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The Physiological Ecology of *Mytilus californianus* Conrad

2. Adaptations to Low Oxygen Tension and Air Exposure

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Summary. *Mytilus californianus* regulated its rate of oxygen consumption ($\dot{V}O_2$) during decline in oxygen tension, but did not acclimate $\dot{V}O_2$ when held at 58 mm Hg PO_2. In spite of a capacity to consume oxygen while exposed to air (the average $\dot{V}O_2$ in air being equivalent to 0.74 × the standard rate of oxygen consumption in water), these mussels acquired an "oxygen debt" during aerial exposure which was discharged on subsequent reimmersion. During exposure to air the oxygen tension of the fluid in the mantle cavity was rapidly reduced to approximately 40 mm Hg, in animals both in the laboratory and on the shore. Heart rate was also reduced during air exposure, though not to the point of cardiac arrest. The concentration of ammonia in the fluid of the mantle cavity increased during aerial exposure, but the rate of excretion of ammonia was much lower than during immersion. Observations of mussels on the shore at low tide indicated that dehydration of the tissues was not a serious threat, possibly due to the large volume of fluid retained in the mantle cavity. During aerial exposure some end-products of anaerobic metabolism (alanine and malate) accumulated in the posterior adductor muscle. Malate accumulation was rapid during the first hour of exposure; alanine accumulated more gradually. It is concluded that during aerial exposure *M. californianus* resorts to anaerobiosis in spite of a capacity to extract some oxygen from the atmosphere. This results in a metabolic deficit during each period of low tide which, coupled with the reduced time available for feeding, imposes a physiological stress on mussels distributed on the shore.

Introduction

Mytilus californianus is a common, and in some parts of its range a dominant, member of the intertidal community along the western coast of North America. Studies of the ecology of this species by Dayton (1971), Harger (1972) and Paine (1974) have indicated some of the interactions with both biotic and abiotic components of the environment that contribute to the integrity of this community. These studies have also led to the suggestion (Paine, 1974) that its intertidal distribution represents for *M. californianus* a refuge, with the lower limits of its distribution set by competition and predation, and its upper limits determined by physiological stress due to such factors as high temperatures, desiccation and the shortage of food. The experiments and observations described in this and in a previous paper (Bayne *et al.*, 1976) were designed to examine the extent to which *M. californianus* from the intertidal zone of the San Juan Islands, Washington, USA, may be subjected to physiological stress on the shore, and to elucidate some of the physiological adaptations which might facilitate such a distribution.

Material and Methods

Mussels were collected on San Juan Island (lat. 48°30′, long. 123°05′) at three sites—Lime Kiln Point, Eagle Point and Cattle Point (see Bayne *et al.*, 1976, Fig. 1). Experiments were carried out at the Friday Harbor Laboratories of the University of Washington. In our previous paper we described briefly the general environmental conditions that these mussels experience.

In the laboratory different experiments were set up to measure various physiological responses to exposure to air; the protocol for these experiments is described in the text. In general, the mussels were exposed to air in glass desiccators, which afforded some control of the relative humidity and temperature. For experiments with animals immersed in water, the flowing water in the laboratory was used. During the period of these experiments, in July and August 1973, the ambient temperature of this water was 13.3 ± 0.7 (standard deviation) °C, and its oxygen tension was slightly below air-saturation, viz. 135 ± 0.3 mm Hg PO_2. In one experiment, referred to as the 'low PO_2' experiment, an equal number of mussels was placed in each of three containers with flowing seawater. In the first of these (the control) the ambient oxygen tension was the same as in the water table, *viz.* 135 mm Hg. In the second and third containers the oxygen tension was reduced by bubbling nitrogen into the inflow water supply to produce PO_2 levels of 94 ± 3.7 mm Hg in container 2 (medium PO_2) and 58 ± 3.5 mm Hg in container 3 (low PO_2). The temperature in this experiment was 13 ± 1.5° C, and the mussels were fed at regular intervals with a mixed culture of flagellate algae (*Isochrysis galbana*, *Phaeodactylum tricornutum* and *Dunaliella* sp.).

In another laboratory experiment, mussels were kept in the seawater table at three ration levels: a) the 'high ration' animals, which were fed continuously with a mixture of algal cells at a concentration of $10{,}000 \pm 1{,}650$ per ml; b) the 'low ration' or starved animals, which were kept in water prefiltered through an in-line 'Aqua-Pure' water filter to provide a particle concentration (naturally-occurring particulate matter) of 770 ± 38 per ml; c) the 'intermediate ration' animals, which were fed continuously with a 50:50 seawater dilution of the algal culture used to feed the high ration animals.

Observations and measurements were also made on mussels in their natural situations on the shore; the details are described in the text. Both in the laboratory and on the shore, a number of different procedures was used, as described below:

1. Oxygen consumption in water was measured as described by Bayne *et al.* (1976). The results are quoted as ml O_2 consumed per hour per animal ($\dot{V}O_2$). In the low PO_2 experiment, individual mussels were subjected to step-wise reduction in the ambient PO_2 by flushing the respirometer flask with water which had been stripped of the required amount of oxygen by exchange with nitrogen gas.

2. Rate of filtration was measured as described by Bayne *et al.* (1976).

3. Frequency of the heart beat was also measured as described earlier, using impedance pneumography.

4. Rate of excretion of ammonia-nitrogen was measured as described by Bayne *et al.* (1976).

5. Oxygen consumption in air was measured using a modified Schollander respirometer. Results are quoted as ml O_2 consumed per hour per animal. Mussels were collected (temperature 12–13° C) before they were uncovered on the shore by the tide, cleaned of epiphytes and debris, and held in the laboratory in water at 13° C for one to 4 hrs. They were then placed individually in respirometer flasks, together with a small volume of water (5–20 ml depending on the size of the animal), which served to maintain both a high relative humidity and a constant vapour pressure in the flask. The respirometers were immersed in the water table (13° C), left undisturbed for 20 min and three readings taken from each flask at 20 min intervals. The readings from each respirometer were then summed to give a value for oxygen consumption by one animal over 1 hr. Control respirometers were also set up, without animals, and only the results from experiments in which these control flasks showed absolute stability (*i.e.* a sequence of three zero readings for 'oxygen depletion' at 20 min intervals) were used for subsequent analysis.

6. The oxygen content of small volumes of liquid: Occasionally (details in the text) samples (0.2–1.0 ml) of fluid from the mantle cavity of individual mussels were analysed for oxygen tension. The samples were withdrawn into a syringe the dead-space of which was filled

with mercury, and quickly injected into a thermostatted cell fitted with an oxygen electrode (Radiometer) for measurement of the partial pressure of oxygen. Frequent calibration of the electrode was necessary, using seawater of known oxygen content (as established by Winkler titration).

7. Chlorinity estimations were carried out by titration with silver nitrate.

8. Osmolality determinations were made using a Fiske Osmometer, with samples analysed in duplicate.

9. Temperatures in the mantle cavities of mussels on the shore were measured with a YSI Telethermometer using fine thermistor probes inserted between the two shell valves.

