

A heading should include your name, the assignment, the course and section, your instructor, and the date.

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2nd Lab Report
Biology 105
Section 02 (Wed. 2-5)
Dr. Tschunko
Nov. 2, 2008

Effect of External pH on Respiratory Movements in Stoneflies

INTRODUCTION

Use headings to outline your report.

Background Information

Background information should touch on relevant topics; here, respiratory movements are discussed.

In order to obtain enough O₂ for aerobic respiration, many organisms perform some sort of respiratory movements to bring more O₂ into the body (Schmidt-Nielsen, 1997, 34). Common examples of such movements are breathing in humans, the movements of the gill covers in fish (Schmidt-Nielsen, 1997, 35) and the "push-ups" performed by some stoneflies (Merritt and Cummins, 1987, 67).

Support your statements with citations. Paraphrase material from your sources, do not quote them directly.

The number of hydrogen ions in water is measured in pH units, which range from 0 to 14 (Keeton, Gould and Gould, 1993, 35). More acidic water has more H⁺ ions and is given a lower pH value; less acidic water has fewer H⁺ ions and a higher pH number (Keeton et al., 1993, 35). Carbon dioxide dissolves readily in water (Smith, 1990, 204). Adding CO₂ to water decreases the pH by forming carbonic acid (Smith, 1990, 204); removing CO₂ increases the pH (Smith, 1990, 204). Because cellular respiration releases CO₂ into the water (or blood), it will thus tend to lower the pH. In humans, this lowering of the pH of the blood as cells respire is used as a cue for the body to initiate breathing movements (Schmidt-Nielsen, 1997, 235).

Text should be double-spaced. Use 12 point fonts and use 1 inch margins around the page. The sample shown here is smaller to allow the annotations to fit on the page.

Note that almost every sentence is backed up by a citation from the literature.

Stoneflies are insects that spend their larval stages in the water. The larval stages, which can last for up to 2 years (Merritt and Cummins, 1987, 65), have been used as indicators of water quality in streams (Surdick and Gaufin, 1978, 3). Stoneflies are normally found in association with clean water (Hilsenhoff, 1977, 6; Hilsenhoff, 1987, 31). Although a number of factors affect their distribution, the limiting factor in freshwater seems to be the availability of oxygen (Olive and Smith, 1975, 34). Stonefly numbers are reduced whenever siltation is present (Surdick and Gaufin, 1978, 8). It is hypothesized that the silt covers the delicate gills (Hilsenhoff, 1987, 6); mayflies, which also have delicate external gills, are similarly affected (Hubbard and Peters, 1978, 8).

Never leave a line or heading "orphaned" at the bottom or top of a page.

Stoneflies increase the flow of water over their gills in times of oxygen stress by doing "push-ups" (Merritt and Cummins, 1987, 67). What cues do the stoneflies use? Olive and Smith (1975, 38) attributed this activity to low oxygen levels, but this begs the question of the ultimate cue. Increasing temperature both increases respiration rates (Schmidt-Nielsen, 1997, 235) and decreases oxygen availability

Don't forget page numbers!

(Smith, 1990, 203). Therefore, an increase in temperature should increase respiratory movements. Carbon dioxide is one product of respiration; in the blood dissolved CO₂ lowers pH (Schmidt-Nielsen, 1997, 235). Also, since low oxygen levels increase anaerobic respiration in tissues (Schmidt-Nielsen, 1997, 450), and one product of anaerobic respiration is lactic acid (Schmidt-Nielsen, 1997, 102), pH might be expected to be lowered even further. In humans, it is well known that respiration is induced by a fall in blood pH (Schmidt-Nielsen, 1997, 235). But what about the stoneflies? Is it a lack of oxygen in the water, an increase in water temperature, or a build up of acid from respiration in their tissues that triggers respiratory movements?

This paragraph goes from the general to the specific. It focuses on the variables being tested, and makes it clear where the current research fits in.

