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Energy metabolism and valve closure behaviour in the Asian clam *Corbicula fluminea*

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Summary

Since its invasion of Europe in the early 1980s, the Asian clam *Corbicula fluminea* has become very abundant in nearly all western river systems. Today this species is one of the most important biomass producers in the River Rhine. Monitoring the valve movements of *C. fluminea* over a period of 2 years revealed a circadian rhythm in summer, with extended periods (10–12 h) of valve closure, predominantly in the morning hours. Altogether valve movements were very scarce, frequently fewer than four movements per individual per day.

Simultaneous measurements of heat dissipation and oxygen consumption (calorespirometry) revealed an intermittent metabolism in the clam. With the onset of valve closure, *C. fluminea* reduced its metabolic rate to 10% of the standard metabolic rate (SMR) measured when the valves were open. Nevertheless, this depressed metabolism remained aerobic for several hours, enabling the clam to save energy and substrates compared to the requirements of the tenfold higher SMR. Only during long-lasting periods of valve closure (more than 5–10 h) did the clams become anaerobic and accumulate succinate within their tissues (2 μmol g^{-1} fresh mass). Succinate is transported into the mantle cavity fluid, where it reaches concentrations of 4–6 mmol l^{-1}. Because this succinate-enriched fluid must pass the gills when the valves open again, we suggest that this anaerobic end product is at least partly reabsorbed, thus reducing the loss of valuable substrates during anaerobiosis. Propionate was also produced, but only during experimental N_2-incubation, under near-anoxic conditions.

The intermittent metabolism of *C. fluminea* is discussed as an adaption to efficiently exploit the rare food supply, saving substrates by the pronounced metabolic depression during valve closure.

Key words: anaerobiosis, bivalve, Asian clam, *Corbicula fluminea*, calorimetry, circadian rhythm, metabolic depression, energy metabolism.

Introduction

Originating in South-East Asia, the clam *Corbicula fluminea* Müller 1774 successfully invaded the North American continent from east to west between 1924 and 1975, and is still considered to be a ‘pest species’ (McMahon, 1983, 2000). It then crossed the Atlantic, presumably within the ballast water of ships, and since the early 1980s has spread all over Europe (Mouthon, 1981). Today *C. fluminea* is found throughout Southern and Western Europe (Bij de Vaate and Greijdanus-Klaas, 1990; Araujo et al., 1993) and has recently been recorded in Great Britain (Aldridge and Müller, 2001) as well as in the Romanian part of the River Danube (Bij de Vaate and Hulea, 2000).

The life history of *C. fluminea* is characterized by typical r-strategic features such as high fecundity, along with short generation times and high growth rates (McMahon, 1983; Meister, 1997). Thus, *C. fluminea* occurs in high abundance wherever it is found, frequently more than 1000 individuals m^{-2} (McMahon, 2000). Meister (1997) reported mean densities of 350–600 individuals m^{-2} in the Rhine, corresponding to a biomass of 1 kg m^{-2}. Thus, *C. fluminea* is one of the most important biomass producers of the Rhine (Kinzelbach, 1995).

Because of its economic impact on artificial water currents such as irrigation channels or cooling water systems of power stations, there have been many investigations into the clam’s reactions to abiotic parameters (for reviews, see Doherty and Cherry, 1988; McMahon, 2000). However, although the clam’s valve movements are used to monitor water-borne stressors (Doherty et al., 1987; Allen et al., 1996), little is known about the animal’s energy metabolism during periods when the shells are closed, nor about the clams’ natural valve closure behaviour throughout the year.

It is generally thought that bivalves with opened valves rely on an aerobic metabolism, mostly fuelled by glycogen. After closing the valves, however, most authors suggest that the enclosed oxygen is spent within a few minutes (Widdows, 1987), since the partial pressure of oxygen seems to decrease rapidly in the mantle cavity, as measured in *Arctica islandica* (Taylor, 1976) and *Mytilus edulis* (Davenport and Woolmington, 1982). Anaerobic energy provision commences as soon as the partial pressure of oxygen falls below between 20 and 50 mmHg (Pörtner et al., 1985). As environmental

anaerobiosis commences, ATP is replenished by degradation of a phosphagen (usually phospho-L-arginine in molluscs) and glycolytic substrate phosphorylations, with lactate and opines (guanidoamino acids) accumulating as electron-accepting end products. Later on, mitochondrial fumarate reduction with concomitant formation of succinate, and the subsequent synthesis of propionate and acetate, provides most of the ATP. The anaerobic ATP yield, which comprises at most 20% of the aerobic energy provision, is not compensated by a Pasteur effect; instead, the energy expenditure of anaerobic molluscs is usually markedly reduced (Grieshaber et al., 1994). A functional anaerobiosis is only demonstrated by some mobile species such as some Pectinidae and Cardiidae, which are capable of vigorous swimming and jumping movements, and is characterized by rapid degradation of a phosphagen, and an increased glycolytic flux, i.e. a Pasteur effect, leading to the accumulation of lactate and/or opines (Grieshaber et al., 1994).

If bivalves become anaerobic during valve closure, even without ambient stressors, as suggested by some authors (Higgins, 1980; Williams et al., 1993; Holopainen and Penttinen, 1993; Sobral and Widdows, 1997), then one must question how these animals fuel inefficient anaerobic pathways with enough substrates to endure long-lasting periods within closed valves. Obviously rapid exhaustion of substrates can only be prevented if the metabolic rate is lowered during anaerobiosis. But even when reduced down to 10% of the standard metabolic rate (SMR), the amount of substrates required to sustain the metabolism anaerobically still equals the amount used during aerobic conditions (Hand and Hardewig, 1996). Thus, the following questions arise. Does *C. fluminea* reduce its metabolism during valve closure and to what extent? And does this species really become anaerobic during valve closure?

In an attempt to answer these questions we continuously monitored the metabolic rate of *C. fluminea* by simultaneously measuring heat dissipation and oxygen consumption of clams maintained in well aerated as well as hypoxic conditions, including numerous consecutive periods of valve closure. Anaerobic end products such as acetate, propionate and the transient intermediate succinate (Grieshaber et al., 1994) were analysed in specimens incubated in aerated artificial freshwater (therein retaining their natural valve-closing behaviour) and, in addition, in specimens incubated in N_2-saturated water. Finally, we recorded the valve movements of *C. fluminea* directly *in situ* in the Rhine over more than 2 years to compare its natural behaviour with that seen in the laboratory.

Materials and methods

Clams *Corbicula fluminea* Müller 1774 (shell length: 10–33 mm) were collected at low water from the Rhine at Düsseldorf (733 km from the river source) using a long-handled sieve. They were sorted from the dredged gravel and taken into the laboratory, where the clams were kept on gravel sediment in 10 l tanks (at 15±0.5°C) containing aerated artificial freshwater (AFW: Neumann, 1960) consisting of 90 mg l^{-1} $MgSO_4.7H_2O$; 8 mg l^{-1} $MgCl_2.6H_2O$; 23 mg l^{-1} NaCl; 28 mg l^{-1} $NaHCO_3$; 15 mg l^{-1} $KHCO_3$; 147 mg l^{-1} $CaCO_3$. Once a week the water was changed and the clams fed with stinging-nettle leaf powder.

