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The nose knows: How tri-trophic interactions and natural history shape bird foraging behavior

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Abstract

THE ECOLOGICAL QUESTION

Do two species of ocean-foraging birds detect and respond to a chemical cue which is linked to their food source, and how might differences in their natural history help to explain any variation in sensitivity?

ECOLOGICAL CONTENT

Foraging, trophic cascades, chemical ecology, behavioral ecology, Antarctic food webs

WHAT STUDENTS DO

This dataset is designed for first-year biology students, but can be altered for upper-level students. After receiving sufficient background on the biology of King penguins, students must make hypotheses regarding the penguins' sensitivity to an odor known to be associated with the primary productivity of the ocean. Students are given multiple data sets and need to analyze the data using a variety of statistical tests. Students work in pairs to examine and analyze the data, and then as a group generate conclusions to develop the larger picture. To give students a chance to apply their knowledge beyond penguins, a second dataset with other seabirds is introduced. Here students must contrast the results of seabirds to this odor against the responses of King penguins. For successful completion of the assignment, students must recognize how differences in the natural history between the two groups of birds might impact their sensitivities towards the odor.

SKILLS

Hypothesis creation, statistical analyses, graph creation, graph interpretation, phylogenetic tree interpretation, manipulating datasets and formulas in MSExcel, MSPowerpoint slide creation, synthesizing knowledge, drawing conclusions

STUDENT-ACTIVE APPROACHES

Guided inquiry, cooperative learning, jigsaw, critical thinking

ASSESSABLE OUTCOMES

Hypotheses creation, statistical results using MSExcel, figure creation using MSExcel, slides produced using MSPowerpoint, answers to questions

SOURCE

Bonadonna, F., S. Caro, P. Jouventin, and G.A. Nevitt. 2006. Evidence that blue petrel, *Halobaena caerulea*, fledglings can detect and orient to dimethyl sulfide. Journal of Experimental Biology 209:2165-2169.

Cunningham, G.B., S. Leclaire, C. Toscani, and F. Bonadonna. 2017. Responses of king penguin *Aptenodytes patagonicus* adults and chicks to two food-related odours. Journal of Avian Biology 48:235-242.

Disciplines

Biology

Comments

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TIEE

Teaching Issues and Experiments in Ecology - Volume 13, April 2018

ISSUES : DATA SET

The nose knows: How tri-trophic interactions and natural history shape bird foraging behavior

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King penguin colony (Photo by Greg Cunningham)

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OVERVIEW OF THE ECOLOGICAL BACKGROUND

The ocean is very large and prey are not everywhere, being dispersed in discrete locations. Prey come in productive patches that predators must find in order to exploit these resources. The distribution of these resources are due to abiotic factors that concentrate energy in certain discrete areas. We are only beginning to fully understand how marine predators identify productive waters.

One possible chemical cue that can be used to identify productive waters is the airborne gas, dimethyl sulfide (DMS). This odor's precursor, dimethylsulfoniopropionate (DMSP), is produced by phytoplankton, the basis of the Antarctic food web. Once DMSP enters into the water from the phytoplankton 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 at shelf-breaks and seamounts, thus acting as indicator of these productive waters. Additionally, the production of DMS increases when zooplankton prey upon phytoplankton. Not surprisingly, zooplankton, and the fish that prey upon them, are both attracted to DMSP. There is evidence that some birds and mammals also detect this odor in the air above productive waters.

The goal of this study is to determine if King penguins use DMS, a scent associated with their prey's prey (hence tri-trophic), as a way of finding productive waters where deep diving is likely to lead to encounters with fish. This study also looks at how sensitivity to DMS develops in King penguin chicks and compares the development of DMS sensitivities against a well-studied seabird, the Blue petrel.

This activity allows students to gain experience with hypothesis creation, statistical analysis, and graphical and written representations of data, as well as develop quantitative skills. Ultimately, students will better appreciate how odors are used by avian predators to find food in a patchy environment.

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DATA SETS

- Blue_petrels.xlsx - This excel file has the data set required to complete the analysis of Blue petrel sensitivity towards DMS. Dr. Francesco Bonadonna gave permission for this data to be included on the TIEE website.
- Lake.xlsx - This excel file has the data set required to complete the analysis of King penguin recruitment on the lake. This data was collected by Dr. Greg Cunningham, co-author, and published as part of Cunningham *et al.* (2016). There are two tabs: one for the DMS presentation and one for the control.
- Sleeping.xlsx - This excel file has the data set required to complete the analysis of the response of sleeping King penguins towards DMS or control. This data was collected by Dr. Greg Cunningham, co-author, and published as part of Cunningham *et al.* (2016). There are two tabs: one for the DMS presentation and one for the control.
- Instructor.xlsx - This excel file contains all of the data (each on its own tab) plus the appropriate graphs that the students should create.

STUDENT INSTRUCTIONS

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 located, often, more than 400 km from their breeding beaches (Bost *et al.,* 2002). Penguins spend long periods of time at sea to forage, interspersed with periods of time on the beach to breed or to provision their young. When commuting, King penguins cover long distances and make shallow dives, since their goal is not to forage. When foraging, King penguins switch from their commuting dives to deeper foraging dives. Foraging King penguins eat a variety of types of fish. Prey is taken at depth (sometimes up to 250m!), and King penguins make a series of dives, with the deepest dives being performed during the daylight hours (Bost *et al.,* 2002).

Although there is some variation, King penguin chicks hatch between the months of January to March. Parents then provision the chicks for the following months in advance of a creching period. During the creching period chicks fast over the winter months and form large assemblages on the beach to survive the intense cold. Following this fasting period chicks are then fed in a second bout from September to December, where they complete development in the creche with other mature chicks. Following this second feeding period, chicks join adults on their early foraging bouts out into the ocean, feeding for themselves for the first time. (see Descamps *et al.,* 2002)

1. *Two common foraging strategies include opportunistic foraging and central place foraging. Investigate each and answer the following.*
    - *Would you consider King penguins as opportunistic or central place foragers?*
    - *What is it about their natural history, described above, that leads you to believe this?*

The ocean is very large, and prey are not everywhere. Prey comes in productive patches that predators must find in order to exploit resources. The distribution of these resources are due to abiotic factors (temperature, water currents, oxygen, nutrients) that concentrate energy in certain discrete 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 oceanic 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 where phytoplankton are found (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 or copepods). The following graph (Figure 1, Dacey and Wakeham, 1986) shows the amount of DMS released by phytoplankton in the presence of krill (black circles) or in the absence of krill (white circles).

