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EFFECTS OF TRANSPORTATION STRESS AND RECOVERY AND SAMPLE TREATMENT ON CA^{2+} AND GLUCOSE CONCENTRATIONS IN BODY FLUIDS OF *ANODONTA ANATINA* (LINNAEUS)

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ABSTRACT Removal of lake mussels from a river and 15–20 minutes storage in river water in a bucket caused Ca^{2+} concentrations in the haemolymph and extrapallial fluid to increase significantly. Further storage and transport of mussels in a small quantity of river water to the laboratory increased the haemolymph and extrapallial fluid calcium concentrations, which were equal to each other and normally about 4.5–6 mmol L^{-1} in summer, to nearly double, about 8–12 mmol L^{-1}. In mussels maintained in the laboratory at 4°C for 2 weeks or 2 months, the elevated calcium concentrations decreased but often remained at about 6–8.5 mmol L^{-1}. If mussels were returned to the river, the calcium concentrations were normalized. In the mantle cavity fluid, Ca^{2+} concentration (which normally was around 4 mmol L^{-1}) rose more slowly, but could reach that of the haemolymph after a transportation stress. During and after the transport of mussels, calcium leakage to the surrounding water was apparent (post-transport leakage of 0.07 μmol g^{-1} h^{-1} was measured over one week).

Glucose concentrations in mussel fluids showed a clear stress reaction when mussels were transferred to the laboratory. Haemolymph and extrapallial fluid glucose concentrations, which normally were very low (50–140 μmol L^{-1}) rose about 5–7-fold, and that of mantle cavity fluid rose about 5-fold. In the laboratory, the elevated glucose concentrations decreased but tended to remain above normal. In a mussel group which had experienced additional stress, haemolymph and extrapallial fluid glucose concentrations remained at the 3-fold elevated level, 300–320 μmol L^{-1}, for at least 2 weeks.

Storage of mussel fluid samples in a freezer significantly decreased measurable Ca^{2+} concentrations due to precipitate formation. Measurable glucose concentrations tended to increase (but not significantly) during as short a time as a few hours storage, particularly when no perchloric acid was added.

KEY WORDS: Lake mussel, *Anodonta anatina*, calcium, glucose, body fluids, transportation stress

INTRODUCTION

The haemolymph calcium concentration in bivalves has frequently been studied with regard to acid stress or emersion (Malley et al. 1988, Pynnönen 1990, 1994, Byrne and McMahon 1991, Byrne et al. 1991). It is a well-known phenomenon that in bivalves experiencing respiratory, metabolic or mixed acidoses $CaCO_3$ reserves are dissolved to buffer the protons and calcium concentration in the haemolymph increases. Fewer empirical studies have been done on the effects of stress on calcium concentrations in the extrapallial fluid or in the mantle cavity fluid. As early as 1935, Dotterweich & Elssner found that, during anaerobiosis, calcium concentration in the extrapallial fluid of *Anodonta cygnea* increased. They concluded that the extra calcium originated from the shell. Later, Crenshaw (1972) analysed ions in the extrapallial fluid of 3 marine bivalves and showed that the calcium concentration changed with the opening and closing of the valves. Heming et al. (1988) studied the effects of emersion and immersion in acid water on the composition of mantle cavity fluid in *Margaritifera margaritifera*. They found that also in the mantle cavity a carbonate buffer system operated, involving reactions with $CaCO_3$ reserves of the mussel and release of CO_2.

Very little is known about the 'natural fluctuation' of Ca^{2+} concentrations in the fluids of bivalves because, in many experiments, control values used for comparison have been obtained from bivalves transported to a laboratory. Transportation conditions, as well as different types of intervention (even handling according to Dietz 1979) can disturb ionic concentrations in bivalve fluids. Due to the fact that experiments will, to an increasing degree, be carried out in a laboratory, it is very important to know how the transportation of bivalves to a laboratory affects their physiology.

The most obvious growing season of the shell of *Anodonta anatina* (L.) in a river in southern Finland is in midsummer but it is not known how long the annual growing season actually lasts (Pekkarinen 1991). In 1994, a study was begun into the seasonal variations in the calcium concentrations of the body fluids of *A. anatina* and how transportation and storage of mussels under laboratory conditions may affect the composition of the fluids. Some of the results have been published in a congress abstract (Pekkarinen and Suoranta 1994), and the seasonal cycle of calcium concentrations in the body fluids of *A. anatina* will be described in another paper (Pekkarinen 1995).

Because sampling of the extrapallial and mantle cavity fluids and subsequent treatment of all the bivalve fluids in the field immediately after emersion are somewhat problematic, different sampling methods were tested, and effects of aging and centrifugation on the fluids were investigated. It was hypothesised that the glucose concentration could be utilized to assess the purity of the fluids: it would be low in the extrapallial fluid and negligible in the mantle cavity fluid. It was discovered that the hypothesis was incorrect but there were indications that the glucose concentration might be useful in indicating stress in this species.

MATERIALS AND METHODS

Experimental Animals and Their Collection

Lake mussels (*A. anatina*) were collected either by dragging the river bottom or, for 'natural' fluid analyses in the field, one at

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a time by hand from depths of about 0.3 to 0.5 m from the Vantaa River in Helsinki (southern Finland) in summer 1994. The sampling was done around noon. Calcium concentration in the river water varied between 13.5 and 19.5 mg L^{-1} (0.34 and 0.49 mmol L^{-1}) on different sampling days. The lengths and status of the mussels, dates of collection and sampling and river water temperatures are shown in Table 1. All parasitized or otherwise diseased (mainly with a pustular disease) (Pekkarinen 1993) mussels were excluded from the calcium and glucose calculations.

'Natural' Bivalve Fluid Samples and Their Transport to the Laboratory

The mussel groups used in the "normal analyses" are indicated with asterisks in Table 1. Sampling was begun within one minute of collecting a mussel. The mussel was wiped with tissue paper, and the haemolymph sample (0.4 to 0.9 mL) was taken with a 19 to 23 gauge needle, fitted to a 1 mL syringe, inserted between the valves into the sinus of the posterior adductor muscle.

Samples of the mantle cavity fluid were taken by forcing the shell valves apart slightly with a knife and allowing a small amount of fluid to drain into an Eppendorf tube.

At first, taking extrapallial fluid samples from unopened mussels by inserting a needle between the valves into the central extrapallial space of one valve was tried. This risked breaking the outer epithelium of the mantle, when the needle was moved in order to assess its position or to enhance free suction of fluid. It was also possible that the wound in the mantle could consequently tear and allow the mixing of mantle cavity fluid with extrapallial fluid. Later, samples of extrapallial fluid were taken after cutting the adductor muscles of the bivalve. Care was taken not to break the mantle of 1 valve. The mantle was wiped with tissue paper, and paper was also used to prevent haemolymph from the adductor muscle entering the mantle cavity. The extrapallial fluid sample was taken by puncturing the mantle.

The fluid samples were transported to the laboratory in styro boxes cooled with freezing blocks. The total time taken for sampling and transport was about 2 to 2.5 hours.

Treatment of the Fluid Samples

The fluid samples were first centrifuged (6000 rev · min^{-1}, 5 minutes) in order to eliminate cells or mud or clay particles. Then, an equal volume of 0.33N perchloric acid was added to the sample aliquots for glucose analysis, and, after mixing, the samples were centrifuged as above. The supernatant was used for the analysis. The glucose analysis was done within 1 day of collection and the calcium analysis (from non-deproteinized supernatants) within a few days (the samples were kept in closed vessels at 4°C). Samples from a few mussel groups used for calcium analysis were stored frozen (−18°C), and a correction factor (Fig. 1) was used in the calculation. Both the colorimetric glucose and calcium analyses were done using kits (Calcium C code No. 997–21809, Wako Chemicals GmbH; Glucose GOD-Perid Methode, Boehringer, Mannheim GmbH).

