Oecologia (Berl.) 22, 211—228 (1976)

The Physiological Ecology of *Mytilus californianus* Conrad

1. Metabolism and Energy Balance

B. L. Bayne[1], C. J. Bayne[2], T. C. Carefoot[3], and R. J. Thompson[4]

[1] Institute for Marine Environmental Research, Citadel Road, Plymouth
[2] Department of Zoology, Oregon State University, Corvallis, Oregon 97331
[3] Department of Zoology, The University of British Columbia, Vancouver, B.C.
[4] Marine Sciences Research Laboratory, Memorial University, St. John's, Newfoundland

Received August 27, 1975

Summary. The rates of oxygen consumption, filtration and ammonia excretion by *Mytilus californianus* have been related to body size and to ration. The rate of oxygen consumption ($\dot{V}O_2$) by individuals while immersed, measured on the shore, resembled rates recorded for mussels starved in the laboratory. $\dot{V}O_2$ by *M. californianus* was relatively independent of change in temperature, with a Q_{10} (13–22° C) of 1.20. In contrast, the frequency of heart beat was more completely temperature dependent [Q_{10} (13–22° C) = 2.10]. Filtration rate showed intermediate dependence on temperature change [Q_{10} (13–22° C) = 1.49] up to 22° C, but declined at 26° C. Both $\dot{V}O_2$ and filtration rate declined during starvation. The utilisation efficiency for oxygen was low (approx. 4%) between 13 and 22° C, but increased to 10% at 26° C. Three components of the "routine" rate of oxygen consumption are recognised and estimated; viz. a basal rate (0.136 ml O_2 h^{-1} for a mussel of 1 g dry flesh weight), a "physiological cost" of feeding (which represented about 6% of the calories in the ingested ration), and a "mechanical cost" of feeding which was three times higher than the physiological cost. The ratio of oxygen consumed to ammonia-nitrogen excreted was low, and it declined during starvation. These data are compared with previously published measurements on *Mytilus edulis*, and the two species of mussel are shown to be similar in some of their physiological characteristics, though possibly differing in their capacities to compensate for change in temperature. For *M. californianus*, the scope for growth was highest at 17–22° C and declined at 26° C; it is suggested that exposure to temperatures in excess of 22° C, as for example during low tides in the summer, might result in a cumulative stress on these populations of mussels by imposing a metabolic deficit which must be recovered at each subsequent high tide. The high "mechanical cost" of feeding imposes a more general constraint on the scope for activity of the species.

Introduction

Mytilus californianus Conrad is distributed along the western coasts of North America, from Baja California to Alaska. Within the bathymetric range of the species (about −30 m to +4 m; Chan, 1973) the mussels occur at highest density in the middle intertidal zone, at approximately +1.5 m to +3.0 m (Paine, 1974). Paine has suggested that this distribution represents a refuge for the species, with the lower limits set by biotic factors of competition and predation, and the upper limits determined by environmental stressors such as extreme temperature, desiccation and the shortage of food. An intertidal distribution of this kind exposes an animal to a variety of potential stressors in addition to those mentioned, including extremes of salinity and the necessity, at low tide, either of withstanding periods without a supply of oxygen, or of gaining oxygen from a medium to which the respiratory apparatus is ill-adapted.

In a series of papers Harger (see review by Harger, 1972) has suggested some of the ways in which these mussels are adapted to wave exposure, including their shapes, strong byssal attachments and clumped distribution, and how they maintain their position in the intertidal community. Important papers by Coe and Fox (1942, 1944) and Fox and Coe (1943) have described relationships between the growth of *M. californianus* and various environmental conditions, primarily food. Rao (1953, 1954) and Segal *et al.* (1953) have reported on aspects of the activity and filtration rates of the species. However, with the exception of a study by Moon and Pritchard (1970), there is little information available on physiological adaptations of these mussels to their intertidal habitat.

We therefore considered that it would be useful to examine certain aspects of the ecology and physiology of *M. californianus* with two particular questions in mind: what physiological characteristics enable the species to thrive in the intertidal zone, and to what extent does this intertidal distribution itself represent a "stress"? In this paper we report experiments on the relationships between rates of oxygen consumption, filtration and excretion, and the frequency of the heart beat, in mussels of various sizes immersed in water at different temperatures and with different levels of available ration. In a subsequent paper we will discuss experiments on the adaptations of *M. californianus* to exposure to air.

Material and Methods

Mussels were observed and collected on San Juan Island, Washington, USA (lat. 48 30′, long. 123 05′), and experiments carried out at the Friday Harbor Laboratories of the University of Washington. On San Juan Island *M. californianus* occurs between +1.2 m and +2.2 m on the shore. At Friday Harbor, mean sea level is +1.5 m relative to chart datum; the mean tidal range is 1.4 m and the diurnal range of the tide is 2.3 m (Connell, 1970). Connell describes the tides as mixed, with one daily tide usually lower than the other. There is a small annual range in surface water temperatures, with monthly means from 7–13° C (Pickens, 1965; Connell, 1970). Mean monthly surface salinities range from 28.5–29.1‰ S, with extremes of 18.3 and 31.8‰ S (Connell, 1970). Dayton (1971) suggested that the effects of different environmental conditions experienced during low tide, such as temperature and humidity, are exaggerated on the San Juan Islands, relative to the outer coasts of the Olympic Peninsula, where *M. californianus* is abundant, because the low spring tides in the summer usually occur around the middle of the day, whereas the low tides in the winter occur at night; intertidal organisms are therefore exposed to the most extreme conditions in both seasons. Air temperatures may be as high as 33° C and as low as −16° C (Connell, 1970). Petersen *et al.* (1974) quote relative humidities at Friday Harbor in the summer ranging from 85% to as low as 20% during periods of easterly winds. Although *M. californianus* may tolerate dilution of seawater to 17‰ S (Fox, 1936), the salinities within the Strait of Juan de Fuca are probably below the optimum for the species (Young, 1941). Individual mussels on San Juan Island are generally small relative to mussels in other, more open-coast habitats, and their distribution on these shores is patchy, with individuals often restricted to cracks and gulleys in the rocks; this is quite unlike the "carpets" of mussels found on more exposed coasts.

