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EFFECT OF CARBONATE IONS (PH) ON THE GROWTH RATE OF *RHINELLA MARINA* TADPOLES

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ABSTRACT

Experiments were conducted to assess the effects of carbonate ions on growth, feeding behaviour and survival in *Rhinella marina* tadpoles. Gosner stage 22 tadpoles were exposed to different pH of carbonic acid, distilled water and pond water for duration of 4 weeks. Pond water had a pH of 7 and distilled water of pH 6.5. Growth rate was significantly higher at higher pH showing a significant difference between the growth in distilled and pond water with carbonic acid. Time spent on feeding was significantly higher in distilled and pond water indicating feeding to be partially responsible towards the growth rate. Survivorship was higher for control animals as compared to those exposed to pH 5.5 and 6.0. It was also observed that tadpoles in lower pH did not swim around a lot. At lower pH tadpoles faced a lot of stress which hindered its development. Stress was brought about by desiccation and lower respiratory rate since carbonic acid contains a lot of hydronium ions and less dissolved oxygen.

Key words: *Rhinella marina tadpoles*, carbonic acid, desiccation, growth, pH, survivorship

INTRODUCTION

Environmental factors play a major role in the development of organisms and favourable environmental conditions ensures that organisms are going to complete metamorphosis in the expected time span. However, not all the time the organisms are able to even complete its metamorphosis due to either factor restricting growth or the conditions have become too lethal for the organisms to survive.

One of the major environmental stresses which the freshwater organisms are facing in their aquatic environment is acidity. Low pH could be derived either from natural or anthropogenic sources which has lethal effects on numerous aquatic animals including amphibians (Rasanen *et al*., 2002). Acidification of the environment leads to a long lasting effect even in water which is circumneutral.

To date, all climatologists have agreed on the consequences of human activities leading to green house gas emissions. This results in global warming hence climate change (Ozdemir and Altindag, 2007). Once carbon dioxide starts to dissolve in aquatic mediums such as the oceans and freshwater, its pH starts to decrease. Carbon dioxide tends to form carbonic acid with water. Climate change can affect marine ecosystems by warming the oceans, sea level rise and decrease in pH due to increase in carbonate ion concentration of the water surface (Ozdemir and Altindag 2007). Increase in dissolved carbon dioxide reduces the amount of oxygen in water.

A study conducted by Beebee (1986) on *Bufo calamita* on lower pH showed that the eggs which survived at pH 4.0 took half the time longer to emerge from their jelly surrounds when compared to higher pH. Under laboratory conditions growth rates of tadpoles at 4.5 were only

60 percent. Tadpoles were placed in different concentrations of sulphuric acid in order to lower the pH. Acidic water can also delay metamorphosis and reduce size among individual tadpoles by stressing the individual tadpoles (Cummins, 1989).

Hence investigation of growth rate of tadpoles (*Rhinella marina*) was carried out in carbonic acid and its length, weight and feeding behaviour were compared with the control animals.

Materials and Methods

The tadpoles of *Rhinella marina* were collected using dip-nets from the University of the South Pacific drain which had the pH of slightly above 7 in September, 2012.Once brought to the lab, 48 of stage 22 tadpoles (Gosner stage) were identified and kept for two days in a glass dish to adapt to laboratory conditions.

Carbonic acid was prepared by dissolving gaseous carbon dioxide in cold distilled water and pH was then adjusted to 5.5 and 6.0 with normal distilled water. Pond water was autoclaved to kill any micro-organisms which could have affected the tadpoles.

Subjects chosen for testing were reared individually in 500 mL beakers and each beaker was covered with gauze netting to prevent tadpoles from jumping out. Temperature range for the tadpoles was from 25-27 ^{0} C. All water salinity was at 1 mg/ml. The amount of dissolved oxygen in distilled water, pH 5.5 pH 6.0 and pond water were 6.08-7.09, 5.37- 5.59, 5.40- 6.08 and 6.0-6.52 mg/L respectively.

Total body length and weight was measured before exposing the tadpoles to various treatments. 12 tadpoles for each setup were exposed to carbonic acid of pH 5.5 and 6.0, distilled water of pH 6.5 and pond water of pH around 7.The pH and temperature was monitored on a 24 hour basis. Water in each setup was changed every three days to avoid accumulation of toxins which could have affected the growth of tadpoles.

Subjects were fed on a weekly basis with decayed leaf of 0.05g. All dead subjects were removed and not replaced since mortality was also part of the results. Measurements such as length were taken using a vernier calliper of 0.01 precision and weight was taken using an electronic balance of 0.001 precision. Feeding behaviour was measured using a stop watch. This was done by monitoring tadpoles in each setup for 5 minutes and timing it on how long it spends on feeding on the decayed leaf.