Experiments on Sample Treatment

For sample treatment experiments, fluid analyses from mussel groups collected on June 20, July 22, July 25, July 28 and August 25 were used (Table 1). Detailed descriptions of the treatments are presented in Figures 1 and 2.

TABLE 1.

Lake mussels (*Anodonta anatina*) from the Vantaa River Used in the experiments of this study in summer 1994.

				All Mussels				Mussels Used in the Results			
Group	Date of Collection	Sampling Date	Water t (°C)	*N*	Length (mm)	Deleted Parasitized	Deleted Otherwise Diseased	Length (mm)	*N*	♂♂/♀♀	Gravid Mussels − or +
1*	1.7.	1.7.	17.5	7	78.5 ± 4.2	–	–	78.5 ± 4.2	7	7/0	–
2	20.6.	4.7.		8	83.8 ± 4.0	–	1	85.0 ± 4.5	7	7/0	–
3*	5.7.	5.7.	19.0	9	81.7 ± 2.0	–	1	82.2 ± 2.2	8	7/1	–
4	20.6.	5.7.		10	80.4 ± 3.8	–	1	80.1 ± 4.2	9	5/4	–
5*	22.7.	22.7.	22.0	24	81.5 ± 1.7	6[a]	2	81.8 ± 2.2	16	13/3	+
		4.8.									
6	22.7.	22.7.		10	77.1 ± 1.9	2	–	77.5 ± 2.4	8	4/4	+
7*	25.7.	25.7.	22.5	10	78.2 ± 3.1	–	1	78.3 ± 3.5	9	5/4	+
8	25.7.	2.8.		9	77.3 ± 3.4	–	3	78.9 ± 4.4	6	4/2	+
9*	28.7.	28.7.	23.0	10	78.4 ± 2.2	1	–	78.6 ± 2.5	9	5/4	+
10	28.7.	28.7.		10	77.2 ± 2.3	2	–	77.1 ± 2.5	8	2/6	+
11	28.7.	28.7.		11	75.0 ± 1.7	1	1	75.4 ± 1.8	9	5/4	+
12	28.7.	28.7.		9	76.2 ± 2.7	–	1	76.2 ± 3.1	8	6/2	+
13	28.7.	10.8.		10	76.9 ± 4.2	–	1	78.2 ± 4.5	9	7/2	+
14	20.6.	24.8.		10	65.8 ± 1.6	–	–	65.8 ± 1.6	10	8/2	–
15*	25.8.	25.8.	16.0	12	83.7 ± 3.2	–	1	84.6 ± 3.3	11	7/4	+
16	25.8.	25.8.		13	75.6 ± 2.5	3	1	75.2 ± 2.9	9	7/2	+
17	25.8.	9.9.		10	68.4 ± 2.6	–	1	69.7 ± 2.5	9	8/1	+
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* Mussel groups used for 'normal' analyses in the field.

[a] Parasitized mussels considered separately.

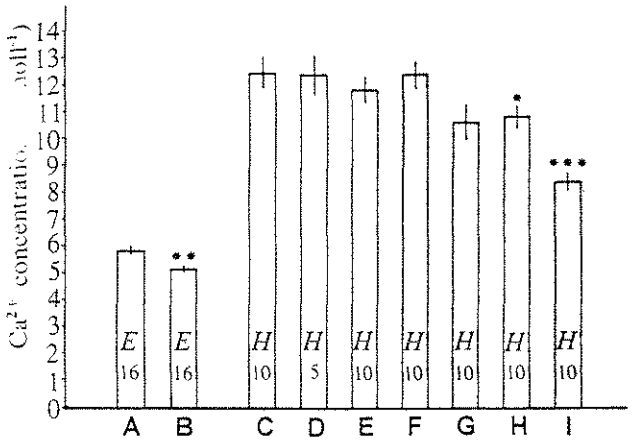

Figure 1. Effect of sample treatment on measurable Ca^{2+} concentration in the extrapallial fluid (*E*) and haemolymph (*H*) of *A. anatina* (results from groups 9–13 = first series and 6 = second series). (A) Refrigerated (4°C) extrapallial fluid centrifuged (6000rpm, 5 min) 2.5 hours after sampling and calcium measured from fresh supernatant. (B) Supernatant stored frozen (−18°C) and calcium measured after 7 weeks. (C) Haemolymph sampled in the laboratory and calcium measured immediately. (D) Calcium measured from the same haemolymph after 2.5 hours' storage in a refrigerator. (E) An aliquot of the haemolymph sample after 2.5 hours' storage centrifuged and calcium measured from the supernatant. (F) Calcium measured from the haemolymph after 1 week storage in the refrigerator. (G) The centrifuged supernatant has been stored in the refrigerator for 1 week before calcium analysis. (H) After 1 week's storage in the refrigerator (see F) haemolymph stored frozen for 6.5 weeks. (I) The supernatant (see G) stored frozen for 6.5 weeks. Numbers of samples are indicated in the lower parts of the columns. Comparison with A and C made with a 2-sided *t* test (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

Transportation of Mussels to the Laboratory and Their Maintenance in the Laboratory

The mussels were placed in buckets containing small amounts of river water (totally or almost totally immersed), transported for about 1 km in a cart and then by bus to the laboratory. This was done during the summer and the temperature of the mussels could not be regulated during transportation. In the laboratory, the mussels were kept in a dark room (at 4°C) in plastic 40-liter aquaria without sediment in running, charcoal-filtered, aerated tap water. The water calcium concentration was 10.8–19.2 mg L^{-1} (0.270–0.479 mmol L^{-1}). Several experiments were done during the summer and they are described as follows.

Recovery Experiment, June 20–July 5

The mussels in groups 2 and 4 (Table 1) had been kept in the laboratory from June 20 but the mussels in group 4 had been returned to the river on July 1. They were put in a net bag on the bottom of the river. The mussels from groups 2 and 4 were sampled on July 4th and 5th, respectively, and their calcium and glucose concentrations were compared with each other. 'Normal' calcium and glucose concentrations from groups 1 and 3 (on July 1 and July 5, respectively) (Table 1) were used for additional comparison.

Experiment with Repeated Sampling and Effect of Sediment, July 22–August 4

Haemolymph samples (0.5 mL) from the individuals in group 5 were taken in the field on July 22 (first sampling, 5_1). These mussels and another mussel group (6) were brought to the laboratory in a small amount of water (partially emersed). The mussels in group 6 were sampled in the laboratory after arrival. The mussels in group 5 were randomly divided into 2 subgroups, 5_a, and 5_b, and were put into buckets, 5_a without sediment and 5_b with clay mud from the river bottom. Aerated, charcoal filtered water (4°C) was intermittently run over the mussels. Dead animals were removed when noticed. The mussels from the subgroups of 5 were sampled again on August 4 (second analysis, 5_2; all fluid samples).