Valve closure behaviour

From August 1997 until August 1999 we continuously recorded the valve movements of *C. fluminea* in the Rhine using two different bivalve monitors. The Dreissena-Monitor® (Envicontrol, Köln, Germany) at Bad Honnef (640 km) was supplied with 34 specimens; the Mosselmonitor® (Delta Consult, Kapelle, Netherlands) at Koblenz (590 km) carried only eight specimens. Both monitors were situated in a bypass that permanently received water directly pumped out of the river. Specimens of *C. fluminea* were attached onto holders with one valve, so that the movement of the other valve could be recorded. Whereas the Dreissena-Monitor® uses the interaction of a Reed Switch and a small magnet, which is glued onto the free moving valve, the Mosselmonitor® measures the distance between two electric coils that are also glued onto the valves (Fig. 1). The signals from each single specimen were recorded using a PC with monitor-specific software. Valve movement data were processed and illustrated with Excel and SigmaPlot software. The use of both measuring systems is well established in water surveillance projects and toxicological behaviour studies (Jenner et al., 1989; Hoffmann et al., 1994; Borcherding and Wolf, 2001). Another Mosselmonitor® was used in a thermostatted chamber inside the laboratory, operated with circulated AFW at 15±0.5°C. After various time intervals, specimens with known valve-closure periods were collected and analysed for anaerobic end products (see below).

Determination of metabolic rates

Heat dissipation and oxygen consumption rates of unfed *C. fluminea* were measured simultaneously between 3 and 27 days in an open-flow system (Fig. 2). A single clam was placed inside the reaction chamber of the perfusion systems (‘2250 111’: 4 ml or ‘2254 001’: 20 ml) within the microcalorimeter [Thermal activity Monitor (TAM) LKB 2277, Thermometric, Järfälla, Sweden], which was kept at precisely 15°C (±0.001°C) and perfused with AFW at flow speeds of 14–20 ml h^{-1} and 30–40 ml h^{-1} for the 4 ml and 20 ml chamber, respectively. The oxygen concentration of the inflowing and the outflowing water was determined using polarographic oxygen sensors (POS: Orbisphere, Geneva, Switzerland), which are part of a respirometer (Twin-Flow 2, Cyclobios, Innsbruck, Austria) situated inside a water bath (at 15±0.5°C). The oxygen concentration and heat signal were continuously recorded *via* the Twin-Flow-Monitor, which also compressed and converted the analogue data. These data were recorded and processed by Cyclobios Software (‘Datgraf Acquisition’ and ‘Datgraf 2.1 Analysis’ © 1993 Michael Reck). Prior to and following each experiment, baseline heat dissipation and oxygen consumption were measured in a blank run without an animal and used to correct the experimental

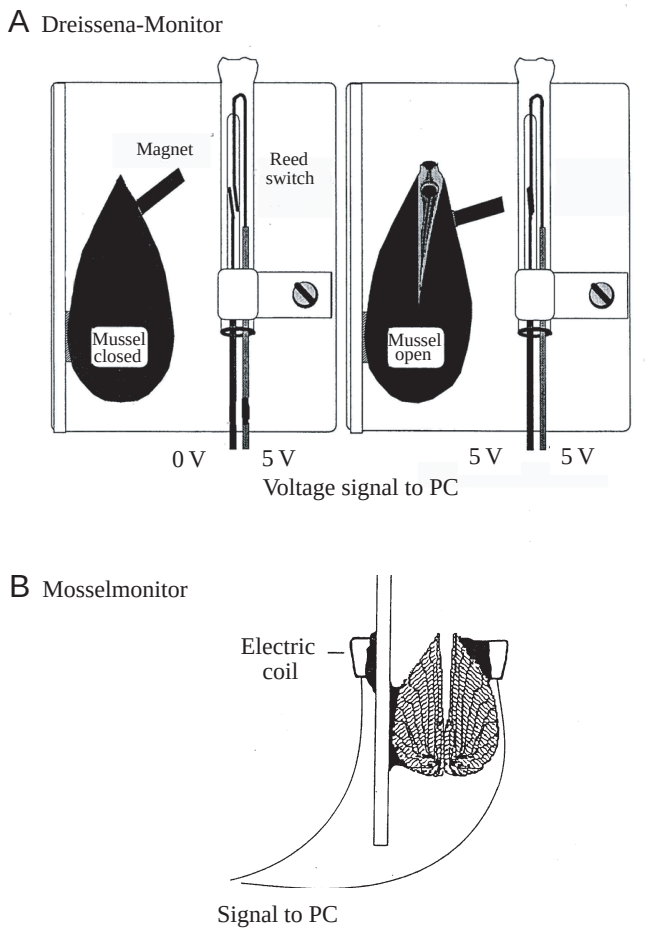

Fig. 1. Measuring principle of the bivalve monitors. (A) The Dreissena-Monitor® (Borcherding, 1992) contained 34 and (B) the Mosselmonitor® 8 measuring units at the same time (Hoffmann et al., 1994). Both figs slightly modified and reproduced with permission.

data. At the end of an experiment the clam's soft body tissue was removed and either used for extracting metabolites or dried at 80°C for 2 days.

Metabolic rate is expressed in J h^{-1} g^{-1} dry mass (heat dissipation) and µmol h^{-1} g^{-1} dry mass (oxygen consumption). Using an oxycaloric equivalent of 450 kJ mol^{-1} O_2 (Gnaiger et al., 1983a), it is possible to compare both rates and thus decide whether anaerobic pathways contribute to the clams' energy provision. If the heat dissipation exceeds the converted value of the oxygen consumption, the difference in energy expenditure can only be the result of anaerobiosis (Gnaiger, 1983b; Widdows, 1987).

Analysis of anaerobic end products

Levels of the main anaerobic end products, acetate, propionate and succinate, were estimated in the adductor muscles, foot, gills and mantle cavity fluid of *C. fluminea*. During near-anoxic incubations, clams were transferred into flasks containing water that had previously been bubbled with nitrogen to guarantee a P_{O_2} of less than 0.4 kPa. Later the

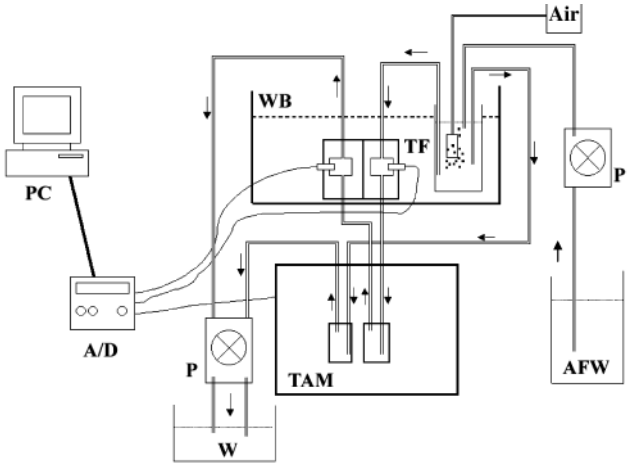

Fig. 2. Flowthrough-system. AFW, artificial freshwater (5 l-reservoir); TF, twin-flow-respirometer with two oxygen sensors and a 500 ml reservoir inside a waterbath (WB; 15°C); A/D, analogue-digital converter inside TF-monitor; P, peristaltic pump; TAM, calorimeter with reaction and reference chamber; W, waste.

nitrogen was bubbled continuously through the incubation water until the clams were removed for preparation of their tissues. To analyse the onset of anaerobic metabolism as well as the occurrence of a prolonged anaerobiosis, incubations lasted 1, 2 and 4 h or 1, 2 and 3 days, respectively. When a clam was removed from the flask, the water enclosed by the valves was collected first, and is referred to below as mantle cavity fluid because it represents the greatest fraction of the collected fluid. Then foot, adductor muscles and gills were dissected and freeze-clamped (Wollenberger et al., 1960). All tissues and the mantle cavity fluid were quickly transferred into liquid nitrogen and stored therein until extracted with perchloric acid (PCA) (see below). Specimens in the laboratory taken out of the Mosselmonitor® after known time periods with closed valves under normoxic conditions were prepared in the same manner.

