Using environmental DNA sampling methods to determine cryptic wetland bird occupancy in Illinois

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Introduction

What is eDNA?
Organisms continually shed DNA from their skin cells, feathers, fur, feces, and/or gametes into their surrounding environments. Environmental DNA, or eDNA, can be extracted from freshwater, marine, and soil samples. eDNA can trace a sample back to the species that produced the sample.

How is eDNA used?
Amphibians, mammals, fish, reptiles and arthropods have been detected with eDNA. DNA of rare and cryptic species, such as Rocky Mountain tailed frogs, Idaho giant salamanders, and Eastern Hellbenders, has been isolated from eDNA samples in freshwater rivers and streams. Thomsen et al. 2012 serendipitously detected migratory birds from marine water samples. Our research focuses on using eDNA to quantify cryptic bird occupancy in wetland patches in Cook and Lake County, Illinois.

Why focus on cryptic wetland birds in Illinois?
Wetland habitat has severely declined in Illinois over the past 200 years. Wetlands patches support over 100 breeding bird species, 15 of which are either state threatened and endangered or globally imperiled. We have poor distribution estimates of rare and secretive rail species. We cannot conserve species unless we know which habitat patches they are using.

Why use eDNA if playback surveys can locate birds?
Birds do not always respond to playback, and eDNA sampling may cause less disturbance and stress than playback to nesting birds.

Questions

(1) Can we use eDNA methods to detect cryptic wetland birds in emergent wetland habitat?
(2) How do eDNA methods compare to traditional survey methods?
(3) Can we use eDNA sampling to track cryptic wetland bird migration?

Results

In the lab
- performed playback surveys using Black Rail, Yellow Rail, King Rail, Virginia Rail, Sora, and Common Gallinule recordings at four sites in NE Illinois
- collected and filtered 1 L water samples at playback survey locations; collected and processed 37 samples, 7 distilled water blanks

In the field
- extracted eDNA from filters using a CTAB extraction protocol
- quantified eDNA extracts on a Qubit 3.0 fluorometer
- amplified 225 bp fragments of avian 16S in the mitochondrial genome
- degenerate primers were developed in June by Dalen et al. 2017
- samples with band size matching Sora rail liver control were purified and sent for sequencing.

Table 1. Sampling locations, water samples collected and playback surveys took place for Black Rail, Yellow Rail, King Rail, Sora, and Common Gallinule. Percentages indicate occupancy estimates from eDNA/PCR results and field observations.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Collection dates</th>
<th>Likey rail eDNA detection</th>
<th>Ringed rail eDNA detection</th>
<th>Rain/rock nDNA detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bristow Beach</td>
<td>05.31.17 - 06.15.17</td>
<td>1/6 (16.6%)</td>
<td>0/10 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>FoxDunes</td>
<td>05.24.17 - 06.31.17</td>
<td>1/5 (20%)</td>
<td>1/6 (16.6%)</td>
<td>0/3 (0.0%)</td>
</tr>
<tr>
<td>PaulDouglas</td>
<td>06.17</td>
<td>1/4 (25%)</td>
<td>1/8 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>OrlandGrassland</td>
<td>05.24.17 - 06.13.17</td>
<td>4/9 (44.4%)</td>
<td>7/9 (77.8%)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

- Amount of eDNA obtained from samples does not significantly vary by site or over time during spring migration and breeding, but more sampling is needed to confirm these results.
- Sample contamination can be reduced using adequate levels of human blocking primer during PCR.
- eDNA sampling methods can increase occupancy estimates obtained using playback recordings.
- Pending sequencing results, eDNA methods can likely be used to identify multiple cryptic, declining rail species.

Open Questions and future directions

- On what timescale does bird DNA persist and degrade in emergent wetlands? Do eDNA concentrations shift significantly after storms?
- Can we use eDNA to estimate rail abundance in addition to occupancy?
- How robust are eDNA diversity estimates compared to passive point count methods?

Acknowledgements & Contact

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References