Mussels were collected from Lime Kiln Point, Eagle Point and Cattle Point (Fig. 1) in July 1973, and brought to the laboratory, where they were kept in the seawater table. In one experiment, mussels of various sizes (shell lengths between 3 and 14 cm) were divided into two groups and each held at ambient salinity and oxygen tension, but with water temperature controlled to 13 ± 1.5° C. One of these groups was fed continuously with a mixed culture of the unicellular algae *Isochrysis galbana*, *Phaeodactylum tricornutum* and *Dunaliella* sp., which were grown individually in batch cultures and mixed in the feeding reservoir

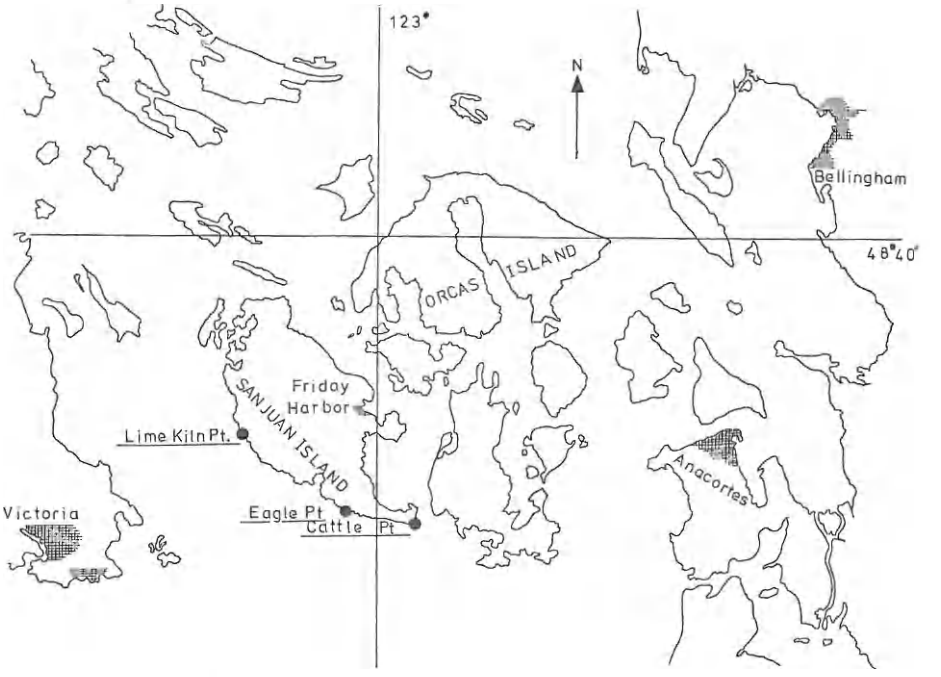


Fig. 1. Sketch map of the San Juan Islands, Washinton, USA, showing the three localities from which *Mytilus californianus* were collected

before being dosed to the mussels. The other group was starved; the water inflow to the tank was filtered by passing it through an in-line "Aqua Pure" water filter. Average particle concentrations in the water of these two groups of mussels were as follows:

Fed animals: $10{,}000 \pm 1{,}650$ particles ml^{-1}
Starved animals: 770 ± 38 particles ml^{-1}

We have determined that 10^6 cells of such a mixed algal culture weighed 0.088 mg; using a calorific conversion of 5.6 cals mg^{-1} (Widdows and Bayne, 1971), the calories available to these two groups of mussels will have been:

Fed animals: 4.93 cals l^{-1}
Starved animals: 0.38 cals l^{-1}

Mussels were sampled at intervals from this experiment for measurement of their rates of oxygen consumption, filtration rates and rates of excretion of ammonia-nitrogen. Animals from the "fed" group were also used to establish the effects of temperature change on the three physiological processes mentioned, as well as on the frequency of the heart beat. Other animals which were maintained at a medium ration (a 50:50 seawater dilution of the algal culture fed to the "fed" group) were used only for the measurement of their rates of excretion.

The rates of oxygen consumption of mussels of various sizes were also measured on the shore under three conditions: 1. Animals were collected from tide-pools (ambient temperature 25–26° C) and measured at 26° C. 2. These same animals were then placed directly in water at 13° C (to simulate the flooding tide entering the tide-pool) and their rates of oxygen uptake measured at this temperature. 3. Individuals whose immediate past thermal history included at least 11 hrs of uninterrupted submergence at 12–13° C were measured at 13° C.

Measurement of Oxygen Consumption on the Shore. Mussels were placed in water of the desired temperature in Kilner flasks. One water sample of 20 ml was taken, the flask sealed without trapping air, and a second sample taken after 20–45 min depending on the size

of the animal. The samples were analysed directly for oxygen content by the Winkler procedure, modified for use with small samples.

Measurement of Oxygen Consumption in the Laboratory. Animals were removed from the experimental trays and placed individually in glass respirometer flasks described by Bayne (1971a). After at least 30 min, at a water flow rate of 60–90 ml min^{-1} at constant temperature, the water flow was stopped and oxygen consumption determined from the rate of decline of the oxygen tension, as monitored with a Radiometer oxygen-electrode. All values for oxygen consumption, referred to as $\dot{V}O_2$, will be quoted as ml O_2 h^{-1}.

Measurement of Heart Beat. Thin copper electrodes were implanted through small holes in the shell, to lie alongside the pericardium on each side. The electrodes were coupled to an impedance pneumograph linked to a multi-channel pen recorder. Impedance changes caused by the beating of the heart were amplified to give a direct recording of the heart rhythm (Hoggarth and Trueman, 1967).

Measurement of Filtration Rate. Animals were placed in the glass respirometer flasks and after at least 30 min at a flow rate of 60–90 ml min^{-1}, water samples of the inflow and outflow of the flask taken for counting, with a Coulter Counter, the concentrations of particles larger than 3 μm in diameter. The difference in particle concentrations between inflow and outflow, together with the measured rates of water flow, were used to calculate the filtration (or clearance) rate as litres of water cleared of particles per hour.

Measurement of the Rate of Excretion of Ammonia-N. Animals were placed in 0.2–1.0 l of membrane-filtered seawater, the volume depending on animal size. After at least 30 min this water was gently siphoned off and replaced with freshly filtered water. After a further 2–4 hrs, samples of water were removed, including samples from flasks not containing an animal, and analysed for ammonia-nitrogen content by the phenol-hypochlorite method as described by Grasshoff and Johannsen (1972). Excretion rates, referred to as $\dot{V}NH_4$, were calculated as the difference between the ammonia-N contents of the water from flasks with and without an animal, and will be quoted as μg $NH_4 \cdot N$ h^{-1}.

The physiological rates were all related to the dry flesh weight of the animals, as determined after drying for 24 hrs at 90° C. Statistical procedures used in the analysis of the data follow Snedecor and Cochran (1972).

