## Introduction

In this module we will explore statistical approaches for evaluating whether the mean of a population differs from a specific value or whether the means of two populations differ from one another. Unlike the previous module, our focus here will be on data that is distributed continuously, such as weight, height, or length. For instance, we might be interested in evaluating whether two populations of garter snakes differ in average length. Or, perhaps we would like to evaluate whether a specific treatment, such as predator exclusion, caused the average feeding rates of a prey species to be increased relative to an un-manipulated control. In cases like these, it is possible to quantify how certain we are about the mean of a population by calculating a *confidence interval*, and to evaluate whether the means of two populations differ significantly by using a *t-test*.

### **Practical Background**

#### Confidence intervals:

We begin by developing the idea of a confidence interval. The goal of a confidence interval is to predict the range of a variable that has a probability, 1 - p, of containing the true mean of a future experiment with a sample size of n. From a practical standpoint, the first step in calculating a confidence interval is to calculate the sample mean,  $\overline{Y}$ , and also the sample standard deviation, s. Because the distribution of sample means is well described by the t-distribution, we can begin calculating our confidence interval by first looking up the critical value of t from the appropriate table, and then multiplying it by the quantity  $s/\sqrt{n}$ . Adding the product of these quantities to the sample mean,  $\overline{Y}$ , yields the high end of the confidence interval whereas subtracting this product from the sample mean yields the low end of the confidence interval:

$$\bar{Y} - t_{\alpha/2, n-1} \frac{s}{\sqrt{n}} \le \mu \le \bar{Y} + t_{\alpha/2, n-1} \frac{s}{\sqrt{n}}$$

$$\tag{1}$$

As we stated previously, *s* is the sample standard deviation, *n* is the sample size, and  $t_{\alpha/2,n-1}$  is the critical value of the t-distribution for a specified Type I error rate,  $\alpha$ , or significance level. Although simple, the confidence interval is a very useful tool, and can be used to evaluate whether or not the mean of a population differs significantly from a specified value (e.g., zero).

#### The t-test:

The confidence interval described in the previous section allows us to make statements about the mean of a single population. Another common problem that arises time and time again in population biology is evaluating whether the means of two different populations differ from one another. The t-test provides us with the statistical tools to address this problem.

Mechanistically, as long as we can safely assume the variances of the populations we hope to compare are equal, we can calculate the critical value of the test statistic, *t*, upon which the t-test relies as follows:

$$t_{\alpha,n_1+n_2-2} = \frac{\bar{Y}_1 - \bar{Y}_2}{\sqrt{\left(\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2}\right)\left(\frac{n_1+n_2}{n_1n_2}\right)}}$$
(2)

where  $\overline{Y}_i$  is the sample mean for population i,  $s_i^2$  is the sample variance for population i, and  $n_i$  is the sample size for population i. Next, we just compare this test statistic to the critical value of the *t*-distribution with  $n_1 + n_2 - 2$  degrees of freedom and significance level,  $\alpha$ .

### Assumptions and Limitations<sup>1</sup>

- The variable under study should have equal variance in both populations
- The variable under study should be normally distributed within both populations

<sup>1</sup> When analyzing real data, you should always test to make sure these assumptions are satisfied. If they are not, alternative approaches or transformations of the data should be applied. However, this is beyond the scope of this course. For analyses conducted in this course, you may simply assume that that these assumptions are satisfied.

### Worked examples:

### <u>Confidence interval</u>

Problem: As part of your job as a biologist with USFWS, you have been assigned to a project evaluating the potential for a recently identified population of the New Zealand Mud Snail, *Potamopyrgus antipodarum*, to become invasive within high mountain lakes in Idaho. One of your first tasks is to estimate the population growth rate of this species using data collected from 12 different lakes, each of which contains an independent population. Because previous research has demonstrated that this snail is likely to become invasive only when its intrinsic rate of increase exceeds r = 0.02, your goal is to use your data to determine whether or not this is the case within Idaho's mountain lakes. The table below includes the estimated growth rate for each of the 12 populations.

