# Habitat Preferences of *Artemia franciscana*

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## Introduction

*Artemia franciscana* (brine shrimp) inhabit saline lakes and temporary ponds. Nauplii larvae hatch from resting eggs in the spring. The response of a mobile animal, such as *Artemia*, to variation in a single environmental factor is often investigated through the use of a habitat preference experiment. In this experiment, *Artemia* will be exposed to wide range of habitats or intensities of a single factor (e.g. temperature, light, pH), given sufficient time to select a given habitat, and then sampled to determine the preferred condition. The experiment will attempt to answer if *Artemia* is indifferent to the factor under study or does it actively select a specific condition and what is the preferred condition. This exercise is used to re-enforce hypothesis testing and the use of chi square goodness of fit statistical test.

## Student Outline

*Artemia franciscana* (brine shrimp) inhabit saline lakes and temporary ponds. Nauplii (larvae) hatch from resting eggs in the spring. Temperature is the principal factor controlling hatching. There are 14 naupliar stages, with sexual maturity being reached at the 12th stage. Development to sexual maturity takes about 3 weeks, and adults live for several months.

*Artemia* populations are usually dominated by parthenogenic (asexually reproducing) females. The eggs they lay are thin-shelled and do not require fertilization. They rapidly hatch to produce more females. At certain times parthenogenically produced eggs (unfertilized eggs that develop into new individuals) give rise to both males and females. Often the appearance of males can be related to a single factor, such as declining temperature, or increasing salinity as a temporary pond begins to dry up in the summer. A different kind of egg is produced by sexual reproduction. It is a thick-walled resting egg. These eggs do not hatch immediately. They fall to the bottom and are able to tolerate both freezing and drying. In fact, resting eggs require a period of drying or low temperature before they will hatch. These eggs are what you get when you buy "Sea Monkeys®", and that is why they are "absolutely guaranteed to grow"; in the wild some resting eggs can stay dormant for many years if not decades!

The response of a mobile animal, such as *Artemia*, to variation in a single environmental factor is often investigated with a habitat preference experiment. In this kind of experiment, the organism under study is exposed to a range of habitats or intensities of a single factor (e.g. temperatures from 10 °C to

30 °C), it is given sufficient time to select any given habitat, and then sampled to determine the preferred condition. If all conditions are made equally available to the organism, then the one that is most frequently selected is assumed to be preferred habitat, and the one that is presumably most favorable for survival and/or reproduction in the wild.

Habitat preference experiments can be conducted in two ways. Firstly, the experimental chamber can be divided into distinct areas, each with a different condition (plot design). Secondly, the experimental chamber can be designed to provide a continuum of variation in a factor like light or temperature (gradient design).

In both designs, it is necessary for the experiment to have a control chamber in which there is no treatment. This allows us to evaluate the possibility that uncontrolled variables influence the outcome of the experiment. Slight tilts to laboratory benches or differences in room lighting are two examples of uncontrolled variables. For example, if you only had one chamber in which you varied temperature and you found that *Artemia* spent most time in the warmest part of the chamber, then you might conclude that high temperature was the preferred condition. However, if it so happens that the warmest part of the chamber was also the brightest (because it was facing a window for example), then temperature may have nothing to do with your results. By using a control chamber with the same temperature throughout and the same orientation as the experimental chamber, we can eliminate the source of this error. If *Artemia* simply prefer bright conditions, then results would be expected to be similar between control and experimental chambers. However, if the effect is truly a temperature effect, then we would expect to see evidence of habitat preference in the experimental chambers only.

**Objectives:**

- To determine the habitat preference of an aquatic organism, *Artemia franciscana* (= the North American version of *Artemia salina*).
- To learn to formulate and test hypotheses about the possible responses of the *Artemia* to gradients of pH, temperature, and light intensity.
- To use a $\chi^2$ (chi-square) statistical test on frequency (or count) data to determine whether your experimental results are statistically significant.

