DNA Barcoding Assessement of Avian Biodiversity

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Abstract

The MNSA, or Marine Nature Study Area in Oceanside, Long Island, is a 52-acre environmental preserve unique for its avian species. In this study, we aim to survey the avian diversity of the site with a focus on DNA barcoding. Various stool, feather, cartilage and other bird samples were collected and purified using a modified DNeasy spin-column protocol from Qiagen. These samples were then PCRed using two special avian primers: BirdF1 and COIbirdR2, which were run through a gel to check for success. The successful products were sent out for sequencing. Results were uploaded and analyzed through DNA Subway, which revealed the identity of our samples through BLASTN. Brants were the most common sample, followed by the mourning dove, lesser scaup, hooded merganser and rock dove. Our results were confirmed through a comparison to the MNSA's local bird observation statistics and the migratory pattern and habits of these birds provided by the Cornell Ornithology Laboratory.

Introduction

What interested us most about the MNSA was its proximity to a major city (NYC) but also its status as a nature reserve. According to the eBird project, more than 260 species have been identified in the Oceanside Marine Nature Study Area (eBird, 2021). This was an easily accessible region which had a wide range of species we could evaluate.

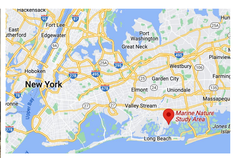
Our main goals:

- **Evaluate** the species diversity of the region through DNA barcoding methods and compare with MNSA to evaluate how well our sampling represented the communities found there.
- **Determine** the efficacy of using DNA barcoding, especially on a vertebrate species like avians

We began by collecting a variety of types of samples from different parts of the MNSA, recording exactly where each was found. We then used DNA barcoding to identify the species of bird each sample originated from, creating a list of species and the habitats each sample was found in. Using the pre-existing eBird database, we compared the expected species found with the actual species found and formed an idea of how the habitats in the MNSA compare to similar habitats in other locations.

Materials and Method

1. Sample Collection



Sample Collection at the **Marine Nature Study Area** (500 Slice Dr, Oceanside, NY 11572) On December 10th, 2022

Collection of 25 samples, including feathers, cartilage, stool and skeletal samples from unknown birds.

Figure 1. Location of each collected sample, numbered, labeled and photographed on the spot. Photos provided GPS data.

2. DNA Extraction/Purification

- Use of Proteinase-K and a modified Qiagen DNeasy Tissue protocol (Qiagen, 2020; Lijtmaer, 2023)
- For feathers, an additional DTT solution and longer incubation time was given to break down keratin
- DNA was collected through filtering samples through a spin column using a series of buffer solutions and centrifugation
- All buffer solutions: ATL → Al → AW1 → AW2 → AE.

3. PCR Sampling

- Primers came in concentrated/powder form.
- Primers rehydrated by adding the nmol concentration number time 10 = amount of water to add in µL to the primers.

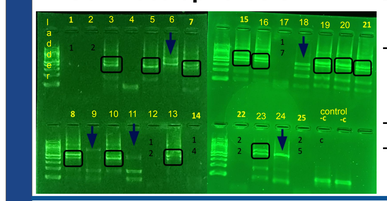
Two Methods for PCR

Four-Primer Approach	Two-Primer Approach

- Good for more fragmented DNA, which could be due to exposure to the elements
- Shorter overlapping sequences are easier to amplify
- Cresol red mix with all four primers (BirdF1, CO1BirdR2, AvMiR1, AvMiF1)
- ***However***, our DNA was in relatively good condition
	- Forward and backward primers BirdF1 and CO1BirdR2
	- Cresol red mix (668µL dH_2O, 460µL Cresol Red, 6 µL BirdF1, 6 µL CO1BirdR2
	- For each PCR Tube
	  - 12.5 µL Taq 2x
	  - 10.5 µL Cresol Red Mix
	  - 2 µL template DNA
	- Thermocycler program

94°C for 1 min, 25 cycles (94°C for 1 min, 45°C for 1.5 min, 72°C for 1.5 min), 35 cycles (94°C for 1 min, 55°C for 1.5 min, 72°C for 1.5 min), 72°C for 5 min	**94°C for 1 min, 5 cycles (94°C for 1 min, 45°C for 40 s, 72°C for 1 min), 35 cycles (94°C for 1 min, 51°C for 40 s, 72°C for 1 min), 72°C for 5 min**

4. Gel Electrophoresis



- Ran at 130 V for 20 minutes
- Boxed regions = successful PCR with primer pairs = successful sequences
- Blue arrows = unclear
- Sent out to Azenta sequencing

5. DNA Barcoding

- Sequences uploaded to DNA subway. Using the blue line, sequences were trimmed, paired and manually edited for errors.
- Samples run through BLASTN and other uploaded sequences to identify the bird of origin or the samples.

