Improved Environmental DNA Reference Library Detects Overlooked Extralimital Marine Fishes in New Jersey, USA

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Supplementary Material

Supplementary File 1. Modified Qiagen PowerSoil DNA extraction protocol.

Supplementary Figure 1. DNA yield vs extraction interval.

Supplementary Figure 2. DNA yield by site, date.

Supplementary Figure 3. *Rhinoptera bonasus, R. brasiliensis* COI barcodes from GenBank, this study.

Supplementary Figure 4. Maximum reads/species vs. detections.

Supplementary File 1. Modified Qiagen PowerSoil protocol.

1. -Tissue: Add 100 µl C1 solution to PowerBead tube.

   -Filter: Add 180 µl C1 solution to PowerBead tube.

2. -Tissue: Place tissue fragment (not larger than 4 mm^3) in PowerBead tube.

   -Filter: Using a single-edge razor and plastic petri dish as a cutting surface, fold filter in half, keeping residue surface inside, and trim off the outer circumferential portion that was not exposed to the filtrate. Then cut into pieces approximately 2-3 mm x 10 mm. Place cut-up filter into PowerBead tube with forceps. Between filters, rinse razor, forceps, and gloves thoroughly with tap water and dry completely with paper towel. Use a new petri dish for each filter.

3. Place on vortexer platform on high for 20 min. Watch that device does not fall off bench.

4. Centrifuge at 10,000*g* for 1 min.

5. Using a P200 set at 155 µl, transfer 3 aliquots of supernatant (total volume 465 µl) from PowerBead tube to a 2 ml Collection Tube. You can stick pipette tip down deeply along the tube wall, as beads and filter fragments are too large to go into pipette.

6. Add 100 µl of Solution C2 and 100 µl of Solution C3. Vortex 10 seconds.

7. Incubate at 4°C for 5 min (put rack in refrigerator/cold room).

8. Centrifuge at room temp 1 min at 10,000*g*.

9. Transfer 625 µl supernatant to a new 2 ml Collection Tube.

10. Add 1 ml Solution C4 to the supernatant. Invert 20 times to mix.

11. -Load 675 µl onto a Spin Filter and centrifuge at 10,000*g* for 1 min at room temp.
    -Discard flow through.
    -Add another 675 µl to Spin Filter and centrifuge at 10,000*g* for 1 min.
    -Discard flow through.
    -Load the remaining supernatant onto Spin Filter and centrifuge at 10,000*g* for 1 min.
    -Discard flow through.

12. Add 500 µl Solution C5 to Spin Filter and centrifuge for 30 secs at 10,000*g*. Discard flow through.

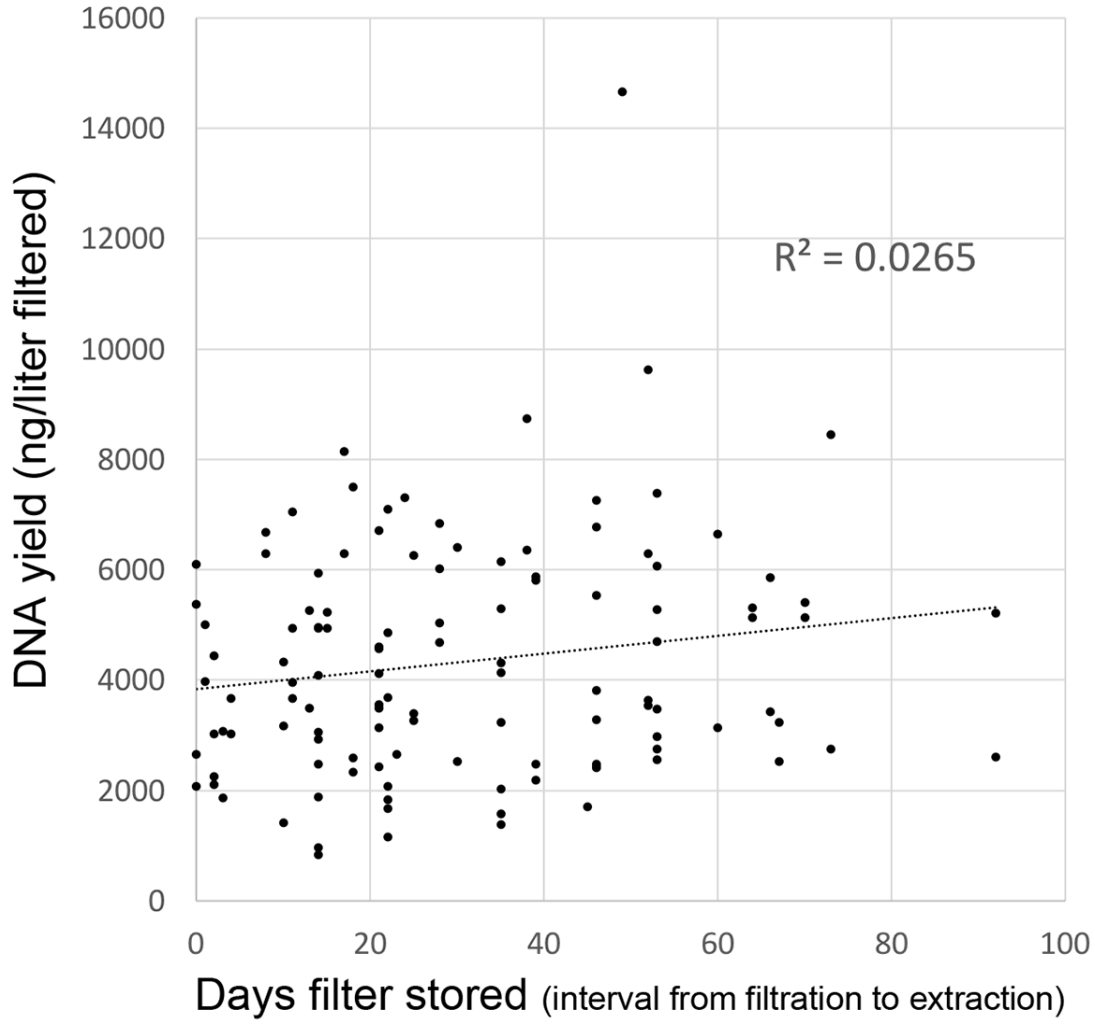
13. Centrifuge for 1 min at 10,000*g*.