10. For all biochemical estimations, the mussels were quick-frozen in a mixture of absolute ethanol and dry ice and stored in a deep-freeze at −20° C. When required for analysis (usually 1 day, and never more than 4 days, later), each animal was broken open and the tissues quickly dissected out. The posterior adductor muscle was removed, weighed and, when still frozen, minced with a razor and transferred to ice-cold 95% ethanol (5 ml per gram weight of tissue) for homogenisation in an allglass homogeniser. The digestive gland was removed, together with the stomach (which was scraped free of its contents) and placed in pre-weighed test-tubes for weighing and digestion with 30% NaOH. The mantle, containing predominantly gonadal tissue, was cut free from the pallial muscle and treated as described for the digestive gland.

The homogenate of the adductor muscle was centrifuged (10,000 × *g*) for 10 min at 0° C. The supernatant was then taken for analysis of L-malate and L-alanine (see below) and the pellet placed in a boiling tube with 5 ml of 30% NaOH. The adductor muscle pellet, the digestive gland and the mantle tissue were all separately solubilised in 30% NaOH in a boiling-water bath for 30 min. 95% ethanol (× 5 the volume of the solubilised tissue) was added, and the mixture allowed to stand overnight at 6° C for precipitation of the extracted glycogen. The contents were then centrifuged at 10,000 × *g* for 10 min (0° C), the pellet collected and the supernatant left for a further 3–12 hrs at 6° C, followed by further centrifugation. For each tissue the two pellets were pooled, dissolved in distilled water, made up to 100 ml, and 1 ml taken for the assay of glycogen content by the phenol/sulphuric acid method of Dubois *et al.* (1956).

The original supernatants from the homogenates of adductor muscle in ethanol were reduced to dryness *in vacuo* on a rotary evaporator at 40° C. The residue was stored at 4° C for 1–4 days prior to dissolution in 4 ml of double-distilled water. These samples were then analysed for L-malate and L-alanine by methods described in Bergmeyer (1965).

Results

Exposure to Reduced Oxygen Tensions while Immersed

a) The Rate of Oxygen Consumption

Five mussels of similar size (2.119 ± 0.136 g dry flesh weight), which had been kept in the laboratory at 135 mm Hg PO_2 for between 7 and 22 days, were individually subjected to a decline in oxygen tension (average rate of decline 20 mm Hg h^{-1}) and their rates of oxygen consumption measured four or five times during the reduction in PO_2 (Fig. 1). Mangum and Van Winkle (1973) examined a number of statistical models which may be used to describe this relationship between VO_2 and PO_2. For reasons which are argued fully elsewhere (Bayne and Livingstone, in press), we have accepted a simple hyperbolic expression as providing the best-fit to our data. This expression (Tang, 1933) is:

$$\text{Rate of oxygen consumption } (VO_2) = \frac{PO_2}{K_2 + (K_1 \cdot PO_2)}.$$

The values for K_1 and K_2 (calculated from the linear form of this expression, *viz.*: $PO_2/VO_2 = K_1 + K_2 \cdot PO_2$) for the five individual mussels are given in Table 1, and the curves that result from these expressions are plotted in Fig. 1. Bayne

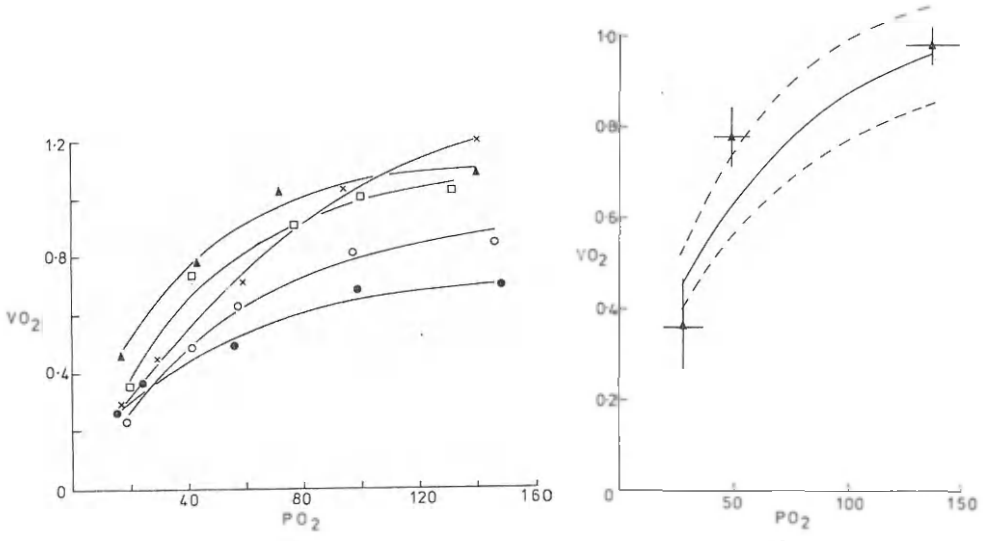

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Fig. 1. The rates of oxygen consumption by *Mytilus californianus* (VO_2; ml O_2 h^{-1}) at different oxygen tensions (PO_2; mm Hg). The different symbols represent values for five individuals. The curves are plotted according to the equation $VO_2 = \frac{PO_2}{K_1 + (K_2 \cdot PO_2)}$, with values for K_1 and K_2 taken from Table 1

Fig. 2. The rates of oxygen consumption by *Mytilus californianus* (VO_2; ml O_2 h^{-1}) at different oxygen tensions (PO_2; mm Hg). The curve (± 95% confidence limit indicated as dashed lines) is drawn according to the equation $VO_2 = \frac{PO_2}{(40.1 + 0.74 \cdot PO_2)}$ and describes the average response of five individuals held at 135 mm Hg PO_2 (see Table 1). ▲ mussels held at 55 mm Hg PO_2 and measured at 48, 27 and then at 135 mm Hg PO_2, with one standard deviation for both VO_2 and PO_2 indicated as bars

(1971) proposed the use of an index, K_1/K_2, as a means of assessing the extent of respiratory independence of ambient oxygen tension. The mean value for this index in five experiments with *M. californianus* was 62.5 ± (standard deviation) 34.3, which compares with values for *M. edulis* which range from 20 to 62 (unpublished data). This analysis suggests that *M. californianus* are well able to maintain aerobic gas exchange at reduced oxygen tension.

Other mussels were held for between 8 and 13 days at reduced PO_2 (mean 58 mm Hg) in the low PO_2 experiment and the rates of oxygen consumption of four individuals then measured, firstly at 48 mm Hg, then at 27 mm Hg, and finally on recovery to a higher oxygen tension (135 mm Hg). The results are plotted in Fig. 2 together with the range of VO_2 values recorded for the control animals maintained at 135 mm Hg. A comparison of experimental and control mussels suggests that *M. californianus* did not acclimate VO_2 to reduced oxygen tension, as was reported for *M. edulis* by Bayne (1975).

The five control animals, which had been maintained at 135 mm Hg PO_2, and exposed for between 2 and 3 hrs to oxygen tensions less than 20 mm Hg, were

Table 1. Values for K_1 and K_2 in the expressions $VO_2 = PO_2/K_1 + (K_2 \times PO_2)$, for five *M. californianus* subjected to reduced oxygen tensions. VO_2, ml O_2 consumed h^{-1}; PO_2, mm Hg

Animal no.	K_1	K_2	Sums of squares of deviations ($\times 10^2$)
1	45.96	1.07	0.474
2	46.98	0.77	3.008
3	32.98	0.68	1.619
4	57.85	0.45	0.603
5	22.65	0.71	1.394
Mean	40.08	0.74	
± S.e.	± 5.36	± 0.099	

recovered to the normoxic condition and VO_2 measured for comparison with values determined before excursion at low PO_2. The mean values (±1 standard deviation) were as follows:

1. Before excursion at low PO_2: $VO_2 = 0.965 \pm 0.028$ ml O_2 h^{-1}.
2. After excursion at low PO_2: $VO_2 = 1.156 \pm 0.092$ ml O_2 h^{-1}.

