Name: ________________________ Pre-Lab: Animal Behavior/Scientific Method

1) (3 pts) You just saw a dog food commercial on TV that states that dogs prefer Alpo over Kal-Kan. Think about how you could conduct an experiment to test whether or not this statement is true. State your null and alternative hypotheses. What response variable would you use in your experiment?

null hypothesis:

alternative hypothesis:

response variable:

2. (2pts) Based on the chart on page Behavior-8 what is the P (heads=9)? Show your calculations.

3) (5 pts) Suppose that you are trying to determine if a coin you have is biased towards flipping more or less heads than the expected 50%. Thus,

H_0 = the coin is 'fair', that is #Heads = #Tails

H_A = the coin is biased, that is #Heads ≠ #Tails

Take a coin and flip it 16 times and record the number of heads and tails you flip. Using these data and the graph on page Behavior-8, determine the probability that your coin is biased. Show your work. (see hints on the next page if you are having difficulty)

HINTS for answering prelab question #3

1. First flip a coin 16 x's get the number of head/number of tails in 16 trials

2. Based on your trial can you reject the null hypothesis?
Determine whether or not to reject the null hypothesis of the coin is fair- that is the whether or not the coin you used is biased.

Even though you would expect it to land on heads 50% of the time- each time you flip the coin it is an independent trial. So by **chance** you may not get the result of 8/16. Then how do you determine if the result you got is just by chance?

You can do this by looking at a binomial distribution a histogram of results of a coin toss generated by a previous class on page Behavior-8. Where does the number of heads you got fit on that curve?

You can determine the probability what you observed is due to chance alone (which would mean you can't reject the null hypothesis). e.g. What is the Probability that by chance alone I flipped a coin 16X's and 13/16 times it was heads-

Looking at the distribution:
$P(\geq 13) = (0+1+0+0+0) / 48 = 1/48 = 0.021$ or there is a 2.1% chance of seeing that result by chance which is less than the 5% level generally used in science to reject a hypothesis.

My confidence level is 100%- 2.1= 97.9% that my result is not due to chance and that my coin is biased. Thus I reject the null hypothesis that the coin is fair.

Animal Behavior and the Scientific Method

Objectives
To compare and appreciate animal diversity, through the lens of behavior.
To consider the diversity of behavioral adaptations which have evolved in response to selection pressures from different physical and social environments.
To understand the major components of the scientific method.
To generate testable hypotheses, design experiments to test hypotheses, and analyze results from an experiment.

Introduction:
Animal behavior

Broadly defined, behavior is the sum of an organism's responses to stimuli in its environment (*Campbell Ch. 51*). Behavior is what an organism does. After detecting another organism, for example, an animal may respond by attacking it, fleeing from it, attempting to eat it, courting it, freezing in place, or ignoring it, just to name a few of the possible behavioral responses depending on the identity and perhaps behavior of that other organism.

Most animals possess far different sensory abilities and live in drastically different habitats than the humans studying them. Animal behaviorists study their subjects by carefully observing and experimentally analyzing behavior patterns. In your study of animal behavior in lab today, consider both the proximate and ultimate causes of the behaviors you observe.

Proximate causes include the immediate sensory, physiological, and biomechanical events that led to the behavior. For example, did the organism use chemical, visual, or electrical cues to detect the stimulus, and what kind of nervous or hormonal events did it trigger in the organism?

Ultimate causes refer to the adaptive value and evolutionary origin of the behavior. In other words, how does this behavior help the survival and reproductive success of the organism, and what is the pattern of behavior in the species' ancestors?

To illustrate, a sparrow feeding on the ground will respond to a stalking cat by taking flight. Part of the **proximate** cause of this behavior might be the sight or sound of the approaching cat stimulating sensory receptors that in turn trigger nervous impulses that lead to muscle contractions in the wings. The **ultimate** cause, the adaptive value, might include fleeing from a predator to avoid being eaten.

In today's lab you will be investigating orientation behavior.

