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Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure

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Abstract

Physiological responses of two bivalves (*Mytilus edulis* L. and *Cardium edule* L.) to intertidal conditions were studied. Specimens were collected from S. W. England in autumn/winter, 1980, and acclimatized to either intertidal or subtidal regimes before measurement of rates of heat dissipation (\dot{q}) and oxygen uptake (\dot{v}_{O_2}) during 5 h of air exposure, and rates of \dot{v}_{O_2} , particle clearance, ammonia excretion, and food-absorption efficiency during 7 h of reimmersion. Subtidal individuals were either intermittently or continuously fed in order to distinguish the effects of periodic food supply from the effects of air exposure. Specimens of *M. edulis* had low aerial rates of \dot{q} (14 to 20% of aquatic rate), and \dot{q} was greater than the energy equivalent of \dot{v}_{O_2} , indicating that they were largely anaerobic. In contrast, *C. edule* "air-gaped" and had higher aerial rates of \dot{q} and \dot{v}_{O_2} (50 to 75% of aquatic rate). There were behavioural and metabolic differences in the responses of intertidally and subtidally acclimatized mussels and cockles to air exposure. Intertidal individuals of both species were more quiescent, had lower aerial rates of \dot{q} and \dot{v}_{O_2} , and showed a conditioned response at the "expected" time of reimmersion. The reduction in aerial rate of \dot{q} was an energy-saving mechanism and the payment of the "oxygen-debt" within 2 h of reimmersion represented a significant "cost". The "heat increment" associated with feeding and digestion was estimated as 15 to 17% of the oxygen uptake by *M. edulis* during all stages of recovery. *M. edulis* adapted to the intertidal regime by reducing its time-averaged aerial and aquatic rates of ammonia excretion. In contrast, *C. edule* maintained a high aerial and aquatic rate of ammonia excretion. The clearance rates of *M. edulis* recovered rapidly (0.5 to 1.5 h) following reimmersion, whereas those of *C. edule* recovered more slowly, particularly for the subtidal individuals

following acute exposure (>4 h). There was no evidence of increased clearance rate or absorption efficiency by intertidal individuals to compensate for the loss of feeding time. Intertidally acclimatized individuals of *M. edulis* and *C. edule* had more energy available for growth (scope for growth) integrated over a 12 h period and higher growth efficiencies than subtidal individuals subjected to acute air exposure and intermittent feeding regimes. This was the result of reduced aerial and aquatic rates of energy expenditure, a relatively small "cost" in terms of the payment of "oxygen-debt" and a rapid recovery of clearance rate following reimmersion.

Introduction

Sessile bivalve molluscs inhabiting the intertidal zone are subjected to regular periods of air exposure, the duration of which depends on their distribution on the shore and on the form of the tidal cycle. Consequently, they experience a restriction on the time available for feeding, large fluctuations in temperature, and may suffer desiccation.

Many studies have examined the physiological, behavioural and biochemical adaptations of bivalves to periods of hypoxia and anoxia in water and gas (Boyden, 1972; Gäde, 1975; Bayne *et al.*, 1976a; Bayne and Livingstone, 1977; de Zwaan, 1977; Livingstone and Bayne, 1977; Widdows *et al.*, 1979; Famme *et al.*, 1981; Nicchitta and Ellington, 1983; Pamatmat, 1983). Bivalves are known to utilize anaerobic metabolic pathways when tissues are deprived of oxygen, either as a result of shell-valve closure or environmental hypoxia/anoxia (reviewed by de Zwaan, 1977). In the majority of investigations, however, the bivalves have been subjected to environmentally unrealistic conditions of acute and usually prolonged (>12 h) exposure to either air or nitrogen gas. These studies, although contributing to our understanding of the funda-

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mental adaptive physiological and biochemical mechanisms operating under hypoxia and anoxia, do not provide information on the responses of bivalves to ecologically realistic conditions of tidal cycles of aerial exposure.

Gillmor (1982) concluded that several species of high-intertidal bivalves showed improved growth relative to species occurring lower on the shore, based on an analysis of growth rates and exposure time on the shore and in a laboratory tidal simulator. Approximately half of the improved intertidal performance was due to energy conservation (reduction of energy demand), and half to energy supplementation (suggested to include the continuation of digestion and absorption of food during intertidal exposure). Griffiths (1981) showed that freshly collected high-shore individuals of *Choromytilus meridionalis* had reduced metabolic costs relative to low-shore mussels; however, there was no enhancement of filtration rate or absorption efficiency in high-shore specimens (Griffiths and Buffenstein, 1981).

The objectives of our present study have been: firstly, to compare the physiological responses of two littoral bivalves, *Mytilus edulis* which typically closes its shell valves when air-exposed, and *Cardium edule* which typically gapes during aerial exposure. Secondly, to compare the physiological responses of subtidally acclimatized individuals during and after acute air exposure, with responses of specimens acclimatized to tidal cycles of air exposure; any differences presumably reflecting adaptation to intertidal exposure. And thirdly, to determine the effects of intermittent feeding by subtidal individuals in order to distinguish the effects of periodic availability of food from the effects of air exposure. The hypothesis of a possible relationship between the size of the "oxygen debt payment" and energy expenditure (e.g. heat increment or "specific dynamic action"; Brett and Groves, 1979) associated with the onset of feeding and digestion immediately following immersion, was also examined.

Materials and methods

Specimens of *Mytilus edulis* L. (dry tissue mass = $0.983 \text{ g} \pm 0.045 \text{ SE}$) were collected from the mid-littoral zone of Whitsand Bay (Cornwall, England) in October 1980, and *Cardium edule* L. (dry tissue mass = $0.385 \text{ g} \pm 0.021 \text{ SE}$) from the mid-littoral zone of St. John's Lake in the Tamar estuary (Cornwall, England) in December 1980. Mussels and cockles were carefully cleaned and were held in a system of recirculating seawater at 32‰ S and seawater/air temperatures of 15 °C. Cockles were maintained out of sediment and supported in their natural orientation by rings made from silicone tubing. Previous studies (Newell, 1977) concluded that neither clearance nor oxygen-consumption rates by *C. edule* were significantly affected by their presence in, or out of, sediments. Both species were fed an algal diet of *Phaeodactylum tricornutum* ($3.5 \times 10^6 \text{ cells l}^{-1} = 0.14 \text{ mg dry mass l}^{-1}$).

Individuals were acclimatized to one of three experimental conditions for at least 14 d:

Intertidal. Individuals were acclimatized to an aerial exposure regime (12 h cycle of 5 h in air and 7 h in water). During the initial 2 to 3 wk they were fed an algal diet (0.14 mg l^{-1}). After determining physiological responses of individuals ($n = 10$) to this experimental condition, these mussels were starved for a period of 4 d, during which time they were provided with "ashed sediment" in order to clear algal food from their digestive system. Measurements of the physiological responses of these starved intertidal mussels were then carried out (intertidal – starved). Finally, this group of intertidal mussels was fed at a higher algal ration level (0.21 mg l^{-1}) for a further 5 d and their physiological responses were again determined (intertidal – high-ration).

Subtidal/intermittently fed. Individuals were acclimatized to subtidal conditions but were fed intermittently (7 h every 12 h).

Subtidal/continuously fed. Individuals were acclimatized to conditions of immersion and continuous feeding.

After acclimation to subtidal conditions the physiological responses of individuals ($n = 10$) were measured before, during, and after an acute period (5 h) of air exposure.

Direct calorimetry

The microcalorimeter used was a double-twin heat-flow instrument designed by Pamatmat (1983) and used by us in other studies of aerial and anoxic heat dissipation by intertidal invertebrates (Shick, 1981; Shick *et al.*, 1983). Calibration by applying various known voltages across a $0.98 \text{ k}\Omega$ precision resistor in the sample chamber gave the static calibration constant of $25.4 \mu\text{W } \mu\text{V}^{-1}$. The instrument baseline was continuously monitored by the other twin half of the calorimeter and was found not to vary during the experiments. The calorimeter was operated in a water bath regulated at $15^\circ\text{C} \pm 0.01^\circ\text{C}$ located in a constant temperature room ($15^\circ\text{C} \pm 1.5^\circ\text{C}$). Average rates of heat dissipation (mW) during the last 3 h of air exposure were adjusted to a standard body mass (approximately the mean dry tissue mass of experimental individuals); 1 g dry tissue mass for *Mytilus edulis* and 0.3 g for *Cardium edule*. The mass-specific rate, \dot{q} (mW) was converted into joules h^{-1} by multiplying by 3.6 (Gnaiger, 1983a). Rates of heat dissipation in the various experimental groups were compared using Anova and Scheffé's method for multiple contrasts (Zar, 1974).

Respirometry

Rates of oxygen uptake (\dot{V}_{O_2}) were measured before, during and after exposure to air.

Aerial (=emersed) rates of oxygen uptake were determined at 15 °C in constant-pressure respirometers (Spencer-Davies, 1966) in which humidity was kept high by the addition of a small volume of filtered seawater.

Aquatic rates of oxygen uptake were determined by placing individuals in temperature-equilibrated (15 °C) respirometers (500 ml vol) containing seawater at near air-saturation (155 mm Hg). The rate of decline in oxygen partial-pressure (pO_2) was measured by means of an oxygen electrode (E5046) connected to a Radiometer blood-gas analyzer (PHM72 Mk 2). In order to prevent reduced pO_2 or accumulated excretory products modifying the rate of oxygen consumption, the seawater in the respirometer was flushed out when the pO_2 had declined to ~ 120 mm Hg and replaced with fresh air-saturated seawater.

All rates of oxygen uptake ($1 \text{ ml } O_2 = 44.6 \mu\text{mol } O_2$) were converted to heat dissipation by applying a generalized oxycaloric equivalent of 20.3 J ml^{-1} (455 kJ mol^{-1}).