There is a small, but significant, difference between these values, signifying the presence of an oxygen debt.

b) The Rate of Excretion of Ammonia

The rates of excretion of ammonia-nitrogen by animals held at ambient oxygen tension in the water-table (the controls) and those held at reduced PO_2 (58 mm Hg) were measured at intervals over 16 days (Fig. 3). The control animals had a constant excretion rate for the first few days of the experiment, but reduced this rate after 7–16 days to about 33 μg $NH_4 \cdot N$ h^{-1}. This value is similar to the results of our other experiments, reported earlier (Bayne *et al.*, 1976). The animals at low PO_2 showed a rapid decline in their rates of excretion, and by day 3 these rates were at a steady-state of 16 μg $NH_4 \cdot N$ h^{-1}.

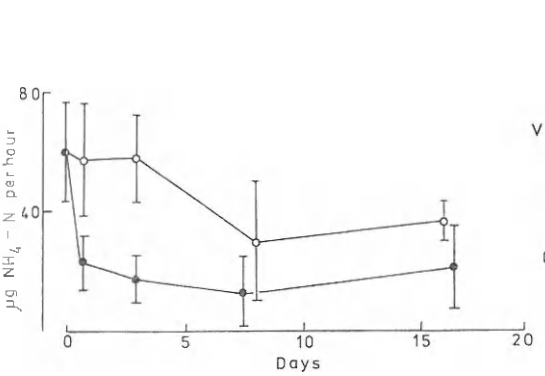
c) The O:N Ratio

Table 2 shows the calculation of the O:N ratio (*i.e.* the ratio by atomic equivalents of oxygen consumed to ammonia-N excreted) for these experiments. The value calculated for mussels at 135 mm Hg is similar to results published earlier (Bayne *et al.*, 1976). At reduced PO_2 the O:N ratio increased.

Exposure to Air—Laboratory Experiments

a) The Rate of Oxygen Consumption in Air

There was a large variance in the determinations of VO_2 in air (Fig. 4), probably due in part to variations in the degree of 'gape' of the shell valves during the measurements. Nevertheless, most individuals did consume some oxygen from the atmosphere, and regression analysis indicated a significant association



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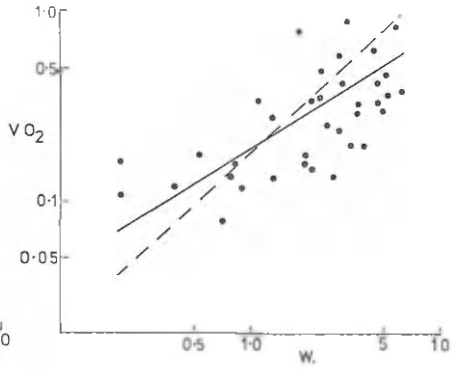

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Fig. 3. The rates of excretion of ammonia-nitrogen by *Mytilus californianus*. ○ mussels at 135 mm Hg PO_2; ● mussels at 58 mm Hg PO_2. Mean values for five animals ± one standard deviation

Fig. 4. The relationship between the rate of oxygen consumption (VO_2; ml O_2 h^{-1}) and dry flesh weight (W; grams) for *Mytilus californianus* in air. See text for explanation of the solid and dashed regression lines

Table 2. The O:N ratio of *Mytilus californianus* held at 137 and 58 mm Hg PO_2, at 13°C

Oxygen tension (mm Hg)	Rate of oxygen consumption (ml O_2 h^{-1})	Oxygen consumption (µg atoms O_2 h^{-1})	Rate of excretion of ammonia-N (µg $NH_4 \cdot N$ h^{-1})	Rate of excretion of ammonia-N (µg atoms $NH_4 \cdot N$ h^{-1})	O:N
135	0.965	85.1	33.3	2.38	35.8
58	0.781	61.9	16.7	1.19	52.0

between the rate of oxygen consumption (VO_2: ml O_2 h^{-1}) and body size (W: dry flesh weight in grams):

$$VO_2 = 0.204\ W^{0.417}\ (n = 35;\ r = 0.559).$$

This regression (which is plotted as a solid line in Fig. 4) was compared by covariance analysis with the regression of VO_2 against W for mussels which had been starved for 16–23 days whilst immersed in water at 13° C (Bayne *et al.*, 1976). There was no significant difference between the coefficients (b in the expression $VO_2 = aW^b$) of these two regressions, but the difference between the constants, a, was significant at $P = 0.05$. In order to facilitate subsequent comparisons between 'immersed' and 'air exposed' rates of oxygen consumption, the data for VO_2 in air were fitted by a regression with a value for b (0.648) which had been found acceptable for describing immersed VO_2 as a function of size (dashed line in Fig. 4). This analysis therefore yielded two equations relating VO_2 to W, in air and water, at 13° C:

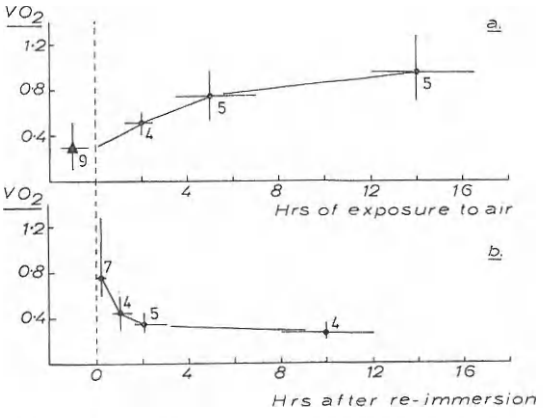

Fig. 5. (a) The rate of oxygen consumption ($\dot{V}O_2$; ml O_2 h^{-1}) by *Mytilus californianus* before (▲) and immediately after (●) various periods of exposure to air. (b) The rate of oxygen consumption by *M. californianus* after 14 hrs exposure to air, showing the decline that represents the payment of an 'oxygen debt'. All values are means (with range of $\dot{V}O_2$ and time indicated as bars) for the numbers of preparations as indicated

1. Air : $\dot{V}O_2 = 0.172\, W^{0.648}$.
2. Water: $\dot{V}O_2 = 0.233\, W^{0.648}$.

The rate of oxygen consumption by *M. californianus* in air of high humidity was therefore estimated to be 0.74× the standard rate of oxygen uptake in water at the same temperature.

b) The Rate of Oxygen Consumption in Water, before and after Exposure to Air

Eight animals of similar size (2.11 ± 0.087 g dry flesh) were exposed to air for various periods of time in the laboratory, and their rates of oxygen consumption measured immediately before and at intervals after the exposure period. All measurements were made at 13° C; during exposure to air the temperature was 19° C and the relative humidity was 81%. On reimmersion after exposure, each animal was allowed 2 min to clear water from the mantle cavity before the respirometer was sealed for recording the rate of depletion of oxygen.

