Gut microbiome composition, not alpha diversity, is associated with survival in a natural vertebrate population

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Gut microbiome composition, not alpha diversity, is associated with survival in a natural vertebrate population

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Abstract

Background: The vertebrate gut microbiome (GM) can vary substantially across individuals within the same natural population. Although there is evidence linking the GM to health in captive animals, very little is known about the consequences of GM variation for host fitness in the wild. Here, we explore the relationship between faecal microbiome diversity, body condition and survival using data from the long-term study of a discrete natural population of the Seychelles warbler (*Acrocephalus sechellensis*) on Cousin Island. To our knowledge, this is the first time that GM differences associated with survival have been fully characterised for a natural vertebrate species, across multiple age groups and breeding seasons.

Results: We identified substantial variation in GM community structure among sampled individuals, which was partially explained by breeding season (7% of the variance), and host age class (up to 1% of the variance). We also identified significant differences in GM community membership between individuals that survived, versus those that had died by the following breeding season. Individuals that died carried reduced abundances of beneficial taxa in the bacterial order *Clostridiales*, but increased abundances of taxa that are known to be opportunistic pathogens (e.g. members of the *Chloroflexi* and *Propionibacteriales*). However, there was no association between GM alpha diversity (the diversity of bacterial taxa within a sample) and survival to the next breeding season, or with individual body condition. Additionally, we found no association between GM community membership and individual body condition.

Conclusions: These results demonstrate that components of the vertebrate GM can be associated with host fitness in the wild, although whether changes in bacterial abundance contribute to, or are only correlated with, the differential survival observed remains unclear. Importantly, it suggests that components of the GM may be under selection, and, thus, could have the potential to influence the evolution of host species living in natural populations.

Keywords

Gut microbiome, microbial diversity, fitness, life history, *Acrocephalus sechellensis*

Background

Almost all eukaryotic organisms accumulate diverse communities of microorganisms that, over evolutionary time, have become an integral part of the host's ecology and biological function [1]. In vertebrates, the gut microbiome (GM) consists of a complex community of microbes, including bacteria, archaea, viruses and microbial eukaryotes, which can play an important role in host processes such as digestion, behaviour, development and immunity [2–5]. As such, experimental studies have shown that GM disruption can have a significant impact upon the health and survival of a wide range of host organisms in captivity [2, 6–8]. For example, a reduction in GM diversity or an imbalance in GM composition (dysbiosis) has often been linked to poor host health and the onset of disease [8–10].

Captive organisms harbour very different, often depauperate, microbial communities relative to individuals living in natural populations [6, 11, 12]. Indeed, the rewilding of captive mice (*Mus musculus*) causes a rapid shift in the composition and complexity of the GM which, in turn, alters the host's immune system and susceptibility to disease [13, 14]. Wild animals are exposed to highly complex and dynamic environmental pressures which are often poorly represented in captive systems, but that could interact with, or override, the impact of the GM on the host [15]. Furthermore, high levels of inbreeding in captive animal lines results in host genetic homogeneity, which can artificially reduce GM diversity in these populations relative to those in the wild [15, 16]. As a result, it is unclear whether the relationships between GM variation and host health that are observed in captivity are representative of those that arise in wild populations. Additionally, whether GM variation is linked to host fitness components, such as survival, in wild populations is largely unknown, meaning that we have a relatively poor understanding of the evolutionary significance of the GM.

The emergence of high-throughput sequencing technologies, in combination with the development of effective, non-invasive sampling techniques, has resulted in a recent proliferation of studies investigating the GM of wild animals. Several studies have demonstrated interspecific differences in

GM characteristics [17–19], and also variation between groups [20–22] or individuals [23–25] within the same natural population. Most of these studies have focussed on investigating the drivers of intraspecific variation in the bacterial component of the GM, identifying a suite of environmental factors that can influence this, including habitat quality and dietary differences [24–26], as well as host-related traits, such as age, sex, and host genotype [23, 27, 28]. However, very few studies have investigated the consequences of individual GM variation for host health and fitness (survival and/or reproductive success) in natural populations.

A small number of studies have explored interactions between GM composition and host infection in the wild [25, 29, 30], however, there is conflicting evidence over whether, and how, GM diversity influences host condition. For example, one study on great tits (*Parus major*) demonstrated a positive relationship between GM species richness and nestling body mass [31], while other studies on the same (and other) species have shown the opposite effect, suggesting that there could be costs to maintaining a diverse microbiome in young birds [32, 33]. We are only aware of two studies that have investigated the link between GM characteristics and survival in wild vertebrate species. In great tit nestlings, a negative, time-lagged association between GM alpha diversity and body mass was identified; while, separately in the same study, a reduced body mass was associated with a lower probability of successful fledging [33]. Similarly, in a study on adult blue tits (*Cyanistes caeruleus*), reduced species richness and the presence of pathogenic *Campylobacter* species in the GM was associated with reduced annual survival, although gel electrophoresis was used to assess bacterial community structure in this instance, which limited further resolution [34]. Further studies that investigate associations between GM and fitness components across different life stages, and in other host species, are essential if we are to understand the evolutionary and ecological importance of the GM.

The main barrier to studying fitness in natural systems is the need for detailed longitudinal monitoring of individuals and accurate measures of fitness components. This requires accurate and complete parentage assignment and that measures of survival are not confounded with individual dispersal

away from the study site. The Seychelles warbler (*Acrocephalus sechellensis*) - an insectivorous passerine endemic to the Seychelles Archipelago - provides an excellent model system for studying fitness in the wild. The entire warbler population on Cousin Island has been intensely monitored since 1985, with the majority of individuals colour ringed (> 96% since 1997), enabling comprehensive longitudinal monitoring of behaviour, annual fitness, and life-history parameters [35–37]. The population is closed, with virtually no inter-island movement, meaning that accurate measures of survival can be achieved [38]. Since 2017 a non-invasive method of sampling the Seychelles warbler GM, via the collection of faecal matter, has been routinely used [39]. Amplicon sequencing of the bacterial component of samples taken across three breeding seasons has previously demonstrated that GM diversity varies substantially across individuals within the Cousin population, and is associated with host immunogenetic variation, age, and seasonal differences [39].