Purpose

The purpose of this experiment was to determine if lowered pH levels are responsible for inducing respiratory movements in stoneflies. Further, the experiment was an effective way to learn about pH, how to measure pH with a digital instrument, and how to determine correlation from a set of data.

The purpose section should make it clear exactly what you expected to learn from the experiment, both in terms of science and in terms of technique. Write this section in the **past** tense (you have already done the experiment).

Hypothesis

Under uniformly high oxygen concentrations and at uniformly cool temperatures, stoneflies in lower pH water will have a greater number of respiratory movements.

The hypothesis should be a clear, concise statement (or statements) predicting what you expect to find in the experiment. Write this section in the **future** tense.

PROCEDURES

A total of 77 stoneflies of the genus *Macroneuria* were collected from Baker Run, a second-order stream in the Wayne National forest. The specimens were collected by kick-screen from a site at N39° 28.560' and W81° 19.838' (as determined by a Sony Pyxis GPS unit) on April 1, 1997. The specimens were placed in a bucket with water, substrate, leaves, and other organisms from the stream and returned to the lab, where they were held in a 40-liter glass aquarium. The substrate, leaves, and other stream organisms were also added to the aquarium to provide food and refuge for the stoneflies. The total volume of stream water was 24 liters; deionized water was added as needed to maintain a constant water level.

The procedure section should explain where you got your experimental organisms, what conditions they were kept in, etc.

Don't leave out details that might be crucial to someone else replicating the experiment. Use of deionized Vs. tap water is one example.

The aquarium was illuminated by a single 75-watt incandescent light bulb placed 30 cm above the aquarium. Light was measured using an Extech Model 101 light meter. The incandescent bulb provided 300 lumens of light at the water's surface. The light was controlled by a timer which was set to turn the light on at local dawn and off at local dusk. The tank also received indirect light from overhead fluorescent room lights (100 lumens) which were turned on occasionally during the day. Some light (50 lumens) reached the tank from north-facing windows on the far side of the room. The tanks were held at room temperature, which was monitored

Specify model numbers for equipment whenever possible. Some equipment might have a well-known inaccuracy that could affect results.

Don't forget page numbers!

Note that this is not a result of the experiment being reported here, but merely an experimental condition. Never report experimental results in the procedures section.

The entire procedures section should be written in the past tense, passive voice. Avoid using I, we, us, our, and so on.

It sometimes helps to imagine yourself hovering over a team of nameless people carrying out the experiment, and recording the procedures as a story of what they did.

There are some unnecessary details. The reader does not need to know that the test tubes were located on the front bench, or that you recorded your results "in your lab notebook".

When an experiment is repeated, you only need to tell the reader what was different. Don't repeat yourself if you can avoid it.

Don't forget page numbers!

(every 10 seconds by a Vernier Systems DC-T temperature probe connected to a DTK 486 computer via a Vernier Systems MLPI interface. The data was recorded with the Vernier Systems Logger Pro software. Temperature over the course of the holding period fluctuated from 22° C to 25° C.

Aeration was supplied by a Challenger I air pump and a single 6 cm long airstone. Oxygen levels in the water were measured by a Vernier Systems dissolved oxygen probe using the same interface, computer, and software described above. Oxygen levels were monitored every 10 seconds by the software, and never fell below 7 mg/l. The airstone was placed at one end of the tank to create a current across the substrate on the bottom of the tank. Current speed at the bottom was estimated visually to be about 0.3 m/s.

The respiration experiments were carried out on April 8, 1997 from 3:30 to 5:30 p.m. The experimental apparatus consisted of a white plastic dishwashing tub measuring 50 cm long by 30 cm wide by 20 cm deep. Water was withdrawn from the holding tank and used to fill the tub to a depth of 10 cm. No substrate was added. The tub was then placed in a larger (70 cm by 50 cm by 30 cm) plastic bus tray, which had 5 cm of crushed ice lining its bottom. Aeration, temperature monitoring, and dissolved oxygen monitoring were all the same as those used in the holding tank. In addition, a Vernier Systems pH probe was employed to monitor pH. Lighting was provided by the overhead room lights and incident light from the windows; light levels were 125 lumens at the start of the experiment, and 103 lumens at the end, as measured at the surface of the water.