**Exercise A: Formulation of Hypotheses**

We will ask two separate questions about the response of *Artemia* to three factors (pH, light intensity, and temperature). They are:

i) Is *Artemia* indifferent to the factor under study or does it actively select a specific condition?
ii) What is the preferred condition?

Acidity (pH) is one of many chemical factors that determine whether a given lake is suitable for a given organism. *Artemia* obviously has a requirement for lakes with high salinity, but other factors may be important in determining whether it occurs in a lake as well. For example, saline lakes are often basic but rarely acidic. We might hypothesize that *Artemia* has a requirement for high pH (basic), or is intolerant of low pH (acidic).

Light intensity decreases exponentially with depth. It has a direct influence on the distribution of the *Artemia* food source, floating single celled algae (phytoplankton) because high light intensity causes rapid photosynthesis and congregation of the algae near the surface. *Artemia* might be expected to respond in the same way, *i.e.*, choose conditions with the most food available, but under bright light

*Artemia* is more easily found by predators. Given this situation, movement into regions of bright light might be selected against.

Temperature gradients are often found in lakes in summer, with the highest temperatures at the surface. Temperature affects respiratory rate and general activity level. As temperature increases so does respiration, allowing greater activity and more rapid growth. However, this also causes more rapid depletion of food reserves.

Before you begin your experiment you should formulate hypotheses about the possible responses of *Artemia* to each of the three factors (pH, light intensity and temperature). Each bench should discuss and develop their own hypothesis for their part of the experiment.

**Exercise B: Experiment Procedures**

The experiment tests the response of the first naupliar stage of *Artemia franciscana* to gradients of light, temperature and pH. Each treatment will be carried out at a different bench. One bench will run a control in which the procedure will be exactly the same as the gradient treatment, but no gradient will be applied.

Each bench will be supplied with: two pieces of flexible plastic tubing (light, pH control) or neoprene tubing (temperature) attached to meter sticks; rubber stoppers or serum caps to seal the ends of the tubes; glass beads to aid in the mixing of the tubes; clamps to isolate animals in different parts of the gradient; a culture of *Artemia franciscana* hatched overnight from eggs; a set of beakers to collect the *Artemia* after the experiment is completed; petri dishes with a grid.

Students should run two replicate treatments, simultaneously. Make sure to always align your tubes so that the zero (0) on the meter stick starts on the left and the hundred (100) is to the right. In all cases however, the 0 – 25 cm section will be referred to as Sec.1, the 25 – 50 cm section will be referred to as Sec 2, the 50 –75 cm section will be referred to as Sec 3 and the 75 – 100 cm section will be referred to as Sec 4.

*Light Treatment:*

1. The tubes at your bench have a 25 cm section covered with black electrical tape (Sec. 4), 25 cm covered with translucent tape (Sec. 3), and 50 cm clear (Sec. 2 & 1). Select one of the tubes and stopper the end with a rubber stopper. (Repeat for the other tube.)
2. Mix the *Artemia* culture well. Holding one of the tubes vertically, add the culture solution of *Artemia* to the open end of the tube along with one of the glass beads. Be sure the tube is completely full and seal the end with a rubber stopper. A little of the culture should run over and there should be no air bubbles in the tube. Gently turn the tube over end-to-end to make sure the *Artemia* are evenly distributed (the glass beads will help with the mixing). (Repeat for the other tube.)
3. Place the tubes side by side, horizontally on the lab bench so that the opaque (black) sections (Sec. 4) are to the right side of the room. Make sure to align your tubes so that the zero (0) on the meter stick starts on the left and the hundred (100) is to the right.
4. Place clamps at the 25, 50, and 75 cm marks of both tubes. Make sure the clamps are just around the tubes and not under the meter stick. Do not tighten the clamps.

