressed in terms of a one gram "standard" mussel. Results (table VIII) showed no significant difference at the 95% probability level between the means of the oxygen consumption values for the two groups (t-test).

Thus whereas in fish increased food availability leads to a higher metabolic level, the latter is independent of food concentration in *Mytilus*, provided the threshold value is exceeded. This is explicable in the following terms. The work done by the filtration apparatus of a bivalve is unlikely to be a function of food availability, because the ciliary tracts on the gill of an actively feeding animal are required to operate continuously regardless of particle concentration in the water. It seems likely (section I) that a considerable part of additional oxygen used during active metabolism comprises the cost of ventilation i.e. bringing food into contact with the gills. In contrast, active metabolism in fish involves locomotion in search of food, which must represent a significant part of the total metabolism. This component is likely to vary according to availability and
Table VII. The effect of food particle concentration on oxygen uptake.

<table>
<thead>
<tr>
<th>Food concentration</th>
<th>Animal</th>
<th>Dry weight (g)</th>
<th>Oxygen uptake (ml h⁻¹)</th>
<th>Standardised oxygen uptake (ml g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low 1,000-2,000 cells ml⁻¹</td>
<td>1</td>
<td>2.615</td>
<td>0.40</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.930</td>
<td>0.40</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.658</td>
<td>0.36</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.563</td>
<td>0.36</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2.008</td>
<td>0.36</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.627</td>
<td>0.40</td>
<td>0.29</td>
</tr>
<tr>
<td>High 7,000-10,000 cells ml⁻¹</td>
<td>1</td>
<td>2.099</td>
<td>0.40</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.702</td>
<td>0.44</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.959</td>
<td>0.29</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.227</td>
<td>0.44</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.898</td>
<td>0.47</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>2.202</td>
<td>0.40</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Mean standardised oxygen uptake (ml g⁻¹ h⁻¹) ± S.D.: -

Low 0.26 ± 0.03
High 0.25 ± 0.04

t ratio = 0.256
Students t at P = 0.05 for 10 df = 2.228

Table IX. Energy cost of amino acid loss by fed animals into surrounding medium. Nitrogen loss values (Bayne, unpublished data) converted into calorific equivalents by assuming that only the commoner amino acids are lost, and in equal proportions. Weight corrections from Hammen (1968).

<table>
<thead>
<tr>
<th>Dry weight (g)</th>
<th>Amino acids lost per day (cal)</th>
<th>Metabolism measured (T) (cal day⁻¹)</th>
<th>Amino acid loss as percentage of T</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.13</td>
<td>9.80</td>
<td>11.5</td>
</tr>
<tr>
<td>1.0</td>
<td>7.04</td>
<td>61.5</td>
<td>11.4</td>
</tr>
<tr>
<td>2.0</td>
<td>11.36</td>
<td>107</td>
<td>10.5</td>
</tr>
</tbody>
</table>
density of food (Kerr, 1971a,b), thus accounting for the observed dependence of metabolic level on food concentration in fish.

In Mytilus, therefore, for any given conditions of season and temperature, the T-line exhibits stability with regard to food concentration, provided the threshold value for filtration activity is exceeded.

Hammen (1968) has drawn attention to the fact that marine bivalves may lose free amino acids into the surrounding medium. Bayne (personal communication) has observed this phenomenon in mussels kept under the same experimental conditions as those used for the empirical model described here. Any such outflow of amino acids represents a loss of assimilated energy which is not included in the measured metabolism T. Table IX gives estimates of energy lost in this way, based on Bayne's unpublished data. These figures represent a maximum underestimate of T by 10 or 11 per cent which is independent of weight. Amino acid loss will therefore not affect the weight relationships defined in the model.

**Filtration rate**

The relationship between filtration rate and dry flesh weight is shown in fig. 13, and resembles that determined by Theede (1963). It describes a power function for animals below one gram dry weight (weight exponent 0.40), but above one gram the rate at which filtration increases with weight becomes considerably reduced. This is presumably associated with heterogonic growth of the gills, and has considerable influence on the energy balance of mussels of different weights, since small animals have a greater food input relative to oxygen uptake than do large animals.

In a discussion of factors affecting ingested ration in fish
Fig. 13 Filtration rate related to dry flesh weight.
Ivlev (1961) introduces the concept of a reduction in grazing efficiency as food availability increases. This is clearly relevant to the present consideration of energy balance in Mytilus, and it is therefore important to determine the relationship between filtration rate and food particle concentration. Richman (1958) demonstrated that feeding rate in the crustacean Daphnia pulex is independent of food availability, and commented that other authors have obtained similar data for marine zooplankton. With regard to filter feeding bivalves reports are conflicting (Jørgensen, 1966), but Dral (1967) comments that latero-frontal ciliary activity is reduced in dense suspensions, suggesting that filtration rate may be reduced.

Results from active metabolism experiments in section I indicate that filtration rate in Mytilus is independent of particle concentration over the range employed. Bayne (personal communication) and Widdows (personal communication), using identical experimental procedures, have drawn similar conclusions to these. In order to obtain filtration rate data over the entire physiological range (500 - 25000 Tetradesmis cells ml\(^{-1}\)), several mussels of uniform length were selected and filtration rate measured at a water flow 50 ± 4 ml min\(^{-1}\), using three values of particle concentration. Results, expressed in terms of a one gram "standard" mussel, are presented in fig. 14 and confirm that filtration rate is independent of food particle concentration.

**Assimilation efficiency**

As discussed in section I (fig. 9) assimilation efficiency is a decreasing linear function of food concentration. It remains to determine whether assimilation efficiency varies with weight. This is unlikely, since assimilation efficiency depends to a great extent on the surface area to volume ratio of the digestive system.
Fig. 14  Filtration rate at various concentrations of food.
and the rate of movement of material by its ciliary tracts, both of which are presumably constant for all adult animals, regardless of size. Nevertheless, an experiment was carried out to investigate the possibility of variation in assimilation efficiency with size.

Three groups of animals were maintained at a food concentration of 7000 - 10000 cells ml\(^{-1}\). Each group contained mussels of uniform length, but the mean length of the individuals in any one group differed from that of the animals in any other group. At intervals of two or three days, samples of faeces were taken from each group and assimilation efficiency determined by Conover's (1966) method. The experiment was repeated using the same animals but at a particle concentration of 1000 - 2000 cells ml\(^{-1}\). Results (table X) indicate that assimilation efficiency is independent of size at both particle concentrations used (t-test, 5% probability level).

**Ingested ration**

Fig. 15 shows that ingested ration \( R \) is a linear function of food particle concentration for all values of dry flesh weight. At any given food concentration, the ration obtained increases disproportionately to flesh weight. There is no decrease in "grazing efficiency" at high food levels, in contrast with Ivlev's (1961) results for fish. Nevertheless, there must be a limiting factor which imposes a critical value for particle concentration above which ingested ration stabilises or falls. Above food concentrations of 20,000 cells ml\(^{-1}\) *Tetraselmis*, mussels tend to close their valves and produce pseudofaeces. Under these conditions, filtration rate measurements are technically difficult and impossible to interpret biologically, since a proportion of the filtered material is not taken into the gut. It is not clear
Table A: The effect of size on assimilation efficiency.

a) Food concentration 10,000 - 12,000 cells ml⁻¹

<table>
<thead>
<tr>
<th>Group</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean length (cm) ± S.D.</td>
<td>6.30 ± 0.16</td>
<td>5.39 ± 0.13</td>
<td>4.58 ± 0.13</td>
</tr>
<tr>
<td>Assimilation efficiency (%)</td>
<td>15.1</td>
<td>10.0</td>
<td>6.9</td>
</tr>
<tr>
<td>mean</td>
<td>17.2</td>
<td>18.1</td>
<td>14.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-test</th>
<th>t-ratio</th>
<th>tabulated t at P = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large v. medium</td>
<td>0.168</td>
<td>2.447</td>
</tr>
<tr>
<td>Large v. small</td>
<td>0.501</td>
<td>2.447</td>
</tr>
<tr>
<td>Medium v. small</td>
<td>0.578</td>
<td>2.447</td>
</tr>
</tbody>
</table>

b) Food concentration 3,000 - 4,000 cells ml⁻¹

<table>
<thead>
<tr>
<th>Group</th>
<th>Large</th>
<th>Medium</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>61.6</td>
<td>50.2</td>
<td>59.5</td>
</tr>
<tr>
<td></td>
<td>52.7</td>
<td>46.3</td>
<td>33.6</td>
</tr>
<tr>
<td></td>
<td>64.0</td>
<td>82.2</td>
<td>50.2</td>
</tr>
<tr>
<td></td>
<td>73.0</td>
<td>71.8</td>
<td>60.6</td>
</tr>
<tr>
<td>mean</td>
<td>62.8</td>
<td>62.6</td>
<td>51.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>t-test</th>
<th>t-ratio</th>
<th>tabulated t at P = 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large v. medium</td>
<td>0.021</td>
<td>2.447</td>
</tr>
<tr>
<td>Large v. small</td>
<td>1.571</td>
<td>2.447</td>
</tr>
<tr>
<td>Medium v. small</td>
<td>1.091</td>
<td>2.447</td>
</tr>
</tbody>
</table>
Fig. 15  Relationship between ingested ration and food particle concentration.

Fig. 16  Relationship between assimilated ration and food particle concentration.

(10^6 cells = 0.059 mg dry organic matter = 0.370 cal)
whether the filtration mechanism breaks down totally at a critical particle concentration, or gradually decreases in efficiency. The effectiveness of the system is clearly limited by the quantity of food material which the gill ciliary tracts can carry in any given time. Visual observations, in this case somewhat subjective, suggest that the filtration apparatus is completely inhibited in dense suspensions. At all events, no reliable data can be provided for particle concentrations above 20,000 cells ml⁻¹.

**assimilated ration**

Assimilated ration pR is an increasing function of food concentration from zero to a maximum value which occurs at 12,500 cells ml⁻¹ for all weights (fig. 16). The larger the animal, the steeper the rise in assimilated ration. This is because larger gills obtain more food in any given time than do smaller ones, but assimilation efficiency is size independent. The influence of size is similar to its effect on ingested ration (fig. 15).

An interesting feature is the similarity between the parts of the assimilated ration curves and the curves obtained by Ivlev (1961) for ingested ration of fish. There is evidence that the assimilation efficiency of fish remains constant over the range of available ration commonly used in experimental work (Paloheimo and Dickie, 1965). If true, this means that for fish the relationship between ingested ration and available ration is similar to that between assimilated ration and food concentration, the latter curve being displaced from the former by a factor (1−p) where p is assimilation efficiency. The assimilated ration curves for fish and *Mytilus* are therefore similar. Regulation of energy input occurs within the digestive system in *Mytilus*, rather than at the ingestion stage, as seems to occur in fish.
The decrease in assimilated ration in dense food suspensions requires comment. This decline may be only apparent, resulting from errors in estimation of assimilation efficiency at high particle concentrations, but if real it suggests that the digestive system may become saturated. Furthermore, there is a particular value of food concentration (12,500 Tetraselmis cells ml\(^{-1}\)) at which the processes concerned with energy input are operating at maximum efficiency.

The particle concentration at which assimilated ration begins to decrease is close to the maximum tolerable by the animal, so from this aspect alone it is perhaps not surprising that the system begins to break down. It is evident that a fundamental difference exists between the fish and the filter feeding bivalve in that high food concentrations do not appear to restrict the feeding mechanism of the former, whereas in the latter the feeding apparatus is inhibited or even inactivated.

**Scope for growth**

The energy balance of an animal under any given set of conditions may be determined by subtraction of total metabolism \( T \) from assimilated ration \( P_R \). The value obtained may be positive, when surplus energy is available for growth or reproduction; or negative, in which case weight is lost. Surplus energy may be used for growth, deposition of metabolic reserves or gamete production. The concept of a "scope for growth" (Warren and Davis, 1967) is particularly attractive since it provides an acute measurement of excess energy without specifying the use to which it is put. To the physiologist, definition of the term "growth" is a problem and weight measurements, traditionally used as growth
indices, often lack sensitivity in bioenergetic terms. Growth may best be regarded here as the total change in energy value of body material. In this study of *Mytilus*, the "active metabolism" regression (fig. 6) is used for the T-line, and assimilation efficiencies are interpolated from fig. 9. A complete synopsis of the data for scope for growth and also for growth efficiencies is given in table XI.

