

THE EFFECTS OF ANOXIA AND HYDROGEN SULPHIDE ON SURVIVAL, ACTIVITY AND METABOLIC RATE IN THE COOT CLAM, *MULINIA* *LATERALIS* (Say)

SANDRA E. SHUMWAY^{1,2} and TIMOTHY M. SCOTT

Department of Ecology and Evolution, State University of New York, Stony Brook, NY 11794, U.S.A.

and

J. MALCOLM SHICK

Department of Zoology, University of Maine, Orono, ME 04469, U.S.A.

Abstract: The mastrid clam *Mulinia lateralis* (Say) shows ephemeral success in colonizing a variety of marine substrata, most commonly "soupy", reducing muds. Depending on temperature and body size, LT_{50} values during exposure to anoxia and hydrogen sulphide range from 2 to 11 days, at the lower end of the range reported for infaunal bivalve molluscs. Unlike most bivalves, *M. lateralis* maintains high levels of feeding, shell valve, and locomotory activities under anoxia, which may be an adaptation to escape periodic burial in unstable, oxygen-deficient sediment. The rate of metabolic heat dissipation under anoxia is the same as under normoxic conditions, which implies a greatly elevated rate of anaerobic glycolysis (Pasteur effect) during anoxic exposure. This may explain the rather short anoxic survival times in this species, and emphasizes its adaptation to short-term as opposed to chronic oxygen deficiency, which may occur in dense deposit-feeding communities from which *M. lateralis* is excluded.

INTRODUCTION

The coot clam, *Mulinia lateralis* is a widely distributed (Parker, 1975), transient, opportunistic species whose numbers show drastic spatial and temporal fluctuations (Sanders, 1956; Stickney & Stringer, 1957; Levinton, 1970; Rhoads, 1974; Holland *et al.*, 1977). Adaptations which enable this clam to maintain its seemingly tenuous infaunal existence in silt-clay substrata include its high fecundity and very short generation time (Calabrese, 1969), iteroparous reproduction (Calabrese, 1970), and perhaps its small size and low bulk density, which allow support in soft substrata (Levinton & Bambach, 1970).

The physiological adaptations which permit life in anoxic, often unstable, reducing substrata have not been studied in *M. lateralis*. Sessile and infaunal bivalves generally show strong resistance to anoxia (reviewed by Theede *et al.*, 1969), due in part to a reduction in activity and hence in energy utilization (De Zwaan & Wijsman, 1976;

¹ To whom requests for reprints should be addressed.

² Present address: Department of Marine Resources, Marine Resources Laboratory, West Boothbay Harbor, ME 04575, U.S.A.

Pamatmat, 1980), and to the employment of anaerobic pathways of metabolism which are energetically more efficient than classical glycolysis (De Zwaan, 1977). Since the occurrence of anoxia in the habitat is often coupled with the presence of highly toxic hydrogen sulphide, tolerance to anoxia alone is insufficient in assessing the capacity for survival in such environments (Theede *et al.*, 1969), and anoxia-resistant macrofauna generally show a correspondingly large tolerance of hydrogen sulphide (Theede *et al.*, 1969; Shick, 1976; Hiroki, 1977, 1978; Groenendaal, 1979, 1980, 1981; Grant, 1981).

In the present paper we describe the physiological responses of *M. lateralis* (Say) to environmental anoxia. Included are data on tolerance to anoxia in the presence and absence of hydrogen sulphide, the effects of anoxic exposure on subsequent oxygen uptake rates, and shell valve activity, rates of filtration and rates of metabolic heat dissipation under normoxic and anoxic conditions.

MATERIALS AND METHODS

Specimens of *Mulinia lateralis* were collected from Port Jefferson Harbor, New York using a Petersen-type grab. The sediment had the strong odor of hydrogen sulphide, and few other macroinvertebrates were present. The clams were maintained in the laboratory in re-circulating Ocean aquaria at 28‰ *S* and pH 7.5. They were fed a mixed algal diet – *Thalassiosira pseudonana* (Hustedt), *Monochrysis lutheri* (Droop), *Isochrysis galbana* (Parke) – during a 3-wk temperature-acclimation period prior to their use in experiments.

