

## Laboratory studies of the effects of Mollusca on alkalinity of their freshwater environment

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A series of aquaria containing various combinations of water, sediments, and molluscs was used to investigate the effects of molluscs on alkalinity of the water. Live molluscs acidified the water, and dead decomposing molluscs were associated with an increase in alkalinity in the aquaria. Aquaria containing dead molluscs had more stable alkalinity concentrations than other aquaria when all received additions of natural acid rain (pH 4.1). Nonmolluscan invertebrates liberated acid-neutralizing materials from the sediments but the source was quickly depleted. A hypothesis of temporal disjunction between periods of molluscan acid and base production is given which indicates a potential role for molluscs in the sources and cycling of carbonates in acidifying environments.

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Une série d'aquariums contenant des combinaisons variées d'eau, de sédiments et de mollusques a servi à mesurer les effets des mollusques sur l'alcalinité de l'eau. Les mollusques vivants acidifient l'eau, alors que les mollusques morts en décomposition entraînent une augmentation de l'alcalinité dans les aquariums. Les aquariums qui contenaient des mollusques morts avaient des concentrations alcalines plus stables que les autres aquariums après l'addition d'eau de pluie acide naturelle (pH 4,1). Les invertébrés autres que les mollusques favorisaient la libération des substances neutralisantes contenues dans les sédiments, mais la source de ces substances s'est trouvée vite tarie. Il est possible qu'il y ait une rupture temporelle entre les périodes de production des substances acides et basiques chez les mollusques; cette hypothèse permet de croire au rôle que pourraient jouer les mollusques comme sources et agents recycleurs des carbonates dans les milieux en voie d'acidification.

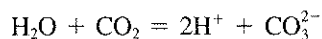
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### Introduction

Recent studies have shown that benthic macroinvertebrates can greatly influence the chemical conditions of their immediate environment, usually through their metabolic activities or interaction with the sediments (Galleg 1979; Hultberg and Anderson 1982; Lawrence *et al.* 1982). Green (1980) showed that certain molluscs may contain as much as 50% of the total lake calcium within their shells and tissues and because of this the activities and population dynamics of these molluscs may play a large role in the concentration and cycling of calcium in the lake.

Another component of molluscan shells is carbonate, and past studies have shown that in low-alkalinity environments molluscs synthesize the necessary carbonates from metabolic carbon dioxide and water (Wilbur 1972; Campbell and Boyan 1976; Simkiss 1976). If this is true, then it is possible that molluscs may provide a source for carbonate in their environment as well as participate in carbonate cycling processes.

If molluscan carbonates are formed from carbon dioxide there must be a concomitant release of acid because the negative carbonate ion cannot be formed from neutral carbon dioxide without the liberation of protons;



Molluscs should, therefore, produce acid during the process of shell formation, above and beyond that expected for any heterotrophic organism. Once the molluscs die the synthesized carbonates should be released and add to the carbonate pool of the environment. In this way the molluscs may play a role in the sources, cycling, and storage of carbonates.

At this point a few terms of major interest to this study

should be defined. Alkalinity is a measure of the balance between hydroxide, carbonate, and bicarbonate ions on one hand and protons on the other hand (Harvey *et al.* 1981). Used in this study, alkalinity is the excess of the above-mentioned anions over the protons, as measured by the total inflection-point method, using a Gran titration (Stumm and Morgan 1970). Acid-neutralizing capacity encompasses alkalinity and also includes other chemical species (certain metals) and processes (adsorption, absorption, etc.) that tend to consume protons and in general raise the pH (Harvey *et al.* 1981). As such, any change in the alkalinity will result in a change in the acid-neutralizing capacity but the reverse is not necessarily true.

The term "acidification" has many possible interpretations, the most common of which is an increase of hydrogen ion activity (decline in pH). Pure water has a pH of approximately 5.6 owing to equilibration with carbonic acid (Wetzel 1975), and water with a pH below this is considered to be acidified (Harvey *et al.* 1981). Because alkalinity represents a balance between the ions noted above, acidification can also be defined as a decrease in alkalinity, and this is the interpretation that is used throughout this study. Alkalinity was chosen because it measures the carbonate-system component of acid-neutralizing capacity and any carbonates synthesized by, or cycled through, the molluscs would become a part of the alkalinity.