14. Place Spin Filter in a clean 2 ml Collection Tube.

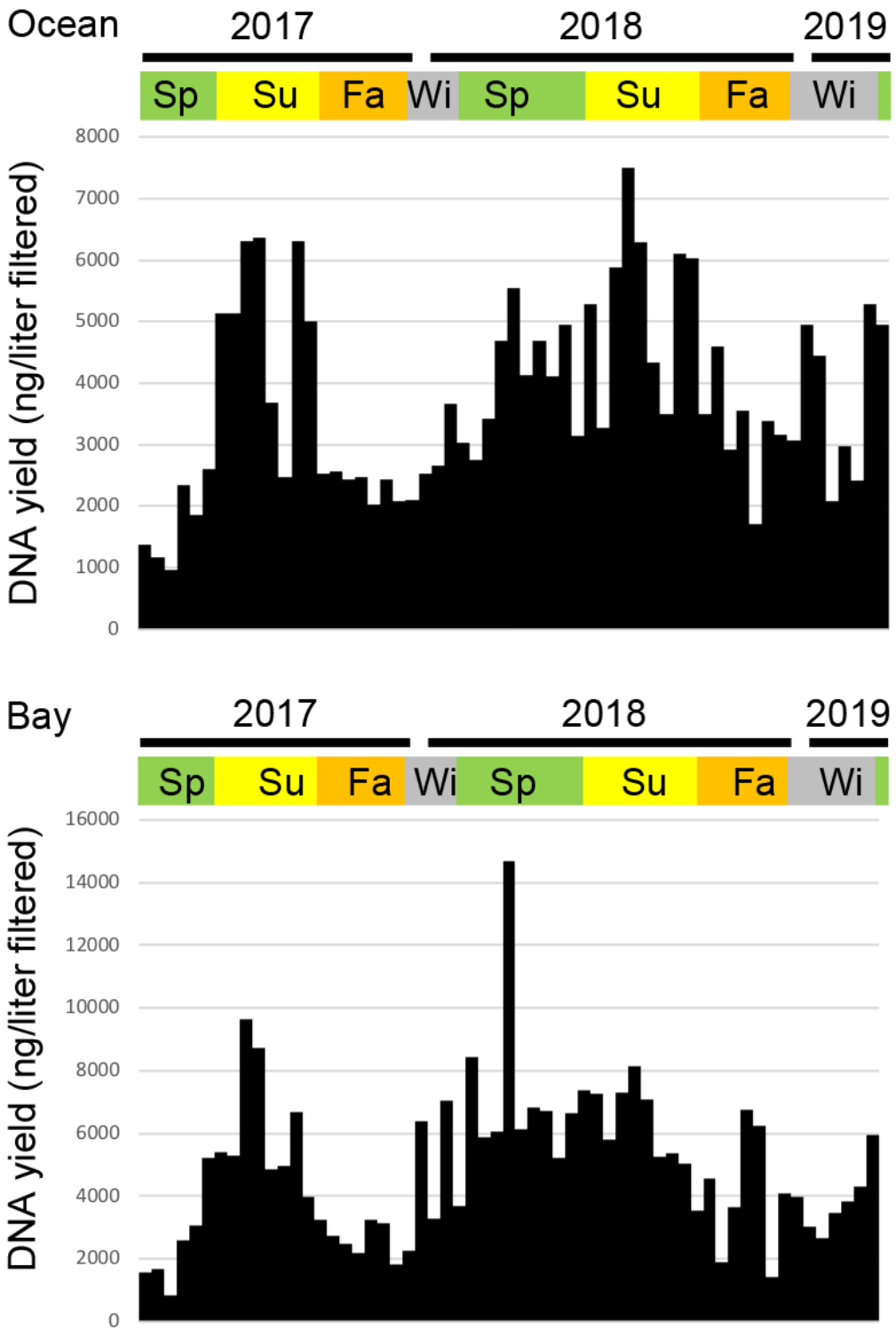
15. Add 100 µl Solution C6 to the center of the white filter (don't touch filter with pipette tip).