5. Place the vertical wood partition at the 25 cm mark of the side by side tubes. Sec. 1 (the 0 to 25 cm section), which should be a clear section of tubing, will be on the other side of the barrier and will receive the bright light.
6. Arrange a bright lamp so it is even with the clear end of the tubes (over Sec. 1) and about 1 meter above them, so that heat is not introduced as a factor. Your tubes will have 4 different light chambers: dark or no light (Sec. 4); translucent light (Sec. 3); room light (Sec. 2) and bright light (Sec. 1).

*Temperature Treatment:*

1. The neoprene tubes on your bench are completely black since an infrared lamp is used as the heat source and we do not wish to introduce light as a second factor. Select one of the tubes and stopper the end with a rubber stopper. (Repeat for the other tube.)
2. Mix the *Artemia* culture well. Holding one of the tubes vertically, add the culture solution of *Artemia* to the open end of the tube along with one of the glass beads. Be sure the tube is completely full and seal the end with a rubber stopper. A little of the culture should run over and there should be no air bubbles in the tube. Gently turn the tube over end-to-end to make sure the *Artemia* are evenly distributed (the glass beads will help with the mixing). (Repeat for the other tube.)
3. Place the tubes side by side, horizontally on the lab bench. Make sure to align your tubes so that the zero (0) on the meter stick starts on the left and the hundred (100) is to the right.
4. Place clamps at the 25, 50, and 75 cm marks of both tubes. Make sure the clamps are just around the tubes and not under the meter stick. Do not tighten the clamps.
5. Fill a plastic bag with crushed ice and seal the end. Place three layers of paper towels and then the bag of ice over the middle of Sec. 1 (the 0 – 25 cm section) of the two side-by-side tubes.
6. Cover Sec. 4 (the 75 – 100 cm section) of the two side-by-side tubes with three layers of paper towel. Place a heat lamp about 30 cm above the paper towel covered Sec. 4.

*pH Treatment (students must wear safety glasses and dissecting gloves for this experiment):*

1. The tubes for the pH treatment are totally clear. Select one of the tubes and stopper the end with a serum cap. (Repeat for the other tube.)
2. Mix the *Artemia* culture well. Holding one of the tubes vertically, add the culture solution of *Artemia* to the open end of the tube along with one of the glass beads. Be sure the tube is completely full and seal the end with a serum cap. A little of the culture should run over and there should be no air bubbles in the tube. Gently turn the tube over end-to-end to make sure the *Artemia* are evenly distributed (the glass beads will help with the mixing). (Repeat for the other tube.)
3. Place the tubes side by side, horizontally on the lab bench. Make sure to align your tubes so that the zero (0) on the meter stick starts on the left and the hundred (100) is to the right.

4. Place clamps at the 25, 50, and 75 cm marks of both tubes. Make sure the clamps are just around the tubes and not under the meter stick. Do not tighten the clamps.
5. Using the plastic syringe provided, *slowly* inject 1 ml of 0.5 % HCl (hydrochloric acid) into the serum caps in the ends of Sec. 4 (the 75 – 100 cm section). Using the other plastic syringe slowly inject 1 ml of 0.5 % KOH (potassium hydroxide, a base) into the ends of Sec. 1 (the 0 – 25 cm section). Be careful not to spill the HCl or the KOH on your clothes or skin, if you do spill the HCl or KOH inform your TA and wash the area well with tap water.
6. Familiarize yourself with the pH meter so that you will be able to make the required pH readings when the experiment is terminated.

*Control Treatment:*

1. The tubes for the control are totally clear. Select one of the tubes and stopper the end with a rubber stopper. (Repeat for the other tube)
2. Mix the *Artemia* culture well. Holding one of the tubes vertically, add the culture solution of *Artemia* to the open end of the tube along with one of the glass beads. Be sure the tube is completely full and seal the end with a rubber stopper. A little of the culture should run over and there should be no air bubbles in the tube. Gently turn the tube over end-to-end to make sure the *Artemia* are evenly distributed (the glass beads will help with the mixing). (Repeat for the other tube.)
3. Place the tubes side by side, horizontally on the lab bench. Make sure to align your tubes so that the zero (0) on the meter stick starts on the left and the hundred (100) is to the right.
4. Place clamps at the 25, 50, and 75 cm marks of both tubes. Make sure the clamps are just around the tubes and not under the meter stick. Do not tighten the clamps.