Here, we use faecal samples taken across six consecutive breeding seasons to investigate whether GM variation is linked to individual differences in condition and survival in the Seychelles warbler. First, we assess whether variation in GM alpha diversity and composition is associated with body condition. Bacterial alpha diversity may be positively associated with body condition, for example if greater species richness translates into increased functional capacity and resource availability for host growth and immunity [31]. Alternatively, high GM diversity may be costly to maintain [32] or, more rarely, be indicative of poor health and GM instability [40] and thus, may be negatively associated with body condition. Specific bacterial taxa may also enhance or reduce host condition, depending on the extent to which they are beneficial or pathogenic to the host [6]. Second, we test whether GM variation is associated with individual survival. We hypothesise that high GM diversity, as a general cause and consequence of good health in captive systems [4, 9], will be associated with a greater probability of survival to the next breeding season. However, the opposite relationship could also occur, if there are costs associated with maintaining a diverse microbiome. We also expect GM composition to differ between individuals that survived compared to those that die by the next breeding season if this corresponds to altered levels of pathogenic or beneficial bacterial families.

Methods

Study species and sample collection

The study was carried out on the population of Seychelles warblers inhabiting Cousin Island (29 ha; 04°20' S, 55°40' E). This stable population consists of *ca* 320 adult individuals [41], nearly all of which (> 96%) have been ringed with a unique combination of a British Trust for Ornithology (BTO) metal ring and three plastic colour rings [42]. Seychelles warblers are long-lived, with a median life expectancy at fledgling of 5.5 years and a maximum recorded lifespan of 19 years [43, 44]. Population monitoring takes place twice a year, during the major (June-September) and minor (January-March) breeding seasons, respectively [45]. As the annual resighting probability of adult individuals is very high (98% ± 1%) [46] and inter-island dispersal is virtually absent [38], individuals not seen during a breeding season can be confidently assumed to be dead. Faecal sampling took place in the major breeding periods of 2017 - 2019, and the minor breeding periods of 2018 - 2020. As it was not possible to carry out the population census for the major breeding season of 2020 (due to the Covid-19 pandemic), information on the survival of individuals sampled in the minor breeding period of 2020 is not known.

The population of warblers on Cousin Island is structured into *ca* 115 territories which are defended year-round [41]. Seychelles warblers are insectivorous and take the majority of their insect food from leaves; thus, for each territory, an index of territory quality was calculated based on the number of insect prey available, the territory size, and foliage cover present in that breeding season [47]. For territories with missing quality measures in a season, quality was calculated as the average from the preceding and following breeding periods [as in 48].

During each breeding season, individuals were caught using mist nets and birds were weighed (± 0.1 g) using a 50 g Pesola balance. Right tarsus length (± 0.1 mm) was measured using vernier callipers. A blood sample was taken via brachial venipuncture and DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Crawley, UK) according to the manufacturer's instructions. Molecular sexing

was subsequently carried out using a PCR-based method [44, 49]. Each individual was classified into one of the following age classes based on a combination of hatch date, behavioural observations, and eye-colour, which changes from grey in fledglings to red-brown in adult individuals [47]: nestling (in the nest), fledgling (1-3 months), old fledgling (3-5 months, and less reliant on parents for food), sub-adults (5-12 months), or adults (>12 months).

To sample the GM, captured birds were placed into a disposable, flat-bottomed paper bag containing a sterilised weigh boat protected by a metal grate; this set-up follows an established protocol [39, 50] and allows faecal matter to fall into the tray whilst minimising the possibility of contamination, for example, from the bird's surface. Each bird was removed from the bag after defecation (or after 30 minutes). A sterile flocked swab was used to collect faecal samples into a sterile microcentrifuge tube containing 1 ml of absolute ethanol. During sample collection, control swabs were also taken from empty collection bags and from field worker's hands to capture possible sources of contamination. All samples were stored at 4°C for the remainder of the field season, before transferring to -80°C for long-term storage. Over the course of six consecutive sampling seasons, 546 faecal samples were collected from 326 individuals on Cousin Island.

DNA extraction from faecal samples and sequencing

The DNeasy PowerSoil Kit (Qiagen) was used to extract total genomic DNA from all faecal and control samples, according to a modified version of the manufacturer's instructions [see 39]. Two negative extraction controls (using blank sterile swabs) were also carried out per extraction kit. Positive controls were extracted from a D6300 Microbial Community Standard (ZymoBIOMICS) to enable extraction quality and the reproducibility of sequencing to be established. In addition, ten randomly selected faecal samples were extracted twice to assess the repeatability of the extraction method. A Qubit dsDNA High Sensitivity Assay kit (Invitrogen) was used to quantify DNA concentration and samples were submitted for 16S rRNA gene amplicon sequencing at the NEOF Centre for Genomic Research, Liverpool. In total, 638 samples were sent for sequencing; this included 27 control samples (11 collection controls, 10 negative extraction controls, and 6 positive

192 controls) and 611 faecal samples which included 55 samples that were sequenced twice (either in the
193 same run or across different runs) as well as 10 repeat extractions. The universal primers 515F and
194 806R [51], which amplify the V4 region of the 16S rRNA gene, were used to generate amplicon
195 libraries [see 39 for details]; these were sequenced across four separate Illumina MiSeq runs.
196
197 **Data Processing**
198 Sequences were imported into QIIME2 2019.10 [52] for processing. The DADA2 plugin [53] was
199 used to truncate forward and reverse sequences at 240 base pairs, and trim 13 base pairs from the 5'
200 end of reads to remove low quality base calls. Amplicon Sequencing Variants (ASVs) were inferred
201 for each sample, followed by dereplication, paired-end joining, and the removal of chimeras. Files
202 from the four separate sequencing runs were then merged, resulting in a total of 35,905,397 reads
203 (mean per sample = 56278 ± 52,716 SD). A mid-point rooted phylogeny was constructed using
204 MAFFT [54] and the Fast Tree approach [55]. ASVs were taxonomically classified by training a
205 naïve-Bayes classifier on the SILVA reference database 132 for 16S sequences. Sequences classified
206 as chloroplast or mitochondria were removed, leaving 34,480,836 reads in total. One negative control
207 and one faecal sample contained no reads after this filtering step, leaving 636 samples in total. One
208 ASV assigned to the genus *Delftia* was removed from all samples sequenced in the first run, as it
209 made up ~90% of reads in the extraction control for this run but was largely absent from samples in
210 other runs. Similarly, ASVs assigned to the genus *Limnobacter* and the family *Veillonellaceae* were
211 also removed from samples, as these were abundant in the negative extraction controls of runs three
212 and four, respectively. All singleton reads were also removed from the dataset, as these represent
213 possible sequencing contaminants. Eight unique ASVs were identified in each of the positive control
214 samples; these corresponded taxonomically to the eight bacterial isolates making up the microbial
215 community standard. The final sample metadata, ASV and taxonomy tables were all exported from
216 QIIME2 into R 4.0.2 [56] and were further processed using *phyloseq* 1.32.0 [57]. Before conducting
217 downstream analysis, sequences were filtered to remove all non-bacterial sequences, as well as ASVs
218 that were unassigned at phylum level. Samples were further filtered to remove all control samples.
219 There were 55,664 ASVs in the remaining 610 samples. Sample completeness and rarefaction curves

were generated using the R package *iNEXT* 2.0.20, with 50 bootstrap replicates per sample [58]. Sample completeness plateaued at approximately 10,000 reads (Additional file 1: Fig S1); therefore, all samples with fewer than 10,000 reads were excluded from downstream analyses (23 samples).