2. *Summarize the findings from Figure 1 for the 24-hour period that the data is collected. Write your summary in the space to the right of the figure.*

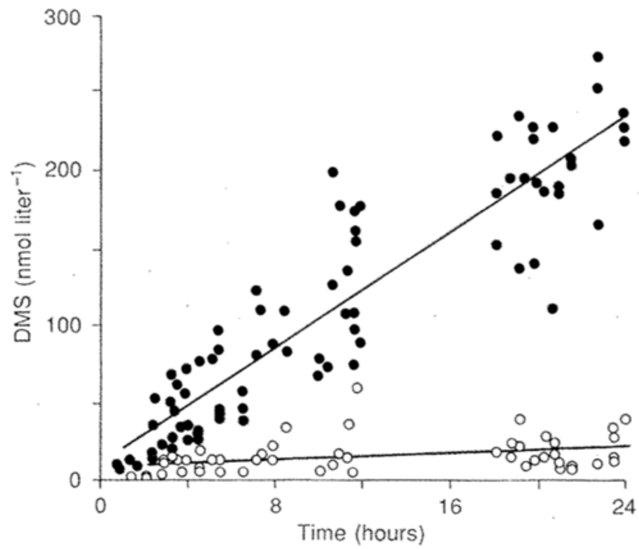

Figure 1: DMS production by phytoplankton while being grazed (black circles) or not (white circles) by zooplankton. Taken from Dacey and Wakeham (1986).

Many marine organisms eat zooplankton, including fish, and seabirds such as penguins.

3. *Given this food web, do you expect fish to be attracted to the scent of DMS(P)? Why or why not? Where would this cue be encountered, in the air or in the water?*

4. *Given this food web, how might King penguins use the scent of DMS? Under what conditions could DMS be encountered? How would the DMS be encountered? What behavior would the penguin initiate in these waters?*

PROCEDURE:

Recall that airborne DMS levels spike when phytoplankton are consumed by zooplankton. Given King penguin's prey of choice and evolutionary position relative to other bird species that have demonstrated DMS sensitivity (we'll discuss this more later), you will examine the role DMS may play in the foraging behavior of the King penguins and their close relatives. In Activity 1 you will investigate whether King penguins can detect DMS, Activity 2 you will assess whether the addition of DMS to the environment changes penguin abundance, suggesting that they are attracted to DMS, and finally in Activity 3 you will investigate DMS detection in a closely related species. For Activities 1 and 2 you will use datasets modified from the publicly available dataset originally from Cunningham *et al.* (2017). For Activity 3, you will use a dataset from Bonadonna *et al.* (2006).

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 birds respond to the odor. The researchers examined responses to DMS in adults and chicks.

5. *Given the natural history of adults and chicks described above, generate a hypothesis regarding response to DMS in adults and chicks.*

Hypothesis, king penguin adults:
Hypothesis, king penguin chicks:

6. *In the hypotheses you wrote, was there a difference in the response of chicks and adults to DMS? Would you expect a difference in response to DMS in adults compared to chicks? Why or why not?*

Your group will evaluate the response to DMS in adults and chicks. In pairs you will investigate either adult or chick responses, and then compare your results with your group. 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.

7. *What odor would the control deployment be in this experiment?*

Open the data file: "Sleeping.xlsx" and navigate to the appropriate tab (either chicks or adults). Next, you'll use a graph to illustrate the differences in response between penguins exposed to the control chemical and DMS.

8. *Describe a type of figure you would create (i.e. bar graph, scatter plot, pie chart) to compare responses. Will you be graphing raw data or the averages of each treatment? Justify your answers to both questions.*

Due the nature of the data collected, it is appropriate to illustrate responses using a histogram. As part of your analyses, you will also need to report descriptive statistics for each treatment group, including the average, standard deviation, and standard error for the response to treatment.

To calculate your descriptive statistics, you can use MSExcel formulas (described below) or simply obtain the information from Descriptive Statistics from the Data Analysis ToolPak.

- Create a new table with your averages, sample size (n), standard deviation (SD), and standard error (SE) in the empty columns to the right of your data. See below.

	Control	DMS
Mean		
Sample size (n)		
Standard deviation (SD)		
Standard error (SE)		

- Use MSExcel formulas to complete your calculations. Use =AVERAGE() and =STDEV() for computing the average and standard deviation, respectively. Either count up your data points or use =COUNT() to calculate your sample size.
- MSExcel does not have a formula for SE, so you will need to create your own. Recall that SE = SD√n, in MSExcel you can use =()^0.5 or =SQRT() to calculate square root, your formula then becomes SE = SD/(n^0.5) or SE=SD/SQRT(n). Enter your formula in the SE cell =SD/(n^0.5).

9. *Average, standard deviation, and standard error are common descriptive statistics use in data analysis. What do each tell us about our dataset?*

To draw your figure (chart) using MSExcel, you will create a frequency table of the responses (counts) for each treatment and then graph the distribution. Alternatively, you can use the Histogram function from the Data Analysis ToolPak.

- Create a new table with Counts of each response category to the treatment

Response	Control	DMS
0		
1		
2		

- 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. Use this formula to complete your table counts.

Using your counts, draw your figure (chart) using MSExcel. Highlight the Control and DMS columns in your new counts table (including title), then choose "Insert" from the menu at the top. Choose clustered Clustered Column. You will be able to modify your figure and add X-axis labels (using your Count column) under the "Chart Design" tab → Select Data, and label your axes using the "Add Chart Element" button.

10. *Looking at your figure and descriptive statistics, is there a significant difference in the average response to the control and experimental odors (DMS)? From your figure can you tell if this is a biologically significant difference?*

Traditionally, we would use a t-test to compare averages between two group. However, since the Porter method collects categorical scores (i.e numbers that are discrete and not continuous) and the resulting data are not normally distributed, we will need to use a nonparametric test 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 this 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 assess differences between treatments using the Mann-Whitney *U*-test analysis available through VassarStats: Website for Statistical Computation (http://vassarstats.net/).

- In the "Site Map" window scroll down to and click on the hyperlink menu "Procedures Applicable to 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 MSExcel 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. Your values should appear in a single column within each 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 autofilled Data.

- Your output will include Mean Ranks for Sample A and Sample B, U_A, z-ratio, and $P_{(1)}$, and $P_{(2)}$.

Record your $P_{(1)}$ value, this is your p-value: _________

- *P*-values can range from 0 to 1. Scientists typically use *p*-values < 0.05 as a cut-off to conclude that the null hypothesis is rejected. 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, which suggests that the frequency distributions between the two groups are statistically different.

Share your results with the other group at your table and answer the following questions.

11. *Based upon your statistical analysis, can adult King penguins detect DMS? Provide evidence to support your conclusion.*
    - ***Put your answers to this question in the appropriate cells of the table associated with question #28***.