Leakage Experiment, July 25–August 2

The mussels in group 8 were brought to the laboratory on July 25 and put in individual jars with charcoal-filtered tap water (5 × mussel weight). The jars were not closed tightly and the water was not aerated. The calcium concentration in the water was measured 6 times during the 8-day experiment. The average amount of calcium leakage over 7 days was calculated by using the results from 6 healthy mussels. No more than two of the nine mussels survived for 8 days, and thus haemolymph samples could be taken only from these two. Initial events in mussel haemolymph calcium

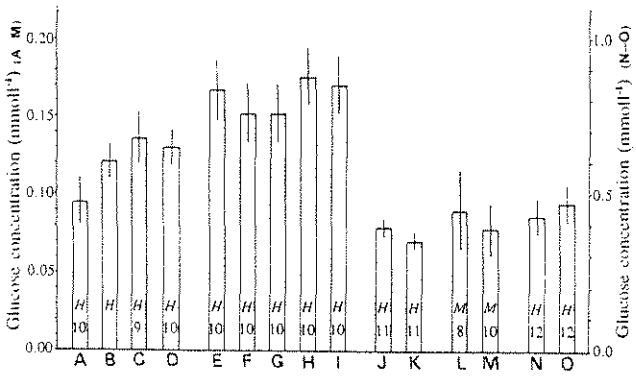

Figure 2. Effect of sample treatment on measurable glucose concentration in the haemolymph (*H*) and mantle cavity fluid (*M*) of *A. anatina* (results from mussel groups 7, 14, 15 and 16, see Table 1; different experiment series separated by broader intervals). (A) Protein precipitant (0.33N perchloric acid) added immediately to haemolymph (1:1) in the field, and the sample centrifuged (6000rpm, 5 min) after transporting to the laboratory (2.5 hours) and glucose measured from the supernatant (stored at 4°C) on the same day. (B) Haemolymph centrifuged after cold storage (4°C) for 2.5 hours, and perchloric acid added and supernatant used for glucose analysis. (C) The haemolymph supernatant (see B) stored 2 days in the refrigerator before addition of perchloric acid and glucose analysis from the supernatant. (D) The haemolymph supernatant (see B) stored frozen (−18°C) for 2 weeks before adding perchloric acid and glucose analysis. (E) Haemolymph from laboratory-maintained mussels diluted immediately with distilled water (1:1), and glucose measured. (F) Fresh 0.33N solution of perchloric acid added (1:1) to haemolymph and glucose measured from supernatant. (G) As F but perchloric acid solution several months old. (H) Haemolymph centrifuged after 2.5 hours' cold storage and diluted with distilled water (1:1) before glucose analysis. (I) As H but perchloric acid and centrifugation instead of addition of distilled water. (J) As H. (K) As I. (L and M) As J and K but mantle cavity fluid as material. (N) As B but mussels brought to the laboratory (y scale at the right). (O) Haemolymph supernatant stored in a refrigerator for 1 day before addition of perchloric acid and glucose analysis. Two-sided *t* test did not reveal significant differences in any test groups.

concentration were hypothesised from results of groups 7 and 12 (see Table 1 and next paragraph).

Handling Stress Experiment, July 28–August 10

Fluid samples from the mussels in group 9 were taken in the field as the 'normal' samples (immediately after collecting) (Table 1). The mussels of group 10 were allowed to rest in a bucket of river water for 15 to 20 minutes and the mussels from group 11 were allowed to rest for 1.5 hr before sampling. The mussels in groups 12 and 13 were then brought to the laboratory. The mussels from group 12 were sampled about 5 hr after collection (warmed to the ambient air temperature, about 27°C). The remaining mussels (group 13) were kept in an aquarium for 13 days and then sampled (10th of August).

Long-Term Laboratory Maintenance, June 20–August 24

The mussels (group 14) were kept in the laboratory from June 20 to August 24.

Late Summer Experiment; Experiment Particularly to Study the Effect on Glucose Concentration, August 25–September 9

The mussels in group 15 were sampled in the field (August 25), those in group 16 at arrival at the laboratory and the mussels in group 17 after keeping in the laboratory for 15 days (on September 9).

Statistical Analyses

The results of measurements are presented as mean ± SEM. The calcium and glucose concentrations in different groups were compared with a 2-sided *t* test. p values <0.01 were considered as significant.

RESULTS

Sample Treatment

When samples of extrapallial fluid (kept at 4°C) were centrifuged 2.5 hours after collection and some aliquots of the supernatants were immediately used for calcium determination and other aliquots were stored at −18°C for seven weeks before calcium determination, it was found that the measurable calcium concentration decreased significantly ($p < 0.01$) during freeze-storage (Fig. 1A,B).

Apparent calcium concentrations in non-centrifuged haemolymphs did not change during either the 2.5 hour or 1 week storage of the fluid at 4°C (Fig. 1C,D,F). An additional storage period (6.5 weeks) at −18°C somewhat decreased the calcium concentration ($p < 0.05$) (Fig. 1H).

If the haemolymph was centrifuged after a 2.5 hour storage time (to simulate the waiting time normally resulting from the sampling of a group of mussels and the transportation of the samples to the laboratory) and calcium was measured in the cell-free supernatant, the concentration showed a slight decrease (Fig. 1E) but the decrease was not significant even after 1 week's storage of the supernatant at 4°C (Fig. 1G). If the supernatant was stored at 4°C for 1 week, kept at −18°C for 6.5 weeks and then the calcium was measured, a very significant decrease ($p < 0.001$) was observed (Fig. 1I). Frozen samples had become turbid and a precipitate was observed on the inner surfaces of the vessel walls.

Apparent glucose concentrations in the fluids tended to increase, although not significantly, during the storage of the fluids at 4°C as native samples or the storage of deproteinized supernatants from the fluids (Fig. 2). The highest increase was apparent within the first few hours after sampling. Immediate addition of the deproteinizing agent (0.33 N perchloric acid, 1:1) to haemolymph prevented the rise in glucose concentration.

Effects of Transporting Mussels to the Laboratory and Maintenance in the Laboratory

Calcium Concentrations

Throughout the study, calcium concentrations in the haemolymph and extrapallial fluid did not differ from each other. Instead, in most mussel groups there was a significant positive correlation between them.

Transportation of the mussels to the laboratory resulted in about 2-fold higher calcium concentrations in the haemolymph and extrapallial fluid (see the Ca^{2+} increases at the sites pointed to by bold black arrows in Figs. 3A,B). Keeping the mussels in the laboratory for 2 weeks only partially normalized these increased values (Figs. 3A,B). The calcium concentration in the mantle cavity fluid was usually significantly lower than that of the haemolymph but clearly higher than that of the surrounding water. During the transportation, the calcium concentration in the mantle cavity fluid rose to similar levels as in the haemolymph (Fig. 3B), and, during the maintenance of the mussels in the laboratory, it decreased to near normal level, with some indications of overshooting in the experiments in early July (Fig. 3A) and early August (Fig. 3B). Even after 2 months' maintenance of mussels in the laboratory, the calcium concentrations in the haemolymph and extrapallial fluid remained above the normal level (Fig. 3B, broken arrows).

When the mussels were returned to the river in a net bag, the increased calcium concentrations in the haemolymph and extrapallial fluid returned to normal but decreased significantly below the normal value ($P < 0.01$) in the mantle cavity fluid (Fig. 3A, arrows with white-cored heads).

In the mussel group for repeated sampling (group 5), there were originally in the first ('normal') sampling (the second bold black arrow in Fig. 3A) 24 animals. Later, dissection revealed that there were 6 parasitized mussels and two suffered from a severe pustular disease (Table 1). Although the mean haemolymph calcium concentration among 5 parasitized mussels did not differ from that of 16 healthy mussels, all parasitized mussels were excluded from the results throughout the whole study.