The average $\dot{V}O_2$ before exposure to air was 0.287 ml O_2 h^{-1} (Fig. 5a). Immediately after air-exposure, oxygen consumption increased: to 0.507 ml O_2 h^{-1} after 2 hrs of exposure; 0.753 ml O_2 h^{-1} after 5 hrs of exposure, and to 0.953 ml O_2 h^{-1} after 14 hrs in air. Rates of consumption declined rapidly with time on re-immersion (Fig. 5b; animals exposed to air for 14 hrs) and reached values similar to the rates recorded before air-exposure within 3 hrs.

An estimate of the accumulated oxygen debt, defined empirically as the increase in oxygen uptake in water following exposure to air, was calculated by linear interpolation between maximum values for $\dot{V}O_2$, recorded immediately after re-immersion, and the steady-state value of 0.287 ml O_2 h^{-1} reached 3 hrs later. The results (Table 3) suggest that the oxygen debt is related non-linearly to the duration of aerial exposure.

Table 3. Estimates of the 'oxygen debt' repayed by *Mytilus californianus* after various periods of exposure to air

Time of exposure to air (hrs)	Estimated cumulated oxygen debt (ml O_2)
2	0.283
5	0.406
14	0.606

c) Heart Frequency, and the Oxygen Tension in the Mantle Cavity, before, during and after Exposure to Air

Experiment 1. Each of four mussels was fitted with a cannula of polyethylene tubing, inserted through the pedal gape into the mantle cavity in such a way as to allow samples of fluid to be drawn from the cavity with a hypodermic syringe. These animals were also fitted with thin copper electrodes for recording heart-beat (see Bayne *et al.*, 1976). With the animals immersed in water (11.9° C; salinity 31.8° $^0/_{00}$; PO_2 135 ± 0.3 mm Hg), a sample of fluid was taken from the mantle cavity of each individual for determination of the oxygen tension, and a record obtained of the heart beat. The mussels were then exposed to air (12.2° C; relative humidity 75%) by siphoning off the water in the chamber. Samples (0.2 ml) of the mantle cavity fluid were taken at three intervals over the following 6 h, and recording of the heart beat continued. The total volume of fluid in the mantle cavity of each of these animals was approximately 12 ml, so that less than 10% of the fluid was removed for analysis during the experiment. The animals were then re-immersed by flooding the chamber with water, the heart beat recorded continuously over the next 10–20 min, and one sample of mantle cavity fluid per individual taken for PO_2 analysis.

The results are shown in Fig. 6. The frequency of heart beat declined over the first 1–2 hrs of exposure to air and reached minimal values of five or six beats per minute (Fig. 6a). Cardiac arrest was not observed, and heart rhythm was maintained throughout the exposure period. On re-immersion there was a period of 2–5 min during which heart frequency remained low, but over the following few minutes heart beat increased rapidly to normal values with some evidence of over-shoot (Fig. 6b). The oxygen tension of the fluid in the mantle cavity was three to six mm Hg below the ambient tension of the water during immersion. On exposure to air, the mantle cavity PO_2 declined, reaching a steady-state of 40 mm Hg at or within 60 min (Fig. 6a). This value was maintained for the remainder of the period of exposure to air, but recovered to normal values within two minutes of re-immersion (Fig. 6b).

Experiment 2. The protocol for this experiment was similar to experiment 1, but measurements of the PO_2 of the mantle cavity fluid, and of the frequency of heart beat, were made over a shorter time-course following exposure to air. The results are plotted in Fig. 7. The heart-beat frequency increased during the first 5 min of air-exposure, and then declined over the next 10 min. The steady-state value of 40 mm Hg PO_2 in the mantle cavity fluid was reached within 10–15 min of exposure. The period of increased heart rate coincided with a PO_2 of between 100 and 60 mm Hg in the mantle cavity.

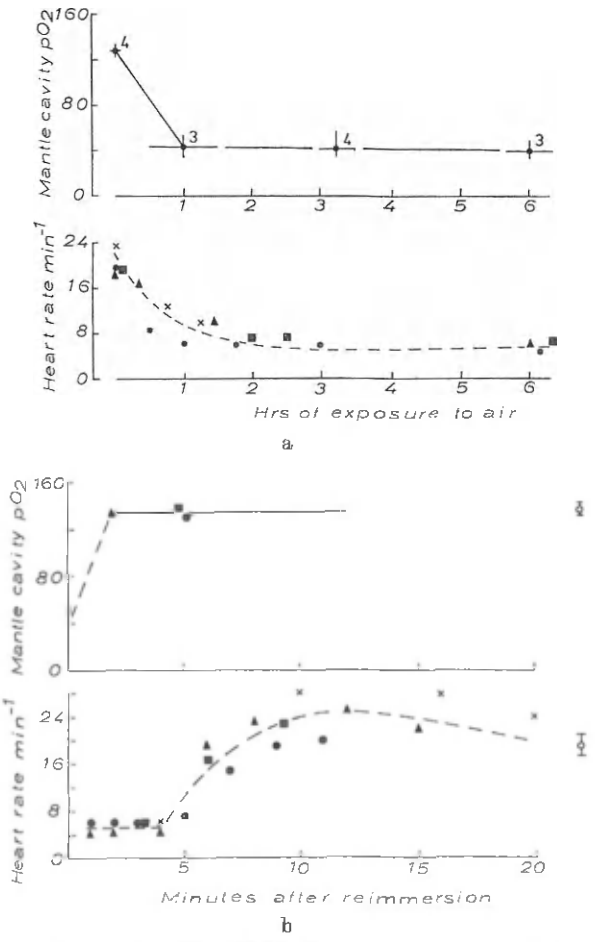

Fig. 6. (a) The oxygen tension (mm Hg PO_2) in the mantle cavity, and the frequency of heart beat (beats min^{-1}) in *Mytilus californianus* during exposure to air. Numbers in the figure indicate the number of measurements used to calculate the means (with range of PO_2 and time indicated as bars). Different symbols represent different individuals. (b) The oxygen tension (mm Hg PO_2) in the mantle cavity, and the frequency of heart beats (beats min^{-1}) in *Mytilus californianus* on re-immersion following 6 hrs of exposure to air. The different symbols represent values for different individuals. ○ mean values (± standard deviation) for mussels before exposure to air

d) Filtration Rate before and after Exposure to Air

Two mussels were fitted with electrodes for recording heart beat, and placed in separate respirometer flasks (volume 550 ml) at 14.5° C with a flow of water of 130 ml min^{-1}. After 1 hr their filtration rates were measured at an inflow particle concentration of 8,500 ml^{-1}. Their rates of oxygen consumption were also measured during immersion, and heart-beat frequency monitored continously. The vessels were then drained of water and the animals exposed to air for 1 hr at 14.5° C, relative humidity at 87.5%. At the end of this period the vessels were

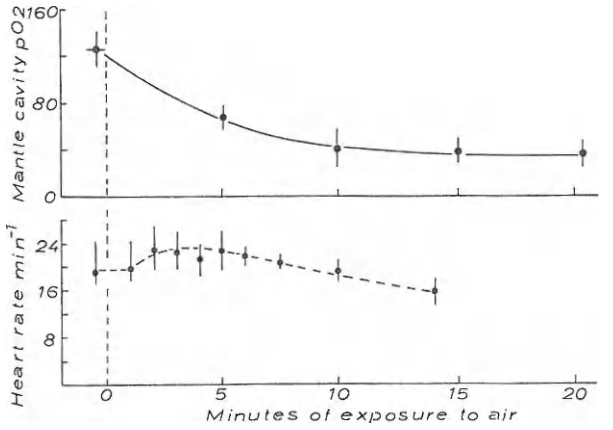

Fig. 7. The oxygen tensions (mm Hg PO_2) in the mantle cavity, and the frequency of heart beat (beats min^{-1}) in *Mytilus californianus* during 15–20 min exposure to air. Values plotted are means ± the range for five individuals

