**113 Lab Learning Objectives**

**Week 12: Information and Natural Selection lab #7**

Learning Objectives for Environmental Information and Natural Selection

*Skills*

* Use statistics to determine the level of significance of data.
* Prepare graphical representations of data and its variance.

*Cognitive*

* Employ a scientific approach to answer biological questions and test hypotheses.
* Analyze experimental data and reach logical conclusions.
* Construct a generalizable explanation linking genetically defined taste capacity to natural selection and evolution.
* Identify possible sources of environmental information that communicates the level of toxicity for plant tissues.
* Design an experiment to use model organisms to extrapolate potential toxicity of a compound or mixture.

**Week 12: Information and Evolution Lab #5**

Learning Objectives for Bacterial Evolution

*Skills*

* Pipet accurately.
* Work with bacterial cells using sterile technique.
* Make dilutions of stock solutions.

*Cognitive*

* Employ a scientific approach to answering biological questions and test hypotheses.
* Describe the big idea of evolution based on lab experiences.
* Explain how antibiotic resistant bacteria can appear quickly in the population.
* Design directed evolution process to select antibiotic resistant bacteria.
* Formulate an hypothesis how antibiotic resistant bacteria evolve outside the laboratory.
* Propose a mechanism that allows bacteria to evolve rapidly when exposed to antibiotics.

**Bio113 Week 12**

Before you come to lab

1) Look up the information about TAS2R38 human gene (three links on lab web page).

2) Read about MRSA and why evolution of drug resistance is a medical concern.

3) Answer each of these four questions in two sentences or less.

A) How widespread is TAS2R38 gene among animals?

B) What is a null hypothesis? What is the right way to use a p value?

C) What is MRSA and why should you be concerned about it?

D) What human behavior leads to antibiotic resistance in human pathogens?

**Week 12**

In Lab

**Evolution: Directed Evolution of Antibiotic Resistance in Bacteria (a 6 week project)**

4) Analyze your results from your last directed evolution experiment.

5) Design your final experiment to determine whether your bacterial cells have evolved antibiotic resistance. You will want to test all 4 antibiotics using the disc method. Each group will use their frozen stock of parental (prior to directed evolution) bacterial cells on LB plates without antibiotic. You will want to compare the evolved strains of bacteria with the original strain of bacteria. How well does each stain grow in the presence of all four antibiotic disks? Remember to use the negative control disk too. This is your final experiment that you will present in your final oral presentation. You will have 9 plates to use.

**Information and Evolution: Genetics of Perceiving Danger Module (an 8 week project)**

6) Discuss brine shrimp toxicity data and conduct statistical analysis of your data. Did you find any statistically significant results? How will you indicate which results are significant and which ones are not? Can you have smaller or bigger statistical differences?

7) Produce the graphics you will need for your final written lab report on tasting and toxicity with regards to natural selection.

8) Optional review session once everyone has finished the two lab modules.

**Do the plant extracts contain any toxins?**

 You have extracted compounds from two plant tissues and we want to know if there are any significant survival differences with the four treatments (two tissues, two doses). We are going to use the Fisher Exact statistical test online at: <<http://www.quantitativeskills.com/sisa/statistics/fisher.htm>>.

Generate a electronic or paper table that looks like the one below. We have provided you with some simulated data so you can walk through the sample analysis process and know that you have done the test properly. Replace the simulated data with your own data.

|  |  |  |  |
| --- | --- | --- | --- |
| Treatment | # Alive | # Dead | Notes |
| Water control |  |  | Should add to 40; obtain results of water control from another group |
| high methanol  |  |  | Should add to 40; obtain results of methanol control from another group |
| low methanol |  |  |
| Tissue A low concentration | 31 | 9 | = 40 (1st set of tubes #4-7 pooled) |
| Tissue A high concentration | 21 | 19 | = 40 (1st set of tubes #8-11 pooled) |
| Tissue B low concentration |  |  | = 40 (2nd set of tubes #4-7 pooled) |
| Tissue B high concentration |  |  | = 40 (2nd set of tubes #8-11 pooled) |