Results

Figure 2. Map of identified results. We had 16 successful matches out of 25
Photos courtesy of Michael Farina, Director of MNSA.

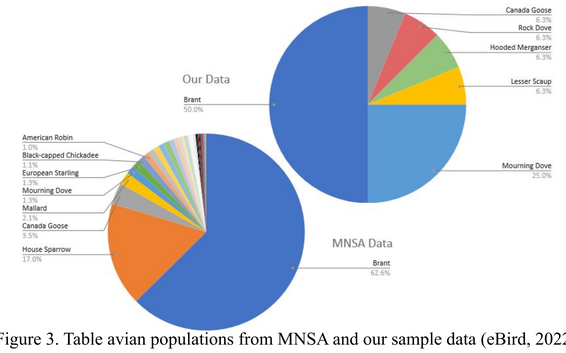


Figure 3. Table avian populations from MNSA and our sample data (eBird, 2022)

Discussion

Lab Evaluation

- ➢ 11/15 Feather samples produced results ~73% success rate
- ➢ 4/8 Skeletal/soft tissue samples succeeded ~50% success
- ➢ 0/2 stool samples succeeded - 0% success rate

Stool contains white chalk-like particles of uric acid crystals in them. When not scraped off entirely, uric acid could interfere with our DNA extraction procedure and degrade the DNA.

Evaluation of Identified Birds

Our final sample count turned up eight brants, four mourning doves, one Canada goose, one rock dove, one hooded merganser, and one lesser scaup. The lesser scaup, notably, is a migratory bird and would normally be as far south as Central America during December, which is the time we collected our samples. However, the detail that our sample was a skeletal sample and not necessarily fresh leads us to believe that this data is correct and that we found the skeleton of a bird that had died weeks to months earlier.

We are unsurprised by the high brant population — noted in Table 1, birdwatching data from the Marine Nature Study Area reports thousands of brants throughout the month of December. With the exception of the lesser scaup and hooded merganser, the other species we found were also common to the area at that time period, each reporting hundreds or thousands of sightings in Nassau.

The lesser scaup, rock dove, and hooded merganser samples were all dead birds, which may have been moved from their previous locations, best exemplified by an unfortunately failed sample that was found nestled behind a tree, perhaps intentionally placed there by a predator. We noted, however, that the four mourning doves were *only* found on the edges of the wooded region of the MNSA and no more than 100 feet from the water. This tracks with eBird's description, which reports that the species "avoids dense forest, but [can be] found on forest edges." The general dispersal of brants also tracks with their tendency to stick to salt marshes and coastal regions during the winter. The strangest result we found was the presence of a rock dove, which is typically found in cities and on farmland, but the nearby presence of NYC and large urban rock dove populations justifies this.

Of note is that one of our samples, a feather found in a lightly forested area, appeared to come from both the Mourning dove and Brant depending on which sequence we used. Given that our first sequence set was abbreviated and generally poor data, we chose to use the second set's data and declared said sample to be a mourning dove, *Zenaida macroura*.

We recognize limitations inherent to our sampling method. By sampling noticeable feathers on the ground and visible soft tissue samples, we biased our results towards larger, more brightly-plumed birds, and did not collect nearly as many samples from smaller, plainer species. We also may have missed out on possible samples from waterfowl, which spend less time on land and thus were less likely to be represented.

Concluding Remarks

We are not surprised by our results. Future experiments building off of these results could sample a wider range of locations and species and run multiple batches of PCR in the case that lab errors result in poor data, as they did in our case. Experimenters should note the ineffectiveness of stool samples compared to feather samples, and either focus entirely on analyzing feathers or develop specific techniques for stool. The use of DNA barcoding and Ljitmaer's procedure was a great success, and we believe that at a large enough scale, the use of these techniques could help more accurately characterize the local biodiversity of a region.

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