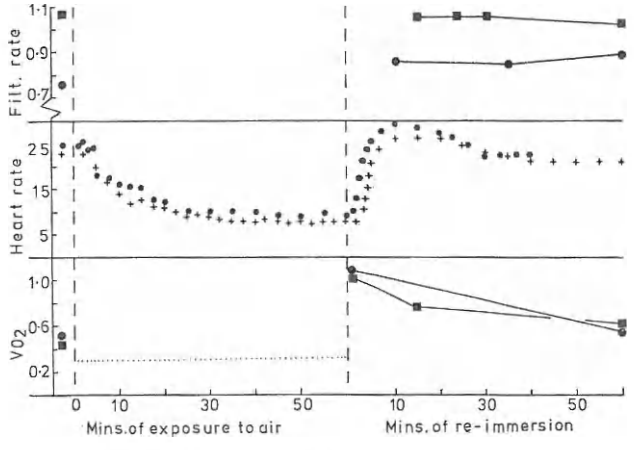

Fig. 8. Filtration rate (l h^{-1}), frequency of heart beat (beats min^{-1}) and rate of oxygen consumption (VO_2; ml O_2 h^{-1}) for two mussels, *Mytilus californianus*, before, during and after exposure to air

again flooded with water, and heart rate, filtration rate and VO_2 measurements taken at intervals over the next hour.

The determinations of the frequency of heart beat and the rate of oxygen consumption (Fig. 8) confirmed our earlier observations (Figs. 5 and 6). The rate of filtration did not alter significantly after air-exposure. If this pattern of a quicker heart beat and a maintained rate of filtration (the latter established for two animals only) should prove to be valid generally, and assuming filtration rate to be equivalent to ventilation rate in this case (see Bayne *et al.*, 1976), the relative 'ventilation : perfusion' ratio (which is an index relating ventilation to heart rate, and which is itself inversely proportional to the efficiency of oxygen

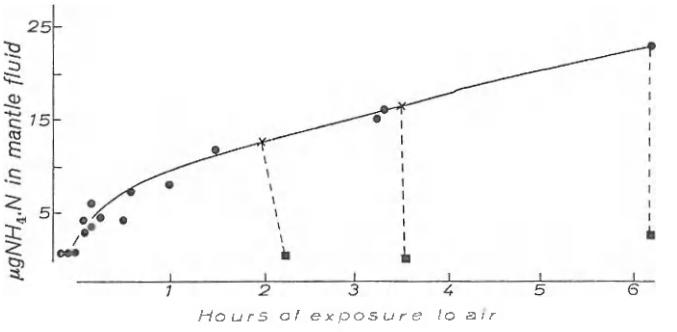

Fig. 9. The total ammonia-nitrogen content of the mantle fluid of specimens of *Mytilus californianus* during exposure to air (●) and on subsequent re-immersion (■). × indicates the time at which some individuals were immersed

utilisation; Bayne *et al.*, 1976) is reduced during re-immersion after exposure to air. This would result in an increased utilisation efficiency for oxygen, so facilitating an increased rate of oxygen consumption during repayment of the oxygen debt.

e) The Excretion of Ammonia

Four animals were each fitted with a cannula through the pedal gape. Samples of mantle cavity fluid were taken during immersion, at intervals over 6 h of subsequent exposure to air (14.5° C; relative humidity 78%), and within minutes of re-immersion following 2, 3.5 and 6 hrs of air-exposure. The fluid samples were analysed for ammonia content. At the end of the experiment the volume of the mantle cavity of each animal was measured and the results of the ammonia analyses (Fig. 9) expressed as µg $NH_4 \cdot N$ present in the mantle cavity fluid. Because some animals were sampled more than once (though never more than three times), corrections were applied for the volume of fluid removed.

There was a rapid accumulation of ammonia-N in the mantle cavity during air-exposure, from less than 1 µg $NH_4 \cdot N$ per individual during immersion to more than 7 µg $NH_4 \cdot N$ within 1 h of exposure and, in one individual, as high as 22.5 µg $NH_4 \cdot N$ after 6 hrs of exposure to air. On reimmersion, the ammonia concentrations returned to low values within 2 min (dashed lines in Fig. 9).

Exposure to Air—Observations on the Shore

a) The Temperature and the Oxygen Tension of the Fluid in the Mantle Cavity

One ml of fluid was withdrawn by syringe from the mantle cavity of five mussels at Lime Kiln Point and 10 mussels at Eagle Point, and analysed immediately for oxygen content by a micro-Winkler technique. A thermistor probe was inserted into the mantle cavity immediately following withdrawal of the fluid sample, and the temperature noted. These results are recorded in Table 4. Some other measurements (of chlorinity and osmolality) were made at the same time on the same individuals, but for ease of discussion these are recorded in the following section.

Table 4. Some measurements on the fluid from the mantle cavity of *M. californianus* during exposure to air (July 1973)

No.	Length (cm)	PO_2 (mm Hg)	Time exposed to air (hrs)	Tissue temperature (°C)	Comments
1	7.3	28.8	14.5	19.8	Lime Kiln Point; animals at
2	—	—	14.0	19.2	the side of tide-pools, with
3	7.7	32.0	14.5	18.2	*1/5–1/3 total length immersed.*
4	8.5	17.6	3.0	18.2	All animals in direct sunlight,
5	7.9	24.0	15.5	17.0	but none gaping. Tide-pool temp.: 19–22° C. Seawater temp.: 13.2° C
6	7.4	81.6	9.0	25.5	Eagle Point; animals from a
7	9.0	33.6	11.0	23.5	fissure, intermingled with
8	9.4	22.4	11.5	23.5	*Pollicipes*. No standing water;
9	8.9	56.0	10.5	24.5	animals in direct sunlight for
10	8.3	33.6	12.5	24.3	1–2 hrs per day only; no animals gaping. Air temp.: 19° C
11	13.0	28.8	10.5	21.5	Eagle Point; animals from a deep
12	9.7	30.4	10.5	21.5	fissure, without *Pollicipes*.
13	10.4	44.8	10.5	20.5	Animals in direct sunlight for
14	10.3	51.2	10.5	18.6	1–2 hrs per day only; none gaping.
15	8.8	59.2	10.5	21.0	Air temp.: 16° C. Seawater temp.: 10.3° C

There was no correlation between the oxygen tension of the fluid in the mantle cavity and the sample site, the time that the animals had been exposed to air at the time of sampling, or the temperature of the mantle cavity. The mean PO_2 of the mantle cavity fluid was 38.8 ± (standard error) 5.0 mm Hg, which was in good agreement with Moon and Pritchard (1970) and with our own laboratory data. Although none of these animals was gaping at the time of sampling, the lowest recorded value for oxygen tension in the mantle cavity was 17.6 mm Hg, in spite of prolonged periods of air-exposure.