Statistical analyses

Alpha diversity

All samples that remained after filtering were rarefied to a depth of 10,000 reads before calculating alpha diversity metrics, leaving 49,116 ASVs across 587 samples. The metrics Chao1 richness (estimates the number of different bacterial ASVs in a sample) and Shannon diversity (the number of ASVs and the evenness of their abundances within a sample) were calculated using *phyloseq* 1.32.0 [57]. Faith's phylogenetic diversity (PD) was calculated using *picante* 1.8.2 [59]. One sample was removed from all downstream analysis due to having an exceptionally small alpha diversity value; extraction notes suggested that this had been an unusually small sample (586 samples retained). To measure how consistent alpha diversity measurements were across different extractions and sequencing runs, pairwise Euclidean distances were calculated based on the Shannon diversity of samples that had been extracted twice, or instances where DNA from the same extraction had been sequenced twice across sequencing runs (10 and 55 samples, respectively); these distances were then compared to the pairwise distances between different individual samples using a Kruskal-Wallis test, followed by a post-hoc Dunn's Multiple Comparisons test using *FSA* 0.8.32 [60], with Benjamini-Hochberg false discovery rate correction [61].

Following this analysis, duplicate samples were filtered, such that only the sample with the highest read count was retained (leaving 527 samples). Where multiple samples had been taken from the same individual during the same catch, only a single sample was retained. Samples were prioritised if they had been taken from the sterile tray, followed by those from inside of the bag. If both samples were collected from the same location, then the sample with the highest read count was retained (470 samples were retained following filtering).

Body condition – Size-corrected body mass has been used as an indicator of body condition for several vertebrate species [62], including the Seychelles warbler [63, 64]. Thus, body mass was used as a proxy for condition in analyses. Individuals that did not have a corresponding body mass measurement taken at the time of sampling were removed from the dataset (leaving 447 samples). Nestlings were also removed due to a small sample size (12 samples), as well as individuals with a "floater" status that had no assigned territory (eight samples). Two female breeding individuals were also excluded as they were recorded as carrying eggs at the time of sampling which increased their body mass. A total of 425 samples from 296 individuals were retained in the analysis. A linear mixed effects model (LMM) was constructed using *lme4* 1.1.27 [65], with body mass as the response variable and right tarsus length as a covariate to account for structural size differences between individuals. GM alpha diversity and it's squared value, sex, age class (fledgling, old fledgling, sub-adult or adult), territory quality and the sampling field period (Major 2017, Minor 2018, Major 2018, Minor 2019, Major 2019, Minor 2020) were included as fixed effects in the model. The time of sampling (minutes from sunrise at 06:00 am) was also included as a fixed effect, as it influences body mass in the Seychelles warbler [63]. Bird ID, territory ID and observer ID were all included as random intercepts to control for the non-independence of samples. Continuous predictors were centred and scaled to a mean of 0 and standard deviation of 0.5, using *arm* 1.11.2 [66]. Separate models were run using Shannon diversity, Chao1 richness or Faith's PD as the measure of GM alpha diversity, to check if results were consistent across metrics. Chao1 richness and Faith's PD were both log-transformed in analyses to improve residual fit. Biologically relevant interactions were also included in the model but were removed sequentially (in order of least significance), followed by the squared terms, if they were not significant, to enable interpretation of the first-order effects. The R package *car* 3.0.10 [67] was used to calculate Variance Inflation Factors (VIFs); VIFs were <3 for all terms in the model. The R package *DHARMA* 0.4.1 [68] was used to carry out model diagnostics. Marginal and conditional R^2 values were calculated using the r.squaredGLMM function in the package *MuMIn* 1.43.17 [69].

Survival – The 470 samples were filtered to remove 72 samples taken in 2020 as these samples had no follow-up census in the next breeding season to assess bird survival. Where multiple samples were available from the same bird over different field periods, only the last sample was included, as there were too few individuals with multiple samples to control for pseudoreplication in the model (<30% of individuals had multiple samples once 2020 samples had been removed). Samples from nestlings and floaters were also removed, as above. A final total of 264 samples/individuals were included in the analysis (226 individuals that survived, 38 individuals that died). A Generalised Linear Model (GLM) with a binomial error structure and logit link function was constructed using stats 4.0.2 [56]. Survival to the next breeding season was included as a binary response variable (0 - died, 1 - survived). GM alpha diversity and its squared term, bird age class (fledgling, old fledgling, sub-adult, or adult), sex, and territory quality were included as independent variables. Sampling year was also included, to control for differences in survival probabilities between years [48]. VIFs were <3 for all model terms, continuous variables were centred and scaled, and interactions were removed sequentially if not significant to interpret the first-order effects.

Beta diversity analysis

The unrarefied reads (filtered to remove samples with <10,000 reads) were used. Samples were processed to remove exceptionally rare taxa that could disproportionately influence beta diversity metrics. As such, ASVs were excluded if they had fewer than 50 reads in total across all samples and/or were present in less than 2% of samples. This retained 3,057 ASVs across 587 samples. ASV abundances were then transformed using the Centered Log Ratio (CLR) transform function in *microbiome* 1.12.0 [70]. The CLR transformation produces values that are scale invariant (i.e. not influenced by differences in library sizes across samples) and controls for the compositional nature of microbiome datasets [71]. The consistency of beta diversity estimates across different extractions and sequencing runs was assessed as described for Shannon diversity, but using pairwise Euclidean distances calculated using the CLR transformed ASV abundances.