*All Treatments:*

1. Use the labels provided to label 8 beakers, which you will use to collect the contents of your tube.
2. Allow your tubes to sit undisturbed for 30 minutes after the gradient has been applied. This means undisturbed, especially for the clear tubes in which the *Artemia* will be disturbed by the movements of large objects, i.e. you.
3. Leave the lab and formulate your hypotheses if you have not already done so.
4. After the 30 minutes, use six hands to tighten the clamps on the tubes, all at the same time. Try not to move the tubes as you tighten the clamps. Be careful as you tighten the clamps, sometimes the rubber stoppers or the serum caps pop off!
5. Empty the tubes, section by section into the appropriately labeled beakers, rinsing each section with the wash bottle as necessary. For the Temperature and pH benches: immediately measure and record the temperature or pH as each section is emptied, then rinse as necessary.
6. Place each beaker on ice for approximately 5 minutes. Distribute the beakers among your group and count the number of *Artemia* in each section. Use a dissecting microscope at low power. Empty one-eighth or less of the contents of the beaker at a time into a small gridded petri dish. It

is far faster and more accurate to make several small counts than to try and do it all in a few big shots! It will help to place the petri dish on a sheet of white paper. Hand held counters are available for use. Begin your count along the top axis of the grid and work across the petri dish.

7. Record the number of *Artemia* in each section of the tube in the table provided by your instructor. You will also post your data in the class data table on the blackboard or the overhead in the Lab.
8. Clean up your bench. Make sure to rinse out your beakers and petri plates. Put everything back the way you found it.
9. You will use the class data for your calculations. Note that because you have sampled two tubes simultaneously you will have to calculate the total of the two tubes. You will use this total number of *Artemia* for your analysis.
10. You will use a $\chi^2$ test (using the total values) as outlined below (in Analysis and Interpretation), to test the hypotheses you made about the *Artemia's* habitat preference.

*Exercise C: Analysis and Interpretation*

In a previous lab, you learned how to use a *t*-test to determine whether 2 sample means of continuously distributed variables (like leaf thickness and leaf area) were different enough to be considered significantly different from each other. The *t*-test is appropriate for continuous data that tends to be normally distributed, but it cannot be applied to data that is collected as counts or frequencies, like the data you have collected for this lab. For the analysis of frequency or count data, we often use a statistical test based on a new statistic called $\chi^2$ (or "chi-square"). Like the *t*-statistic, the $\chi^2$-statistic has a known distribution that varies with sample sizes, so we can use it like the *t*-test to determine the probability of getting our observed count data by chance. The same rule applies here as it did for the *t*-test; we calculate a value for our statistic from our observed data, and if the probability associated with that value is $\leq 0.05$, then we will reject the (statistical) null hypothesis and state that our results are statistically significant.

There are two common ways of using a $\chi^2$-statistic. First, if we want to compare our observed frequency data to any theoretical or expected distribution, we can use the $\chi^2$-statistic in a so-called goodness of fit (GOF) test. It tests to see how well your observed data fits an expected distribution. For example, if you tossed a coin randomly 200 times and got 150 heads and 50 tails, you could compare that observed distribution to an expected 50:50 distribution with a GOF test to see what the chances are of obtaining your observed distribution by chance. Secondly, we can use a $\chi^2$-statistic in a so-called contingency table analysis (also called a test of independence) to compare two observed distributions with each other to see if they should be considered to have come from different populations. For example, we could compare a distribution of frequencies from an experimental setup to one from a control setup (like you will for this lab) to see if the experimental treatment had a significant effect on our response variable (which in this case is the number or frequency of *Artemia* in different sections of your tubes). This is called a test of independence because it examines whether the frequencies of your response variable are independent of some experimental or predictor variable (like temperature or light).