Oxygen deficient sea water was prepared by bubbling with pure nitrogen at the appropriate temperature. Residual oxygen was always <4 mm Hg as determined with a calibrated pO₂ electrode (Radiometer, Copenhagen). Hydrogen sulphide concentrations were as follows: 50 (to allow comparison with previously published data), 161, 322, 463 and 644 mg · l⁻¹. They were prepared by adding Na₂S · 9H₂O to deoxygenated sea water (to prevent oxidation) and allowing it to dissolve, utilizing the bubbling nitrogen for agitation. Three species of hydrogen sulphide (H₂S, HS⁻ and S²⁻) exist in sea water, depending on the pH (Goldhaber & Kaplan, 1975; Powell *et al.*, 1979), but for the purposes of these experiments no distinction will be made between the species. The pH was adjusted to 7.5–7.7 by addition of 0.1 N HCl.

Groups of 20 individuals of two size classes of clams (> 10 mm and < 5 mm total length) were exposed to anoxia and to anoxia in the presence of hydrogen sulphide in sealed 1-liter glass jars at temperatures of 10, 20, and 30 °C. Preliminary experiments indicated that once the ability to retract the foot or siphons was lost the clams could not be revived. Survival was assessed periodically as the ability or lack thereof to retract the siphons and/or foot in response to agitations of the sealed containers. Results are expressed as percent survival. Five replicates of the anoxia experiments (*n* = 100) and three replicates of the anoxia/hydrogen sulphide experiments (*n* = 60) were carried out at each temperature.

Oxygen uptake (\dot{V}_{O_2} ; $\mu\text{l O}_2 \cdot \text{h}^{-1}$) in recently-fed specimens was monitored at 20 °C using a Radiometer pO₂ electrode in a closed system as described previously (Shumway, 1981). The reported \dot{V}_{O_2} values were measured at pO₂ values above 80% air saturation. The clams were subsequently allowed to deplete completely the available oxygen and left under these anoxic conditions for 12 h. The anoxic sea water was then replaced with aerated sea water and oxygen uptake was re-measured. Preliminary observations indicated that *M. lateralis* remained very active (siphons and foot extended) during anoxic exposure thus experiments were devised to assess the extent of this activity.

Clearance rate was monitored by means of a Coulter Counter Model B. Individual clams were placed in beakers with suspensions of *T. pseudonana* at a concentration of 10^5 cells · ml⁻¹. Clearance rate was calculated according to Newell (1979, p. 461):

$$m = M/t(\log_e \text{conc}_0/\text{conc}_t)$$

where m is the filtration rate of a single animal; t is the time interval, M is the volume of the suspension, conc_0 is the initial concentration at time zero and conc_t is the concentration after time t . Filtration rate was measured under both normoxic and anoxic conditions at 20 °C.

Shell valve activity was monitored under both normoxic and anoxic conditions in sea water in glass bowls to facilitate viewing. Anoxic conditions were maintained by continuous bubbling with N₂. Food was present in both conditions as it was found previously that the most regular movements were elicited in this way (Shumway, in prep.). Clams were attached by one valve to small pieces of slate using dental cement; the other valve was cemented to a strain gauge, and changes in resistance were displayed on a chart recorder (Djangmah *et al.*, 1979).

Total heat dissipation by unfed clams was monitored under both normoxic and anoxic conditions in a double-twin heat flow calorimeter (Pamatmat, 1979, in press; Shick, 1981) operated at 15 °C. Clams were incubated individually in 10 ml of air saturated water with ≈ 25 ml of air above the water in a sealed vial. Ventilation by the clam kept the water well mixed, and the atmospheric oxygen in the closed vial was sufficient to maintain the sea water pO₂ above 75% air saturation during an 8-h measurement, as calculated from \dot{V}_{O_2} . Anoxic incubations were in 35 ml N₂ purged water (initial pO₂ < 3 mm Hg). The sequence of treatments (air saturated or N₂-purged sea water) was randomized in the paired comparisons, but the animal always was allowed to recover in air saturated sea water for 48 h before re-use.

