The Journal of Experimental Biology 213, 1471-1476

doi:10.1242/jeb.040493

Effect of acute exposure to low environmental calcium on respiration and locomotion in *Lymnaea stagnalis* (L.)

Sarah Dalesman* and Ken Lukowiak

Department of Physiology and Pharmacology, University of Calgary, HSC 2104, 3330 Hospital Drive NW, Calgary, AB, Canada T2N 4N1

*Author for correspondence (sarah.dalesman@ucalgary.ca)

Accepted 19 January 2010

SUMMARY

Environmental calcium is a major factor affecting the distribution of freshwater gastropods. Whilst the effects on growth and morphology are fairly well understood, little is known about how calcium availability affects other aspects of gastropod biology. *Lymnaea stagnalis* (L.) is considered a calciphile and exhibits reduced growth and survival in environments containing less than 20 mg l^{-1} Ca^{2+}. Many freshwater systems exhibit fluctuations in calcium concentration over time: where calcium levels are normally high there may be periods of low [Ca^{2+}], for example following periods of flooding. Here we examined the effects of acute periods of low (20 mg l^{-1}) environmental calcium on the physiology and behaviour of *L. stagnalis*, specifically measuring how locomotion and respiration differ between high calcium (80 mg l^{-1}) and low calcium (20 mg l^{-1}) environments. We found that in a low calcium environment crawling speed is reduced, and that this coincides with an increase in cutaneous respiration, indicating that the increased metabolic demands of calcium acquisition at low [Ca^{2+}] reduce the energy available for locomotion. Conversely we found a decrease in aerial respiration in hypoxic conditions in the low calcium relative to the high calcium environment. In conclusion, we found that acute exposure to low environmental calcium has a highly significant effect on locomotion and respiration, which may have consequences for snail fitness when no morphological effects are apparent.

Key words: environmental calcium, locomotion, *Lymnaea stagnalis*, pulmonate, respiration.

INTRODUCTION

Calcium availability is considered to be one of the major limiting factors affecting the distribution of many freshwater aquatic organisms including molluscs (Boycott, 1936; Macan, 1977; Okland, 1983; Briers, 2003). Molluscs rely on calcium for growth of their shell and so are highly dependent on calcium availability for survival, demonstrating reduced growth rate, survival and reproductive output in low calcium environments (Harrison et al., 1970; Young, 1975a; Madsen, 1987; Zalizniak et al., 2009). In addition, in low calcium molluscs demonstrate thinning of the shell, potentially making them easier prey and more susceptible to damage (Boycott, 1936; Young, 1975a; Madsen, 1987; Lewis and Magnuson, 1999; Glass and Darby, 2009; Zalizniak et al., 2009). In high calcium environments molluscs can demonstrate induced shell thickening in the presence of predators, potentially reducing predation mortality; however, this induced response is prevented when calcium availability is limited (Rundle et al., 2004; Czarnoleski et al., 2006). Widespread decline of calcium has occurred in freshwater systems in North America and Europe, primarily due to leaching of calcium cations from soil in the surrounding catchment areas following prolonged exposure to acid rain (Evans et al., 2001; Huntington, 2005; Watmough et al., 2005; Keller, 2009). This decline is likely to impact on both the growth and survival of the species present, and may also alter species composition favouring those that tolerate low calcium environments (Jeziorski et al., 2008).

Freshwater gastropods have historically been separated into two broad groups: (1) those considered soft-water species, that can obtain the majority of their calcium requirements from food sources and thus survive well in low calcium environments and (2) those that require at least 20 mg l^{-1} of environmental calcium and are unable to satisfy their calcium requirements from their food, referred to as calciphiles (Boycott, 1936; Young, 1975a; Young, 1975b; Madsen, 1987). For example Van Der Borght and Van Puymbroek demonstrated that *L. stagnalis*, considered a calciphile species, obtains approximately 80% of its calcium requirements from the environment rather than food sources (Van Der Borght and Van Puymbroek, 1966). Calcium uptake from the water appears to be an active process in aquatic gastropods; for example, uptake is increased within 6 h of damage to the shell in *Indoplanorbis exustus* (Vaidya and Nagabhushanam, 1980). In *L. stagnalis* calcium uptake in high environmental calcium is thought to be due to the effect of an electrochemical gradient between the haemolymph and external [Ca^{2+}], where passive transport of calcium from the external media can occur above 20 mg l^{-1}, whereas below this level no gradient exists, so all calcium uptake requires energy, and at 15 mg l^{-1} Ca^{2+} in the external environment net uptake of calcium is 50% of that at 40 mg l^{-1} Ca^{2+} (Greenaway, 1971).