Results

Rates of Oxygen Consumption

The results of the oxygen consumption determinations were examined by regressions of $\dot{V}O_2$ against dry weight of flesh (W), and by co-variance analysis (Table 1). Of the measurements on the shore, there was no significant difference between the rates of consumption recorded at 13° C after the animals had experienced higher temperatures and those measured for animals whose immediate thermal history included a long period at 13° C. The results at the lower temperature were therefore combined and the following common regression equation derived:

$$\dot{V}O_2 = 0.227 W^{0.483} \quad (n = 65).$$

The measurements at 26° C on the shore failed to show a correlation between $\dot{V}O_2$ and dry flesh weight ($n = 11$; $r = -0.017$), probably due to the small sample size and the narrow range of values for dry weight (the mean and standard deviations for weight were 4.09 ± 1.23 g). Consequently, an average rate of oxygen consumption at 26° C was calculated as 2.048 ± 0.367 ml O_2 h^{-1}. From the expression given above to describe $\dot{V}O_2$ at 13° C, an animal of 4.09 g dry weight would have an oxygen consumption rate of 0.448 ml O_2 h^{-1}. The Q_{10} value for this temperature increment (13–26° C) is therefore 3.55.

In the laboratory, measurements of oxygen consumption rates by mussels of different sizes were made under three conditions: 1. Animals which had been

Table 1. Regression analysis by least squares of the rates of oxygen consumption (ml O_2 h^{-1}) by *Mytilus californianus* of different dry flesh weights (in g)

Conditions	Regression		Standard error of b	Correlation coefficient	Degrees of freedom
	Constant (a)	Coefficient (b)			
Shore					
13° C [a]	0.187	0.600	0.169	0.536	31
13° C [b]	0.234	0.441	0.076	0.775	32
13° C [c]	0.227	0.483	0.070	0.658	64
Laboratory					
13° C: fed	0.559	0.562	0.075	0.857	21
13° C: starved	0.216	0.818	0.090	0.696	18

[a] Previous thermal history included 1–4 hrs at 24–26° C.
[b] Previous thermal history included more than 11 hrs at 13° C.
[c] Combined analysis of all shore values at 13° C.

fed in the main experiment at 13° C were measured at 13° C. 2. Animals which were starved at 13° C for between 16 and 23 days were measured at 13° C. 3. Animals which were fed at 13° C (as in 1. above) were measured at 13° C, and the temperature of the water bath in which the respirometer was immersed was then slowly raised (3° C h^{-1}) and further oxygen consumption determinations made at 17.5, 22 and 26° C. The temperature was then rapidly lowered to 14° C and another measurement of VO_2 taken.

The analysis of the measurements obtained under procedures 1. and 2. (above) is shown in Table 1. The regression of VO_2 against W for fed and starved mussels at 13° C had significantly different regression constants (the value of a in the allometric expression $VO_2 = aW^b$), but values for the regression coefficient b were not significantly different at $P = 0.01$. A common regression coefficient was therefore determined and the following expressions established to describe the relationships between VO_2 and W in the laboratory at 13° C:

1. Fed animals: $VO_2 = 0.542\ W^{0.648}$ $(n = 22)$
2. Starved animals: $VO_2 = 0.233\ W^{0.648}$ $(n = 19)$

The rates of oxygen consumption recorded for fed animals will be referred to as "routine" rates, those for starved animals as "standard" rates (Bayne *et al.*, 1973). These regressions are plotted, together with the line describing VO_2 as measured on the shore at 13° C, in Fig. 2. An analysis of co-variance was carried out to compare the regressions of VO_2 on W at 13° C as measured on the shore and in the laboratory. The result (Table 2) demonstrated no significant difference between the two regressions for mussels on the shore and those which were starved in the laboratory.

The common regression coefficient (b) of 0.648 was used to transform the VO_2 measurements made at different temperatures to a standard "metabolic body size", *i.e.* $VO_2/W^{0.648}$. These transformed values are plotted in Fig. 3 to illustrate the acute effects of temperature change on the respiration rates of

Table 2. A co-variance analysis of regressions of rates of oxygen consumption (ml O_2 h^{-1}) by *Mytilus californianus* of different dry flesh weights (in g) on the shore and in the laboratory (starved animals only), together with the results of regression analysis by least squares of all these data pooled

Source of variation	Degrees of freedom	Sums of squares	Mean square	*F*	*P*
1. Difference between coefficients *b*	1	0.2698	0.2698	3.916	<0.05
2. Difference between constants *a*	1	0.2815×10^{-5}	0.2815×10^{-5}	0.394×10^{-4}	>0.05
3. Total	82	5.7814			

Pooled regression for all shore and laboratory (starved animals) data at 13° C

Regression		Standard error of *b*	Correlation coefficient	Degrees of freedom
Constant (*a*)	Coefficient (*b*)			
0.255	0.502	0.068	0.632	85

mussels acclimated to 13° C. The Q_{10} values for each increment in temperature were:

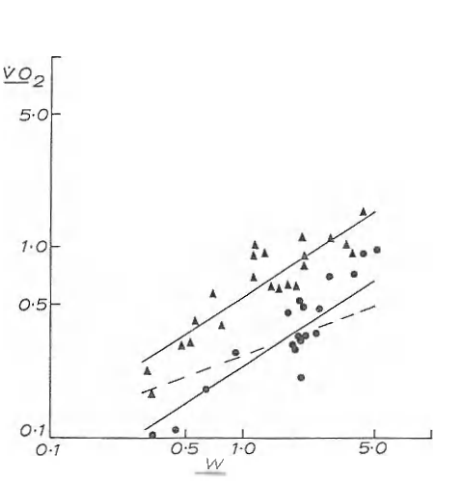
13	–17.5 °C:	$Q_{10}=1.00$
17.5	–22° C:	$=1.47$
22	–26° C:	$=2.08$
13	–26° C:	$=1.41$

Animals which had been held for 3–5 hrs at temperatures in excess of 22° C, and then quickly returned to water at 14° C, did not differ in their rates of oxygen consumption at 14° C from mussels recorded at 13° C before exposure to the higher temperatures. In this respect field and laboratory data were in agreement; there was no marked undershoot of the respiration rate when animals suddenly experienced a large drop in temperature.

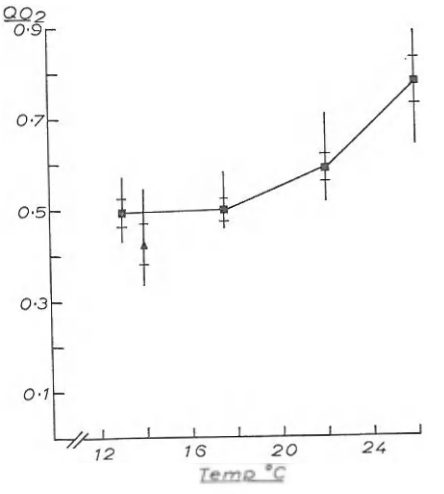
The main discrepancy between the shore and laboratory measurements of the rates of oxygen uptake is the high value recorded at 26° C on the shore, which is reflected in the high Q_{10} (13–26° C) of 3.55 compared with a Q_{10} in the laboratory of 1.41. Of course mussels on the shore and in the laboratory experienced different thermal regimes, and further study is needed to clarify how this may have affected respiration rate at high temperature. However, the present data indicate that a rise in temperature from 13° C (mean ambient water temperature) to 26° C (maximum recorded temperature in the tide-pools) will result in an increase in the rate of oxygen consumption. The results of the laboratory experiments further suggest that this is due primarily to temperatures exceeding 22° C, since between 13 and 22° C rise in temperature had little effect on the rates of oxygen uptake.

