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What's in the diet? DNA-based analysis for qualitative and quantitative assessment of animal diet

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Summary

The study of animal diets plays a pivotal role in ecology, offering insights into trophic interactions, energy flow, and nutrient cycling within ecosystems. By analyzing what animals eat, researchers can understand species specialization and predator-prey dynamics, all of which are crucial for conservation and ecosystem management. Diet analyses are also vital for managing invasive species, predicting environmental changes, and assessing ecosystem health.

Ecologists use various techniques to analyze animal diets, including non-genetic and genetic methods. Non-genetic approaches, such as visual examination of stomach contents, feces, and field observations, provide direct insights but can be labor-intensive and biased by digestion rates. Genetic approaches, like DNA sequencing of scat or stomach contents, allow precise identification of consumed species, facilitating both qualitative and quantitative dietary assessments.

This thesis delves into DNA-based techniques used to study animal diets, and investigates its strengths and limitations. DNA-based approaches, particularly DNA metabarcoding, have revolutionized dietary studies by offering non-invasive and precise identification of consumed species. However, these advanced methods face challenges like incomplete reference databases, non-standardized protocols and issues with quantitative accuracy. In this thesis I focused on developing standardized protocols for quantitative analysis using digital droplet PCR (ddPCR) to improve the accuracy of rare diet constituent quantification and address contamination and secondary consumption in qualitative genetic dietary assessments, thereby enhancing our understanding of complex dietary interactions in ecological research.

Chapter 1 begins by outlining the diverse applications of diet studies across various ecological research fields. It then delves into the methodologies of diet analysis, categorizing them into genetic and non-genetic approaches. The chapter proceeds to discuss the challenges associated with non-genetic methods, highlighting how genetic (DNA-based) qualitative and quantitative techniques can address these issues. The introduction concludes with a discussion of the current limitations and knowledge gaps in DNA-based diet studies and outlines my research aims to address these challenges.

In Chapter 2, a robust method for quantification of seed DNA in the diet of wood mice was developed. Here, we especially looked into the methodological biases of DNA extraction, PCR inhibition and marker choice (PCR assay and sensitivity) on target DNA quantification in wood mouse feces. Biological biases, as the effects of digestion, age and sex of the wood mouse, and the effect of other diet components in their diet on target DNA quantities were additionally investigated. Furthermore, gut transition times for target DNA were obtained for the wood mouse, observing a gut transition time between 8 and 24 hours for 95% of target DNA. We observed that DNA extraction needed optimization, as standard extraction kits prevented reliable quantification. Additionally, by using droplet digital PCR we prevented PCR inhibition as far as possible. Next, different DNA markers, targeting different parts of the chloroplast, influenced target DNA detectability. However, all markers showed higher target DNA content for higher seed numbers. Although we tried to prevent methodological biases as far as possible, DNA degradation, due to random digestive processes, still complicated robust quantification somewhat as the target DNA quantities showed large variation within mice fed the same number of seeds. However, we did find that biological factors; age, sex and other diet constituents did not alter the digestion patterns and thus quantification. Overall, the newly developed, sensitive DNA-based approach allowed for minimally invasive and robust quantification of small diet constituents in feces, which would otherwise be undetectable with traditional non-genetic methods.

In Chapter 3, the abovementioned method was tested in a realistic field scenario. Biological factors as meal size and digestibility and their effect on target DNA quantity in wood mice feces were further studied here. Additionally, we investigated the possibilities of relating and converting target DNA to actual number of diet items eaten. We observed that increasing meal size also increased the variability of target DNA found in the feces of wood mouse. This further illustrated that, although robust quantification techniques were established, using feces as starting material which is always subject to digestive processes, DNA quantification can only be reliable when sufficient number of feces samples are analyzed to accommodate for random DNA degradation. Doing so, we succeeded to obtain statistically significant differences of target DNA when mice were fed varying seed intake numbers. This enabled the development of calibration curves relating target DNA numbers to seed number fed. Subsequently, these curves were applied in a field trial to quantify seed intake in a wild wood mouse population. We detected seed DNA in the fecal samples of the wood mice caught in the field, which resembled a seed intake of up to 1 seed (although with large confidence intervals due to digestion variation). Nevertheless, this was the first-ever study to quantify seed intake in a realistic field scenario using a DNA-based analysis, showing that accurate seed intake estimates can be obtained given a high enough sample number.

In Chapter 4, the aim was to look at the contribution of small prey species to the diet of lions. Moreover, we discussed the bias that is introduced by non-genetic methods towards larger prey species compared to diet estimation with qualitative DNA metabarcoding. We inferred that the sensitivity of DNA-based diet analysis aids detection of small taxa compared to non-genetic methods, but this sensitivity also means that it will detect species that may have not been eaten by the animal of focus. In our study, we found substantial presence (8% of all prey items) of mesopredator DNA in the fecal samples of lions, which we interpret as over-marking by mesopredators. Furthermore, ecological transfer by scats attracting coprophagous taxa (mostly insects) which in turn attract small predators, such as insectivores, that feast on these prey species additionally scats may attract osteophagous animals. Via saliva or feeding remains or directly by touching the fecal samples, these small predators can transmit their DNA into the scat of lions. We concluded that field contamination (via overmarking, ecological transfer or secondary predation) is likely to happen very often in terrestrial studies of DNA-based carnivore diet and should be accounted for. A combination of DNA and morphologically based methods which are less prone to field contamination (such as microscopic analysis of undigested matter, e.g. hairs) might be complementary in such cases.

In Chapter 5, after taken knowledge of the biases and considerations in previous chapters and applying the lessons learned we steered away from methodological questions towards an applied ecological question. We harnessed the power of being able to analyze large sample sizes using DNA metabarcoding to enable a direct comparison of lion diet composition and prey preference between four different National Parks (NPs) as a means to study the effect of fencing. We found changing lion diet composition and prey preference over a gradient from fully fenced NP's to completely unfenced NP's. Strikingly, the fenced park also showed almost an opposite pattern in terms of prey preferences for specific prey body weights compared to the unfenced NP. The lions in this small and fenced reserve

showed high preference for smaller prey and avoidance for prey weights that are generally preferred in other NPs. Partly fenced NPs generally showed varying diet results, but were nearly always situated within the preference boundaries set by the prey preferences of the fenced and unfenced NP and showed no particular outliers for certain prey species.

Chapter 6 addresses the challenges and opportunities associated with DNA-based analysis for qualitatively and quantitatively assessing animal diets. Here, I explore the interpretation of DNA-derived diet data and highlight the potential of combining high-throughput sequencing and diagnostic PCR techniques to enhance accuracy. Furthermore I emphasize the need to address methodological, biological, and environmental biases to better match the estimated diet with the true diet. Additionally, I discuss the complementarity of genetic and non-genetic methods, concluding that integrating these approaches can improve dietary assessments. Furthermore, the results of this thesis underscores the importance of continued technological advancements and multidisciplinary methods for accurate diet reconstruction and ecological research, stressing the potential of genetic techniques to provide comprehensive insights into trophic interactions and ecosystem dynamics.