Quantifying Reticulocytes in Sea Birds

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Abstract

Arctic Terns (ARTE) are the longest distance travelers, migrating from above the Arctic Circle in the north to Antarctica in the south every year, while the Long-Tailed Jaegers (LTJA) breed in tundra and migrate through both the Pacific and Atlantic Oceans. Migration is a period of intense exercise as the birds fly, which needs red blood cells to support as they carry the majority of oxygen in the blood stream. The reticulocytes are a developmental stage of red blood cells which can be indicative of aerobic capacity. Higher reticulocytes could indicate the birds have upregulated the production of red blood cells to carry more oxygen. The purpose of this study is quantifying reticulocytes in sea birds. Seventeen breeding birds were captured by in Denali National Park alpine tundra (n= 6 LTJA) and the North Slope of Alaska, above the Arctic Circle (n = 9 ARTE and n = 2 LTJA). Stain was added to the blood samples and then blood smears were prepared for 2-3 microscope slides per bird. Reticulocytes were identified and counted under the microscope (100x). The goal was to count 1000 cells per slide, however the quality of several slides was low and the average total count of cells was 833 (+- 217) for ARTE and 759 (+-213) for LTJA. Reticulocytes as a proportion of the total cells counted averaged 2.21 (+- 1.02) % for ARTE and 3.22 (+- 1.02)% for LTJA. The results were lower than we expected compared with other species. Further research with larger sample sizes and better slides quality would help us better understand these patterns.

Introduction

- Quantifying Reticulocytes in Sea Birds is the main purpose of this study.
- Blood samples were collected from Arctic Terns and Long-Tailed Jaegers in Alaska during the summer of 2018.
- Arctic Terns (ARTE) are the longest distance travelers, migrating from above the Arctic Circle in the north to Antarctica every year. Less is known about the migration of the Long-Tailed Jaeger (LTJA), and this collection was part of a different project quantifying migration patterns in the Pacific Ocean, but they breed in tundra and migrate through both the Pacific and Atlantic Oceans.
  - Migration is a period of intense exercise as the birds fly. Red blood cells are important to this process as they carry the majority of oxygen in the blood stream.
- I analyzed microscope slides of blood smears from the samples to count the percentage of reticulocytes
  - The reticulocytes is a developmental stage of red blood cells which can be indicative of aerobic capacity.
  - Higher reticulocytes could indicate the birds have upregulated the production of red blood cells to carry more oxygen.
- This is a time intensive project and requires some moderate skill with a microscope.

Figure 1. Arctic Terns (ARTE).

Figure 2. Long-Tailed Jaeger (LTJA).

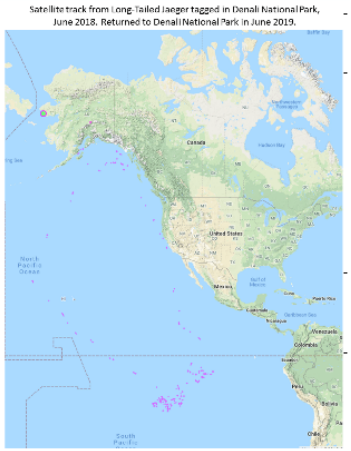

Figure 3. Example of Long-Tailed Jaeger Track.

Materials and methods

- Dr. Fowler captured 17 breeding birds in Denali National Park alpine tundra (n= 6 LTJA) and the North Slope of Alaska, above the Arctic Circle near the town of Nuiqsut (n = 9 ARTE and n = 2 LTJA).
- Blood samples were collected from branchial veins and stained with methelyene blue stain (Richard-Allen Scientific, 89029). 15 ul whole blood + 5 ul stain, mix and wait for 15 minutes before smearing on 2-3 slides/bird.
- Counted with oil immersion microscopy.
- Attempted 1000 cells total per slides and calculated:
  - Percentage of cells that were reticulocytes.
  - Percentage of Coefficient of Variation (CV) (Standard Deviation/Mean) between duplicate slides of same individual counted.
  - Average percentage of CV by species.
  - Compared average percentage of reticulocytes by species using a T-test (Calculated in R studio).

Results

Problems:

- Due to the poor quality of the slides, I could not count the reticulocytes as planned and some slides were totally destroyed with crystal squares which made it hard to see.

