

## Studies on the pH tolerance of freshwater snails

Ciara O'Sullivan<sup>1</sup>, Bernard Fried<sup>2</sup>, and Joseph Sherma<sup>1</sup>

<sup>1</sup>Department of Chemistry, <sup>2</sup>Department of Biology, Lafayette College, Easton PA 18042, USA

### ABSTRACT

The environment in which animals can live is dictated by several abiotic and biotic factors. One important factor, especially for aquatic animals, is pH. The optimal pH is vital to ensure the occurrence of important biological processes. Enzymes work best at certain pH values, and the environmental pH may affect the enzyme effectiveness. The medically important, aquatic snail *Biomphalaria glabrata* has been studied extensively in regards to its behavior and biochemistry. Our review examines the pH range at which *B. glabrata* can survive optimally and the effect pH has on the metabolism of *B. glabrata*. Based on previous studies on the pH tolerance of *B. glabrata* both in the field and the laboratory, these snails tolerate a relatively broad pH range. This paper also examines the literature on pH tolerance in laboratory and experimental studies on selected freshwater snails with major emphasis on *B. glabrata*. Lastly, a biochemical study on the effects of subjecting the *B. glabrata* snail to a wide range of pH values showed that such changes did not alter the composition of the snail lipids as determined by high-performance thin layer chromatography (HPTLC).

**KEYWORDS:** freshwater snails, pH, *Biomphalaria glabrata*, *Pomacea insularum*, HPTLC, acid-base tolerances

### INTRODUCTION

Studying the environment in which organisms live is important to be able to predict the optimal growth and survival of plants and animals in an ever-changing global climate. In an aquatic

environment there are numerous biotic and abiotic factors that determine the survival and distribution of organisms including temperature, light intensity, salinity, and pH. Living in an optimal pH environment helps to assure that the organisms vital biological processes are working effectively. This particular variable, i.e. pH, has not been studied as much as other important environmental variables. There is relatively little experimental literature available on this topic especially in regards to the pH requirements of freshwater snails. This is probably because pH is a difficult variable to study experimentally. Biological events influence the pH substantially by way of a variety of metabolic processes.

Experimental studies on the pH tolerance of freshwater snails both in the field and in the laboratory are relatively sparse. Our recent studies have been concerned with the biochemical consequences of various biotic and abiotic factors that influence freshwater snails, particularly the economically and medically important snail *Biomphalaria glabrata*. In this paper we review the available literature on the important abiotic factor, pH, as it relates in general to the survival of freshwater snails and in particular to *B. glabrata* snails. We review the pertinent literature on the topic and include our laboratory research on the pH tolerance of *B. glabrata* recently performed in our laboratory.

### Field studies

An important indirect method of determining the pH ranges of freshwater snails is to take samples of the water where these organisms are found in the wild. Snails will only be found in areas where

the pH is tolerable. Sampling a wide selection of field sites will give a pH range where these snails are found. Several workers have used this method to determine pH field values, as well as other environmental factors that have an impact on freshwater snails. Because each project used different sample sites, the recorded pH ranges varied from study to study as stated in Malek [1]. He also reported that the pH of the water in any particular location had pH fluctuations throughout the course of a day. Malek [1] found that freshwater snails usually have a pH tolerance of 6.0-9.0 with some exceptions. In the Belgian Congo, Gillet and Wolfs [2] found that species of *Biomphalaria* and *Bulinus* snails survived in lakes with pH up to 9.2. In a study by Okland [3] in Norway, pulmonate snails were not found in lakes where the pH was less than 5.2. This was attributed to an elevated concentration of  $Al^{3+}$  present in the more acidic waters and not strictly to the higher concentration of  $H^+$ . The effect of pH on other solutes in the water is important in considering the survival tolerance of freshwater snails. The pH needs to be studied in conjunction with the other solutes present in the water. In a comprehensive study on the habitat of *B. glabrata*, Sturrock [4] studied 124 sites in St. Lucia and found that the mean pH value was 7.2 with a range of 5.6-8.9. From numerous studies in various parts of the world, we can determine where these snails are best able to survive in the wild.

### Laboratory studies

There have not been many laboratory studies on the pH tolerance of freshwater snails published, but the available ones were helpful in our selection of a method for such studies. In a study reported by Deschiens [5], *Biomphalaria glabrata* and *Bulinus truncatus* showed a pH tolerance of 4.5-10.0. Fox [6] did an experiment on pH in which he examined how pH influenced the impact of Bayer 73, a molluscicide, on *B. glabrata*. He found that at extreme pH values (4.0-5.0 and 9.7-9.9), more Bayer 73 was needed to achieve 100% snail mortality, suggesting that the organisms had altered their metabolic processes at the extreme pH ranges in order to survive. This further supports the work of Okland [3] who noted that pH does not directly affect the survival of snails

but has an important influence over other ions in solution. The most critical study influencing our work was the dissertation of Ramakrishnan [7] on the tolerance of the apple snail (*Pomacea insularum*) to various environmental conditions including pH ranges. The dissertation chapter on pH served as the experimental basis for our research. Ramakrishnan [7] found that *P. insularum* snails survived at a pH range of 4.0-10.0 for 28 days. The apple snail is in a different taxon than the *B. glabrata* snail, but we would expect similarities in pH tolerance for both *P. insularum* and *B. glabrata*.