Frequency of Heart Beat

The heart beats of five individuals were recorded, simultaneously with their rates of oxygen consumption, during gradual increases in temperature from



2



3

Fig. 2. The rates of oxygen consumption by *Mytilus californianus* at 13° C (VO_2; ml O_2 h^{-1}) plotted as a function of dry flesh weight (W; g). ▲ fed animals; ▪ starved animals. Regression lines are plotted according to equations in the text; the dashed regression line describes VO_2 for mussels measured on the shore at 13° C

Fig. 3. The rates of oxygen consumption by *Mytilus californianus* (QO_2; ml O_2 $h^{-1}/W^{0.848}$) at different temperatures. Values plotted as means ± total range of observations, with 1 standard deviation indicated as a bar. ▪ measurements taken as temperatures increased; ▲ measurements taken on decrease of temperature from 26 to 14° C

13 to 17.5, 22 and 26° C, followed by an abrupt return to 14° C. The results (Fig. 4) showed an almost linear increase in heart rate with rise in temperature. Values for Q_{10} were as follows:

13	–17.5° C:	$Q_{10} = 2.39$
17.5	–22° C:	$= 1.88$
22	–26° C:	$= 1.68$
13	–26° C:	$= 1.97$

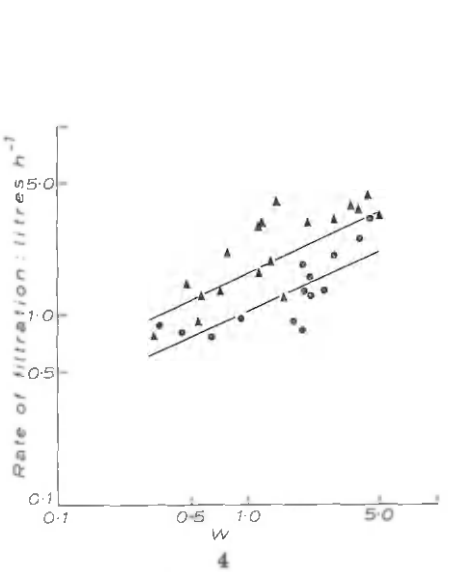
This sensitivity of heart rate to increases in temperature was in contrast with the relative independence of VO_2 from temperature within the range 13–22° C.

Rates of Filtration

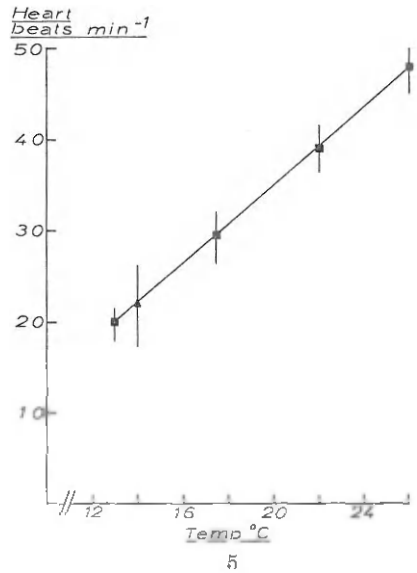
The rates of filtration by both "fed" and "starved" mussels of different size were measured at 13, 17.5, 22 and 26° C. The relationship between filtration rate (FR: l of water filtered free of particles per hr) and body size (W: dry weight of flesh in g) are described by the following expressions (see Fig. 5):

1. Fed animals: $FR = 1.61\ W^{0.468}$ $(n = 18)$
2. Starved animals: $FR = 1.02\ W^{0.416}$ $(n = 14)$

An analysis of co-variance revealed that the regression constants (1.61 and 1.02), but not the coefficients, in these equations were significantly different at $P = 0.05$.



4



5

Fig. 4. The rates of filtration by *Mytilus californianus* at 13° C (l h^{-1}) plotted as a function of dry body weight (W; g). ▲ fed animals; ● starved animals. Regression lines are plotted according to equations in the text

Fig. 5. The frequency of heart beat of *Mytilus californianus* (beats min^{-1}) as a function of temperature. The values plotted are means ± total range of observations. ■ measurements taken as temperature increased; ▲ measurements taken on decrease of temperature from 26 to 14° C

The data were therefore re-analysed with a common regression coefficient (0.434) and the following expressions accepted as describing the relationship between rate of filtration and body size for mussels at 13° C:

1. Fed animals: $FR = 1.64\ W^{0.434}$
2. Starved animals: $FR = 1.04\ W^{0.434}$

Table 3. The rates of filtration by *Mytilus californianus* at different temperatures, together with calculated values for Q_{10}. The rates have been referred to a "standard body size" of $W^{0.434}$, where W is dry flesh weight in grams and 0.434 is the regression coefficient in the expression of filtration rate as a function of body size

Temperature (°C)	Filtration rate (l h^{-1})	Temperature range (°C)	Q_{10}
13	1.89		
17.5	2.48	13–17.5	1.82
22	2.71	17.5–22	1.22
26	1.60		
14[a]	1.84		

[a] Animals returned to 14° C after experiencing step-wise increase in temperature from 13–26° C (see text).

The regression coefficient of 0.434 was then used to transform the measurements of rates of filtration at different temperatures to a common value for "metabolic body size" ($FR/W^{0.434}$): these values are listed in Table 3. From 13 to 22° C the rate of filtration increased with rise in temperature, and then declined at 26° C.

Relationships between Oxygen Consumption, Filtration and Heart Beat Frequency

Relationships between VO_2 and FR may be considered in terms of the volume of oxygen that is delivered to the respiratory surfaces by the ventilation current, and the efficiency with which this oxygen is removed (the utilisation efficiency). To describe this relationship from our results, we must assume that the rates of filtration approximate to the true ventilation rate; this assumption will only be met if filtration efficiency is high. Our data suggest that particles of the size used in these experiments (3–8 μm diameter) were indeed retained by *M. californianus* with 80–100% efficiency, in agreement with the observations of Vahl (1972) on *M. edulis*. Oxygen utilisation efficiencies were therefore calculated (Table 4). Efficiency did not change significantly between 13 and 22° C, but increased from an average 4.4% to 10.4% at 26° C.