Outputs (μV) from the thermopiles surrounding the active chamber and from the reference twin were recorded continuously for 8 h using Keithley 150B microvolt ammeters and a two-channel recorder. Data from the first 3 h, during which the instrument re-equilibrated thermally, were discarded. Individual power-time curves (thermograms) were integrated using a Zeiss Videoplan digitizer and μV converted to $-Q_H$ (total heat production) using the static calibration constant of $25.4 \mu\text{W} \cdot \mu\text{V}^{-1}$ for this calorimeter. The instrument baseline was checked immediately before and after each measurement using vials containing only filtered sea water.

RESULTS

No significant differences were found between replicates and the data within survival experiments were pooled. *M. lateralis* exposed to anoxia exhibited an expected decline in survival time as the experimental temperature increased (Fig. 1). There was no

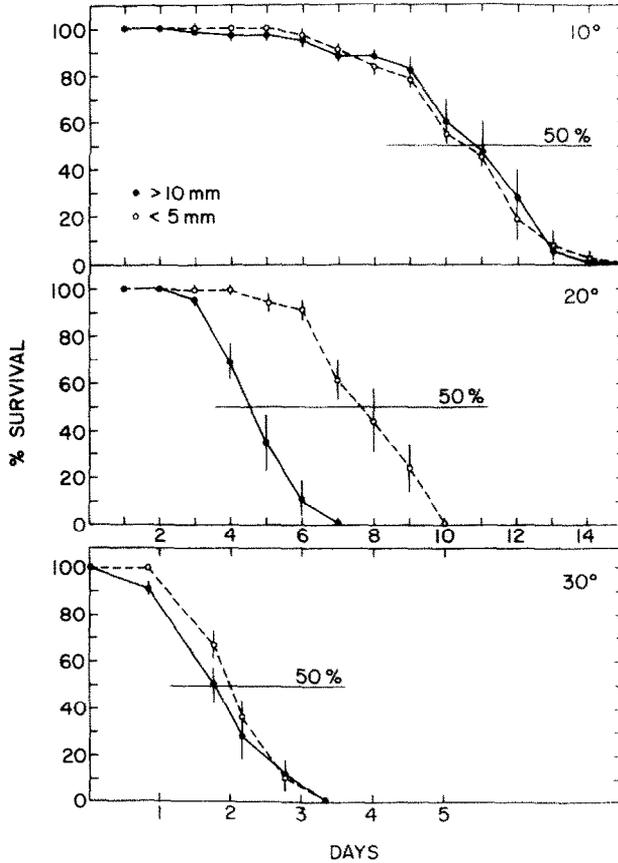


Fig. 1. Survival under anoxia in *Mulinia lateralis* at three temperatures: $n = 100$ individuals in each size class; vertical bars indicate ± 1 SE; LT_{50} is indicated on each graph.

difference in survival time (LT_{50} ; days) between large and small clams at the temperature extremes (10 and 30 °C). At 20 °C, smaller individuals showed a greater tolerance to anoxia than large specimens (LT_{50} values of ≈ 7 and 4 days, respectively).

As the concentrations of hydrogen sulphide increased, the survival time at 10 °C decreased, the effect being more pronounced in large animals (Fig. 2). LT_{50} values ranged from 6 days (> 10 mm; 0.21 mM $Na_2S \cdot H_2O$) to ≈ 4 days (all animals; 2.68 mM $Na_2S \cdot 9H_2O$). A similar response occurred at 20 °C (Fig. 3) where survival

time decreased with increased hydrogen sulphide concentrations, although there was no apparent difference in survival time between the two size groups. It appears that upon reaching the higher concentrations of hydrogen sulphide there is a point at which further increases in concentration do not significantly alter the LT_{50} in small clams. In addition, survival time for both size classes was shorter at 20 °C than at 10 °C.