Fig. 17 shows the relationship between scope for growth (pR-T) and dry flesh weight for several values of food concentration. Below 5000 cells ml\(^{-1}\), (pR-T) is a decreasing linear function of weight, since increased weight results in a disproportionately higher metabolic rate T compared with the gain in assimilated ration pR. At 5000 cells ml\(^{-1}\), however, scope for growth is constant for animals of 100 to 500 mg dry weight, then decreases at greater weight values. Above 5000 cells ml\(^{-1}\), 500 mg is the optimum weight for growth. This suggests that there is an interaction between weight and food availability which does not necessarily favour the smallest animal. The mechanisms underlying such an interaction are not clear, but the explanation must lie in the relationship between filtration rate and dry weight. It is possible that the food level at which the filtration mechanism breaks down may be weight dependent. There is no direct evidence for this, but on some occasions large animals (approximately 2 grams dry weight) have been seen to filter normally at 25000–30000 cells ml\(^{-1}\).

**Growth efficiency - the k-line**

The efficiency with which food ration is converted into body tissue is usually termed growth efficiency. Growth per unit ingested
Table XI. Synopsis of data used to construct growth model

<table>
<thead>
<tr>
<th>Dry flesh weight (mg)</th>
<th>Food concentration (cells ml⁻¹)</th>
<th>Number of cells ingested per day (x 10⁶)</th>
<th>Weight of cells ingested per day (mg)</th>
<th>Calories ingested per day</th>
<th>Assimilation efficiency</th>
<th>Calories assimilated per day</th>
<th>Total metabolism (ml oxygen per day)</th>
<th>Total metabolism (calories per day)</th>
<th>Scope for growth (calories per day) K₁</th>
<th>Gross growth efficiency K₂</th>
<th>Net growth efficiency K₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>100</td>
<td>18.7</td>
<td>2.47</td>
<td>0.82</td>
<td>0.62</td>
<td>3.46</td>
<td>3.12</td>
<td>-6.7</td>
<td>-1.93</td>
<td>-2.14</td>
<td>-0.61</td>
</tr>
<tr>
<td>1</td>
<td>250</td>
<td>37.4</td>
<td>2.47</td>
<td>1.24</td>
<td>1.81</td>
<td>6.92</td>
<td>6.09</td>
<td>-3.7</td>
<td>-0.83</td>
<td>-0.61</td>
<td>-0.09</td>
</tr>
<tr>
<td>2</td>
<td>500</td>
<td>37.4</td>
<td>2.47</td>
<td>3.61</td>
<td>1.81</td>
<td>10.1</td>
<td>8.90</td>
<td>-3.5</td>
<td>-0.17</td>
<td>-0.21</td>
<td>-0.17</td>
</tr>
<tr>
<td>5</td>
<td>1000</td>
<td>137</td>
<td>9.03</td>
<td>18.7</td>
<td>18.1</td>
<td>50.6</td>
<td>37.4</td>
<td>4.32</td>
<td>20.5</td>
<td>-16.9</td>
<td>+0.23</td>
</tr>
<tr>
<td>10</td>
<td>2000</td>
<td>547</td>
<td>36.1</td>
<td>36.1</td>
<td>18.1</td>
<td>101</td>
<td>56.6</td>
<td>-36.1</td>
<td>-0.36</td>
<td>-0.54</td>
<td>-0.54</td>
</tr>
</tbody>
</table>
Fig. 17 Scope for growth related to dry flesh weight and food particle concentration (cells μl$^{-1}$).
ration is gross efficiency \( (K_1) \), growth per unit assimilated ration being net efficiency \( (K_2) \). For a given type of food, Paloheimo and Dickie (1965, 1966b) claim that in a number of experiments using various species of fish, growth efficiency is related to ration by an exponential function:

\[
K_1 = \frac{\mu}{A} = e^{-a -bR}
\]

where 'a' and 'b' are constants. A similar relationship exists for \( K_2 \), but in fisheries biology it is \( K_1 \) which usually merits attention. Such a function implies that growth efficiency is a maximum at minimum food concentration, decreasing by a constant fraction \( e^{-b} \) for each unit increase in the amount consumed per unit time. In this analysis, growth efficiency is independent of size of the fish.

The \( K \)-line is the fundamental expression of the Paloheimo and Dickie model. The next step in the analysis of growth and metabolism of \textit{Mytilus} is therefore to compute \( K \)-lines and determine to what extent the model is applicable to the filter feeding bivalve.

**Gross Growth Efficiency (\( K_1 \))**

Fig. 18 relates gross efficiency \( K_1 \) to ingested ration \( R \). At very low ration, \( K_1 \) is negative i.e. the animal is out of balance, but small increases in \( R \) result in greatly improved growth efficiencies. When \( K_1 \) is zero, energy input is equal to total metabolism, and \( R \) is then a measure of maintenance ration. \( K_1 \) reaches a maximum value before becoming a decreasing function of \( R \).

The \textit{Mytilus} \( K \)-line therefore differs in two respects from \( K \)-lines described for fish by Paloheimo and Dickie (1965, 1966b). First, in the mussel the \( K \)-line has a negative as well as a positive phase. Secondly, \( K_1 \) in \textit{Mytilus} reaches a maximum value at an optimum
Fig. 16  The effect of increasing ingested ration on gross efficiency of growth ($K_1$).
ration. Using sockeye salmon, Brett et al. (1969) have produced very similar K-lines to those derived here for the mussel, and suggest that the K-line described by Paloheimo and Dickie (1965, 1966b) is associated with supra-optimal feeding levels only. Kerr (1971b) derives equations in which K for fish increases hyperbolically with ration. This is precisely the relationship determined here for Mytilus (figs. 18, 19) using experimental methods. Further support for such relationships between growth efficiency and ration comes from work on crustaceans. Corner and Cowey (1968), commenting on the wide range of K values quoted for planktonic Crustacea, remark that K is likely to vary with food concentration, being small at low ration and rising to a maximum before decreasing at high ration. Reeve (1963) draws the same conclusions from experiments with Artemia salina. Richman (1958) describes similar observations using Daphnia pulex and Slobodkin (1962) demonstrates an optimum algal concentration for growth efficiency, also in D. pulex.

There is considerable evidence, therefore, that the Mytilus K-line may be of more general application. It is likely that the data analysed by Paloheimo and Dickie (1965, 1966b) comprise measurements made at comparatively high ration levels, which would account for a K-line of zero or negative slope, whereas in the analysis of Mytilus measurements were made over the entire physiological range for ration. A second possibility is that conventional growth measurements using weight changes are not sufficiently acute for calculation of K values at low ration.

Fig. 20 represents the relationship between ingested ration K and all values of log K where K is positive. This provides a direct comparison with the K-line derived by Paloheimo and Dickie (1965, 1966b), but in Mytilus the decrease in K at high ration is
Fig. 19  Gross efficiency of growth ($K_1$) related to the reciprocal of ingested ration $R$. 
Fig. 20 The relationship between ingested ration and all values of log gross growth efficiency $K_1$ where $K_1$ is positive.
not exponential, neither is it a power function of ration. The rate of decline in $k_1$ increases considerably as the maximum tolerable ration is approached, due to the saturation of the feeding and digestive systems in the mussel.

Figs. 16 and 19 indicate that the $K$-line is size dependent. This is in contrast to the findings of Faloheimo and Diokkie (1966b) and reflects once again the relationship between filtration rate and weight in *Mytilus*, since the $T$-line is similar in fish and bivalve. The $K$-line (fig. 18) may be conveniently transformed by relating $K_1$ to the reciprocal of ingested ration $R$ (fig. 19).

Between zero ration and maximum $K_1$ the relationship between $K_1$ and $R$ is hyperbolic for animals of any weight, but $K_1$ falls as ration increases further. The smaller the mussel, the greater the growth efficiency for any given ration $R$, and the greater the maximum value of $K_1$. Jørgensen (1952) also found an inverse relationship between $K_1$ and size for *Mytilus edulis*. The ration at which growth is most efficient, i.e. the optimum ration, is an increasing function of weight (fig. 19), reflecting the greater energy input required to offset total metabolism of a large animal.

Net growth efficiency ($K_2$).

The relationship between net growth efficiency and assimilated ration describes a perfect hyperbola (fig. 21). Hyperbolic functions are characteristic of systems which become saturated, in this case the digestive system of *Mytilus*. At any given assimilated ration, $K_2$ is a decreasing function of weight. The maximum value of $K_2$ varies from 75% for a 0.1 gram animal to zero for a 2 gram mussel.

Fig. 22 shows that net growth efficiency of an individual of given weight increases disproportionately to food concentration,
Fig. 21 The relationship between net growth efficiency $K_2$ and assimilated ration.
Fig. 22. The effects of dry flesh weight and food availability on net growth efficiency $K_2$. 
which is an important observation from the standpoint of commercial cultivation. For instance, if a mussel receiving 10,000 particles ml\(^{-1}\) is given a further 5,000 cells ml\(^{-1}\), the resulting increase in \(z_2\) is insignificant. The greater the food concentration, the greater the conversion efficiency, but the lower the rate of increase.

Conclusions

Fig. 23 represents a summary of changes in energy balance of a 1 gram mussel at 15°C over the physiological range of food particle concentration. The difference between total metabolism (respired energy) and assimilated ration is a measure of scope for growth, which is negative at cell concentrations below the maintenance value (5,000 cells ml\(^{-1}\)) and positive at food levels in excess of maintenance ration. Subtraction of assimilated ration from ingested ration represents non-utilised input, which is an increasing function of particle concentration. This non-assimilated energy is lost to the *Mytilus* population in the form of faecal pellets and as pseudofaeces. Nevertheless, it is potentially available to the detritivores of the benthic community, and in this sense the mussel population may be said to provide a trophic link between the benthic and planktonic communities. The growth model proposed here offers a means of quantifying this concept in future modelling of the benthic community as a whole.

The empirical growth model clearly has potential in obtaining acute measurements of energy balance under a variety of laboratory controlled conditions. This is particularly true of the K-line, which provides a sensitive index of environmental stress. At present
Fig. 23 Representation of changes in the energy balance of a one gram mussel over the physiological range of food particle concentration.
only two variables have been examined for *Mytilus*, namely body
weight and food availability, but further physiological dimensions
remain to be analysed. For instance, the effects of spatial
distribution of food merit attention (Ivlev, 1961; Kerr, 1971a,b).
Prey size influences the K-line of fish (Paloheimo and Dickie, 1966b;
Beamish and Dickie, 1967; Kerr, 1971a,b), so that an investigation
of particle nature and size in terms of the K-line in filter feeders
would be worthwhile.

In fish, the T-line, but not the K-line, is temperature
dependent (Paloheimo and Dickie, 1965, 1966a,b). Since Widdows and
Dayne (1971) have analysed the influence of temperature on oxygen
uptake and energy balance of *Mytilus* under similar experimental
conditions to those described here, it should prove possible to
generate K-lines for different degrees of thermal stress and integrate
them into the model described. Less detailed at present is work
on salinity stress, but Wilson (personal communication) has recorded
salinity effects on oxygen consumption of *Mytilus*.

Such experimental methods may be applied equally well to
bivalve larvae. In addition to describing energy relations in
various larval stages and relating changes to stress factors,
the empirical model may serve most usefully in determining the
effects which stress imposed on adults may produce in terms of the
energy balance of the filial generation. This link between the
stressed adult and the degree of success of the next generation
is now a fundamental problem in physiological studies of stress in
bivalves.
SECTION III

Synthesis and utilisation of energy reserves
INTRODUCTION

One of the most significant features of the biology of many marine invertebrates, at least in temperate regions, is their ability to synthesise and store energy reserves during periods of food availability (Giese, 1959, 1966) and then to utilise these at times when food may be scarce. Such seasonal patterns of metabolism are often closely integrated with a well defined reproductive cycle, as in Mytilus edulis (Daniel, 1921, 1922; Williams, 1969), in which gametes gradually mature during winter and spring, spawning taking place from late spring onwards (Chipperfield, 1953).

The experiments and analyses described in this section were carried out to investigate seasonal changes in the utilisation of metabolic reserves and the effects of nutritive stress on these seasonal patterns, particularly during the period of gamete maturation. The common mussel Mytilus edulis is a convenient animal for such experiments because of its ease of culture, its almost ubiquitous distribution and because the invasion of the mantle by the developing gonad facilitates the separate analysis of germinal and somatic tissues. In addition, the mussel is of commercial importance and there is an extensive literature on the biology of the species.

The work described here falls into two distinct parts. First, some preliminary experiments were carried out in 1969, forming part of a more extensive programme on stress physiology of Mytilus (Hayne and Thompson, 1970). These initial studies concentrated on the biochemical composition of the gonad. In 1971 a second series of
experiments was carried out in which other tissues were examined as well as the gonad, and in greater detail. Attention was particularly focussed on the digestive gland, not only because of its obvious significance as a link between assimilated food and the blood system but also in view of suggestions (Sastry, 1966) that the bivalve digestive gland may regulate nutrient distribution to other parts of the body.