Body condition - Samples were filtered as above (see alpha diversity analysis), leaving a total of 425 samples from 296 individuals. A Principal Components Analysis (PCA) was carried out using a Euclidean distance matrix calculated from the CLR-transformed ASV abundances. The PC1 scores, which explained 7% of the variance in GM community structure, were extracted for each sample and analysed in an LMM. The LMM was constructed with body mass as the response variable and right tarsus length as a covariate. PC1 scores were included as a fixed effect in the model, along with sex, age class (fledgling, old fledgling, sub-adult, or adult), territory quality, time of day of sampling, and the sampling field period (Major 2017, Minor 2018, Major 2018, Minor 2019, Major 2019, Minor 2020). Bird ID, territory ID and observer ID were all included as random intercepts. Interactions between PC1 scores, age and sex were also tested, but were eliminated from the model if not significant to interpret the first-order effects. A second analysis was also performed using ASV abundances that were instead transformed using the Phylogenetic Isometric Log Ratio transformation (PhILR) in the R package *philr* 1.14.0 [72]. This transformation controls for the compositionality of the data but also preserves information about the relatedness of ASVs, thus providing a phylogenetically aware measure of beta diversity [72]. The PCA analysis and LMM were performed as for CLR transformed abundances, and the PC1 scores explained 21% of the variance in GM composition in this instance.

Survival - To identify compositional differences in the GM between individuals that survived versus those that died, sequencing and catch duplicates were removed, as were samples taken from nestlings, floaters and those collected in 2020. The remaining samples were also filtered to retain the latest sample per individual (as described above). Post-filtering, 264 samples containing 2,900 ASVs were retained (226 individuals that survived, 38 individuals that died). A Euclidean distance matrix was calculated using the CLR-transformed ASV abundances. Differences in GM composition across samples were then visualised via PCA. To quantify differences in beta diversity between groups of samples a Permutational Analysis of Variance (PERMANOVA) was performed using the *adonis2* function within the R package *vegan* 2.5.7 [73, 74], with 9,999 permutations. Survival to the next breeding season (yes, no), individual age class (fledgling, old fledgling, sub-adult, adult), sex, and the

corresponding sampling field period (Major 2017, Minor 2018, Major 2018, Minor 2019, Major 2019) were included as predictors. The function *betadisper* was used to check for the homogeneity of group dispersion values [73, 74]. Pairwise PERMANOVA analyses were conducted using pairwiseAdonis 0.0.1 [75] - the Benjamini and Hochberg method [61] was used to correct *P* values for multiple testing as part of this method. A second analysis was also performed using a Euclidean distance matrix calculated using PhILR-transformed ASV abundances to assess whether communities were phylogenetically distinct.

To establish whether specific ASVs were differentially abundant across groups of individuals, an Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) was carried out, using the *ANCOMBC* 1.1.5 package in R [76]. Differential abundance was tested between groups of individuals that survived, versus those that died, whilst controlling for age class, sex and sampling season. As part of ANCOM-BC, the Benjamini and Hochberg method was used to correct *P* values for multiple testing [61]. A cut-off of $P_{adj} < 0.05$ was used to assess significance.

Results

Gut microbiome variation

Following the removal of control samples and those that had fewer than 10,000 reads, the number of high-quality reads per sample ranged from 10,979 to 744,600, across 586 samples. Reads were clustered into 55,664 ASVs, with a mean of 383 ± 277 (SD) ASVs per sample. Both alpha and beta diversity metrics showed high levels of similarity across extraction and sequencing repeats, with pairwise Euclidean distances between samples extracted and/or sequenced twice being significantly lower than those measured between pairs of different samples ($P_{adj} < 0.001$ in Dunn's multiple comparison tests, Additional file 1: Fig S2). Following the removal of extraction, sequencing, and catch duplicates, 470 samples remained which contained a mean of 368 ± 253 (SD) ASVs per sample after rarefying to 10,000 reads.

Consistent with a previous study on the Seychelles warbler [39], faecal samples were dominated by the phyla *Proteobacteria* (mean relative abundance = 43% ± 24% SD), *Firmicutes* (26% ± 24%), and *Actinobacteria* (15% ± 13%). However, despite the dominance of these taxa, there was also substantial inter-individual variation in GM composition across all age-classes (Fig 1). For full details of the core microbiome see [39].

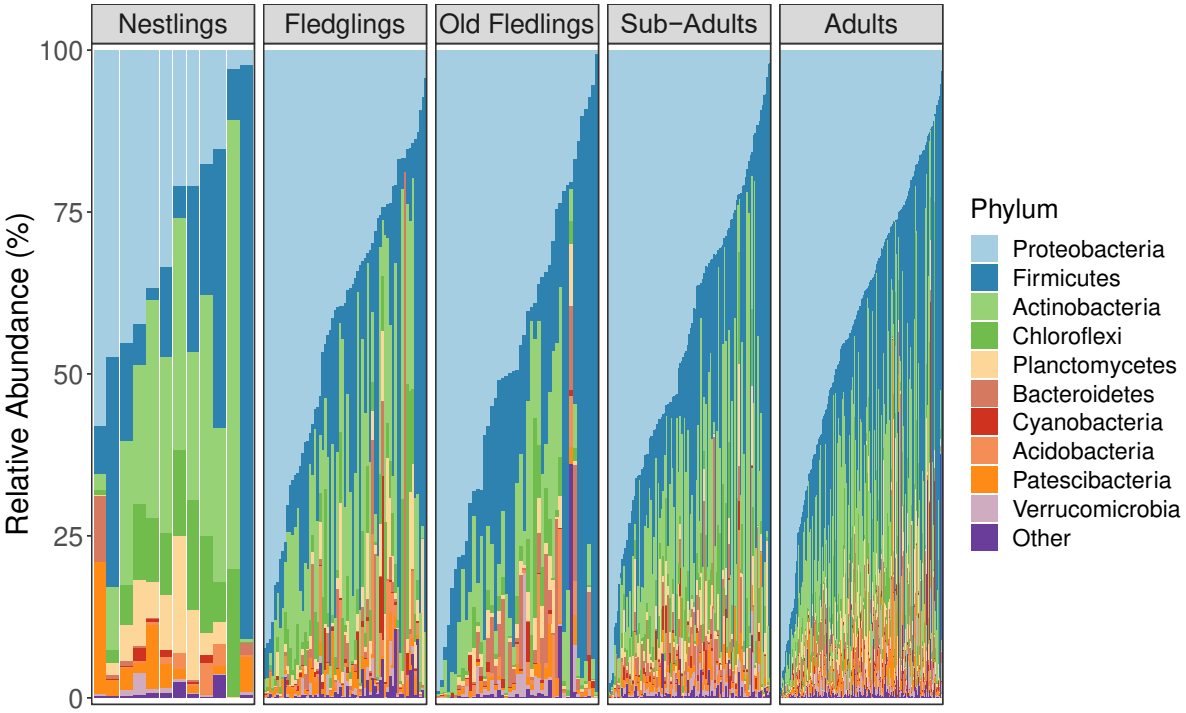


Figure 1. The relative abundance (%) of bacterial phyla in Seychelles warbler gut microbiome samples. Each vertical bar represents a separate faecal sample. N = 470 samples from 370 individuals in total: nestlings = 12, fledglings = 65, old fledglings = 45, sub-adults = 107 and adults = 241 samples, respectively. Phyla with a median relative abundance of less than 1% are collapsed into the category "Other". Bars are categorised by the individual's age class at time of sampling.