The main purpose of this study was to investigate any effects that molluscs may have on the alkalinity concentration of their immediate environment. This was accomplished with a series of laboratory experiments that utilized water, sediment, and benthic macroinvertebrates from a low-alkalinity lake in south central Ontario.

### Materials and methods

The water, sediment, and benthic organisms used in the following series of experiments were all taken from Dickie Lake, near Dorset, Ontario. This lake has very low alkalinity concentrations (mean of

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10 samples from April to October 1980 = 8.2 mequiv.  $\cdot L^{-1}$ , data provided by the Ontario Ministry of the Environment, Dorset, Ontario) and is thought to have had these low levels for many years (P. J. Dillon, personal communication). Four experiments were run at room temperature, with a 9 h light : 15 h dark cycle and with periodic aeration.

#### Experiment 1

The primary aim of the first experiment was to determine if live molluscs reduce the alkalinity of (and hence acidify) their immediate environment. Individual *Campeloma decisum* from Dicke Lake were placed in 250-mL beakers of lake water. Alkalinity of the water from four replicates of treatment and control (no snails) was measured periodically over an 81-h period. Alkalinity was determined by Gran titration (Stumm and Morgan 1970). The beakers were loosely covered and evaporative losses were negligible.

#### Experiment 2

The second laboratory experiment was designed to investigate the role of molluscs in influencing the alkalinity of their immediate environment over a relatively long time period using conditions that simulated the natural environment more closely than those in the first experiment.

Twelve 6-L aquaria were set up with three replicates of each of the following treatments (in this, and the following experiments, 2 L of lake water and 0.5 L of sieved lake sediment were used as indicated; dead molluscs had been heat killed and included both body tissues and shells when added to the microcosms; all components added to the system were distributed randomly among the indicated treatments): (i) water only, (ii) water and sediment, (iii) water and sediment with 10 live *Ammicola limosa*, 10 live *Campeloma decisum*, and 20 live *Pisidium casertanum*, (iv) water and sediment with 10 dead *A. limosa*, 10 dead *C. decisum*, and 20 dead *P. casertanum*. Alkalinity was measured periodically over 36 weeks. Using a siphon, 100-mL aliquots were taken from approximately middepth in each microcosm. Aquaria were loosely covered to allow gaseous exchange and evaporative and sampling losses were made up with deionized water. Because of this, some dilution of alkalinity could have occurred but this would have been equal across all treatments.

#### Experiment 3

The third experiment was designed to test the hypothesis that molluscs and other benthic organisms could influence the alkalinity of the water, even when external inputs of acids and acid-neutralizing materials occurred. A secondary purpose was to discover if molluscs affect the alkalinity differently than other benthic organisms.

Twelve 6-L aquaria were set up with three replicates of each of the following treatments: (i) water and sediment, (ii) water and sediment with 10 live *A. limosa*, 10 live *C. decisum*, and 20 live *P. casertanum*, (iii) water and sediment with 10 dead *A. limosa*, 10 dead *C. decisum*, and 20 dead *P. casertanum*, (iv) water and sediment with five dragonflies (*Gomphus* sp.) and five mayflies (*Ephemera* sp.). Both of these species are large active burrowers and were observed actively disturbing the sediments throughout the experiment. Aquaria were periodically acidified with a mist of acid rain (pH 4.1, natural rain collected near Dorset, Ontario) or neutralized with a mist of "neutral rain" (pH 5.6, natural acid rain adjusted with 0.1 N sodium hydroxide). The rain was added as a mist from a plastic squeeze bottle at a rate of 20 mL  $\cdot$  day $^{-1}$  for 5 consecutive days out of 7, during the weeks indicated in Fig. 3.