12. *Based upon your statistical analysis, can King penguin chicks detect DMS? Provide evidence to support your conclusion.*
    - ***Put your answers to this question in the appropriate cells of the table associated with question #28***.

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

As a group, create a single powerpoint slide. Include:

- Figure (and a way to note significance (if it exists)) showing adult's responses to DMS and Control, and figure caption (include your descriptive statistics here).
- Figure (and a way to note significance (if it exists)) showing chick's responses to DMS and Control, and figure caption (include your descriptive statistics here).
- One concluding statement about your group's results.
- Save the powerpoint slide as you will be adding to it in the following exercises and sharing with your instructor.

14. *Based on your investigations above, have you demonstrated that King penguins use DMS as a foraging chemical cue? Why or why not?*

Activity 2: Are King penguins attracted to DMS?
To further investigate the influence of DMS on King penguin foraging behavior researchers deployed the potential infochemical into a small lake behind the King penguin colony. Both adult and mature chicks have been regularly observed swimming in the lake. Researchers compared changes in bird prevalence on the lake in response to slicks of DMS dissolved in vegetable oil and a control. The slicks were deployed on the lake upwind from the colony, and deployment was separated by at least 24 hours. For each deployment, the number of penguins swimming were reported before the slick and at 30 second intervals for 30 minutes after each slick was deployed. Six DMS deployments, and six control deployments were made. Additionally, researchers measured abiotic factors at the time of deployment, including average wind speed and average air temperature.

15. *What do you think would be an appropriate control deployment?*

Similar to the previous exercise you will be investigating the Lake data set as a group, but pairs will either investigate either the response to the DMS or control deployment. Applying your knowledge from above about adult King penguins' response to DMS, predict the changes in King penguin abundance in the lake over the observation time after deployment of the treatment.

16. *If King penguins use DMS as an infochemical during foraging, then I expect after DMS deployment the King penguin abundance on the lake will: (circle one)*

    increase *decrease* *not change*

17. *If King penguins do not use DMS as an infochemical during foraging, then I expect after DMS deployment the King penguin abundance on the lake will: (circle one)*

    increase *decrease* *not change*

18. *Do you expect the King penguin abundance to change in response to the control treatment? Why or why not?*

Now that you have your predictions, open the data file: "Lake.xlsx" and navigate to the appropriate tab appropriate (either DMS or Control). Graphically illustrate how King penguin abundance changed over time after your treatment. Recall that the data collected was the number of birds in the lake every 30 seconds for a total of 30 minutes.

19. *Describe the type of figure you plan on creating (i.e. bar graph, scatter plot, pie chart). Will you be graphing raw data or average (of the 6 trials per treatment)*

of each treatment? Justify your choice for the type of figure you'll create and the type of data you'll plot.

Create your figure. Based on the information and conclusions above, highlight the appropriate variables and create a figure (chart). Again, use the Insert heading. Make sure that your dependent variable is on the x-axis and your independent variable is on the y-axis. Recall that you can use the "Add Chart Element" button on the top left when you are under the "Chart design" heading to label aspects of your chart.

- Using the same "Add Chart Element" button, add a trendline, and print the R^2, and trendline equation on your graph. Click on "More trendline options" to add all three components. What do these elements tell us about our data?
  - A trendline is an estimate of the best fit line to describe the relationship between the two variables. Trendlines are generated in MSExcel using least-squares regression for linear relationships.

  - R^2 is known as the coefficient of determination and has values that range from 0 to 1. The R^2 describes the degree to which the data is spread around the best fit line. Scientists often use this value to describe the amount of variation explained by the independent variable. For example, "Precipitation explained 22% of the variation in plant growth" (R^2=0.22).

Record your R^2: _______

  - The trendline equation describes the slope and intercept of the trendline, recall y=mx+b. The slope indicates magnitude and direction of the relationship between the two variables. Variables are said to be *directly* correlated if as one variable increases in value the other variable also increases. Variables are *indirectly* (or inversely) correlated when as one variable increases in value, the other decreases.

Record your regression line equation: __________________

Is the relationship between the two variables direct or indirect?

- Notice that there is no *p*-value associated with your R^2 or regression line. We will need to do a linear regression analysis to get a *p*-value. To do this you will need to have the Data Analysis ToolPak Add-in in MSExcel.
  - If it is not already under your Data heading (all the way to the right), then you will need to add it. To add the ToolPak, go to the Tools pull down menu, select Excel Add-ins…, check the Analysis ToolPak, and click Ok.
- Run a Linear Regression Analysis using the Data Analysis ToolPak (under the Data heading). Be sure to highlight the appropriate X and Y columns, and to click headings if your column titles are included in your selection. The null

hypothesis in Linear Regression Analysis is that the correlation coefficient is 0, suggesting that there is no relationship between the two variables.
- Your *p*-value indicates statistical association between the two variables in your analysis. A *p*-value greater than 0.05, the conventional cut-off, indicates that there is insufficient evidence that there is a relationship between the two variables. Conversely, a *p*-value of 0.05 or less indicates a significant association between the two variables.

Once you have completed the figure and regression analysis for your treatment, compare your results with the other group and answer the following questions as a group (but record your own answers below).

20. *As time went by, how did the population of King penguins change in response to the DMS treatment? Describe the strength and statistical significance of the correlation. What is the implication of this finding?*

21. *As time went by, how did the population of King penguins change in response to the control treatment? Describe the strength and statistical significance of the correlation. What is the implication of this finding?*

22. *Aside from the treatment (DMS or Control), what other factors could explain the differences in the number of birds on the lake that you observed in your analysis.*

As a group, add a second powerpoint slide to your presentation. Include:

- The figure showing the response of King penguins to DMS deployment on the lake, and figure caption.
- The figure showing the response of King penguins to Control deployment on the lake, and figure caption.
- One concluding statement about your results as a group.
- Save the powerpoint slide as you will be adding to it in the following exercises and sharing with your instructor.

23. *Now, based on all of your investigations above, are you convinced that King penguins use DMS as a foraging chemical cue? Why or why not? What type of further experiment might be conducted to support your answer?*
    - ***Put your answers to this question in the appropriate cells of the table associated with question #28***.

Activity 3: Are other sea birds 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, showing the relationships among many types of birds.