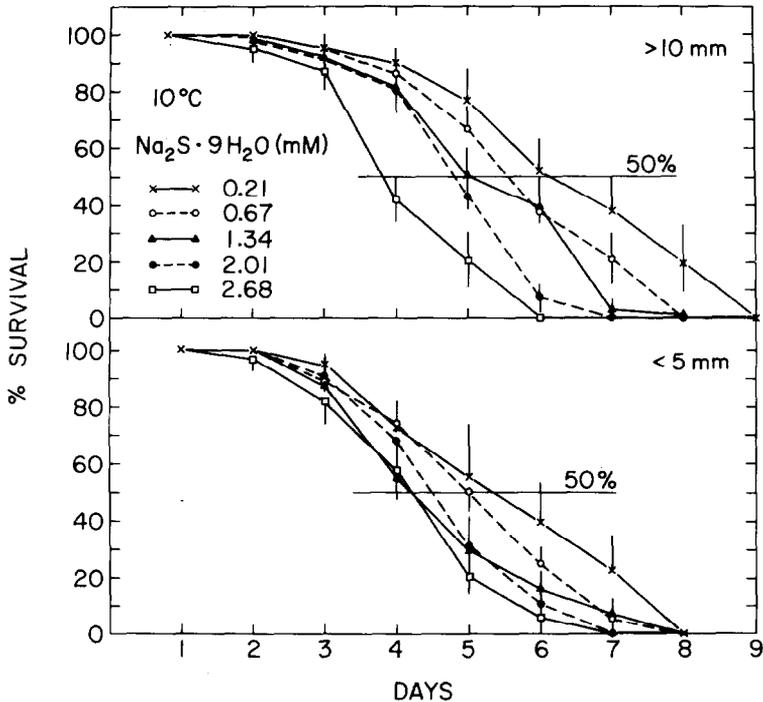


Fig. 2. Survival under conditions of anoxia and various concentrations of hydrogen sulphide at 10 °C: each curve is the result of 60 individuals; vertical bars indicate ± 1 SE; LT_{50} is indicated on each graph.

Oxygen uptake rate in relation to body size is shown in Fig. 4. The data have been fitted to the equation $\dot{V}_{O_2} = aW^b$ where W is dry tissue weight in g, and a and b are constants. The data represent paired observations for animals before and after exposure to 12 h anoxia. Covariance analysis indicates a significant post-anoxic elevation of \dot{V}_{O_2} ($P < 0.0001$). Under pre-anoxic conditions, \dot{V}_{O_2} increases with the 0.645 power of body weight whereas post-anoxia the same rate increases with the 0.489 power; although this difference is not statistically significant ($0.10 > P > 0.05$), the tendency is toward a greater increase in \dot{V}_{O_2} after anoxic exposure in small animals than in large ones, post-anoxic/pre-anoxic $\dot{V}_{O_2} = 1.95 W^{-0.156}$.

Filtration rates did not differ between individuals exposed to anoxic conditions and those exposed to normoxic conditions. As seen in Fig. 5, there is considerable variance in the data, a function of the clams' highly variable behavior.

