Review of: "Noninvasive sampling for comparisons of wildlife microbiomes may be more reliable than sampling trapped animals"

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Review of Turjeman et al. 2022 Crane rDNA review 3-31

The authors should be applauded for using a metagenomic approach for evaluating the impact of sample collection procedures in ecological and conservation biology studies. It is a sensitive and broad contextual method to characterize systems-based changes in biological models. What the authors found however was that it is extremely sensitive and their results suggest significant biological changes take place after such field activities. However, the actual long-term impact can only be determined with further assessment of specific endpoints.

The authors use 16S rRNA metagenomics to analyze crane fecal microbiome samples collected using two different methods: non-invasive collection from fields recently visited by cranes and samples from trapped and sedated birds. They found large differences in microbial community composition between the two sample collection methods. Alpha and beta diversity and core microbiomes differed consistently and dramatically between the samples using the different methods. The authors speculate that both stress and administering sedation to the birds could be major factors affecting fecal microbiome community composition.

It's ironic that most microbiome studies try to highlight differences between samples derived from different experimental conditions while the authors here would welcome overall equivalence in bacterial community structure between the two collection methods in order to validate noninvasive sampling. In addition, community differences seem oddly opposite of what one might typically expect: alpha diversity increased among anthropogenically impacted captured birds while anaerobic versus aerobic bacterial contamination increased in the noninvasive samples. The authors are careful to use state-of-the-art statistical techniques for analyzing compositional metagenomic data bringing into even sharper relief the differences between the sample methods. Quickly eyeballing the differentially abundant taxa in the Figure 3 heatmap tells the whole story in a nutshell, that there are major differences in the genus level core microbiomes between invasive and noninvasive collected samples.

This analysis is only a small part of a much larger project studying the microbiome of cranes migrating annually from Russia to Israel [Pekarsky et al. 2021]. That study included gut microbiome analysis of birds feeding in fields at different times of the year in different locations, with supplemental feeding, no supplemental feeding or both. The sheer number of potentially confounding factors in that study seemed to make it practically impossible to predict microbial community structure. Likewise, the Turjeman et al. study is subject to many potential confounding factors that affect the microbiome in the captured specimens that it's impossible to show whether noninvasive versus invasive sampling more accurately reflects the true crane gut microbiome even if comparisons between the two sampling methods may be valid. These studies do offer the possibility of characterizing many potential impacts such as change of anesthetic to a less potent alternative, double checking types of restraints, etc to see how to minimize these microbiome changes. They also could determine further time course of microbiome changes and characterize both acute as well as chronic impacts.

Conservation biology studies like these could benefit greatly by a switch to deep whole genome shotgun metagenomic sequencing given the continued decrease in shotgun sequencing costs per sample. It's clear that the authors have taken 16S targeted analysis as far as possible and need to include functional analysis only available with nextgen metagenomic sequencing. Besides eliminating potential variable region and primer biases it opens the door to functional pathway analysis to help explain differences between invasive versus noninvasive collection methods. Even greater benefits might be seen in evaluating metagenome assembled genomes (MAGS) at species and strain level including pathogenicity, antimicrobial resistance and mobile genetic elements.

Pekarsky, Sasha, et al. "Drivers of change and stability in the gut microbiota of an omnivorous avian migrant exposed to artificial food supplementation." *Molecular Ecology* 30.19 (2021): 4723-4739.