Metabolites were extracted according to Beis and Newsholme (1975). Tissues were ground with a mortar and pestle under liquid nitrogen and added to 200 µl (foot) or 150 µl (adductors and gills) of 0.6 mol l^{-1} PCA, which was approximately three times the volume of the tissues. Mantle cavity fluid was diluted by a factor of two with 0.6 mol l^{-1} PCA. Then the extracts were homogenized and centrifuged at 14 000 ***g***, and the supernatant neutralised with 5 mol l^{-1} KOH and centrifuged again. The remaining supernatant was stored at –20°C until analysed.

Succinate, which indicates the onset of anaerobiosis in mitochondria (Pörtner et al., 1985), was determined enzymatically according to Beutler (1985). Succinate-CoA-synthetase (SCS), an enzyme needed for this assay, is no longer available commercially, but can be purified according to methods described by Buck et al. (1985) and Wolodko et al. (1994). The SCS that we used in this study was generously provided by Dr William Wolodko (University of Alberta, Edmonton, Canada).

Acetate and propionate were measured by gas chromatography using a WCOT-column heated at 100°C (fused silica, coating CP-wax 58, FFAP-CB, 25 m × 0.53 mm i.d.; Varian, Darmstadt, Germany). To separate acetate and propionate, the gas chromatograph (GC; CP-9001, Varian) was operated with a split-splitless injector (270°C) and a flame ionisation detector (FID, 300°C). Prior to the injections, the extracts were diluted with an equivalent amount of 1% HCl to convert the acids into their undissociated form. Samples (1 µl) were injected manually using a 10 µl gas-tight syringe (type #1801, RNE; Hamilton, Bonaduz, Switzerland).

Statistics

For normally distributed data we used one-way analysis of variance (ANOVA); otherwise we used the Kruskal–Wallis one-way ANOVA on ranks, to identify significant differences ($P<0.05$) between samples and controls, marked by an asterisk. Statistical analyses were performed with SigmaStat 1.0 (Jandel Scientific, Erkrath, Germany).

Results

Valve closure behaviour

Whereas the Dreissena-Monitor® only distinguishes between the open and closed states of the clam, the Mosselmonitor® also estimates the gape of the valves. However, other than rare small-scale movements, the Mosselmonitor® recordings of the clams' valve movements revealed hardly any positions other than wide open or completely closed (Fig. 3A). At both measuring sites, the monitors revealed that *C. fluminea* rarely closed its shells more than twice a day, but then usually stayed closed for about 5–10 h. Longer closing times of up to several days were also occasionally recorded, mainly at cold water temperatures during the winter.

During the warmer months, the clams showed a circadian rhythm of valve opening, with valves open in the afternoon and predominantly closed at night. Some specimens exhibited a strikingly regular pattern with one period of closed and one period of opened valves each day, each period lasting for about 10–12 h. If the clams were disturbed, for example during the maintenance of the monitor (Fig. 3A), a break in this rhythm could occur. The clams did not always close their valves in such a synchronous pattern (Fig. 3A), but for most of the clams monitored in the Rhine this rhythmic pattern persisted from the end of April until mid-October, with a daily minimum at night (most animals closed at around 1:00 h to 4:00 h) and a maximum at late afternoon (most animals open between 16:00 h to 18:00 h). Fig. 3B shows the magnitude of valve opening of the eight clams at Koblenz during a week in June 1998. Each value is the mean of a total of 420 readings taken every minute during each hour for each of the eight specimens for 7 successive days (7×60 readings per hour per

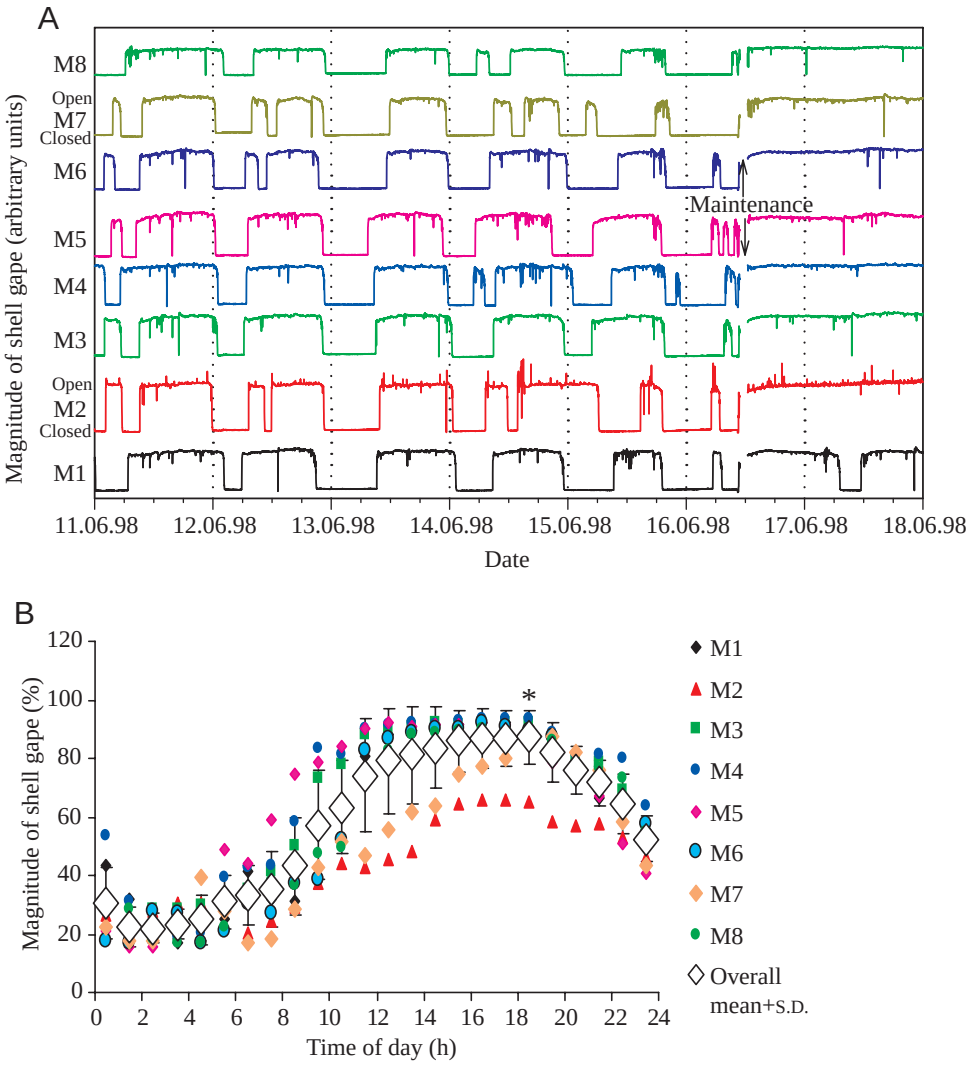

Fig. 3. Valve movements of the eight specimens (M1–M8) in Koblenz during 11–17 June 1998, recorded by the Mosselmonitor®. Water temperature varied between 19.1°C and 22.4°C. (A) Continuous recordings of individual magnitudes of shell gape, in arbitrary units. For each trace, closed is the baseline level. Dotted lines mark the change of date (0:00 h). Periods of monitor maintenance are indicated. (B) Individual means of the magnitude of shell gape at hourly intervals throughout the day, calculated from the maximum of arbitrary units (in A) set as 100%, minimum as 0%. White diamonds are mean values ± S.D. of the eight clams at each hour. *Minimum values, from 2:00 h to 3:00 h (22%), and maximum, from 18:00 h to 19:00 h (87%), differ significantly.