At Eagle Point the temperatures in the mantle cavity were higher than air temperature, by an average of 5.1° C in the animals associated with *Pollicipes* and 4.6° C in the animals in the deep fissure. At Lime Kiln Point, however, temperatures in the mantle cavity were similar to the ambient air and tide-pool temperatures. These animals were attached at the side of the tide-pools, with from 1/5 or 1/3 of their lengths immersed in the water of the pool.

b) The Chlorinity, Osmolality and Temperature of the Mantle Cavity Fluid, and the Proportion of the Mantle Cavity Occupied with Fluid during Exposure to Air

Animals at four different habitats on the shore were examined. The results are listed in Table 5, together with the mean sizes of the animals examined and the estimated lengths of time that the animals had been exposed to air at the time of sampling.

Table 5. Various measurements taken on the mantle cavity fluid of *M. californianus* from four different habitats; results quoted as mean ± standard deviation

Habitat	Time exposed to air (hrs)	[Cl] as % of seawater	Osmolarity as % of seawater	Tissue temp. (°C)	% of mantle cavity occupied with fluid	Mean length (cm)
1. Edge of tidepool; sunny but cool ($n=6$)	14–15	105.2 (31)	100.0 (1.4)	17.9 (0.6)	—	8.03 (0.24)
2. Crevices; shaded and cool ($n=11$)	9–12	105.0 (0.9)	102.4 (0.2)	22.1 (0.7)	—	9.75 (0.47)
3. Horizontal rock face; sunny and hot ($n=25$)	4– 7	117.7 (0.9)	110.0 (0.5)	24.3 (0.1)	65.4 (3.3)	8.58 (0.34)
4. Vertical rock face; foggy and cool ($n=12$)	3– 7	100.4 (0.7)	98.1 (0.5)	17.7 (0.4)	74.2 (2.5)	9.58 (0.29)

Habitat 1 = Lime Kiln Point. Habitats 2 and 4 = Eagle Point. Habitat 3 = Cattle Point.

Table 6. The concentrations of glycogen in the digestive gland, adductor muscle and mantle tissues of *M. californianus* held in the laboratory at 135 ('control'), 94 ('medium') and 58 ('low') mm Hg PO_2

Condition	Mean concentration of glycogen ± standard deviation [mg g (fresh weight)$^{-1}$]		
	Digestive gland	Adductor muscle	Mantle
1. Controls:			
9 days	7.13 (1.155)	7.10 (0.631)	7.35 (1.556)
13 days	5.05 (0.883)	4.30 (0.508)	10.43 (1.329)
18 days	6.00 (0.868)	4.40 (0.762)	10.90 (2.201)
23 days	5.24 (1.336)	4.68 (0.308)	6.46 (1.557)
2. Reduced PO_2:			
a) Medium			
2 days	22.17 (6.690)	8.69 (1.225)	15.15 (4.930)
8 days	9.79 (0.285)	5.20 (0.305)	10.63 (0.845)
16 days	7.50 (1.905)	5.02 (0.245)	14.91 (5.945)
b) Low			
2 days	17.08 (5.625)	7.99 (1.870)	14.12 (1.355)
8 days	10.33 (0.765)	5.09 (2.260)	12.69 (2.285)
16 days	8.805 (1.505)	—	11.91 (3.845)

Should any loss of water from the mantle cavity occur by evaporation, this would result in an increased osmo-concentration of the mantle fluid, and a concomitant dehydration of the tissues. Animals on the vertical rock face at Eagle Point (habitat 4, Table 6) were sampled on a foggy and cool day. These animals showed no evidence of increased osmo-concentration, or chloride content, of the mantle cavity fluid. Although, on average, 26% of the fluid had been lost from

the mantle cavity of these individuals, this presumably occurred as a result of drainage through the pedal gape. Five of these animals were noted as showing 'slight gape' and the remainder as 'not gaping'. Mantle cavity temperature was similar to air temperature.

The mussels at the edge of tide-pools at Lime Kiln Point (habitat 1) also showed little evidence of increased osmo-concentration of the mantle cavity fluid, and the temperature in the cavity was similar to air temperature. The humidity in the micro-habitat of these animals was probably high.

In crevices at Eagle Point (habitat 2), where the animals were shaded from the sun, some increase in osmo-concentration occurred. These crevices were noted at the time as 'dry', but they clearly afforded some protection from desiccation, possibly by reducing air movement around the mussels. Tissue temperatures were higher than air temperature.

The animals on the horizontal rock face at Cattle Point (habitat 3) gave most indication of possible stress. There was a significant increase in the osmo-concentration and chloride content of the mantle cavity fluid (resulting in an estimated 10% desiccation of the animal tissues) and high mantle cavity temperature. On average, 35% of the fluid of the mantle cavity had been lost in these individuals (maximum recorded value was 80%) although only two animals were recorded as 'slightly gaping' and the remainder as 'not gaping'.

c) Ammonia in the Mantle Cavity Fluid

The concentration of ammonia-nitrogen in the fluid of the mantle cavity was measured only on animals from the vertical rock face at Eagle Point sampled on a cool and foggy day after 3–7 hrs of exposure to air. The average of 12 measurements was 2.07 $\mu g\ NH_4 \cdot N \pm$ (standard deviation) 0.118 per ml, equivalent to 24.6 $\mu g\ NH_4 \cdot N \pm 1.40$ total in the available mantle cavity fluid. These mussels averaged 7.74 ± 0.32 grams dry weight of tissue, and the estimated rate of production of ammonia-N during air-exposure was therefore 1.03 $\mu g\ NH_4 \cdot N \pm 0.092\ g^{-1}\ h^{-1}$. These values are similar to those recorded in the laboratory during exposure to air, and they suggest a depressed (relative to rates during immersion), but nevertheless significant, degradation of nitrogenous substrates at low tide.

Biochemical Determinations

a) Glycogen Levels in the Tissues

The glycogen levels were determined in the tissues of animals maintained under two sets of conditions. Firstly, mussels which were kept in the laboratory at three different ration levels (see "Material and Methods") were sampled at 9, 13, 18 and 23 days and the digestive gland, posterior adductor muscle and mantle tissues were analysed separately. These results are listed in Table 6 as 'controls'. Secondly, mussels were kept for 16 days at two levels of oxygen tension ('low PO_2' experiment), a medium (94 mm Hg) and a low (58 mm Hg) level, and the three tissues analysed at 2, 8 and 16 days. These results are listed in Table 6 as 'reduced PO_2'.

Glycogen levels showed no differences due to the ration level at which the animals were kept. The results also indicated no differences due to time in the

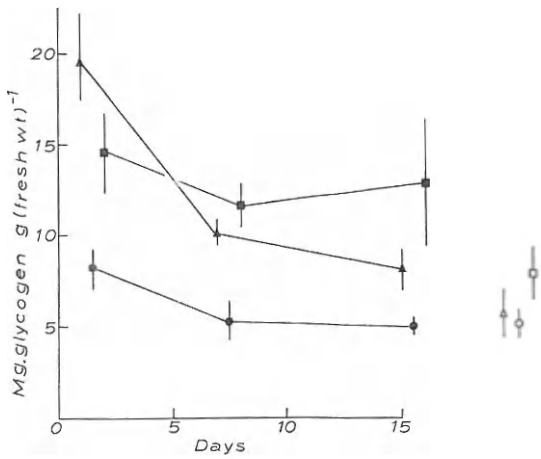

Fig. 10. The concentration of glycogen (mg g (fresh wt)$^{-1}$) in the mantle (■, □), digestive gland (▲, △) and adductor muscle (●, ○) of *Mytilus californianus* held at reduced oxygen tension (filled symbols) and at 135 mm Hg PO_2 (open symbols)