A. PRELIMINARY SERIES

MATERIALS AND METHODS

Two experiments were done in this 1969 series, the first from February 3rd to April 3rd, the second from May 6th to July 7th. In both cases animals of uniform length were selected and 30 mussels were maintained in each of two 8.25 l polythene buckets with flow rate 50 ± 10 ml min⁻¹. Tetraselmis was dosed into one bucket at 10⁴ cells ml⁻¹ ("high ration") and into the other at 10³ cells ml⁻¹ ("low ration"). Mussels were withdrawn at intervals during the course of these experiments for determination of a gonad index (May-July experiment only) and for biochemical analysis. Initial values were determined within two days of sampling the field population. Animals taken from the field will hereafter be referred to as "field animals".

Gonad index

Ten mussels were opened by cutting the adductor muscle and a piece of mantle tissue examined for determination of the gonad index by a method developed by Bayne (1963). The tissue was squashed on
a slide and classified as one of the following stages: 1) mantle is thick but no gametes are observable; 2) mantle is thick and gametes are observed in the squash, but the sperm are not active in seawater and the seminal vesicle of the oocyte does not disappear on release into seawater; 3) the gametes are activated on release into seawater, i.e. the sperm are active or the oocytes lose the seminal vesicle; 4) the mantle is thin and no gametes are visible (stage 0). The numbers of animals that fall into each category are multiplied by the number of the stage and the sum divided by the number of animals in the sample. This yields an index that ranges from 0 for fully spawned or "spent" mussels to 3 for ripe animals in the pre-spawning stage (Chipperfield, 1953). This gonad index suffers from not recognising a "partially spawned" stage (Lubet, 1955) in which some, but not all of the gametes have been released. However, such a condition is recognisable by the patchy appearance of the mantle and may be recorded as a partially spawned stage 3.

Two points require emphasis. First, the gonad index provides information relating to the stage of development of the gametes but not to the number of gametes present. Secondly, the numerical values are not on a linear scale, and must be considered as semi-quantitive only.

Preparation of tissue for analysis

Mantle tissue was separated from non-mantle tissue in six animals. Mantles from paired mussels were pooled to give three units of tissue for analysis. An identical procedure was adopted for non-mantle tissue. Six units, three mantle and three non-mantle, were thus obtained from each experimental condition at each sampling time. The tissues were homogenised in a cooled Potter-Alvahjem homogeniser and dried to constant weight at 90°C. Portions were taken from the
dried homogenates for determination of nitrogen and carbohydrate.

**Protein determination**

Total nitrogen was determined by Kjeldahl digestion followed by distillation in a Markham still, collection in boric acid and back titration against H/70 sulphuric acid. Protein values are given as nitrogen x 6.25.

**Carbohydrate determination**

Total carbohydrate was estimated as glucose equivalents by the phenol-sulphuric acid method (Dubois et al., 1956) after homogenising the tissue with cold 5% trichloroacetic acid containing 0.1% silver sulphate (Barnes and Heath, 1966) and boiling to ensure hydrolysis of all polysaccharides and oligosaccharides.

**RESULTS**

**Gonad index and dry weight**

Figure 24 represents the gonad index at various times of the year and during the May-July experiment. Changes in dry weight are also shown. In both high and low ration groups, gonad index decreased from 2.8 at the start of the experiment to 2.2 after 31 days in the laboratory. There was no further change in gonad index in either group during the remainder of the experiment (total 59 days). Both groups showed a weight loss (total flesh weight) during the first month of stress, but no subsequent loss. The low ration group experienced the greater decrease in weight.

These results suggest that the maintenance energy requirement of the animals was not being met in either culture. It also seems
Fig. 24  Top: Gonad index. Circles - field values. Triangles - May - July culture.
Bottom: Gonad dry weight, May - July culture.
Open symbols - starved culture.
Filled symbols - fed culture.
likely that the stress imposed by the culture conditions further increased the natural maintenance requirement. Both high ration and low ration cultures showed very similar decreases in gonad index and dry weight, which suggests that non-nutritive stress factors were contributing to these changes. The animals in both groups experienced a temperature rise of 5°C when brought into the laboratory. Subsequent work by Middows and Bayne (1971) has shown that temperature increase represents a significant stress to Mytilus in laboratory cultures; furthermore, mussels can acclimate either completely or partially within fourteen days of being subjected to a warm temperature stress. These results are entirely compatible with those described here.

Biochemical composition

The results of the analyses for carbohydrate and protein in the mantle and non-mantle tissues are presented in fig. 25 (February - April experiment) and fig. 26 (May - July experiment). In each case there were decreases in carbohydrate and protein (expressed as percentages of dry weight) which were greater in the mantle than in the non-mantle. Carbohydrate was lost more rapidly than protein. These results are consistent with the view that the mantle serves as a site for the storage of reserve materials as well as for the production and maturation of gametes.

In the February - April experiment, the initial temperature stress (10°C above ambient) was greater than in the May - July experiment (5°C above ambient), accounting for the more extensive losses of carbohydrate and protein in the earlier culture. For instance, in February - April non-mantle protein was maintained at the initial level for 40 days but then declined, whereas through-
Fig. 25 Carbohydrate and protein values, expressed as percentages of dry weight, during February - April culture.
Circles = mantle, triangles = non-mantle. Open symbols = starved culture, filled symbols = fed culture. Mean ± range.
Fig. 26 Carbohydrate and protein, expressed as percentages of dry weight, during the May - July culture. Circles - mantle, triangles - non-mantle. Open symbols - low ration, filled symbols - high ration. Mean + range.
out the 61 days of the May - July experiment there was no decrease in non-mantle protein as a percentage of dry weight. Furthermore, the decrease in mantle carbohydrate was greater in February - April than in May - July, although carbohydrate losses from the non-mantle were similar in the two experiments.

The weights of carbohydrate and protein were computed for the May - July experiment (table XII). There was no significance difference in mantle carbohydrate between high ration and low ration animals after 61 days, but a marked difference in the carbohydrate of the non-mantle. Mantle protein declined significantly more in low ration animals but there was no significant difference in non-mantle protein. In table XIII the weights of carbohydrate and protein lost are listed as percentages of the observed overall dry weight losses.

The balance between carbohydrates and proteins in the tissues may be expressed as the carbohydrate : protein ratio by weight. This ratio refers carbohydrate to unit weight of protein. C : P curves for high and low ration animals in the two experiments are presented in fig. 27. The curves illustrate the loss of carbohydrate in both mantle and non-mantle. In the February - April experiment, the C : P ratio decline was much greater in the mantle than the non-mantle. Furthermore, an equilibrium was established between losses of carbohydrate and protein. During the May - July experiment, however, the decrease in C : P ratio was very similar in both mantle and non-mantle and there was no evidence of equilibrium after 61 days. The loss of non-mantle carbohydrate relative to protein in May - July was associated with the maintenance of a high gonad index (2.2) during this period. Although low ration individuals utilised more of their body reserves than did high ration animals, the C:P ratio was controlled to similar values by both groups. This occurred
### Table XI11 Mean weights (± standard deviation) of carbohydrate and protein in the May–July experiment. Probability values in the t-test are given for seven comparisons; where the differences are significant at the 5% level, the probability value is underlined.

<table>
<thead>
<tr>
<th>Days</th>
<th>Ration level</th>
<th>Carbohydrate</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mantle</td>
<td>Non-mantle</td>
</tr>
<tr>
<td>1</td>
<td>High &amp; Low</td>
<td>6.8±0.7</td>
<td>17.3±3.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.8±0.9</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>61</td>
<td>High</td>
<td>0.3±0.0</td>
<td>2.4±0.9</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.3±0.1</td>
<td>0.9±0.3</td>
</tr>
</tbody>
</table>

**Statistical analysis (t-test):**
- Total carbohydrate (61 days): high ration v. low ration < 0.001
- Total protein (61 days): high ration v. low ration < 0.05
- Mantle protein (61 days): high ration v. low ration < 0.001
- Non-mantle carbohydrate (61 days): high ration v. low ration < 0.05
- Non-mantle protein (61 days): high ration v. low ration > 0.05
- High ration: mantle protein (31 days) v. mantle protein (61 days) > 0.05
- Low ration: mantle protein (31 days) v. mantle protein (61 days) < 0.001

### Table XIII Total weight loss and loss of carbohydrate and protein in the May–July experiment.

<table>
<thead>
<tr>
<th>Days</th>
<th>Ration level</th>
<th>Total weight loss (mg)</th>
<th>Carbohydrate loss % of total</th>
<th>Protein loss % of total</th>
<th>Unaccounted loss mg % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>High</td>
<td>130</td>
<td>19.3</td>
<td>14.8</td>
<td>113</td>
</tr>
<tr>
<td>61</td>
<td>Low</td>
<td>173</td>
<td>22.6</td>
<td>13.1</td>
<td>143</td>
</tr>
</tbody>
</table>
Fig. 27  Carbohydrate : protein ratios during preliminary experiments.
Circles = mantle, triangles = non-mantle.
Open symbols = low ration, filled symbols = high ration. Mean + range.
in mantle and non-mantle.

It appears, therefore, that under the conditions of nutritive and temperature stress represented in these experiments, *Mytilus edulis* rapidly mobilised carbohydrate reserves from the mantle to meet the energy requirement. As stress continued, proteins were also utilised and reserves of both carbohydrate and protein in non-mantle tissues called upon. Animals with similar initial levels of reserves utilised these in similar proportion although the absolute level of utilisation was higher in the low ration groups. These and other experiments (Bayne and Thompson, 1970) showed that in autumn and winter the changes in balance, but not in absolute quantities, of carbohydrate and protein in the body were similar in laboratory cultures to the changes occurring in the field population.

B. MAIN SERIES

INTRODUCTION

The second series of experiments was carried out to provide further information about the utilisation of food reserves by *Mytilus edulis* in response to long term laboratory stress. Particular attention was paid to the mantle (containing the gonad) because the preliminary experiments had shown this tissue to be an important source of reserve material. In terms of fecundity and spawning success of the population, the implications of a depletion of gonad reserves are obvious. For this reason, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) have been estimated to give measurements of gamete concentration and rate of protein synthesis respectively.
Emphasis was placed on stress effects in the ripe gonad. The biochemical compositions of male and female mantles were separately analysed in this series. Lipid was also measured, since there is evidence that gonad lipid varies seasonally in *M. edulis* (Lubet and Longcamp, 1969; Williams, 1969) and *M. galloprovincialis* Lamarck (Bourcart et al, 1964), suggesting a possible role for lipid as a stored energy reserve.

A further objective was to examine seasonal and stress-induced changes in the digestive gland. There is little information concerning the role, if any, of the bivalve digestive gland in storing metabolic reserves. Reid (1969) has suggested that in the horse clam *Tresus cupax* Gould digestive gland lipid may serve as an energy store which is depleted when food is scarce. Sastry (1966) and Sastry and Blake (1971) have shown that material stored in the digestive gland of *Aequipecten irradians* Lamarck is transferred to the gonad during gametogenesis. The digestive tissue of *Mytilus edulis* may also have a storage function and may therefore be involved in the utilisation of reserves during stress.

These experiments also demonstrate structural changes in the digestive tubules resulting from long-term stress. It is not the purpose of this work to describe details of digestive tubules' histology and fine structure. Several accounts exist for various bivalve species (Yonge, 1926; Sumner, 1966a, 1966b; Owen, 1970; Bowen and Davies, 1971; Pal, 1971), although there appears to be no information for the genus *Mytilus*. The bivalve digestive tubule possesses two cell types distinguishable by light microscopy, the digestive cell and the basophil cell. Electron microscope studies have shown that the digestive cell possesses various cytoplasmic inclusions which are involved in a cycle of food breakdown (Owen,
and that the basiphil cell is characterised by a well developed endoplasmic reticulum (Owen, 1970; Pal, 1971). The function of the basiphil cell is not certain. According to Yonge (1926) the basiphil cell is an undifferentiated type which replaces degenerating digestive cells. Mansour (1946), however, regards the basiphil cell as fully differentiated with a secretory function. More recent studies (Summer, 1966; Owen, 1970; Pal, 1971) have shown that in some species two distinct forms of basiphil cell occur in the digestive tubule. One of these is flagellated and has much less endoplasmic reticulum than the other, non-flagellated, type. Summer (1966) has separated these two forms of basiphil cell histochemically in some freshwater bivalves. Thus there are some indications that basiphil cells may possess both functions previously attributed to them. The present work provides evidence that the structure of *Mytilus* digestive tubules is very similar to that described for other bivalves. Description of basic structure will therefore be kept to a minimum and only those features which are important in relation to stress will be discussed in detail.