- **Orientation** behavior helps an organism locate the most favorable environment currently available to it. Orientation behavior includes taxis, movement directly toward or away from a stimulus. "Positive taxis" refers to movement toward a stimulus while "negative taxis" refers to movement away from a stimulus. Prefixes such as photo-, chemo-, and thermo- describe the nature of the taxis. For example, an animal that approaches light is positively phototaxic.

- **Antagonistic behavior**. In addition to the need to find a favorable place to live, animals often find themselves in conflict with other organisms. For example, two bears may attempt to use the same profitable location along a stream to catch fish. Behaviors associated with conflict situations are known as agonistic and include both aggressive (attack or threatening) and submissive (retreat and avoidance) behaviors. Agonistic behavior often involves displays to make the animal appear larger or threatening, and in many cases (but not all), a conflict ends without serious injury or death. These threats and displays often help an animal to maintain a territory or social position in which it has predominant access to resources such as space, food, and mates.

The Scientific Method

The information contained in your biology textbook results from thousands of scientific investigations. Scientists express curiosity about the world and ask questions that address their desire and often society's pressing need for knowledge. Most scientists follow the same general procedure, called the **scientific method**, for formalizing questions and seeking information to address them.

The scientific method typically begins with observations of a pattern or process that suggests one or more corresponding questions to the observer. A scientist attempts to formulate alternate answers for the questions called **hypotheses**. The process of formulating a hypothesis often starts with an initial or informal hypothesis that the scientist reformulates into a formal hypothesis that ideally produces unambiguous predictions that can be tested with future experiments or observations. Formal hypotheses strive to yield mutually exclusive predictions that do not overlap with predictions from competing hypotheses. Scientists usually express formal hypotheses in two forms: a null hypothesis and one or more alternate hypotheses.

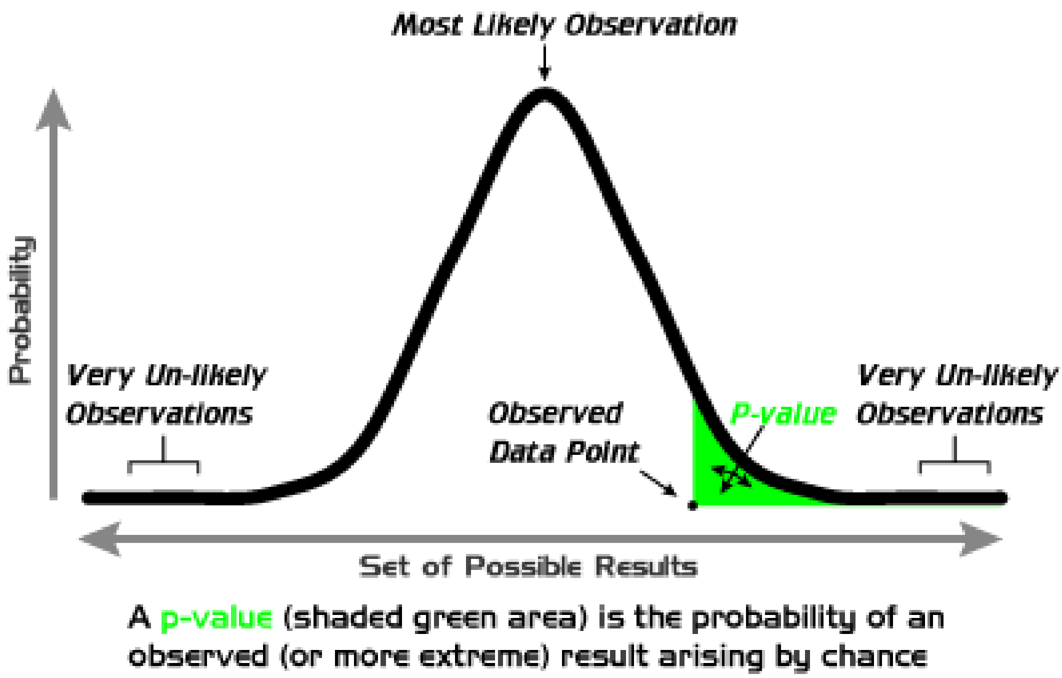
A **null hypothesis (H_0)** predicts "no difference" or "no effect" between two or more experimental conditions. For example, in an experiment to test the effectiveness of a flu shot, the null hypothesis would predict that people that received the flu shot are just as likely to contract the flu as people who did not receive the shot (i.e., no difference between the two experimental groups).