Thirteen mussels in group 5 survived to the second sampling after about 2 weeks (Fig. 3A, early August); the immediate after-transport maximal value was obtained from an additional group (group 6). Of the parasitized mussels, two survived and both diseased mussels survived. There were only 9 healthy mussels remaining to be used in the comparison of the first and second samples. Group 5_2 was further divided into 2 subgroups (5_a kept without sediment and 5_b kept in natural river sediment in the laboratory), and the numbers of healthy mussels surviving in the subgroups were five and four, respectively. Calcium concentrations in the haemolymph and the extrapallial fluid were slightly higher (but not significantly) in the group without sediment. It has to be noted that in all of group 5_2 calcium concentrations were very variable: between 1.66 and 15.38 mmol L^{-1} in the haemolymph

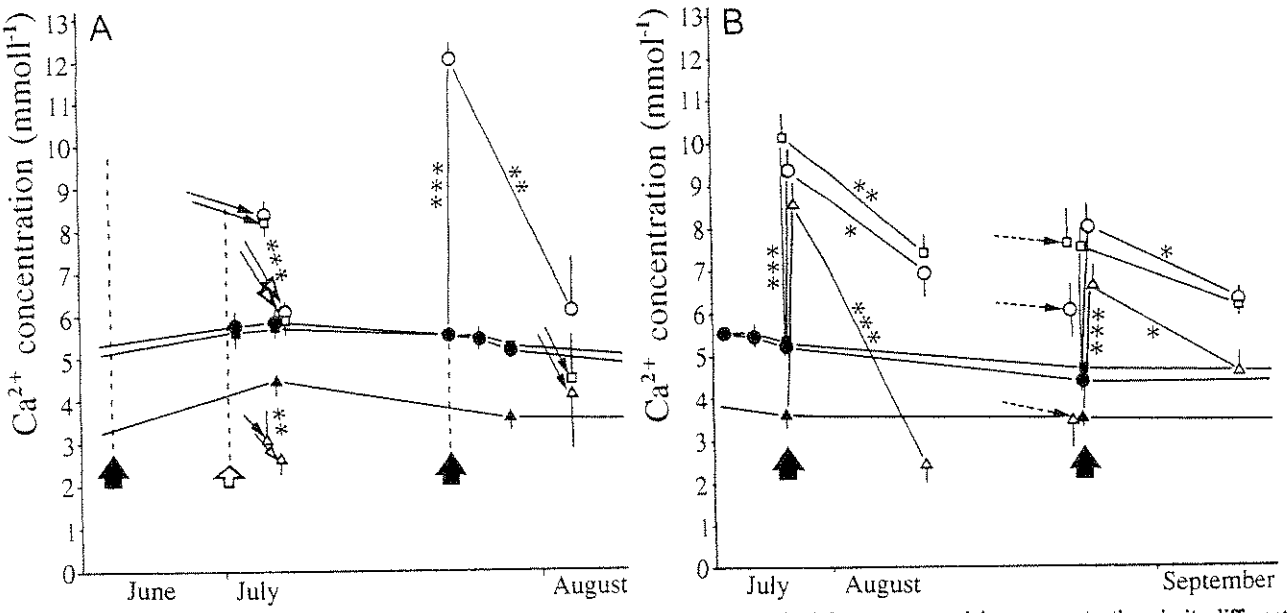

Figure 3. Effects of transport to (bold solid arrows) and maintenance of *A. anatina* in the laboratory on calcium concentrations in its different fluids in midsummer (A) and late summer (B). Solid symbols (●–● = haemolymph, ■–■ = extrapallial fluid, ▲–▲ = fluid in mantle cavity) indicate values measured in the field and open symbols, respectively, values measured in the laboratory (means with one SEM or two, or sometimes SEMs are hidden by the fluid symbols). Hypothetical courses are indicated with thin arrows (in long-term experiment with broken thin arrows, Fig. 3B, August). The values measured after returning mussels to the river (Fig. 3A, open bold arrow) are indicated with thin arrows with white-cored heads (in early July). Comparison of concentrations was made by 2-sided *t* test (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$).

(reflected in the large SEMs in Fig. 3A, the last values of the graph). In the parasitized mussels, the variation was similar.

In a transportation experiment (group 5), it was noticed that mussels leaked calcium into the transporting water. Therefore, a separate leakage experiment was done (using mussel groups 7, 8 and 12) (Fig. 4). After transporting the mussels (group 8) to the laboratory, the calcium concentration in the water (5 × mussel weight) in 6 jars (1 mussel per jar) increased tenfold, from 0.27 mmol L^{-1} to 2.77 ± 0.26 mmol L^{-1} by day 7 and to 3.33 ± 0.16 mmol L^{-1} by day 8. During 7 days, the mussels leaked calcium into the water at a rate of 485 ± 36 μg g^{-1} h^{-1} (0.072 μmol h^{-1}). Only 2 mussels survived until day 8, and their haemolymph

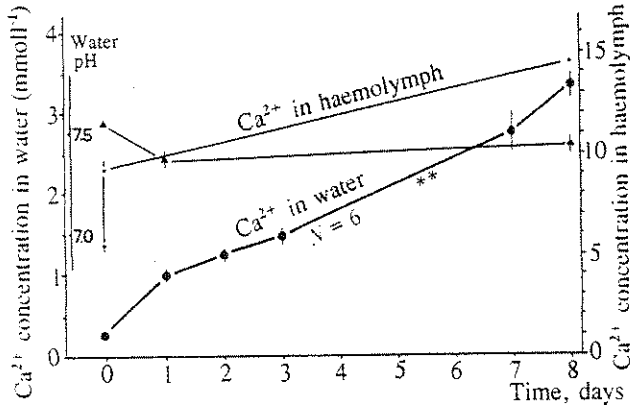

Figure 4. Calcium leakage experiment (mussel group 8). Change of calcium concentration in 5-fold mussel weight of stagnant (charcoal-filtered, 4°C) tap water during 8 days post mussel transport to the laboratory. Initial events in mussel haemolymph calcium concentration were hypothesized from results of groups 7 and 12. Mussel mortality in group 8 was greatest around day 7; the haemolymph calcium value in day 8 is from 2 surviving mussels.

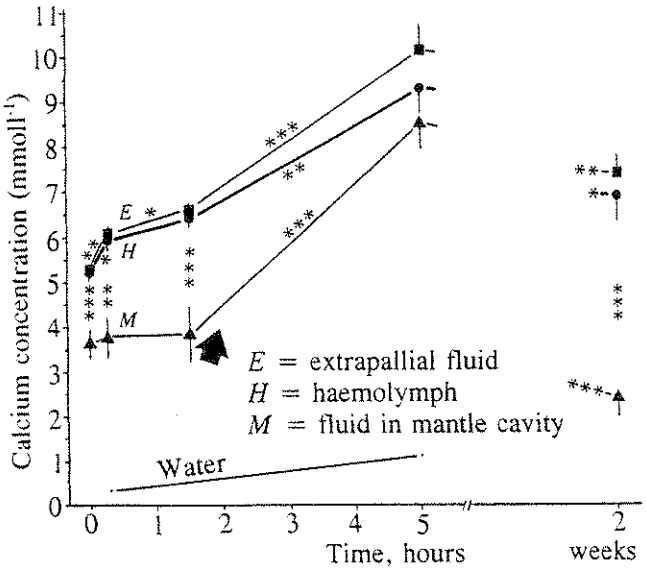

Figure 5. Handling stress experiment. Change of calcium concentrations in fluids of *A. anatina* as a function of time after collecting the mussels from the river and keeping them in river water in a bucket (groups 9–11) and after transporting (bold solid arrow) them (group 12) to and maintaining them in the laboratory (at 4°C) for 2 weeks (group 13). Calcium concentration was also measured in the transporting water (the lowest line). Statistical analyses as in Figure 3.