**MATERIALS AND METHODS**

Estimates were made of RNA and DNA in the foot, digestive gland and mantle of animals taken from the field at intervals during 1970 and 1971. Male and female mantle tissue was analysed separately. At various times during this period laboratory cultures of starved mussels were maintained at 15°C for up to six months. Nucleic acid estimations were carried out periodically on these cultures.
From May 1970 to September 1971, more comprehensive analyses were carried out on digestive glands from field animals. At each sampling, the digestive glands from ten mussels were excised, dried at 100°C and weighed. Each weight was expressed as a percentage of mantle-free dry weight, this ratio being termed the "digestive gland index". Mantle weight was excluded from the calculation of the digestive gland index, otherwise seasonal changes in the former would obscure the true seasonal pattern of digestive gland weight changes relative to somatic tissue. Similar digestive gland indices have previously been used for molluscs (Lawrence et al., 1969; Sastry, 1966; Sastry and Blake, 1971; Waber, 1971).

During the 1971 April-September culture, a group of mussels fed at 2000 Tetraselmis cells ml⁻¹ was also maintained. In both starved and fed groups comprehensive analyses were made at intervals. Digestive gland, mantle and remaining somatic tissue were excised from ten animals from each group, then separately dried and weighed. Carbohydrate, protein, lipid and ash were estimated in the dry digestive gland and mantle, nucleic acids in the wet tissue. Male and female mantles were separately analysed. Studies were also made of structural changes in the digestive tubules during this experiment.

Estimation of nucleic acids

RNA and DNA were extracted by a procedure modified from Munro and Fleck (1966). Approximately 0.1 to 0.2 g wet tissue was excised from the freshly killed animal and placed on ice. The tissue was broken up in 2 ml ice-cold water by means of ultrasonic disintegration. Temperature was maintained between 0°C and 4°C throughout. Protein and nucleic acids were then precipitated by addition of 5 ml 13% trichloroacetic acid (TCA) and allowing the sample to stand for 10
minutes in ice. The centrifuged precipitate was washed with 10% TCA, then incubated with 4 ml 0.3M potassium hydroxide for two hours at 37°C. According to Munro and Fleck (1966) more drastic hydrolysis results in solubilisation of some DNA. The two hour incubation period was determined experimentally, giving optimum ribose yields in the mussel tissues used here.

After treatment with alkali, the samples were cooled in ice, 2.5 ml 1.2M perchloric acid (PCA) added to each and the tubes left to stand for 15 minutes at 0° - 4°C. The resulting precipitate, containing protein and RNA, was centrifuged and washed with 1.2M PCA. The supernatant and washings, containing ribose from RNA hydrolysis, were pooled and made up to a known volume (usually 10 ml). 1.5 ml samples were analysed for ribose by the orcinol reaction (Dische modification, described by Ashwell (1957)), using D-ribose (Sigma grade) as standard. Results were expressed as ribose per unit dry weight of tissue, or as ribose per unit deoxyribose, referred to as an "RNA : DNA ratio". The ratio used here is not a true absolute value, since the orcinol reaction measures purine bound pentose sugar only. The RNA : DNA ratio used does, however, reflect relative changes in the absolute, unknown, value.

The precipitate containing DNA and protein was incubated with 2 ml 1.2M PCA at 70°C for 20 minutes. This time was found to give an optimum yield of deoxyribose in all tissues concerned. Use of stronger acid or higher temperatures may result in unacceptable protein levels in the extract (Munro and Fleck, 1966). The precipitate was centrifuged, washed and discarded. Supernatant and washings were made up to a known volume (usually 5 ml). Deoxyribose was estimated in 2 ml of this volume by the diphenylamine...
method (Giles and Myers, 1969), using 2-deoxy-D-ribose (Sigma grade) as standard. Results were expressed as deoxyribose per unit dry weight of tissue.

Measurements of nucleic acids in the digestive gland may be complicated by the presence of food matter. The extraction procedure, however, precipitates RNA and DNA in sequence, and any free pentoses are discarded. Only pentose sugars bound in nucleic acid molecules are estimated. When pieces of digestive tissue were taken for analysis, care was taken to avoid the stomach, which lies in the centre of the digestive gland, so that there was no possibility of taking intact algal cells. Furthermore, histological and electron microscope studies indicated that no viable cells were present in the tubule lumen. There is thus strong evidence that nucleic acid measurements in the digestive gland reflect events in the tubule cells and are not affected by the presence of food.

determination of carbohydrate, protein, lipid and ash

Carbohydrate was estimated by the phenol-sulphuric acid method and protein by kjeldahl distillation, both as previously described (preliminary series).

Lipid was determined gravimetrically after extraction by a modification (Holland, personal communication) of the Bligh and Dyer method (1959). Approximately 40 to 100 mg dry tissue were homogenised in 1 ml water using a Potter-Elvehjem homogeniser. The latter was then rinsed with 3.75 ml 1:2 V/V chloroform : methanol in two stages, the washings being added to the aqueous homogenate. This mixture was vigorously shaken (rotamixer) for one minute, then allowed to stand for 15 minutes. 1.25 ml chloroform
and 1.25 ml water were then added and the mixture again shaken for one minute. The resulting emulsion was centrifuged and separated into two phases, with a solid protein layer at the interface. The aqueous phase was discarded and the organic phase (containing lipid) dried at 40°C - 50°C before weighing.

Ash was determined by combustion of dry tissue at 450°C.

On all sampling occasions, the sum of the four independent estimations lay between 90% and 95% of the digestive gland dry weight. The remaining 5%-10% comprised nucleic acids, components not estimated and experimental error. It is suspected that most error was incurred in the determination of carbohydrate by the phenol-sulphuric acid method.

Histology and fine structure

For routine histology, pieces of digestive gland were fixed in Bouin's fluid, washed with several changes of 70% ethanol, dehydrated, cleared in cedarwood oil and embedded in paraffin wax. Sections were cut at 5 μm with a Spencer microtome and stained with haematoxylin and eosin.

For high resolution histology and for electron microscopy, small pieces of tissue (approximately 1 mm³) were fixed for 30 minutes in 2.5% glutaraldehyde buffered at pH 7.2 with 0.05M sodium cacodylate and containing 0.15M sucrose. The tissue was washed in buffer containing 0.30M sucrose, then postfixed for two hours in 1% osmium tetroxide buffered at pH 7.4 with sodium veronal acetate (Palade, 1952). Specimens were then rinsed in buffer, dehydrated and embedded in araldite.

Thick plastic sections (0.5 - 1.0 μm) were cut on a Huxley Mk. II ultramicrotome and stained with a mixture of methylene
blue and azure II (equal parts). Wax sections and thick plastic sections were examined with a Zeiss photomicroscope.

Ultrathin sections (silver or grey interference colours) were mounted on formvar-coated copper grids and stained with a 30% solution of uranyl acetate in methanol (7 minutes) followed by lead citrate (7 minutes; Reynolds, 1963) before examination with an AEI 802 electron microscope.

RESULTS

Digestive gland index

During early summer the digestive gland was full of food and unusually green in colour, presumably as a result of feeding on phytoplankton cells. The digestive gland index reached a maximum of 27-34% at this time before decreasing during late summer to a value of approximately 20% which was maintained throughout autumn and winter. In a starved culture started in July there was no change in digestive gland index, whereas in April the index decreased sharply, reflecting the warm temperature stress (10°C above ambient) in April and the absence of temperature stress in July. During the July-September culture, the digestive gland index recovered to the field value in fed animals but not in starved ones.

According to Sastry (1966) the digestive gland index of Aequipecten irradians also decreases from a maximum in May to a minimum in July. Lawrence et al. (1965) have shown that the size of the digestive gland in the chiton Katharina tunicata is influenced by the amount of food available; the digestive gland shrinks during starvation and may have a storage function. These results
Fig. 28  Digestive gland index and somatic dry weight (less digestive gland).
Open circles - field values, filled circles - starved cultures, crosses - fed culture.
Mean ± S.D.
are consistent with the present work on the bivalve *Mytilus edulis*.

**Somatic weight**

The seasonal pattern in somatic weight (less digestive gland) is also shown in Fig. 28. Growth occurs between May and August, corresponding with Baird's (1966) data based on whole flesh weight measurements of west coast mussels.

Somatic weights of animals in laboratory cultures, like their digestive gland indices, decrease much more in April than in July, due to the greater temperature stress in spring. Extensive somatic weight losses in April may also result from conservation of gametes at this time (see DNA data). Approximately 50% of the initial somatic tissue weight was lost after five months stress, which agrees closely with figures provided by Fox and Coe (1943) for starved *Mytilus californianus*.

**Digestive gland nucleic acids**

The digestive gland RNA : DNA ratio (Fig. 29) shows a pronounced peak during the spring (April 1970, May 1971). In both cases the maximum values occurred immediately after the onset of spawning and coincided with the maximum digestive gland index. Thus when food is abundant the digestive gland is large and protein synthesis increases. This may represent enzyme secretion, growth of digestive tissue, or both. Sutcliffe (1970) has described the use of RNA measurements as estimates of growth in some invertebrates. Digestive gland protein reached its highest level at the time of maximum RNA : DNA ratio (Fig. 31).

During all laboratory cultures protein synthesis (indicated by RNA : DNA ratio) was reduced below field values. Animals starved in April were more adversely affected than those starved
Fig. 29  RNA : DNA ratios in digestive gland, foot and female mantle.
Open circles - field values, filled circles - starved cultures, crosses - fed culture.
Mean ± S.D. (digestive gland and foot).
Mean ± range (female mantle).
in January, although the temperature stress was similar. This may result from relative inactivity in the digestive gland during midwinter (figs. 28, 29, 31) and depletion of digestive gland reserves to maintain viable gametes in the mantle in April. In July, when there was no temperature stress, the reduction in RNA : DNA ratio was greater than in January, when stress was a maximum. It appears that the mechanisms controlling protein synthesis in the digestive gland are more susceptible to stress during the period when large quantities of carbohydrate and protein reserves are being laid down (fig. 31) than during the time of apparent inactivity in winter.

**Mantle RNA**

The female mantle showed a rise in RNA : DNA ratio from February to April in both years (fig. 29). Maximum protein synthesis was greater than in the digestive gland and represents yolk deposition in the maturing oocytes. There is considerable evidence that yolk synthesis occurs at this time in *Mytilus edulis*. First, sections of female mantles in March revealed a large number of immature follicles but few follicles containing mature eggs. Secondly, autoradiographs of female mantles fed $^{14}C$ labelled Tetraselmis cells in March indicated that a considerable proportion of the activity in the gonad was localised in the yolk regions of those mature eggs which were present (experiments in progress). Thirdly, the gonad index was approximately 1.0 in December and 2.0 in March, so that yolk deposition could not have occurred before midwinter.

Female mantle RNA : DNA was maintained at a maximum for a very short time before decreasing at the onset of spawning. Minimum values were observed between July and October, a resting phase in terms of gametogenesis but a period of accumulation of
reserves, associated with a low metabolic rate (Bayne and Thompson, 1970). At this time, RNA : DNA values were similar to those in the digestive gland.

During the laboratory culture started in January 1970, RNA : DNA ratios approximated field values for five weeks but were unable to attain the maximum field value recorded in May. In February 1971, however, the RNA : DNA ratio decreased immediately animals were brought into the laboratory. During the May-September 1970 and April-June 1971 cultures, the decreases in RNA : DNA ratio were similar to field changes, despite the absence of spawning in the stressed groups. This suggests either that no further protein synthesis is possible in ripe animals brought into the laboratory, or that such synthesis is unnecessary. At all events, existing mature eggs are maintained for several weeks in laboratory cultures.

In July, the RNA : DNA ratio in the mantles of stressed females was very similar to the field value. This situation continued until the following January, when the field RNA : DNA ratio had risen but the stressed animals were unable to increase protein synthesis.