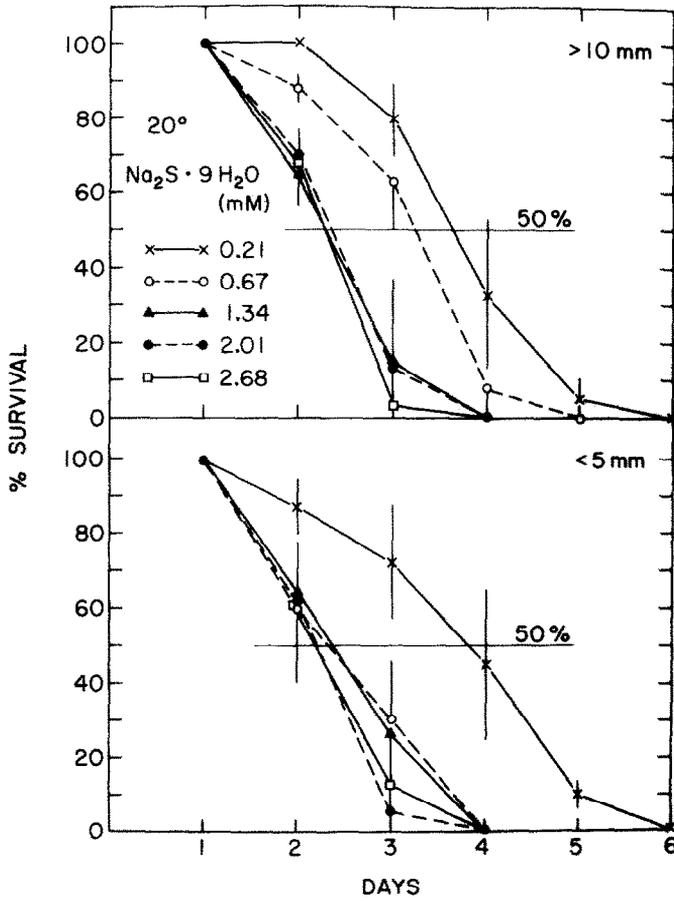


Fig. 3. Survival under conditions of anoxia and various concentrations of hydrogen sulphide at 20 °C: each curve is the result of 60 individuals; vertical bars indicate ± 1 SE; LT_{50} is indicated on each graph.

TABLE I

Measured rates of heat dissipation ($-\dot{Q}_H$; $mW \cdot g^{-1}$) in *Mulinia lateralis* under normoxic and anoxic conditions at 15 °C: paired observations for 10 individuals; values are average rates for the last 5 h of 8-h exposure periods.

Clam number	Dry weight (g)	Aerobic $-\dot{Q}_H$	Anaerobic $-\dot{Q}_H$
1	0.0130	6.00	4.80
2	0.0300	2.32	2.43
3	0.0261	1.92	1.42
4	0.0229	3.26	2.48
5	0.0120	3.00	4.74
6	0.0141	4.22	3.89
7	0.0183	3.19	3.41
8	0.0252	1.83	2.02
9	0.0145	5.59	5.00
10	0.0268	2.12	2.25
	\bar{X} 0.0203	3.35	3.24
	SE 0.0021	0.47	0.41

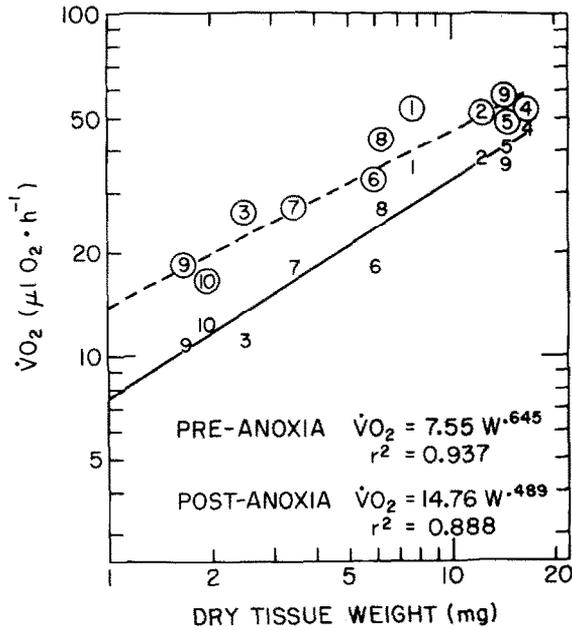


Fig. 4. The effect of exposure to anoxia for 12 h on oxygen uptake in *Mulinia lateralis* at 20 °C: solid line represents pre-anoxic rate; broken line and circled numbers represent the same individuals after 12 h exposure to anoxia.