Bayne (1971b) calculated a ventilation:relative perfusion ratio for *M. edulis* and found it to correlate with utilisation efficiency. The estimate of "relative perfusion" for use in this ratio requires a measure of the amplitude, as well as the frequency, of the heart beat. We did not record amplitude of heart beat in these experiments with *M. californianus*. However, Widdows (1973b) found no temperature-related changes in heart amplitude in *M. edulis*. Assuming this to hold also for *M. californianus*, ratios of filtration rate to frequency of heart beat were calculated (Table 4). The ratio declined during rise in temperature between 13 and 26° C, and a decrease in the ratio correlated with an increase in utilisation efficiency.

Whether or not there is a coupling between the ventilation and the perfusion of the gill surfaces during short-term exposures to increases in temperature, the utilisation efficiency for oxygen is held relatively stable, or slightly increased. In this way an increase in the animal's demand for oxygen can be met. At 26° C, however, there is a decline in filtration (and ventilation?) rate which, although

Table 4. Oxygen utilisation by *Mytilus californianus* at different temperatures, and a "ventilation: perfusion ratio" (see text)

Temperature (°C)	Oxygen made available by the ventilation current (ml O_2 h^{-1})	Oxygen utilisation efficiency (%)	$\frac{\text{Ventilation rate}}{\text{Heart rate}} \times 10^2$
13	11.4	4.4	9.4
17.5	13.8	3.6	8.4
22	13.7	4.2	6.9
26	7.5	10.4	3.3
14[a]	10.9	3.9	9.0

[a] Animals returned to 14° C after 3–5 hrs at temperatures in excess of 22° C.

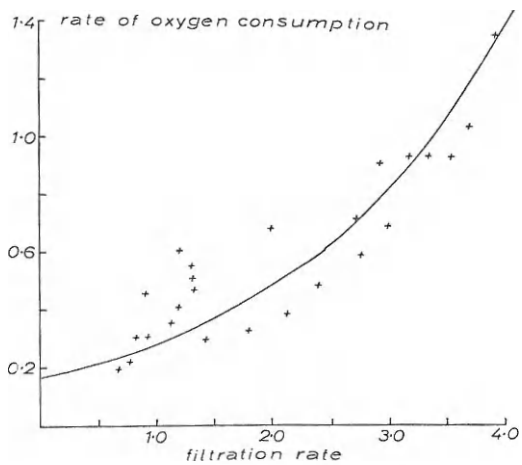


Fig. 6. The relationship between the rate of oxygen consumption (ml O_2 h^{-1}) and the filtration rate (l h^{-1}) in *Mytilus californianus* at 13° C

it helps to bring about an increase in utilisation efficiency, probably signifies the incipient collapse of the gas exchange mechanism, should the temperature increase further.

Components of the Rate of Oxygen Consumption

From the many measurements of oxygen consumption and filtration at 13° C, 25 values were extracted which represented near-simultaneous determinations of the two physiological rates on animals of approximately 1 g dry flesh weight. These measurements covered a range for $\dot{V}O_2$ from 0.2 to 1.4 ml O_2 h^{-1}, and from 0.6 to 3.9 l h^{-1} filtration rate (Fig. 6). Analysis by least squares suggested that the relationship between these two values is exponential, and can be described by the expression:

$$\dot{V}O_2 = 0.17\, e^{(0.531FR)} \quad (n = 25;\ r = 0.818)$$

where $\dot{V}O_2$ is the rate of oxygen consumption (ml O_2 h^{-1}) and FR is the filtration rate (l water h^{-1}). From this relationship, the increase in $\dot{V}O_2$ was calculated for increments of 1 l h^{-1} in filtration rate (Table 5). When converted to caloric equivalents (1 ml O_2 consumed = 4.80 calories), this calculation gives an indication of the cost of increasing the rate of filtration, or of ventilation. This cost accelerates as the volume of water to be moved through the mantle cavity increases, and it is this which, presumably, imposes an ultimate restriction on the scope for activity. Values for the rates of oxygen consumption by "fed" and by "starved" animals (Fig. 2) suggest a "routine scope for activity" (Bayne *et al.*, 1973) of ×2.3, accompanying an actual increase of filtration rate of ×1.6. It is interesting that, in response to a rise in temperature, the maximum increase recorded for filtration rate (Table 3) was ×1.4.

In the relationship between the rate of oxygen consumption and the rate of filtration (Fig. 6) the Y-intercept, or value for $\dot{V}O_2$ at zero filtration rate,

Table 5. The "cost" (in calories per hour) to *Mytilus californianus* of increasing filtration rate. Calculations relate to an animal of 1 g dry flesh weight, at 13° C, as based on data in Fig. 6

Increment in filtration rate (litres water h^{-1})	Increment in rate of oxygen consumption (ml O_2 h^{-1})	Caloric equivalent of increment in oxygen uptake (calories h^{-1})
0–1	0.117	0.562
1–2	0.198	0.950
2–3	0.332	1.594
3–4	0.556	2.669

is an estimate of the rate of metabolism of an inactive animal. This value will vary according to the nutritional condition of the individual; *i.e.* the presence of food in the digestive tract, and the resulting processes of digestion and assimilation (the "specific dynamic action"), will impose a metabolic demand that is not reflected in the activity-related costs of metabolism. We can therefore distinguish between a "mechanical" and a "physiological" cost of feeding. The former refers to the energy costs of ciliary action on the gills and the labial palps, which force the water over the filtering surface, and provide the filtration mechanism itself. By definition, this mechanical cost is not included in the estimate of energy expenditure at zero activity. The "physiological" cost refers to the energy utilised in digestion and assimilation, and to the ". . . non-utilised energy freed through deamination and other processes" (Warren and Davis, 1967).

If the rates of oxygen consumption and filtration for starved animals (of standard size) are inserted into the equation,

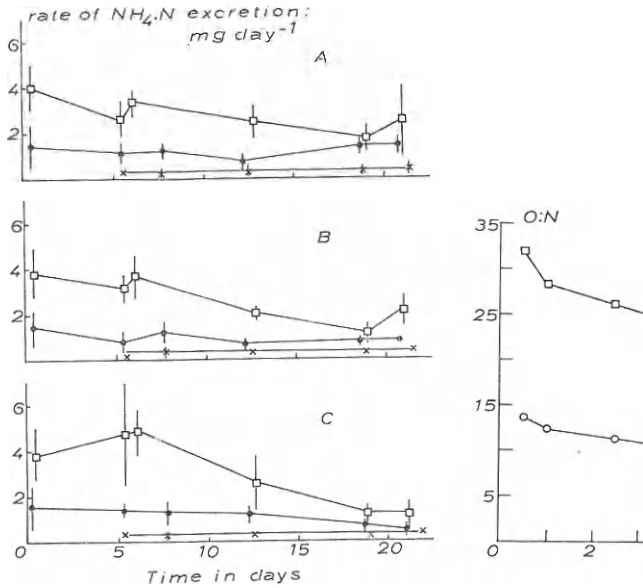
$$VO_2 = ke^{(0.531FR)}$$

the value for k reflects the basal metabolic rate. When values for fed animals are inserted, the value of k is an estimate of the sum of the basal metabolic rate and the physiological cost of the particular ration. These values are as follows:

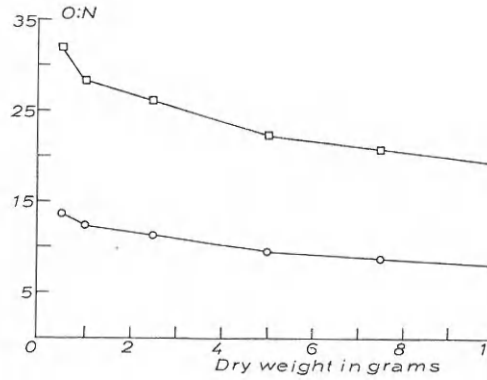
1. Fed animals: 0.232 ml O_2 h^{-1}
2. Starved animals: 0.136 ml O_2 h^{-1}

The physiological cost may therefore be represented as (0.232–0.136) = 0.096 ml O_2 h^{-1} or 0.461 cals h^{-1}. The average ration available to the fed animals in these experiments (see "Material and Methods") was 4.93 cals l^{-1}. At an average filtration rate of 1.64 l h^{-1} the ingested ration was therefore 8.08 cals h^{-1}, and the physiological cost was equivalent to 5.7% of the ingested ration.

It is possible therefore to identify tentatively three components of the metabolic rate of these mussels, as "indexed" by their rates of oxygen consumption. These are: the basal rate, being the metabolic rate of a starved individual showing zero activity; the physiological cost of feeding and the mechanical cost, which is estimated as the difference between the routine metabolic rate and the sum of the basal rate and the physiological cost, and which includes the activity-related and other aerobic energy demands. For an animal of 1 g dry flesh weight at 13° C in our experiments, these values are:



7



8

Fig. 7 A—C. The rates of excretion of ammonia-nitrogen by *Mytilus californianus* as a function of time spent in the laboratory. (A) Animals at high ration. (B) Animals at medium ration. (C) Animals starved. ▫ animals 4.13 ± 1.035 (s.d.) g dry flesh weight; • animals 1.53 ± 0.309 g dry flesh weight; × animals 0.55 ± 0.130 g dry flesh weight. Values are mean plus total range of three determinations

Fig. 8. The ratio, by atomic equivalents, of oxygen consumed to ammonia-nitrogen excreted (O:N ratio) in *Mytilus californianus* of different sizes when fed (▫) and when starved (▫)

Basal rate:	0.136 ml O_2 (or 0.653 cals) h^{-1}
Physiological cost:	0.096 ml O_2 (or 0.461 cals) h^{-1}
Mechanical cost:	0.310 ml O_2 (or 1.488 cals) h^{-1}

The mechanical cost was, by these calculations $3.2\times$ the physiological cost and accounted for 59% of the routine oxygen consumption.

The Rate of Excretion of Ammonia-Nitrogen

Mussels were grouped as large [4.13 ± 1.035 (standard deviation) g flesh dry weight], medium (1.53 ± 0.309) and small (0.55 ± 0.13) individuals, taken from high, medium and low (=starved) ration levels, and their rates of excretion measured at irregular intervals over 23 days (Fig. 7). The largest animals reduced their excretion rates between days 7 and 18, and this was most marked under starvation conditions. The medium-sized and small animals showed negligible change in their excretion rates over time.

The excretion rates for all animals from days 12 to 23 were used to calculate mean values for each condition. There was no clear effect due to ration, however, so the values were pooled for an assessment of excretion rate as a function of dry flesh weight (in g):

$$V\mathrm{NH_4} = 23.88\ W^{0.817}$$

where VNH_4 is the rate of excretion of ammonia-N in μg h^{-1}. However, it is to be expected that the numerical values of both the parameters in this expression will vary with season.

The rates of excretion of ammonia-N were used, together with the rates of oxygen consumption, to calculate the ratio, by atomic equivalents, of oxygen to nitrogen, or O:N ratio, for fed and starved individuals of different sizes. This ratio is an index of the catabolic balance between carbohydrate, lipid and protein substrates in the body (Corner and Cowey, 1968; Bayne, 1975). The results (Fig. 8) show a reduced O:N during starvation, and a decline with increase in animal size.

Discussion

In 1937 Whedon and Sommer reported values for the rates of oxygen consumption by *Mytilus californianus* sampled in San Francisco. We have extracted from their Table 1 21 estimates, all taken within the temperature range 17–21° C and covering a range of total dry weight from 6–59 g. Analysis of these values by least squares yields the following regression equation:

$$VO_2 = 0.625\ W^{0.84} \quad (n = 21;\ \ r = 0.88)$$

where VO_2 is ml O_2 consumed h^{-1}, and W is the dry flesh weight in grams. The agreement between this expression and our own measurements is reasonable, considering the marked differences in material and technique. Also in 1937, Fox *et al.* measured the filtration rates (rates of water propulsion) of *M. californianus* using suspensions of finely ground $CaCO_3$; our values are in good agreement with these. Rao (1953) used suspensions of colloidal graphite (diameter 5–15 μm) to measure the filtration rates of *M. californianus* from different latitudes. He recorded a mean value for animals from Friday Harbor, weighing approximately 6 g dry flesh, of 3.7 l h^{-1} (at 16° C); our analysis predicts a rate for such an animal at 13° C of 3.5 l h^{-1}. Pickens (1965) illustrated rates of heart beat (actually the pulsation rate of the plicate membranes which, according to the author, is numerically the same as the true heart rate) for *M. californianus* from Friday Harbor of approximately 30 and 50 beats min^{-1} at 17 and 25° C, respectively. From our results, predicted rates of beat for such animals are 28 and 48 beats min^{-1}. All of these comparisons between our results and previously published values for various physiological processes in *M. californianus* therefore indicate an acceptable level of agreement.

It is of interest also to compare the responses of *M. californianus* to changes in temperature and ration with results obtained, using similar procedures, in experiments with *M. edulis* (Bayne, 1973a, b; Widdows, 1973a, b; Bayne *et al.*, 1973).