Foot nucleic acids

The foot is an example of a tissue unlikely to have a storage function. RNA : DNA ratios are highest in December - February, falling to minimum values during summer (fig. 29). At all times of year, protein synthesis was less in laboratory cultures than in field animals. This can be interpreted in terms of increased byssus secretion during adverse winter weather conditions and little necessity for byssal gland activity in the laboratory.
Mantle DNA

DNA values in mantles of field and stressed mussels are presented in fig. 30. In the male, DNA per unit weight increased from October onwards, but most rapidly after December, and represented primordial germ cell divisions and subsequent spermatogenesis. A full complement of gametes was present by February or March and retained until receipt of a suitable spawning stimulus (Bayne, 1965). Under natural conditions, this stimulus is a gradual rise in water temperature (Chipperfield, 1953). Although DNA synthesis in the male gonad was complete in March, the gonad index (based on sperm motility) did not reach its maximum of 3.0 until April or May, immediately before spawning. Thus sperm DNA is completely synthesised by February or March and the gonad index is then 2.0 i.e. sperm visible but not motile. The sperms then attain motility and become viable, the gonad index rising to 3.0 at spawning. There is no increase in the number of gametes during this period. Spawning in this population takes three months to complete, in contrast to the three or four weeks described as normal by Chipperfield (1953).

The mantles of males starved in January 1970 showed an initial rise in DNA concentration compared with field values, but this reached a maximum which was much lower than the value attained by animals in the field population. During January 1971, however, DNA per unit weight decreased in starved males. Gametogenesis began earlier in that year and the DNA content of field animals was greater at the time mussels were brought into the laboratory than at the corresponding time in the previous year.

If males with ripe gonads are stressed in the laboratory, the DNA concentration is maintained for four weeks before gamete
Fig. 30  Deoxyribose in mantle tissue.
Open circles – field values, filled circles – starved culture, crosses – fed culture. Mean + range.
resorption begins; there is no spawning. There is apparently no difference between starved and fed animals. Histological evidence (not presented here) confirms that resorption of gametes eventually takes place and gonad structure breaks down. According to Fox and Coe (1943) gametes in ripe gonads of starved Mytilus californianus may be retained in a viable state for several months before cytolysis begins.

During summer, when there was no temperature stress and no gametogenesis, DNA levels in male mantles of laboratory cultures were similar to field values.

In field animals and in laboratory cultures, DNA concentration in the female mantle increased from January to April 1970 as oogenesis progressed. The rapid decrease in DNA per unit weight during April coincided with the maximum value in RNA : DNA ratio, i.e. protein synthesis (fig. 29). An increase in yolk deposition with no increase in DNA (i.e. cell numbers) will result in a reduction in DNA per unit weight. The DNA concentration therefore increases during gametogenesis and falls during vitellogenesis, reaching a minimum just before spawning. When spawning occurs, a large amount of yolk and other cytoplasmic material is lost relative to DNA, so that DNA concentration in the gonad increases once again. During late summer 1970, the DNA level in the female mantle decreased, probably as more oocytes matured. This is consistent with observations of the duration of the spawning period.

Throughout winter 1971 the gametogenesis cycle was repeated. During a laboratory culture commenced in April 1971, no spawning occurred in either starved or fed animals, so there was no increase in DNA per unit weight, in contrast to the field data. There is an apparent anomaly regarding the situations in April 1970 and April
1971, but it is not known whether the 1970 mussels spawned.

**Composition of digestive gland**

Results from digestive gland analyses (less nucleic acids) are presented in fig. 31. Protein comprises 50% of digestive gland dry weight during autumn and early winter, rising gradually to a maximum of 60% in April, which corresponds with peak protein synthesis (fig. 29). There is a rapid reduction in protein to a minimum of 40% in June; this is associated with carbohydrate synthesis. Protein then rises once again to 50% of total dry weight in late summer.

Total carbohydrate accounts for 15-20% by weight of the digestive gland during winter. This value falls to a minimum of 4% in late April, immediately after spawning commences, then rises very rapidly to 32% in June. During late summer the carbohydrate level decreases to 6% (August) before increasing once more in September.

There are also seasonal changes in the amount of lipid present in the digestive gland. This contrasts with the work of Daniel (1922), who comments on the high lipid content of *Mytilus edulis* digestive tissue but failed to detect any seasonal variation. Reid (1969) found no changes in digestive gland lipid of the horse clam *Tresus capax*, a winter spawner. The present study of *H. edulis* shows clearly that digestive gland lipid decreases from between 14% and 18% of total dry weight in early winter, to a minimum of 11% during late winter when the final stages of gametogenesis, including vitellogenesis, are occurring. From March onwards, lipid rises to a maximum of 31% in July, then decreases in September.

There is a curious reciprocal relationship between carbohydrate and lipid during summer, the significance of which is at present obscure. These summer changes in digestive gland composition,
Fig. 31  Biochemical composition of the digestive gland.
Open circles = field values, filled circles = starved culture, crosses = fed culture. Mean ± S.D.
however, lend strong support to the seasonal pattern of metabolism postulated by Bayne and Thompson (1970) for *Mytilus*. During June and July, carbohydrate synthesis and catabolism of lipid result in a low respiratory quotient (Bruce, 1926) and a high oxygen : nitrogen ratio. Metabolic rate rises in autumn as body protein and lipid increase; the O : N ratio decreases and the respiratory quotient increases as carbohydrate becomes the dominant substrate.

Ash comprises 6%–9% of the digestive gland dry weight throughout the winter, but rises sharply to 11%–15% during March and April, i.e. during and immediately after spawning. This implies a low organic content and "poor condition". Ash returns to normal values during May.

During the laboratory cultures maintained between April and September 1971, there was no difference between the responses to stress of starved and fed animals in terms of digestive gland composition. Protein increased initially, associated with very small decreases in carbohydrate, lipid and ash. Between May and September there was no change in carbohydrate or lipid, despite pronounced fluctuations in field animals. Protein, however, decreased gradually throughout this period and ash correspondingly increased to account for almost 25% of the total dry weight of the digestive gland after five months stress.

Carbohydrate : protein and lipid : protein ratios (fig. 32) emphasise the pattern of metabolism in the digestive gland. There is a decrease in C : P ratio during late winter. L : P also declines but this occurs earlier than the reduction in C : P. During summer there is a reciprocal relationship between C : P and L : P.
Fig. 32 Carbohydrate : protein and lipid : protein ratios in the digestive gland.
Open circles - field values, filled circles - starved culture, crosses - fed culture.
Composition of mantle

No detailed seasonal data are available, but Bayne and Thompson (1970) have shown that mantle carbohydrate is high in late summer and in winter. Mantle reserves are utilised during gametogenesis. Lipid levels reach a maximum in the ripe gonad and a minimum after spawning in *Mytilus edulis* (Lubet and Longcamps 1969; Williams, 1969) and *M. galloprovincialis* (Bourcart et al., 1964). Bayne and Gabbott (unpublished data) have identified similar changes in mantle lipid of *M. edulis* from the population used in the present work. The mantle analyses described here for laboratory cultures begun in April 1971 were therefore carried out when carbohydrate was low but lipid at a maximum. Results are plotted in fig. 33.

Mantle dry weight decreased steadily, with no difference between starved and fed animals. More than 80% of the initial mantle weight was lost after five months stress, compared with the 50% decrease in somatic weight (fig. 28).

At this time there was a distinct difference in mantle composition between male and female mussels. In the female, lipid was higher and protein correspondingly lower than in the male. Protein comprised 80% of the male mantle by dry weight. These differences are interpreted in terms of the presence of yolk (lipoprotein) in the female and large quantities of nucleoprotein in the male.

During stress, the male mantle lost more protein proportionately than the female, which conserved protein and lipid and utilised carbohydrate initially. This may be a mechanism to conserve mature eggs with their high yolk content.
Fig. 33  Dry weight and biochemical composition of male and female mantles during stress.
Open circles - initial values (field), filled circles - starved culture, crosses - fed culture.
Mean ± S.D.
Calorific losses during stress

Biochemical analysis of tissue provides information about the percent dry weight composition of that tissue at any given time. This is of limited value in any attempt to understand the utilisation of energy reserves during stress. Combining weight losses and biochemical analyses enables one to calculate calorific losses in various tissues. This is the most meaningful approach to the problem, and has been adopted for the starved animals in the April-September culture 1971. The following calorific equivalents were used:

- Protein \(5.50 \text{ cal mg}^{-1}\)
- Carbohydrate \(4.10 \text{ cal mg}^{-1}\)
- Lipid \(9.30 \text{ cal mg}^{-1}\)

Mean values were used for the percent composition of the gonad, since there were differences between male and female. Somatic tissue was weighed but not analysed, so results from non-mantle analyses in the preliminary series were used.

The calculations (table XIV) show that after five months starvation, 72% of the total energy loss resulted from protein catabolism, 20% from lipid and 8% from carbohydrate. Mantle tissue contributed 51% of this energy, the digestive gland 15% and the remaining somatic tissue 34%. Thus the mantle and digestive tissue together provided two thirds of the total energy loss after five months starvation. Protein accounted for nearly three-quarters of the total loss. According to Bayne (personal communication) the oxygen : nitrogen ratio for starved animals at this time is approximately 30. From theoretical considerations protein catabolism accounts for 60% of the total calorific loss when \(O : N\) is 30 (assuming that carbohydrate and lipid are utilised in equal
Table XIV  Calorific losses during the April - September starved culture.

<table>
<thead>
<tr>
<th>Tissue</th>
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<td>1097</td>
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<td>5446</td>
<td>100</td>
</tr>
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</table>

Percentage of total loss contributed by each component: 8 20 72 100

Estimated respiratory loss (from Middows and Bayne, 1971) 5400
Oxygen uptake was not monitored, but Widdows and Bayne (1971) provide data for standard metabolism of mussels kept in the laboratory at the same time as the culture discussed here, and at the same temperature. The agreement between total metabolism over five months based on this estimate and the measured total calorific loss from the tissues is remarkably close (table XIV). Amino nitrogen loss, however, was not estimated but is known to be significant (Bayne, personal communication). Nevertheless, the degree of agreement suggests that measurements of both total metabolism and calorific loss from tissues provide accurate estimates of the same phenomenon, i.e., utilisation of energy reserves during stress.

Structure of digestive tubules

Wax sections and thick plastic sections (plate 1) show that the digestive tubules of field animals (April 1971) are well organised. Digestive cells and basophil cells are readily distinguishable, particularly in wax sections stained with haematoxylin and eosin. The basic structure is similar to that described by Yonge (1926) for lamellibranchs in general. A significant feature is the large number of darkly staining inclusions in the basal regions of the digestive cells. Granules are visible in the tips of many basophil cells.

Plate 2 illustrates the fine structure of the basophil cell, which is very similar to that of Anodonta cygnea (Sumner, 1966b), Cardium edule (Owen, 1970) and Mya arenaria (Pal, 1971). Its characteristic features are an extensive endoplasmic reticulum with numerous free ribosomes (see also plate 4), an active Golgi
body (plate 3), microvilli at the tip of the cell at its boundary with the tubule lumen, and the presence of granules at the cell apex. These granules are regarded by many authors as secretory in function. All available evidence indicates that the basiphil cell is equipped for extensive protein synthesis.

Digestive gland fine structure (plates 2, 4) is also similar to that described for other bivalves by Sumner (1966b) and Owen (1970). The cytoplasm contains various inclusions associated with an intracellular digestive cycle. Most prominent are the large structures referred to here as "macrovesicles" (MV), which contain moderately electron dense material and occur most commonly near the apex of the digestive cell. These macrovesicles are visible under the light microscope. They appear to correspond approximately to the "type 1" and "type 2" vesicles described by Owen (1970) in Cardium. Equally conspicuous are smaller inclusions termed "microvesicles" (mv) which are very numerous, tend to occur in the basal region of the cell and are identical with Owen's (1970) "type 4" vesicle. These microvesicles are the densely staining bodies which are visible in thick plastic sections.

Plate 5 illustrates detail of the apex of an active digestive cell. Discrete, uniform food particles from the lumen are channelled between the microvilli of the cell. The particles presumably pass across the cell membrane by pinocytosis (Owen, 1970) and form a large number of pinocytic vesicles (pv), which appear to coalesce, giving rise to macrovesicles.

The effects of four weeks starvation are illustrated in plates 6-8. Basiphil cells are much less prominent (plate 6) than in tubules from field animals, appearing smaller, fewer in number and very densely stained. The endoplasmic reticulum of many basiphil
colls shows pathological changes (plates 7, 8) becoming arranged into a series of concentric sheets. The Golgi body is not active. The reduction in volume of the cell probably accounts for its increased density in both light microscope and electron microscope preparations.

Some digestive cells appear completely empty after four weeks starvation (plate 6); others only partly empty and a few show little change from the normal condition. There is, however, considerable reduction in the density of the basal cytoplasm of the digestive cells. Macropores are still present in most cells and are even more prominent than in normal tissue (plate 1), due to the reduction in cytoplasm density. The most striking feature, however, is the scarcity of microvesicles. There is considerable variation among tubules in the extent to which necrosis has occurred, but cell breakdown is particularly evident in wax sections, which are much more representative of the tissue than are thick plastic sections, due to the larger area available for observation.