Freshwater gastropods may experience considerable fluctuations in calcium concentration in natural conditions over the period of a year. For example in Canada the Gravenhurst–Barrie region of Ontario showed maximum fluctuations of 2.0 to 13.2 mg l^{-1} in 'soft' water, 2.8 to 32 mg l^{-1} in 'medium' water environments and 46 to 100 mg l^{-1} in 'hard' water areas (McKillop and Harrison, 1972). Williams found greater than 10-fold fluctuations of 6.2 to 101.0 mg l^{-1} Ca^{2+} at study sites in central Africa (Williams, 1970) and *L. stagnalis* populations in drainage ditches on the Somerset Levels, UK, experience 8-fold changes in [Ca^{2+}] from 23 to 185 mg l^{-1} (S.D., unpublished). These studies used data collected every 1–3 months, and give no indication of the rate of change in calcium concentration experienced by freshwater gastropods. Macan

found 3-fold fluctuations of 8–30 mg l^{-1} in tarns in the English Lake District (Macan, 1950), and provides the most fine-scale measurements of data available, demonstrating 2- to 3-fold declines in environmental calcium in less than 1 week following periods of heavy rainfall, although daily measurements were not taken. This type of environmental fluctuation is rarely taken into account when sampling gastropod populations; however, it may have highly significant effects on the physiology of the snails.

Lymnaea stagnalis alters respiration and locomotory behaviour in response to acute environmental variation in temperature (Sidorov, 2003; Sidorov, 2005) and predator presence (Orr et al., 2007). However, the effects of environmental calcium concentration on snail behaviour and physiology have not previously been considered. Looking back in the literature we have been struck by how the levels of calcium in the environment alter the occurrence of *L. stagnalis*. For example, in the McKillop and Harrison study mentioned above (McKillop and Harrison, 1972) only a few *L. stagnalis* were found in soft water environments, while in hard water locations the abundance was over 6-fold greater. There was also a lack of larger adults in the soft water locations, indicating the possibility of slower growth rates and a lack of survivorship in adult snails (McKillop and Harrison, 1972). Piggott and Dussart also found that *L. stagnalis* actively select high calcium environments, independent of whether they have previously been held in high or low calcium water, and so can potentially rapidly sense the calcium content of the environment (Piggott and Dussart, 1995).

Thus, we decided that we needed to determine whether differing levels of environmental calcium altered behaviours that we typically study in *L. stagnalis*. As a first step we focused our attention on both aerial and cutaneous respiration as well as speed of locomotion. We report here that acute exposure of snails to low levels (20 mg l^{-1}) of environmental calcium significantly altered both forms of respiration and also locomotion. These data will serve as a foundation for future experiments aimed at determining whether such low levels of environmental calcium serve as a potent stressor for the snail.

MATERIALS AND METHODS

Adult *L. stagnalis*, 25±1 mm spire height, were raised from stock originally obtained from Vrije Universeit in Amsterdam. This population originated from wild snails collected in the 1950s from canals in a polder located near Utrecht. Adult snails were reared in aquaria filled with de-chlorinated tap water, [Ca^{2+}] of 60±5 mg l^{-1}, in the snail rearing facility at the University of Calgary, and were transferred 1 week prior to experiments into oxygenated artificial pond water (0.26 g l^{-1} Instant Ocean®, Aquarium Systems Inc., Mentor, OH, USA) with additional calcium sulphate dehydrate added to make low (20 mg l^{-1}) [Ca^{2+}] or high (80 mg l^{-1}) [Ca^{2+}] water depending on treatment group. Snails were maintained at room temperature (20±1°C) at a stocking density of 1 snail per litre and fed romaine lettuce *ad libitum*. Romaine lettuce has been used as a food source to successfully rear snails at this facility for several years, and although it contains a source of calcium that the snails would be able to utilise, the calcium content is fairly low (0.36 mg Ca^{2+} per gram of lettuce), and previous work has suggested that *L. stagnalis* obtains the majority of its calcium requirements from the water (Van Der Borght and Van Puymbroek, 1966).

Crawling speed

Individual *L. stagnalis* were taken from the home aquaria and placed in a large Petri dish (14 cm diameter by 2 cm depth) in 200 ml of oxygenated artificial pond water with 20 or 80 mg l^{-1} Ca^{2+}, equivalent to the level they had experienced in their home aquaria during the previous week, giving a depth of 15 mm in the Petri dish, sufficient to fully submerge the snail. On placement of the snails into the Petri dish they generally withdrew into their shells. We did not commence measurements of their crawling speed until the snail re-emerged, when the head and tentacles were fully extended. Once the snail was fully emerged the distance moved was measured over a period of 15 min; a 2 cm×2 cm grid marked on the base of the Petri dish allowed the distance crawled to be estimated by counting the number of squares each snail crossed during the 15 min observation period. The mean travelling speed over 15 min was then calculated in mm s^{-1}. A total of 12 snails were observed in high calcium (80 mg l^{-1}) and 13 snails in low calcium (20 mg l^{-1}).