Shells

After removing the soft-body tissues, the shells of *Mytilus edulis* and *Cardium edule* were placed in the calorimeter and aerial respirometer. A significant rate of oxygen uptake by the shells was recorded, and a low rate of heat production was occasionally detected. *M. edulis*: $\dot{v}_{O_2} = 0.032 \pm 0.002 \text{ ml } O_2 \text{ h}^{-1}$; *C. edule*: $\dot{v}_{O_2} = 0.021 \pm 0.003 \text{ ml } O_2 \text{ h}^{-1}$.

The low rate of heat dissipation by shells could not be accounted for in terms of oxygen uptake when a standard oxycaloric equivalent of $20.3 \text{ J ml}^{-1} O_2$ was applied (the calculated heat equivalent of oxygen uptake was > 16 times the \dot{q}). In addition, the shell \dot{v}_{O_2} was found to be only slightly affected by metabolic inhibitors (KCN and NaN_3) and thorough scrubbing of the shells, which reduced the surface microbial population by 95%, as determined by epifluorescence microscopy. There was no detectable heat dissipation or oxygen uptake by shells aged for 4 to 5 wk. Therefore, this fresh shell component, a non-metabolic process, was subtracted from the aerial rates of \dot{v}_{O_2} .

Excretion

The rate of ammonia excretion before and after aerial exposure was determined by placing individuals in 200 ml of $0.4 \mu\text{m}$ membrane-filtered seawater. The bivalves were incubated for up to 7 h and water was changed every 2 h. Controls of filtered seawater, but without bivalve, were incubated at the same time. At intervals during the recovery from air exposure, samples were taken and analysed in duplicate for ammonia using the phenol-hypochlorite method of Solórzano (1969).

Feeding and digestion

The clearance rates, or the volume of water cleared of particles per hour, were determined before and after air

exposure in a flow-through system (Widdows, 1985), with flow rates of 160 to 190 ml min^{-1} .

Faecal pellets were collected over a 12 h period and the efficiency of absorption of ingested food was estimated according to Conover (1966).

At the end of each experiment, the soft-body tissues of *Mytilus edulis* and *Cardium edule* were removed from their shells, dried at 90°C for 24 h and then weighed. All physiological responses were corrected to a standard body mass, 1 g for *M. edulis* and 0.3 g for *C. edule*.

Results

Mytilus edulis L.

Aerial rates of heat dissipation and oxygen uptake

Intertidal vs Subtidal. Mussels acclimatized to intertidal and subtidal regimes had markedly different rates of total heat dissipation (\dot{q}) and oxygen uptake (\dot{v}_{O_2}) when exposed to air. Specimens continuously fed under subtidal conditions, when subjected to acute air exposure for 5 h, had a significantly higher \dot{q} ($P < 0.05$) ($0.83 \text{ J g}^{-1} \text{ h}^{-1}$) than intertidal mussels ($0.50 \text{ J g}^{-1} \text{ h}^{-1}$), but no measurable \dot{v}_{O_2} (Fig. 1); the intertidal mussels, on the other hand, had a rate of oxygen uptake ($= 0.19 \text{ J g}^{-1} \text{ h}^{-1}$), which represented 40% of the total heat dissipation in air.

The two groups of mussels not only differed in their rate of heat dissipation but also in the pattern of \dot{q} . Typical thermograms of intertidal and subtidal individuals during the last 2.5 h of their normal 5 h of air exposure are presented in Fig. 2. When specimens were held in the calorimeter for 2.5 h beyond the normal reimmersion time, one-third of the intertidal mussels so tested showed increased \dot{q} , with distinct activity peaks within ± 15 min of the anticipated time of reimmersion, suggesting condi-

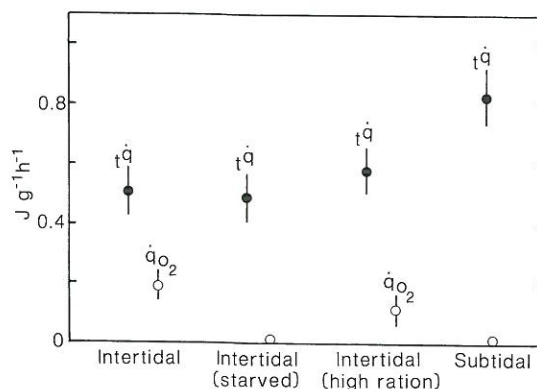


Fig. 1. *Mytilus edulis*. Mass-specific rate of heat dissipation (\dot{q}) and caloric equivalent of oxygen uptake (\dot{q}_{O_2}) by intertidally and subtidally acclimatized mussels during 5 h exposure to air. Intertidal: individuals fed at a concentration of 0.14 mg algal dry mass l^{-1} ; Intertidal (starved): individuals unfed for 4 d; Intertidal (high ration): individuals fed at 0.21 mg algal dry mass l^{-1} for 5 d after starvation; Subtidal: individuals continuously fed at 0.14 mg algal dry mass l^{-1} for 2 to 3 wk. Data points are means \pm SE.

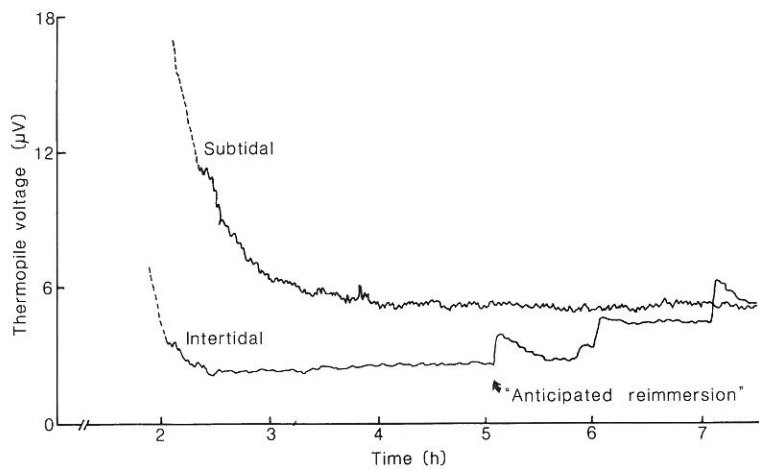


Fig. 2. *Mytilus edulis*. Typical thermograms of intertidal and subtidal mussels during last 2.5 h of their normal 5 h of air exposure. Arrow denotes time when intertidal individuals were normally reimmersed but were left air-exposed within the calorimeter. ($1 \mu\text{V} = 25.4 \mu\text{W} = 25.4 \mu\text{J s}^{-1}$)

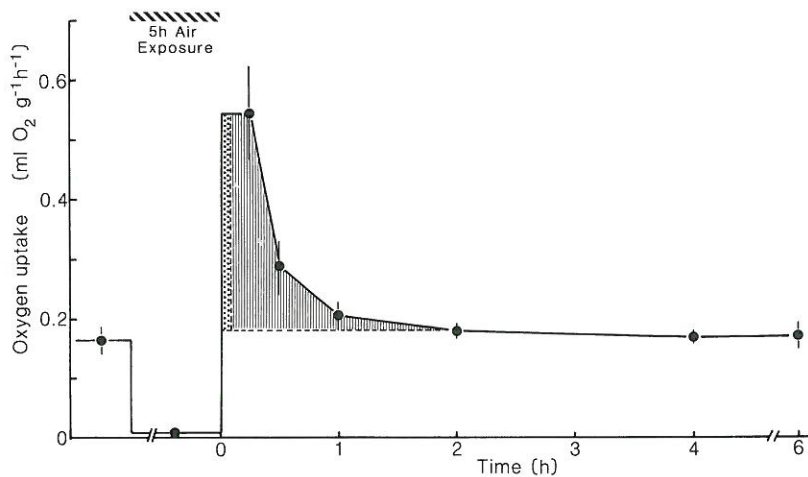


Fig. 3. *Mytilus edulis*. Rate of oxygen uptake by intertidally acclimatized mussels before, during and after 5 h of air exposure. Stippled area represents the $31 \mu\text{l}$ oxygen required to reoxygenate the fluids within the shell valves after air exposure, and vertical shading represents "oxygen-debt payment". Data points are means \pm SE

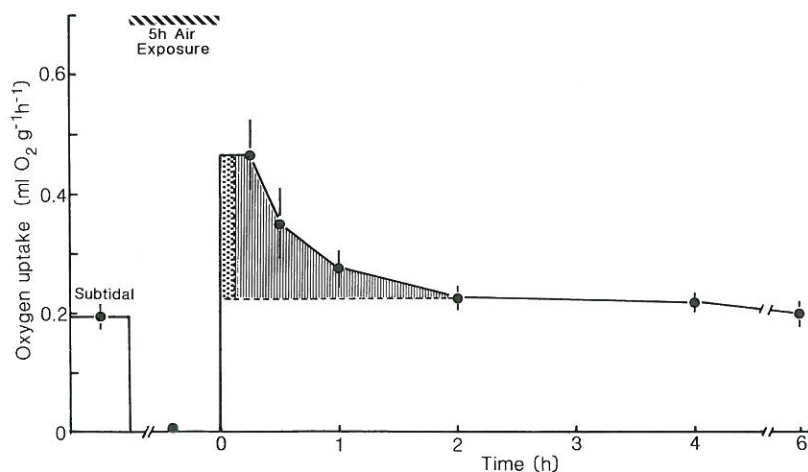


Fig. 4. *Mytilus edulis*. Rate of oxygen uptake by subtidally acclimatized mussels before, during and after 5 h of acute air exposure. Stippled area represents the $31 \mu\text{l}$ oxygen required to reoxygenate the fluids within the shell valves after air exposure, and vertical shading represents "oxygen-debt payment". Data points are means \pm SE

tioning of individuals and "anticipation" of a resumption of normal aerobic processes. The remaining intertidal and all subtidal mussels maintained a relatively constant \dot{V}_{O_2} during this period.