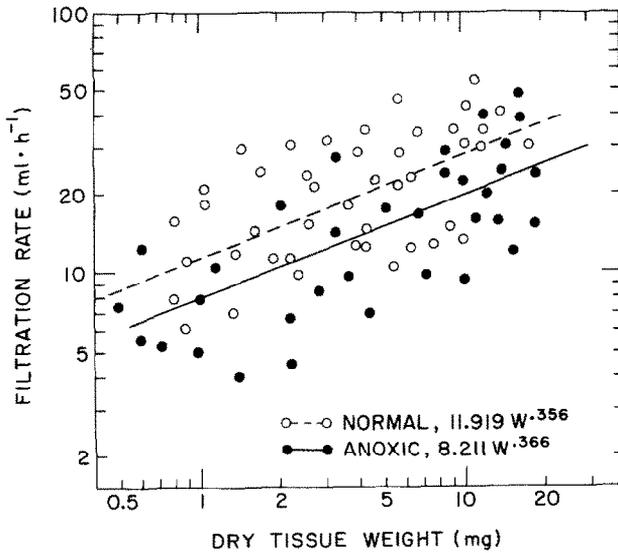


Fig. 5. Filtration rate in *Mulinia lateralis* in relation to body size under normoxic and anoxic conditions at 20 °C: estimates are based on clearance of a suspension of *Thalassiosira pseudonana* at a concentration of 10^5 cells · ml⁻¹.

Fig. 6 shows representative traces of shell valve activity in *M. lateralis*. Each spike corresponds to an abrupt, coughing-like action of the valves. Clams exposed to anoxic conditions exhibited very irregular patterns of movement. Small clams tended to be more active (i.e., had more frequent and higher-amplitude adduction–abduction movements) under anoxia than did larger individuals, although this response was not quantified.

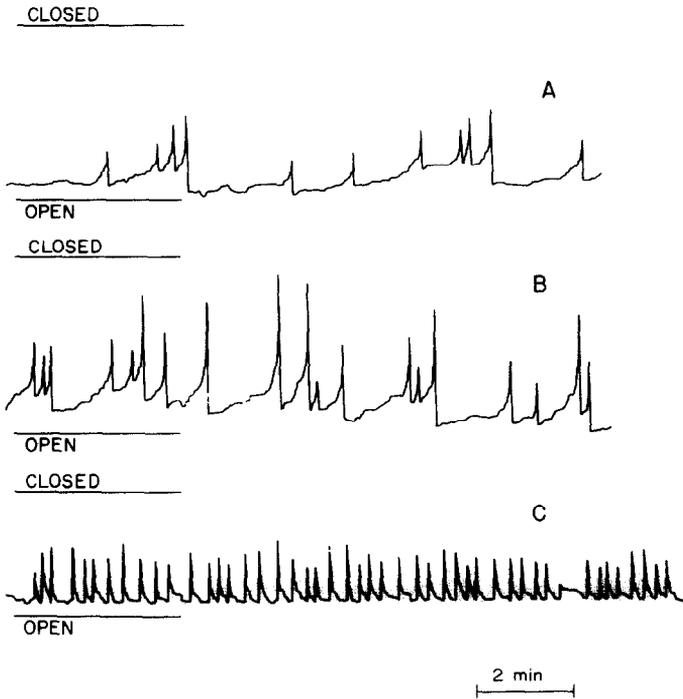


Fig. 6. Traces of shell valve activity in *Mulinia lateralis* during anoxic exposure in the presence of food: A, large animal (> 10 mm); B, small animal (< 5 mm); C, large individual under normoxic conditions; the sensitivity setting of the measuring apparatus was the same for all specimens.

