

Seasonal variation in the stomach microbiota of two sympatrically breeding *Pygoscelis* penguin species at Signy Island, South Orkney Islands

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Abstract

The gut microbiomes of Antarctic penguins are important for the fitness of the host birds and their chicks. The compositions of microbial communities in Antarctic penguin guts are strongly associated with the birds' diet, physiological adaptation and phylogeny. Whilst seasonal changes in food resources, distribution and population parameters of Antarctic penguins have been well addressed, little research is available on the stability or variability of penguin stomach microbiomes over time. Here, we focused on two *Pygoscelis* penguin species breeding sympatrically in the maritime Antarctic and analysed their stomach contents to assess whether penguin gut microbiota differed over three austral summer breeding seasons. We used a high-throughput DNA sequencing approach to study bacterial diversity in stomach regurgitates of Adélie (*Pygoscelis adeliae*) and chinstrap (*Pygoscelis antarctica*) penguins that have a similar foraging regime on Signy Island (South Orkney Islands). Our data revealed significant differences in bacterial alpha and beta diversity between the study seasons. We also identified bacterial genera that were significantly associated with specific breeding seasons, diet compositions, chick-rearing stages and sampling events. This study provides a baseline for establishing future monitoring of penguin gut microbiomes in a rapidly changing environment.

Impact Statement

This study makes a significant contribution to the field by documenting seasonal variations in the stomach microbiota of two sympatrically breeding Antarctic penguins on Signy Island (South Orkney Islands). Our data underscore the complexity of microbial dynamics in a seemingly homogeneous environment and highlight the influence of seasonal changes on penguin gut microbiomes. Notably, despite similar foraging patterns and environmental conditions, distinct seasonal shifts in bacterial diversity were observed in the stomach regurgitates, suggesting a nuanced interplay between diet, environmental factors and microbial communities. By drawing parallels with previous research on alpine accentors and emphasizing the impact of climate-driven alterations in penguin diet and sea ice duration, this study expands the understanding of ecological drivers shaping stomach microbial community composition. Moreover, the identification of specific bacterial taxa associated with diet preferences and chick-rearing stages unveils potential biomarkers and ecological indicators for monitoring environmental

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Abbreviations: BAS, British Antarctic Survey; CCAMLR, convention for the conservation of antarctic marine living resources; CEMP, CCAMLR Ecosystem Monitoring Programme; LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size; OTU, operational taxonomic unit; PERMANOVA, permutational analysis of variance.

The generated sequencing data are available at MG-RAST metagenomics analysis server <https://www.mg-rast.org/mgmain.html?mgpage=project&project=mgp11815> with accession numbers listed in Table S1.

Two supplementary figures and two supplementary tables are available with the online version of this article.

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