Plate 8 also illustrates a cell type additional to the digestive cell and basiphil cell thus far considered. This third type is not commonly seen in normal tissue but becomes prominent in stressed tubules. It is apparently not affected by stress, possessing a healthy endoplasmic reticulum and an active Golgi body. The cell is flagellated (plate 12). Owen (1970) has described a second basiphil cell in Cardium which is flagellated, much less pyramidal than the non-flagellated type and with less endoplasmic reticulum. Mytilus is similar in this respect. Pal (1971) comments that some basiphil cells of Mya are flagellated. In Anodonta there are also two types of basiphil cell (Summer 1966a). One form has a low RNA content, no apical granules and is regarded as immature,
since it also shows mitosis. The second form is rich in RNA, possesses apical granules and is considered to be a mature secretory cell. The role of this flagellated basiphil cell during prolonged stress in *Mytilus* should therefore prove significant.

Wax sections of tubules from animals fed for four weeks show less marked breakdown of digestive cells than in the corresponding starved group. More microvesicles are present. These features are also visible in thick plastic sections (plate 9). Normal digestive activity takes place (plate 10). Secretory cells are in general not as badly affected as in the starved tubules, showing little or no size reduction, little change in endoplasmic reticulum and possessing apical granules.

After eleven weeks starvation, disruption of digestive cells is very extensive (plate 11). The degree of breakdown is variable and some macrovesicles are still present. Although the cytoplasm is comparatively devoid of inclusions, many empty or partly empty microvesicles are visible (plates 11, 12). Very few of the initially abundant non-flagellated basiphil cells occur, although there are a few flagellated forms (plate 12). The latter are not affected by stress and possess a normal endoplasmic reticulum and an active Golgi apparatus.

The corresponding fed group shows less severe degeneration in both digestive cell and non-flagellated basiphil cell (plate 13). Those microvesicles which are present in digestive cells tend to be dense rather than empty, in contrast to those of starved tubules. Furthermore, non-flagellated basiphil cells are present, but all have a pathological endoplasmic reticulum and stain densely, resembling cells from the four weeks samples. Flagellated cells are much more common than in starved tubules, staining readily
in thick plastic sections (plate 13) and possibly replacing degenerate digestive cells.

Five months starvation leads to extensive breakdown of digestive cells (wax sections and thick plastic sections, plate 14) which become empty and lose their structural integrity. There appears to be some fusion of digestive cells. Many macrovesicles are still present. Microvesicles are essentially devoid of contents. Non-flagellated basophil cells show advanced necrosis with total breakdown of the endoplasmic reticulum (plate 15). Digestive cells also show signs of extensive degeneration. The nuclear envelope invaginates and its component membranes may separate. Microvesicles are often empty or nearly so, becoming similar to the "type 3" vesicles described by Owen (1970) in Cardium.

The structure of tubules from mussels fed for five months (plate 16) is very different to that of starved tubules. Wax sections show that there is structural integrity with the exception of a few degenerating tubules. Discrete digestive cells are present. The general appearance is similar to that of normal tubules from field animals. Digestive cells contain numerous dense vesicles similar to microvesicles already described (plate 16). These vesicles have a curious two component structure (plate 17) which must be associated with the use of a Tetraselmis monoculture as food. Finally, flagellated basophil cells are common (plate 17).

Any experimental study of structural changes in a system over a period of time poses difficulties in adequate sampling, since multiple sampling on each occasion involves an impractical work load. Consideration of this problem is commonly evaded by histologists and particularly by electron microscopists. In the
present investigation of *Mytilus* digestive tissue, general trends are identified in the effects of laboratory stress. Analysis of the various changes in terms of a precise time course is not possible. In the mollusc digestive cell the situation is further complicated by the intracellular digestion cycle associated with normal feeding. Both autophagic and heterophagic bodies (De Duve and Wattiaux, 1966) are likely to be present in the same cell, particularly under stress conditions. Furthermore, digestive cells have a limited life and breakdown is continuously occurring in normal tissue (Purchon 1971).

According to Purchon (1971) the means by which degenerate digestive cells are replaced is obscure. Yonge (1926) regarded the basophil cell as an immature digestive cell because of its high mitotic rate. Similar conclusions were reached by Sumner (1966a) and Mix and Sparks (1971), whereas Owen (1970) and Pal (1971) found no evidence that basophil cells of either type could or could not give rise to digestive cells. The present study of *Mytilus* strongly suggests that the flagellated basophil cell may differentiate into a mature digestive cell under suitable conditions. The extensive breakdown of digestive cells in this experiment disturbed the normal equilibrium between cell types with the result that the replacement of degenerate digestive cells in fed cultures became more obvious than in previous studies of non-stressed bivalves.

There is a correlation between digestive gland index (fig. 28) and tubule structure during stress. After four weeks the digestive gland index decreased by a similar amount in starved and fed mussels. Both groups showed structural degeneration which was slightly more extensive in the starved animals. During prolonged laboratory culture the digestive gland index of starved mussels remained low and tubule structure continued to break down. In the fed group,
however, the digestive gland index recovered to the field value and the digestive cells regained their structural integrity. In a study of the effects of ionising radiation on the digestive tubules of *Crassostrea gigas* Thunberg, Mix and Sparks (1971) found that digestive cell necrosis was very advanced two months after gamma-irradiation. Subsequent repair of digestive cells was effected by division of basophil cells. The time course of these degenerative changes and the evidence of tubule recovery lend support to the present *Mytilus* study. Stress induced degenerative changes in the digestive tubules cells themselves do not affect the capacity of the tissue for recovery.

The degenerative changes which occur in the ultrastructure of stressed digestive tubules are typical of those induced by starvation of animal cells in general (Ratcliffe, 1967). In particular, the endoplasmic reticulum is capable of rapid and drastic changes in form (Fawcett, 1959). Protein secretory cells are particularly susceptible to starvation. For instance, in the venom system of the hymenopteran parasitoid *Masonia vitripennis* Walker it is the secretory cell of the acid gland which rapidly degenerates on starvation and is reduced in volume (Ratcliffe and King, 1970). Other cells of the system show minor adverse effects only.

DISCUSSION

The digestive gland of *Mytilus edulis*, like the mantle, shows marked seasonal variations in biochemical composition, suggesting a possible role in the storage of metabolic reserves. Lawrence
et al. (1965) and Sastry (1966) have postulated a flow of digestive gland reserves into the gonad during gametogenesis in the chiton _Katharina tunicata_ and the bivalve _Aequipecten irradians_, but the evidence is tenuous. In _M. edulis_ there is no change in digestive gland index, total carbohydrate or lipid during winter, suggesting either that metabolism is low or that the system is in equilibrium throughout winter. The slight fall in lipid content during late winter may not be biologically significant, at least in terms of utilisation of energy reserves, because there is the difficulty of separate identification of structural lipids and those which may be metabolically labile (Giese, 1966). From February until spawning, however, there is a significant reduction in digestive gland carbohydrate. Organic content is low i.e. "condition" poor. There are thus indications that reserves may be transferred from the digestive gland to the metabolically active gonad during late gametogenesis, and may have particular significance in vitellogenesis. Nutrients must of course enter the mantle via the digestive gland, but the important point is to establish whether there is utilisation of reserves already accumulated in the digestive tissue.

There is no doubt that digestive gland material is rapidly utilised during stress, whereas remaining somatic tissue and mante tissue is metabolised gradually. Recovery of normal digestive cell structure and digestive gland index in fed mussels after an initial temperature stress is essential to survival under these conditions, since all metabolic processes are ultimately dependent upon the functional integrity of the digestive gland. Replacement of disrupted digestive cells is remarkably rapid even under normal conditions (Purchon, 1971).

If the gonads are ripe, both male and female mussels are able
to retain large numbers of viable gametes for several weeks under stress conditions, despite loss of weight from the mantle. After four to eleven weeks starvation and further weight loss, DNA levels fall rapidly and extensive breakdown of gametes occurs. Protection of gametes from stress effects is clearly advantageous to the success of the population.

In most of the laboratory cultures there was little or no difference between starved and fed mussels in terms of biochemical composition and weight loss (excepting the digestive gland index). There are three identifiable contributory factors. First, warm temperature stress is more significant than nutritive stress in determining the initial responses of mussels brought into the laboratory (Middone and Bayne, 1971). Secondly, fed animals have a high metabolic rate which may result in the utilisation of much of the calorific input during feeding. Thirdly, biochemical indices such as the carbohydrate : protein ratio are controlled to similar values under various degrees of stress. Nevertheless, there are differences between starved and fed mussels in the structural changes within the digestive tissue. These may reflect more subtle stress effects which are not revealed by biochemical analysis. Williams (1969) measured the biochemical composition of mussels in an attempt to determine the effect of the parasite Mytilicola intestinalis Steuer, but found no difference between parasitised and non-parasitised animals. This approach, therefore, is not useful in determining stress indices, not because analyses are insensitive but because parameters such as the C : P ratio tend to be regulated during stress. Analysis of biochemical composition is however useful for the identification of seasonal patterns of metabolism, for instance to determine the nature, quantity and site
of energy reserves, and in studies of gametogenesis, particularly its synchronisation with other events. If combined with weight data, such analyses may also provide precise measurements of calorific losses which are particularly meaningful in determining the utilisation of reserves from various sites during sublethal environmental stress.

One of the stated aims of this work is to identify meaningful indices which are descriptive and predictive of stress. The biochemical parameters examined, particularly the C : P ratio, do not meet this requirement, whereas certain physiological parameters afford acute, reliable and practical measurements of sublethal stress. Scope for growth (section II) and the oxygen : nitrogen ratio (Bayne and Thompson, 1970) are particularly useful and will be used in future work, not only with *Mytilus edulis* but with other marine organisms in a variety of stress situations.

The extensive protein catabolism during stress is particularly interesting in terms of the high loss of amino-nitrogen recorded by Bayne (personal communication) in the same population of *Mytilus edulis* under similar conditions. Nitrogen loss at this time may account for an additional metabolic demand of half the standard oxygen uptake. In these circumstances the use of oxygen uptake as an index of metabolism leads to an underestimate of standard metabolic rate by 30-35% of the true value. Thus two features, namely the results of tissue analyses and the high loss of amino nitrogen, suggest that nitrogen metabolism plays a major role in stress physiology. This has been amply documented for zooplankton (Corner and Gowey 1968). Classical accounts which associate high glycogen levels in bivalves with reliance on carbohydrate reserves are therefore oversimplified, but the full significance of amino nitrogen loss is not yet understood and is under investigation.
PLATES
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Plate 1

Digestive tubules from field animals. The tubules are well organised. Microvesicles are prominent and numerous (mv).
Plate 2

Fine structure of the basiphil cell (BC) of a field mussel. Two adjacent basiphil cells are shown, flanked by digestive cells (DC). The basiphil cells are active, possessing apical granules (ag), prominent Golgi bodies (Gb) and extensive endoplasmic reticulum (ER).
Plate 3

Active Golgi body from a basiphil cell (field animal).
Large numbers of vesicles (v) are being synthesised which may give rise to the larger granules (g). These granules are similar or identical to those observed at the cell apex (plate 2). Note also the abundance of free ribosomes (r).
Basal regions of a digestive cell (DC) and a basophil cell (BC) from a field animal. The basophil cell possesses a very extensive endoplasmic reticulum (ER) and numerous free ribosomes (r). The digestive cell Golgi (Gb) is active.
Plate 5

Apical regions of three digestive cells from animals feeding in the field. Food particles (fp) are being directed between microvilli (mi) before pinocytosis apparently takes place.
Plate 6

Digestive tubules from animals starved for four weeks. Some tubules show advanced necrosis (DT), but the degree of degeneration is variable, particularly in the digestive cells (DC). The basiphil cells (BC) are considerably affected by stress, becoming very dense and losing structural integrity in some places. Microvesicles (mv) are fewer than in field mussels (plate 1).
Plate 7

Fine structure of the digestive cell (DC) and basophil cell (BC) after four weeks starvation. Note particularly the relatively empty cytoplasm at the apices of the digestive cells (compare field animals, plate 5) and the pathological nature of the basophil cell endoplasmic reticulum.
Basophil cells of the flagellated (FC) and non-flagellated (BC) type. The flagellum of the former is not visible here but may be seen in other sections (plate 12). The endoplasmic reticulum of the non-flagellated cell is markedly affected by the four weeks starvation but the flagellated type remains healthy and possesses an active Golgi body (Gb).
Plate 9