'control' values. The mean (± standard deviation) levels were therefore calculated for each tissue as follows:

Digestive gland:	5.84 ± 1.06 mg gram fresh weight^{-1}
Adductor muscle:	5.12 ± 0.55 mg gram fresh weight^{-1}
Mantle:	7.79 ± 1.66 mg gram fresh weight^{-1}

The results of the 'low PO_2' experiment show a decline in the glycogen level in the digestive gland and in the adductor muscle, though not in the mantle, during the experiment. However, there was no apparent difference due to ambient oxygen tension. Consequently, the values were pooled to arrive at mean estimates of the glycogen level in the three tissues, and plotted against time in Fig. 10, together with the 'control' values. Interpretation is made somewhat difficult by the lack of glycogen estimations prior to day 9 in the 'controls'. Nevertheless, the indications are that the mussels utilised significant amounts of glycogen from the digestive gland and adductor muscle, regardless of the oxygen tension. This utilisation was particularly marked during the first week, after which some accommodation to the laboratory conditions occurred. Glycogen in the mantle was not utilised in this way, and it appeared that the mussels increased their stores of glycogen between the first (mid-July) and the second (early August) determinations. There was no indication of more marked utilisation of glycogen at low than at high ambient oxygen tension.

b) The Concentration of Alanine and Malate in the Posterior Adductor Muscle

L-Alanine and L-malate in the posterior adductor muscle were analysed in two experiments; malate alone was determined in a third experiment.

Experiment 1. Mussels were sampled on days 9, 14 and 19 from the main stock of animals maintained and fed in the laboratory, and their adductor muscles

Table 7. The concentration of alanine and malate in the posterior adductor muscle of *M. californianus* held in the laboratory under different conditions (see text)

Condition	Mean concentration	Standard deviation
I. Alanine [μM g (fresh wt)$^{-1}$]		
1. Controls	2.78	0.552
2. Air exposure (expt. 1)		
2 days	6.00	0.560
4 days	6.25	0.874
3. Reduced PO_2 (expt. 2)		
a) Medium		
2 days	7.02	0.690
9 days	2.48	0.402
b) Low		
2 days	10.45	3.330
9 days	5.81	0.287
II. Malate (μM g (fresh wt)$^{-1}$]		
1. Controls	0.090	0.032
2. A) Air exposure (expt. 1)		
2 days	0.254	0.076
4 days	0.276	0.085
2. B) Air exposure (expt. 3)		
0 hrs	0.250	0.137
1 hrs	1.051	0.269
6 hrs	0.647	0.072
3. Reduced PO_2 (expt. 2)		
a) Medium		
2 days	1.069	0.290
9 days	0.154	0.002
16 days	0.159	0.085
b) Low		
2 days	0.139	0.071
9 days	0.305	0.028
16 days	0.559	0.205

analysed individually as described in "Material and Methods". On the same sample dates, some individuals were removed from the main trays and exposed to air (temperatures varied between 20 and 26° C, relative humidity between 62 and 83%) for 2 and 4 days, after which their adductor muscles were dissected for analysis. There was no significant change in alanine or malate concentrations over time in the animals while immersed and, in Table 7, all the values for both alanine and malate have been pooled to provide a single estimate of each substrate in these 'controls'. We have also pooled the estimates from animals exposed to air, in order to present two values each for alanine and malate, one value for animals air-exposed for 2 days and one for animals exposed for 4 days. The trend for both substrates was clear: alanine and malate both accumulated in the adductor muscle by the second day of exposure, and their concentrations remained high over 4 days.

Experiment 2. Mussels were maintained at two levels of oxygen tension, in the 'low PO_2' experiment. The adductor muscles of these animals were assayed after 2, 9 and 16 (malate only) days (Table 7). At the medium oxygen tension (94 mm Hg) both alanine and malate increased markedly in the adductor muscle over 2 days, and then declined close to control values; malate remained at low concentration to day 16. At low PO_2 (58 mm Hg) however, the two substrates behaved differently. Alanine increased almost by a factor of four by the 2nd day and then declined by day 9. Malate increased gradually in concentration over 16 days.

Experiment 3. Mussels were immersed in the laboratory water-table for 2 hrs after collection from the shore. Malate in the adductor muscle was then estimated in some animals ("0 hrs" in Table 7); the remainder of the animals were exposed to air (22° C; 78% relative humidity) for 1 and for 6 hrs prior to estimation of malate levels. The results (Table 7) indicated a rapid, very marked accumulation of malate in the adductor muscle after 1 hr of exposure to air, and a subsequent decline to 6 hrs.

Discussion

Mytilus californianus is capable of the partial regulation of its rate of oxygen consumption (VO_2) at reduced oxygen tensions (PO_2), a property shared with many other species of bivalve (Bayne, 1967; Mangum and Burnett, 1975; Taylor, 1975). At a PO_2 as low as 20 mm Hg these mussels are able to maintain a rate of oxygen consumption that is higher than the 'standard' rate recorded for individuals that have been starved for long periods in the laboratory (Bayne *et al.*, 1976). Nevertheless, at reduced PO_2, the rate of aerobic metabolism is depressed below the routine rate, which is the rate typical for fed animals in water at full air-saturation with oxygen. Unlike *Mytilus edulis* (Bayne, 1975), *M. californianus* did not acclimate VO_2 when kept at 58 mm Hg PO_2 for between 8 and 13 days. Under these conditions anaerobic metabolic pathways (Hochachka and Somero, 1973; De Zwaan *et al.*, 1975) are invoked, probably in order to maintain redox balance in the tissues and to make up the energy deficit that results from their prolonged inability to meet their routine demand for oxygen. This is illustrated, in our data, by the accumulation of alanine, which is an end-product of anaerobiosis in bivalves (Stokes and Awapara, 1968; Livingstone, 1975), in the adductor muscles of mussels held at low PO_2 for 2 and for 9 days (Table 7). Livingstone and Bayne (unpublished) have observed a more transient utilisation of anaerobic pathways by *M. edulis* during exposure to low PO_2; in this species, respiratory acclimation to the depletion of oxygen occurred, and the time-course of accumulation and subsequent removal of the end-products of anaerobiosis coincided with the period necessary for respiratory acclimation to be completed. In *M. californianus*, however, there was no indication of acclimation of VO_2, and alanine accumulated and remained at high concentration over the 9 days of the experiment. Only further study can elucidate whether these differences are truly specific, or to what extent they are due to seasonal factors.

Whereas accumulation of alanine can, with some assurance, be taken to indicate anaerobic metabolism in mussels, changes in the concentration of malate are more difficult to interpret in this context. The first stages in anaerobic glycolysis in bivalves involve the carboxylation of phospho-enol-pyruvate to oxalo-

acetate and subsequent reduction to malate. These conversions are sufficient to maintain redox balance in the cells (Hochachka and Mustafa, 1972). Under certain circumstances, as for example during short-term exposure to air, or when subjected to water at 'medium PO_2' (see Table 7) anaerobiosis may proceed only as far as the accumulation of malate, so ensuring the maintenance of redox balance. In different circumstances, for example during longer periods of air exposure, or at lower immersed oxygen tensions, malate may be metabolised further to succinate. There is some indication in our data to suggest that malate accumulates during short-term or partial hypoxia, but is reduced in concentration when the animal is forced deeper into anaerobiosis.