24. *Find the Sphenisciformes (penguins) on Figure 2, and then using the relationships between penguins and other birds, propose another group of birds you would expect to be responsive to DMS. Why did you choose this group? Check in with your instructor regarding your choice.*

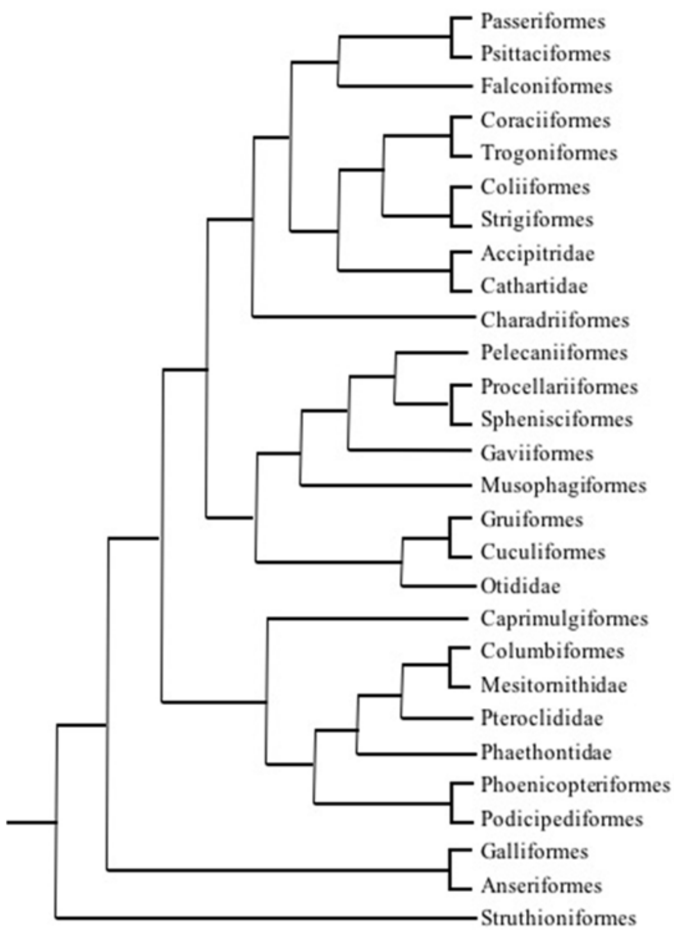

Figure 2. Phylogenetic tree of a selection of birds showing their evolutionary relationships. Redrawn from Hackett et al. (2008)

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 (***There is a row in the table below (Question #28) where you can enter this fact***). 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 (Figure 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 as to which arm they choose to spend time in. In their study, they tested chicks with either the scent of DMS or control.

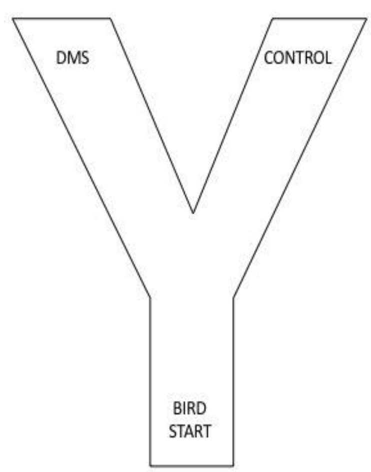

Figure 3. Example of a Y-maze

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.

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

It's now time to look at the data. 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 collected data compares the number of chicks choosing the arm associated with DMS against 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.

26. *The researchers chose not to include the 'no choice' chicks in their final analyses. As a result, your analysis will not include a comparison of the number of birds choosing DMS (or control) against the number of birds not making a choice. Do you think this is appropriate approach to analyzing this data? Why or why not?*

Open the data file: "Blue_petrels.xlsx" and create a figure comparing bird choice and treatment. This is a chance to apply some of the skills you have practiced earlier in the activity.

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 MSExcel to carry out the binomial test. For this test we need to know the number of chicks that moved towards the DMS 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)
0.5 = null hypothesis that chicks are equally likely to choose either arm
FALSE = data is not cumulative
Note: You can use =COUNTIF() to determine the number of chicks that moved towards the DMS rather than counting by hand.

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 observed results are consistent with your expectation. However, if the *p*-value is less than or equal to 0.05, again following convention, then you can conclude that the observed results significantly deviate from predicted, and thus reject the null hypothesis.

Record your p-value: ________

Compare your results with the other groups.

27. *Based upon your analysis, are Blue petrel chicks attracted to DMS? Support your answer using the results of your statistical analysis.*
    - ***Put your answers to this question in the appropriate cells of the table below***.

As a group, add a third powerpoint slide to your presentation. Include:

- The figure showing the number of birds that respond to DMS and Control, including a figure caption and a way to note statistical significance (if it exists)
- One concluding statement about your results as a group.
- You should now have three slides: (1) Sleeping King penguin study results, (2) Lake King penguin study, and (3) Blue petrel Y maze results. Send your completed set of powerpoint slides to your instructor. Be sure that the file is labeled with all group member names. Your powerpoint should also have an opening slide which includes a title, the names of the students in the group, the date and the course number. The title should summarises the activities.

We now want to put our results together to compare the responses of penguins and procellariiforms towards DMS and how their life history might explain these sensitivities.

28. *You've created the following table as we've answered questions throughout this activity.*

Question	Answer	Support
#11 (Sleeping): Are King penguin adults able to detect DMS?		
#12 (Sleeping): Are King penguin chicks able to detect DMS?		
#22 (Lake): Are King penguins attracted to DMS?		
Results of Nevitt (2000): Are adult Blue petrels attracted to DMS?		
#27 (Y-maze): Are Blue petrel chic attracted to DMS?		

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

29. *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 King penguin and Blue petrel adults and chicks explain the sensitivities, or lack thereof, that we see with respect to DMS?*

Science is a never-ending process. Each experiment gives rise to new questions and new ideas, which feed into new hypotheses to be tested.

30. *What would you study next to further understand the role that DMS plays in seabird foraging?*

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NOTES TO FACULTY

General instructions to Faculty

The overall goal of this study is for students to understand how a chemical cue, associated with primary productivity in the ocean, can be used as an infochemical by multiple trophic levels of the oceanic food web. Students start off by answering questions that help them to understand how this infochemical is produced, and we have guiding questions that lead them to understand how the scent might be used by different species.

The activities that students will work through are data heavy and rich in quantitative skill development, and as such students may be initially put off by the amount of statistics that it involves. To that end, we have included specific instructions for the students and instructors on how to maneuver through the datasets with ease. Since the study is split into three activities, instructors should feel free to use as much or as little as is warranted given time constraints and the strengths of their students. The entire set of activities together can be run in a laboratory setting, while if desired the first two activities could be run in a class setting with the third activity assigned as homework. We have two different versions for activity 1. The activity that is included in the main document is at the level of a lower-division course. It involves using MS Excel and an available online statistical package (VassarStats) to analyze the data. The version of activity 1 that is found in the Appendix tackles the non-parametric analysis by hand, and we presume that this version is appropriate for upper-division students. Both versions end at the same point, so whether the faculty member chooses the version here or in the Appendix, they will follow the same overall path.