Aerial respiration

Lymnaea stagnalis are bimodal breathers obtaining oxygen through either cutaneous respiration (i.e. directly through the skin, see below) or aerial respiration *via* a lung (i.e. gas exchange with the atmosphere) (Lukowiak et al., 1996; Orr and Lukowiak, 2010). To perform aerial respiration, the snail must surface and open its pneumostome (respiratory orifice) while contracting and relaxing the appropriate respiratory muscles. Aerial respiratory behaviour is driven by a 3-neuron CPG whose sufficiency and necessity have been demonstrated (Syed et al., 1990; Syed et al., 1992). To increase aerial respiratory behaviour we made the pond water hypoxic (<1% O_2 saturation) by vigorously bubbling N_2 through 500 ml of either high or low Ca^{2+} pond water in a 1 l beaker for 20 min before the introduction of snails. Snails were first acclimated to the beaker for 10 min, followed by a 30 min breathing observation period. N_2 was gently and continuously bubbled throughout to maintain hypoxic conditions.

Snails were tested in groups of 10 individuals in each beaker; this number has been found to enable a larger number of individuals to be processed at one time, without contact between individuals disrupting breathing patterns. Snails were individually labelled a minimum of 48 h prior to the experiments by gluing a small number printed on waterproof paper onto the shell above the position of the pneumostome. This allowed individual snails to be easily identified during the breathing trials and also tracked in their home aquaria.

Individual aerial respiration rate was tested first in the calcium concentration in which the snail had been held for the previous week. The snail was then transferred to the alternative calcium concentration for 24 h and re-tested at this new concentration. A total of 30 snails experienced the high calcium concentration first and 30 experienced the low calcium concentration first. Occasionally snails did not demonstrate aerial respiration during the course of the experiment, in which case they were excluded from analysis. This resulted in a total of 29 snails in the high to low concentration group and 28 snails in the low to high concentration group being used for data analysis.

Cutaneous respiration

As mentioned above, in addition to being able to respire by exchanging the contents of their lung with the atmosphere (i.e. aerial respiration), snails are also able to respire cutaneously. That is, dissolved gasses in the water are able to cross the skin. In eumoxic conditions (i.e. >20% O_2 saturation) cutaneous respiration predominates (Lukowiak et al., 1996).

Respirometry was carried out to assess whether calcium concentration was altering the basic metabolic rate of *L. stagnalis*. Glass spice jars, 75 ml volume, were used as respirometry chambers as they have an excellent seal on the lid of the jar allowing

measurements to be taken in an enclosed system. This size of container allowed the snail to fully extend and move, whilst providing a small enough volume to accurately measure oxygen consumption over a relatively short period of time. A hole was drilled in the lid to allow a rubber stopper holding the electrode to be inserted into the top of the jar. A stainless steel mesh platform was formed in the jar, 1 cm from the base, so that a small magnetic stirrer could be placed in the base, ensuring that water mixing was occurring throughout without disturbing the snail. Water, either low or high calcium, was aerated vigorously for a minimum of 20 min to give a reading of 100% oxygen saturation prior to the start of each trial. Aeration was ceased and the respirometry chamber was fully flushed with water to make sure there were no trapped air bubbles; an individual snail was then placed in the chamber and allowed to acclimate for 10 min. This period was found to be sufficient to allow all snails used to fully emerge from their shells. The oxygen consumption of each snail was then measured using a Fibox 3 oxygen meter (PreSens Precision Sensing GmbH, Regensburg, Germany) and analysed using OxyView software (PST3-V5.32 02/2004 PreSens Precision Sensing GmbH) taking oxygen readings every 15 s for a further 15 min; this was then adjusted to account for electrode oxygen consumption measured when no snail was present prior to analysis. Respirometry experiments were carried out at 20°C.

The oxygen consumption of each snail was initially measured in the calcium concentration in which it had been held for the previous week. Each individual was then transferred into the alternative calcium concentration for 24 h and its oxygen consumption was measured at this new calcium level. A total of 30 snails were used to measure cutaneous respiration rate, with 15 experiencing high calcium and 15 experiencing low calcium conditions initially.

Statistics

Crawling speed and snail spire height of animals used were analysed as a two-sample *t*-test in SPSS 17.0 (SPSS Inc. Chicago, IL, USA). Aerial breathing and rate of cutaneous respiration were both analysed using repeated measures ANOVA in SPSS, and tested for equal variance using Mauchly's test for sphericity. For aerial breathing the within-subject factor was calcium level (two levels: high *vs* low), and between-subject factors were treatment block (three levels: 1–3) and which calcium concentration they experienced first (two levels: high *vs* low). The rate of oxygen consumption (average slope for each snail) was also used to compare cutaneous respiration between calcium concentrations, the within-subject factor was calcium concentration (two levels: high *vs* low) and between-subject comparison was which calcium concentration they experienced first (two levels: high *vs* low).