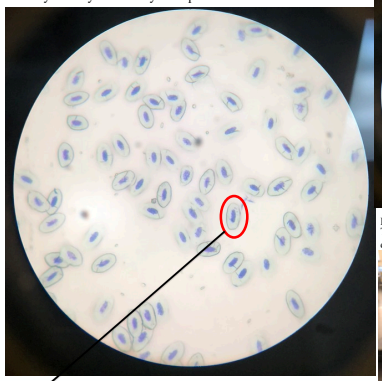

Figure 4. Good example for reticulocytes. Recognize the cell as a reticulocytes with more than five spots out of nucleus. This picture is not super clear due to poor photo quality, but it is significantly to see spots.

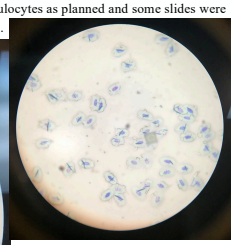

Figure 5. Example of poor-quality slide. This picture shows how those crystal squares destroyed the cells under 100× lens.

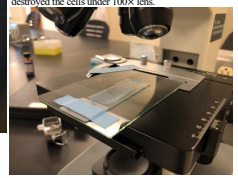

Figure 6. The use of glass stage.

In order to have a clear view of reticulocytes, I usually looked for cells on the edge of slides. When I zoomed in with 100× lens, the slides would fall out of the microscope's stage. I used a glass stage to deal with this problem (shown in Figure 6.).

Data:

- Instead of 1000 cells per slides, I tried my best to count as much as I could.
- The averages of total cells counts were 833 for ARTE and 759 for LTJA.
- The SD of percentage of reticulocytes and total cells, and percentage of CV (Coefficient of Variation) are shown below (Table 1).

	% reticulocytes (±SD)	Average Total Cells counted (±SD)	Mean %CV between duplicate slides of same individual
ARTE	2.21±1.02	833±217	19.05%
LTJA	3.22±1.02	759±213	9.35%

Table 1. The averages of the percentage of reticulocytes and total cells, the SD of percentage of reticulocytes and total cells, and percentage of Coefficient of variation.

- % CV of ARTE was so high, which means there was more variability between different slides.
- % CV of LTJA was in the reasonable region.
- There is one very high ARTE value which may be skewing the average higher, closer to the average of the LTJA. This could reduce our ability to detect a difference between these groups. With more samples, the trend of higher LTJA might be statistically significant.
- Figure 7 illustrates the different patterns between species.
- The % reticulocytes is not different between species when compared with a t-test (the t-test values are a t= -1.6365, df = 8.6434, and p value= 0.1375).

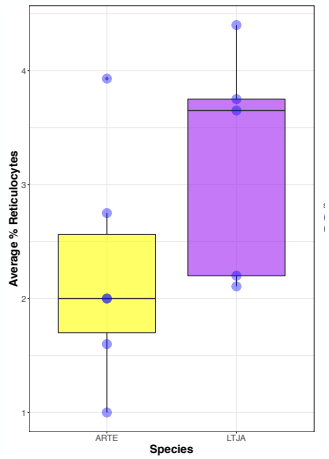

Figure 7. The boxplot shows the average % reticulocytes between species.

Conclusions

- Species are same but we had these issues:
  - Slides quality.
  - Low sample size.
- Our species had low levels, with non-significant differences, but maybe with more samples we could detected differences.
  - Compared to other species, including breeding starlings with 7-15% of reticulocytes (Fowler et al, 2018), breeding zebra finches with 10-15%, (Wagner et al, 2008), and migrating bar-tailed godwits with 3.8-4.5% (Landys-Ciannelli et al 2002), our two kinds of sea birds have lower percentage of reticulocytes.
  - Based on our data, both species have low percentage of reticulocytes (shown in Table 1), indicating that they are responding to a need for red blood cells by producing more.
- High aerobic capacity is necessary for their long-distance flying.
  - Long-Tailed Jaeger (LTJA) has a non-significant trend for more reticulocytes, which indicates they may be producing more red blood cells during breeding, despite not migrating as far as Arctic Terns (ARTE) .
- Given the delay between finishing their migration and the time of capture, this could indicate a difference in recovery time between species.

Literature cited

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Acknowledgments

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For further information

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