The pH of the water for the first trial was 7.6; this was the same as the pH in the holding tank. Once the water in the experimental tank had stabilized at a temperature of 8° C, 4 *Macroneuria* were randomly chosen and removed from the holding tank. The insects were allowed to acclimate for a period of 10 minutes. Each student in the team of 4 chose one of the individual insects to monitor. At the end of the acclimation period, a laboratory timer was set to sound an alarm every minute. The number of "push-up" style respiratory movements made by each individual stonefly was recorded every minute for 5 minutes. After the observation period, the stoneflies were transferred to a temporary holding tank, and the experiment was repeated with a second and a third group of 4 stoneflies.

After the first 12 stoneflies had been observed and removed, the pH in the tank was lowered using nitric acid until the pH level was 6.0. An additional 12 stoneflies were observed, using the same protocol as was used for the pH 7.6 observations. Likewise, the pH was lowered two more times, to pH 5.0

The fate of the experimental organisms is not necessarily an important part of a lab report, but it can be included. On the other hand, if you dumped them down the sink....

Don't forget to give details on how you analyzed the data. Different software packages may give different results, so be sure to name the software you used to prepare graphs and run statistical tests.

The results section is written in the past tense.

Report any monitoring you did of experimental conditions early on. Here, temperature, pH, and oxygen levels were all part of the hypothesis.

Be sure to title all your tables and figures. A table is a set of numbers, a figure is any kind of graph, illustration, photograph, etc.

Paste tables in directly from Excel.

It is not enough to list the data in a table or graph it out. You need to explain it as well. Always refer to your tables or graphs in the text.

Don't forget page numbers!

and pH 4.0, and the observations were repeated. Different sets of stoneflies were used for each of the different pH levels. A total of 48 stoneflies were used. At the conclusion of the experiment, all stoneflies and other insects taken from the stream were returned to the stream and released.

Statistical and graphical analysis of the data was done using Gateway E-3000 200 MHz Pentium computers running Microsoft Excel 97. The software package was used to calculate an ANOVA table and run t-tests on the data. In addition, Excel was used to produce a scattergraph plotting respiratory rate Vs. pH, and to calculate a trendline for this graph.

RESULTS

Oxygen levels in all experiments remained between 13.5 and 13.7 mg/l. Likewise, temperature remained very constant, ranging between 7.8 and 8.3° C throughout. The pH values remained within 0.1 units of the nominal pH for each of the experiments.

In terms of the number of respiratory movements per minute, there were differences between the average number of movements for each of the groups. These are summarized in Table 1.

Table 1 - ANOVA table for number of respiratory movements per minute in all trials.

SUMMARY

<i>Groups</i>	<i>Count</i>	<i>Total Number of "push-ups"</i>	<i>"push-ups" per minute (average)</i>	<i>Variance</i>
pH 7.6	60	298	5.0	2.0
pH 6	60	369	6.2	2.0
pH 5	60	414	6.9	3.8
pH 4	60	558	9.3	4.4

ANOVA

<i>Source of Variation</i>	<i>SS</i>	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P-value</i>	<i>F crit</i>
Between Groups	602.4125	3	200.8	66.3	4.18758E-31	2.642849495
Within Groups	715.5833333	236			3.0	
Total	1317.995833	239				

As can be seen in Table 1, the average number of movements was 5.0 in the pH 7.6 trials, 6.2 in the pH 6 group, 6.9 in the pH 5 experiment, and 9.3 in the pH 4 experiment. When analyzed using Microsoft Excel 97's ANOVA function, the results show a significant difference between the groups ($p = 4.2 \times 10^{-31}$). Since the *F* value returned by the computer (66) is much greater than the *F-critical* value (2.6), we can reject the null hypothesis that the average number of "push-ups" is

The results section does not contain any conclusions. Don't tell the readers what the results mean at this point.