Alkalinity was measured weekly on 100-mL aliquots taken with a siphon from approximately middepth. Additions of "rain" made up for both evaporative and sampling losses of water from the microcosms. After 18 weeks, live molluscs and burrowers were removed from their respective aquaria and alkalinity was monitored for 3 more weeks. The burrowers were placed into the aquaria containing previously undisturbed sediments and alkalinity was measured for 3 more weeks.

#### Experiment 4

The main purpose of the final laboratory experiment was to discover if burrowing molluscs would have a different effect on the alkalinity

than would nonburrowing molluscs.

Nine 6-L aquaria were set up with three replicates of each of the following treatments: (i) water and sediment, (ii) water and sediment with 30 live *A. limosa*, 4.0–4.5 mm in length, (iii) water and sediment with 30 live *C. decisum*, 5.0–6.0 mm in length. Alkalinity was measured weekly for 8 weeks on 100-mL aliquots taken with a siphon. Adult *C. decisum* are much larger than adult *A. limosa* and animals of the indicated size range were chosen to make the treatments as similar as possible.

Despite repeated attempts, it proved impossible to determine the alkalinity of the sediments used in these experiments. Because of this it is not possible to calculate what proportion of the alkalinity in the entire system was attributable to the activities of the organisms, and only changes in alkalinity concentrations in the water can be compared between treatments. Although it may be possible to determine the carbonate content of the sediments, this would only represent a portion of the sediment alkalinity and would only indicate what was potentially available. Neither of these estimates would greatly clarify the results of these experiments.

As used here, the term microcosm refers to an artificial system that attempts to simulate natural conditions as closely as possible (Funk 1963). The first experiment was, therefore, less a microcosm than the following three. The final three experiments were designed to simulate conditions in certain areas of Dickie Lake and used molluscan densities and community structure, and water and sediment quality that occurred naturally in that lake. Variables such as temperature and dissolved oxygen were not continuously monitored during these experiments but the initial experimental conditions simulated natural conditions and there was no reason to suspect that these would have changed radically during the course of the experiments. Passive exchange with the atmosphere and periodic aeration of the tanks provided sources for oxygen and sinks for carbon dioxide similar to those found under natural conditions. The use of natural unsterilized sediments would have incorporated a natural microbial flora and fauna into the aquaria, allowing the development and maintenance of whole-system biochemical cycles similar to those in nature. This flora and fauna was incorporated in all treatments using sediments and could not have been responsible for any of the differences noted, except where "water-only" treatments were compared with treatments using sediments. The animals were not artificially fed during any of the experiments but in the final three experiments molluscs were observed feeding on the detritus that was present and on the algae that developed on the sides of the aquaria. Because none of these variables were measured the above-stated ideas must remain assumptions but the fact that the majority of organisms remained alive, even after 36 weeks in the second experiment, suggests that the conditions in the aquaria were at least tolerable for the resident biota.

## Results

The preliminary laboratory experiment, using live snails and no sediment, indicated that over a 4-day period the alkalinity of lake water exposed to the atmosphere gradually increased (Fig. 1). In beakers containing water and single, live snails alkalinity increased during the first 9 h then declined to initial levels after 81 h, when the experiment was terminated.

Figure 2 shows that aquaria containing dead molluscs, live molluscs, and undisturbed sediments had changes in alkalinity of the water over a 36-week period. Decomposing molluscs (tissue and shell) appeared to cause a brief rise in alkalinity, the alkalinity then declined to zero before returning to higher levels. Live molluscs were associated with a total depletion of alkalinity until a few individuals died (week 25, Fig. 2), when alkalinity began to increase. In aquaria with undisturbed sediments the alkalinity of the water was completely depleted after 8 weeks. Alkalinity of water alone (control) stayed relatively constant over 36 weeks with only a gradual decline.