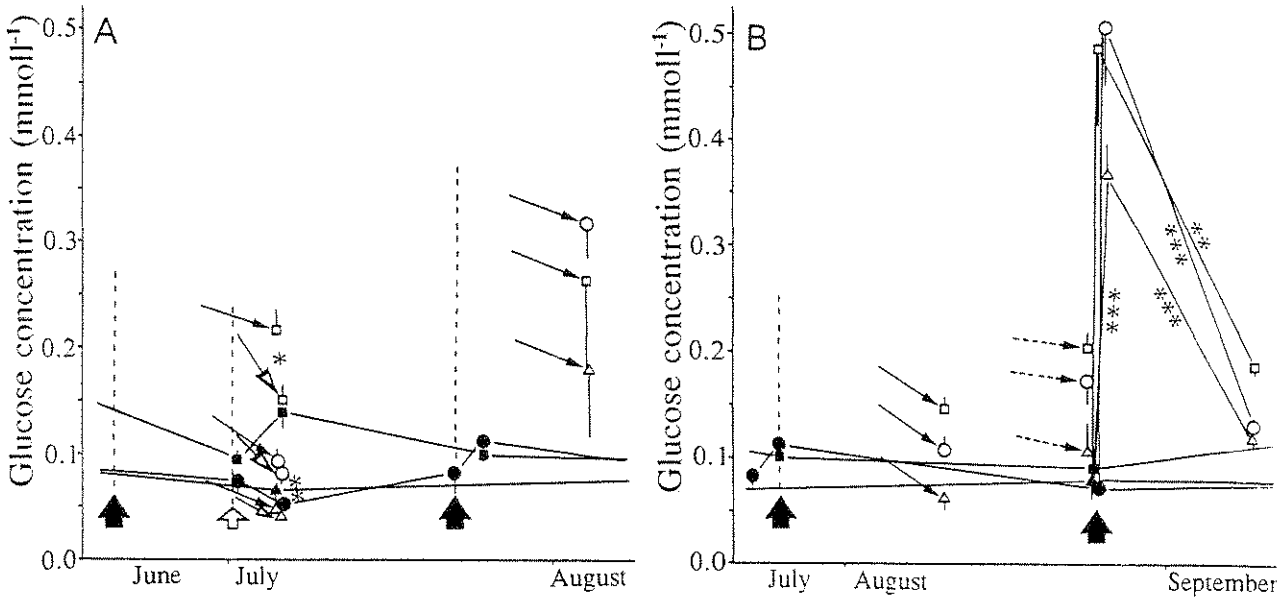

Figure 6. Effects of transport to (bold solid arrows) and maintenance in the laboratory of *A. anatina* on glucose concentrations in its different fluids (●–● = haemolymph, ■–■ = extrapallial fluid, ▲–▲ = fluid in mantle cavity) in midsummer (A) and late summer (B). Hypothetical courses are indicated with thin arrows. Bold open arrow = returning of mussels to the river; the values after it are marked with thin arrows with white-cored heads. See Figure 3.

calcium concentrations were 11.63 and 16.82 mmol L^{-1}. The mussels died with the foot extended.

The handling stress experiment (using mussel groups 9–13) (Fig. 5) was done in order to see whether just collection and 15–20 minute waiting time in a bucket of river water affected the calcium concentrations in the mussel fluids and how fast the calcium concentrations rose. A waiting time of 15–20 minutes caused a significant increase in the calcium concentrations of the haemolymph and extrapallial fluid, and further waiting and transporting of the mussels caused further increase in the concentrations. The calcium concentration in the mantle cavity fluid did not increase until transportation, and, after the transportation, it was as high as that of the haemolymph. The calcium concentration in the transporting water rose from 0.34 (concentration in the river water) to 1.14 mmol L^{-1}. After a 2-week maintenance of mussels in the laboratory, the calcium concentration in their mantle cavity fluid had decreased to below the concentration measured at the beginning of the experiment ($p < 0.05$) (Fig. 5). The same experiment is summarized in Figure 3B (late July–early August).

Glucose Concentrations

In an experiment in late summer 1994 (Fig. 6B, late August–early September), glucose concentrations in the haemolymph and extrapallial fluid rose to 5–7-fold during transport of mussels to the laboratory. In the fluid of the mantle cavity, a very significant increase in glucose concentration was also observed. After keeping mussels in the laboratory for 2 weeks, glucose concentrations in the haemolymph sometimes remained above normal values (Fig. 6A, both experiments; and 6B, second experiment) and sometimes approached the original values (Fig. 6B, first experiment). In the experiment with repeated sampling (Fig. 6A, late July–early August), the glucose concentration remained 3 times the original value. Even after a 2-month maintenance of mussels in the laboratory, haemolymph glucose concentration was significantly higher than that of mussels in the wild (Fig. 6B, broken arrow in late August). In the extrapallial fluid of mussels, particularly, glucose concentration remained at an elevated level after 2-week or 2-month maintenance of the mussels in the laboratory (Figs. 6A and 6B, all experiments). Returning of mussels to the river enhanced restoration of the normal glucose concentrations in the haemolymph and extrapallial fluid (Fig. 6A, arrows with white-cored heads).

DISCUSSION

Sampling of the Fluids and Their Treatment

Haemolymph samples of bivalves are usually taken from either the pericardium or the sinuses of the adductor muscles or the foot (Crenshaw 1972, Fyhn and Costlow 1975). In this experiment, the haemolymph samples were taken from the sinus of the posterior adductor muscle. It is possible that, at the puncture site, some haemolymph can leak and run over the adductor muscle into the mantle cavity and contaminate the mantle cavity fluid. Also th forceful pulling apart of the shells in order to drain the mantl cavity fluid can break mantle tissue resulting in contamination by some haemolymph and can expose the marginal extrapallial space. The fluid volume in this space is, however, minimal. Mucus from the mantle surfaces may accompany the mantle cavity fluid. Horohov et al. (1992) also considered drainage of the mantle cavity fluid of *Dreissena polymorpha*, although providing a large volume, problematic. They observed that failure to drain the mantle cavity fluid in smaller zebra mussels could contaminate the haemolymph sample if the syringe needle passes beyond the pericardial region. The extrapallial fluid of *A. anatina* was collected last, through the mantle. Moreover, the attachment sites of the mantle to the shell have to be intact, or otherwise the extrapallial fluid may be contaminated by haemolymph or mantle cavity fluid.

As early as 1930, de Waele found that exposure of the blood of *Anodonta cygnea* to air caused precipitation of a thin film of calcium carbonate and protein. This is not surprising since bivalve

haemolymph is saturated with respect to aragonite (Potts 1954). De Waele (1930) stated that the precipitate formed as a result of loss of carbon dioxide leading to a rise in pH. The fluids of *A. anatina* tended to become somewhat cloudy during storage, particularly when stored frozen. Crenshaw (1972) found a similar phenomenon in fluids of marine bivalves even 10 minutes after the fluid collection. Attempts by Crenshaw to clarify the samples by centrifugation or by pressure or vacuum filtration were only partially successful. Calcium concentrations in these partially clarified samples were lower than in the samples diluted immediately after their collection. Crenshaw also studied non-dialysable macromolecules (protein and mucopolysaccharides) of the mussel fluids, and he suggested that, in the extrapallial fluid, calcium is bound to glycoprotein. In this present study, the mussel fluids were centrifuged (to remove possible contamination by cells) about 2.5 hours after sampling, and, therefore, some calcium may have been lost if it had precipitated during this time. The calcium concentration of haemolymph did not, however, decrease significantly during this period. In a later experiment in winter (unpublished), it was found that the calcium concentration in extrapallial fluid did not change if it was measured either immediately after sampling and centrifugation or after a 2.5 hour delay and centrifugation. Centrifugation was particularly important for the mantle cavity fluid samples, because there was often mud in the cavity. Because storage of frozen supernatants of mussel fluids caused a significant loss of calcium due to precipitation on the inner wall of the storage vessel, calcium analysis after freeze-storage of mussel fluids cannot be recommended.