In contrast, the **alternate hypothesis (H_A)** predicts a difference or effect of the experimental condition. For the flu shot example, an alternate hypothesis might predict that there will be a difference between people who received the flu shot compared to those who did not in the likelihood of contracting the flu.

After formulating the hypotheses, scientists devise experiments or additional observation protocols to collect the data needed to test the hypotheses. The data typically require analysis, often statistical, before they can be judged against the predictions. If the data fail to support the predictions of the hypothesis, that hypothesis is rejected. If the data support the hypothesis, the hypothesis is not rejected. The scientist could then say that the results of the experiment support the hypothesis. However, the results do not prove that the hypothesis is true. Additional experiments need to be conducted that address the hypothesis in different ways. Several different experiments each in support of the hypothesis would strongly suggest that the hypothesis is true. Moreover, additional experiments may reveal that the hypothesis seems to hold under certain conditions but not others.

Statistical testing:
Statistical tests take into account the amount of variation within the samples and the size of the samples to allow you to estimate the probability that the results you obtained could have been due to chance events alone. A significant difference is one that is greater than would be expected by chance. Later in this lab, we will introduce the binomial test that will be used for statistically testing today's experiments
The decision about whether to accept or reject the null hypothesis is based on the **p value** obtained from the statistical test.

p value is the probability of obtaining a test statistic at least as extreme as the one observed. It is a measure of the likelihood of the hypothesis. The smaller the p value is the less likely the hypothesis is. In science the null hypothesis is usually rejected if **$p < 0.05$**



One more issue that can undermine the ability of your data to support your hypothesis is that of **confounding factors**. An experimental confound produces an alternate causal explanation for the observed results. For example, if you wanted to test the effects of heat on the movement of an animal and used a light to produce that heat, you could not say whether the animal was responding to heat or light even if your results were statistically significant.

Experimental Organisms

In today's lab, you will investigate taxis in red worms (*Eisenia foetida)*, and you will work in groups to design, conduct, and analyze a formal experiment. Because of the advanced nature of the statistical procedure required to analyze your experimental data you will use red worms to have ample number of subjects available for experimentation and therefore be able to conduct a statistical analysis.

Think about the proximate and ultimate causes of the behavior when designing your experiment.

Red worms

Red worms belong to the phylum Annelida which contains about 15,000 species. Like other earthworms, red worms lead primarily subterranean lives and consume organic, often decaying, matter in the soil. Red worms inhabit particularly moist soils rich in organic matter such as compost heaps and gardens. In fact, they are the primary worm used for composting. Worms have no lungs and must absorb oxygen through their skin, a process that requires their skin to stay moist. Your behavioral experiments will investigate taxis in these worms. Recall that orientation behaviors such as taxis function to place an organism in an environment favorable for survival and reproduction. When designing your experiment, you should think about the natural habitat of earthworms and the cues that they may use to detect the suitability of different areas.

Hypothesis testing with red worms

Based on the description of red worm natural history presented above, you will formulate hypotheses, design an experiment to test your hypotheses, conduct an experiment, and statistically examine the results. Equipment and supplies are available for your experiment. Talking to your TA or glancing at the supplies and equipment available may help inspire your creative thinking. For example, think about the habitat of the worms; you may want to examine what environmental cues they use to select where to live and feed. Say you think that temperature may represent an important influence in the lives of red worms (you cannot use this experiment).