Additions of acid rain to the third set of aquaria had some

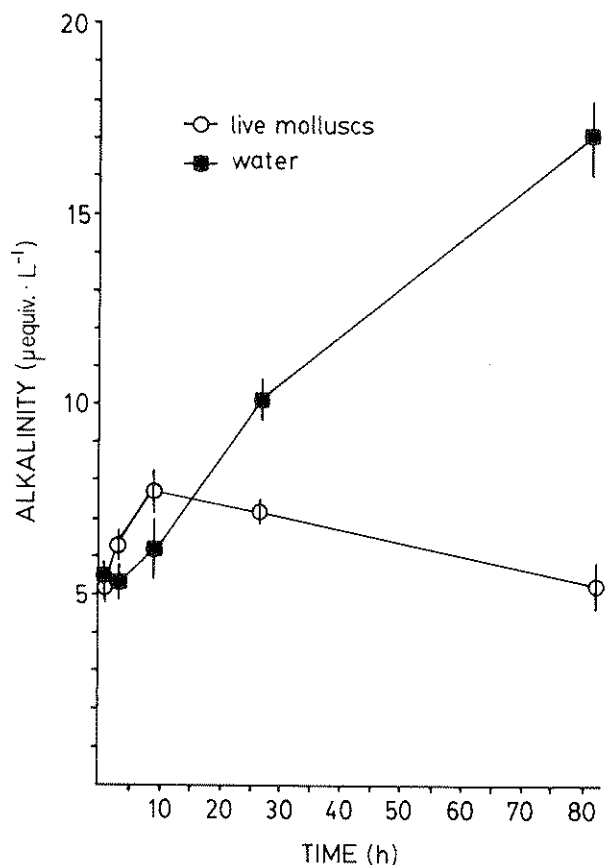


FIG. 1. Plot of alkalinity versus time for the first laboratory experiment, using 250-mL beakers of lake water and live snails. Vertical lines are standard errors.

noticeable effects on the patterns established from the previous experiments. Dead molluscs once again greatly increased the alkalinity but these levels declined each time acid rain was added. During periods of no external acid inputs alkalinity increased (Fig. 3). The occurrence of the U-shaped curve noted in the second experiment was not evident here. Alkalinity was again depleted in treatments with live molluscs but it did not decline to zero or lower this time. Alkalinity of water over undisturbed sediments soon became depleted. Alkalinity in aquaria with nonmolluscan burrowers (dragonflies and mayflies) rose briefly then became completely depleted after 15 weeks. During the first 2-week period of no external acid inputs alkalinity began to rise in the "burrower" aquaria but cessation of acid inputs later in the experiment did not bring about a similar response.

When animals were removed from some aquaria (week 18) alkalinity remained constant. Introduction of burrowers to previously undisturbed sediments resulted in a brief rise in alkalinity followed by a decline. When dead molluscs were removed alkalinity remained high for the following 3 weeks.

In the final experiment there appeared to be no difference between the alkalinity changes of water in aquaria containing *A. limosa* (epibenthic) and *C. decisum* (burrowing) (Fig. 4) and in both cases the alkalinity of the water declined with time, relative to controls. Once again the alkalinity of water overlying undisturbed sediments was totally depleted after 8 weeks.