## EXPERIMENTAL

### Snail maintenance in our laboratory

Adult *B. glabrata* snails ranging in shell diameters from 12-17 mm were obtained from Dr. Fred A. Lewis, Head, Schistosomiasis Laboratory, Biomedical Research Institute (Rockville, MD, USA) and used within 1 month of receipt. The snails were maintained in glass jars each containing 800 mL of de-ionized (DI) water. Appropriate amounts of 1.0 M or 0.01 M HCl and NaOH solutions were added to the jars to obtain a known pH value. There were 3 snails per jar, and the snails were fed *ad libitum* on Romaine lettuce. The water was changed each time the pH was checked i.e., about twice a day. The initial pH values tested in order to determine a tolerance range were 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 with DI water as the control. The snails placed in solutions with a pH of 2.0 and 12.0 immediately retracted into their shells and were dead within 1 hr. In the next set of survival trials, a pH of 3.0 and 11.0 were tested; snails in these trials did not die immediately. Within 24 hr, however, all of the snails in the jar at pH 11.0 were dead. After several trials we found that within 2 hr, the pH of most solutions would fluctuate considerably. We also found that at the more extreme pH values, there was not as much fluctuation in the pH.

### High performance thin layer chromatography (HPTLC) analysis

To determine any biochemical consequences of maintaining snails at different pH values we examined snails maintained at pH values of 4.0 and 10.0 for 48 and 72 hr to note if there were any alterations in neutral and polar lipids using

**Table 1.** Weight percentage [mean  $\pm$  standard error (S.E.)] of polar Lipids in the DGG of snails.

Time	Phosphatidylcholine			Phosphatidylethanolamine		
	4.0	DI	10.0	4.0	DI	10.0
48 hr	0.6 $\pm$ 0.2	0.7 $\pm$ 0.3	0.07 $\pm$ 0.01	0.10 $\pm$ 0.03	0.13 $\pm$ 0.01	0.5 $\pm$ 0.4
72 hr	0.6 $\pm$ 0.1	0.4 $\pm$ 0.3	0.62 $\pm$ 0.09	0.11 $\pm$ 0.03	0.13 $\pm$ 0.09	0.16 $\pm$ 0.06

**Table 2.** Weight percentage (mean  $\pm$  S.E.) of neutral lipids in the DGG of the snails

Time	Free sterols		
	4.0	DI	10.0
48 hr	0.07 $\pm$ 0.04	0.14 $\pm$ 0.05	0.12 $\pm$ 0.08
72 hr	0.08 $\pm$ 0.05	0.10 $\pm$ 0.04	0.11 $\pm$ 0.06

HPTLC- densitometry. Snails in DI water were used as controls for this experiment. Snail's digestive gland-gonad complexes (DGGs) were collected after either 48 or 72 hr in test or control solutions. Snail bodies were extracted from their shells by lightly tapping the shell with a hammer and removing the shell with forceps. Using fine scissors, the DGG was then separated from the body and used in analysis. Using the Folch *et al.* method [8] samples (0.0294-0.1348 g) were extracted in 2:1 chloroform-methanol in a 7 mL capacity Wheaton (Millville, NJ, USA) glass homogenizer. To separate and remove nonlipophilic material, 4 parts chloroform-methanol (2:1) for every 1 part Folch *et al.* wash (0.88% KCl, w/v, in DI water) was added. The aqueous top layer was removed and discarded. The lipophilic samples were then placed in a warm water bath and dried by a stream of nitrogen gas. To obtain sample scan areas within the HPTLC calibration curves, residues were reconstituted in 0.500-1.348 mL of chloroform-methanol 2:1 as necessary and stored at -20°C until used.

Details of methods for standard solution preparation from Matreya, Inc. (Pleasant Gap, PA USA) neutral and polar standard mixtures; standard and sample solution application to plates with a Drummond (Broomall, PA) 10  $\mu$ L digital microdispenser; ascending plate development in a CAMAG (Wilmington, NC USA) HPTLC twin trough chamber; and neutral and polar lipid zone detection with phosphomolybdic acid and cupric sulfate-

phosphoric acid spray reagents, respectively, were the same as described in Counihan *et al.* [9]. The plates used were Analtech, Inc. (Newark, DE USA) 10 x 20 cm HPTLC-HLF (Catalog No. 61927) containing 19 scored channels and a preadsorbent spotting area at the bottom. The mobile phases were petroleum ether-diethyl ether-glacial acetic acid (80:20:1) for neutral lipids and chloroform-methanol-deionized water (65:20:1) for polar lipids. The lipids were quantified by slit-scanning densitometry in the absorbance-reflectance mode with slit dimensions 4.00 x 0.45 mm Micro and scanning rate 20.0 mm/second. The tungsten light source was set at 610 nm for neutral lipid determination, and the deuterium light source was set at 370 nm for the polar lipids. The winCATS software automatically created polynomial calibration curves (standard zone masses versus peak areas) and interpolated sample zone masses based on their bracketed peak areas. The percentage mass of each lipid was calculated using the equation:

$$\text{Percent lipid} = [w \times R \times \text{dilution factor} \times 100] / [\text{Initial DGG sample mass} (\mu\text{g})]$$

Where  $w$  = lipid mass ( $\mu\text{g}$ ) of sample interpolated from calibration curve,  $R$  = reconstituted volume ( $\mu\text{L}$ )/spotted volume ( $\mu\text{L}$ ). In samples with peak areas outside of the bracketed zone, samples that were either diluted or concentrated in order to alleviate this problem have this extra factor included in the calculation. Statistical analysis of the data was conducted using Microsoft Excel's version of the Student's  $t$  test to determine the statistical significance of the quantitative data. A value of  $P < 0.05$  was considered significant.

All of the snails, with the exception of one in the 48 hr DI jar survived until termination of the experiment. Of the neutral lipids analyzed, only free sterols were reported because the other lipids were not detected in sufficient amounts to be quantified in order to gain insight into the effect

of pH on the occurrence of these compounds in the snails in either control or test samples. Based upon our statistical analysis, neither cholesterol, phosphatidylcholine nor phosphatidylethanolamine were significantly different as a function of different pH values (Tables 1 and 2).

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