Within the temperature range 13–22° C the routine rate of oxygen consumption (routine VO_2) by *M. californianus* was relatively independent of temperature, with a Q_{10} of < 1.5; between 22 and 26° C the Q_{10} increased to > 2.0 (Fig. 3). In contrast, *M. edulis*, in response to sudden changes in temperature, has a very high Q_{10} for routine VO_2, *viz.* Q_{10} (10–20° C) = 3.12 (Widdows, 1973a). The filtration rate of *M. californianus* was also relatively more independent of temperature increases up to 22° C than is the filtration rate of *M. edulis* (*cp.*

Table 6. A comparison of the sensitivity to increase in temperature of heart beat frequency in two species of *Mytilus*. Values for *M. californianus* were read from Fig. 4. Values for *M. edulis* were taken from Widdows (1973b)

Temperature interval (°C)	Q_{10}	
	M. californianus	*M. edulis*
15–20	2.00	2.71
20–25	1.74	2.36

Table 3 in this paper and Table II in Widdows, 1973a). In Table 6, a comparison is made between the Q_{10} values for frequency of heart beat in *M. californianus* and *M. edulis*. As with the other physiological processes, the heart beat of *M. californianus* is apparently less sensitive to temperature increase than the heart beat of *M. edulis*.

These inter-species comparisons are based on the measurement of larger individuals in the case of *M. californianus* than of *M. edulis*, and may therefore be in error should there be a size-dependent variation in Q_{10}. In addition, the comparisons are based on animals in different gametogenic condition. However, the comparisons are based on average responses by individuals of average size for each species, and they do suggest a greater degree of temperature-independence in the eco-physiology of *M. californianus* than of *M. edulis*, a finding which correlates positively with the extremes of temperature known to exist over the geographic ranges of these two species.

In spite of these differences in the quantitative aspects of physiological response in the two species, there is a basic qualitative similarity in their integrated responses to temperature increase. In *M. californianus*, oxygen utilisation efficiency increased from 4.4% at 13° C to 10.4% at 26° C; in *M. edulis* utilisation efficiency increased from 4.5% at 10° C to 12.0% at 25° C (Widdows, 1973b). The "water convection requirement" (the volume of water ventilated past the respiratory surfaces per unit of oxygen utilised; see Dejours, 1972) decreased with rise in temperature in both species. It is likely that in both species the "acute" response (as distinct from any possible "acclimation" responses) to an increase in temperature consists of an acceleration of heart beat frequency which, coupled with a less-marked increase in filtration (= ventilation) activity, results in a maintenance, or slight increase, in oxygen utilisation efficiency, and the regulation of oxygen consumption at a rate which is, in part, independent of the temperature change.

The two species of mussel are also similar in their responses to starvation, which causes a decline in both the rate of oxygen consumption and filtration rate (Bayne, 1973b). In *M. edulis*, starvation also results in a disruption of the steady-state pattern of the rate of ammonia excretion, and a consequent change in the O:N ratio. In *M. californianus*, ammonia excretion rates did not alter considerably with starvation, but the O:N ratios declined as a result of reduced $\dot{V}O_2$. The O:N ratio was also reduced in larger relative to smaller individuals. This may be associated with season, stage of gametogenesis, or the amounts

Table 7. Components of a simple energy budget for *Mytilus californianus* at four temperatures and two ration levels in the laboratory. Values for feeding rate and respiratory loss in starved individuals assumed to have the same Q_{10} as in fed individuals, and the measurements taken at 13° C weighted accordingly to give estimates at other temperatures

Temperature (°C)	Ration condition	Ingested ration (cals day^{-1})	Assimilated ration (A)[a] (cals day^{-1})	Respiratory loss (R) (cals day^{-1})	Scope for growth (A-R) (cals day^{-1})
13	Fed	223.62	134.17	61.79	72.4
17.5		293.43	176.06	61.79	114.3
22		320.65	192.39	73.53	118.9
26		189.31	113.58	98.61	15.0
13	Starved	9.48	7.58	26.56	−19.0
17.5		12.40	9.92	26.56	−16.6
22		13.59	10.87	31.58	−20.7
26		8.02	6.42	42.29	−35.9

[a] Assimilation efficiencies (used to derive assimilated from ingested ration) were based on our own estimates for fed animals (efficiency = 0.6) with the assumption based on Thompson and Bayne (1974), that the efficiency at lower ration was higher (efficiency = 0.8).

of glycogen in the tissues. At the time of these experiments many of the animals had spawned, either on the shore or in the laboratory soon after collection, and the glycogen level in the tissues was low, averaging 31.0 mg per gram flesh weight (Bayne *et al.*, 1976). When in this condition, mussels have a high rate of protein catabolism, signified by a low O:N ratio.

The capacity to maintain metabolic rate relatively stable during short-term increases in temperature is a useful adaptation for an intertidal animal, and is indeed characteristic of many littoral species (Newell, 1970). Primarily, maintenance of a stable respiration rate is indicative of the conservation of metabolic reserves under conditions of potential stress. The same observation can be made for the reduction in respiration rate during starvation. These aspects of ecophysiological adaptation are well illustrated by calculation of the "scope for growth" (Warren and Davis, 1967; Widdows and Bayne, 1971; Bayne *et al.*, 1973; Thompson and Bayne, 1974). In Table 7 the scope for growth of *M. californianus* at various temperatures and at two rations has been calculated from laboratory data. Over the temperature range 13–22° C the mussels maintained a significant positive balance in calories available for growth, when fed at a ration level equivalent to between 4 and 6% of body weight per day. At 26° C however, due to a much increased rate of oxygen consumption and a decline in filtration rate, the scope for growth was considerably reduced. At a ration level equivalent to only 0.1–0.2% of body weight per day, and in spite of the reduced respiration rates, the scope for growth was negative at all temperatures tested, and markedly so at 26° C.

These calculations of scope for growth suggest that at 26° C *M. californianus* from the San Juan Islands are approaching their thermal lethal limit. We did not examine the capacity of these mussels to acclimate to a maintained temperature change. During acclimation to high temperatures *M. edulis* is able

gradually to recover from a negative to a positive scope for growth (Widdows and Bayne, 1971). However, it is unlikely that *M. californianus* at Friday Harbor would experience temperatures in excess of 22° C for long periods; they are more likely to be exposed to such high temperatures for short periods during summer low tide. Under such conditions the mussels may suffer periods during each day when their scope for growth is reduced, so that the cumulative effect represents a significant stress, relative to other individuals which do not experience such high temperatures and do not have periods of the day when food is not available.

Our measurements of the rates of oxygen consumption by *M. californianus* at 13° C indicated a closer resemblance between rates typical of animals on the shore and animals which were starved, rather than fed, in the laboratory. The available ration for fed animals in the laboratory was rather high, possibly as much as two or three times the maintenance ration (*i.e.* the ration at which weight is neither lost nor gained; see Thompson and Bayne, 1974). Either oxygen consumption by the animals on the shore was depressed by the technique employed in its measurement, or the ration available to these animals was below optimum. Only further experiments can help to clarify this.