RESULTS

Since *L. stagnalis* are considered to be calciphiles (i.e. they need $\geqslant$20 mg l^{-1} of environmental calcium to survive and prosper) we hypothesised that acute exposure to a low calcium environment (20 mg l^{-1}) for 1 week would alter a number of their basic behaviours relative to snails held in a high calcium environment (80 mg l^{-1}). We first examined crawling speed. We found that snails maintained in aquaria containing a low concentration of environmental calcium had a significantly lower crawling speed than snails that had been maintained in aquaria with high environmental calcium (Fig. 1; t=3.05, P=0.006, d.f.=22). This difference was not related to snail size as spire height (distance from the apex to the outer aperture margin) did not differ significantly between those maintained for 1 week at 80 mg l^{-1} (25.59±0.56 mm) and those maintained for 1 week at 20 mg l^{-1} (25.43±0.51 mm). Thus we conclude that exposure to low environmental calcium for 1 week is sufficient to reduce the speed of locomotion.

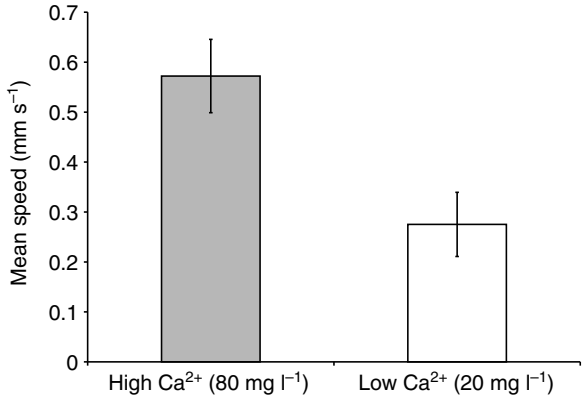

Fig. 1. Mean ± s.e. crawling speed of *Lymnaea stagnalis* held in either a high or a low calcium environment for 1 week prior to testing.

As snails maintained for 1 week in aquaria containing a low concentration of environmental calcium (20 mg l^{-1}) locomote significantly slower than those maintained in 80 mg l^{-1} calcium, we tested the hypothesis that there would be a significant difference in the time spent performing aerial respiration in hypoxic conditions. Snails maintained at low environmental calcium levels spent significantly less time performing aerial respiration (total breathing time over 30 min) than when they were maintained in a high calcium environment (Fig. 2; $F_{1,51}$=5.696, P=0.021). There was no effect of the order in which they experienced high or low environmental calcium ($F_{1,51}$=0.031, P=0.860), nor any effect of treatment block ($F_{2,51}$=0.586, P=0.560) or interaction between these factors ($F_{2,51}$=0.409, P=0.667). We therefore conclude that when snails are maintained in a low calcium environment they show a reduction in both locomotion and aerial respiration.

Having shown that snails maintained in the low calcium environment have a significantly lower rate of aerial respiration we tested whether there would also be an effect of the high *vs* low calcium environment on cutaneous respiration. In contrast to our finding with aerial respiration, we found that the rate of oxygen consumption was significantly higher when snails were maintained in the low calcium environment than in the high calcium

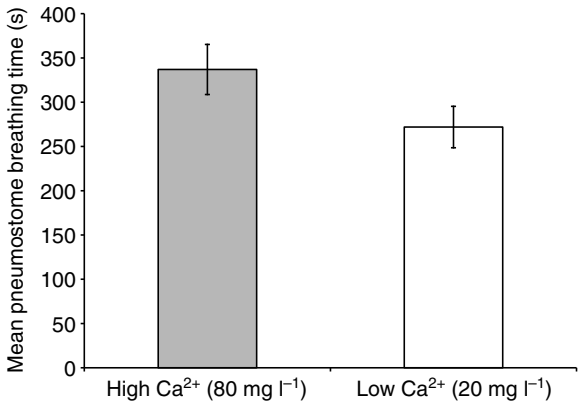

Fig. 2. Total mean ± s.e. pneumostome breathing time over 30 min in high and low calcium environments.

environment (Fig. 3; $F_{1,28}$=5.219, P=0.030) irrespective of which calcium treatment they experienced first.

DISCUSSION

Lymnaea stagnalis is a calciphile (Boycott, 1936; Young, 1975a); that is, this species of snail requires a minimum concentration of 20 mg l^{-1} environmental calcium, below which it suffers from reduced growth and survival. We therefore set out to determine whether a number of important behaviours were altered by acute (1 week) exposure to low environmental calcium. We found locomotion, aerial respiration and cutaneous respiration were all significantly altered by exposure to low environmental calcium.