specimen). This figure clearly demonstrates that despite some differences in behaviour during the week (e.g. after maintenance of the monitor) and between the specimens, the mean magnitude of valve closure of these eight animals (white diamonds) followed an impressive circadian rhythm. Moreover, *C. fluminea* also showed circadian valve movements in the laboratory, despite being held under constant conditions without any light. But in contrast to the valve closure behaviour recorded *in situ* in the Rhine, clams inside the laboratory did not move synchronously at all. Rather, each clam followed its own rhythm for up to several weeks, with different periods and phases, until the rhythm was completely lost.

During winter the valve movements of *C. fluminea* almost ceased and the clams stood open for much longer periods, frequently up to several days. Nevertheless, the periods during which the valves remained closed lengthened too, especially on very cold days. At water temperatures below 5°C some clams remained closed inside their valves for more than a week without any movement at all (Fig. 4).

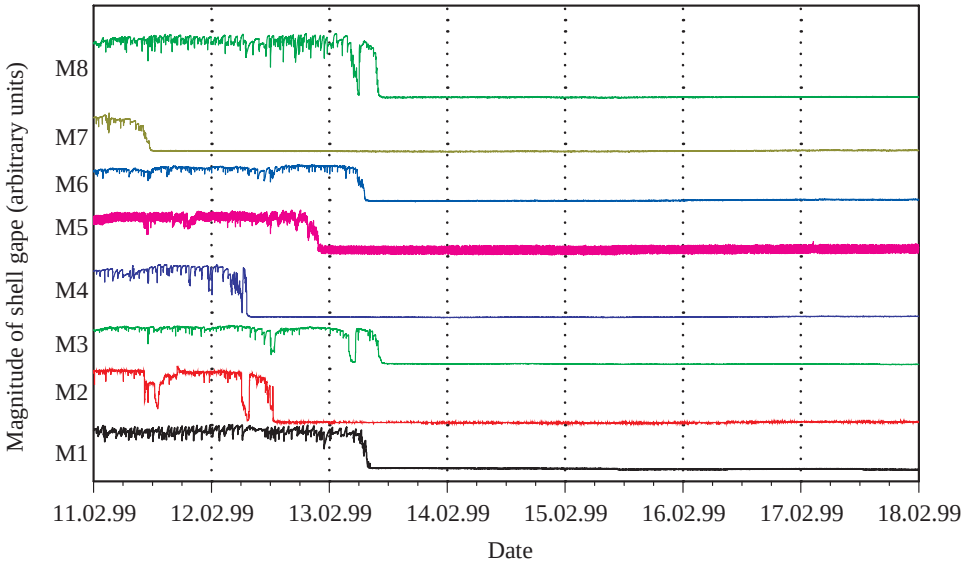

Fig. 4. Valve movements of the eight specimens (M1–M8) in Koblenz during 11–17 February 1999, recorded by the Mosselmonitor®. Water temperature varied between 3.1°C and 4.3°C. Continuous recordings of individual magnitudes of shell gape, in arbitrary units. For each trace, closed is the baseline level. Dotted lines mark the change of date (0:00 h).

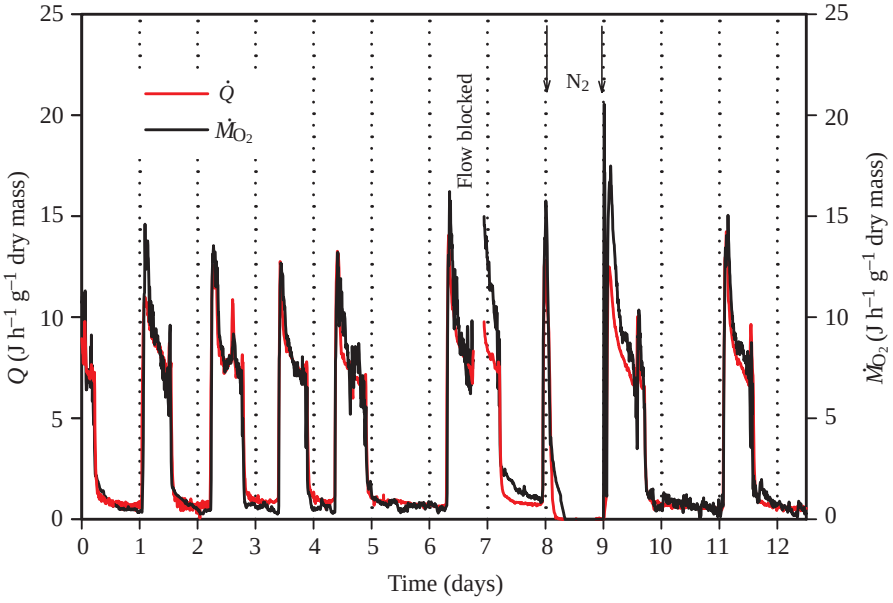

Fig. 5. Metabolic rates of *C. fluminea* (J h^{-1} g^{-1} dry mass) over a time period of nearly 2 weeks, measured as heat dissipation $\dot{Q}$ (red) and rate of oxygen consumption $\dot{M}_{O_2}$ (transformed *via* the oxycaloric equivalent: 450 kJ mol^{-1} O_2; black), recorded in artificial freshwater at 15°C. During the eighth day the water was bubbled with nitrogen.

Energy metabolism of C. fluminea

Heat dissipation $\dot{Q}$ and rate of oxygen consumption $\dot{M}_{O_2}$ mirror the valve closure behaviour of *C. fluminea*. Its intermittent metabolism changed rapidly between periods of high and low metabolic rate (Fig. 5). Unfortunately, a visual control of valve opening inside the calorimeter was impossible, but because of the great similarity in the patterns of valve movement recordings and metabolic rates, it is highly likely that the two are closely correlated. The valve closing behaviour in the Mosselmonitor® (Fig. 3) was similar to that in the calorimeter (Fig. 5) with alternating periods of about 10–12 h. Also, a circadian rhythm frequently occurred (e.g. first 5 days in Fig. 5) in the metabolic rates, with one period at a high metabolic rate and one period of depressed metabolism within a 24 h period. During such periods the metabolic rate was at least ten times higher (8.74±0.44 J h^{-1} g^{-1} dry mass for the specimen in Fig. 5) than during the depressed periods (0.78±0.12 J h^{-1} g^{-1} dry mass).