Gut microbiome diversity and body condition

There was no significant association between the alpha diversity of GM samples (Shannon diversity) and individual body condition, measured as size-corrected body mass ($P = 0.450$, Additional file 1:

Table S1). Results were very similar when Chao1 richness or Faith's PD were used as the alpha diversity metric in models (Additional file 1: Table S1). As shown previously [63], body condition was significantly greater in males and adult individuals (Additional file 1: Table S1). Body condition also increased significantly over the course of the day and varied across the different sampling field periods (Additional file 1: Table S1).

A PCA analysis of CLR-transformed ASV abundances was performed to determine how GM composition differed across individuals. The PC1 axis explained 7.11% of the overall variance in bacterial community structure. PC1 scores were subsequently used in a regression analysis to establish whether GM beta diversity was associated with individual body condition. There was no significant relationship between PC1 scores and individual body condition ($P = 0.453$, Additional file 1: Table S2). This was also the case when the PC1 scores from a PCA on PhILR-transformed ASV abundances were used for analysis ($P = 0.842$, Additional file 1: Table S2); in this instance the PC1 axis explained 21.28% of the variation in GM structure. Together, these results suggest that there is no association between GM community structure and individual body condition in the Seychelles warbler.

GM alpha diversity and survival

There was no significant association between GM alpha diversity (measured as Shannon diversity) and the probability that an individual survived to the next breeding season ($P = 0.758$, Table 1). This result was robust, regardless of whether Shannon diversity, Chao1 richness or Faith's PD were used as alpha diversity metrics (Additional file 1: Table S3). Old fledglings (3-6 months old) had a lower probability of survival compared to adult individuals ($P = 0.033$, Table 1) and sub-adults ($P = 0.02$ in a Tukey's HSD post-hoc test), which is consistent with previous findings in the Seychelles warbler [48].

Table 1. A Generalised Linear Model investigating the association between gut microbiome alpha diversity (Shannon diversity) and survival in the Seychelles warbler. Significant ($P < 0.05$) predictors are shown in bold. Reference categories for categorical variables were as follows: adult (age class), female (sex) and 2017 (sample year). $N = 264$ samples/individuals were included in the analysis (226 individuals survived, 38 individuals died by the next breeding season).

Predictor	Estimate	SE	*z*	*P*
Intercept	**1.515**	**0.610**	**2.483**	**0.013**
Shannon	-0.117	0.378	-0.308	0.758
Age class				
Fledgling	-0.275	0.564	-0.487	0.626
Old fledgling	**-1.073**	**0.503**	**-2.134**	**0.033**
Sub-adult	0.954	0.583	1.637	0.102
Sex (Male)	-0.484	0.372	-1.300	0.193
Territory quality	1.246	0.699	1.782	0.075
Sample Year				
2018	0.599	0.724	0.827	0.408
2019	0.326	0.744	0.439	0.661

GM beta diversity and survival

There was a significant difference between the GM composition of individuals that survived, versus those that had died by the next breeding season, in a PERMANOVA analysis of Euclidean distances obtained using CLR-transformed ASV abundances ($F_{1,262} = 1.511$, $P = 0.004$; Fig 2, Table 2). Furthermore, analysis of PhILR-transformed abundances, which retain information on the

phylogenetic relatedness of ASVs, confirmed that GM communities were phylogenetically distinct between the two groups of individuals ($F_{1,262} = 2.397$, $P = 0.008$, Table 2, Additional file 1: Fig S3). Survival explained 0.6% and 0.9% of the variation in GM community structure across individuals in the CLR and PhILR analyses, respectively (CLR $R^2 = 0.006$ and PhILR $R^2 = 0.009$, Table 2). Importantly, betadisper tests showed that, in both cases, the PERMANOVA results were caused by differences in the mean location of samples rather than differences in GM variability between the two groups (CLR betadisper: $F_{1,262} = 0.319$, $P = 0.575$; PhILR betadisper: $F_{1,262} = 0.630$, $P = 0.429$).