During prolonged exposure to low PO_2 the oxygen:nitrogen ratio increased (Table 2) and, although our interpretation here cannot be unequivocal, glycogen appeared to decrease (Table 6). These changes may be related, as an increased catabolism of carbohydrate, if it were to occur in the absence of a concomitant increase in protein catabolism, would result in a rise in the O:N ratio. However, under the conditions of our experiments, *viz.* animals with a relatively low store of glycogen, immediately after spawning, and subjected to hypoxia, the O:N ratio may be unreliable as an indicator of gross catabolic balance in the tissues. Protein catabolism may proceed normally at this time, but the resulting ammonia may be involved in synthetic reactions linked with anaerobiosis, for example the amination of pyruvate and/or of α-keto-glutarate (De Zwaan and Van Marrewijk, 1973).

During stress in the laboratory there was a decline in the glycogen content of the digestive gland and adductor muscle, but in the mantle the glycogen level remained stable. Gabbott (1975) has suggested that the mantle tissue of *M. edulis* may be either glycolytic or gluconeogenic, depending on season and the cycle of gametogenesis, but that it cannot carry out both functions at the same time. During the period of our experiments (July/August) the mussels were apparently synthesising glycogen in the mantle, following spawning, and only the glycogen in the digestive gland and the adductor muscle was 'available' for catabolism during stress. The existence of a mechanism for isolating the effects of stress from the mantle tissue during a period of glycogen synthesis may be a fundamental biochemical adaptation in mussels.

In recent years evidence has accumulated that intertidal bivalves can extract oxygen from the air (Kuenzler, 1961; Boyden, 1972; Coleman, 1973). In all cases for which the calculation is possible (Table 8), the rate of oxygen consumption in air is equivalent to approximately 0.7 times the standard rate in water. This capacity to respire in air is naturally linked with the ability to maintain the shell-valves slightly apart ('shell gaping') and so afford contact between the atmosphere and the fluid in the mantle cavity. On San Juan Island we did not observe a very high proportion of animals to be gaping during aerial exposure on the shore, although in the laboratory, where the humidity was higher, gaping was common. At high humidity *M. californianus* extracts oxygen from the air. However, under these conditions the rate of aerobic metabolism is depressed below the standard rate, and the animals acquire a large oxygen debt which is discharged during subsequent immersion (Table 3). The biochemical evidence (Table 7) suggests that anaerobic metabolism occurs to a significant extent during exposure to air.

Table 8. Equations relating the rates of oxygen consumption (VO_2, ml O_2 h^{-1}) to dry flesh weight (W; g) in three species of bivalve, in air and in water

Species (and authority)	Allometric equation	Temperature (°C)	Rate in air as a %age of rate in water for animals 1 g in weight
1. *Cardium edule* (Boyden, 1973)			
Air	$VO_2 = 0.131 W^{0.440}$	15	65.5
Water	$VO_2 = 0.200 W^{0.439}$	15	
2. *Modiolus demissus* (Kuenzler, 1961)			
Air	$VO_2 = 0.240 W^{0.590}$	20	
Water	$VO_2 = 0.380 W^{0.380}$	20	63.2
3. *Mytilus californianus* (this paper)			
Air	$VO_2 = 0.172 W^{0.648}$	13	
Water	$VO_2 = 0.233 W^{0.648}$	13	73.8

Within 1 hr of exposure there is a considerable accumulation of malate in the adductor muscle, and with more prolonged exposure both malate and alanine reach apparent steady-state concentrations in excess of the 'control' situation during immersion.

As the tide recedes past the mussels on the shore, therefore, the oxygen in the mantle cavity is quickly depleted (see also Moon and Pritchard, 1970) and, with gaping, diffusion of oxygen from the atmosphere maintains an equilibrium oxygen tension of about 40 mm Hg in the mantle fluid. The effective "diffusion distance" for oxygen from the mantle cavity fluid to the blood is presumably increased considerably at this time, due to reduced ventilation of the gill surfaces, and the rate of oxygen consumption is depressed. Anaerobic glycolysis is invoked as a result of the reduction in oxygen uptake, and ammonia excretion is considerably reduced. During air exposure the frequency of the heart beat is also reduced. Coleman and Trueman (1971) examined the different patterns of bradycardia typical of *Mytilus edulis* and *Modiolus modiolus* during air exposure, and concluded that *Mytilus* shows a more controlled response, as illustrated by a maintained heart rhythm and shell gape. By these criteria *M. californianus* also shows a controlled adaptation to intertidal air exposure.

When mussels are exposed to air on the shore, there is an increase in body temperature. In at least two situations (see Tables 4 and 5) the body temperatures were 4–5° C higher than the air temperature. This is typical of many intertidal animals (Newell, 1970), but Fyhn, *et al.* (1972) found that tissue temperatures in the stalked barnacle *Pollicipes polymerus* at Eagle Point on San Juan Island stabilised during exposure to 2–5° C below the rock temperatures, due to evaporative cooling. An increase in body temperature during low tide may be expected to increase the basal energy demand of the mussels, and so accentuate the requirement for anaerobiosis, although results presented earlier (Bayne *et al.*, 1976) showed that the temperature sensitivity of the rate of aerobic metabolism while immersed was not as great as may be expected on strict physicochemical grounds.

During exposure to air there is also a loss of water from the mantle cavity of *M. californianus*, presumably by leakage through the pedal gape. This loss may be as high as 20–30% of the available fluid in the mantle cavity, but it does not lead to desiccation of the tissues. Indeed, a loss of this kind may facilitate gas exchange with the atmosphere by exposing a larger surface area of mantle cavity fluid to the air. Only in one situation (Cattle Point; see Table 5) did we find evidence of a rise in the osmolality of the fluid in the mantle cavity above that of seawater, but this did not amount to more than a 15% increase in the most extreme case. Presumably the relatively large volume of water in the mantle cavity can serve as a reservoir "... which allows evaporative water loss to proceed during intertidal air exposure without reaching lethal limits of desiccation" (Fyhn *et al.*, 1972, referring to *Pollicipes*).

We conclude from this study that although *Mytilus californianus* exhibits a variety of physiological adaptations to its intertidal habit, some physiological stresses are apparent. Possibly the most significant of these is the inability to meet the standard metabolic demand by aerobic means. Oxygen extraction from the air, which is probably feasible only at relatively high humidities, is not adequate to maintain standard metabolic rate, and processes of anaerobic metabolism are required. Although the pathways of anaerobiosis in bivalves may be more efficient in the production of ATP than is 'classical' anaerobic glycolysis (Hochachka and Somero, 1973), the yield of ATP is not as great as in normal aerobic metabolism. There is stress also as a result of the limited time available for feeding. This is evidenced, not only as a restriction in the time for which feeding is possible, but also in the requirement, at each period of immersion, to 'make up' for the energy deficit acquired during air exposure. The scope for growth (see Bayne *et al.*, 1976) is not simply reduced in proportion to the feeding time that is lost, but in proportion also to the large oxygen debt that is accumulated during low tide. Finally, survival intertidally imposes a requirement on the mussels for limiting the extent to which protein catabolism can proceed normally, with consequent excretion of ammonia, during exposure to air. Either the rate of protein and amino-acid breakdown must be reduced, or the resulting ammonia must be involved in synthetic pathways associated with anaerobiosis. Nitrogen metabolism during periods of aerial exposure is a topic that requires further study.

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