We first found that the speed of locomotion was significantly reduced in snails exposed to low environmental calcium (20 mg l^{-1}) compared with snails exposed to the high (80 mg l^{-1}) calcium environment. Anecdotal observations expressed in a previous study (Zalizniak et al., 2009) are in line with our findings in that they state that another species of snail, *Physa acuta*, also has reduced speed in water lacking calcium. A question that has to be addressed is whether the significantly faster speed that we observed in the high calcium environment is abnormally fast for *L. stagnalis*. In two recent studies the speed of crawling locomotion was determined to be 0.47 mm s^{-1} on Teflon plates (Aono et al., 2008) and 0.51 mm s^{-1} on silicone plates (Miyamae et al., 2010). The conditions in which the snails were maintained prior to both these studies had a similar calcium concentration to our high calcium environment and both produced similar crawling speeds to those found in our high calcium conditions. However, Ormshaw and Elliott obtained a considerably slower speed of locomotion (0.17–0.33 mm s^{-1}), comparable to our results in low calcium conditions, despite maintaining their snails in a high calcium environment (80 mg l^{-1}) (Ormshaw and Elliott, 2006). These authors measured locomotion using groups of 10 snails in a single chamber (Ormshaw and Elliott, 2006); we believe that this creates problems in measuring locomotion rate as snails will interact with one another, and this interaction 'slows' them down. Thus we conclude that the rate of locomotion we measured using *L. stagnalis* in the high calcium environment is not abnormally fast, and falls within the typical crawling rate for this species obtained in the laboratory when maintained in high environmental calcium. We therefore conclude that the speed we observed in the low calcium environment is significantly slower than the speed that this species is capable of achieving.

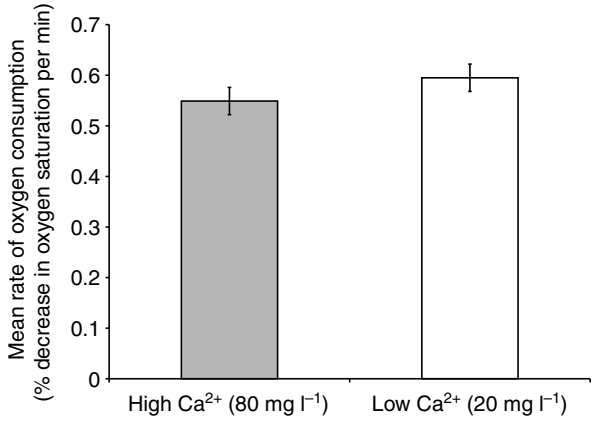

Fig. 3. Mean ± s.e. rate of oxygen consumption per minute *via* cutaneous respiration in high and low calcium environments.

We suspect that the slower speed observed in the low calcium snails is due to the increased metabolic demands on *L. stagnalis* in a low calcium environment, where a greater proportion of energy is required for calcium acquisition, reducing the energy available for motility. Greenaway suggested that calcium uptake in solutions of 20 mg l^{-1} Ca^{2+} or less is against the electrochemical gradient, and therefore only active uptake is occurring (Greenaway, 1971). Above 20 mg l^{-1} Ca^{2+}, calcium uptake is likely to occur in part *via* a passive electrochemical gradient, and evidence that net uptake above 40–60 mg l^{-1} Ca^{2+} plateaus suggests that the energy requirement to obtain calcium at this level of environmental concentration is diminished (Greenaway, 1971). Consistent with this notion that snails are slower because of the increased metabolic demand imposed by the need to actively take up calcium from pond water are the data we obtained measuring cutaneous respiration. The significant increase in cutaneous respiration rate found here in low environmental calcium relative to when snails were maintained in the high calcium environment indicates an increased metabolic demand.

We did not, however, observe an increase in aerial respiration in the snails from the low calcium environment compared with those in the high calcium environment. These data appear to contradict the cutaneous respiration data and our hypothesis that the slower speed observed in the low calcium environment was due to having less energy available as a result of the demand to actively take up calcium from the environment. To explain why the snails have a significantly lower total breathing time in the low calcium compared with the high calcium environment we have to remember that in the apparatus used to measure aerial respiratory behaviour (i.e. a 1 l beaker filled with 500 ml of pond water) snails have ample space to move around, and our impression based on casual observation is that snails move around more in the high calcium water than in the low calcium water. Thus, the reduced time spent on aerial respiration in the low calcium environment may be accounted for by a reduction in movement decreasing oxygen demands in hypoxic conditions. Consistent with this view it must also be remembered that movement in the chambers used to measure cutaneous respiration, whilst not prevented, is limited due to the small chamber volume, giving a closer approximation to the basal metabolic rate.