This set of activities can be used in MS Excel or R. It is currently written for use in MS Excel, therefore students should feel comfortable manipulating data and creating graphs in MS Excel prior to this activity. The suite of MS Excel tutorials through HHMI is a good place to send students that need a refresher (http://www.hhmi.org/biointeractive/spreadsheet-data-analysis-tutorials).

Another potential stumbling point is the interpretations from the phylogenetic tree because students may not be familiar with phylogenies or they may come in with variety of misconceptions about evolution. A brief primer on how to interpret phylogenies (i.e. defining nodes and branches, identifying common ancestors, and noting that species that are more closely related tend to share more characteristics) can alleviate any issues by ensuring all students have the necessary background to be successful.

The bulk of this activity focuses on how King penguins locate their distant foraging grounds. King penguins forage in the Antarctic Polar Frontal Zone, which is located 100s of km from their breeding islands. We do not know how King penguins locate this area, or how they recognize particularly productive waters once encountered. Dimethyl sulphide (DMS) might be a cue that they can use to identify these waters.

The first step in the study has the students demonstrating that King penguins can detect DMS. They do this by analyzing data from a study done at Ratmanoff Beach on Kerguelen Island. Here a colony of more than 100,000 birds live and breed. The technique employed was a modified Porter test (Porter et al. 1999). In the Porter method, a chick is lulled into a sleep-like state using the warmth from an incandescent light bulb. Scents are then puffed onto their bills and their response is scored. In the study the students are examining, birds were found naturally sleeping on the beach and odors were presented beneath the bird's bills while asleep. King penguins responded significantly more to the DMS presentation than to the control presentation, suggesting that they could detect the scent.

Students also look at the response of King penguin chicks to DMS. Interestingly, the chicks do not respond. This may be because the chicks have never been to sea and therefore have never encountered the scent. When the chicks do eventually head out on their maiden foraging trips, they likely accompany experienced adults and chicks likely learn the significance of DMS through their experiences at sea.

In the second activity, students examine data in which researchers deployed DMS or a control slick onto a lake that was located behind the breeding colony and counted the number of birds that recruited into the lake. This experiment mirrored research conducted by Wright et al. (2011) that deployed DMS on the ocean as a slick and counted the number of African penguins (*Spheniscus demersus*) that were recruited. In the dataset that the students will be working with, King penguins recruited more to the lake in the presence of DMS than in the presence of control, suggesting that DMS is attractive.

Depending on the available time and the skillset of the students, you may only get the King penguin work completed. If time permits, or perhaps as follow up homework, the discussion can be extended to discussing how DMS sensitivities develop in penguin's closest living relatives, the Procellariiformes (albatrosses, petrels and shearwaters).

The Procellariiformes are well studied with respect to avian olfaction and it is well established that they use DMS as an aid in foraging. Their natural history, however is quite different from penguins. In many procellariiforms, such as petrels, the parents over-feed the chicks and then abandon them to complete development on their own. This presents a unique difficulty for the chicks since they develop underground in a burrow, theoretically cut off from external environmental cues that might help them to successfully forage as adults. The emerging picture with petrels is that they likely imprint on odors learned while in the burrow, so that when they leave the burrow they already have learned some salient cues.

In the third activity, students analyze results from a Y-maze study conducted with Blue petrels. The researchers put either DMS or a control in the different arms of the maze and then looked at how the Blue petrel chicks responded. The chicks chose the arm of the maze associated with DMS more than the control arm.

In our module, students develop the skills to needed to analyze data using three different statistical tests (Mann-Whitney U test (essentially a non-parametric t-test), simple linear regression, binomial test), graph their results, and interpret and synthesize information. They also draw conclusions based upon natural history and phylogenetic analysis.

Answers to questions in the pre-assignment.

1. ***Two common foraging strategies include opportunistic foraging and central place foraging. Investigate each and answer the following. Would you consider King penguins as opportunistic or central place foragers? What is it about their natural history, described above, that leads you to believe this?*** Opportunistic foraging exists when predators travel through their habitat, with little preference for one prey over the other under many circumstances. These predators will eat as they go, instead of skipping over less productive regions in favor of more productive areas. A central place forager travels back and forth from a home base (in this case, their breeding island) to a distant productive foraging patch. Therefore, King penguins, based upon the description contained in the Background section, are indeed central place foragers. A key point to highlight here is that foraging penguins must be able to differentiate these productive patches from unproductive waters. They should commute through the unproductive waters, and only dive deep in productive waters.

2. ***Summarize the findings from Figure 1 for the 24-hour period that the data is collected. Write your summary in the space to the right of the***

figure. The graph clearly shows that in the presence of zooplankton, the amount of DMS released by the phytoplankton increases. It is important for the students to understand that this is because as the zooplankton are foraging upon the phytoplankton, they are breaking open the cells, thus releasing more DMSP, which is converted to DMS.

3. ***Given this food web, do you expect fish to be attracted to the scent of DMS(P)? Why or why not? Where would this cue be encountered, in the air or in the water?*** We expect fish to be attracted to DMS(P). Since the presence of the fish's prey, zooplankton, cause increases in the DMS(P) levels. Obviously, this cue is detected in the water.

4. ***Given this food web, how might King penguins use the scent of DMS? Under what conditions could DMS be encountered? How would the DMS be encountered? What behavior would the penguin initiate in these waters?*** King penguins can use peaks of airborne DMS, encountered at the surface of the water, as an indicator of waters where there is lots of zooplankton, and, presumably, fish. Since DMS levels spike in the presence of zooplankton, and some fish are attracted to DMS(P) (DeBose et al. 2008), a penguin would switch from its commuting dives (shallow) to its foraging dives (deep).

<u>Answers to questions in the assignment.</u>

5. ***Given the natural history of adults and chicks described above, generate a hypothesis regarding response to DMS in adults and chicks.*** For the adult King penguins, we expect students to be able to recognize that these birds should be sensitive to DMS. Thus, an appropriate hypothesis might be that: ***King penguin adults are able to detect DMS, a food related odor.*** The student response to the chick hypothesis is more difficult to predict. If the student was reading with great detail, they might notice that the chicks make their early foraging trips with the adults, suggesting that the chicks might not know where to go or how to get there or what cues to use to find productive grounds. Thus, an appropriate hypothesis might be: ***King penguin chicks cannot detect DMS.*** However, if a student thinks that the sensitivity to DMS might be innate, they may write a hypothesis that looks similar to the adult one, above.