Stored mussel fluid samples showed slightly but not significantly increased glucose concentrations. This is in accordance with the observations by Wijsman and Maaskant (1982) on glucose contents in stored tissues of *Lymnaea stagnalis*. Glycoproteins, mucopolysaccharides, or even glycogen contamination from the punctured adductor muscle or the mantle, may be responsible for this. Therefore, a protein precipitant should be added to the sample as soon as possible. Perchloric acid, in addition to removing glycoproteins, denatures enzymes which could release glucose.

Transportation of Mussels and Their Maintenance in the Laboratory: Effect on Calcium Concentrations

If freshwater mussels experience metabolic, respiratory or mixed acidosis, for instance as a consequence of emersion, $CaCO_3$ reserves are then dissolved to buffer the protons (Dotterweich and Elssner 1935, Byrne and McMahon 1991, Byrne et al. 1991). Both Ca^{2+} and HCO_3^- increase in the haemolymph. The ratio $\Delta HCO_3^-/\Delta Ca^{2+}$ depends on whether mussels are ventilating or not. CO_2 formed from HCO_3^- passes freely out from an open mussel but extra Ca^{2+} remains for a longer time. It has been thought that the Ca^{2+} and HCO_3^- are taken from the shell (Dotterweich and Elssner 1935, Crenshaw and Neff 1969, Byrne and McMahon 1991, Byrne et al. 1991,) or from the calcified concretions which freshwater bivalves generally have in their tissues (Machado et al. 1988). The concretions are, however, mostly composed of calcium phosphate (in addition to an organic matrix) (Silverman et al. 1983, Pynnönen et al. 1987, Pekkarinen and Valovirta 1994), and only a small portion of mantle calcium is exchangeable (Jodrey 1954, Istin and Maetz 1964).

During transportation, the mussels may have been partially emersed due to the small amount of water, and the shells may have been partially closed in response to the vibratory stress. The water temperature also rose to near that of the surrounding air. The calcium concentrations in the haemolymph and extrapallial fluid increased concomitantly. This is in accordance with the finding of Coimbra et al. (1993) that *A. cygnea* equilibrates calcium concentration between the haemolymph and the extrapallial fluid. Calcium concentration in the mantle cavity fluid of *A. anatina* is significantly lower than that in the haemolymph, but clearly higher than that of the surrounding water. This suggests that the mantle cavity is a compartment partially separated from the surrounding water. Matsushima and Kado (1982) also found that mantle cavity fluid of *Anodonta woodiana* was more similar to blood than pondwater. Because Scheide and Dietz (1984) reported that they had observed blood even being expelled from the extended foot of *Ligumia subrostrata*, especially when the mussels were handled, it is possible that the mantle cavity fluid of *A. anatina* contained haemolymph. During transportation, the mussels leaked additional calcium into the mantle cavity and so the calcium concentration in the mantle cavity fluid became close to that in the haemolymph. Mussels are thought to actively take in calcium through some epithelia (Coimbra et al. 1993). Specialized cells in the gill have been attributed to ion transport (Dietz and Findley 1980, Kays et al. 1990). Calcium moves to the extrapallial space by diffusion, and the epithelial cells of the shell side of the mantle are an order of magnitude more permeable to Ca^{2+} than the cells bordering the mantle cavity (Kirschner and Sorenson 1964, Coimbra et al. 1988). This would have the effect of conserving calcium. The permeability barrier of the outer mantle epithelium (Neff 1972) may be modulated seasonally (Coimbra et al. 1988). In the handling experiment, a small lag time in the rise of the calcium concentration in the mantle cavity fluid may have resulted from a permeability barrier.

The calcium concentrations in the haemolymph and extrapallial space of the mussels resting in a bucket of river water rose in only 15 minutes. Whether the extra calcium originated from the shell or from the calcified granules in the tissues is not known. Dietz (1979) also found that in *Margaritifera hembeli* acclimated to pond water in the laboratory and transferred to a small container of pond water (100 mL), sodium influx increased over several hours and then stabilised to normal. Thus minimal handling stress is enough to change ionic concentrations in fluids of some bivalves. Serotonin and cAMP are involved with regulation of sodium (Dietz et al. 1982, 1985), and sodium transport shows both seasonal and diurnal changes (Nemcsók and Szász 1975, Graves and Dietz 1980). Endogenous and exogenous calcium concentrations can also interfere via effects on adenylate cyclase (Scheide and Dietz 1984). Diurnal changes were excluded during this study by sampling mussels at the same time of the day. It has also been shown that the pH and calcium concentration in the extrapallial fluid of clams and mussels fluctuates with the opening and closing of the valves (Crenshaw and Neff 1969, Crenshaw 1972, Gordon and Carriker 1978). In the extrapallial fluid of *Mercenaria mercenaria*, changes in calcium concentration and pH were observable, reflecting a 10 to 15 minute opening-closing rhythm (Crenshaw 1972). This rhythm could not be taken into consideration in the present study. In the *A. anatina* in this study, the mean calcium concentration in the mantle cavity fluid did not change during the mussels' 1.5-hour wait in a bucket of river water (handling experiment). This means that there was not yet any net leakage of calcium from the mussels, or that the mussels excreted any accumulated calcium from the mantle cavity. The concomitant increase

of calcium concentration both in mantle cavity fluid and surrounding water during the transportation of the mussels suggests net leakage and only partial shell closure.

Leakage of calcium from post-transport *A. anatina* in a leakage experiment was of a similar order as for *M. hembeli* recorded by Heming et al. (1988) after exposure of the mussels to acid water. During 1 week, average leakage from *A. anatina* was 0.07 μmol h^{-1} g^{-1} (total fresh weight) and from *M. hembeli* 0.03 μmol h^{-1} g^{-1} (the leakage from *M. hembeli* was recorded in acid water, pH 5.25). In *A. anatina* the most rapid leakage occurred during the first day after transporting the mussels to the laboratory. The net efflux of calcium may have partially resulted from hampering the mussel's active calcium uptake by the low water temperature in the laboratory. *A. grandis simpsoniana*, after a 6-day emersion, showed a substantial calcium efflux during reimmersion, 5.85 μmol^{-1} h^{-1} per g soft part dry weight (Byrne and McMahon 1991) (0.6 μmol g^{-1} h^{-1}, assuming that soft part dry weight is 20% of fresh weight and fresh weight is 50% of total mussel weight) (Pekkarinen 1993).

Machado et al. (1988) found that keeping *A. cygnea* in acidic water led to the formation of a calcified pellicle on the inner surface of the shell. This indicates that, in freshwater unionaceans, calcium might be taken from the calcified concretions of the tissues for buffering. On the other hand, it has been assumed that protons are pumped into the extrapallial fluid during acidosis (Machado et al. 1990, Hudson 1993) leading to dissolution of the shell. The exact time when the calcified pellicle is formed is not known. After stress, for instance after emersion or acid exposure, bivalves may experience a transient alkalosis (Byrne and McMahon 1991, Pynnönen 1994). CO_2 is easily ventilated even from partially exposed mantle edges but the elevated calcium concentration persists longer, leading to imbalance of ions. Therefore extra calcium has to be eliminated from the haemolymph. Part of it may leak into the surrounding water and part may be precipitated onto the shell. Increased ventilation movements may have caused slight over-shooting of the decrease in calcium concentration in the mantle cavity fluid in some of the experiments in this study. This over-shooting was more pronounced in the mussels which were returned to the river.

