**FACULTY NOTES**

The nose knows: How tri-trophic interactions and natural history shape bird foraging behavior. An introduction to statistical analysis in animal behavior research.

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**Abstract**

Students investigate the role of olfaction and infochemicals on bird foraging behavior through two different quantitative activities where they generate hypotheses, create figures, conduct data analyses, and draw conclusions.

**Description**

These activities were designed to develop students’ quantitative skills, and are applicable in both introductory biology and upper division ecology and animal behavior courses.

Activity 1 focuses on how King penguins locate their foraging grounds, hundreds of kilometers from their breeding areas using dimethyl sulphide (DMS) as a chemical cue. For this activity, students generate hypotheses from background information and analyze data collected using a scaled categorical score of how King penguin adults and chicks respond to the presentation of DMS odor. Students conduct descriptive statistics, a t-test using MS Excel Analysis Toolpak, and a Mann-Whitney U test using VassarStats: Website for Statistical Computation. Students also create a bar chart to visually present results and practice properly labeling figures, in addition to interpreting statistical analyses.

Activity 2 examines how DMS sensitives developed in the penguin’s closest living relative, the Procellariiformes, who have a different natural history than King penguins. For this activity, students generate hypotheses from background information and analyze data from a Y-maze experiment conducted on Blue petrel chicks with either DMS or a control odor in each arm of the maze. Students create a figure, conduct a binomial statistical test using MS Excel, and interpret resulting p-values. Finally, students are asked to draw conclusions about how the differences in the natural history between the two groups of birds might impact their sensitives toward DMS in chicks versus adults.

**Learning 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

**Change Notes**

This module was adapted from its original form as a publication in Teaching Issue and Experiments in Ecology -Volume 13, April 2018 by Kaitlin M. Bonner and Gregory B. Cunningham. Initially designed as a 3-hour lab in Animal Behavior for an Introductory Biology course, I have adapted it and implemented it during an hour-long class period for an upper-division Animal Behavior course (40 students). Initially 3 activities, I have reduced it to two, reduced the number of questions students must complete for assessment, eliminated the creation of a PowerPoint, and revised the grading rubric assessment. Because the number of activities were reduced, the original Activity 2 was removed along the regression analysis component of the activity, however I added in a t-test, so students would gain experience conducting both parametric and non-parametric tests. These activities are designed and written to be conducted in MS Excel. However, I did allow students to use another statistical program of their choice to conduct the analyses if they preferred (hence the data files are in .csv format). I also created a three-question reading quiz that I gave at the beginning of class using an iClicker, to ensure students came to the class adequately prepared (the nose knows reading quiz.pptx). I also found it helpful to show a short video taken by Greg Cunningham during data collection, so students could visualize how the data were collected (The nose knows video.mpg). I have also included results of an evaluation of the using iClickers, so you can get a sense of how far the students got during class time, versus how much time they spent on the activity at home, as well as their perceptions of how helpful the activity was in general for developing their quantitative skills (The nose knows evaluation.pdf).

**Data Files**

Sleeping\_adults.csv: This csv file has the data required to complete Activity 1; the response of sleeping King penguin adults towards DMS or control. These data were collected by Dr. Greg Cunningham, published in Cunningham et al. (2017), and used in this activity with permission from Dr. Cunningham.

Sleeping\_chicks.csv: This csv file has the data required to complete Activity 1; the response of sleeping King penguin chicks towards DMS or control. These data were collected by Dr. Greg Cunningham, published in Cunningham et al. (2017), and used in this activity with permission from Dr. Cunningham.

Blue\_petrels.csv: This csv file has the data required to complete the analysis of Blue petrel sensitivity toward DMS. These data were collected by Dr. Francesco Bonadonna, published in Bonadonna et al. (2006), and used in this activity with permission from Dr. Bonadonna.

Sleeping\_adults instructor.xlsx: Completed analyses for Activity 1, including separate worksheets for descriptive statistics, t-test output, and figures.

Sleeping\_chicks instructor.xlsx: Completed analyses for Activity 1, including separate worksheets for descriptive statistics, t-test output, and figures

Blue\_petrels instructor.xlsx: Count of the number of DMS and Control for Activity 2, including figure

**Data Sources**

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.

**ANSWERS TO QUESTIONS IN ASSIGNMENT**

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?*

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.

1. *Which dataset are you examining (adults or chicks)?*

Students should state either adults or chicks. This allows you to know what the correct answers should be for future questions.