The separate integration of metabolic rates during periods with open and closed valves (Fig. 6) clearly demonstrates that during

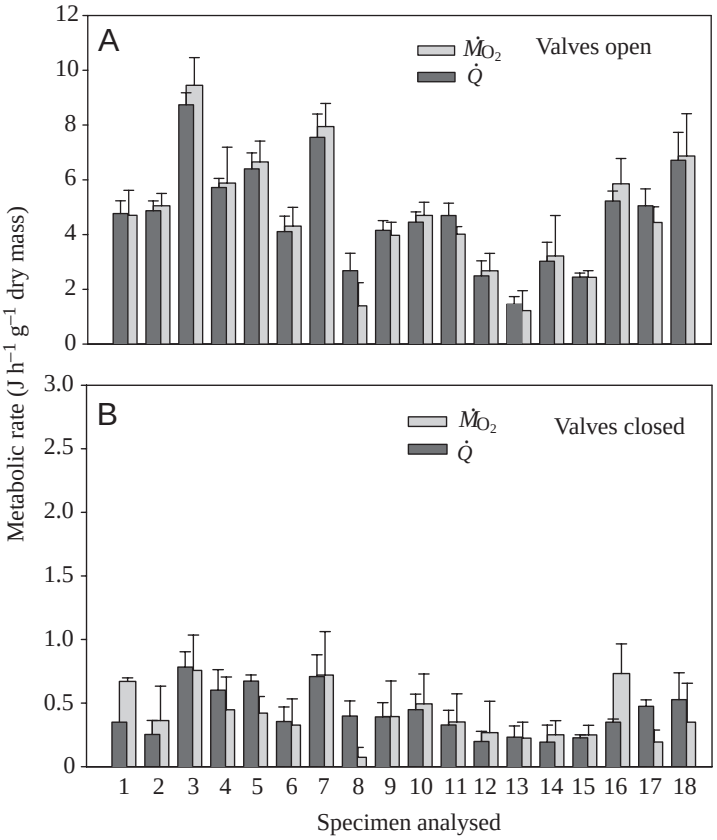

Fig. 6. Comparison of metabolic rates [J h^{-1} g^{-1} dry mass] of *C. fluminea* measured by direct (heat dissipation) and indirect (rate of oxygen consumption) calorimetry. Integration of periods with open (A) and closed (B) valves in artificial freshwater at 15°C. Heat dissipation ($\dot{Q}$; dark grey bars), oxygen consumption ($\dot{M}_{O_2}$; light grey bars). Each pair of bars (means ± S.D.) represents one of the 18 specimens used for calorespirometry, with open (A) and closed (B) valves. The oxycalorific equivalent = 450 kJ mol^{-1} O_2.

valve closure *C. fluminea* depressed its energy metabolism to less than 10% of the standard metabolic rate (SMR) measured when the valves are open. For the 18 specimens investigated by calorespirometry the mean metabolic rate with closed valves accounted for 9.3±2.7% (measured as heat dissipation) or 9.1±3.5% (measured as oxygen consumption) of the rate with opened valves. This relative reduction of metabolism during valve closure was independent of mass, whereas the dissipated heat at both levels followed an allometric relationship and could be calculated as:

$$MR_{open} = 13.87x^{0.52} \ (r^2=0.78)$$

and

$$MR_{closed} = 1.65x^{0.46} \ (r^2=0.56) \ ,$$

where MR = heat dissipation in μW and x = dry mass in mg.

Comparing heat dissipation and oxygen consumption *via* the oxycaloric equivalent (450 kJ mol^{-1} O_2; Gnaiger et al., 1989), there is no doubt that the clams' metabolism was completely aerobic during periods when the valves were open (Figs 5, 6A). Although in most cases the differences were still very small during periods with closed valves, occasionally the heat dissipated exceeded the corresponding value of oxygen consumption (Figs 5, 6B). Thus, it is likely that anaerobiosis contributed to the clams' metabolism during these periods.

Anaerobic end products

When incubated in near-anoxic conditions, *C. fluminea* expressed a kind of anaerobiosis frequently described for other bivalves (De Zwaan et al., 1976; Isani et al., 1995). Propionate levels in both the foot (0.13±0.09 μmol g^{-1} fresh mass) and the adductor muscles (0.25±0.11 μmol g^{-1} fresh mass) were already significantly increased after 1 h of N_2 incubation compared to the controls (0.04±0.04 μmol g^{-1} fresh mass and 0.05±0.05 μmol g^{-1} fresh mass, respectively). After more than 24 h of incubation the concentration in all tissues as well as in the mantle cavity fluid was significantly elevated, reaching a maximum plateau after the second day of incubation of approximately 1 μmol g^{-1} fresh mass for the tissues and 1 μmol ml^{-1} in the mantle cavity fluid (Fig. 7A; foot, 1.19±0.87 μmol g^{-1} fresh mass; adductors, 1.07±0.66 μmol g^{-1} fresh mass; gills, 0.78±0.18 μmol g^{-1} fresh mass; mantle cavity fluid, 0.97±0.87 μmol ml^{-1}). Acetate, in contrast, did not accumulate in the tissues; after at least 24 h of N_2 incubation only the concentrations inside the mantle cavity fluid were significantly elevated (at 24 h, 0.28±0.19 μmol ml^{-1}; at 48 h, 0.55±0.43 μmol ml^{-1}; controls, 0.11±0.04 μmol ml^{-1}). Concentrations of succinate, on the other hand, reached a significantly elevated level in all tissues within the first 2 h of N_2 incubation (foot, 1.13±0.51 μmol g^{-1} fresh mass; adductors, 2.90± 0.97 μmol g^{-1} fresh mass; gills, 0.75±0.28 μmol g^{-1} fresh mass) and remained at this level during prolonged incubations (Fig. 7B). However, succinate also accumulates inside the mantle cavity fluid, and this has not been previously demonstrated in clams. During the first hour of hypoxic incubation, succinate concentrations in the mantle cavity fluid had already increased to 1.19±0.91 μmol ml^{-1} and remained significantly increased thereafter. In fact, it appeared as if the succinate concentration in the mantle cavity fluid had not reached maximum levels, even after the longest (72 h) incubations (Fig. 7B).

Unlike the N_2-incubated animals, clams removed from the Mosselmonitor® (laboratory constant conditions, see above) without hypoxic stress, did not produce propionate, even after prolonged periods of valve closure of up to several days. In contrast to propionate, during 'voluntary' valve closure the amounts of succinate accumulated by *C. fluminea* in its tissues were equal to those accumulated in the N_2-incubation experiments (Fig. 8), e.g. after 2–3 days of valve closure, succinate levels were 1.85±0.49 μmol g^{-1} fresh mass (foot), 2.26±0.58 μmol g^{-1} fresh mass (adductors) and 1.18± 0.53 μmol g^{-1} fresh mass (gills). But the onset was delayed; there were no elevated concentrations of succinate in the tissues after 2–5 h of 'voluntary' valve closure: 0.56± 0.18 μmol g^{-1} fresh mass (foot), 1.48±0.47 μmol g^{-1} fresh