6. ***In the hypotheses you wrote, was there a difference in the response of chicks and adults to DMS? Would you expect a difference in response to DMS in adults compared to chicks? Why or why not?*** See the response in #5.

7. ***What odor would the control deployment be in this experiment?*** The control for this experiment is the presentation of propylene glycol.

8. ***Describe the type of figure you plan on creating (i.e. bar graph, scatter plot, pie chart) and justify your choice. Will you be graphing raw data or the averages of each treatment? Explain your answers to both questions.*** For this data set, a bar graph or box plot could be created. A bar graph of averages allows for the average response to the odors to be plotted and visually compared. However, a bar graph is not actually the appropriate choice given the actual data. Since the data is not normal and is analysed using non-parametric statistics, it would be more appropriate to use a box plot. A perusal of the literature, however, would show that researchers who use the Porter method to test the responses of chicks towards odors, commonly plot their data as a bar graph with the SE. This is likely because the bar graph is a more intuitive way of understanding the data (and better understood by readers) than a box plot due to the zeros in the data. Given this, we've chosen to represent the data using a frequency distribution graph.

9. ***Average, standard deviation, and standard error are common descriptive statistics use in data analysis. What do each tell us about our dataset?*** The average, or arithmetic mean, is the most common metric used to quantify the middle of the frequency distribution created by your data points, also known as the central tendency. Standard deviation provides a measure of the spread of the data points around the mean. Standard error is considered a measure of accuracy of the sample mean.

10. ***Looking at your figure and descriptive statistics, is there a significant difference in the average response to the control and experimental odors (DMS)? From your figure can you tell if this is a biologically significant difference?*** Students will point out that there is a difference in means in the adult King penguin comparisons, and none in the chicks. They will commonly state that it is a significant difference in the adults, but not for the chicks. However, conclusions about differences between groups must be supported by statistics.

11. ***Based upon your statistical analysis, can adult King penguins detect DMS? Provide evidence to support your conclusion.*** Adult King penguin can detect DMS. This is demonstrated by a p-value less than 0.05 in the Mann-Whitney U test.

12. ***Based upon your statistical analysis, can King penguin chicks detect DMS? Provide evidence to support your conclusion.*** King penguin chicks

cannot detect DMS. This is demonstrated by a p-value greater than 0.05 in the Mann-Whitney U test.

13. ***Speculate on the differences in responses between the adults and chicks. What might explain/cause the differences observed?*** Adult King penguins have foraged in the past and have likely learned the association between spikes in DMS and the productivity of the ocean. Chicks, having never been to sea and likely never having smelled DMS before, do not respond to it since they likely do not know the significance of DMS. This is an opportunity to point out a difference in a behavioral versus a physiological assay. A physiological assay, such as an EOG (electro-olfactogram, where electrical activity is recorded from the olfactory nerve), might show that a bird can indeed detect the odor physiologically. But the behavioral assay shows that the bird does not respond to it.

14. ***Based on your investigations above, have you demonstrated that King penguins use DMS as a foraging chemical cue? Why or why not?*** We have not demonstrated that King penguins use DMS as a foraging cue. All we have done is demonstrate that they can detect this odor. Other studies with seabirds and DMS have deployed the odor as slicks over the ocean and showed that birds recruit to the slick. These other studies suggest that some birds use DMS as a foraging cue. The point of the next exercise is to try and figure out if King penguins are attracted to DMS, like these at-sea studies in other birds.

15. ***What do you think would be an appropriate control deployment?*** Since the DMS was dissolved in vegetable oil for its deployment, the control deployment would be the release of a slick of just vegetable oil.

16. ***If King penguins use DMS as an infochemical during foraging, then I expect after DMS deployment the King penguin abundance on the lake will:*** If King penguins use DMS as a foraging cue, King penguin numbers should increase on the lake. A student might point out, reasonably, that since penguins aren't foraging in the lake that the penguins might not interpret the presence of DMS as a foraging cue and therefore the numbers might not change. The answer of 'decrease' would not be acceptable since there is nothing in the description of the lake and how they use it that would explain this response.

17. ***If King penguins do not use DMS as an infochemical during foraging, then I expect after DMS deployment the King penguin abundance on the lake will:*** If King penguins do not use DMS as a foraging cue, then you would expect no change in the numbers across time.

18. ***Do you expect the King penguin abundance to change in response to the control treatment?*** King penguin numbers should not change in response to the vegetable oil slick. The scent of vegetable oil is neither attractive nor repulsive to King penguins, that we know of.

19. ***Describe the type of figure you plan on creating (i.e. bar graph, scatter plot, pie chart). Will you be graphing raw data or average (of the 6 trials per treatment) of each treatment? Justify your choice for the type of figure you'll create and the type of data you'll plot.*** In this case we are going to make a scatter plot with a trendline, plotting the number of birds on the Y-axis against the time since deployment on the X-axis. This would allow us to see the changes in the lake's population over time. With respect to a student graphing either the raw data, or the average data, there is not specific right answer. If a student were to compare the plot of the average data against the plot of the raw data they might reason that the trends are easier to see in the average data. In the original manuscript, the average data was plotted for this reason. This is, however, a great time to point out to students that how researchers display data (averages) is not always the same as how the data is analysed (raw). Researchers display their data in the way that tells the most understandable story to the reader.

20. ***As time went by, how did the population of King penguins change in response to the DMS treatment? Describe the strength and statistical significance of the correlation. What is the implication of this finding?*** In response to the DMS deployment, the numbers of King penguins significantly increased over time (R^2=0.10, $p<0.05$). The implication of this is that DMS is an attractive cue for the penguins, even in situations where they are not foraging.

21. ***As time went by, how did the population of King penguins change in response to the control treatment? Describe the strength and statistical significance of the correlation. What is the implication of this finding?*** In response to the control deployment, the numbers of King penguins actually significantly decreased over time (R^2=0.07, $p<0.05$). This is an unexpected result since one would have expected the numbers of penguins to not change over time. The original manuscript did not attempt to explain this, but rather just focused on the increase in the numbers of penguins in response to the DMS. This is an opportunity to discuss how researchers, in presenting data, focus on the best story and sometimes do not introduce discussions to which there is no clear answer.

22. ***Aside from the treatment (DMS or Control), what other factors could explain the differences in the number of birds on the lake that you observed in your analysis.*** Many answers are possible here. In the original study, the researchers also collected environmental data (temperature, humidity, wind speed, and wind direction). The experiment was always performed at the same time of day. The hope here is that the students can reason that without taking these sorts of factors into consideration, that something else other than the deployment might be causing the penguins to be in the lake.