The power-time curves of normoxic and anoxic *M. lateralis* were qualitatively similar. Rates of heat dissipation ($-\dot{Q}_H$) were generally steady, and the frequency of shell valve activity was apparently too high (or the associated enthalpy changes too small) to be discerned as individual peaks in the calorimetric recordings. A few anoxic clams showed small peaks of heat dissipation (10% above the steady level) with a frequency of ≈ 10 min, probably corresponding to probing activities by the foot, which were seen in the laboratory. There was no significant difference (paired *t*-test, $P < 0.001$) in the rate of heat dissipation between specimens under normoxic conditions and those held under anoxia (Table I).

DISCUSSION

Depending on body size and ambient temperature, *M. lateralis* tolerates anoxia for 2 to 11 days, with the presence of hydrogen sulphide reducing survival time. Theede *et al.* (1969) reported LT_{50} values for three species of infaunal bivalves (*Cyprinia islandica*, *Scrobicularia plana*, and *Mya arenaria*) ranging from ≈ 21 to 55 days under anoxia at 10 °C. In the presence of $50 \text{ mg} \cdot \text{l}^{-1} \text{Na}_2\text{S} \cdot \text{H}_2\text{O}$ (0.21 mM) these times were reduced to 15 and 42 days. Rao & Kutty (1968) showed that two species more closely related to *Mulinia lateralis* (*Donax faba* and *D. cuneatus*) tolerated anoxia for 1.7 and 3.3 days, respectively, and Brafield (1963) reported that *Macoma balthica* survived only 2 or 3 days of anoxia. Thus, the anoxic tolerance of *Mulinia lateralis* is unexceptional and falls toward the lower end of the range among infaunal bivalves.

The behavior of *M. lateralis* during oxygen deficiency is relatively uncommon among bivalve molluscs. Although behavior varies interspecifically, the usual response to low oxygen conditions is a drastic reduction of activity, which in turn decreases the energy requirements of the individual (Brafield, 1963; Bayne *et al.*, 1976; De Zwaan, 1977; Pamatmat, 1980). *M. lateralis*, however, maintains high levels of feeding and activity during exposure to anoxia (see Figs. 5 and 6).

For an animal routinely exposed to periodic environmental anoxia, it is probably advantageous to maintain normal or near normal levels of activity, which in *M. lateralis* may serve two functions. First, it may allow the clam to resurface if buried in the highly unstable mud substratum. This response was previously described in the ecologically similar heterodontid clam *Macoma balthica* (Brafield, 1963) and we have also observed *Mulinia lateralis* to emerge from the sediment as ambient pO_2 is lowered (Shick, pers. obs.). The "coughing" behavior observed during anoxic exposure in the laboratory may serve to cleanse the gills and mantle cavity of fine sediment (cf. Yonge, 1944), which in nature is the likely cause of oxygen deficiency for this clam. In this sense it is interesting that small specimens of *Mulinia*, i.e., those most likely to become buried and most subject to suffocation by disturbed sediments (see Levinton, 1970; Levinton & Bambach, 1970), tend to be more active than large specimens during anoxia (see Fig. 6). Second, the ability to feed during routine anoxic exposure would appear to be a benefit. However, the tendency for smaller animals to have a larger ratio of post-anoxic/pre-anoxic \dot{V}_{O_2} ("oxygen debt") is not associated with a concomitant increase in feeding rate. Thus they do not enjoy any greater energetic advantage than larger clams by remaining active, which suggests that their greater activity relates more to short-term survival than to steady-state energy intake.

This maintenance of feeding and other normal activities under anoxia implies a high anaerobic energy demand, which we have confirmed using direct metabolic calorimetry: the rate of anaerobic heat dissipation is the same as that under air-saturated conditions (Table I). The maintenance of a high rate of heat dissipation under prolonged environmental anoxia is uncommon among bivalves. In *Mytilus edulis* for example, rates of aerobic heat dissipation are from 6 to 15 times greater than anaerobic rates (Pamatmat,