Digestive tubules from mussels fed for four weeks in the laboratory. There is some variation in the extent to which degeneration has occurred but there are many basophil cells (BC) which often contain apical granules (ag).
Normal digestive activity at the apices of digestive cells from mussels fed for four weeks. Compare with plate 5 (field animals) and plate 7 (four weeks starved).
Plate 11

Digestive tubules after 11 weeks starvation. There is severe breakdown of digestive cell structure. A few microvesicles (mv) are still present, but they tend to be empty or partly so.
Plate 12

A flagellated basophil cell (PC) from an animal starved for eleven weeks. The cell has a characteristic endoplasmic reticulum (ER) (see also plate 8) and an active Golgi body (Gb).
Plate 13

Digestive tubules from mussels fed for 11 weeks. Degeneration is less extensive than in the corresponding starved group (plate 11).
Plate 14

Digestive tubules showing almost total breakdown after five months starvation.
Plate 15

Advanced necrosis after five months starvation. The non-flagellated basophil cells (BC) are completely degenerate and the endoplasmic reticulum has broken down. The nucleus and cytoplasm of the digestive cells (DC) also show pathological changes.
Plate 16

Digestive tubules from a mussel fed for five months in the laboratory. Considerable recovery has occurred and there are numerous microvesicles (mv) present. The organisation of the tubules contrasts with that of the starved group (plate 14).
Plate 17

Microvesicles (mv) within the digestive cells after five months feeding. Part of a flagellated basophil cell is also shown.
SECTION IV

Distribution of assimilated radiocarbon and radiophosphorus within tissues and tissue fractions
INTRODUCTION

Quantitative biochemical analyses (section III) provide information about the energy reserves laid down and utilised in various tissues at different times of the year and under sublethal stress conditions. They do not give any indication of the dynamics of nutrient transfer from the food source to the tissues, or from one biochemical component to another within a particular tissue.

The experiments described in this fourth section were carried out to determine the distribution of nutrients in various tissues and tissue fractions using radionuclide tracer techniques. Integrated data from such tracer studies and from quantitative biochemical estimations should provide a detailed picture of metabolism under various conditions.

Radionuclide tracers have not been employed extensively in studies of bivalve metabolism, although Hobden (1969) examined the uptake of iron ($^{59}$Fe) by Mytilus edulis and Sastry and Blake (1971) investigated the effect of temperature on the fate of $^{14}$C - leucine injected into the digestive gland of Aequipecten irradians. Incorporation of $^{32}$P into tissues of a variety of bivalves (including M. edulis) has been described by Allen (1962, 1970).

In the present work using Mytilus, incorporation of both $^{14}$C and $^{32}$P into the tissues was studied simultaneously, after non-specific labelling of the food cells (Tetraselmis). Food carbon labelled in this way will become incorporated into carbon skeletons throughout the mussel. $^{14}$C is therefore a suitable radionuclide for preliminary studies on synthesis and utilisation of reserves.
Studies using $^{32}$P were carried out for the following reasons. First, $^{32}$P affords a convenient measure of activity in nucleic acids without requiring their separation from protein in the protein-nucleic acid fraction. This is particularly important in the mantle during gametogenesis. Secondly, there is some information available for $^{32}$P distribution in *M. edulis* and other bivalves (Allen, 1962; 1970). Thirdly, phosphorus metabolism is interesting in itself. In particular, there is evidence (own unpublished observations) that phospholipids comprise a high proportion of the total lipid in *Mytilus*. Finally, both $^{14}$C and $^{32}$P distribution may be studied in the same experiment without difficulty, since the energy emission spectra of the two radionuclides are sufficiently separate to facilitate dual isotope analysis of a single doubly-labelled sample.

**MATERIALS AND METHODS**

Labelled *Tetraselmis* cells were prepared as follows. Approximately 200 to 300 ml fresh culture were centrifuged and the cells resuspended in 100 ml fresh seawater medium before addition of 0.1 - 0.2 mCi sodium bicarbonate NaH$^{14}$CO$_3$ and 0.1 - 0.2 mCi sodium orthophosphate NaH$_2$$^{32}$PO$_4$ (Radiochemical Centre, Amersham). Labelled cultures were stirred continuously and used between three and seven days of inoculation, during which period 90% of the total $^{32}$P activity in the culture was located in the algal cells.

Mussels were brought into the laboratory in January, June and September 1970. Within 24 hours of arrival, two groups of ten animals were each placed in 600 - 800 ml seawater and labelled *Tetraselmis* culture added gradually over a period of four hours.
Subsequently both groups were held at 15°C ± 1°C for up to 50 days, one group starved, the other fed unlabelled *Tetraselmis* at 2000 - 3000 cells ml⁻¹. Animals were taken at intervals for analysis, two mussels from each group on each occasion.

Digestive gland, mantle, adductor muscle and foot were dissected out and fractionated by the Schmidt - Thannhauser method as modified by Runro and Fleck (1966). Small pieces of tissue (100 - 200 mg wet weight) were broken up in 2 ml ice-cold water by an ultrasonic disintegrator and 5 ml cold 13% TCA added. The resulting precipitate was centrifuged and washed twice with water. The supernatant and washings were made up to 10 ml. This fraction is referred to as the "acid soluble" fraction and comprises glycogen plus compounds of low molecular weight.

Lipids were removed from the precipitate with 3 ml 90% ethanol (containing 1% sodium acetate to prevent solubilisation of nucleic acids) followed by three extractions with 2 ml 3:1 V/V 90% ethanol : ether. The extracts were pooled and made up to 10 ml with 90% ethanol.

The remaining solid, comprising protein and nucleic acids, was solubilised by digestion with a mixture of 0.5 ml perchloric acid and 1 ml hydrogen peroxide. The colourless digest was made up to 5 ml or 10 ml with water. During the January - February experiment, RNA and DNA were separated from protein in mantle tissue using the methods described in section III.

One ml of each extract was added to 9 ml scintillation fluid (butyl-PBD scintillant in dioxaq/solvent; Kobayashi and Maudsley, 1969). The samples were placed in a liquid scintillation counter ("Tritomat", Isotope Developments Ltd.) for determination of $^{32}$P and $^{14}$C by dual isotope analysis (Kobayashi and Maudsley, 1969).
Results were expressed as counts per second per mg dry weight after appropriate corrections for quenching and decay.

RESULTS

Data from $^{14}$C analyses are expressed in three ways. First, total counts per unit weight are presented for each of the four tissues in figs. 34 and 35A, without distinction between tissue fractions. Secondly, counts per unit weight in each fraction are shown in figs. 35B and 36, no distinction being made between the four tissues. Finally, $^{14}$C activity in the digestive gland and mantle is illustrated in figs. 37 and 38 respectively.

Distribution of $^{32}$P is discussed in two tissues only, the digestive gland (fig. 39) and the mantle (fig. 40).

$^{14}$C distribution in tissues

In September (fig. 34A) the digestive gland lost $^{14}$C activity initially, but after seven days there was no further loss per unit weight. Activity was constant in mantle, foot and adductor muscle throughout the experiment. There appeared to be an initial decrease in activity in the digestive gland which probably represented replacement of losses due to turnover in other tissues during the acclimation period, after which there was considerable retention of $^{14}$C in the digestive gland.

Fig. 34B indicates a continuous loss of $^{14}$C from the digestive gland in January, yet the labelled pools in other tissues were lower than in September. The prolonged depletion of digestive gland material may function to replace reserves lost from other sites,
Fig. 34  Distribution of $^{14}C$ in tissues during the September and January experiments.

Circles - digestive gland, squares - mantle, triangles - adductor muscle, inverted triangles - foot.

Open symbols - starved, filled symbols - fed. Mean + range.
thus maintaining them at a constant level. Alternatively, digestive tissue $^{14}$C activity may be lost entirely by metabolism within the digestive gland itself, but this is very unlikely. Total metabolism (measured by oxygen uptake) was highest in January, at the onset of gametogenesis (Widdows and Bayne, 1971), and temperature stress was at a maximum. Either or both of these factors could account for loss of material from the digestive gland without a corresponding increase in $^{14}$C in other tissues.

Loss of labelled carbon from the digestive gland in June (fig. 35A) was similar to the September condition, but there was more accumulation in other tissues than in September or January. Maximum activity occurred after four days in mantle, foot and adductor. There was little loss of $^{14}$C overall, which may be correlated with low oxygen uptake at this time (Widdows and Bayne, 1971) in contrast to winter metabolism.

$^{14}$C distribution in fractions

Figure 35B illustrates the loss of activity from the acid soluble fraction in starved animals (June experiment) in contrast to the fed group where the initial decrease was followed by a period when the activity level was maintained. Extensive protein synthesis occurred in fed mussels. Lipid synthesis was observed in both starved and fed animals but was greater in the former. Thus starved mussels were synthesising more lipid than protein, fed animals more protein than lipid. This may be a mechanism to ensure that a starved animal maintains as large a lipid pool as possible for subsequent use under unfavourable conditions, whereas a fed individual is able to channel a greater proportion of the available energy into protein. This does not imply that a fed mussel in these circumstances has
Fig. 35  Distribution of $^{14}$C in tissues (top) and tissue fractions (bottom) during the June experiment.

less total lipid than a starved one; radionuclide tracer data indicates the relative degree of use of various metabolic pathways. A proportion of the acid soluble fraction is glycogen, but lipid appears to be more extensively synthesised at this time.

In September (fig. 36A) activity in the acid soluble fraction decreased rapidly from a high value to reach equilibrium after approximately 15 days. The lipid fraction accumulated $^{14}C$ to a much lesser degree than in June. Protein synthesis was considerable as it was in June. The initial loss of $^{14}C$ from the acid soluble fraction (and to a lesser extent the lipid fraction) may represent replenishment of the protein pool, assuming that there was a turnover of $^{14}C$ protein. Respiration must also account for a proportion of the activity lost. The equilibrium attained may be correlated with the 15 day acclimation period shown for Mytilus by Widdows and Bayne (1971).

Figure 36B shows considerable losses of $^{14}C$ from the acid soluble fractions in starved and fed animals throughout the January experiment. Activity in lipids was initially high, especially in the starved group, but decreased at a similar rate to that in acid soluble fractions. Incorporation of $^{14}C$ into proteins and nucleic acids was much greater in fed than in starved mussels. These differences between starved and fed animals in the relative degree of lipid and protein synthesis are similar to those observed in June. In winter there is a very high turnover of material in the body as a whole (particularly in the lipid and acid soluble fractions) which is associated with high metabolism during gametogenesis.

$^{14}C$ distribution in digestive gland

The digestive gland merits particular attention because it
Fig. 36  Distribution of $^{14}$C in tissue fractions during the September and January experiments.
Circles - acid soluble, squares - lipid, triangles - protein/nucleic acids.
Open symbols - starved, filled symbols - fed. Mean ± range.
represents the site of food assimilation into the animal, and also because it has a role in the storage of energy reserves (section III).

In September (fig. 37A) there was a very rapid loss of activity from the digestive gland during the first ten days of the experiment (see also fig. 34A). Depletion of accumulated $^{14}C$ occurred in all three fractions. The amount of labelled carbon in digestive gland protein was high and its initial turnover very rapid, possibly representing enzyme synthesis.

Figure 37B illustrates the approximately exponential decrease in the acid soluble fraction which occurred in January, especially in starved mussels. The activity in this fraction was reduced after 40 days to less than 5% of its original value, representing a considerable turnover of the small molecule pool. Lipid synthesis was also extensive in both groups, but $^{14}C$ activity was lost steadily after its initial accumulation. Activity in the protein also reached a maximum after four to seven days before decreasing. This continuous depletion of digestive gland pools throughout the January experiment contrasts with the September experiment and may be correlated with high metabolism in winter.

The June experiment (fig. 37C), which lasted for ten days only, also showed a loss of $^{14}C$ from the acid soluble fraction in starved and fed mussels. There was considerable activity in lipid throughout the experiment, but as in September the initial high $^{14}C$ levels in protein subsequently decreased.

$^{14}C$ distribution in mantle

Figure 38A illustrates results from the September experiment. Activity in the acid soluble fraction of the mantle was initially high, decreasing during the first five days of the experiment before
Fig. 37  Distribution of $^{14}$C in digestive gland fractions.
Circles - acid soluble, squares - lipid,
triangles - protein/nucleic acids.
Open symbols - starved, filled symbols - fed.
Mean + range.
Fig. 38 Distribution of $^{14}$C in mantle fractions.
Open symbols – starved, filled symbols – fed. Mean + range.
equilibrating. Protein synthesis was considerable, but lipogenesis low. Protein and lipid pools were maintained at constant levels throughout the 50 day period.