### Discussion

To construct their shells, molluscs either extract carbonates

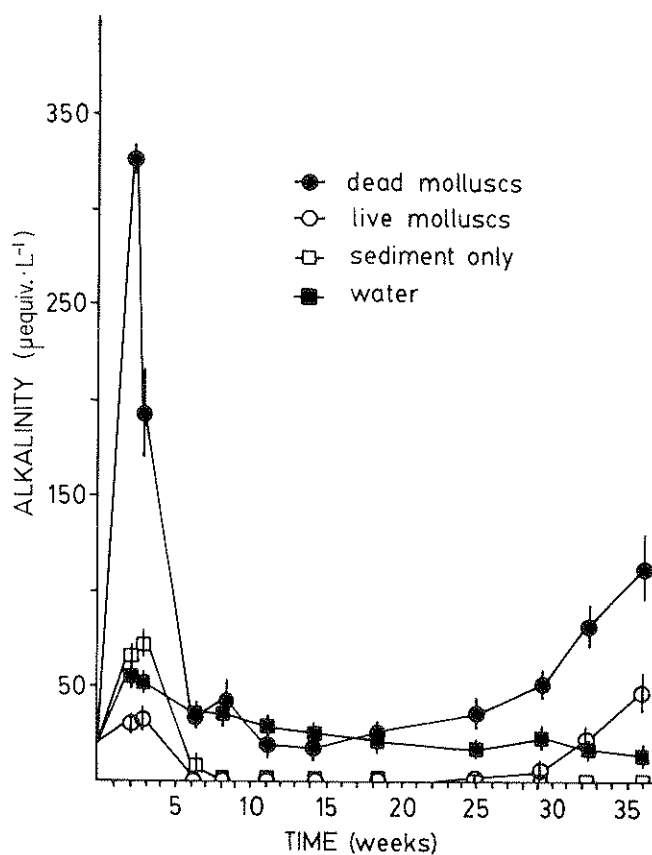


FIG. 2. Plot of alkalinity versus time in 6-L aquaria with various combinations of water, sediment, and molluscs. Vertical lines are standard errors.

from their environment (Van der Bronght and Van Puymbroek 1965; Hunter and Lull 1977) or form them de novo from metabolic carbon dioxide (Wilbur 1972; Simkiss 1976) (Eq. 1).



When carbonate sources are low, as in the present study, carbonate synthesis from carbon dioxide would appear to be the only mechanism possible. This, of course, assumes that the calcium requirements can also be satisfied but Mackie and Flippance (1983) have indicated that at least some molluscs can accumulate the necessary calcium from their food, and are not dependent directly on bedrock sources of calcium.

Carbonates stored in the body and shell could add to the carbonate levels of the water, and possibly of the sediments also, once the molluscs die and the tissues and shell begin to degrade (Eq. 2).



It would, therefore, appear that the molluscs are able to create new carbonates (from the carbon in their food) and that these carbonates could be continually renewed so long as the populations continued to turn over. Synthesized carbonates would be stored in the molluscan tissues and shells during life, then released to the environment after death and decomposition. Once freed, these new carbonates would be available to neutralize acidic substances in the environment.

Of course for each carbonate ion formed, two hydrogen ions would also be liberated (Eq. 1). These hydrogen ions, if released directly to the environment, would result in increased

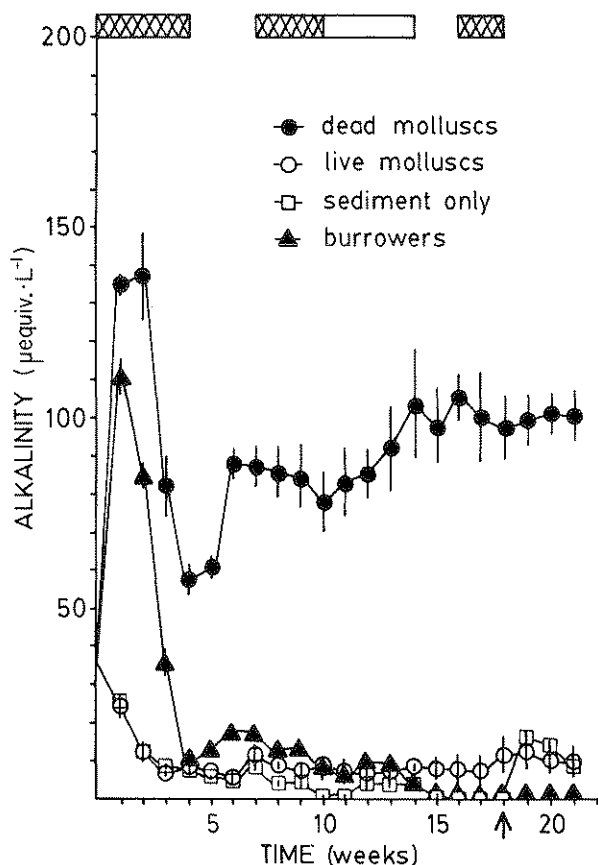


FIG. 3. Plot of alkalinity versus time in 6-L aquaria with various combinations of water, sediment, and benthic organisms. Vertical lines are standard errors. Hatched bars indicate periods of "acid rain" addition. Hollow bar shows period of "neutral rain" addition. Some animals were removed and (or) transferred at week 18 (arrow) (see text).