Effect of ration. The 4 d non-feeding period has no significant effect on the \dot{V}_{O_2} of intertidal mussels, but the heat

equivalent of aerial \dot{V}_{O_2} was reduced to zero (Fig. 1). The resumption of feeding, at a higher ration level, resulted in a slightly higher \dot{V}_{O_2} and a measurable \dot{V}_{O_2} (Fig. 1), but neither were significantly different from the original \dot{V}_{O_2} and \dot{V}_{O_2} measurements (intertidal). Levels of aerial \dot{V}_{O_2} in intertidal mussels, therefore, had little or no significant effect on \dot{V}_{O_2} .

Aquatic rates of oxygen consumption and oxygen-debt payment

Intertidal vs subtidal. Immediately following reimmersion, there was an elevated rate of oxygen uptake, approximately two to three times higher than the steady rate prior to air-exposure in both intertidal and subtidal mussels (Figs. 3 and 4). This initial high rate of oxygen uptake was followed by a rapid decline, and a constant rate was maintained after ~ 2 h. The "oxygen-debt payment" by both intertidal (acclimated) and subtidal (acute) individuals, 0.20 and 0.18 ml oxygen respectively, was completed within 2 h of reimmersion. The term "oxygen-debt payment" refers to the enhanced oxygen uptake or overshoot during an anaerobic-aerobic transition following a period of hypoxia or anoxia induced by environmental conditions or strenuous exercise when an "oxygen deficit" or "oxygen debt" is contracted (di Prampero, 1981).

An important component of the observed enhanced oxygen uptake was the reoxygenation of the total fluids within the shell valves of air-exposed mussels. The total internal fluid (volume = 12.5 ml for an individual of 1 g dry mass) can be divided into two basic compartments, the mantle cavity water (4.0 ml) and the intra- and extracellular body fluid which is obtained by difference. Assuming that the pO_2 of the intracellular fluid was in equilibrium with the blood, which increased from 50 to 100 mm Hg (blood pO_2 data from de Zwaan *et al.*, 1983), then $15.6 \mu\text{l O}_2$ would be required to reoxygenate this compartment. The mantle cavity water, however, was assumed to be in equilibrium with the blood during aerial exposure (50 mm Hg), and upon reimmersion was replaced by water with a pO_2 of 155 mm Hg, thus accounting for $15.4 \mu\text{l O}_2$. Therefore, the total oxygen required to reoxygenate the fluids within the shell valves was $31 \mu\text{l O}_2$. This component represented 15 to 19% of the total "oxygen-debt payment" and is illustrated by the stippled area in Figs. 3 and 4.

The steady-state aquatic $\dot{v}O_2$ by subtidal mussels before and after acute air exposure was $\sim 14\%$ higher than the intertidal group, but similar to the intertidal "high-ration" group.

Effect of ration. The short-term effects of starvation (4 d) and feeding (5 d) on the $\dot{v}O_2$ and the size of the oxygen-debt payment by intertidal mussels were studied. The results (Table 1) indicate that there was a significant (paired-sample *t* test) effect of ration on the maximum rates of oxygen uptake immediately after reimmersion ($P < 0.01$), on the size of the oxygen-debt payment ($P < 0.05$) and on the steady state rate of $\dot{v}O_2$ between 2 and 6 h of reimmersion ($P < 0.01$).

The oxygen consumption by continuously fed subtidal mussels was $0.193 \pm 0.017 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ and was not significantly different from the $\dot{v}O_2$ in the intertidal "high-ration" group (0.20 ± 0.02) and the intermittently fed subtidal mussels (0.192 ± 0.007 and $0.198 \pm 0.010 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ during the non-feeding and feeding period, respectively).

Table 1. *Mytilus edulis*. Effect of ration on size of oxygen-debt payment and steady-state aquatic rate of oxygen uptake by intertidal mussels. Starved: unfed for 4 d; High-ration: fed $0.21 \text{ mg algae l}^{-1}$ for 5 d. Values are means \pm SE, $n = 10$

	Starved	High-ration	% increase
Maximum rate of oxygen uptake ($\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$)	0.40 ± 0.07	$0.46 \pm 0.05^{**}$	15%
Size of oxygen debt minus oxygen-resaturation component (ml O_2)	0.12	0.14*	16%
Steady-state aquatic rate of oxygen uptake ($\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$)	0.17 ± 0.02	$0.20 \pm 0.02^{**}$	17%

Paired sample *t*-test, * $P < 0.05$; ** $P < 0.01$

Ammonia excretion

Intertidal vs subtidal. There was a marked difference between the rates of ammonia excretion by intertidal and subtidal mussels following reimmersion. Subtidal individuals immediately began excreting ammonia at a rate similar to VNH_4 ($\mu\text{g NH}_4 \text{ g}^{-1} \text{ h}^{-1}$) before acute air exposure. The rates of ammonia excretion reached a maximum after 2 h and at no time was the rate significantly different from the pre-exposed VNH_4 (Fig. 5).

Intertidal mussels had a significantly lower (50%) rate of ammonia excretion than the subtidal group. VNH_4 in both groups gradually increased to maximum rates 2 to 6 h after reimmersion. Both intertidal and subtidal individuals showed a very small pulse of ammonia release within the initial 15 min after reimmersion, but there was no evidence of a significant flushing-out of ammonia from the body immediately after air exposure or during the following 7 h. The total ammonia excreted by intertidal and subtidally acclimatized *Mytilus edulis* during a period of 5 h in air and 7 h of reimmersion was 14 and 65% (respectively) of the expected total ammonia production during an equivalent period of time (12 h) in water.

Effect of ration. There was no significant difference between the rate of ammonia excretion by intermittently fed ($8.2 \mu\text{g NH}_4 \text{ g}^{-1} \text{ h}^{-1} \pm 2.0 \text{ SE}$) and continuously fed ($6 \mu\text{g NH}_4 \text{ g}^{-1} \text{ h}^{-1} \pm 1.35 \text{ SE}$) subtidal mussels.

The rates of ammonia excretion by "starved" and "high-ration" groups of intertidal mussels show that ration interacts with aerial exposure. Thus, the reduced rate of ammonia excretion as a result of adaptation to intertidal conditions, including reduced ration intake, is raised by increasing the ration level (average rates of ammonia excretion during immersion: "starved" group, $3.11 \pm 0.37 \text{ SE}$; "high-ration" group, $4.6 \pm 0.46 \mu\text{g NH}_4 \text{ g}^{-1} \text{ h}^{-1}$).

Feeding and digestive efficiency

The clearance rates of mussels acclimatized to intertidal and subtidal conditions are presented in Table 2. Following

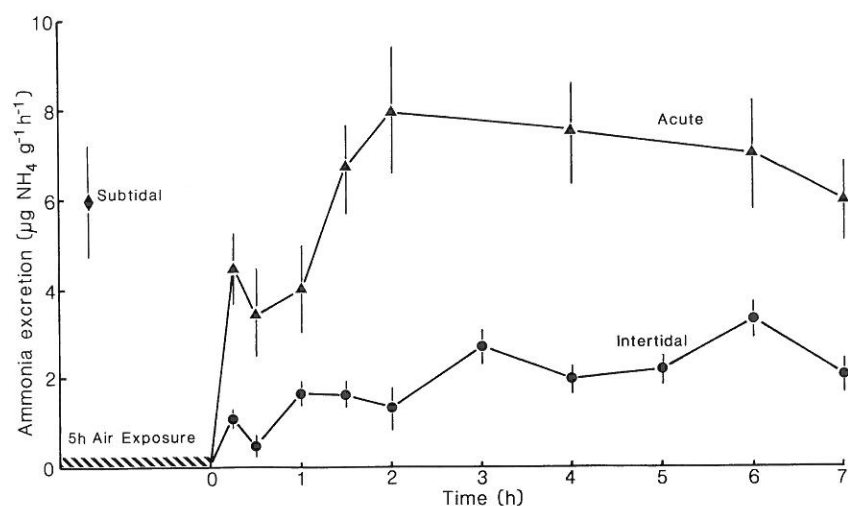


Fig. 5. *Mytilus edulis*. Rate of ammonia excretion by subtidally acclimatized mussels before (diamond) and after (triangles) 5 h acute air exposure. Rate of ammonia excretion by intertidal individuals during the 7 h period of reimmersion is also shown. Data points are means \pm SE

Table 2. *Mytilus edulis*. Clearance rates (litres $g^{-1} h^{-1}$) and food absorption efficiencies of mussels in response to aerial exposure (intertidal-fed; subtidal-acute air exposure; intertidal-high ration, after 4 d starvation and 5 d feeding at high ration) and to intermittent/continuous feeding regimes (subtidal groups). Values are means (\pm SE), $n = 10$

Condition	Time (h) after reimmersion/feeding							Absorption efficiency (%)							
	0.5	1.5	2.5	3.5	4.5	5.5	6.5								
Intertidal (fed)	3.47 (0.16)	4.17 (0.15)	4.26 (0.18)	4.27 (0.18)	4.53 (0.17)	4.58 (0.11)	4.78 (0.13)	64 (4)							
Subtidal (acute air exposure)	3.93 (0.50)	4.50 (0.25)	4.76 (0.26)	4.67 (0.22)	4.74 (0.27)	3.73 (0.50)	4.61 (0.25)	60 (3)							
Intertidal (high ration)	4.71 (0.19)	4.60 (0.31)	4.69 (0.14)	4.75 (0.21)	5.11 (0.16)	5.01 (0.18)	5.59 (0.12)	55 (4)							
Subtidal (intermittent feeding -7 h)	4.65 (0.39)	4.50 (0.46)	4.74 (0.10)	4.62 (0.15)	4.73 (0.16)	4.58 (0.19)	4.61 (0.17)	65 (3)							
Time (h)															
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	<i>t</i>	<i>t</i> +2	<i>t</i> +4	<i>t</i> +6	<i>t</i> +8										
Subtidal (continuous feeding)		4.16 (0.36)	4.23 (0.31)	4.10 (0.32)	4.43 (0.18)	4.34 (0.18)		66 (2)							

air exposure, there was a rapid opening of the valves and a resumption of ventilation within 30 min in both groups. The clearance rates of both intertidal and subtidal mussels were > 3 litres $g^{-1} h^{-1}$ within 30 min of reimmersion, but there was a further significant increase ($P < 0.01$) between 0.5 and 1.5 h. After 1.5 h, the clearance rates were maintained at a steady rate. Intertidal mussels starved for 4 d and then fed for 5 d at a higher ration level ($0.21 \mu g l^{-1}$) did not show an increase in clearance rate between 0.5 and 1.5 h (Table 2).