Regardless of which test we use, the calculation for the $\chi^2$-statistic is the same, as follows:

$$\chi^2 = \sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

We will illustrate a goodness of fit test and a contingency table analysis using the hypothetical sample data from Table 1.

**Table 1.** Hypothetical data of the response of *Artemia* to three factors

| | sec. 1 | sec. 2 | sec. 3 | sec. 4 | row totals |
|---|---|---|---|---|---|
| control | 23 | 24 | 25 | 28 | 100 |
| treatment A | 41 | 19 | 13 | 11 | 84 |

We would expect that the numbers of *Artemia* in the control chamber would be randomly distributed, in which case the numbers in each section would not differ significantly from an equal distribution (25:25:25:25). They look pretty close, but now we will use the GOF to assign a probability that they are equal (just like we used the *t*-test to determine the probability that 2 samples came from the same population). If you think about this for a minute, you might anticipate that the $\chi^2$-value from these data will be low, and the probability that they are equal will be high; we are likely to accept the null hypothesis in this case. From our data in Table 1 we see that the total number of *Artemia* is 100. We have divided our tubes into four sections, so that each section of the control should be expected to have 25 individuals. We can transfer this information to another table (Table 2) that allows us to calculate $\chi^2$ easily:

**Table 2.** Calculation of $\chi^2$ from hypothetical *Artemia* data

| | sec. 1 | sec. 2 | sec. 3 | sec. 4 | row totals |
|---|---|---|---|---|---|
| expected (E) | 25 | 25 | 25 | 25 | 100 |
| Observed (O) | 23 | 24 | 25 | 28 | 100 |
| O – E | –2 | –1 | 0.0 | +3 | |
| $(O - E)^2$ | 4 | 1 | 0.0 | 9 | |
| $(O - E)^2/E$ | 0.16 | 0.04 | 0.0 | 0.36 | |
| $\chi^2 = \sum(O - E)^2/E$ | $\chi^2 = 0.16 + 0.04 + 0.0 + 0.36$ | | | | $\chi^2 = 0.56$ |

The $\chi^2$-value for the example control is 0.56. The actual probability associated with this value (for this sample size) can be determined from a table of continuous $\chi^2$-values, and is 0.91, 91% (an extremely high probability as expected!), but we often just compare the calculated value with a critical value, just like we did for the *t*-test in a previous lab. Before we can check our tables for the critical $\chi^2$-value, we must calculate a corrected sample size for the analysis called the degrees of freedom (*df*). The degrees of freedom for the $\chi^2$-test are calculated as the number of frequency categories minus one. In our example there are 4 categories, therefore :

$$df = 4 - 1$$
$$df = 3$$

Knowing our degrees of freedom, the calculated $\chi^2$ value of 0.56 is compared with a critical value using an α-value of 0.05. From chi square critical value table, we see that the critical value is 7.81 at $\alpha = 0.05$ and $df = 3$. As expected, our calculated value of 0.56 is much less than the critical value of

7.81, and so we would fail to reject the null hypothesis that all sections of the control were equally preferred, or that the observed frequencies did not differ from the expected random or chance values. Note: This example is based on hypothetical data for the control, and your control data may differ from these substantially.

Now we will use the data in Table 1 to perform a $\chi^2$-contingency table analysis, or $\chi^2$-test of independence. Instead of comparing the *Artemia* frequencies in the control tubes to an expected distribution, now we will compare them to the frequencies in the treatment tubes to test the null hypothesis that the *Artemia* frequencies are not contingent on (or are independent of) the treatment. This test is a little tougher to do than the GOF test in that the expected (null) frequencies are not as obvious. Now we use the column and row totals to generate the expected frequencies, which we need to calculate the $\chi^2$-value. Note that we don't use any of the frequency information in the separate columns (tube sections) or rows (control, treatment) to generate the expected null frequencies. This is because the expected frequencies are those that would be expected if the *Artemia* frequencies were completely independent or unaffected by the treatment.