The lack of an effect of the order in which snails experienced either high or low calcium environments on both cutaneous and aerial respiration, despite 7 days acclimation in the first instance and only 24 h in the second, suggests that the effect of calcium concentration in the environment on respiration is rapid. Data on the ability of *L. stagnalis* to maintain haemolymph calcium concentrations in extremely low [Ca^{2+}] suggest that this behavioural change is not due to an internal change in haemolyph [Ca^{2+}] (Greenaway, 1971; Dewith et al., 1987; Grosell and Brix, 2009); indeed, *L. stagnalis* is able to maintain haemolymph [Ca^{2+}] in the complete absence of environmental calcium for at least 10 days (Dewith, 1977). There is evidence that *L. stagnalis* is able to sense environmental calcium levels and orientate towards higher concentrations (Piggott and Dussart, 1995), so it is possible that this directly controls respiratory behaviour and locomotion based on external [Ca^{2+}] rather than internal haemolymph [Ca^{2+}]. As yet, however, there is no direct evidence that *L. stagnalis* is able to sense [Ca^{2+}] in the external environment.

Previous work has highlighted the effect of environmental calcium on growth and reproduction in aquatic gastropods, demonstrating that prolonged deficits in calcium availability can reduce fitness through reduced growth and reproductive output (Harrison et al., 1970; Young, 1975a; Madsen, 1987; Zalizniak et

al., 2009). Furthermore, reduced levels of calcium in the environment can also prevent shell thickening upon predator detection, thereby preventing gastropods from showing induced morphological defences (Rundle et al., 2004; Czarnoleski et al., 2006). Here we have shown that even acute periods of low calcium can impact on metabolism and activity in *L. stagnalis*. A reduction in activity levels may reduce the ability to find food or mates, but could also potentially reduce the ability to respond to predators. Previous work has demonstrated that aquatic gastropods respond to predation threat by moving into habitat that offers greater protection, either under shelter or crawling above the water line (Turner et al., 1999; Dalesman et al., 2006). Reduced activity levels following acute exposure to low [Ca^{2+}] may decrease the ability of *L. stagnalis* to respond behaviourally to predator cues, and hence increase vulnerability to predation.

Whilst there is considerable evidence that environmental calcium levels have a significant impact on aquatic gastropod distribution much of this data is based on mean levels in the environment or measurements at single time points (e.g. Boycott, 1936; Briers, 2003). Data where multiple measurements have been taken suggest that environmental calcium can fluctuate considerably over the course of a year (Macan, 1950; Williams, 1970; McKillop and Harrison, 1972) (S.D., unpublished). The data presented here suggest that fluctuations where calcium levels are reduced to 20 mg l^{-1} in environments where the calcium level is normally higher, for example during times of flooding (Williams, 1970), can have an important impact on gastropod physiology and behaviour. It is important to note at this stage that the change from high to low calcium concentration was immediate in our study, whilst in nature changes are likely to occur more gradually potentially allowing for acclimatisation to occur. Currently there are no data available indicating the rate of change from high to low calcium experienced in natural conditions, and this is likely to vary considerably between habitats, though 3-fold declines in environmental calcium have been recorded to occur within 1 week following a period of heavy rainfall (Macan, 1950).

In addition to fluctuations over short time scales there is considerable evidence that environmental calcium is declining in freshwater systems in North America and Europe. A 35% decline in environmental calcium available in some freshwater systems has occurred over the past 2 decades (Jeziorski et al., 2008; Keller, 2009). Exposure to acid rain has leached available calcium cations, normally present in the soil, from many watersheds and despite significant declines in acidity, calcium levels have yet to recover, leading to low calcium input from runoff into freshwater habitats (Watmough et al., 2005; Keller, 2009). In addition to this, a warming climate may lead to increased growth rates of trees and a tendency for coniferous species to be replaced by deciduous species, both of which are predicted to increase the depletion rate of available calcium from soils (Huntington, 2005). Long term depletion of environmental calcium is likely to result in aquatic gastropod populations experiencing longer or more frequent exposure to a low calcium environment when fluctuations occur. Where levels are normally lower there may be the potential for local adaptation to reduced [Ca^{2+}] as found in *Daphnia galeata* (Rukke, 2002). As yet there is no evidence for this type of local adaptation in aquatic gastropods, though natural variation in physiological responses to environmental calcium concentration does appear to exist (Hunter and Lull, 1977; Dussart and Kay, 1980). Work with laboratory reared animals where the environmental conditions are closely controlled is required to assess whether these differences are indeed due to genetic races.

ACKNOWLEDGEMENTS

We would like to thank Doug Symes for providing laboratory facilities for the respirometry work and two anonymous reviewers for constructive comments on the manuscript. This study was funded by an Alberta Heritage Foundation for Medical Research postdoctoral fellowship awarded to S.D.; the Canadian Institute for Health Research supports K.L. and the laboratory.