acidity. The synthesis of "new" carbonates which are locked into the bodies and shells of living molluscs is, therefore, an acid-producing process (Wilbur 1972; Campbell and Boyan 1976). Several possible mechanisms of carbonate deposition have been proposed in the literature but in every instance free protons or proton precursor compounds (e.g.,  $\text{NH}_4^+$ ) are hypothesized to be released (Campbell and Speeg 1969; Campbell and Boyan 1976; Wilbur 1976). Theoretically, the total acid produced during life should equal the total carbonate released after death (on a chemical equivalence basis and considering only the processes of carbonate formation and decomposition).

To a certain extent the results obtained here substantiate these ideas. Snails held in individual containers of low-alkalinity water were associated with a decline of alkalinity (and hence acidification), while water alone experienced an increase of alkalinity (this increase in alkalinity of water exposed to the atmosphere has been attributed to contamination by airborne dust (P. J. Dillon, personal communication)). The snails therefore produced alkalinity-consuming materials, and the apparent lag time (9 h) before acid production began may represent a period of acclimatization before normal physiological processes resumed after the animals had been transferred to the containers. This acidification by live molluscs was noted in all four experiments and it should be noted that the decline in alkalinity was not due to respiratory carbon dioxide. As defined previously, alkalinity is the balance between acid-

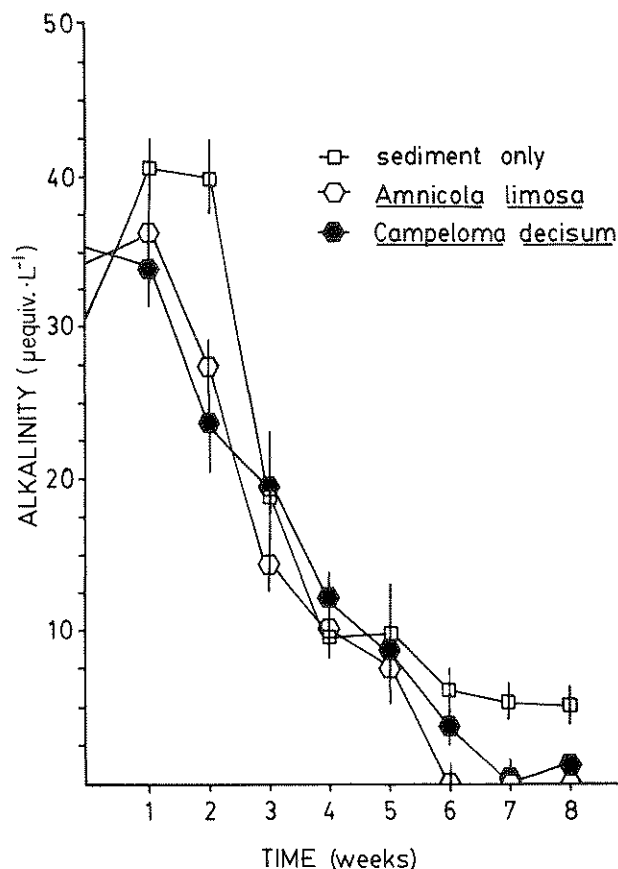


FIG. 4. Plot of alkalinity versus time in 6-L aquaria containing *Amnicola limosa* or *Campeloma decisum*. Vertical lines are standard errors.

neutralizing ions of the carbonate system and protons, and dissolution of carbon dioxide in water would increase the proton and bicarbonate levels by stoichiometrically equal amounts (Eq. 1).

Dead molluscs (tissues and shells) were always associated with an increase in alkalinity, as could be expected from the dissolution of the carbonaceous shells. In the first experiment, however, alkalinity rose briefly, declined to near zero, and then rose once again. This suggests that shell carbonates were not being released at a continuous rate. This is at present unexplainable but it did not happen again in the third experiment.