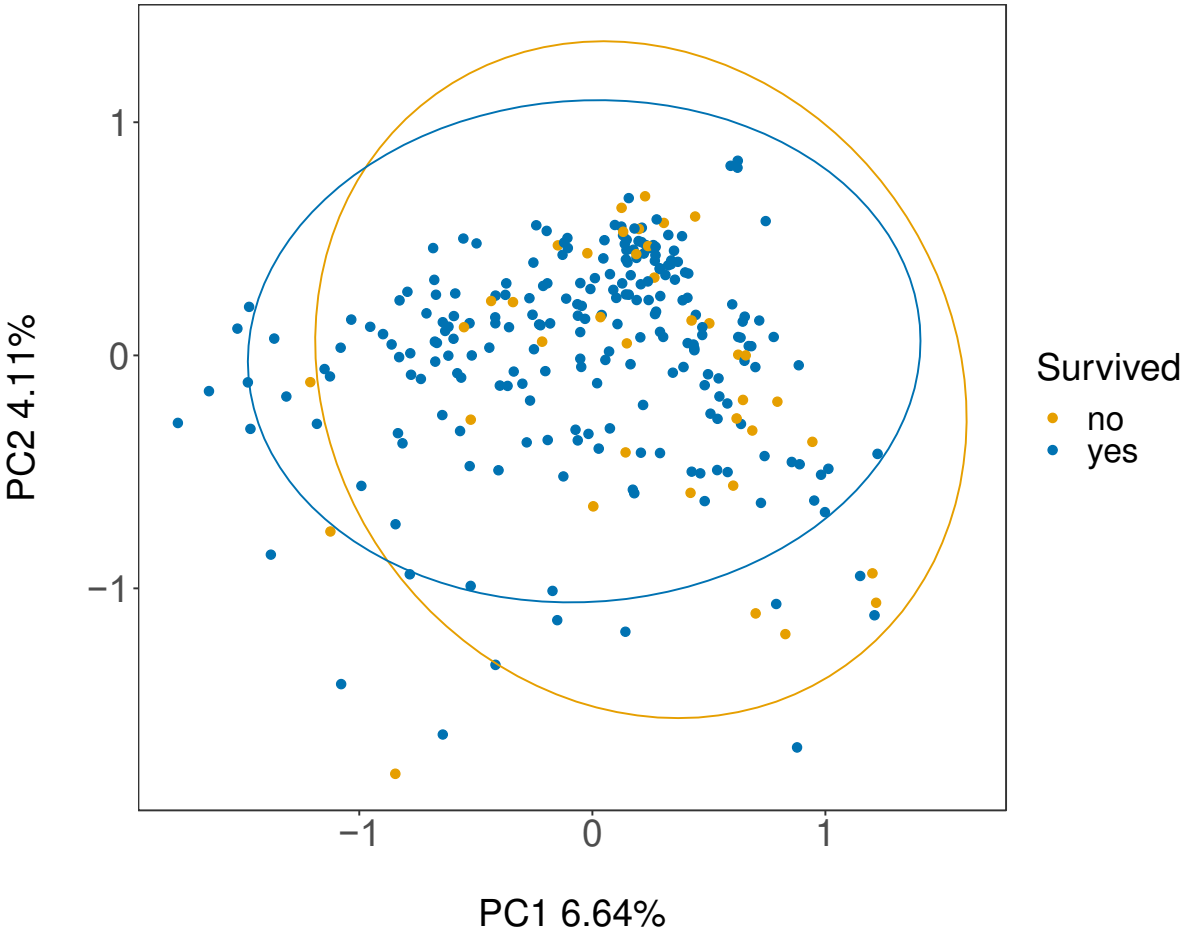


Figure 2. Principal Components Analysis (PCA) of Euclidean distances between the gut microbiomes of Seychelles warblers that survived (blue) versus those that had died (yellow) by the next breeding season. Each point represents a sample taken from a different individual. Euclidean distances are

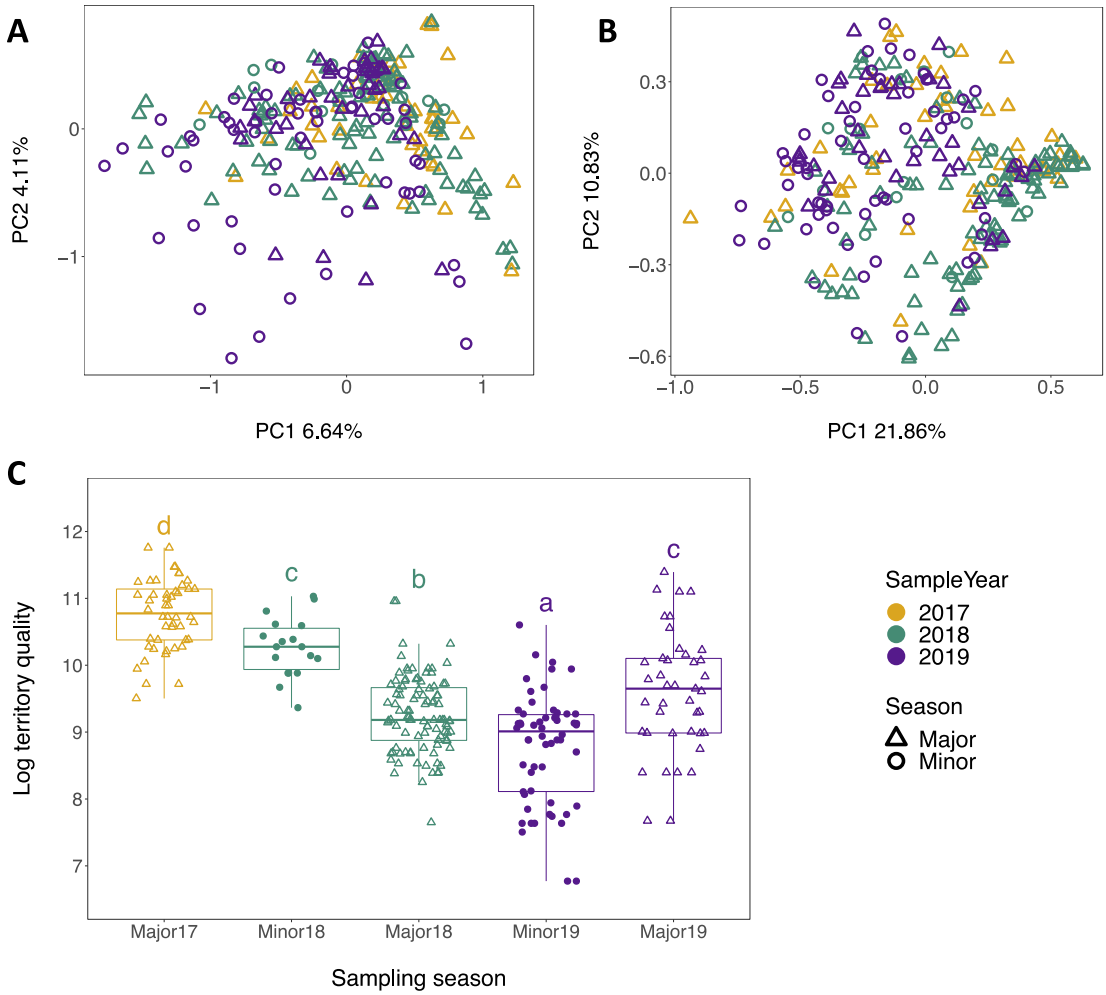
based on CLR-transformed abundances of ASVs. Ellipses denote 95% confidence intervals. Principal components one and two explained 6.64% and 4.11% of the variation in GM community structure, respectively. $N = 264$ samples/individuals were included in the analysis (226 individuals survived, 38 individuals died).

Table 2. PERMANOVA analysis of gut microbiome distances in the Seychelles warbler. Euclidean distances were calculated based on either CLR or PhILR transformed Amplicon Sequencing Variant (ASV) abundances. Significant predictors ($P < 0.05$) are shown in bold.