REFERENCES

Aono, K., Fusada, A., Fusada, Y., Ishii, W., Kanaya, Y., Komuro, M., Matsui, K., Meguro, S., Miyamae, A., Miyamae, Y. et al. (2008). Upside-down gliding of *Lymnaea*. *Biol. Bull.* **215**, 272-279.
Boycott, A. E. (1936). The habitats of fresh-water Mollusca in Britain. *J. Anim. Ecol.* **5**, 116-186.
Briers, R. A. (2003). Range size and environmental calcium requirements of British freshwater gastropods. *Global. Ecol. Biogeogr.* **12**, 47-51.
Czarnoleski, M., Kozlowski, J., Kubajak, P., Lewandowski, K., Muller, T., Stanczykowska, A. and Surowka, K. (2006). Cross-habitat differences in crush resistance and growth pattern of zebra mussels (*Dreissena polymorpha*): effects of calcium availability and predator pressure. *Arch. Hydrobiol.* **165**, 191-208.
Dalesman, S., Rundle, S. D., Coleman, R. A. and Cotton, P. A. (2006). Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Anim. Behav.* **71**, 789-797.
Dewith, N. D. (1977). Evidence for independent regulation of specific ions in hemolymph of *Lymnaea stagnalis* (L). *Proc. Kon. Ned. Akad. Wet. C.* **80**, 144-157.
Dewith, N. D., Kok, T. P. and Vanderschors, R. C. (1987). Relationships between apparent net H^+ excretion and Na^+, Ca^{2+} and Cl^- uptake in the pulmonate freshwater snail *Lymnaea stagnalis*. *Comp. Biochem. Phys.* **87A**, 671-675.
Dussart, G. and Kay, R. (1980). Relationship between water chemistry and respiration rate in several populations of *Lymnaea peregra* Müller (Gastropoda: Mollusca). *Hydrobiologia* **69**, 57-65.
Evans, C. D., Cullen, J. M., Alewell, C., Kopacek, J., Marchetto, A., Moldan, F., Prechtel, A., Rogora, M., Vesely, J. and Wright, R. (2001). Recovery from acidification in European surface waters. *Hydrol. Earth Syst. Sci.* **5**, 283-297.
Glass, N. and Darby, P. (2009). The effect of calcium and pH on Florida apple snail, *Pomacea paludosa* (Gastropoda: Ampullariidae), shell growth and crush weight. *Aquat. Ecol.* **43**, 1085-1093.
Greenaway, P. (1971). Calcium regulation in the freshwater mollusc, *Limnaea stagnalis* (L.) (Gastropoda: Pulmonata). I. The effect of internal and external calcium concentration. *J. Exp. Biol.* **54**, 199-214.
Grosell, M. and Brix, K. V. (2009). High net calcium uptake explains the hypersensitivity of the freshwater pulmonate snail, *Lymnaea stagnalis*, to chronic lead exposure. *Aquat. Toxicol.* **91**, 302-311.
Harrison, A. D., Williams, N. V. and Greig, G. (1970). Studies on the effects of calcium bicarbonate concentrations on the biology of *Biomphalaria pfeifferi* (Krauss) (Gastropoda: Pulmonata). *Hydrobiologia* **36**, 317-327.
Hunter, R. D. and Lull, W. W. (1977). Physiologic and environmental factors influencing the calcium-to-tissue ratio in populations of three species of freshwater pulmonate snails. *Oecologia* **29**, 205-218.
Huntington, T. G. (2005). Assessment of calcium status in Maine forests: review and future projection. *Can. J. For. Res.* **35**, 1109-1121.
Jeziorski, A., Yan, N. D., Paterson, A. M., DeSellas, A. M., Turner, M. A., Jeffries, D. S., Keller, B., Weeber, R. C., McNicol, D. K., Palmer, M. E. et al. (2008). The widespread threat of calcium decline in fresh waters. *Science* **322**, 1374-1377.
Keller, W. B. (2009). Limnology in northeastern Ontario: from acidification to multiple stressors. *Can. J. Fish. Aquat. Sci.* **66**, 1189-1198.
Lewis, D. B. and Magnuson, J. J. (1999). Intraspecific gastopod shell strength variation among north temperate lakes. *Can. J. Fish. Aquat. Sci.* **56**, 1687-1695.
Lukowiak, K., Ringseis, E., Spencer, G., Wildering, W. and Syed, N. (1996). Operant conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. *J. Exp. Biol.* **199**, 683-691.
Macan, T. T. (1950). Ecology of fresh-water mollusca in the English Lake District. *J. Anim. Ecol.* **19**, 124-146.
Macan, T. T. (1977). *A Key to the British Fresh- and Brackish-Water Gastropods with Note on their Ecology*. Ambleside: The Freshwater Biological Association.
Madsen, H. (1987). Effect of calcium concentration on growth and egg laying of *Helisoma duryi*, *Bulinus africanus* and *Bulinus truncatus* (Gastropoda: Planorbidae). *J. Appl. Ecol.* **24**, 823-836.
McKillop, W. and Harrison, A. (1972). Distribution of aquatic gastopods across the interface between the Canadian Shield and limestone formations. *Can. J. Zool.* **50**, 1433-1445.
Miyamae, Y., Komuro, M., Murata, A., Aono, K., Nishikata, K., Kanazawa, A., Fujito, Y., Komatsu, T., Ito, D., Abe, T. et al. (2010). Contrary effects of octopamine receptor ligands on behavioral and neuronal changes in locomotion of *Lymnaea*. *Biol. Bull.* **218**, 6-14.
Okland, J. (1983). Factors regulating the distribution of freshwater snails (Gastropoda) in Norway. *Malacologia* **24**, 277-288.
Ormshaw, J. and Elliott, C. (2006). Octopamine boosts snail locomotion: behavioural and cellular analysis. *Invertebr. Neurosci.* **6**, 215-220.
Orr, M. and Lukowiak, K. (2010). Sympatric predator detection alters cutaneous respiration in *Lymnaea*. *Comm. Integr. Biol.* **4**, 1-4.
Orr, M. V., El-Bekai, M., Lui, M., Watson, K. and Lukowiak, K. (2007). Predator detection in *Lymnaea stagnalis*. *J. Exp. Biol.* **210**, 4150-4158.
Piggott, H. and Dussart, G. (1995). Egg-laying and associated behavioural responses of *Lymnaea peregra* (Müller) and *Lymnaea stagnalis* (L.) to calcium in their environment. *Malacologia* **37**, 13-21.
Rukke, N. A. (2002). Tolerance to low ambient calcium shows inter-population differences in *Daphnia galeata*. *J. Plankton Res.* **24**, 527-531.