Again, explain what statistical tests you used and how they were calculated. The reader should have enough information to repeat your statistical procedures. Also, list any critical assumptions or specifications, such as what α level you consider to be significant. If other scientists or mathematicians made similar assumptions or specifications, cite them to support your choices.

equal in each of the pH treatments (McClave and Dietrich, 1979, 353). The *P-value* of 4.2×10^{-31} means that the probability that the differences between the pH treatments are due to random or natural fluctuations is very small, much less than the 5% chance that most scientists are willing to take when evaluating this sort of data (McClave and Dietrich, 1979, 154). Table 2 (below) shows the results of Student's T-tests comparing the means of each of the groups against all of the other groups. This was a single-tailed test for unequal variances using Microsoft Excel's t-test function. At an $\alpha = 0.95$, all of the groups are significantly different from one another. According to McClave and Dietrich (1979, 290) significantly different means that there is less than a 5% chance that the differences seen between two averages is due to chance or natural fluctuations. The p-values in Table 2 show the probability that the differences are due to random fluctuations not related to the pH treatments for each pair of treatments. The closest two means were for the pH 6 and pH 5 trials, and even here the difference between the means was significant at an α of 0.99, which is a higher standard than the normally used α value of 0.95 (McClave and Dietrich, 1979, 154).

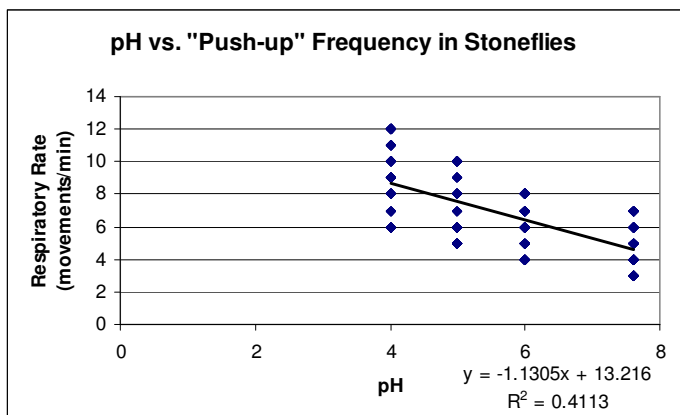
Don't forget titles!

Table 2. Results of Single-Tailed T-tests on all trials

T-tests:	p-value
pH 7.6 Vs pH 6	5.20826E-06
pH 7.6 Vs pH 5	4.28743E-09
pH 7.6 Vs pH 4	2.5661E-24
pH 6 Vs pH 5	0.008364619
pH 6 Vs pH 4	2.1336E-16
pH 5 Vs pH 4	1.00158E-09

In order to examine the possibility of a correlation between pH and the number of respiratory movements, a scattergraph was prepared in Microsoft Excel 97. The graph, along with the computer-generated trendline, is shown below as Figure 1.

Paste charts in directly from Excel. Each chart should have a comprehensive title and a legend (if needed). Avoid superfluous legends; if you only have one data set there is no need for a legend.



A figure caption should contain enough information to allow the reader to determine what the figure is about.

Figure 1 - pH Vs. "Push-up" Frequency in Stoneflies. A trendline has been drawn through the data points; its equation is shown in the lower right-hand corner of the graph. There was an *n* of 48 observations.

In Figure 1, it is clear that as pH increases, the number of respiratory movements decrease. The slope of the line is -1.1, and the degree of correlation is 0.411. Since there were 4 discrete pH values used in this experiment, the values seem "stacked up" over those pH values.

DISCUSSION

Conclusions

The results of this experiment clearly support the hypothesis that a lower pH will result in more respiratory movements. The mean number of respiratory movements for each lower pH increased significantly over that of the next higher pH, as is shown in Tables 1 and 2. Further, the trendline shown in Figure 1 is also consistent with this interpretation of the results.