In mussels kept in the laboratory for 2 weeks, calcium concentrations only partially returned to normal. The degree of normalization may depend on the season, the stage of reproductive cycle and the extent of stress experienced by mussels. In an experiment which was done when the river water temperature was highest and female mussels were at the beginning of gravidity, the mussels were punctured twice, and this may also have, due to leakage of haemolymph from the wound, increased mortality. Calcium concentrations in the survivors varied greatly: high in some and in others, possibly moribund individuals, very low.

The mussels in most experiments in this study were kept in aquaria with no sediment in which to burrow. This may have stressed the mussels along with the very low, unseasonal temperature. Heat and cold stresses have been shown to change serotonin concentrations in the mussel central nervous system (Stefano et al. 1978). However, Pekkarinen has noticed that clams (*Macoma balthica*, unpublished) can be maintained in the laboratory for longer periods more successfully at lower temperatures.

Transportation of Mussels and Their Maintenance in the Laboratory: Effect on Glucose Concentrations

There is evidence that molluscs have hyperglycemic neurohormones (Lubet et al. 1976, Hemminga et al. 1985, Robbins et al. 1990). In an arcid clam, during anaerobiosis, the blood plasma glucose concentration rose from 86 μmol L^{-1} to 228 μmol L^{-1}, or even to 500 μmol L^{-1}, during 96 hr of anaerobiosis (de Vooys et al. 1991, de Zwaan et al. 1995). The blood glucose of sea hares (*Aplysia dactylomeda*) responded to relatively small temperature or salinity changes or to short duration air exposures (Carefoot 1994). Even handling for blood sampling caused hyperglycemia 30–40 minutes after sampling in a snail: the blood glucose rose about 6.5-fold within 40 minutes (Marques and Falkmer 1976). A similar rise was found in the haemolymph and extrapallial fluid glucose concentrations of *A. anatina* during transportation in this study. The rise may partially result from handling stress, hypoxia and temperature change.

It has to be noted that glucose concentrations in the haemolymph and extrapallial fluid may remain above the normal level in mussels maintained in the laboratory. In this study, the temperature in the laboratory was low and the mussels were maintained without sediment and food in darkness. Blood sodium in bivalves is regulated by serotonin through cAMP (Scheide and Dietz 1984). Sodium is involved in glucose transport at least in the gills of *Crassostrea gigas* (Bamford and Gingles 1974). Such factors (e.g., temperature) which affect the sodium pump may also affect glucose metabolism. Constant light has been shown to dampen the diurnal variation of sodium net flux in *Corbicula fluminea* and it may also dampen the entire metabolic level (McCorkle-Shirley 1982). In this study the mussels were kept in constant darkness, and the effects of prolonged darkness are not known. Starvation and anaerobiosis are known to decrease exogenous glucose utilisation while glycogen degradation increases (Zaba et al. 1981).

The glucose values obtained for *A. anatina* in the field analyses in this study were very low compared with the posttransport values of the same species or with blood sugar concentrations reported in many other molluscs (Goddard and Martin 1966). Thus any stressed state of laboratory-kept mussels has to be taken into account if physiological experiments are to be done with such mussels. *A. anatina* could be a good bivalve model to study stress effects and the regulation of glucose concentration.

LITERATURE CITED

Bamford, D. R. & R. Gingles. 1974. Absorption of sugars in the gill of the Japanese oyster, *Crassostrea gigas*. *Comp. Biochem. Physiol.* 49A: 637–646.

Byrne, R. A. & B. R. McMahon. 1991. Acid-base and ionic regulation, during and following emersion, in the freshwater bivalve, *Anodonta grandis simpsoniana* (Bivalvia: Unionidae). *Biol. Bull.* 181:289–297.

Byrne, R. A., B. N. Shipman, N. J. Smatresk, T. H. Dietz & R. F. McMahon. 1991. Acid-base balance during emergence in the freshwater bivalve *Corbicular fluminea*. *Physiol. Zool.* 64:748–766.

Carefoot, T. H. 1994. Effects of environmental stressors on blood-glucose levels in sea hares, *Aplysia dactylomeda*. *Mar. Biol.* 118:579–583.

Coimbra, J., J. Machado, P. L. Fernandes, H. G. Ferreira & K. G. Ferreira. 1988. Electrophysiology of the mantle of *Anodonta cygnea*. *J. Exp. Biol.* 140:65–88.

Coimbra, A. M., K. G. Ferreira, P. Fernandes & H. G. Ferreira. 1993. Calcium exchanges in *Anodonta cygnea*: barriers and driving gradients. *J. Comp. Physiol.* B 163:196–202.

Crenshaw, M. A. 1972. The inorganic composition of molluscan extrapallial fluid. *Biol. Bull.* 143:506–512.

Crenshaw, M. A. & J. M. Neff. 1969. Decalcification at the mantle-shell interface in molluscs. *Am. Zool.* 9:881–885.

de Vooys, C. G. N., A. de Zwaan, J. Roos, E. Carpene & O. Cattani. 1991. Anaerobic metabolism of erythrocytes of the arcid clam *Scapharga inaequivalvis* (Bruguiere): effects of cadmium. *Comp. Biochem. Physiol.* 98B:169–175.

de Waele, A. 1930. Le sang d'*Anodonta cygnea* et la formation de la coquille. *Mém. Acad. R. Belg. Cl. Sci., deuxim serie* 10, fasc. 3:1–52 + plates I–V.

de Zwaan, A., G. Isani, O. Cattani & P. Cortesi. 1995. Long-term anaerobic metabolism of erythrocytes of the arcid clam *Scapharca inaequivalvis*. *J. Exp. Mar. Biol. Ecol.* 187:27–37.

Dietz, T. H. 1979. Uptake of sodium and chloride by freshwater mussels. *Can. J. Zool.* 57:156–160.

Dietz, T. H. & A. M. Findley. 1980. Ion-stimulated ATPase activity and NaCl uptake in the gills of freshwater mussels. *Can. J. Zool.* 58:917–923.

Dietz, T. H., J. I. Scheide & D. G. Saintsing. 1982. Monoamine transmitters and cAMP stimulation of Na transport in freshwater mussels. *Can. J. Zool.* 60:1408–1411.

Dietz, T. H., W. L. Steffens, W. T. Kays & H. Silverman. 1985. Serotonin localization in the gills of the freshwater mussel, *Ligumia subrostrata*. *Can. J. Zool.* 63:1237–1243.

Dotterweich, H. & E. Elssner, 1935. Die Mobilisierung des Schalenkalkes für die Reaktiosregulation des Muscheln (*Anodonta cygnea*). *Biol. Zbl.* 55:138–163.

Fyhn, H. J. & J. D. Costlow. 1975. Anaerobic sampling of body fluids in bivalve molluscs. *Comp. Biochem. Physiol.* 52A:265–268.

Goddard, C. K. & A. W. Martin. 1966. Carbohydrate metabolism. *In: Physiology of Mollusca II*. K. M. Wilbur & C. M. Youne (ed.), pp. 275–308. Academic Press London.

Gordon, J. & M. R. Carriker. 1978. Growth lines in a bivalve mollusk: subdaily patterns and dissolution of the shell. *Science* 202:519–521.