Predictor	*df*	R^2		*F*		*P*	
		CLR	PhILR	CLR	PhILR	CLR	PhILR
Survival	1	0.006	0.009	1.511	2.397	**0.004**	**0.008**
Age class	3	0.013	0.013	1.167	1.209	**0.032**	0.150
Sex	1	0.004	0.006	1.226	1.697	0.059	**0.048**
Sampling period	4	0.049	0.070	3.357	4.913	**< 0.001**	**< 0.001**

GM beta diversity also differed significantly between individuals in different age classes, with age class explaining 1.3% of the variation in GM composition across individuals ($R^2 = 0.013$, Table 2). However, this was only when CLR- (and not PhILR-) transformed abundances were used (CLR PERMANOVA: $F_{3,260} = 1.167$, $P = 0.032$; PhILR PERMANOVA: $F_{3,260} = 1.209$, $P = 0.150$, Table 2), indicating that although GM composition differed, the bacterial communities weren't phylogenetically distinct across the different age classes. A post-hoc pairwise PERMANOVA analysis using the CLR-transformed abundances indicated that fledglings had a significantly different GM composition compared to old fledglings, sub-adult and adult birds ($P_{adj} < 0.05$, Table S4). A betadisper analysis indicated that the differences identified in CLR PERMANOVAs could be at least

446 partially driven by differences in GM variability across age classes (CLR betadisper: $F_{3,260} = 3.532$, P
447 = 0.015, Additional file 1: Fig S4). Indeed, a PCA analysis demonstrated that although clusters
448 overlapped for all age classes, the GM of sub-adults had slightly greater levels of variation overall
449 compared to other age classes (Additional file 1: Fig S4). In addition to age class, host sex had a
450 marginally significant effect on GM phylogenetic structure (PhILR PERMANOVA: $F_{1,262} = 1.697$, P
451 = 0.048, Table 2), although it explained less than 0.4% of the variation in GM beta diversity (R^2 =
452 0.004, Table 2).
453
454 The beta diversity of GM samples differed significantly across sampling periods (Fig 3, Table 2 &
455 Additional file 1: Table S4). Post-hoc pairwise tests confirmed that the GM was compositionally
456 distinct between all sampling periods when analysing CLR-transformed ASV abundances (Fig 3A,
457 Additional file 1: Table S4). Similarly, the phylogenetic structure of GM samples differed
458 significantly across sampling periods, with the exception of the major period of 2017 and the minor
459 period of 2018 which were phylogenetically similar (Fig 3B, Additional file 1: Table S4). Differences
460 in GM variability were identified across the different sampling periods when analysing CLR-
461 transformed abundances (CLR betadisper: $F_{4,259} = 3.109$, $P = 0.017$, Additional file 1: Fig S5); there
462 was a slight increase in variability in the minor sampling period of 2019 and lower variability in the
463 major sampling period of the same year (Additional file 1: Fig S5). Interestingly, although mean
464 territory quality differed significantly across all consecutive sampling periods ($P < 0.01$ in a Tukey's
465 HSD posthoc tests, Fig 3C), it was lowest in the minor period of 2019 (Fig 3C). None of the sampling
466 periods were phylogenetically more variable than others (PhILR betadisper: $F_{4,259} = 1.542$, $P = 0.187$).
467 Sampling period explained the largest amount of variation in GM composition across individuals (up
468 to 7% of the total variation), with all other variables explaining a smaller proportion of the overall
469 variance (Table 2).
470
471



472

473 **Figure 3.** Variation in gut microbiome composition across sampling periods in the Seychelles
474 warbler. Principal Components Analysis (PCA) of Euclidean distances calculated using **A)** CLR-
475 transformed ASV abundances or **B)** PhILR-transformed abundances. Each point represents a GM
476 sample taken from a different individual (N = 264 birds). Principal components one and two
477 explained 6.64% and 4.11% of the variation in GM structure in the CLR analysis, and 21.86% and
478 10.83% in the PhILR analysis, respectively. The bottom panel **(C)** shows the territory qualities
479 recorded in each of the field periods (N = 264 territory qualities measured from 94 territories). The
480 boxes span the interquartile (25%-75%) range and the median is marked by a horizontal line.
481 Whiskers extend to 1.5 times the interquartile range. Significant differences in mean territory quality
482 between sampling seasons are represented by different letters ($P < 0.01$ in a Tukey's HSD post-hoc
483 tests). Points are shaped by season (major or minor) and coloured by sampling year.

484

485

486 **Bacterial taxa associated with survival**

487 There were 15 bacterial ASVs that were significantly (P_{adj} < 0 .05, ANCOM-BC) differentially
488 abundant between the GM of individuals that survived versus those that died by the next breeding
489 season (Fig 4, Additional file 1: Table S5). Of these, seven ASVs were significantly more abundant in
490 individuals that survived (Fig 4, Additional file 1: Table S5). These were members of two phyla,
491 namely *Proteobacteria* (four ASVs) and *Firmicutes* (three ASVs). The enriched *Proteobacteria*
492 included one ASV from the order *Desulfovibrionales* (family *Desulfovibrionadaceae*, genus
493 *Desulfovibrio*), two ASVs in the order *Rhizobiales* (family *Rhizobiaceae*, genus *Bartonella)* and one
494 in the order *Rhodospirillales* (family *Rhodospirillaceae*, genus *Pararhodospirillum*) (Additional file
495 1: Table S5). All enriched ASVs in the phylum *Firmicutes* were in the order *Clostridiales*; at the
496 family level, these ASVs were classified as being in the *Lachnospiraceae* (genus *Robinsoniella*),
497 *Ruminococcaceae* (uncultured genus) and *Clostridiales* family XIII (genus *Anaerovorax*) (Additional
498 file 1: Table S5).

499

500 The eight ASVs that were identified as being more abundant in individuals that had died by the
501 following breeding season belonged to three different phyla, namely *Chloroflexi* (four ASVs),
502 *Actinobacteria* (three ASVs) and *Proteobacteria* (one ASV) (Fig 4, Additional file 1: Table S5). All
503 ASVs in the phylum *Chloroflexi* were uncultured members of the bacterial order *Thermomicrobiales*
504 (Additional file 1: Table S5). The enriched Actinobacterial ASVs were classified in the order
505 *Propionibacteriales* (genus *Friedmanniella*), *Solirubrobacterales* (uncultured genus) and
506 *Pseudonocardiales* (genus *Actinomycetospora*), respectively (Additional file 1: Table S5). The
507 enriched ASV in the phylum *Proteobacteria* was classified as a member of the order *Rhizobiales*
508 (genus *Aureimonas*) (Additional file 1: Table S5).