Explanation of conclusions

Several possible factors that might affect the number of respiratory movements were controlled for in this experiment. Light levels, water chemistry (aside from pH), temperature, and handling were the same for all trials. Oxygen levels were maintained at a high level throughout the experiment. The overall oxygen level of at least 13.5 mg/l is much higher than the 6.5 mg/l noted as the environmental requirement for this genus (Surdick and Gaufin, 1978, 175). Lack of O₂ should not have triggered respiratory movements, since there was high oxygen saturation throughout the experiment. Likewise, temperature, which might be expected to increase respiration through an increase in O₂ demand as metabolic rates increase, was held constant in all trials.

While it was not monitored directly, CO₂ concentration is assumed to have remained constant throughout the trials. Any additional CO₂ in the water would have lowered pH; no reduction in pH was seen over the course of the experiment. As far as can be determined, the only difference between the trials (aside from pH) was the change in light levels and the time of day. The time of day may not be trivial if respiratory movements typically increase at a particular time in the day. While this is not implausible, there was no mention of such an effect in my limited review of the literature.

In humans, higher CO₂ levels act on the medulla in the brain to trigger respiration at the lungs (Schmidt-Nielsen, 1997, 235). This allows the blood to vent excess CO₂ into the atmosphere, raising the pH of the blood. The results of this experiment are consistent with such a mechanism; however, other explanations must be considered.

The conclusions section is written in the past or the present tense (which can make for a nice change of pace at this point). The conclusions address the simple question: Was the hypothesis supported or refuted by the experiment? By the way, if a well-thought out hypothesis is not supported by the facts, say so. It will not affect your grade.

The explanation section should explain why you got the results you did. If you made a mistake say so here (but also be sure that the procedures section records what you *did*, not what you were *supposed* to do)!

Bring in the opinions and findings of others to support your claims.

In this section the word "my" appears. Sometimes it is impossible to avoid using the personal pronoun. Particularly if you are taking "blame" for some shortcoming in library research or experimental procedure, the occasional use of a personal pronoun can be refreshing.

In this experiment, nitric acid was used to lower pH. It is possible that the negative ions in this case, nitrogen oxides, might trigger the respiratory movements rather than the H⁺ ions. However, I was not able to find any evidence that nitrogen oxides trigger increased respiration.

Future experiments

The future experiments section should be written in the future tense. It should address any shortcomings of the current experiment (such as increasing the number of replications or fixing a flaw in the experiment's design). It should also describe experiments designed to address new questions raised by the current study.

This experiment should be repeated with several variations to further test the proposition that increased acidity leads to increased respiratory movements. First, the experiment should be repeated with the tests at the lowest pH values occurring first, to control for any effects that might arise due to circadian rhythms. This experiment would have to proceed in a reverse fashion, with the organisms being acclimated to a lower pH, and then raising the pH with a base such as calcium carbonate. If the hypothesis is true, the stoneflies would show more respiratory movements in the first trials at the lower pH values. This experiment would also help determine if the increased respiration noted here was due to the nitrogen oxides in the nitric acid used to lower the pH since the same nitrogen oxide levels would be present throughout these trials.

There may be more than one way to proceed. You might need to outline several experiments. Describe each in some detail.

Another test would more definitively control for the possible effects of the nitrogen oxides; in this experiment the trials carried out here would be replicated, but with another acid, such as HCl, used in place of nitric acid. As in the trials reported here, the organisms should respond to the additional acid with increased respiration. Further controls in this vein would include an experiment where nitric acid and a base would both be added so that nitrogen oxide levels would rise but pH would be unaffected. In this case, respiration rates should not change if in fact nitrogen oxides are not responsible for the change in respiratory rates.

It never hurts to end with some of the questions raised by the research. This last paragraph is more like the traditional "conclusion" of an essay (note that in a lab report we use the term conclusion in a much more restricted way). A concluding paragraph seeks again to put the results of the experiment into a larger context.