Initial hypothesis: Red worms will move from warmer environments into cooler ones.
Response variable: The number of worms in each environment after 3 minutes of choice.
Test situation: Test 16 worms in a dish with a warm side and a cool side.
Null hypothesis: # of worms on warm side = # of worms on cool side
Alternate hypothesis: # of worms on warm side ≠ # of worms on cool side

You should now decide on an initial hypothesis, your response variable, and H_0 and H_A. **Answer questions 1a-c for your lab report and have your TA approve them.**

Experimental design

Next, you need to design your experiment. How will you test your hypothesis? You should keep in mind several factors when designing your experiment. Recall the problem of confounding factors discussed earlier. In the sample experiment on temperature preference in worms, what if you used a lamp to warm one side of the dish? If the worms move away from the source, they may be responding to light rather than heat. Perhaps you decide to use a chemical heat pack or warm water bottle instead.

If you put a worm in the middle of the dish and it moves away from the heat, you still cannot conclude that red worms, in general, are repelled by heat. Why not? Even in the absence of a heat source, you would expect that the worm may move to one side of the dish or the other 50% of the time (like flipping a coin). You must perform repeated trials of the experiment to gain confidence that the movement away from the heat source is not merely due to chance. Think about the following when designing your experiment: where in the dish do you place the worm, how long do you wait to record the result (you need to be consistent between trials), when will you start the timing? **Answer questions 1d and e.**

Data collection

You should now set up your experiment and collect the data. You will perform 16 trials using 16 different individual worms. **Fill out the chart for your lab report (question 1f) as you collect your data.**

Data analysis

To illustrate how to analyze the significance of your data, we need to revisit the sample red worm experiment. To test the red worms' preferences for heat or cool, suppose you performed 16 three-minute trials on 16 different worms and obtained the following data:

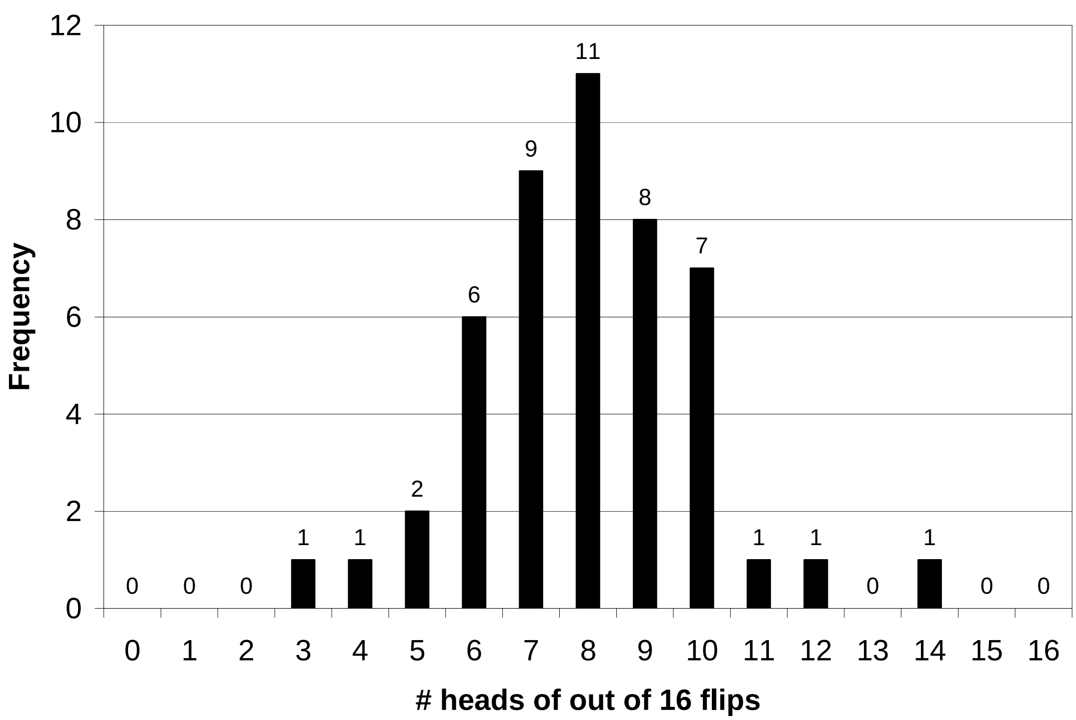
Subject #	**Worm on warm side after 3 min**	**Worm on cool side after 3 min**
1	X	
2		X
3		X
4		X
5		X
6	X	
7		X
8		X
9		X
10		X
11		X
12		X
13	X	
14	X	
15		X
16		X
Total	**4**	**12**