Experiment was terminated on the 28th day.

Data collected was analysed using Kruskal-Wallis test to see for any significance change between the treatment groups.

Results

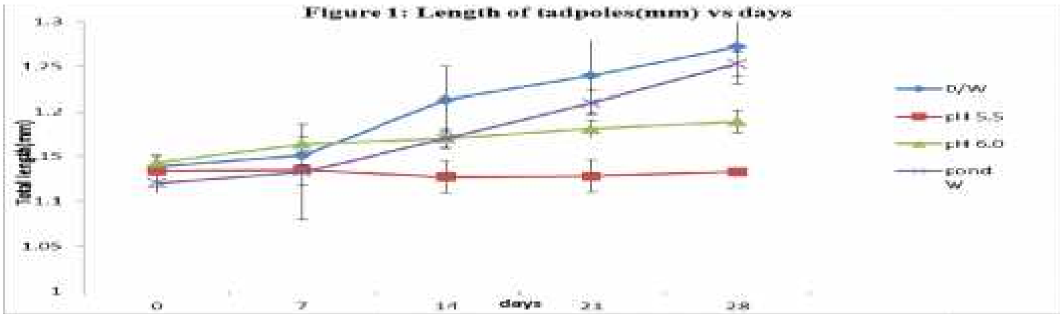
It has been observed that at lower pH the growth rate slows down and even stops (Table 1). The contributing factor towards this is the less amount of time tadpoles spend at low pH on feeding (Table 3). Distilled and pond water subjects showed a gradual increase in the length and the weight of over 28 days. At lower pH like 5.5, there was no growth in terms of length observed and the weight drastically dropped shown in Table 2. At pH 6.0, tadpoles showed gradual slow increase in length and gradual drop in weight.

On all days, temperature was stable, however the amount of dissolved oxygen varied everyday due to the fact that atmospheric oxygen and carbon dioxide kept dissolving and escaping.

Table1: Mean (± SE) length of tadpoles in different treatments over a 28 day period.

Mean Length(cm)	initial	7 days	14 days	21 days	28 days	Mean diff.
Distilled Water	1.14 ± 0.01	1.15 ±0.01	1.21 ±0.04	1.24±0.04	1.27 ±0.04	0.13
pH 5.5	1.13 ±0.02	1.14 ±0.02	1.13 ±0.02	1.13 ±0.02	1.13 ±0.01	0
pH6.0	1.14 ±0.01	1.16 ±0.01	1.17 ±0.01	1.18 ±0.01	1.19 ±0.01	0.05
Pond water	1.12 ±0.01	1.13 ±0.05	1.17 ±0.01	1.21 ±0.01	1.25 ±0.01	0.13

Length data expressed as means (n= 12 at initial). Values indicate a significant change in length in distilled and pond water, reduced growth in pH 6.0 and no growth in pH 5.5.

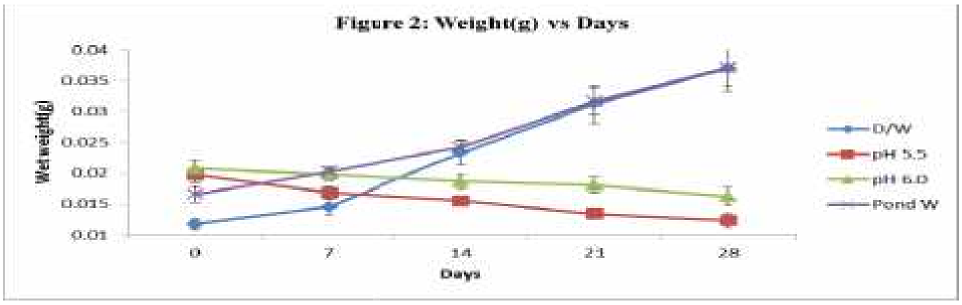


Graphs showing the difference in the length of tadpoles under different treatments. Slow growth is noted for pH 5.5 and 6.0.

Table 2: Mean (± SE) weight of tadpoles in different treatments over a 28 day period.

Mean Weight(g)	initial	7 days	14 days	21 days	28 days	Mean diff.
Distilled Water	0.012 ±0.001	0.015 ±0.000	0.023 ±0.002	0.031 ±0.003	0.037 ±0.004	0.025
pH 5.5	0.019 ±0.001	0.017 ±0.001	0.016 ±0.001	0.013 ±0.000	0.012 ±0.001	-0.007
pH6.0	0.021 ±0.001	0.019 ±0.001	0.019 ±0.001	0.018 ±0.001	0.016 ±0.001	-0.005
Pond water	0.017 ±0.001	0.020 ±0.001	0.024 ±0.001	0.032 ±0.002	0.037 ±0.003	0.021

Weight data expresses as means (n=12 at initial). Values show an increase in weight wet in distilled and pond water. At pH 5.5 tadpoles lost a significant amount of body weight followed by subjects in pH 6.0.