1980; Famme *et al.*, 1981; Shick *et al.*, 1983). [Parenthetically we note that our independent direct and indirect calorimetric measurements of aerobic metabolic rate are in excellent agreement. \dot{V}_{O_2} in starved specimens weighing 20 mg is 0.25 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ at 10 °C and 0.97 ml $\cdot g^{-1} \cdot h^{-1}$ at 20 °C (Shumway, unpubl.); using an average oxycaloric coefficient of -5.58 mW per ml $O_2 \cdot h^{-1}$ (Gnaiger, in press), the median value of 0.61 ml $O_2 \cdot g^{-1} \cdot h^{-1}$ is equivalent to a $-\dot{Q}_H$ of 3.40 mW $\cdot g^{-1}$, and the directly measured aerobic rate in starved clams at 15 °C is 3.35 mW $\cdot g^{-1}$ (Table I).]

The ability of *Mulinia lateralis* to utilize either aerobic or anaerobic metabolism for energy production to support its normal activities must be a prime factor in allowing its existence in periodically anoxic habitats. However, because of the much lower yield of ATP per glucosyl unit in anaerobic than in aerobic metabolism (see De Zwaan & Wijsman, 1976), glycolytic flux must increase to maintain high levels of ATP production under anoxia. Moreover, because of the generally lower caloric equivalent of dissipative ATP turnover in anaerobic (as low as -43 kJ $\cdot mol^{-1}$) than in aerobic metabolism (-80 kJ $\cdot mol^{-1}$) (Gnaiger, 1980), an equal $-\dot{Q}_H$ under both conditions actually implies a greater rate of ATP turnover under anoxia.

Although there are no data for *M. lateralis*, the ecologically similar heterodont *Macoma balthica* has an exceptionally high level of octopine dehydrogenase activity (De Zwaan *et al.*, 1981). This enzyme acts analogously to lactic dehydrogenase and typically is used during "burst" activity or other periods of high energy demand (Livingstone, 1982). Its much higher activity in *M. balthica* than in, e.g., *Mytilus edulis* (which drastically reduces energy demand under anoxia), suggests that the former, like *Mulinia lateralis*, may be geared to maintain a high rate of anaerobic glycolysis, and *Macoma balthica* indeed increases locomotor activity under anoxia (Brafeld, 1963).

The foregoing considerations may also explain the short anoxic survival times of *Mulinia lateralis* (and *Macoma balthica*) relative to most other marine bivalves: by drastically elevating its rate of anaerobic glycolysis (Pasteur effect), it may simply be burning itself out of glycogen. Clams were not fed during the survival experiments, but this would not be the case in nature. Although primarily a filter-feeder, there is evidence that *Mulinia lateralis* can also feed on resuspended sediment (Parker, 1975, p. 91). If as seems likely this species can utilize particulate detritus as well as microalgae, anoxic survival might be prolonged by the ready availability of such food resources in the organically rich silty mud in its habitat (Sanders, 1960) and the demonstrated ability of the clam to feed under such conditions. Obvious future studies include the determination of rates of glycogen depletion and digestion and absorption efficiencies under normoxic and anoxic conditions, and an examination of the pathways of anaerobiosis, in *M. lateralis*.

Despite its relative intolerance of anoxia, *M. lateralis* is an extremely successful colonist of strongly reducing soft substrata (Parker, 1975, p. 87). This seems dependent on the clam's maintaining ready access to the oxygenated water column, which in the absence of mobile macrofauna may be hindered only periodically by storms, increased sedimentation, etc., and easily dealt with by the clam's high activity levels during

short-term anoxia. However, *M. lateralis* appears to be unsuccessful in coping with the effects of chronic sediment destabilization caused by bioturbation activities of mobile deposit feeders (Levinton & Bambach, 1970; Rhoads, 1974, p. 281), and indeed achieves large population sizes only in the absence of such interfering competitors (Parker, 1975, p. 91). The picture that emerges is one of a strongly *r*-selected species having low competitive ability, high fecundity, short generation time, rapid rates of energy turnover, and a delicate energy balance.

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