**The nose knows: How tri-trophic interactions and natural history shape bird foraging behavior**[[1]](#footnote-1)

*An Introduction to Statistical Analysis in Animal Behavior Research*



*King penguin colony (Photo by Greg Cunningham)*

**BACKGROUND**

King penguins (*Aptentodytes patagonicus*) are seabirds of the Order Sphenisciformes, and can be found at sea or on multiple sub-Antarctic islands throughout the Southern Ocean. From a foraging perspective, these birds are particularly impressive as their foraging grounds are often located more than 400 km from their breeding beaches (Bost *et al.,* 2002). Penguins spend long periods of time at sea foraging, interspersed with periods of time on the beach breeding and provisioning their young. King penguins cover long distances and make shallow dives when commuting to foraging sites, but dive deeper when foraging on a variety of fishes. Prey is captured at depth (sometimes up to 250 m), with the deepest dives being performed during daylight hours (Bost *et al.,* 2002).

King penguin chicks usually hatch between the months of January to March. Parents provision their chicks for a few months before the creching period (when the chicks form their own group separate from their parents). During the creching period, chicks fast and form large assemblages on the beach to survive the intense cold during the winter months. Following fasting, chicks are fed again from September to December and complete development in the creche with other mature chicks. After the second bout of feeding, chicks join adults on their early foraging trips out into the ocean, feeding themselves for the first time (Descamps *et al.,* 2002).

When foraging, penguin chicks must learn how to find patches of prey to feed successfully. Prey is distributed in patches because of abiotic factors (temperature, water currents, oxygen, nutrients) that concentrate prey in certain areas. We do not fully understand the cues that penguins use to successfully travel to and from their distant foraging grounds, nor do we understand how productive waters are recognized once encountered.

One possible cue that can be used to identify productive waters is the airborne gas, dimethyl sulfide (DMS). This is an example of an infochemical. Scientists use the term infochemical to describe chemical cues that inform an organism about some element of their environment. This odor’s precursor, dimethylsulfoniopropionate (DMSP), is found in the water when phytoplankton are present (Raina *et al.*, 2013). Phytoplankton are one of the primary producers encountered in the ocean. Once DMSP is released by phytoplankton due to predation, it is converted to DMS. DMS is then volatilized and enters into the air above the phytoplankton patch. It is well established that high levels of DMS are found in productive waters, thus DMS acts as an indicator of phytoplankton presence (Berresheim *et al.*,1989). There is evidence that some birds, turtles, and seals also detect this odor in the air above productive waters (Cunningham *et al.*, 2008; Kowalewsky *et al.* 2006; Endres and Lohmann, 2012).

Phytoplankton are preyed upon by zooplankton, such as krill, a small shrimp-like crustacean. Many marine organisms eat zooplankton, such as fish, seabirds (including penguins), and whales. The amount of DMS released by phytoplankton in the presence of krill (black circles) or in the absence of krill (white circles) is shown (Figure 1).



Figure 1. DMS production by phytoplankton while being grazed (black circles) or not (white circles) by krill (Dacey and Wakeham 1986).

Recall that King penguin’s prey on fish (which eat krill), and are closely related to other bird species that have demonstrated DMS sensitivity. Thus, in this activity, you will examine the role DMS may play in the foraging behavior of King penguins and their close relatives. In Activity 1, you will investigate whether King penguins can detect DMS and in Activity 2, you will investigate DMS detection in a closely related species. For Activity 1, you will use datasets modified from the publicly available dataset originally from Cunningham *et al.* (2017) and for Activity 2, you will use a dataset from Bonadonna *et al.* (2006).

Objectives – Upon completion of the activity, students should be able to:

* Understand why certain statistical analysis are more appropriate for certain datasets
* Conduct statistical tests most common to the study of Animal Behavior, including t-test, Mann-Whitney U and binomial tests
* Determine the most appropriate way of presenting data graphically and to create that figure

Assignment

This is a self-directed activity intended to help you learn how to analyze data for animal behavior research. You will work in pairs, but may each submit the same write up, answering all the questions below. Your write-up is due on D2L BEFORE the beginning of class on the date specified above. Formatting should include Times New Roman font, one-inch margins, and 12-point text size. In addition to the write up, you will add an assessment of your partner’s contributions, privately written, at the end of the assignment.

**ACTIVITY 1**

**Can King penguin adults and chicks detect DMS?**

To establish that King penguin foraging behavior is influenced by DMS, first you need to determine if the adults and chicks respond to the odor.