Subtidal mussels subjected to an intermittent feeding regime had a constant clearance rate throughout the 7 h feeding period. Continuously fed, subtidal individuals also maintained a relatively constant clearance rate.

Intertidal and subtidal mussels fed continuously or intermittently at a food concentration of $0.14 mg l^{-1}$ had

similar food absorption efficiencies (60 to 66%; Table 2). Intertidal mussels fed at a higher ration ($0.21 mg l^{-1}$), after a short period of starvation, had a slightly lower absorption efficiency (55%; Table 2). Reduction in the efficiency of food absorption is a typical response to increasing ration (Bayne *et al.*, 1976 b).

Energy budget and scope for growth

Scope for growth provides a measure of the performance of individual mussels exposed to different experimental conditions. The individual physiological responses were converted into energy equivalents and used in the balanced energy equation (Winberg, 1960) to calculate the scope for growth (Warren and Davis, 1967). The balanced

energy equation is as follows:

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

where C = total consumption of food energy; P = production of both somatic tissue and gametes, R = respiratory energy expenditure, U = energy lost as excreta, and F = faecal energy loss. The absorbed ration, A , is the product of consumption, C , and the efficiency of absorption of energy from the food. Production may then be expressed as:

$$P = A - (R + U).$$

When production, P , is estimated from the difference between the energy absorbed from the food and the energy expenditure via respiration and excretion, it may be referred to as the "scope for growth".

The calculation of C , A , R and U is as follows: C ($\text{J g}^{-1} \text{h}^{-1}$) = clearance rate ($\text{litres g}^{-1} \text{h}^{-1}$) \times algal food concentration ($\text{mg ash-free dry mass l}^{-1}$) \times energy content of *Phaeodactylum tricornutum* (23.5 J mg^{-1} ash-free dry mass); A ($\text{J g}^{-1} \text{h}^{-1}$) = $C \times$ absorption efficiency; R ($\text{J g}^{-1} \text{h}^{-1}$) = $\dot{V}\text{O}_2$ ($\text{ml O}_2 \text{ g}^{-1} \text{h}^{-1}$) $\times 20.33 \text{ J}$; U ($\text{J g}^{-1} \text{h}^{-1}$) = VNH_4 ($\text{mg NH}_4\text{-N g}^{-1} \text{h}^{-1}$) $\times 24.8 \text{ J}$.

The effects of aerial exposure and different feeding regimes on the main components of the energy budget and the scope for growth of intertidal and subtidal *Mytilus edulis* are presented in Table 3 and illustrated in Figs. 6, 7 and 8. The rate of energy expenditure during aerial exposure was 14 and 20% of the routine aquatic rate of energy expenditure by intertidal and subtidal mussels, respectively. Additional energy loss via ammonia excretion was between 1 and 4% of the respiratory energy loss.

The effect of aerial exposure on the scope for growth of intertidal mussels is illustrated in Fig. 6 (shaded area). There was a rapid recovery of feeding and digestion after reimmersion, a concomitant decline in respiration during the first 2 h of reimmersion, and the maintenance of relatively steady rates of feeding and respiration for the remaining period of immersion. Table 3 shows the energy loss during 5 h emersion (R), energy expenditure during

7 h immersion (R'), the energy loss via excretion (U), the energy gain during immersion (A), the scope for growth ($A - [R + R' + U]$) per 12 h cycle, and the net growth efficiency (K_2).

Fig. 7 illustrates the changes in major components of the energy budget and the resultant scope for growth during and after subjecting subtidal individuals to 5 h of acute air exposure. The responses were similar to intertidally acclimated mussels, but the respiratory energy expenditure was higher during air exposure and recovery, thus resulting in a lower scope for growth (Table 3). Intertidal mussels fed at a higher ration level showed a similar pattern of response, but the energy absorbed from this food (A) and the scope for growth ($A - [R + R' + U]$) was higher and proportional to the 50% increase in food available.

The effects of an intermittent feeding regime on the components of scope for growth are illustrated in Fig. 8 and summarised in Table 3. Mussels had a lower scope for growth and net growth efficiency than the intertidal individuals due to the higher respiratory energy expenditure during the immersed non-feeding period. This was only partially compensated by (a) the immediate resumption of feeding when food was available, unlike the gradual increase in clearance rate of mussels after air exposure; and (b) the lack of a significant enhancement of $\dot{V}\text{O}_2$, compared to the large oxygen debt payment/overshoot shown by mussels during the initial 2 h of recovery from air exposure.

Cardium edule L.

Aerial rates of heat dissipation and oxygen uptake

Intertidal vs subtidal. The aerial rate of heat dissipation (\dot{q}) by *Cardium edule* acclimatized to intertidal conditions was significantly lower ($P < 0.05$) than the aerial \dot{q} of subtidal individuals subjected to acute air exposure (Fig. 9). Under all experimental conditions, there was close agreement

Table 3. *Mytilus edulis*. Components of energy budget (units = joules) of 1 g mussels integrated over period of 12 h

Condition	Energy expenditure during 5 h period of non-feeding or aerial exposure (R)	Energy expenditure during feeding period of 7 h (R')	Energy loss via excretion over 12 h (U)	Energy gain during feeding period of 7 h (A)	Scope for growth during 12 h period ($A - [R + R' + U]$)	K_2 (growth efficiency) $\frac{(A - [R + R' + U])}{A}$
Intertidal (fed)	- 2.5	-28.8	-0.3	+ 64	+ 32.4	0.50
Intertidal (high-ration)	- 2.9	-33.0	-0.8	+ 94	+ 57.3	0.61
Subtidal						
Acute air exposure (fed)	- 4.2	-35.2	-1.0	+ 63	+ 22.6	0.36
Intermittently fed	-19.5	-28.5	-2.0	+ 68	+ 18	0.26
Continuously fed	-	-47.0 ^a	-1.7	+ 110 ^a	+ 61.3	0.55

^a 12 h period

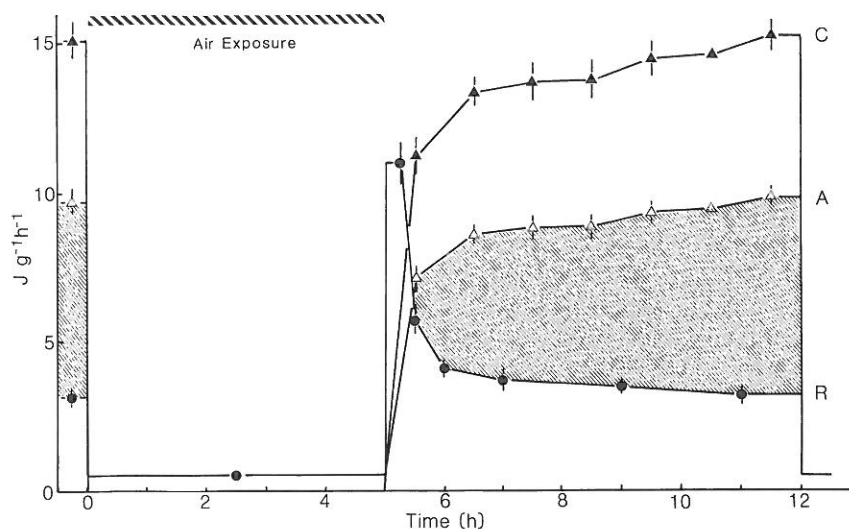


Fig. 6. *Mytilus edulis*. Effect of 5 h air exposure and 7 h reimmersion on components of energy budget of intertidally acclimatized mussels. C: energy consumed; A: energy absorbed; R: energy respired; shaded area represents scope for growth. Data points are means \pm SE

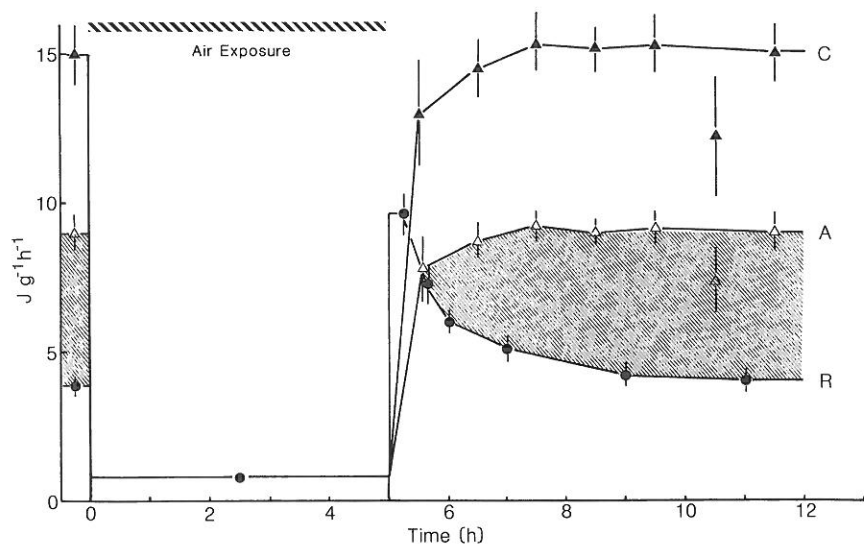


Fig. 7. *Mytilus edulis*. Effect of 5 h acute air exposure and 7 h reimmersion on components of energy budget of subtidally acclimatized mussels. C: energy consumed; A: energy absorbed; R: energy respired; shaded area represents scope for growth. Data points are means \pm SE