Two major points should be noted concerning alkalinity in microcosms containing dead molluscs. First, when only 6 out of 40 molluscs had died, alkalinity of the water began to increase (Fig. 2). Second, only those microcosms containing the dead molluscs were able to replenish alkalinity levels in the water after acid additions were stopped (Fig. 3). The first point indicates that the death of a relatively small proportion of the molluscs supplied enough carbonate to completely neutralize the cumulative acid production of the previous 25 weeks and to create a positive alkalinity balance despite the presence of the remaining live molluscs which should still have been releasing acid. The second point shows that carbonate from the decomposing molluscs supplied enough alkalinity to replenish that which had been consumed by short-term external acid inputs. These studies indicate that live molluscs acidify their immediate environment and that dead molluscs can neutralize acids in their immediate environment. The two points noted above also indicate that if natural environments could tolerate the low-

level acidification that results from molluscan carbonate synthesis then a powerful acid-neutralizing reserve could be built up and become available after some of the molluscs died.

Rooke and Mackie (1984) showed that molluscs grew only between April and October in Dickie Lake. So long as no growth took place few carbonates would be synthesized and hence few protons would be released. Assuming stable molluscan populations from year to year, acid would be released only in late spring, summer, and early autumn but carbonates would be dissolving from the shells of dead molluscs year-round (although at a somewhat slower rate in the coldest periods). It is, however, during late spring, summer, and early autumn that most freshwaters experience a natural decline of hydrogen ion activity (pH increase) (Wetzel 1975). Therefore, the environment may be able to assimilate the molluscan acids as they are released, leaving a supply of carbonate which could later become available to maintain stable alkalinity levels or possibly neutralize short-term but highly acidic inputs, such as occur during spring snowmelt (Dillon *et al.* 1978). Rooke and Mackie (1984), however, also showed that in six low-alkalinity lakes the resident molluscs could at best supply only 24% (and usually much less than 2%) of the alkalinity already present in the water. Clearly, the molluscs could not be expected to be able to turn low-alkalinity lakes into high-alkalinity lakes but the present study shows that the molluscs can greatly affect the alkalinity sources and cycling in their immediate environment.

These experiments also indicate a possible role for the sediments and sediment-animal interactions in alkalinity cycling. In all experiments with undisturbed sediments alkalinity dropped rapidly, indicating that the alkalinity initially present in the water had been neutralized, possibly by binding at the sediment-water interface. This indicates that any alkalinity declines in these experiments could be at least partially attributable to undisturbed portions of the sediments. When the sediments were disturbed by the burrowing of dragonflies and mayflies alkalinity initially increased but was rapidly depleted by external acid inputs. Alkalinity failed to return to "preacidification" levels after the acid inputs were stopped despite the continued disturbance of the sediments by the resident animals. Since there is no immediate reason why these animals should supply alkalinity to the system it seems most probable that they were liberating preexisting alkalinity materials from the sediments (Lawrence *et al.* 1982). Chemical release by bioturbation has been noted in other studies of freshwater acidification (Gallep 1979; Hultberg and Anderson 1982). This storage pool was rapidly depleted and could not provide the same degree of acid-neutralization that was available from the molluscan carbonates. The molluscs could provide a source of alkalinity while the sediments were only a storage compartment for existing alkalinity.

Most molluscs used in these experiments were active burrowers, but alkalinity did not increase when they were allowed to disturb the sediments, as might be expected from the discussion above. The acids produced by the molluscs may have quickly neutralized any newly released alkalinity, but this could not be accurately tested with the present experimental design. The fact that burrowing and nonburrowing molluscs did not produce different effects on alkalinity is at present unexplainable. If biological perturbation did release alkalinity then alkalinity in microcosms with *C. decisum* should have at

least declined more slowly than in microcosms with *A. limosa*. These results may be due to inherent physiological differences or age differences between the two genera used here.

#### Acknowledgements

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