Rundle, S. D., Spicer, J. I., Coleman, R. A., Vosper, J. and Soane, J. (2004). Environmental calcium modifies induced defences in snails. *Proc. R. Soc. B* **271**, S67-S70.

Sidorov, A. V. (2003). Effects of temperature on respiration defensive behavior and locomotion of fresh water snail *Lymnaea stagnalis*. *Zh. Vyssh. Nerv. Deiat. Im. I P Pavlova* **53**, 513-517.

Sidorov, A. V. (2005). Effect of acute temperature change on lung respiration of the mollusc *Lymnaea stagnalis*. *J. Therm. Biol.* **30**, 163-171.

Syed, N. I., Bulloch, A. G. M. and Lukowiak, K. (1990). In vitro reconstruction of the respiratory central pattern generator of the mollusk *Lymnaea*. *Science* **250**, 282-285.

Syed, N. I., Ridgway, R. L., Lukowiak, K. and Bulloch, A. G. M. (1992). Transplantation and functional-integration of an identified respiratory interneuron in *Lymnaea stagnalis*. *Neuron* **8**, 767-774.

Turner, A. M., Fetterolf, S. A. and Bernot, R. J. (1999). Predator identity and consumer behavior: differential effects of fish and crayfish on the habitat use of a freshwater snail. *Oecologia* **118**, 242-247.

Vaidya, D. P. and Nagabhushanam, R. (1980). Calcium regulation in the freshwater snail *Indoplanorbis exustus* during shell repair. *Hydrobiologia* **69**, 29-32.

Van Der Borght, O. and Van Puymbroek, S. (1966). Calcium metabolism in a freshwater mollusc: quantitative importance of water and food supply for calcium during growth. *Nature* **210**, 791-793.

Watmough, S., Aherne, J., Alewell, C., Arp, P., Bailey, S., Clair, T., Dillon, P., Duchesne, L., Eimers, C., Fernandez, I. et al. (2005). Sulphate, nitrogen and base cation budgets at 21 forested catchments in Canada, the United States and Europe. *Environ. Monit. Assess.* **109**, 1-36.

Williams, N. V. (1970). Studies on aquatic pulmonate snails in central Africa 1. Field distribution in relation to water chemistry. *Malacologia* **10**, 153-164.

Young, J. O. (1975a). Preliminary field and laboratory studies on the survival and spawning of several species of gastropoda in calcium-poor and calcium-rich waters. *Proc. Malac. Soc. Lond.* **41**, 429-437.

Young, J. O. (1975b). A laboratory study, using ^{45}Ca tracer, on the source of calcium during growth in two freshwater species of Gastropoda. *Proc. Malac. Soc. Lond.* **41**, 439-445.

Zalizniak, L., Kefford, B. J. and Nugegoda, D. (2009). Effects of different ionic compositions on survival and growth of *Physa acuta*. *Aquat. Ecol.* **43**, 145-156.