First we need to generate the column and row totals, shown below in Table 3.

**Table 3.** Contingency table of observed frequencies of hypothetical *Artemia* data

| | sec. 1 | sec. 2 | sec. 3 | sec. 4 | row totals |
|---|---|---|---|---|---|
| control | 23 | 24 | 25 | 28 | 100 |
| treatment A | 41 | 19 | 13 | 11 | 84 |
| column totals | 64 | 43 | 38 | 39 | 184 (grand total) |

From Table 3, we can now calculate the expected frequencies for each of the 8 positions (the statistician calls these positions the "cells" of the contingency table) by multiplying the column total by the row total and dividing by the grand total GRAND TOTAL. For example for the control/section 1 cell, the calculation would be (64) (100) ÷ 184 = 34.78 and for the treatment A/section 1 cell, the calculation would be (64) (84) ÷ 184 = 29.22. We continue with these calculations for all 8 cells and we create another table of expected frequencies:

**Table 4.** Contingency table of expected frequencies of hypothetical *Artemia* data

| | sec. 1 | sec. 2 | sec. 3 | sec. 4 | row totals |
|---|---|---|---|---|---|
| control | 34.78 | 23.37 | 20.65 | 21.20 | 100 |
| treatment A | 29.22 | 19.63 | 17.35 | 17.80 | 84 |
| column totals | 64 | 43 | 38 | 39 | 184 (grand total) |

Note: The row and column totals and grand total are the same for the observed and expected frequencies - this will always be the case and provides a check on your arithmetic.

The $\chi^2$ to be computed is the same as our first $\chi^2$ equation except that we write it as:

$$\chi^2 = \sum\sum \frac{(\text{observed} - \text{expected})^2}{\text{expected}}$$

We use two summations signs ($\Sigma\Sigma$) to indicate that we sum values of $(O - E)^2/E$ across all rows as well as all columns. For our example:

$$\chi^2 = ((23 - 34.78)^2/34.78) + ((24 - 23.37)^2/23.37) + ((25 - 20.65)^2/20.65) + ((28 - 21.20)^2/21.20) + ((41 - 29.22)^2/29.22) + ((19 - 19.63)^2/19.63) + ((13 - 17.35)^2/17.35) + ((11 - 17.80)^2/17.80)$$

$$= 3.99 + 0.017 + 0.916 + 2.181 + 4.75 + 0.0202 + 1.091 + 2.60$$

$$= 15.565$$

Again, we can determine the probability associated with this value (for the sample size) from a table of continuous $\chi^2$-values, and it is 0.0014. You should now know whether we will accept or reject the null hypothesis based on this, but we will follow our convention here and compare the calculated value to a critical value in chi-square table. We have to take sample size into account by calculating the degrees of freedom as follows:

$$df = \text{(number of rows - 1) (numbers of columns - 1)}$$

Since our table had 2 rows and 4 columns:

$$df = (2 - 1)(4 - 1)$$
$$df = 3$$

Using a critical value of chi-square table, the $\chi^2$ critical value for $df = 3$ and $\alpha = 0.05$ is 7.81. Because the calculated $\chi^2$ is greater than the critical value, the null hypothesis that the *Artemia* frequencies are independent of the treatment is rejected. We would conclude that Treatment A had a significant effect; that is, that *Artemia* appeared to use the sections in the treatment tubes differently than they used the sections in the control tubes.

Now you will need to complete a $\chi^2$-goodness of fit test and a contingency table analysis on your real experimental data.