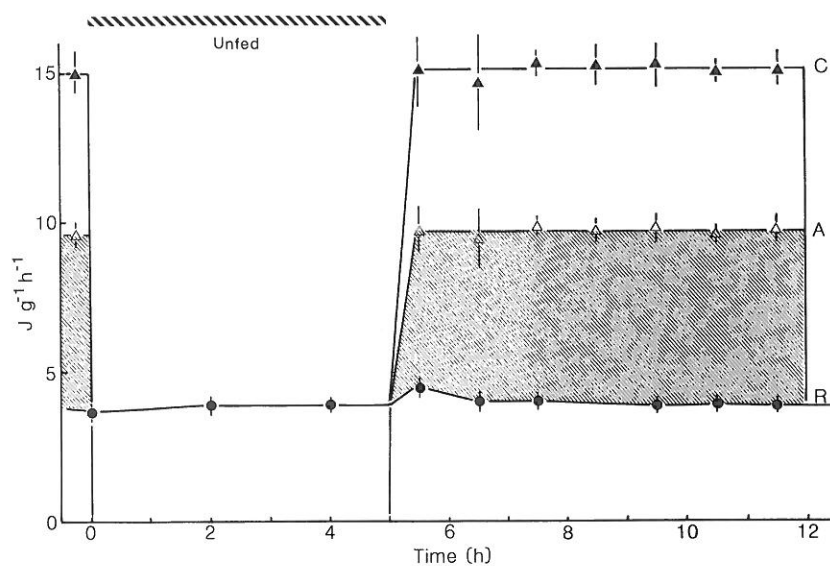


Fig. 8. *Mytilus edulis*. Effect of intermittent feeding regime (5 h unfed every 12 h) on components of energy budget of subtidal mussels. C: energy consumed; A: energy absorbed; R: energy respired; shaded area represents scope for growth. Data points are means \pm SE

between direct (\dot{q}) and indirect (\dot{v}_{O_2}) measurements of aerial rates of metabolic energy expenditure (Fig. 9). The thermogram in Fig. 10, although an atypical response by a subtidal individual, illustrates the effect of a sudden behavioural change (abrupt valve movement and squirting of mantle fluid followed by wide gaping) on the rate of heat dissipation. After 3.75 h of air exposure, there was a sevenfold increase in \dot{q} , probably indicating a switch to aerobic respiration due to air-breathing.

Effect of ration. There was no significant difference between the aerial \dot{q} and \dot{v}_{O_2} of intertidal *Cardium edule* before and after 4 d of non-feeding. There was also no significant difference between the aerial \dot{q} and \dot{v}_{O_2} of

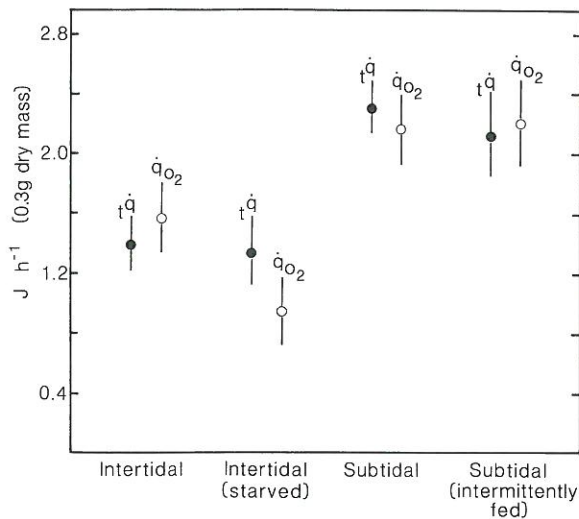


Fig. 9. *Cardium edule*. Mass-specific rate of heat dissipation (\dot{q}) and caloric equivalent of oxygen uptake (\dot{q}_{O_2}) by intertidally and subtidally (acute) acclimatized cockles (standard dry body mass, 0.3 g) during 5 h of exposure to air. Data points are means \pm SE

subtidal *C. edule* from “intermittently fed” and “continuously fed” experimental groups. The greater \dot{q} and \dot{v}_{O_2} by subtidal “intermittently fed” cockles than by intertidal cockles, which received the same ration, was therefore probably due to increased activity of the subtidal individuals during their acute exposure.

Aquatic rates of oxygen consumption and oxygen-debt payment

Intertidal vs subtidal. Upon reimmersion there was a release of air from the mantle cavity, followed by an elevated aquatic rate of oxygen uptake, ~ 1.5 to 1.7 times higher than the steady rate prior to air exposure (Fig. 11). Subtidal individuals subjected to an acute 5 h period of air exposure not only had a higher aerial \dot{q} and \dot{v}_{O_2} compared with intertidal specimens, but also had a higher aquatic \dot{v}_{O_2} upon reimmersion.

“Payment of the oxygen-debt” by intertidal and subtidal individuals was complete within 2 h of reimmersion and the amount of excess oxygen was 0.08 ml oxygen for both conditions.

Effect of ration. The \dot{v}_{O_2} measurements of intertidal starved and fed cockles were performed on different individuals, and therefore the results could not be analysed by the method of paired comparisons. Short-term (4 d) starvation had no statistically significant effect on the steady-state aquatic \dot{v}_{O_2} of intertidal *Cardium edule*, but both the maximum \dot{v}_{O_2} immediately after reimmersion and the size of the “oxygen-debt payment” (0.195 ml O_2 h⁻¹ and 0.064 ml O_2 , respectively) were lower (Fig. 11) than the fed intertidal group (0.235 ml O_2 h⁻¹ and 0.079 ml O_2 , respectively). The \dot{v}_{O_2} of continuously fed subtidal individuals was not significantly different from the steady-state aquatic \dot{v}_{O_2} of the intertidal group.

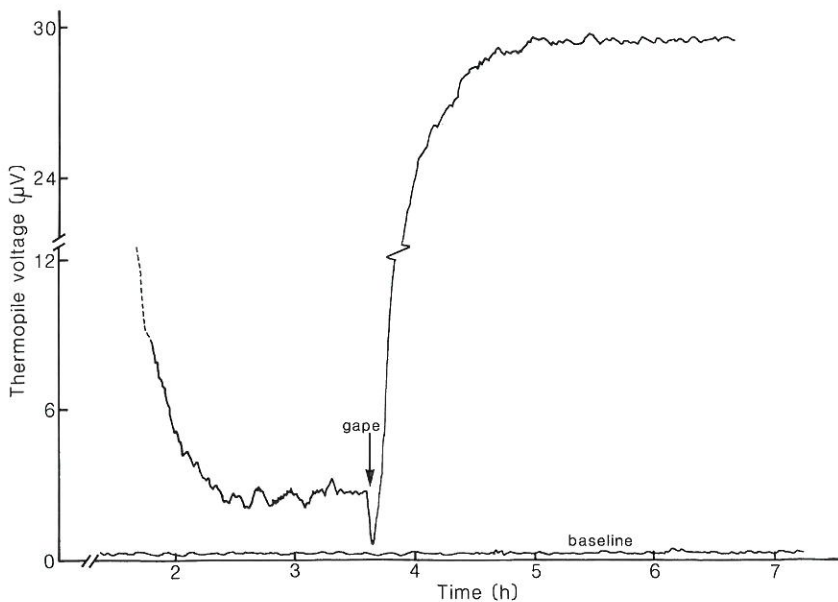


Fig. 10. *Cardium edule*. Thermogram of subtidal individual during acute air exposure. Arrow denotes abrupt valve movement and squirting of mantle fluid followed by wide gaping and ~ 10 fold increase in heat dissipation ($1 \mu V = 25.4 \mu W = 25.4 \mu J s^{-1}$)

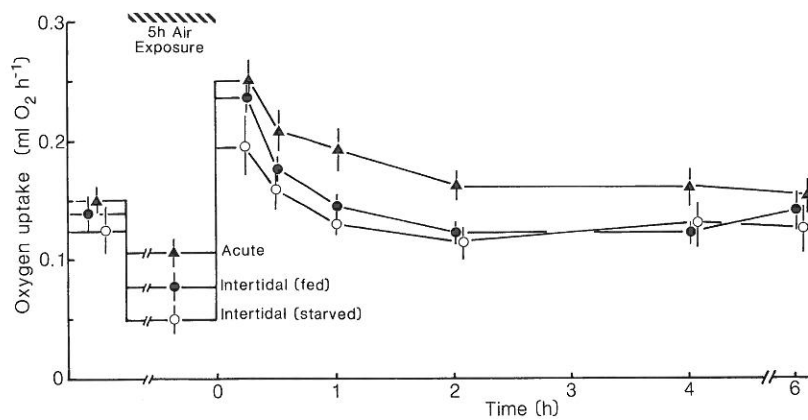


Fig. 11. *Cardium edule*. Rate of oxygen uptake by fed and starved intertidally and subtidally (acute) acclimatized cockles (standard dry tissue mass of 0.3 g) before, during and after 5 h of air exposure. Data points are means \pm SE