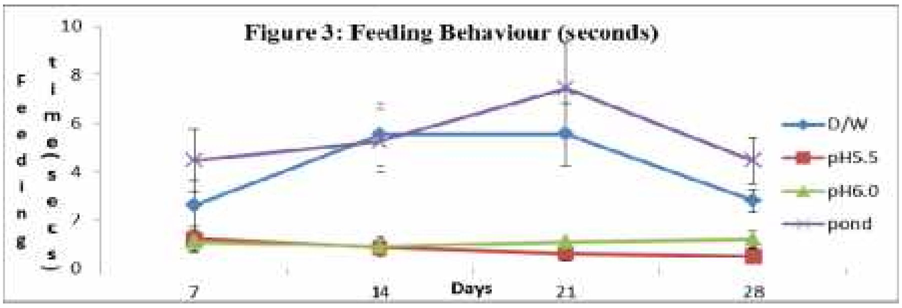


Graphs showing the differences in weight of tadpoles under different *treatments*. Under pH 5.5 and 6.0 the weights drop over a period of 28 days.

Table 3: Mean (± SE) feeding duration of tadpoles in different treatments for 4 weeks.

Mean Feeding(sec)	7th day	14th day	21st day	28th day	Mean time(sec)
D/ W	2.60 ±1.03	5.54 ±1.30	5.54 ±1.30	2.80 ±0.48	4.12
pH 5.5	1.22 ±0.47	0.75 ±0.34	0.63 ±0.32	0.50 ± 0.33	0.77
pH6.0	1.09 ±0.41	0.90 ±0.40	1.10 ±0.04	1.20 ±0.36	0.87
Pond water	4.45 ±1.28	5.27 ±1.31	7.45 ±1.89	4.45 ±0.96	5.41

Feeding data expressed as means (n=12 at initial).Values indicate that tadpoles feeding behaviour reduces at lower pH. Under distilled and pond water, tadpoles spend a lot of time in feeding.



Graphs showing that tadpoles fed well at higher pH and at lower pH their attachment towards food dropped.

Table 4: Survivorship of tadpoles over a span of 28 days under different treatments.

Treatment	Initial number	Number of survivors	Number died
Distilled water (pH 6.5)	12	10	2
Carbonic acid (pH5.5)	12	6	6
Carbonic acid (pH 6.0)	12	8	4
Pond water (pH 7.1)	12	11	1

Numbers indicate that mortality rate is higher under low pH.Drastic loss of subjects were observed under pH 5.5 where only 6 tadpoles were left towards the end of the experiment.

Table 5: Data analysis of length, weight and feeding behaviour.

Kruskal-Wallis test	Length	Weight	Feeding
Chi Square	25.954	37.214	37.438
df	3	3	3
Asym.Sig	0.000	0.000	0.000

The Kruskal-Wallis test confirms that the changes in length, weight and feeding behaviour showed significant differences under different treatments.

DISCUSSION

Any major changes to the aquatic environment have devastating effects on the marine organisms. Changes could be in regards to presence of predators, nutrients, availability of food and most recently pH is being another major factor affecting these lives. Under low pH conditions, the diversity of fish, micro invertebrates, parasite and zooplankton decreases (Marcogliese, 2001).

Tadpoles in carbonic acid of pH 5.5 and 6.0 showed that lower pH affects its development as shown in Tables 1and 2. Acidity lowered the amount of dissolved oxygen in water which eventually lowers the rate of respiration in animals. In this case, the amount of dissolved oxygen decreased since gaseous carbon dioxide was dissolved. As dissolved carbon dioxide increases, the amount of dissolved oxygen decreases. Not only does the dissolved carbon dioxide lower the amount of oxygen but also the pH since carbonic acid is formed when carbon dioxide reacts with water. According to a study conducted by Cummins (1989), low pH had a suppressive effect on all the tadpoles at individual level.

The relationship between the growth rate of tadpoles and dissolved oxygen are formed due to two major reasons. Firstly, the physiological cost of breathing during gill respiration decreases the growth performance. This means that the more energy the tadpoles spend on breathing, the less it can spend on growth. Alternatively, raised buccal ventilation may decline feeding duration by interfering with the feeding function of the same surfaces. For instance, *Xenopus laevis* tadpoles allowed to inhale air developed faster than tadpoles deprived from breathing air. This could be probably due to increased ventilation interfering with feeding (Smith, 1997).