It has proved impossible to date, to measure directly the "work done", and hence its true energy cost, in the ventilation of the mantle cavity by bivalve molluscs. In the absence of suitable direct techniques, to consider the possible "physiological" and "mechanical" costs of activity (Bayne *et al.*, 1973) may be of some help in understanding the capacity of a species for metabolic adaptation to the environment. Our preliminary attempt at such an analysis for *M. californianus* supports earlier suggestions made for *M. edulis* (Bayne *et al.*, 1973) that the costs of ventilation are high, and that they rise exponentially with any increase in the ventilation rate. On the other hand, the physiological costs of even a rather high ration are relatively low. The sedentary habit of mussels may suggest a variety of possible advantages and disadvantages, relative to a less sessile mode of life. But mussels must expend a considerable amount of energy in order to provide a continuous supply of food, and this high energy demand in turn limits the scope for activity to a low value, so imposing an important restraint on the ecological flexibility of the species.

Acknowledgements. This research was funded by a National Science Foundation Grant (GB-29315) to C. J. Bayne, by a National Research Council of Canada Operating Grant No 67-5961 to T. C. Carefoot, and by the Killan Fund of Dalhousie University (Nova Scotia) to R. J. Thompson. B. L. Bayne acknowledges the support of the Natural Environment Research Council, U.K. We are all grateful to Dr. Dennis Willows, Director of the Friday Harbor Laboratories, and to members of his staff, for the provision of facilities and equipment. William Noonan and Roger Smith gave invaluable assistance in the laboratory and on the shore.

References

Bayne, B. L.: Oxygen consumption by three species of lamellibranch mollusc in declining ambient oxygen tension. Comp. Biochem. Physiol. **40** A, 307–313 (1971 a)

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

Bayne, B. L.: Physiological changes in *Mytilus edulis* L. induced by temperature and nutritive stress. J. mar. biol. Ass., U.K. **53**, 39–58 (1973a)

Bayne, B. L.: Aspects of the metabolism of *Mytilus edulis* during starvation. Neth. J. sea. res. **7**, 399–410 (1973b)

Bayne, B. L.: Aspects of physiological condition in *Mytilus edulis* L. with special reference to the effects of oxygen tension and salinity, p. 213–238. In: Proceedings of the 9th European Marine Biology Symposium (H. Barnes, ed.). Aberdeen: Aberdeen University Press 1975

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

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

Chan, G. L.: Subtidal mussel beds in Baja California with a new record size for *Mytilus californianus*. Veliger **16**, 239–240 (1973)

Coe, W. R., Fox, D. L.: Biology of the Californian sea mussel *Mytilus californianus*. I. Influence of temperature, food supply, sex and age on the rate of growth. J. exp. Zool. **90**, 1–30 (1942)

Coe, W. R., Fox, D. L.: Biology of the Californian sea mussel *Mytilus californianus*. III. Environmental conditions and rate of growth. Biol. Bull. **87**, 58–72 (1944)

Connell, J. H.: A predator-prey system in the marine intertidal region. I. *Balanus glandula* and several predatory species of *Thais*. Ecol. Monogr. **40**, 49–78 (1970)

Corner, E. D. S., Cowey, C. B.: Biochemical studies on the production of marine zooplankton. Biol. Rev. **43**, 393–426 (1968)

Dayton, P. K.: Competition, disturbance and community organisation: the provision and subsequent utilisation of space in a rocky intertidal community. Ecol. Monogr. **41**, 351–389 (1971)

Dejours, P.: Comparison of gas transport by convection among animals. Resp. Physiol. **14**, 96–104 (1972)

Fox, D. L.: The habitat and food of the California sea mussel. Bull. Scripps Inst. Oceanogr. **4**, 1–64 (1936)

Fox, D. L., Coe, W. R.: Biology of the Californian sea mussel *Mytilus californianus*. II. Nutrition, metabolism, growth and calcium deposition. J. exp. Zool. **93**, 205–249 (1943)

Fox, D. L., Sverdrup, H. U., Cunningham, J. P.: The rate of water propulsion by the California mussel. Biol. Bull. **72**, 417–438 (1937)

Grasshoff, K., Johannsen, H.: A new, sensitive and direct method for the automatic determination of ammonia in seawater. J. Cons. int. Explor. Mer. **34**, 516–521 (1972)

Harger, J. R. E.: Competitive coexistence among intertidal invertebrates. Amer. Sci. **60**, 600–607 (1972)

Hoggarth, K. R., Trueman, E. R.: Techniques for recording the activity of aquatic invertebrates. Nature (Lond.) **213**, 1050–1051 (1967)

Moon, T. W., Pritchard, A. W.: Metabolic adaptations in vertically-separated populations of *Mytilus californianus* Conrad. J. exp. mar. biol. ecol. **5**, 35–46 (1970)

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

Paine, R. T.: Intertidal community structure. Experimental studies on the relationship between a dominant competitor and its principal predator. Oecologia (Berl.) **15**, 93–120 (1974)

Petersen, J. A., Fyhn, H. J., Johansen, K.: Ecophysiological studies of an intertidal crustacean, *Pollicipes polymerus* (Cirripedia, Lepadomorpha): Aquatic and aerial respiration. J. exp. Biol. **61**, 309–320 (1974)

Pickens, P. E.: Heart rate of mussels as a function of latitude, intertidal height and acclimation temperature. Phys. Zool. **38**, 390–405 (1965)

Rao, K. P.: Rate of water propulsion in *Mytilus californianus* as a function of latitude. Biol. Bull. **104**, 171–181 (1953)

Rao, K. P.: Tidal rhythmicity of rate of water propulsion in *Mytilus* and its modificibility by transplantation. Biol. Bull. **106**, 353–359 (1954)

Segal, E., Rao, K. P., James, T. W.: Rate of activity as a function of intertidal height within populations of some littoral molluscs. Nature (Lond.) **172**, 118–1109 (1953)

Snedecor, G. W., Cochran, W. G.: Statistical methods. Iowa: Iowa State University Press 1972

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

Vahl, O.: Efficiency of particle retention in *Mytilus edulis* L. Ophelia **10**, 17–25 (1972)

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

Whedon, W. F., Sommer, H.: Respiratory exchange of *Mytilus californianus*. Z. vergl. Physiol. **25**, 523–528 (1937)

Widdows, J.: The effects of temperature on the metabolism and activity of *Mytilus edulis* L. Neth. J. sea res. **7**, 387–398 (1973a)

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

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

Young, R. T.: The distribution of the mussel *Mytilus californianus* in relation to the salinity of its environment. Ecology **22**, 379–386 (1941)

Dr. B. L. Bayne
Institute for Marine Environmental Research
67 Citadel Road
Plymouth PL1 3DH, Devon, England