In January (fig. 38B) there was a significant difference between starved and fed animals. Fed mussels were able to channel more $^{14}$C into all the mantle fractions than were starved individuals. There was very little loss of activity from the acid soluble fraction of the fed group, but a gradual loss in the starved animals. Incorporation of $^{14}$C into lipid was greater relative to other fractions than in September. Synthesis of protein and nucleic acids was much greater in fed than in starved animals. Lipid and protein pools were maintained in both groups.

Considerable variation was found in the June experiment data (fig. 38C), but trends are identifiable. Acid soluble fraction activity was maintained, apart from an initial decrease in the fed group. Lipid and protein synthesis in both groups were greater than at any other time of the year, but starved animals incorporated less labelled carbon into protein than did fed ones.

32P distribution in digestive gland

Throughout the 50 days of the September experiment (fig. 39A) 32P was gradually transferred from the acid soluble fraction to lipid. No 32P was detected in the protein-nucleic acid fraction, yet protein synthesis was occurring (fig. 37A). The most likely explanation is that significant amounts of ribosomal RNA were synthesised in the previous summer during the growth period and remained until autumn. Protein synthesis could therefore occur without any incorporation of assimilated 32P into RNA.

During January (fig. 39B) there was considerable loss of 32P
Fig. 39 Distribution of $^{32}$P in digestive gland fractions.
Open symbols – starved, filled symbols – fed.
Mean ± range.
from the acid soluble fraction. Labelled phosphorus was probably incorporated into phospholipid but more rapidly than in September. Some activity was detected in nucleic acids. Phospholipid and nucleic acid pools were maintained; presumably any depletion is made good from the acid soluble fraction.

In June (fig. 39C) $^{32}{\text{P}}$ activity decreased in both acid soluble and lipid fractions, whereas the nucleic acid $^{32}{\text{P}}$ level remained constant.

**Distribution of $^{32}{\text{P}}$ in mantle**

Figure 40A indicates that during September $^{32}{\text{P}}$ was incorporated into both acid soluble and lipid components, but more extensively into the latter, as in the digestive gland (fig. 39A). Equilibrium was attained after 14 days and there was no subsequent depletion of $^{32}{\text{P}}$ from the mantle. No activity was detected in the protein-nucleic acid fraction. This has already been discussed in connection with the digestive gland data.

Phosphorus distribution data were complicated in January (fig. 40B) by the sex of the developing gonad. Differences in $^{32}{\text{P}}$ content of the protein-nucleic acid fractions are interpretable in these terms rather than in terms of the nutritive condition of the animals. Thus activity in nucleic acids was much greater in the male than in the female mantle. Separation of RNA from DNA showed that most of the $^{32}{\text{P}}$ in the male gonad was incorporated into RNA whereas in the female the bulk of the activity was located in RNA. During gametogenesis nucleic acid levels are much higher in the male gonad than in the female (section III), which correlates with the greater degree of incorporation into the male gonad during the January tracer experiment. Activity in the acid soluble fraction
Fig. 40 Distribution of $^{32}$P in mantle fractions.
Circles — acid soluble, squares — lipid, triangles — protein/nucleic acids.
Open symbols — starved, filled symbols — fed.
Mean + range.
was greater in the female than the male. The $^{32}P$ content of the lipid fraction was less than in September in both male and female mantles.

During June, activity in the acid soluble component was much greater than in other fractions (fig. 40c). Some $^{32}P$ was incorporated into lipid but to a lesser degree than occurred in September. The relatively high $^{32}P$ activity in the protein-nucleic acid fraction suggests formation of RNA associated with extensive protein synthesis at this time (fig. 35B).

**DISCUSSION**

A significant feature of these radionuclide tracer experiments is the rapid initial loss of activity from the digestive gland. This depletion of material assimilated by the digestive tissue is most rapid and prolonged in winter, when the gonad is actively metabolising during gametogenesis and when temperature stress in the laboratory is at a maximum. Using $^{14}C$ - leucine injected into the digestive gland of *Aequipecten irradians*, Sastry and Blake (1971) obtained some evidence that the transfer of nutrient reserves to the gonad occurs at the initiation of gonad development, which correlates with events in *Mytilus*.

The digestive gland plays a role in the storage of energy reserves and in the regulation of transfer to other tissues. Thus in autumn and winter the digestive gland contains more activity per unit weight than any other tissue, but the labelled pools in these other sites are maintained. During autumn particularly the
digestive gland reserves of $^{14}C$ and $^{32}P$ are high. Total metabolism and temperature stress are known to be minima at this time. In summer, during the growth period, the labelled pools built up in foot, mantle and adductor are much greater than at other times of the year, whereas the reserves held by the digestive gland are lesser. The rapid turnover of metabolic pools in the digestive gland is characteristic of "active" tissues such as mammalian liver.

In contrast to the digestive gland, labelled pools in mantle, foot and adductor are maintained, especially in summer and autumn. This implies that any losses are made good from the digestive gland, but the turnover in the other three tissues is not known because further transfer of labelled material from the digestive gland almost certainly occurs. It has been established, however, that mantle reserves are utilised during stress (Bayne and Thompson, 1970) and that the mantle loses weight (section III). The only way in which these facts can be reconciled with the maintenance of labelled reserve pools in the mantle is to postulate replenishment from the digestive gland. This interpretation is consistent with the rapid turnover of assimilated radionuclides observed in the digestive tissue.

Considerable quantities of lipid are synthesised by *Pytilus*, particularly in summer. Glycogen is also deposited but in these experiments it was not separated from the low molecular weight compounds in the acid soluble fraction. According to Williams (1969) the lipid content of *Pytilus* flesh increases through the summer. Lubet and Longcamp (1969) found that mantle lipid increases as the gametes mature, decreases at spawning and further increases during summer as the gonad recovers. This seasonal pattern has been confirmed by Bayne and Gabbott (personal communication),
Working with mussels from the Heacham population. Such observations support the conclusions from the radionuclide tracer work, which demonstrates considerable incorporation of material into lipid during winter and especially during summer, but to a lesser extent in autumn. In the digestive gland lipid levels are highest in summer and lowest in winter (section III), yet a large quantity of assimilated label is initially found in digestive gland lipid during winter. This labelled lipid, however, is quickly transferred elsewhere and turnover is rapid. Thus in winter lipid is synthesised in the digestive gland but does not accumulate. Accumulation does occur in summer, when lipogenic pathways are active and lipid is deposited. By autumn, lipid stores are considerable in mantle and non-mantle tissue and are available for use during gametogenesis in winter. Turnover of non-mantle lipid is rapid during winter as fat accumulates in the developing gonad.

Like $^{14}C$, $^{32}P$ is also transferred from the digestive gland to other tissues, especially in summer. Allen (1962, 1970) claims that the digestive gland is the major storage organ for $^{32}P$ in various bivalves, including Mytilus, and that six weeks after assimilation of $^{32}P$ the digestive gland contains more labelled phosphorus than does any other tissue. The present work supports these observations to some extent, but transfer of $^{32}P$ from the digestive gland varies on a seasonal basis. In autumn there is certainly extensive storage of $^{32}P$ in digestive tissue, but in winter a large amount is transferred to the developing gonad and in summer there is rapid distribution of $^{32}P$ to mantle, foot and adductor. According to Kuenzler (1961) and Allen (1962) there is significant release of $^{32}P$ into the surrounding water in Modiolus demissus Dillwyn and Mercenaria mercenaria L. respectively, but this aspect of phosphorus metabolism has not been
investigated in *Mytilus*. The form of the $^{32}P$ stored by the digestive gland is discussed by Allen (1970), who considers that whereas some may be "bound" the bulk of the phosphorus is "labile", presumably inorganic phosphate. The present study indicates that labelled phosphorus is initially high in the acid soluble fraction of *Mytilus* digestive gland, decreasing as transfer to other sites takes place. In winter and summer more digestive gland $^{32}P$ occurs in acid soluble form than in lipid, but the reverse is true in autumn, when most phosphorus stored in the mantle and digestive gland is in lipid form. These phospholipid reserves may provide a source of phosphorus for utilisation in gametogenesis, when the mantle is synthesising large quantities of nucleic acids. Thus in autumn there are high levels of lipid (including phospholipid) available for use in gametogenesis, and this occurs at a time when food is not normally available in quantities sufficient to meet the demand.

In most instances there was no significant difference between starved and fed animals in the pattern of distribution of $^{14}C$ and $^{32}P$. The nutritive condition subsequent to the short period of intake of labelled food generally had no effect on the metabolic fate of the assimilated material. There was, however, one exception. In summer and in winter, starved animals channelled a greater proportion of labelled carbon into lipid than into protein and nucleic acids. The reverse occurred in fed mussels. This was particularly evident in the mantle during gametogenesis, when fed animals synthesised more protein and nucleic acids than did starved ones. In this case starvation appears to alter the relative utilisation of lipogenic and protein synthetic pathways so that more lipid is deposited. This mechanism may reflect the necessity of synthesising lipid reserves for subsequent use when conditions are adverse. The observations are consistent with measurements of oxygen uptake and
nitrogen loss in starved and fed animals. Lipogenic pathways require relatively few reducing equivalents and this may contribute to the lower oxygen consumption of starved animals.

Radionuclide tracer studies of the distribution of assimilated food to various tissues and tissue components of Mytilus edulis under different conditions have provided some basic information concerning the metabolism of this lamellibranch. Such information may be usefully integrated with data obtained from studies of body composition, oxygen uptake, nitrogen loss and other physiological parameters. The seasonal pattern of metabolism is summarised as follows. During autumn, metabolic rate is low and growth has ceased. Carbohydrate and lipid reserves are available, especially in the mantle and digestive tissue. The turnover of material is slow.

Metabolic rate increases throughout early winter and reaches a maximum during January and February, when nucleic acids and proteins are being synthesised in the developing gonad. The mantle tissue is metabolically active at this time and is maintained by utilisation of its own reserves and those of other tissues, especially the digestive gland. Lipid and carbohydrate are catabolised and also some protein. A full complement of gametes is present by early spring and is maintained until receipt of a suitable spawning stimulus. Non-mantle and mantle carbohydrate and lipid are at minimum values immediately after spawning. "Condition" is poor. Spawning is followed by a period of summer growth and an improvement in "condition". Metabolic rate is low but there is considerable deposition of carbohydrate, lipid and protein reserves. Turnover is rapid in the digestive gland and biosynthesis occurs extensively in other tissues. Protein catabolism is low, resulting in an O : N ratio which is higher than at other times of the year. Summer may be regarded as
a period of growth, of accumulation of energy reserves and of recovery after spawning.

In developing a life cycle which depends on a planktotrophic larva, *Mytilus edulis* is committed to gamete development during winter when food is scarce in temperate waters. The problem of deriving sufficient energy for gametogenesis has been overcome by the synthesis of metabolic reserves during summer when conditions are favourable, food plentiful, and spawning essentially completed.


ABSTRACT

Three levels of metabolism have been identified in the mussel *Mytilus edulis* L. Standard metabolism is attained after prolonged starvation. On feeding, there is an initially elevated metabolic rate, termed active metabolism, which is characterised by increases in oxygen uptake, ventilation rate and filtration rate. After three days feeding, oxygen consumption decreases to a value intermediate between standard and active levels; this is defined as routine metabolism.

Integrated measurements of ingested ration, assimilated ration and metabolic rate provide an estimate of energy balance which is a useful index descriptive and predictive of the effects of sublethal stress. Growth efficiency increases hyperbolically with increasing ration to reach a maximum after which efficiency decreases as ration is further raised. The optimum ration for efficient growth is an increasing function of mussel weight.

Gametogenesis in *Mytilus edulis* occurs in winter when food is scarce. Energy for gonad maturation is obtained from reserves built up in summer when food is abundant. The mantle and digestive gland are particularly important storage sites. The digestive gland also regulates the flow of assimilated material to other tissues. During prolonged stress the digestive tubules become considerably degenerate but the tissue possesses the capacity to recover after such periods of stress.
Lipid, carbohydrate and protein are synthesised in summer and metabolic rate is low. During winter, gametogenesis results in an inflated metabolic rate and turnover of metabolic pools is rapid as reserves are utilised. Sublethal stress also leads to catabolism of energy reserves but it is not possible to define the degree of stress in terms of biochemical parameters such as the carbohydrate:protein ratio because they tend to be regulated to similar values which are independent of the severity of the stress experienced.