The data appear to exhibit a trend toward a temperature preference for the worms, but is this result significant? If the data supported the null hypothesis of no difference or no preference, you would expect to see approximately half (n=8) of the worms crawling to the warm side and half to the cool. To test for significance, the results must be compared to the distribution expected by chance.

If a worm exhibited no preference for the warm or cool side, 50% of the time it would travel to warm side and 50% to the cool. Earlier we compared this to the probability of a flipped coin landing heads up (or likewise, the likelihood that it lands tails up). On average, you would expect that a coin flipped 16 times should land heads up about 8 times and tails up about 8 times. But what if you flipped a coin 16 times and it landed heads up 12 times, does this mean the coin was loaded? Not necessarily. Because each flip is an independent event (in other words, the outcome of any given flip does not depend on the outcome of any other flip), by chance alone you could get 12 heads and 4 tails. By chance alone, you could get 16 tails and no heads although highly improbable. Statistics is concerned with calculating the probability that a certain combination of outcomes will occur by chance.

To illustrate, the class as whole will generate a distribution graph for this type of problem. Everyone will flip a coin 16 times and count the number of heads and then repeat with another 16 flips. You will have two "head" counts to report to your TA. We intuitively expect that everyone will get 8 heads and 8 heads, but other outcomes will occasionally occur. The TA will pool the class data to see how often we get unexpected results just by chance. Do this now if you haven't already. **Record the distribution generated by the class in question 1f.** You will use this distribution to test your hypothesis.

Suppose 24 students in your lab each flipped a coin 16 times, counted the number of heads, and then repeated it for another 16 flips. In all, the class would have generated 48 (= 2 X 24) trials of 16 flips. Imagine your class obtained the following distribution (**note**: do <u>not</u> use this distribution to test your hypothesis, use the one generated by your lab section).



What can you determine based on this distribution? We got exactly 3 heads out of 16 flips once in 48 trials. This means the probability that the number of heads=3 is 1/48 or 0.021. Eleven out of 48 flips we got 8 heads so that probability is 11/48 or 0.23. As expected, the probability of getting 8 heads out of 16 flips is much greater than 2 heads out of 16 flips. Based on the distribution, what is the probability of getting 11 *or more* heads in 16 flips? Add the number of times we got 11, 12, 13, 14, 15, and 16 heads and divide by the number of trials. Thus,

$P(\geq 11 \text{ heads in 16 flips}) = (1 + 1 + 0 + 1 + 0 + 0) / 48 = 0.0625$

Returning to the red worm temperature experiment, our mutually exclusive hypotheses were

H_0: # worms on warm side **=** # worms on cool side

H_A: # worms on warn side **≠** # worms on cool side

We can use the above distribution to determine the probability that our results (4 worms on warm side and 12 worms on cool side) could have occurred by chance alone. To determine this, you must look at the two extremes or tails of the distribution (0-4 and 12-16). In other words, what is the probability <u>due to chance alone</u> that 12 of 16 worms would move either to one of two sides? Based on the above distribution:

$P(\leq 4, \geq 12) = (0 + 0 + 0 + 1 + 1) + (1 + 0 + 1 + 0 + 0) / 48 = 4/48 = 0.083$

So, a little over 8% of the time we would expect worms to exhibit this degree of preference for either the cooler or warmer side of the dish just by chance alone. From this we can also calculate a **confidence level**:

$100\% - P(\leq 4, \geq 12) = \%$ confidence

$100\% - 8.3\% = 91.7\%$ confidence

Thus, we are almost 92% confident that the red worms are actually exhibiting a preference for either the warmer or cooler side of the dish.