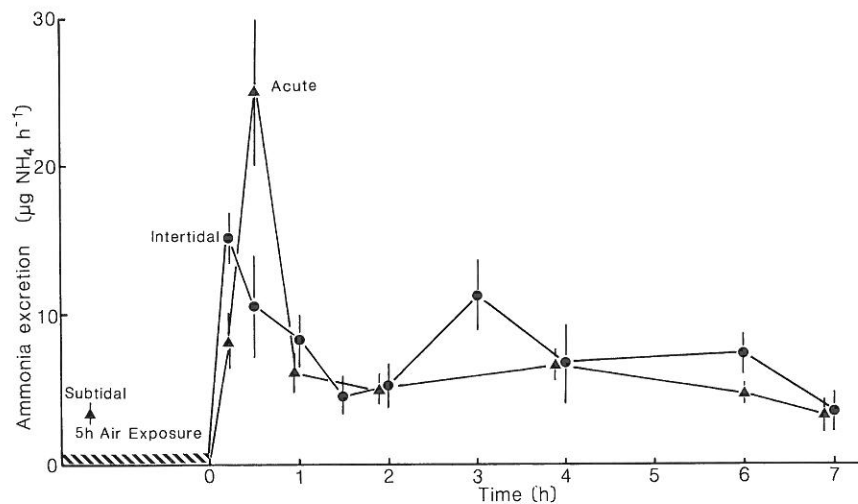


Fig. 12. *Cardium edule*. Rate of ammonia excretion by intertidally (circles) and subtidally (triangles) acclimatized cockles (standard dry tissue mass of 0.3 g) before and after 5 h air exposure. Data points are means \pm SE

Ammonia excretion

Intertidal vs subtidal. Following 5 h of air exposure there was an elevated rate of ammonia excretion by both intertidal and subtidal groups of *Cardium edule* (Fig. 12). Intertidally acclimatized and fed specimens had a maximum rate of ammonia excretion/release ($15 \mu\text{g NH}_4 \text{ h}^{-1}$) within the first 15 min of reimmersion, but the subtidally acclimatized groups did not reach a maximum rate until 30 min after reimmersion. This difference reflected the more rapid opening and recovery of clearance rate by intertidal cockles. The elevated rates of ammonia excretion (> 4 -fold) were reduced to rates similar to pre-emersion rates of excretion within 1.5 to 2 h of reimmersion. The total ammonia released by intertidally and subtidally acclimatized *C. edule* during a period of 5 h of air exposure and 7 h of reimmersion was between 20% (intertidal) and 80% (acute subtidal) higher than the expected total ammonia excretion during the equivalent period of time (12 h) in water.

Effect of ration. There was no statistically significant effect of ration (starved or intermittently/continuously fed) on the rate of ammonia excretion by *Cardium edule*. The intertidal starved group and the subtidal intermittently fed specimens that were acutely exposed had maximum am-

monia excretion rates (mean \pm SE after 30 min reimmersion) of 17.0 ± 2.5 and $14.8 \pm 2.5 \mu\text{g NH}_4 \text{ h}^{-1}$, respectively, and steady-state excretion rates (after 7 h) of 2.8 ± 0.8 and $3.2 \pm 1 \mu\text{g NH}_4 \text{ h}^{-1}$, respectively.

Feeding and digestive efficiency

The clearance rates of intertidally and subtidally acclimatized *Cardium edule* following 5 h of air exposure are summarized in Table 4. Opening of the shell valves was followed by a gradual increase in clearance rate during the first 1.5 h of immersion. The increase in clearance rate between 0.5 and 1.5 h was statistically significant (paired-sample *t* test) for both the intertidal ($P < 0.05$) and the subtidal acute air-exposed ($P < 0.01$) groups. After 1.5 h of reimmersion the clearance rate by intertidal specimens had reached a steady state of 2.5 litres h^{-1} , whereas the subtidal acute air-exposed individuals increased their clearance rate slowly over a period of 5.5 h.

Intermittently fed subtidal *Cardium edule* showed a similar gradual increase in clearance rate during the first 1.5 h followed by a steady rate for the remaining feeding period (Table 4). In contrast, the subtidal, continuously fed individuals did not maintain a constant feeding rate, but showed some evidence of behavioural and feeding rhythms

which resulted in a lower and variable clearance rate for this experimental group. The overall average clearance rate for a 12 h period was 1.77 litres h⁻¹.

Individual rates of faecal production by *Cardium edule* were relatively low, therefore faeces from each experimental condition were collected as three pooled samples. There were no marked differences in absorption efficiencies and the overall average was 0.64 ± 0.04 SE.

Energy budget and scope for growth

The effects of aerial exposure and intermittent feeding on the components of the energy budget and the scope for growth of intertidal and subtidal *Cardium edule* are presented in Table 5 and illustrated in Fig. 13. Specimens acclimated to a tidal aerial exposure regime (Fig. 13a) had

a significantly lower rate of energy expenditure during air exposure, approximately 50% of the aquatic routine rate of energy expenditure. The rate of energy expenditure by subtidal individuals during acute air exposure was higher, and approximately 75% of the aquatic routine rate. Additional energy loss via ammonia excretion was ~ 6% of the energy loss associated with respiration by both intertidal and subtidal groups.

The intertidal *Cardium edule* established a positive scope for growth within 30 min of reimmersion due to the more rapid increase in feeding rate (Fig. 13a). The overall energy balance during the 12 h tidal cycle was positive (scope for growth, Table 5) as a consequence of the lower rate of energy expenditure, both during (*R*) and after (*R'*) the period of air exposure, and the higher rate of energy gain (*A*) due to the higher clearance rate within an hour of reimmersion.

Table 4. *Cardium edule*. Clearance rates (litres h⁻¹) of cockles (0.3 g dry mass) in response to aerial exposure (intertidal-fed and subtidal-acute air exposure) and to intermittent/continuous feeding regimes (subtidal groups). Average food absorption efficiency was $64\% \pm$ SE. Values are means (\pm SE), $n = 10$

Condition	Time (h) after reimmersion/feeding						
	0.5	1.5	2.5	3.5	4.5	5.5	6.5
Intertidal (fed)	1.87* (0.21)	2.50 (0.22)	2.49 (0.16)	2.39 (0.16)	2.41 (0.17)	2.46 (0.18)	2.49 (0.24)
Subtidal							
Acute air exposure	0.68** (0.17)	1.91 (0.24)	2.08 (0.17)	2.24 (0.19)	2.30 (0.21)	2.52 (0.30)	2.51 (0.20)
Intermittent feeding – 7 h	1.04 (0.45)	2.43 (0.30)	2.58 (0.30)	2.43 (0.28)	2.55 (0.29)	2.42 (0.23)	2.47 (0.26)
	Time (h)						
Subtidal	<i>t</i>	<i>t</i> + 2	<i>t</i> + 4	<i>t</i> + 6	<i>t</i> + 8	<i>t</i> + 10	
Continuous feeding	1.45 (0.34)	2.14 (0.43)	2.24 (0.29)	2.11 (0.24)	1.44 (0.27)	1.22 (0.27)	
% of individuals < 0.5 litres h ⁻¹	30%	20%	10%	0	20%	20%	

Paired sample *t*-test, * $P < 0.05$; ** $P < 0.001$ (compared with clearance rate after 1.5 h)

Table 5. *Cardium edule*. Components of energy budget (units = joules) of 0.3 g cockles integrated over period of 12 h

Condition	Energy expenditure during non-feeding or aerial exposure of 5 h (<i>R</i>)	Energy expenditure during feeding period of 7 h (<i>R'</i>)	Energy loss via excretion over 12 h (<i>U</i>)	Energy gain during feeding period of 7 h (<i>A</i>)	Scope for growth during 12 h period ($A - [R + R' + U]$)	K_2 (growth efficiency) $\frac{(A - [R + R' + U])}{A}$
Intertidal (fed)	- 7.0	-19.7	-1.2	+ 34.3	+ 6.4	0.19
Subtidal						
Acute air exposure (fed)	-11.5	-23.8	-1.1	+ 29.5	- 6.9	-
Intermittently fed	-10.1	-27.9	-1.0	+ 35.1	- 3.9	-
Continuously fed	-	-37.3 ^a	-0.6	+ 70.1 ^a	+ 32.2	0.46

^a 12 h period

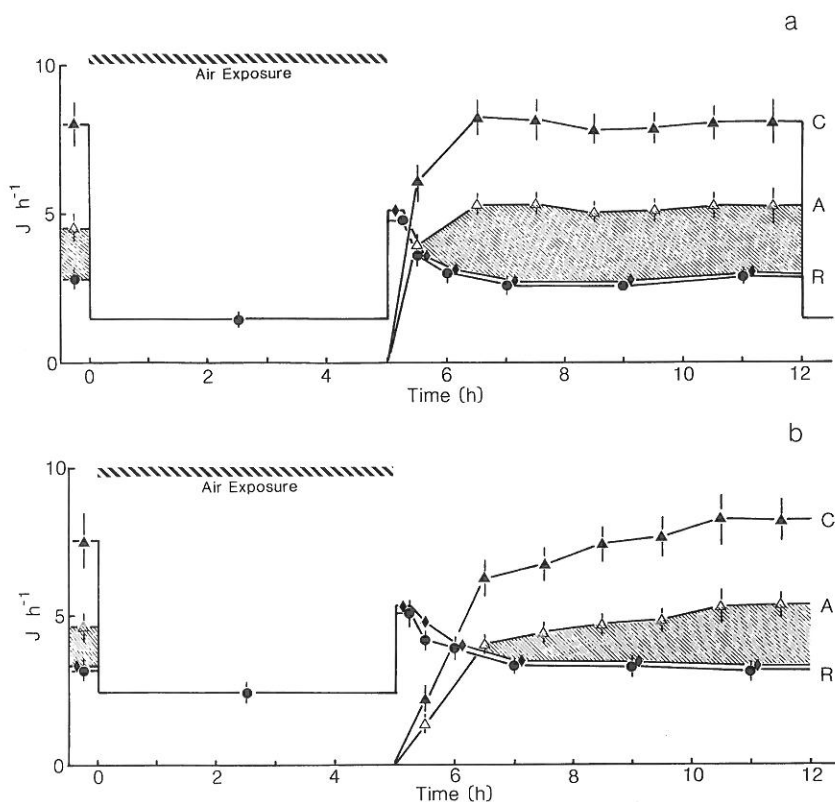


Fig. 13. *Cardium edule*. Effect of (a) 5 h air exposure and 7 h reimmersion of intertidal cockles, and (b) 5 h acute air exposure of subtidal cockles (standard dry tissue mass of 0.3 g) on components of energy budget. C: energy consumed; A: energy absorbed; R: energy respired (circles) and excreted (diamonds); shaded area represents scope for growth. Data points are means \pm SE