23. ***Now, based on all of your investigations above, are you convinced that King penguins use DMS as a foraging chemical cue? Why or why not? What type of further experiment might be conducted to support your answer?*** This study has demonstrated that penguins can detect DMS (sleeping study) and that they are attracted to it in a non-foraging context (lake study). Overall we can say that there is a good indication that this species, similar to other seabirds, may use DMS as a way of identifying productive areas of the ocean, but that further studies, particularly in the ocean, must be conducted. In the ocean, we would expect studies to be done where DMS and control slicks are deployed and the number of penguins recruiting to each are counted. Measurements of DMS levels, particularly in areas where King penguins forage, would be beneficial.

24. ***Find the Sphenisciformes (penguins) on Figure 2, and then using the relationships between penguins and other birds, propose another group of birds you would expect to be responsive to DMS. Why did you choose this group? Check in with your instructor regarding your choice.*** Hopefully, students chose the Procellariiformes since these birds are the closest relatives of the penguins. The Procellariformes, or the tube-nosed seabirds, are represented by albatrosses, shearwaters and petrels. In fact, the concept that DMS is used as a foraging cue by seabirds was first established in Procellariiformes! The close evolutionary relationship between penguins and procellariiforms led early penguin researchers to believe that penguins would use DMS as well. This notion led to the research that we're talking about here. It is well established that some procellariiforms, such as Blue petrels (*Halobaena caerulea*) are attracted to DMS at sea and likely use it as a foraging cue (Nevitt, 2000).

25. ***Given the natural history described above, generate a hypothesis regarding the response of Blue petrel chicks to DMS in a Y-maze.*** Multiple answers are possible here. A student who meticulously read the Procellariiform natural history might realize that Blue petrel chicks do not have the opportunity to learn from their parents and therefore should learn in the

nest. Thus, they would, appropriately, write the hypothesis to be ***Blue petrel chicks are attracted to the scent of DMS in a Y-maze.***

- The lack of response to DMS by the King penguin chicks might lead a student to
- believe that all chicks are insensitive to DMS. If this is the case, students might write a hypothesis such as: ***Blue petrels are not attracted to the scent of DMS in a Y-maze.***

26. ***The researchers chose not to include the 'no choice' chicks in their final analyses. As a result, your analysis will not include a comparison of the number of birds choosing DMS (or control) against the number of birds not making a choice. Do you think this is appropriate approach to analyzing this data? Why or why not?*** We are not interested in comparing the number of birds that chose an arm (either DMS or Control) against the non-choice. This comparison doesn't inform us about the biology of Blue petrels since the he lack of choice does not inform the researchers as to why the bird did not make a choice (other than possibly telling us that a few birds don't like being put in Y-mazes).

27. ***Based upon your analysis, are Blue petrel chicks attracted to DMS? Support your answer using the results of your statistical analysis.*** Blue petrel chicks chose the DMS-scented arm significantly more than the arm associated with control. The p-value for the Binomial test being less than 0.05 indicates this.

28. The table, filled out:

Question	Answer	Support
#11 (Sleeping): Are King penguin adults able to detect DMS?	Yes	Adults responded to DMS deployments more than to control deployments while asleep

#12 (Sleeping): Are King penguin chicks able to detect DMS?	No	There were no significant differences in the chicks' responses to DMS and the control
#22 (Lake): Are King penguins attracted to DMS?	Yes	King penguins recruited to the lake significantly more when DMS was deployed compared to control
Results of Nevitt (2000): Are adult Blue petrels attracted to DMS?	Yes	Blue petrels recruit to DMS slicks at sea
#27 (Y-maze): Are Blue petrel chicks attracted to DMS?	Yes	Blue petrel chicks choose the arm of the Y-maze associated with DMS significantly more than the control arm

29. ***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 King penguin and Blue petrel adults and chicks explain the sensitivities, or lack thereof, that we see with respect to DMS?*** This is the opportunity to put it all together. King penguin chicks develop on the beach and have frequent opportunities to interact with their parents, and also to watch other adults. They likely head out on their maiden foraging trips with more experienced birds. The adults are responsive to DMS, and may use it as a foraging cue. The chicks, however, do not respond to DMS on the beach prior to independently foraging. It is likely that the chicks learn the significance of DMS with respect to productive waters in their early trips with adults. Once they have learned the association between DMS and productive waters, they can successfully forage on their own.

Blue petrel chicks do not have the opportunity to learn from their parents, since the parents abandon the chicks and they complete development on their own. Not surprisingly, Blue petrel chicks are attracted to DMS, suggesting that they might be learning about this cue while still in the burrow. They would then fledge from the nest with an olfactory search image in place. Interestingly, there is some evidence that chicks might even learn about odors while in the egg (Cunningham and Nevitt, 2011)!

30. ***What would you study next to further understand the role that DMS plays in seabird foraging?*** Many answers are possible here. The student answer should be a logical extension of the current work. We outlined a few possible future studies in the answer to question #23.

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ASSESSMENT

Students can be assessed through the questions in the student worksheet. We've also provided a rubric for the powerpoints that the students are assembling throughout the activity (below), a template for the presentations (additional files), and an example presentation (additional files). Instructors can assess powerpoint to determine if students correctly generated their graphs, figure captions, and concluding statements.

		Exemplary	Meets expectations	Missing elements	Elements are absent
Title slide	Succinct and informative title. Includes group member names, date, and course number				
Activity 1	Graph constructed correctly (general appearance)				
	Axes are appropriate and labeled accurately				
	Figure captions are informative				
	Descriptive statistics are accurate and included in figure caption				
	Concluding statement is accurate, but not speculative				
Activity 2	Graph constructed correctly (general appearance)				
	Axes are appropriate and labeled accurately				
	Regression line is oriented correctly				

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	Figure captions are informative				
	Graph includes regression line equation and R^2, p-value is reported in figure caption				
	Concluding statement is accurate, but not speculative				
Activity 3	Graph constructed correctly (general appearance)				
	Axes are appropriate and labeled accurately				
	Regression line is oriented correctly.				
	Figure captions are informative				
	Descriptive statistics are accurate and included in figure caption				
	Concluding statement is accurate, but not speculative				

APPENDIX: Mann-Whitney U test in MS Excel for the "Sleeping.xlsx" data exploration

The following data analysis allows students to perform the Mann-Whitney U test by hand, rather than by hand as it is set up in the main document.

PROCEDURE:
Now, go back to the dataset that you were working with when making your figure and copy the data only into a new sheet. You will be using the Mann-Whitney *U*-test to assess differences between treatments. Follow the detailed instructions below.