*Null Hypothesis = the two treatments are equally toxic.*

 When you go to the online test site, you will see a table that looks like this:

|  |  |  |
| --- | --- | --- |
|  | Col 1 | Col 2 |
| Row 1 |  |  |
| Row 2 |  |  |

You can convert the labels to this:

|  |  |  |
| --- | --- | --- |
|  | Alive | Dead |
| Low A | 31 | 9 |
| High A | 21 | 19 |

When you hit “Calculate” you will see this output:

The total number of cases= 80

The smallest value= 9

The smallest marginal= 28

Use of \* (starred) statistics is advised

The p for exactly this table= 0.01233

**One sided p-values: for p(O>=E):** [Use this value (0.01699) if you predicted high dose would

**p(O>=E): 0.01699 \*** be more toxic than the low dose.]

p(O>E): 0.0046553 [O = observed; E = expected]

mid-p: 0.0108227

**p(O<=E): 0.9953447** [if outcome is the opposite of what you predicted (*e.g.,* low dose was more

 toxic than high dose)]

**Two sided p-values p(O>=E|O<=E):** [Use this if you only predicted the two plant extracts

p= 0.03398 \* (sum of small p's) (the two treatments) would be different.]

p= 0.0123347 (left+right-exact)

p= 0.0216453 (left+right)

p= 0.03398 (double the single sided p)

p(O>E|O<E)= 0.00931 (sum of small p's)

mid-p 0.0216453 (sum of small p's)

For help go to SISA.

Chi squares

(both with 1 degree of freedom):

Pearson's= 5.495 (p=0.019076) \*

LRX= 5.587 (p=0.0181)

**One sided test:** (*e.g.* high dose is more toxic than low dose; null hypothesis = the two treatments are the same.)

 If you hypothesized that one condition was more toxic than the other, AND the data trend in the direction you hypothesized, then use the one-sided p-value that is highlighted (**bold and underlined**) above (*i.e.*, ~0.017). That is the ONLY value you need from the table.

 However, if you hypothesized that one condition was more toxic than the other, AND the data trend in the OPPOSITE direction you hypothesized, then use the one-sided p-value that is **bold**) above (*i.e.*, ~0.99). You could restate your hypothesis and subtract the p value from 1 to get

p = 1 – 0.99 = 0.01.

**Two sided test:** (berries have a different level of toxicity than leaves; null hypothesis = berries and leaves are equally toxic.)

 You might only predict that two conditions are different, but not specifically predict which was more toxic than the other. In that case you would use the two-sided p-value that is underlined above (*i.e.*, ~0.034).

**Full Analysis**

 You will need to perform 10 Fisher Exact tests, making the following comparisons:

water vs. low methanol (what are you testing here?)

water vs. high methanol (what are you testing here?)

low methanol vs. [LOW] Tissue A (what are you testing here?)

low methanol vs. [LOW] Tissue B (what are you testing here?)

high methanol vs. [HIGH] Tissue A (what are you testing here?)

high methanol vs. [HIGH] Tissue B (what are you testing here?)

 [LOW] Tissue A vs. [LOW] Tissue B (what are you testing here?)

[HIGH] Tissue A vs. [HIGH] Tissue B (what are you testing here?)

[LOW] Tissue A vs. [HIGH] Tissue A (what are you testing here?)

[LOW] Tissue B vs. [HIGH] Tissue B (what are you testing here?)

**Written Lab Report**

 Your next graded assignment is to write a paper that incorporates all the data associated with taste, genotyping and shrimp bioassay for toxicity. This paper is due at the beginning of lab on November 19 (no lab on Thanksgiving day). This paper has been a group project until it comes time to write the paper. Each person turns in his or her own single-authored paper. Use CATME peer evaluation to characterize student participation in the collective effort (prior to writing).