*Data Analysis*

1. Test the three null hypotheses that the distributions in the control and the gradient treatments of pH, temperature and light are the same. For each treatment you must generate the appropriate column and row totals in a table so you can calculate your expected values. Use the example calculations above to guide you through your analysis. You will end up calculating a $\chi^2$ value for each treatment (*i.e.* 3 values).
2. Use the results of these tests to interpret the experiment by evaluating the various hypotheses you made for the three gradients used to determine the habitat preferences of *Artemia*.
3. From your measurements you have generated data. Since one of the objectives of this lab is to give you experience using statistics, you should take the data and compute the statistic calculations (based on a significance level or $\alpha$ value of 0.05) as outlined in the lab manual. The statistical tests are used to determine the probability that your results are due to chance; that is, whether the statistical null hypothesis is correct. Once you have tested the statistical null hypothesis, you must then make your own conclusions about whether your data supports or refutes your original biological experimental hypothesis.

*Questions to consider:*

1. Why didn't you use a *t*-test on the *Artemia* data?
2. What were your original biological experimental hypotheses?
3. Is *Artemia* indifferent to the factor under study or does it actively select a specific condition?
4. If there is active selection and an obvious preference, what is the preferred condition?
5. Do the results support or refute your experimental hypotheses?
6. In what ways are preferences for certain environmental factors important in the isolation of species?
7. What differences in gradient responses might be expected between organisms inhabiting areas with little environmental fluctuation and those inhabiting areas with great environmental fluctuation?
8. Having completed the experiment you no doubt have experienced or have an opinion on some of the sources of experimental error or experiment design flaws. Suggest ways to eliminate or reduce these errors or flaws.

## Notes for Instructor

1. Protocol for Culturing *Artemia*

*Artemia are available from most tropical fish stores or they can be order from Brine Shrimp Direct (info@brineshrimpdirect.com).*

*a.* *Artemia* need to be cultured in salt water. You can either purchase Instant Ocean (mix as directed) or use sodium chloride. If using sodium chloride, make a 0.4% salt solution. A 3 L jar or small aquarium can be used.
*b.* Add ~1.25 mL (¼ tsp) *Artemia* eggs to this solution and stir well with a glass rod.
*c.* Place the jar ~30 cm (12 inches) from a bright light source (150 Watt, 125-130 Volt flood lamp on a stand) and aerate the jar with an air stone using a moderate and even flow of air.
*d.* Let this sit in the light and aerate for ~ 18 to 24 hrs.
*e.* Remove the air stone and allow the hatched A*rtemia* to swim towards the light for a ~10 or 15 minutes. Unhatched eggs should sink to the bottom of the jar.
*f.* Siphon off *Artemia* into a 3 L clean jar. Rinse and top up with 0.4% salt water.
*g.* We try to thin out the *Artemia* so that we get between 500 to 700 or so *Artemia* in each beaker per student group, which would mean that the students would be counting about 250 to 350 *Artemia* per tube.
*h.* *Artemia will last longer and you will get a better hatch percentage if eggs are stored in a freezer for a few weeks prior to using*.

2. Materials Needed

- Experimental tubes for each set-up: flexible plastic tubes attached to a meter stick – 2 per bench; for control, light and pH use clear tubing R-1000, ½" ID, 5/8" OD; for temperature use neoprene tubing ½" ID, 5/8" OD.
- Elbow clamps – 6 per bench; pliers (4)
- Serum caps (4) or rubber stoppers (2 per bench)
- Glass beads
- *Artemia franciscana* eggs (→naupli larva) grown in sea water (Instant Ocean)
- 30 ml 0.5% KOH (bottle w/ syringe)
- 30 ml 0.5% HCl (bottle w/ syringe)
- pH meter; small thermometers (4)
- 3 pH buffers (4,7,10)
- 4 pairs safety goggles; 4 pairs dissecting gloves
- 1 stand with clamp & heat lamp; 1 stand with clamp & regular flood lamp
- 4 - 250 ml bottles of rinsing water
- 4 - styrofoam box with crushed ice
- 16 - 50 or 100 plastic beakers
- 1 – 400 ml beaker
- Petri dish with grid – one per student; hand held counter – one per student
- Chi square critical table – one per bench

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