

DNA metabarcoding quantifies relative abundances of arthropod taxa in songbird diets: validation with camera-recorded diets

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Abstract

Ecological research is often hampered by the inability to quantify animal diets. Diet composition can be tracked through DNA metabarcoding of faecal samples, but whether (complex) diets can be quantitatively determined with metabarcoding is still debated and needs validation using free-living animals. This study validates that DNA metabarcoding of faeces can retrieve actual ingested taxa, and most importantly, that read numbers retrieved from sequencing can also be used to quantify relative abundances of dietary taxa. Validation was done with the hole-nesting insectivorous Pied Flycatcher whose diet was quantified using camera footage. Size-adjusted counts of food items delivered to nestlings were used to approximate provided biomass of prey orders and families and subsequently nestling faeces were assessed through DNA metabarcoding. To explore potential effects of digestion, stomach and lower intestine samples of freshly collected birds were subjected to DNA metabarcoding. For metabarcoding with Cytochrome Oxidase subunit I (COI), we modified published invertebrate COI primers LCO1490 and HCO1777, which reduced host reads to 0.03%, and amplified Arachnida DNA without significant changing the recovery of other arthropod taxa. DNA metabarcoding retrieved all commonly camera-recorded taxa. Overall, and in each replicate year ($N = 3$), the relative abundances of size-adjusted prey counts and COI read numbers correlated at $R=0.85$ (CI:0.68-0.94) at order level and at $R=0.75$ (CI:0.67-0.82) at family level. Similarity in arthropod community composition between stomach and intestines suggested limited digestive bias. This DNA metabarcoding validation demonstrates that quantitative analyses of arthropod diet is possible. We discuss the ecological applications for insectivorous birds.

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