1. *Given the natural history of adults and chicks described in the background, generate a hypothesis regarding the response to DMS in adults and another for that in chicks. Would you expect a difference in the response of chicks and adults to DMS? Why or why not?*

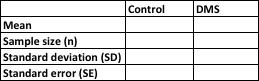
In pairs, you will investigate either adult or chick responses to DMS, then compare your results to another group who investigated the one you did not. Researchers tested olfactory responses of individual birds using a modified Porter method (Porter *et al*.1999), which involved presenting odors to sleeping birds. Birds were either exposed to DMS (dissolved in propylene glycol) or a control odor.  The response to the odor was scored on a 0 - 2 scale with 0 being no response and 2 meaning that the birds woke up.

Open the data file, either “Sleeping\_chicks.csv” or “Sleeping\_adults.csv” and navigate to the appropriate tab (either chicks or adults). You’ll use a graph to illustrate the differences in response between penguins exposed to the control chemical and DMS. As part of your analyses, you will also need to report descriptive statistics **for each treatment group** (DMS and control), including the average, standard deviation, and standard error for the response to treatment. To calculate your descriptive statistics, use Descriptive Statistics from the Data Analysis ToolPak. If you are familiar with another statistical program, such as R software or SPSS, you are welcome to use another program instead of Excel.

If you are feeling rusty with Excel, you might find some Excel tutorials helpful at: (<http://www.hhmi.org/biointeractive/spreadsheet-data-analysis-tutorials>).

To install the Toolpak (Microsoft Excel for Mac 2016), click “Tools”, then “Excel Add-ins”, then “Analysis Toolpak.” To use the Toolpak for summary statistics, select Data -> Data analysis ->Descriptive Statistics then select input range and check summary statistics

1. *Which dataset are you examining (adults or chicks)?*
2. *Create a new table (see below) with your averages, sample size (n), standard deviation (SD), and standard error (SE). Include this table in your write up with a table caption (table captions go above table).*



To draw your figure (chart) using MS Excel, you will create a frequency table of the responses (counts) for each treatment and then graph the distribution. First, you will need to create a table (shown below) with counts of each response category for each treatment.

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Use the =COUNTIF (*range, criteria*) to fill in your table. For example, enter in Response 0 for the control =COUNTIF (A2:A31,“0”) where A is the column of data from the Control treatment we want to count from (range), and “0” is the category we are interested in counting up (criteria).  Thus, the formula will output the number of responses that were zero for the Control treatment. You may realize there are few enough data points to create your table by hand, but if you had thousands of data points, hand calculations would no longer be possible.

Using your counts, draw your figure (chart) using MS Excel. Highlight the Control and DMS columns in your new counts table (including title), then choose “Insert” from the menu at the top.  Select Column chart (the first option under 2D column). Don’t forget to label your axes: “Chart Design” ->“Add Chart Element” ->”Axis Titles and remove the “Chart Title” Excel automatically places over the chart.

1. *Turn in the figure you created here. Include a caption placed below the figure.*
2. *Based on your figure and descriptive statistics, do you think there is a biologically significant difference in the average response to the control and experimental odors (DMS)? Why or Why not?*

Now that you have thought about a biological response, we will examine if there is a statistical one. Traditionally, we would use a t-test to compare averages between two group. To do this, click on “Data” -> “Data Analysis”, -> “t-Test Two-Sample Assuming Equal Variances”. Input your ranges for Variable 1 (control) and Variable 2 (DMS treatment) and leave the alpha value at 0.05. Since we are expecting the DMS treatment to have an effect in only one direction (larger or smaller, but not BOTH), we look at the p-value for a one-tailed t-test. *P*-values can range from 0 to 1. Scientists typically use *p*-values < 0.05 as a cut-off to reject the null hypothesis. Recall that our null hypothesis is that there is no difference between the two groups. You can conclude that there is not sufficient evidence to reject the null hypothesis if your *p*-value > 0.05. However, if your *p*-value < 0.05 then you would reject the null hypothesis in favor of the alternative, which suggests that the frequency distributions between the two groups are statistically different.

If you had three or more groups (instead of two), you would have wanted to conduct an ANOVA (analysis of variance). “Data” -> “Data Analysis” -> “Anova: Single Factor”, then select your data for the input range, and group by columns.