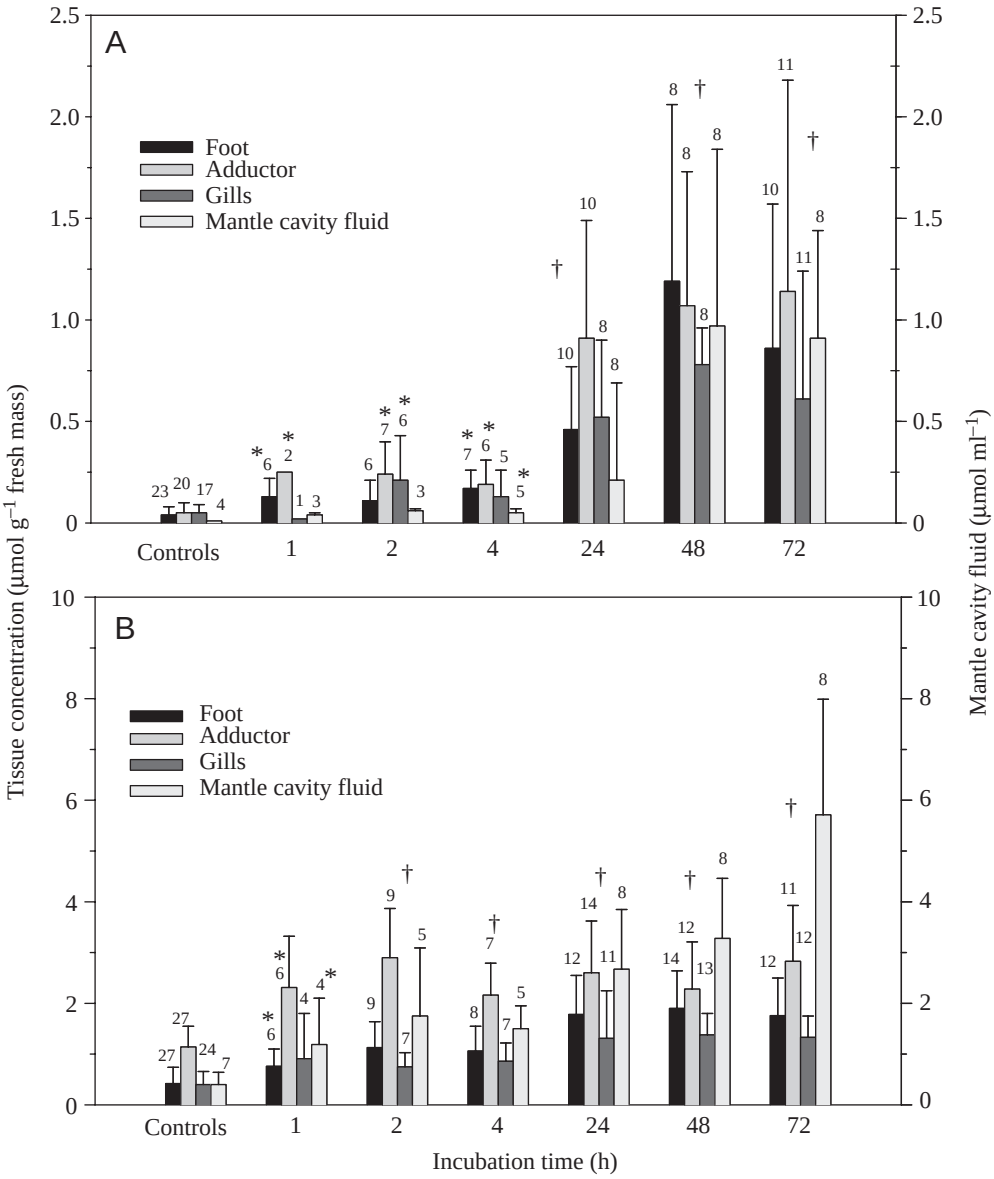

Fig. 7. Concentrations of (A) propionate and (B) succinate in foot, adductor muscles and gills (μmol g^{-1} fresh mass) and in the mantle cavity fluid (μmol ml^{-1}) of *C. fluminea* in controls and after N_2 incubation. Values are means ± S.D.; numbers above the bars indicate the number of animals analysed. *Significant difference (P<0.05) compared to the controls; †significant difference (P<0.05) of a whole group of tissues from the controls.

mass (adductors) and 0.52±0.25 μmol g^{-1} fresh mass (gills). As for the hypoxic incubated specimens, clams with 'voluntarily' closed valves transported succinate from the mitochondria into the mantle cavity, where the concentrations steadily increased up to 5.66 μmol ml^{-1} after more than 4 days of valve closure (Fig. 8).

Discussion

The Asian clam *Corbicula fluminea* is a conspicuous neozoan, which has invaded many freshwater habitats in the United States and in Europe. It is quite common in many European rivers and in the River Rhine it occurs at densities of 350–600 animals m^{-2} (Meister, 1997). Due to this enormous presence in natural freshwater habitats, but also because of its density in man-made water channels and water-supply pipes, there is considerable interest in the behaviour and propagation of *C. fluminea* (reviewed by Doherty and Cherry, 1988). We therefore wished to find out how the clam's energy metabolism contributes to the high fecundity of this animal and thus its wide propagation.

Previous experiments suggested that bivalves remain predominantly open within a suitable environment. However, consistent with some earlier studies on *C. fluminea* (Doherty et al., 1987; Ham and Peterson, 1994; Allen et al., 1996; Tran et al., 2003), this study clearly confirms that extended periods of valve closure, frequently up to 10–12 h, are part of the natural behaviour pattern of this clam (Fig. 3A). Furthermore, in the Rhine its valve movements followed a circadian rhythm from April until October (Fig. 3), accompanied by a 90% decrease in metabolic rate during valve closure. Moreover, the almost exactly matching rates of heat dissipation and oxygen consumption (Fig. 5), as well as the integrated energy demands of the corresponding intervals (Fig. 6) suggested that the clams' energy metabolism remained aerobic almost all the time. However, under near-anoxic conditions, *C. fluminea* did accumulate typical anaerobic end products such as succinate and propionate in its tissues, as well as in the mantle cavity fluid, and the concentrations of these metabolites were already significantly elevated within the first hours of incubation (Fig. 7). But, in contrast to the nitrogen-incubated animals, aerobically incubated specimens removed from the Mosselmonitor® while their shells were closed did not produce propionate, and the onset of succinate accumulation was delayed for several hours (Fig. 8). After more than 2 days of valve closure, however, the concentrations of succinate in the tissues