1. *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).*

Adults

Descriptive statistics for King penguins (*Aptentodytes patagonicus*) adults of each response type ranging from 0 (no response) to 2 (birds woke up) after exposure to dimethyl sulfide (DMS) and a control.

|  |  |  |
| --- | --- | --- |
|   | Control | DMS |
| Mean | 0.4 | 0.8 |
| Sample size (n) | 35 | 35 |
| Standard deviation (SD) | 0.50 | 0.63 |
| Standard error SE) | 0.08 | 0.11 |

Chicks

Mean, sample size, standard deviation, and standard error for King penguins (*Aptentodytes patagonicus*) chicks of each response type ranging from 0 (no response) to 2 (birds woke up) after exposure to dimethyl sulfide (DMS) and a control.

|  |  |  |
| --- | --- | --- |
|   | Control | DMS |
| Mean | 0.6 | 0.6 |
| Sample size (n) | 30 | 30 |
| Standard deviation (SD) | 0.67 | 0.62 |
| Standard error SE) | 0.12 | 0.11 |

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

Adults



Number of King penguins (*Aptentodytes patagonicus*) adults of each response type ranging from 0 (no response) to 2 (birds woke up) after exposure to dimethyl sulfide (DMS).

Chicks



Number of King penguins (*Aptentodytes patagonicus*) chicks of each response type ranging from 0 (no response) to 2 (birds woke up) after exposure to dimethyl sulfide (DMS).

1. *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?*

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.

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 = 0.05, n = 60)*

Adults

p=0.002, alpha=0.05, n=70

Chicks

p=0.5, alpha=0.05, n=60

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.*

Adults

Yes, adults can detect DMS based on the significant p-value of 0.009, alpha=0.05, n=70

There is a 0.9% chance that the data obtained could occur by chance. Students should conclude that the adults do detect DMS because of the very small p-value.

Chicks

No, chicks cannot detect DMS based on a nonsignificant p-value of 0.47, alpha=0.05, n=60

There is a 47% chance that the data obtained could occur by chance. Students should conclude that the chicks do not detect DMS because of the large p-value.

1. *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.

1. *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.***

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



The number of Blue Petrel (*Halobaena caerulea*) chicks that made the choice of control or dimethyl sulfide (DMS) in a Y-maze.

Students may also plot “no choice” birds.

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?*

p=0.005, alpha=0.05, n=20. Blue petrel chicks are attracted to DMS because the p-value is less than the alpha value, so chicks chose the DMS arm significantly more than they would be chance alone. Students should not include “no choice” chicks in analysis since it is stated in the instructions that “researchers chose not to include the ‘no choice’ chicks in their final analyses”.

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?*

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)!

1. *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.).*

Student response to this question is your chance to assess if partners contributed equally, and adjust grades, or at least speak to students, if there was an issue with unequal contributions.

**ASSESSMENT**

Students can be assessed through the questions above using the grading rubric provided here.

**The Nose Knows : Grading Rubric \_\_\_/40 Total points**

**Questions**

1. \_\_/2 Is the hypothesis stated and supported by background information?

\_\_/2 Is the expected difference in response of chicks and adults accurate and supported by evidence?

1. \_\_/1 Is the dataset stated?
2. \_\_/2 Is the table completed correctly and completely?

\_\_/1 Does the table have an informative caption?

1. \_\_/1 Does the figure present the data appropriately?

\_\_/3 Is the figure easy to interpret visually and does it include the use of appropriate axes, axes labels, legend and an informative caption?

1. \_\_/2 Is there a biologically significant difference stated and evidence to support the conclusions?
2. \_\_/2 Is p-value correct and presented with alpha value and sample size?
3. \_\_/2 Is p-value correct and presented with alpha value and sample size?

\_\_/2 Are reasonable conclusions drawn from statistical results and evidence to support conclusions?

1. \_\_/1 Is there a reasonable explanation for the differences observed?
2. \_\_/2 Is the hypothesis stated and supported by background information?
3. \_\_/1 Does the figure present the data appropriately?

\_\_/3 Is the figure visually pleasing with axis labels and an informative caption?

1. \_\_/2 Is p-value correct and presented with alpha value and sample size?

\_\_/2 Is appropriate evidence given to support the stated conclusion?

1. \_\_/2 Is sufficient background information on natural histories of both species given to support conclusions drawn?

**Overall**

\_\_/1 Are egregious spelling or grammatical mistakes avoided?

\_\_/1 Is the meaning of the prose clear?

\_\_/1 Were directions for font, spacing, margins etc. followed?

**Partner contributions**

\_\_/4 Had a cooperative attitude, interest in assignment, took an active role, contributed to half of assignment from start to finish

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