16. Centrifuge for 30 secs at 10,000*g*. Usually recover ~95 µl. Store at -20°C.

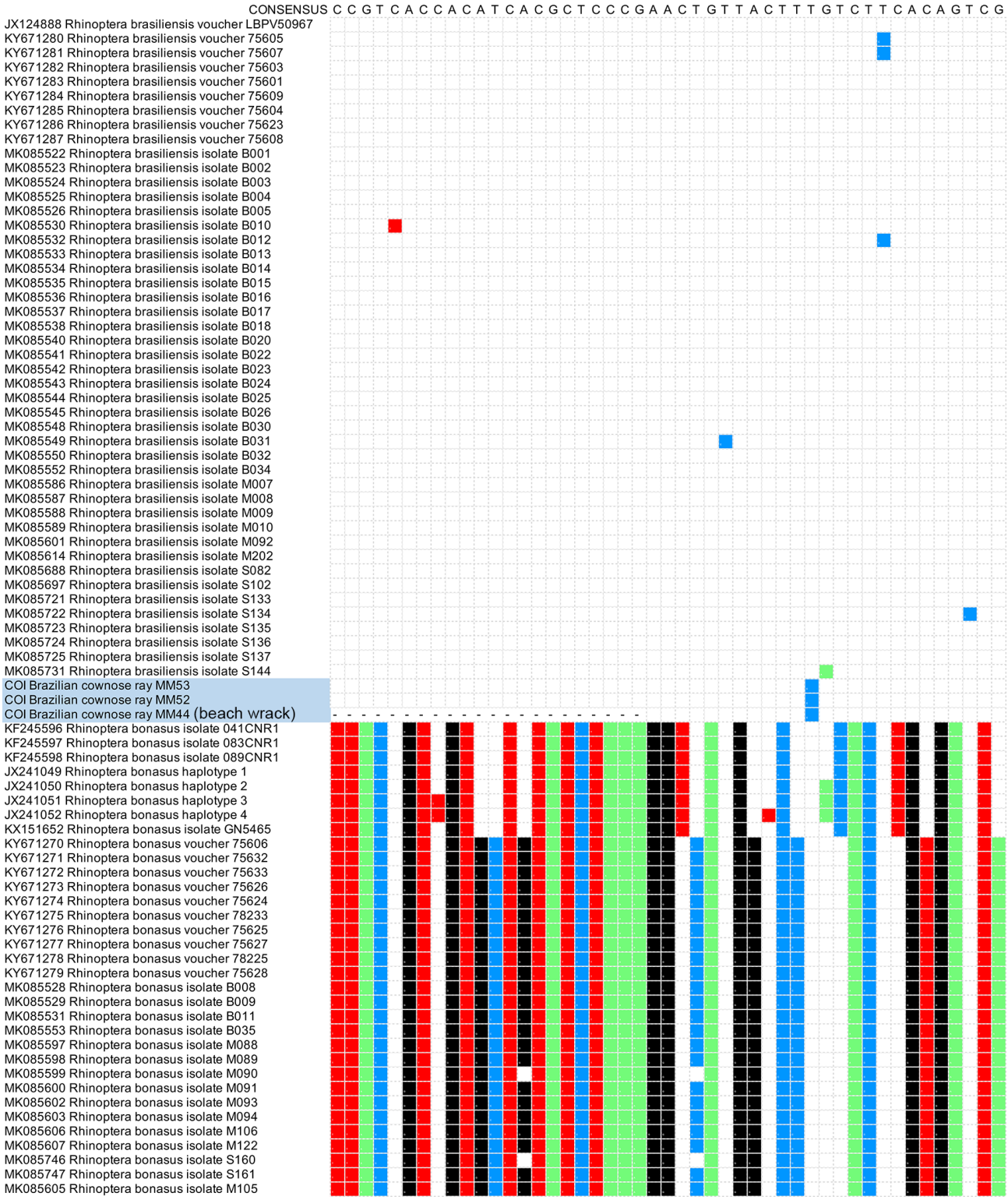
Supplementary Figure 1. DNA yield vs. filter storage interval.



Supplementary Figure 2. DNA yield from field samples by date collected.



Supplementary Figure 3. Comparison of *Rhinoptera brasiliensis* sequences obtained in this study (highlighted in blue) with *R. brasiliensis* and *R. bonasus* COI barcodes obtained from GenBank. Conserved sites not shown. Colors indicate nucleotides that differ from consensus (A=green, C=blue, G=black, T=red).



Supplementary Figure 4. Maximum reads vs. detections for each species. The apparent dynamic range, i.e., the fold-difference between the lowest read number from single-detection species (shown in red) and the highest read number from more common species (shown in blue), is indicated at right of each graph. For single-detection species, maximum and minimum reads are the same.