The second explanation is when the desire of breathing air results in behaviours that decreases the amount of time spent on feeding as seen in figure 3. A study by Smith (1997) on effect of aeration on amphibians showed that the proportion of tadpoles seen at the surface was more in non-aerated containers than for tadpoles from aerated containers. This behaviour reduces the amount of time spent on feeding since tadpoles are spending more time on the surface in gasping for oxygen.

This idea is further supported by Pierce and Montgomery (1989) whereby they also investigated the effect of short -term acidification on the tadpoles' growth rate. Tadpoles' behavioural changes were observed such as reduced attraction to food odours or the level of activity which was also reduced at low pH. Less feeding indicates lower rates of food being assimilated in the gut hence contributing towards reduced growth.

At lower pH, weight is also reduced due to tadpoles losing water in acid water .Through observation it is evident that the other contributing factor towards weight loss is reduced feeding (refer to figure 3). Tadpoles in the acid medium were less active and most of the time was seen lying at the bottom of the beaker. Reduced growth rate under acid conditions in the larvae of amphibians has been attributed to the disrupted sodium balance. However the relationship between the body ionic content and feeding in amphibians is not known and more work needs to be done to learn the actual relationship (Rasanen *et al.*, 2002).

Since respiration is one of the major factors which get affected at lower pH it has always been evident that gills perform an important role in osmoregulation in amphibian larvae. The presence of a mucous secretion which covers the whole gill surface has already been observed in the gills of fish when exposed to acid stress. This secretion is also released under normal conditions to facilitate the exchange of gases. So under low pH the cells of the external layer degenerate leaving a residual structure. Hence the ultra structural and histological changes observed could interfere with respiratory and osmoregulation processes (Brunelli and Tripepi, 2005).

Table 4 shows that mortality rate was also very high at pH 5.5 followed by pH 6.0. One of the reasons could have been the reduced feeding time at lower pH which must have contributed towards lower metabolic rate. A study conducted on acid tolerance of Natterjack toad development by Beebee (1986) stated that longer development time increased the mortality through higher risks of desiccation. In addition, amphibians accessed to low pH may experience immunosuppression followed by microbial disease resulting in mortality (Simon *et al*., 2002).

Hence survival of tadpoles in carbonated medium is a challenge since it not only reduces the level of dissolved oxygen but also the pH. Both factors are detrimental for the tadpoles' survival and in future lives of this amphibian would be at risk.

CONCLUSION

Reduced feeding time contributes towards reduced physical activity indicating slow intracellular activity. This plays a vital part in the growth of the tadpoles since insufficient oxygen and glucose is being able to reach the cells. Carbonic acid contains low amount of dissolved oxygen which interferes with respiration. It also contains a lot of hydronium ions which makes tadpoles lose a lot of water from its body. This interferes with the tadpoles normal cellular processes which eventually lead towards cell death. At lower pH this damages a lot of cells including the cells responsible in gill respiration. Due to this fact, mortality rate is higher at low pH. Climate change can eventually change the population structure of amphibians in years to come since acidity selects against aquatic organisms.

RECOMMENDATIONS

A greater depth of study could have been conducted if the metamorphosis of the tadpoles were observed till the last stage starting from the egg stage. It was very difficult to spot eggs for this study since the cold season restricted toads from mating.

Since the study was only conducted for four weeks, the growth rate of tadpoles was observed while the tadpoles were in Gosner stage 22. It would have been more interesting to observe the growth rate while the tadpoles were developing from one stage to another.

Further study would include the egg stage till the tadpoles have developed into toadlets and are ready to leave water for respiration. Detailed analysis of the growth of tadpoles to adulthood in different pH would have given more results in regards to any deformities and swimming behaviour.

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APPENDIX

Table I: Range of environment to which tadpoles were exposed to.

Treatment	Dissolved oxygen (mg/l)	Temperature(^{0}C)	Salinity(mg/ml)
Distilled water	**6.08-7.09**	**25-27**	**1.000**
pH5.5	**5.37- 5.59**	**25-27**	**1.000**
pH 6.0	**5.40- 6.08**	**25-27**	**1.000**
Pond water	**6.0-6.52**	**25-27**	**1.000**

Materials

- Carbondioxide gas cylinder
- DO meter
- Distilled water
- Salinity meter
- thermometer
- 48 500 mL pyrex beakers
- gauze nets
- rubber bands
- venier calliper
- balance
- 5 L conical flask
- Dropper
- tissue paper