	Population											
	1	2	3	4	5	6	7	8	9	10	11	12
r	-0.02	0.03	0.02	0.04	-0.01	0.01	0.05	-0.02	0.03	0.06	0.09	0.01

Solution: How can we proceed to answer the biological question using this data? A simple and straightforward approach is to calculate a confidence interval for the growth rate of the snail. The first step toward this goal is to calculate the sample

mean,  $\overline{Y}$ , which for the data shown above is equal to 0.024. Next, we calculate the sample standard deviation,  $s = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \overline{Y})^2}{n-1}}$ , which is equal to 0.033 for the snail growth rate data. Using the standard significance level  $\alpha = 0.05$  and the fact that our sample size, n, is equal to the number of lakes, 12, we can then look up the critical value of the t statistic which is equal to  $t_{0.025,11} = 2.201$ . Finally, substituting the appropriate values into formula (1) shows that the 95% confidence interval for snail growth rate is given by:

$$0.024 - 2.201 \frac{0.033}{\sqrt{12}} \le \mu \le 0.024 + 2.201 \frac{0.033}{\sqrt{12}}$$

which equals:

 $0.003 \leq \mu \leq 0.045$ 

and includes the critical value of the growth rate, r = 0.02. Thus, if we were to conduct this experiment over and over again, the true growth rate of the population would fall within this interval 95% of the time. The logical conclusion to make in light of this result is that we cannot rule out the possibility that the true growth rate of the snail population exceeds the critical threshold.

#### <u>T-test</u>

Problem: Building on the previous study, you wish to evaluate whether the castrating trematode, *microphallus*, which infects and sterilizes sexual *Potamopyrgus antipodarum* snails could be used to control the snail population. To this end, you collected estimates of snail population growth rate from 12 additional lakes inoculated with the castrating trematode. The snail growth rate data for these additional lakes is shown in the following table:

	Infected Population											
	1	2	3	4	5	6	7	8	9	10	11	12
r	-0.03	0.03	-0.03	-0.06	-0.01	0.04	0.01	-0.04	0.02	0.02	0.03	0.01

Based on this new data, does it appear that the castrating trematode, *Microphallus,* alters the growth rate of the invasive snail, *P. antipodarum*?

Solution: In order to answer this question, we need to evaluate whether or not snail growth rate is significantly reduced in the presence of the parasite. Because our goal here is to compare the means of two populations, we employ the t-test. The first step is to calculate the test statistic, t, given by equation (2). If we arbitrarily label uninfected populations using the subscript 1, then the work we did in the previous problem already yielded some of the necessary calculations. Specifically, we know that  $\bar{Y}_1 = 0.024$ ,  $s_1^2 = 0.00109$ , and  $n_1 = 12$ . Next we need to determine these same quantities for the snail populations exposed to the parasite

which we will label using the subscript 2. Following the exact same procedures as before, we find that  $\bar{Y}_2 = -.00083$ ,  $s_2^2 = 0.00104$ , and  $n_2 = 12$ . Substituting all of these values into expression (2) yields the following value for the test statistic, t:

$$\frac{0.024 + .00083}{\sqrt{\left(\frac{(11)(0.00109) + (11)(0.00104)}{22}\right)\left(\frac{24}{144}\right)}} = 1.864$$

Now we need just compare this value to the critical value of t for our chosen  $\alpha$  and degrees of freedom. Assuming we wish to use the standard value  $\alpha$ =0.05, the table of critical values for the student's t-distribution shows that the critical value is  $t_{0.025,22} = 2.074$  because this is a two-tailed test. Because the value we calculated for our test statistic, t = 1.864, is less than this critical value,  $t_{0.025,22} = 2.074$ , we cannot reject the null hypothesis that the growth rate of the snail population is identical in the presence and absence of the parasite. Thus, our data provides no support for the efficacy of the trematode parasite as a biological control agent.