Score	Treatment
0	Control
1	Control
2	Control
1	Control
0	Control
0	Control
1	Control
2	Control
2	DMS
1	DMS
1	DMS
0	DMS
0	DMS
2	DMS
1	DMS
1	DMS

Data set

Score	Treatment
0	Control
0	Control
0	Control
0	DMS
0	DMS
1	Control
1	Control
1	Control
1	DMS
1	DMS
1	DMS
1	DMS
2	Control
2	DMS
2	DMS
2	DMS

Sort data by score

Score	Treatment	Rank
0	Control	1
0	Control	2
0	Control	3
0	DMS	4
0	DMS	5
1	Control	6
1	Control	7
1	Control	8
1	DMS	9
1	DMS	10
1	DMS	11
1	DMS	12
2	Control	13
2	DMS	14
2	DMS	15
2	DMS	16

Assign a rank to each score

Using the diagram above and instructions below, prepare your dataset and assign initial ranks to the scores.

- Sort your data by the score. Highlight all data columns and under the Data header choose Sort, then Sort by Score column (by value, from A → Z). Make sure that the “My columns has headers” button in the top right is checked. This will sort the dataset by Score from lowest to highest value.
- Assign a numerical rank to each score starting with 1 (lowest rank). See above Figure for clarification.
- You’ll notice that you have multiple measurements with the same Score. However, since they have the same Score they should have the same rank. Therefore, we need to calculate the mid-rank scores to adjust for tied measurements.

Score	Treatment	Rank
0	Control	1
0	Control	2
0	Control	3
0	DMS	4
0	DMS	5
1	Control	6
1	Control	7
1	Control	8
1	DMS	9
1	DMS	10
1	DMS	11
1	DMS	12
2	Control	13
2	DMS	14
2	DMS	15
2	DMS	16

To determine the mid-rank, calculate for each score category...

Count (n) = 5
Sum = (1+2+3+4+5) = 15
New rank = 15/5 = 3

Count (n) = 7
Sum = (6+7+8+9+10+11+12) = 63
New rank = 63/7 = 9

Count (n) = 4
Sum = (13+14+15+16) = 5
New rank = 58/4 = 13.5

Score	Treatment	New Rank
0	Control	3
0	Control	3
0	Control	3
0	DMS	3
0	DMS	3
1	Control	9
1	Control	9
1	Control	9
1	DMS	9
1	DMS	9
1	DMS	9
1	DMS	9
2	Control	13.5
2	DMS	13.5
2	DMS	13.5
2	DMS	13.5

Using the diagram above and the instructions below, assign mid-ranks for all tied measurements.

- Calculate mid-rank scores for each categorical score (0, 1, 2). The mid-rank score is based on the total number of data points with that rank divided by the sum of the ranks. Follow the diagram above and assign a new rank to each score.
- Enter the New Ranks for each score. It is easiest to do this by adding a new column to the right called “New Rank”. After you have completed the “New Rank” column, delete the “Rank” column because it is no longer of use.
- Now that you have completed the ranking process we will need to calculate rank-sums for each treatment and then calculate *U*.

Score	Treatment	New Rank
0	Control	3
0	Control	3
0	Control	3
1	Control	9
1	Control	9
1	Control	9
2	Control	13.5
0	DMS	3
0	DMS	3
1	DMS	9
1	DMS	9
1	DMS	9
1	DMS	9
2	DMS	13.5
2	DMS	13.5
2	DMS	13.5

Now calculate ....

Control treatment:
R_1 = Sum (Control ranks) = 49.5
n_1 = Sample size = 7

DMS treatment:
R_2 = Sum (DMS ranks) = 82.5
n_2 = Sample size = 9

Using the diagram above and the instructions below, calculate the rank-sum (*R*) and sample size (*n*) for each treatment using the New Rank scores.

- First, resort your dataset by Treatment. Highlight all data columns and under the Data header choose Sort, then Sort by Treatment column (by value, from A → Z). Again, make sure that the "My columns has headers" button in the top right is checked. This will sort the dataset by Treatment with Control first then DMS data measurements.
- Calculate the rank-sum for each treatment. Treatment one is the Control, therefore the rank-sum for the Control treatment is R_1 and the rank-sum for the DMS treatment is R_2. To calculate the rank-sum, simply sum the ranks for each treatment. Use the formula =SUM() in MSExcel. In the example above the Control rank-sum (R_1) = 3+3+3+9+9+9+13.5 = 49.5.

Record your values:

Control rank-sum (R_1) = ________

DMS rank-sum (R_2) = ________

Calculate the *U*-statistic.

- To calculate *U* for each you will need to know the sample size (*n*) of each treatment. Simply count up the total measurements in each treatment to get n for each. Again, n_1 is the sample size of the Control treatment, whereas n_2 is the sample size for the DMS treatment. Use the formula =COUNT() in MSExcel. In the example above, the Control sample size (n_1) =7.

Record your values:

Control sample size (n_1) = ________

DMS sample size (n_2) = ________

- Calculate U_1 and U_2, each represents an estimate as to the number of times a data point in one group is smaller than a data point in the other group. Use the equations below.

  $U_1 = n_1n_2 + n_1(n_1+1)/2 - R_1$

  $U_2 = n_1n_2 + n_2(n_2+1)/2 - R_2$ or $U_2 = n_1n_2 - U_1$

Continuing with our example above, $R_1 = 49.5$, $n_1 = 7$, $n_2 = 9$, to calculate U_1 U_1=(7*9)+(7(7+1)/2)+49.5 = 41.5. U_2 would then be (7*9)-41.5 = 21.5.

Record your values:

Control U_1 = ________

DMS U_2 = ________

- Choose the larger of the above calculated U_1 and U_2 as your test statistic, U. For our example above $U = U_1 = 41.5$.

Record your U-statistic: ________

- Since our sample size is fairly large, we cannot use the Mann-Whitney U-distribution table to determine critical values for significance. Therefore, we will need to calculate a Z-statistic. Use the equation below.

$Z = (2U - n_1n_2) / (\sqrt{} \, (n_1n_2\,(n_1 + n_2 + 1) / 3)$

Again with our example, Z= ((2*41.5)-(7*9))/ √ ((7*9) (7+9+1) / 3) = 1.06. In MSExcel you can use SQRT() or ^0.5 to square root a value in your formula.

Record your Z-statistic: ________

- Once you have your Z-statistic you can use the standard normal (Z) distribution table to determine the probability of sampling a value greater than or equal to your Z-statistic. You can also calculate the p-value in MSExcel using the formula =1-NORM.DIST(x, 0, 1, TRUE) where x is the Z-statistic you calculated above.

Record your p-value: _________

- P-values can range from 0 to 1. Scientists typically use p-values < 0.05 as a cut-off to conclude that the null hypothesis is rejected. 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, which suggests that the frequency distributions between the two groups are statistically different.

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