1. *Report your p-value for your t-test output.? When you report a p-value, you should also report your alpha value and sample size (e.g., p=xxx , alpha = 0.5, n = 60)*

Although we just conducted a t-test, there a number of assumptions that must be met in order for this test to be valid. After some more data exploration, the scientists realized that the assumption of normally distribution data (bell-shaped curve), required for a t-test, was not met. They concluded that a nonparametric test was a better choice to investigate the differences between the control and DMS-exposed populations. The Mann-Whitney *U*-test is a nonparametric test that compares frequency distribution of two groups, but instead of using raw data it uses ranks of the data points. The null hypothesis of the Mann-Whitney U-test is that the frequency distribution of ranks is the same between the two groups. Thus, if rank scores are unequally distributed such that one group has consistently higher ranks, then we would conclude that there is a significant difference between the two groups.

Now, go back to the dataset that you were working with when making your figure. You will now assess differences between treatments using the Mann-Whitney *U*-test analysis available through VassarStats: Website for Statistical Computation (<http://vassarstats.net/>).  (Note: you would use the Kruskal Wallis test if you had three or more groups – this is also available through VasserStats). Again, if you prefer a different statistical program, feel free to use it here.

* Click on the hyperlink menu on the left menu bar “Ordinal Data”, then click on the “Mann-Whitney Test” hyperlink.
* A pop-up window will appear to enter in the sample sizes of each treatment before moving on to the statistical test page. Your Control treatment is Sample A and your DMS treatment is Sample B. Enter in the sample sizes you determined above for each in the prompts.
* After you enter your sample size information, you will be directed to the Mann-Whitney webpage.  Please read through the description of the test.
* Scroll down to the “Import Raw Data” window.  From your MS Excel file, copy the Control values and paste them into the Sample A Raw Data Cell,  do the same with the DMS treatment and Sample B Raw Data Cell.
* Click the link to the right of your values, “Import data into data cells”. This will autofill the “Raw Data for…” in the “Data Entry” heading.
* Under the “Data Entry” heading, click the “Calculate from Raw Data” button at the bottom of your auto filled data.
* Your output will include Mean Ranks for Sample A and Sample B, UA, z-ratio, and P(1), and P(2).

1. *Record your P(1) value. Based upon your statistical analysis, can adults or chicks (whichever you examined) detect DMS?  Provide evidence to support your conclusion.*

Now meet with another group who examined the other dataset. Share your results with that group and answer the following question.

1. *Speculate on the differences in responses between the adults and chicks. What might explain/cause the differences observed?*

**ACTIVITY 2**

**Are other seabirds sensitive to DMS?**

Let’s think about sensitivity to DMS more broadly. We’re curious about what other types of birds might use DMS as a foraging cue. Based upon analysis of nuclear DNA, Hackett et al. (2008) have released the most recent phylogenetic tree (Fig. 2), showing the relationships among many types of birds. If you find the Sphenisciformes (penguins) on the phylogenetic tree, you will see that the most closely related group is the Procellariiformes.



Figure 2.  Phylogenetic tree of a selection of birds showing their evolutionary relationships.  Redrawn from Hackett et al. (2008)

Procellariiform (albatrosses, shearwaters and petrels) natural history is varied, but can be quite different from that of King penguins. Whereas King penguins do not make a nest, but hold their lone egg on their feet throughout incubation, many Procellariiforms dig burrows in excess of 1m deep where they lay their egg. Procellariiform chicks, similar to penguins, are periodically fed by parents returning from their foraging trips. Interestingly, many Procellariiform adults over-feed their chicks in the final days of development, such that a chick’s weight grossly exceeds that of their parents. Once this weight is reached in a chick, the parents stop provisioning their offspring and leave the burrow to forage for themselves. The chicks use this extra weight to fuel their final days of development on their own, and then fledge from the burrow without aid from their parents. Chicks, therefore, do not learn how to forage from their parents, but must be able to successfully forage independently from their first day out of the burrow.

Nevitt (2000) demonstrated that Blue petrels are attracted to DMS. Cunningham et al. (2003) were the first to show that Blue petrel chicks are sensitive to DMS. To do this, they used the Porter method (Porter et al. 1999) and tested sleeping chicks. As we’ve discussed before, however, showing that an animal is sensitive to an odor is not the same as showing that the odor is attractive to them. Bonadonna et al. (2006) tested the responses of Blue petrel chicks to DMS using a technique called a Y-maze (Fig. 3). In a Y-maze, odors are placed in the arms of the Y and the chick is placed at the base. Chicks are allowed to investigate the maze, and eventually make a choice one arm. In their study, Bonadonna et al. (2006) tested chicks with either the scent of DMS or control.