Graves, S. Y. & T. H. Dietz. 1980. Diurnal rhythms of sodium transport in the freshwater mussel. *Can. J. Zool.* 58:1626–1630.

Heming, T. A., G. A. Vinogradov, A. K. Klerman & V. T. Komov. 1988. Acid-base regulation in the freshwater pearl mussel *Margaritifera margaritifera:* effects of emersion and low water pH. *J. Exp. Biol.* 137:501–511.

Hemminga, M. A., J. J. Maaskant, W. Koomen & J. Joosse. 1985. Neuroendocrine control of glycogen mobilization in the freshwater snail *Lymnaea stagnalis*. *Gen. Comp. Endocrinol.* 57:117–123.

Horohov, J., H. Silverman, J. W. Lynn & T. H. Dietz. 1992. Ion transport in the freshwater zebra mussel, *Dreissena polymorpha*. *Biol. Bull.* 183:297–303.

Hudson, R. L. 1993. Bafilomycin-sensitive acid secretion by mantle epithelium of the freshwater clam, *Union complanatus*. *Am. J. Physiol.* 264:R946–R951.

Istin, M. & J. Maetz. 1964. Perméabilité au calcium du manteau de lamellibranches d'eau douce étudiée à l'aide des isotopes ^{45}Ca et ^{47}Ca. *Biochim. Biophys. Acta* 88:225–227.

Jodrey, L. H. 1954. Studies on shell formation. III. Measurement of calcium deposition in the shell and calcium turnover in mantle tissue using the mantle-shell preparation and Ca^{45}. *J. Exp. Biol.* 31:398–407.

Kays, W. T., H. Silverman & T. H. Dietz. 1990. Water channels and water canals in the gill of the freshwater mussel, *Ligumia subrostrata:* ultrastructure and histochemistry. *J. Exp. Zool.* 254:256–269.

Kirshner, L. B. & A. L. Sorenson. 1964. Calcium movement across the isolated clam mantle. *Fed. Proc.*, 23, Part I, No. 87.

Lubet, P., P. Herlin, M. Mathieu & F. Collin. 1976. Tissu de réserve et cycle sexuel chez les lamellibranches. *Haliotis* 7:59–62.

Machado, J., J. Coimbra, C. Sá & I. Cardoso. 1988. Shell thickening in *Anodonta cygnea* by induced acidosis. *Comp. Biochem. Physiol.* 91A: 645–651.

Machado, J., K. G. Ferreira, H. G. Ferreira & P. L. Fernandes. 1990. The acid-base balance of the outer mantle epithelium of *Anodonta cygnea*. *J. Exp. Biol.* 150:159–169.

Malley, D. F., J. D. Huebner & K. Donkersloot. 1988. Effects on ionic composition of blood and tissues of *Anodonta grandis grandis* (Bivalvia) of an addition of aluminum and acid to a lake. *Arch. Environ. Contam. Toxicol.* 17:479–491.

Marques, M. & S. Falkmer. 1976. Effects of mammalian insulin on blood glucose level, glucose tolerance, and glycogen content of musculature and hepatopancreas in a gastropod mollusk, *Strophocheilus oblongus*. *Gen. Comp. Endocrinol.* 29:522–530.

Matsushima O. & Y. Kado. 1982. Hyperosmoticity of the mantle fluid in the freshwater bivalve, *Anodonta woodiana*. *J. Exp. Zool.* 221:379–381.

McCorkle-Shirley, S. 1982. Effects of photoperiod on sodium flux in *Corbicula fluminea* (Mollusca: Bivalvia). *Comp. Biochem. Physiol.* 71A:325–327.

Neff, J. M. 1972. Ultrastructure of the outer epithelium of the mantle in the clam *Mercenaria mercenaria* in relation to calcification of the shell. *Tiss. Cell* 4:591–600.

Nemcsók J. & A. D. J. Szász. 1975. Seasonal alterations of Ca-, K-, Na-, and Cl-ions in the haemolymph of *Anodonta cygnea* L. A simple, rapid determination of Ca- and Cl-ions. *Ann. Biol. Tihany* 42:73–80.

Pekkarinen, M. 1991. Annual ring formation in some unionacean shells in the river Vantaanjoki (southern Finland). *Bivalve Stud. Finland* 1:10–19.

Pekkarinen, M. 1993. Reproduction and condition of unionid mussels in the Vantaa River, South Finland, *Arch. Hydrobiol.* 127:357–375.

Pekkarinen, M. 1995. Seasonal changes in calcium and glucose concentrations in different body fluids of *Anodonta anatina* (L.) (Bivalvia: Unionidae). Submitted.

Pekkarinen, M. & R. Suoranta. 1994. Effects of transport and laboratory conditions on calcium and glucose concentrations in different fluids of *Anodonta anatina* (Bivalvia: Unionidae) in the growing season. *The 10th National Meeting on Physiol. Physiol. Toxicol. in Aquat. Animals, Lammi, Finland, December 1–2, 1994*. (Abstract)

Pekkarinen, M. & I. Valovirta. 1994. Calcium, iron and other constituents of *Margaritifera margaritifera* (L.) (Bivalvia: Margaritiferidae). *The 10th National Meeting on Physiol. Physiol. Toxicol. in Aquat. Animals, Lammi, Finland, December 1–2, 1994*. (Abstract)

Potts, W. T. W. 1954. The inorganic composition of the blood of *Mytilus edulis* and *Anodonta cygnea*. *J. Exp. Biol.* 31:376–385.

Pynnönen, K. 1990. Physiological responses to severe acid stress in four species of freshwater clams (Unionidae). *Arch. Environ. Contam. Toxicol.* 19:471–478.

Pynnönen, K. 1994. Hemolymph gases, acid-base status, and electrolyte concentration in the freshwater clams *Anodonta anatina* and *Unio tumidus* during exposure to and recovery from acidic conditions. *Physiol. Zool.* 67:1544–1559.

Pynnönen, K., D. A. Holwerda & D. I. Zandee. 1987. Occurrence of calcium concretions in various tissues of freshwater mussels, and their capacity for cadmium sequestration. *Aquatic Toxicol.* 10:101–114.

Robbins, I., F. Lenoir & M. Mathieu. 1990. A putative neuroendocrine factor that stimulates glycogen mobilization in isolated glycogen cells from the marine mussel *Mytilus edulis*. *Gen. Comp. Endocrinol.* 79: 123–129.

Scheide, J. I. & T. H. Dietz. 1984. The effects of calcium on serotonin-stimulated adenylate cyclase in freshwater mussels. *Biol. Bull.* 166: 594–607.

Silverman, H., W. L. Steffens & T. H. Dietz. 1983. Calcium concretions in the gills of a freshwater mussel serve as a calcium reservoir during periods of hypoxia. *J. Exp. Zool.* 227:177–189.

Stefano, G. B., L. Hiripi & E. J. Catapane. 1978. The effects of short and long term temperature stress on serotonin, dopamine and norepinephrine concentrations in molluscan ganglia. *J. Thermal Biol.* 3:79–83.

Wijsman, T. C. M. & J. J. Maaskant. 1982. Tissue glucose in the pond snail *Lymnaea stagnalis:* a pitfall for unsuspecting biologists. *Comp. Biochem. Physiol.* 71B:293–295.

Zaba, B. N., P. A. Gabbott & J. I. Davies. 1981. Seasonal changes in the utilisation of ^{14}C- and ^{3}H-labeled glucose in a mantle tissue slice preparation of *Mytilus edulis* L. *Comp. Biochem. Physiol.* 70B:689–695.