In contrast, subtidal *Cardium edule* subjected to acute air exposure did not achieve a positive scope for growth until after 1.5 h of reimmersion, due to the slow increase in feeding rate (Fig. 13b). The overall energy balance (scope for growth) during the 12 h period was slightly negative (Table 5) as a result of the relatively high rate of energy expenditure both during (R) and immediately after (R') air exposure and the reduced rate of energy intake (A).

Continuously fed subtidal *Cardium edule*, although more erratic in their behaviour and feeding activity than any other experimental group, maintained a positive scope for growth and a relatively high growth efficiency (Table 5). The intermittently fed *C. edule*, however, had 40% less food available per 12 h and had similar rates of feeding and energy expenditure as the subtidal acute air-exposed individuals, and consequently a similar negative scope for growth.

Discussion

In order to interpret the responses of suspension-feeding bivalves to intertidal conditions it is necessary to differentiate between the effects of air exposure *per se* and the effects of an intermittent feeding pattern imposed by the tidal regime. In this study, the possible effects induced by differences in available ration can be eliminated by comparing air-exposed individuals (intertidal and acute-subtidal) with subtidal, intermittently-fed individuals, and intertidal "high ration" mussels with subtidal continuously-

fed mussels. Our results demonstrate that there are considerable intergeneric differences in the physiological responses of bivalve molluscs to air exposure, and also that *Mytilus edulis* and *Cardium edule* show some adaptation of metabolic and excretory processes to a 12 h cyclic aerial-exposure regime; this adaptation is in response to air exposure rather than to changes in available ration.

Aerial rates of energy expenditure (\dot{q}) by *Mytilus edulis* were low and ~ 14 to 20% of the aquatic rate of energy expenditure, based on \dot{v}_{O_2} measurements. Recently, Shick and Widdows (1981) have shown that there is good agreement between the routine aquatic rates of heat dissipation and oxygen consumption by *M. edulis*. Furthermore, Pamatmat (1980) reported that the anaerobic rate of heat dissipation by *M. edulis* was approximately 7 to 18% of the aerobic aquatic \dot{q} over a period of 24 h of anoxia, and Griffiths (1981) found that aerial \dot{v}_{O_2} of *Choromytilus meridionalis* was $\sim 11\%$ of the aquatic \dot{v}_{O_2} . In contrast, the aerial rate of energy expenditure (\dot{q}) by *Cardium edule* was relatively high, ~ 50 to 75% of the mass-specific aquatic heat dissipation based on oxygen uptake (\dot{q}_{O_2}). These aerial rates of energy expenditure by *M. edulis* and *C. edule* relative to the aquatic rates were similar to those reported previously by Widdows *et al.* (1979).

The results, based on direct and indirect calorimetry (respirometry), suggest that aerial rates of oxygen uptake by bivalves should be interpreted with caution, because there are occasions when the recorded rate of oxygen uptake may not reflect the measured rate of total heat

dissipation, and the oxycaloric coefficients for aerobic catabolism (-19.6 to -21.4 J ml $^{-1}$ O $_2$) are therefore inappropriate. Oxygen uptake will provide an underestimate of total energy expenditure when an animal is largely anaerobic, and an overestimate when there is oxygen uptake which is not involved in aerobic catabolism. In the present study, a significant component of the aerial rate of oxygen uptake was found to be associated with the shells, and this was only slightly affected by metabolic inhibitors and could not be accounted for in terms of heat dissipation (i.e., the caloric equivalent of \dot{v}_{O_2} was greater than the measured \dot{q}). A possible explanation for this phenomenon of "excess oxygen uptake" may be the oxygen-consuming process of sclerotization and tanning of the periostracum on the edge of the shell (Gordon and Carriker, 1980; Waite and Andersen, 1980; Waite, 1983). The relatively small "shell component" was therefore subtracted from all the aerial rates of oxygen uptake by *Mytilus edulis* and *Cardium edule*.

The aerial \dot{q} by *Mytilus edulis* was greater than that predicted from the aerial \dot{v}_{O_2} (Fig. 1), based on oxycaloric coefficients for aerobic catabolism (-19.6 to -21.4 J ml $^{-1}$ O $_2$), thus confirming the involvement of anaerobic processes during periods of air exposure and valve closure reported in both laboratory (Widdows *et al.*, 1979) and field (Brinkhoff *et al.*, 1983) studies. In *Cardium edule*, however, the aerial rates of \dot{q} were very similar to the calculated rates of catabolic heat dissipation (Fig. 9), based on the aerial \dot{v}_{O_2} and using the average oxycaloric coefficient. This implies that the metabolic processes were largely aerobic in this gaping bivalve, thus confirming previous laboratory studies (Widdows *et al.*, 1979). Although *C. edule* is known to be capable of extended periods of anaerobiosis under artificial anoxic conditions (Gäde, 1975; Meinardus and Gäde, 1981), there is no biochemical evidence of anaerobiosis in specimens of *C. edule* collected from the intertidal zone (Brinkhoff *et al.*, 1983).

The behavioural and metabolic differences between intertidal and subtidal *Mytilus edulis* show that there is some adaptation and conditioning to an intertidal regime. Thermograms for *M. edulis* (Fig. 2) demonstrate the effect of behavioural conditioned response on \dot{q} at the time of reimmersion. When individuals were left in the calorimeter beyond the normal aerial exposure time, there was a marked increase in \dot{q} coinciding with the anticipated time of reimmersion in approximately one-third of the individuals tested. Pamatmat (1983) has shown similar changes in heat dissipation associated with behavioural responses and changes in aerial \dot{v}_{O_2} by *Geukensia demissa* (= *Modiolus demissus*). Although a correlation exists between valve gape and increased aerial rates of heat dissipation and oxygen uptake, it is unknown whether the behavioural responses determine metabolic activity or whether differences in energy demand during aerial exposure elicit different behavioural patterns.

The lack of oxygen uptake in air by starved intertidal *Mytilus edulis* and its maintenance in intertidal mussels

suggests that some aspect of digestion, absorption, or assimilation of food continues during exposure to air and is an oxygen-requiring process. The decline in aerial \dot{v}_{O_2} after starvation in *Cardium edule* supports this idea. The absence of aerial oxygen uptake in subtidal *M. edulis*, despite their receiving the same ration as intertidal (high-ration) mussels, was due to the behavioural response of the former, i.e., shell-valve closure during what was for them an unnatural period of emersion. Thus, acclimatization to intertidal conditions may include the enhancement of the ability to continue the aerobic processing of a ration ingested during the immersion period.

Following valve closure during air exposure, *Mytilus edulis* becomes largely anaerobic, significantly reducing the overall energy dissipation as measured in terms of \dot{q} . Periodic reliance on anaerobiosis as an energy-saving mechanism has been suggested earlier by de Zwaan (1977) and Pamatmat (1980). Anaerobiosis in bivalves yields a possible 4.7 to 6.4 moles ATP mol $^{-1}$ of glycogen in comparison with a total yield of 37 moles ATP mol $^{-1}$ of glycogen that is completely oxidized to CO $_2$ and H $_2$ O (Gnaiger, 1983b), and a Pasteur effect is typically absent (de Zwaan, 1977). However, the partially oxidized substrates accumulated during anaerobiosis are not necessarily lost to the individual, but are oxidised or participate in glyconeogenesis upon resumption of aerobic metabolism (Gnaiger *et al.*, in preparation).

In this study, we have estimated not only the "energy saving" during air exposure but the "cost of recovery" (the overshoot in \dot{v}_{O_2} known as the payment of oxygen-debt) by *Mytilus edulis* and *Cardium edule* following reimmersion (Tables 3 and 5). In comparison with the intermittently-fed subtidal individuals, the acute-air-exposed mussels saved 7 J or 14% of the total energy expenditure and the intertidal mussels saved 15.7 J or 33% of the total energy expenditure over the entire 12 h period. Acute-air-exposed specimens of *C. edule*, when compared with the intermittently-fed subtidal individuals, saved 2 J or 5% of the total energy expenditure, whereas the intertidal cockles saved 10 J or 28%. Differences in available ration did not significantly affect the total energy expenditure by intermittently fed and continuously fed subtidal individuals of *M. edulis* and *C. edule* over a period of 12 h (Tables 3 and 5). The higher aerial \dot{q} and \dot{v}_{O_2} by "intermittently fed" subtidal than by intertidal individuals, which received the same ration, was probably due to increased activity during acute emersion in the former, again indicating the energy saving afforded by quiescence in intertidally acclimatized animals.