Is this a statistically significant result? Where is the cutoff between statistical significance (reject H_0 but not H_A) and no statistical significance (reject H_A but not H_0)? The cutoff varies somewhat among disciplines but often is set at either $P \leq 0.1$ or $P \leq 0.05$. These respectively correspond to 90% and 95% confidence levels. $P \leq 0.05$ is the more typical standard and the one that we will use here. From our sample experiment, $P = 0.083$ which is greater than 0.05 so we must reject H_A but not H_0 and conclude that our results did not support the hypothesis that red worms show a preference between warm and cool sides of the dish. Note that the rejection of a hypothesis is somewhat subjective though. If we set our significance level cutoff at $P \leq 0.1$, we would have rejected our null hypothesis and concluded that our data support the alternate hypothesis. In practice, scientists use tables of probability calculated for a very large number of coin flips.

<u>The Effects of Sample Size</u>

Sample size can influence whether results show statistical significance. In our sample experiment, 75% (12/16) of the red worms displayed a preference for the cool side of the dish. Yet, this result was not $p<0.05$ the standard for rejecting the null hypothesis, this result did not reach statistical significance, and we could not reject our null hypothesis. If instead we had tested 100 red worms and 75 had displayed a preference for the cool side (= 75% as before), would this be significant at $P \leq 0.05$? Using statistical tables generated for just this purpose, we would find that $P < 0.0001$, a *highly* significant result, and could quite confidently state that out data supported our alternative hypothesis that red worms displayed a temperature preference. Although we used a series of coin flips to illustrate a frequency distribution based on two equal outcomes (heads or tails), mathematicians and statisticians have generated standardized probability distributions which scientists use to check the significance of their results. As a side note, our sample coin-flip distribution above fairly well simulates the true distribution based on two outcomes of equal probability in an experiment with 16 trials.

Complete questions 1h-j based on the results of your red worm experiment.

Lab report

• Must be typed; handwritten reports will not be accepted. Hand-drawn and labeled drawings are fine; photographs are not acceptable. All drawings must indicate size.
• Due next week at the start of the lab session you are currently in. This is a firm deadline.
• Although you will perform these activities as a group, each member of the group must turn in an individual lab report. Each person's report must be in his or her own words as much as possible.
• Your lab report will be written up as a formal lab report on the Red worm experiment must contain:

Introduction: background with references.
(a) State your initial hypothesis

(b) State your null and alternate hypotheses

H_0:

H_A:

TA's initials

Methods:

(c) What response variable will you use to study the behavior of this species?
How many trials?

(d) Describe your experimental design. Include important details about your organisms, the physical setup, number of subjects, duration of each trial, etc.

Results:

(e) Fill in the blank chart with your data. Label the chart appropriately for your experiment.

Subject			Comments or Notes
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
Total			

Why is it important to use 16 different individuals instead of testing the same subject 16 times?

(f) Fill in the distribution your class generated by coin flipping and label the axes.

0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

(g) Using the class coin flipping distribution above as a basis for your statistical test, how likely is it that the results from your red worm experiment are due to chance? Show your calculations.

(h) Should you reject your null hypothesis? Explain. What is your confidence level? What do your data suggest about your hypotheses?

Discussion

(i) Offer a discussion of your experiment. What do your results demonstrate about the behaviors and organisms you observed? What do your results suggest about the proximate cause(s) of the behaviors you observed in response to your experimental manipulations? What do think may be the ultimate cause(s) of the behaviors you observed.

(j) Are there potential confounding factors in your experimental design? If so, explain.