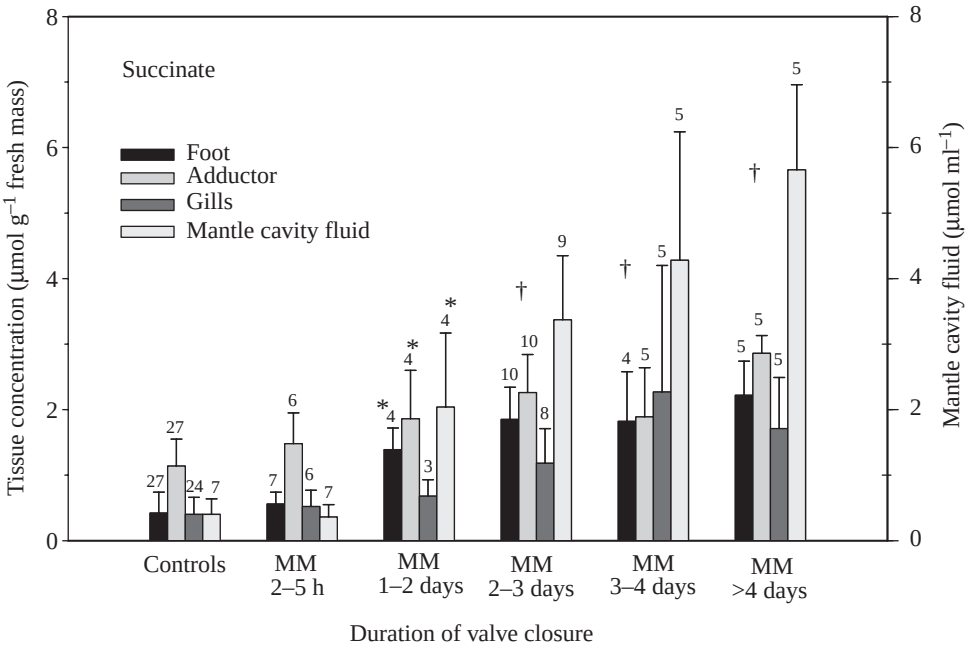

Fig. 8. Concentrations of succinate in foot, adductor muscles and gills (µmol g^{-1} fresh mass) and in the mantle cavity fluid (µmol ml^{-1}) of *C. fluminea* in controls and after different times within 'voluntarily' closed valves. Clams were taken out of the Mosselmonitor® (MM), where they experienced a permanent flow of well-aerated artificial freshwater at 15°C without light. Values are means ± S.D.; numbers above the bars indicate the number of animals analysed. *Significant difference (P<0.05) compared to the controls; †significant difference (P<0.05) of a whole group of tissues from the controls.

Table 1. *Aerobic metabolism continues for hours inside* C. flumina *with closed valves (two examples)*

C. fluminea (mass)	200 mg DM=1.4 g FM	1000 mg DM=7 g FM
Volume (ml)		
Mantle cavity fluid	0.5–1.0	1–2
Tissue[a]	1.4	7
Normoxic O_2 content at 15°C (µmol O_2)[b]	0.60–0.76	2.5–2.8
Oxycalorific equivalent (J)[c]	0.27–0.34	1.1–1.3
Metabolic rate at 15°C[d]	19 µW=0.07 J h^{-1}	40 µW=0.14 J h^{-1}
Duration until O_2 is depleted (h)	4–5	8–9

DM, dry mass; FM, fresh mass.
[a]1 g FM tissue=1 ml.
[b]O_2 content is 0.315 µmol ml^{-1}.
[c]Oxycalorific equivalent=0.45 J µmol^{-1} O_2.
[d]From the equation $MR=1.65x^{0.46}$, where MR=heat dissipation (µW) and x=dry mass (mg).

(2–3 µmol g^{-1} fresh mass) and mantle cavity fluid (4–6 µmol ml^{-1}) in the specimens also reached similar values to those measured under conditions of hypoxic stress, even though the animals were still in aerobic water.

To confirm our assumption that *C. fluminea* saves energy during valve closure in normoxic water, we calculated how long the enclosed oxygen could fuel energy metabolism during voluntary valve closure. Assuming approximately normoxic conditions at the moment of valve closure and knowing the volume of the mantle cavity fluid as well as the fresh mass of the soft body, it is possible to estimate the amount of oxygen trapped inside the closed valves. From the oxycaloric equivalent and the known metabolic rate occurring during valve closure, it can be calculated that *C. fluminea* remains aerobic for approximately 4–9 h after valve closure, depending on the size of the individual (Table 1). This result confirms our assumption that larger specimens hardly become anaerobic at all during the usual daily period of valve closure.

The metabolic rate of *C. fluminea* declines to 10% during valve closure, which is similar to the 90% reduction in heat dissipation observed for *Pisidium amnicum* and *Sphaerium corneum* by Holopainen and Penttinen (1993). Because these authors could not demonstrate any significant difference in the metabolic rates of specimens with closed valves under normoxic and anoxic conditions, they concluded that these Pisiid clams quickly became anaerobic after valve closure. By contrast, our measurements for *C. fluminea* provide clear evidence that this clam is able to remain aerobic for several hours during valve closure. The tremendous reduction in the clams' metabolism after closing the valves saves a great amount of energy and substrates compared to the tenfold higher metabolic rate when the valves are open. Such a large decrease in metabolic rate, down to 10% or less of the standard metabolic rate (SMR), which is defined as the metabolic rate at rest, constant temperature, normoxia and without food (Grieshaber et al., 1994; Hulbert and Else, 2000), has

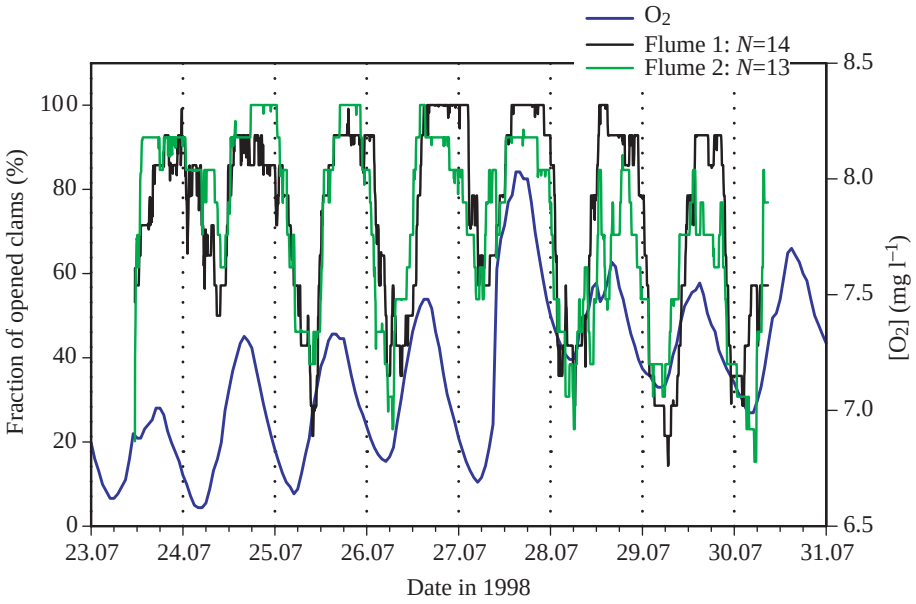

Fig. 9. O_2 content (O_2-data from North Rhine–Westphalia State Environment Agency) and valve closure behaviour of *C. fluminea* (27 specimens in parallel) inside the Rhine at Bad Honnef during July 23–31 1998, recorded by the Dreissena-Monitor®. Illustrated are the proportions of opened clams inside the two parallel flumes of the monitor. Dotted lines indicate the change of date, 0:00 h, water temperature varied between 22°C and 24°C.

frequently been described among invertebrates facing severe stress (for a review, see Guppy and Withers, 1999). A reduced metabolism needs less energy and fewer substrates, which is especially important if an animal has to rely on inefficient anaerobic pathways (Hochachka and Guppy, 1987; Grieshaber et al., 1994). However, the ability to close the valves and thus protect the animal from any major environmental stress, such as predators, water pollution or contamination of the gills, along with the concomitant reduction in the metabolic rate in an aerobically respiring animal, saves an enormous amount of energy and can be particularly beneficial during periods of starvation.

In addition to *C. fluminea* (Allen et al., 1996; Tran et al., 2003) and the Pisiid clams *Sphaerium corneum* and *Pisidium amnicum* (Holopainen and Penttinen, 1993), long periods of valve closure in conditions that are not obviously stressful, lasting at least several hours, have also been reported for several other bivalve species, e.g. *Anodonta cygnea* (Salanki, 1965), *Crassostrea virginica* (Higgins, 1980), *Mytilus edulis* (Kramer et al., 1989) and *Dreissena polymorpha* (Borcherding, 1992). Unfortunately, continuous and simultaneous direct recordings of heat dissipation, oxygen consumption and biochemical studies during such ‘voluntary’ valve closures are lacking in these species.