During the recovery from air exposure there was a significant oxygen-debt payment by both species, in spite of *Cardium edule* respiring aerobically during aerial exposure. A significant oxygen-debt payment by *C. edule* had also been observed by Widdows *et al.* (1979), the size of which increased with temperature. In the present study, the size of the oxygen-debt payment increased with ration, therefore a component of the overshoot in oxygen uptake appears to be related to digestion and food absorption

processes (heat increment or specific dynamic action, SDA), in addition to the restoration of high energy intermediates, catabolic and anabolic metabolism and the oxygen resaturation of haemolymph, tissue water, mantle water and possible "oxygen stores" (Gnaiger *et al.*, in preparation; Shick *et al.*, in preparation).

The difference between the rate of oxygen uptake by starved and fed mussels provided an estimate of the energy associated with feeding and digestive processes, the "heat increment" or "SDA". It represented a constant proportion (15 to 17%; Table 1) of the total oxygen uptake during all stages of the recovery process. Under steady state conditions, it formed 4% of the ingested ration, which is in close agreement with previous estimates of "heat increment" for *Mytilus edulis* (Bayne and Scullard, 1977 b) and *M. californianus* (Bayne *et al.*, 1976 a). The estimated physiological cost of feeding, digestion and assimilation by *M. edulis* was therefore equivalent to $0.03 \text{ ml O}_2 \text{ h}^{-1}$ or $1.34 \mu\text{mol O}_2 \text{ h}^{-1}$ and similar to that calculated by Hawkins *et al.* (1985).

The size of the oxygen-debt payment was similar for intertidal and subtidal specimens in intraspecific comparisons of *Mytilus edulis* and *Cardium edule*. Consequently, there was no additional energy saving by intertidally acclimatized individuals through a lower "cost of recovery". De Vooy and de Zwaan (1978) recorded a difference in the size of the oxygen-debt payment by sublittoral and littoral *M. edulis*, and found that the size of the oxygen-debt payment increased with air exposure up to 8 h. However, they reported that the size of the oxygen-debt payment was significantly less than the theoretical amount of oxygen required for the complete oxidation of accumulated succinate and propionate. This is in contrast to the work of Gnaiger *et al.* (in preparation), who found that the size of the oxygen-debt payment was considerably larger than the theoretical estimate of the oxygen required for the catabolic-anabolic coupled oxidation and participation in glyconeogenesis of accumulated anaerobic end-products. This discrepancy may be explained by the technique adopted by de Vooy and de Zwaan (1978) for measuring \dot{v}_{O_2} . The rate of oxygen consumption by a rack of mussels (20 to 30 kg) was determined by measuring the pO_2 of seawater before and after flowing over the rack, but there was no allowance for diffusion of oxygen at the air-water interface above the mussels, and this is likely to result in a significant underestimate of the \dot{v}_{O_2} and the size of the oxygen-debt payment. Also, although de Vooy and de Zwaan (1978) suggested the possibility of glyconeogenesis during recovery, they did not calculate its contribution to a potentially seven times smaller oxygen-debt payment.

The conversion of the oxygen-debt payment to rates of catabolic heat dissipation during recovery, using the oxy-caloric equivalent of $20.3 \text{ J ml}^{-1} \text{ O}_2$, is probably a further example of an overestimation by indirect calorimetry, because Shick *et al.* (in preparation) have recently demonstrated that the rate of heat dissipation, measured by direct calorimetry, was less than predicted from the measure-

ment of \dot{v}_{O_2} . The discrepancy is largely due to a lower overall oxy-caloric equivalent during recovery, which includes not only the exothermic component of aerobic catabolism but also the conservative (endothermic) processes of glyconeogenesis and restoration of tissue ATP and phosphoarginine (Gnaiger *et al.*, in preparation).

The rate of ammonia excretion during the recovery period was a function of adaptation to an intertidal regime and the species' mode of respiration during air exposure. Intertidal and acute-air-exposed *Mytilus edulis* closed their valves, albeit incompletely, during air exposure and reduced their rates of ammonia excretion to 14 and 65% of the subtidal aquatic rate, respectively, over the 12 h period. Unlike the rate of oxygen uptake, there was no overshoot in ammonia excretion during recovery, rather a conservation of ammonia at a time of year (winter) when the rate of ammonia excretion was at a seasonal minimum (Bayne and Scullard, 1977 a; Widdows, 1978). De Zwaan *et al.* (1983) showed that during 5 h of acute air exposure (in winter), *M. edulis* accumulated $\sim 100 \mu\text{M NH}_4$ or a total of $9 \mu\text{g NH}_4$ in 5 ml of blood. Blood ammonium concentration declined rapidly following reimmersion, but based on the present data only $\sim 4 \mu\text{g NH}_4$ was excreted during the first hour, thus indicating conservation or ammonia fixation by *M. edulis*. However, experiments carried out in the summer (Widdows, unpublished data) have shown a different response with a distinct overshoot in ammonia excretion by *M. edulis* immediately after reimmersion. De Vooy and de Zwaan (1978) have reported a similar overshoot in ammonia excretion by *M. edulis* which was proportional to the exposure time.

The greater conservation of nitrogen by intertidal relative to subtidal mussels (Table 3, Fig. 5) may also be related to the balance of aerobic and anaerobic catabolism during emersion. By remaining partially aerobic during exposure, intertidal mussels could rely on glycogen and lipid catabolism and thus spare protein. Conversely, becoming totally anaerobic during emersion precluded lipid catabolism by subtidal mussels, which in the absence of sufficient readily available glycogen stores, may have necessitated their use of protein as an energy source and accounted in part for their higher rate of ammonia excretion (van Waarde, 1983).

In contrast, *Cardium edule* gaped during the period of air exposure, showed an overshoot in ammonia excretion following reimmersion, which when integrated over a 12 h period was equivalent or slightly higher than the rate of ammonia excretion by the subtidal individuals. Similarly, Jordan and Valiela (1982) demonstrated that *Geukensia demissa*, a "shell-gaper" during 8 to 17 h of aerial exposure per day, maintains a daily rate of ammonia excretion independent of exposure time.

The calculation of the energy available for growth, based on a physiological analysis of the energy budget, highlights not only some of the important differences between intertidally and subtidally acclimatized individuals, but also the differences between species, and provides a useful summary of the overall performance. Intertidal

mussels adapted to 5 h of air exposure every 12 h cycle by reducing their aerial and aquatic rates of energy expenditure and ammonia excretion, but only at a small extra cost in terms of the payment of an oxygen-debt during reimmersion. The energy acquired by suspension-feeding during immersion was sufficient to achieve a scope for growth and K_2 growth efficiency (Table 3) nearly two-fold higher than the subtidal mussels fed intermittently at an equivalent ration level. Comparison between the intertidal (high-ration) group and the subtidal continuously-fed group of mussels shows that, in spite of the slightly lower energy gain by the former, the scope for growth values were similar and the growth efficiency higher due to reduced energy expenditure by the intertidal group over the 12 h period. The subtidal group of mussels, when subjected to acute air exposure, had a lower scope for growth and growth efficiency than the intertidal mussels due to higher aerial and aquatic rates of energy expenditure. Therefore, *Mytilus edulis* has the capacity to adapt to intertidal conditions, maximising the energy available for growth, primarily by conserving energy during air exposure. There was no evidence of compensation for loss of feeding time by increasing feeding rate or absorption efficiency.

In contrast to *Mytilus edulis*, air exposure caused a very marked reduction in the scope for growth and growth efficiency of *Cardium edule* due to the maintenance of a relatively high rate of energy expenditure during air exposure and a slower recovery of feeding rate following reimmersion. However, there was evidence that acclimatisation to an intertidal regime reduced both aerial and aquatic rates of energy expenditure, thus increasing the energy available for growth. Two factors were responsible for the lower scope for growth values for *C. edule* compared with *M. edulis*; firstly, the smaller body mass (0.38 g dry mass) for adult cockles; and secondly, the more marked seasonal cycles in their physiology. In the winter, *C. edule* enters a relatively quiescent period in terms of reproductive activity, clearance rate, respiration and growth (Newell and Bayne, 1980), whereas *M. edulis* maintains a relatively high level of feeding and respiration (Widdows, 1978) and gametogenic activity (Lowe *et al.*, 1982).

The rather different behavioural and metabolic responses to emersion by *Mytilus edulis* (shell-valve closure and reliance primarily on anaerobic metabolism) and by *Cardium edule* (shell-valve gaping and maintenance of aerobic respiration) may be interpretable more in terms of habitat than energetics. Although both bivalves experience similar durations of intertidal exposure, the infaunal habitat of *C. edule* minimizes desiccation stress and so permits air-gaping and maintenance of aerobic respiration at nearly the aquatic rate. Although this results in a higher energy utilisation during the entire tidal cycle, this may be offset by the increased food availability and phytoplankton productivity characteristic of cockle beds (Johnstone, 1899). Adoption of the epibyssate habit by *M. edulis* exposes the mussel directly to air, placing a premium on

behaviours (shell-valve closure and quiescence) to conserve water during intertidal exposure. The concomitant heavy reliance on anaerobiosis and the energy savings so afforded may be of further benefit in the mussel's less productive hard-bottom community. In these contexts, it is interesting that the mussel *Geukensia demissa*, which typically is partially buried in the mud of highly productive salt marshes, gapes its shell valves and remains largely aerobic during emersion (Keunzler, 1961; Lent, 1968; Nicchitta and Ellington, 1983; Pamatmat, 1983), whereas *M. californianus*, a mussel living on hard substrate, resorts to anaerobiosis in spite of a capacity to extract some oxygen from the air during intertidal exposure (Bayne *et al.*, 1976b).

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