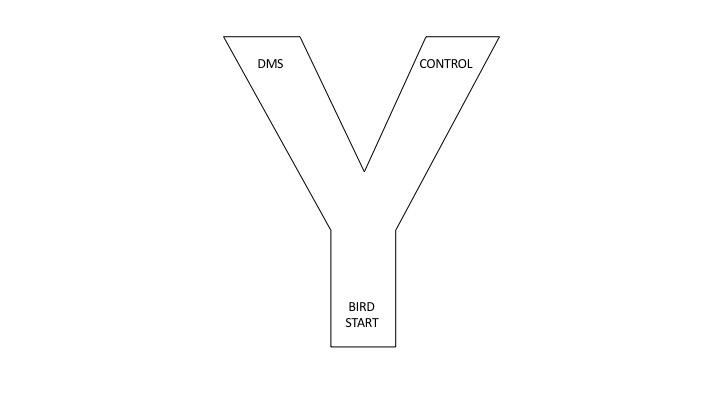


Figure 3.  Example of a Y-maze used in Bonadonna et al. (2006)

The goal of the next part of this assignment is to determine if Blue petrel chicks are attracted to DMS and to compare these results to the penguin analyses you have already completed.

1. *Given the natural history described above, generate a hypothesis regarding the response of Blue petrel chicks to DMS in a Y-maze.*

For their experiment, Bonadonna et al. (2006) put DMS (dissolved in propylene glycol) in one arm of the maze and propylene glycol in the other arm of the maze (the control). Their data compare the number of chicks that chose the arm with DMS with the number of chicks that chose the control. Some chicks did not make a choice and spent the entire testing period in the entranceway. The researchers hypothesized that the chicks were likely stressed by the testing apparatus and chose not to include the ‘no choice’ chicks in their final analyses.

Open the data file: “Blue petrels.csv” and create a figure comparing bird choice and treatment. Remember that you do not need a chart title, but you do need a figure caption and axes labels. You can use =COUNTIF() to determine the number of chicks that moved towards the DMS rather than counting by hand.

1. *Turn in the figure you created here. Include a caption placed below the figure.*

Before you can draw conclusions about your hypothesis, you will need to do a statistical analysis to determine if Blue petrel chicks are attracted to DMS. The binomial test is used when a variable has two possible character states, in this case chicks either move towards the DMS arm or the control arm of the Y maze. The null hypothesis is that chicks are equally likely to move towards either arm, thus we expect roughly 50% of the chicks to move towards the DMS arm and 50% to move towards the control arm of the Y maze.

We can use MS Excel to carry out the binomial test. For this test we need to know the number of chicks that moved towards the DMS arm compared to total number of chicks tested. Use the formula =BINOM.DIST(s, t, 0.5, FALSE), where…

s = number of chicks that chose the DMS arm

t = the total number of chicks tested (number of trials) – remember to exclude the chicks with “no choice”

0.5 = null hypothesis that chicks are equally likely to choose either arm

FALSE = data is not cumulative

The binomial test generates a *p*-value.  If your *p*-value is greater than 0.05, then you would fail to reject the null hypothesis and conclude that the chicks are equally likely to move towards either arm. If the *p*-value is less than or equal to 0.05, then you would conclude that the observed results differ significantly from the null hypothesis, and thus reject the null in favor of the alternative hypothesis.

Feel free to compare your results with another group.

1. *Report your p-value here. Remember when reporting a p-value, you should always report your alpha and sample size. Based upon your analysis, are Blue petrel chicks attracted to DMS?  Why or why not?*

Given these differences and similarities in responses to DMS, answer the following:

1. *Given what you have learned about how and when chicks and adults interact in King penguins and Blue petrels, how does the natural history of each species explain the sensitivities, or lack thereof, that we see with respect to DMS?*
2. *Please complete this question separately from your partner. Describe you and your partner’s contributions to the assignment. Consider if he/she had a cooperative attitude, interest in the assignment, took an active role, and contributed half of the assignment from start to finish. Additionally, please list the percentages that you and your partner contributed and explain why you contributed more or less (e.g., me 75%, my partner 25%. My partner took no role in the second activity, and did not respond when I tried to contact her etc.).*

**LITERATURE CITED**

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1. Adapted from *TIEE*, Volume 13 © 2018 – Kaitlin M. Bonner, Gregory B. Cunningham, and the Ecological Society of America. *Teaching Issues and Experiments in Ecology* (*TIEE*) is a project of the Committee on Diversity and Education of the Ecological Society of America (<http://tiee.esa.org>). [↑](#footnote-ref-1)