Finally, one cannot help but wonder at the implications of this study for organisms exposed to acid rain. If further tests support the hypothesis, it would be instructive to examine stoneflies in streams to see if stoneflies living in acidified streams show higher respiration rates due to the acid. If so, it could be predicted that the higher respiration rates would lead to lower production rates in such streams (since the respiratory movements waste energy that could otherwise go into growth), and a corresponding decrease in food for fish. In fact, it has been shown (Kharman, Frankel and Jackson, 1993, 345) that fish production is dropping in streams affected by acid rain. This effect is seen even in streams where fish reproduction and physiology do not seem to be directly affected by the increased acidity (Kharman et al. 1993, 348). Perhaps the decreased fish production is due to loss of energy in the system caused by increased respiratory movements of prey invertebrates such as stoneflies?

LITERATURE CITATIONS

The Literature Cited section should contain a list of all the works actually cited in the text. Hint: As you proofread, be sure that every citation in the text has an entry in this section, and that every entry here is cited in the text.

Hilsenhoff WL 1977. Using a biotic index to evaluate water quality in streams. Technical Bulletin Wisconsin Department of Natural Resources, Number 132. 22pp.

Hilsenhoff WL 1987. An improved biotic index of organic stream pollution. The Great Lakes Entomologist. 20:31-39.

Hubbard MD, Peters WL. 1978. Environmental requirements and pollution tolerance of Ephemeroptera. U.S. Environmental Protection Agency, Document 600/4-78-061.

Keeton WT, Gould JL, Gould CG. 1993. Biological Science, 5th ed. New York (NY): W.W. Norton & Co.; 1194p.

Note the distribution of primary, secondary and tertiary titles. Remember - you cannot use your class textbooks or the lab manual. No web pages, either!

Kharman GIA, Frankel LP, Jackson JJ. 2002. Effects of acid deposition on two Adirondack streams. New York Academy of Science Proceedings. 86:341-347.

McClave JT, Dietrich II FH. 1979. Statistics. San Francisco (CA): Dellen Publishing Company. 681p.

Merritt RW, Cummins KW, editors. 1987. An introduction to the aquatic insects of North America, 2nd ed. Dubuque (IA): Kendall/Hunt Publ. Co. 722p.

For a paper written in 2008, there are not enough up-to-date articles. Old articles may still be relevant, but a good paper will also contain the most up-to-date research.

Olive JH, Smith KR. 1975. Benthic macroinvertebrates as indexes of water quality in the Scioto River Basin (Ohio). Ohio Biological Survey New Series Bulletin. 5(2). Columbus (OH): The Ohio State University Press. 87p.

Schmidt-Nielsen K. 1997 Animal physiology: adaptation and environment, 6th edition. Cambridge (GB): Cambridge University Press. 560p.

Smith RL. 1990. Ecology and field biology, 4th ed. New York (NY): Harper & Row. 922p.

Surdick RF, Gaufin AR. 1978. Environmental requirements and pollution tolerance of Plecoptera. U.S. Environmental Protection Agency, Document 600/4-78-062.

Final note: This is a sample lab report, not an actual one. We would be pleased to see such a report from a senior; obviously we expect less from a beginning student. In particular, this paper is longer than would be expected for a beginning student, and the statistical analysis and library work also go beyond what we would expect at that level. Still, at any level, we expect to see clear, concise writing following the proper formats, proper grammar and spelling, the proper information presented in the proper sections, and strict attention paid to following the directions. Many of the statements and sources used here are fictitious; don't use this as a reference. The introductory section probably should have had a paragraph on cellular respiration, but that was cut out because it was too similar to one aspect of the lab report for the yeast lab in 105. The following page is an example of what the report would look like in a form ready to turn in (i.e. without the annotations). Properly formatted, this lab report is 14.5 pages long. That is probably too long for a 105 lab report, but not excessively so.