As revealed by the analysis of anaerobic end products, *C. fluminea* nevertheless does become anaerobic during extended periods of valve closure (Fig. 8), presumably after the enclosed oxygen is depleted. To our surprise, however, succinate produced anaerobically is only processed into propionate under conditions of artificial hypoxia (Fig. 7). The production of propionate, *via* methylmalonyl-CoA, is the most efficient anaerobic pathway, yielding more ATP than during anerobic glycolysis and removing most protons from the given storage substrate (Hochachka and Guppy, 1987; Grieshaber et al., 1994). Moreover, during ‘voluntary’ valve closure of more than a day, *C. fluminea* transports the accumulating succinate out of the tissues, instead of processing it further into propionate. Perhaps protons are discharged by cotransport with succinate into the mantle cavity. Regardless of the mechanism, succinate levels accumulated in the mantle cavity fluid clearly exceed the concentration inside the tissues (Figs 7 and 8). We think this is another strategy for saving valuable substrates under anaerobic conditions, because some of this succinate may be reabsorbed when the valves open again. The morphology of *C. fluminea* ensures that the succinate-enriched pallial water has to pass the gills before leaving the clam. Its mantle is closely connated, with only narrow slits for the foot and the adductor muscles (Kraemer, 1979). In addition, the typical gill structure of the Eulamellibranchia ensures that the whole water current has to pass the gill tissues *via* the mantle cavity.

But what are these energy and substrate savings needed for? So far all the data portend to some kinds of unfavourable conditions underlying these energy-saving strategies. First of all, the need to save energy depends on the energy balance of the metabolism, characterised by the available food and stored substrates on the one hand and the costs of growth, reproduction and sustenance on the other. There is only one reason that could explain the need for saving substrates: the clams are unable to get enough food out of the water to fulfil their requirements. But how is this possible while they exhibit such high productivity? A closer look at their main food supply, the phytoplankton, reveals that it has declined during recent years. Data from the North Rhine–Westphalia State Environment Agency reveal that chlorophyll *a* concentrations at Bad Honnef were mostly less than 10 μg l^{-1} (Table 2), especially after the spring bloom had disappeared. According to Foe and Knight (1985), *C. fluminea* is already food-limited at chlorophyll *a* concentrations below 20 μg l^{-1}. Since 1993, such high amounts are only found during the annual algal bloom in spring. However, these chlorophyll concentrations (Table 2) derive from weekly samples taken around 10:00 h and they may be higher in the afternoon. In the absence of better data on chlorophyll *a* concentration, the hourly available oxygen concentration may be used to indicate the diurnal character of the phytoplankton concentrations inside the river. Moreover, the O_2-maxima fit perfectly with the times that *C.*

Table 2. *Chlorophyll a concentration in the Rhine at Bad Honnef*

	[Chlorophyll *a*] (μg l^{-1})		
Year	Whole year	CW 10–20	CW 25–35
1990	20.7	37.1	35.7
1991	22.8	32.8	34.9
1992	19.2	33.6	21.8
1993	12.1	43.4	4.0
1994	7.3	15.3	10.3
1995	7.0	14.2	9.4
1996	9.1	33.3	2.7
1997	6.4	18.7	2.8
1998	**3.0**	**4.3**	**3.1**
1999	8.4	15.1	12.0
2000	3.7	10.4	2.2

Values are means of whole years and the algal blooms in spring (calendar weeks CW 10–20) and summer (CW 25–35). Data from North Rhine–Westphalia State Environment Agency.

fluminea kept its valves opened, indicating that the clams only filter feed during those hours of a day where the highest amount of food is available (Fig. 9). Nevertheless, the diurnally changing oxygen tension might be involved in sustaining their circadian rhythm, although ambient oxygen concentrations seemed to be too high (always above 60% O_2 saturation) to impose any serious stress on the bivalves.

Further support for a feeding-dependent rhythm comes from the work of Borcherding (1992) and Williams et al. (1993), who reported similar circadian rhythms in another freshwater species, *Dreissena polymorpha*, and an Australian intertidal clam, *Austrovenus stutchburyi*, respectively. Feeding experiments at a constant oxygen supply revealed that both bivalves altered their rhythm of valve closure according to the shift of food supply (Borcherding, 1992; Williams and Pilditch, 1997). So it seems that the available food rather than the ambient oxygen tension controls the rhythm. It is also well known that bivalves lengthen their periods of valve closure under poor nutritional conditions (Higgins, 1980; Williams et al., 1993). Finally, Borcherding (1992) demonstrated that the rhythm in *Dreissena polymorpha* vanished if sufficient food was supplied. Hence, the difference between the chlorophyll *a* maxima in the afternoon and minima during the nights seems to be high enough to induce the circadian oscillation in the valve closure behaviour of *C. fluminea*. Especially at relatively high temperatures during the summer, the daily chlorophyll maxima alone deliver enough food for the clams to sustain their expensive metabolism, and it seems to be more economic to shut it down at night. Nevertheless, there must be components of endogenous rhythm as well, because those rhythms persisted, for at least some days, under the constant conditions in the laboratory (Williams and Pilditch, 1997; this study). However, further work is needed to confirm the influence of food and/or oxygen concentrations on circadian rhythms of bivalves, as well as the mechanisms involved in the control of endogenous, circadian rhythms.

But what is so expensive in the clam's SMR that it is worthwhile depressing metabolism and isolating itself from the environment instead of increasing the amount of food obtained from the water current? Without considering controversial opinions on bivalves' ability to control their filtration rate or to sort food particles of different nutritional values (Jørgensen, 1996; Ward et al., 1997; Bayne, 1998), there is no doubt that the gills dissipate a great part of the metabolic energy because they are extremely densely covered by different kinds of cilia (Clemmesen and Jørgensen, 1987; Riisgård and Larsen, 2000). Thus, in addition to the most important energy consumers such as protein turnover, ion regulation and the proton leak over the inner mitochondrial membrane, each comprising approximately 20% of the oxygen consumption of the SMR (Hochachka and Guppy, 1987; Brand et al., 2000; Pakay et al., 2002), the gills are another important target for saving energy during a metabolic depression. Bivalve gills are highly developed organs whose main function is the filtration of food particles, with gas exchange now regarded as less important (Jørgensen, 1990). Especially under conditions of low food supply it seems reasonable to cut down the costs of filter feeding by valve closure, because inside closed valves the cilia are thought to cease beating (Newell and Branch, 1980; Jørgensen, 1990). This is presumably relevant for every bivalve species, but *C. fluminea* seems to coordinate the times of valve closure with those of low food availability and *vice versa* through its circadian rhythm, so that the concomitantly depressed metabolism can be sustained aerobically for about 4–9 h (Table 1).

We conclude that by adopting an intermittent metabolism, rhythmically coordinated during the summer and accompanied by an approximately tenfold reduced aerobic metabolic rate during valve closure, efficient exploitation of the available food resources is guaranteed. Thus, saving substrates during periods of low food supply and ongoing aerobic energy metabolism during normal periods of valve closure may support *C. fluminea*'s high productivity, namely its rapid growth and high fecundity.

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