509

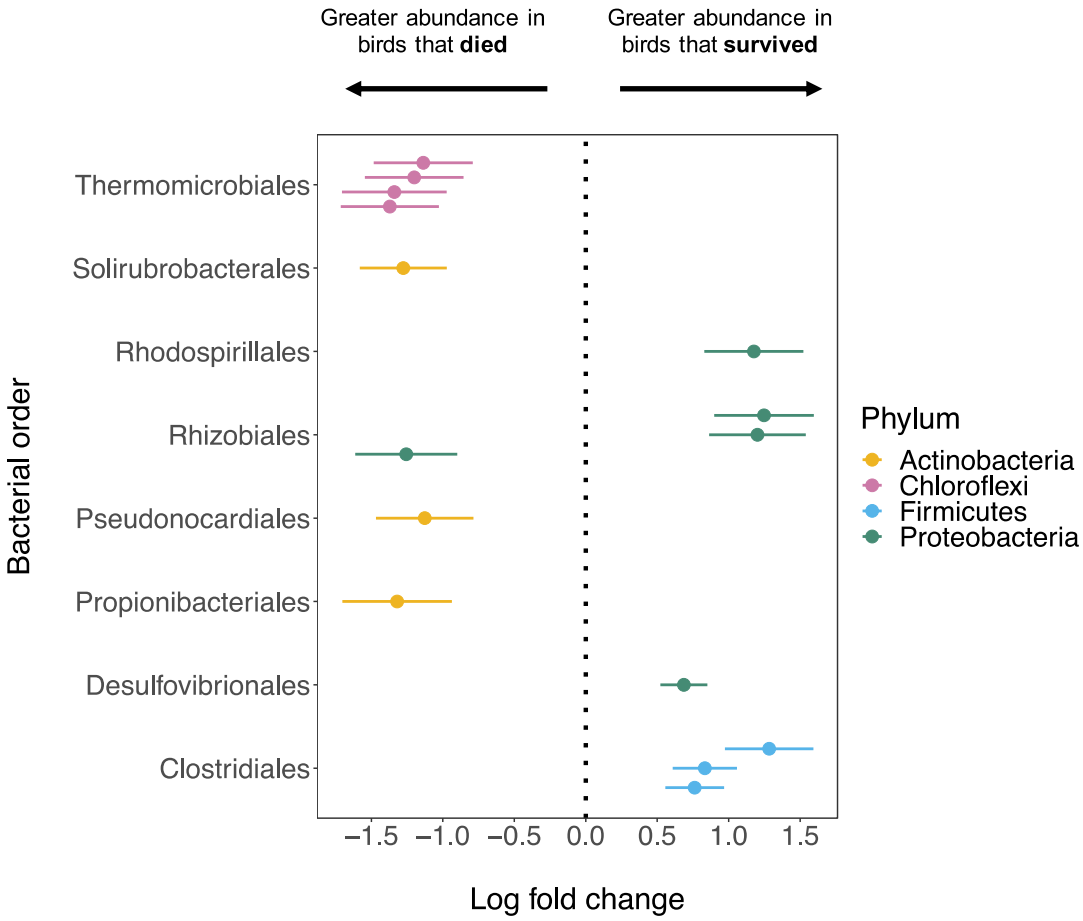


Figure 4. Differentially abundant Amplicon Sequencing Variants (ASVs) in the gut microbiome of Seychelles warblers that survived, versus those that died by the next breeding season. Points represent the log fold change (effect size) of individual ASVs - only those with significant effect sizes (P_{adj} < 0.05) are shown. A positive log fold change indicates that an ASV is more abundant in individuals that survived (right), and a negative log fold change indicates a higher abundance in individuals that died by the next season (left). Bars represent 95% confidence intervals derived from the ANCOM-BC model. ASVs are classified by bacterial order on the y-axis and are coloured by phylum. Results of differential abundance tests and ASV taxonomies are presented in full in Table S5.

Discussion

In this study, we use data from a closed, island population of Seychelles warblers, to investigate the association between GM variation, host condition, and survival. Results show that there is substantial variation in GM diversity across individuals within the population on Cousin Island. While there was no association between GM alpha diversity and body condition or survival, we did identify significant differences in GM composition between individuals that survived, versus those that died by the next breeding season, with several bacterial taxa being differentially abundant between the two groups. The composition of the GM was also associated with seasonal variation and, to a lesser extent, with an individual's sex and age.

Gut microbiome diversity and body condition

Previous studies investigating the relationship between GM alpha diversity and individual condition have shown mixed results, with both positive [23, 31, 77] and negative [32, 33, 78] relationships, as well the absence of an association [79, 80] being identified across various wild and captive vertebrate host species. However, these studies often focus on early life stages, despite the fact that body mass can be an important predictor of fitness in adult individuals [63, 81, 82]. Additionally, several of these studies used antibiotics to artificially alter GM diversity [32, 78] and so it is unclear how well these relationships hold in natural, unmanipulated populations. We found no relationship between body condition and GM alpha diversity in the Seychelles warbler, sampled across four different age classes post-fledging. Furthermore, the relationship between GM beta diversity and body condition was not significant, although we acknowledge that beta diversity, in this instance, was measured as the principal component scores from a PCA, which themselves only explain a portion of the microbial community structure (7.11%). Additionally, there was no relationship between GM beta diversity and body condition when taking the phylogenetic relatedness of ASVs into account; in this instance the principal component scores explained ~20% of the community structure. This suggests that individuals with different body condition did not carry consistently different, or phylogenetically distinct, bacterial communities.

There are several possible explanations for the lack of an association between GM characteristics and body condition (measured as size-corrected body mass) in the Seychelles warbler. Birds, (and other flying organisms) are under strong selection for lower body mass to improve flight efficiency [83]; it has been suggested that this pressure may extend to the need to reduce microbial biomass in the intestinal tract [84]. Indeed, many bird species have reduced gut lengths and shorter food retention times compared to non-flying vertebrates [85]. An increased rate of intestinal paracellular absorption compensates for this by enabling greater quantities of simple nutrients to be absorbed by the bird's own cells [85]. Together, these adaptations may have reduced reliance on microbial metabolism and, consequently, the potential for bacteria in the gut to strongly influence physical traits that impact flight in birds - such as body mass [84]. This may be particularly pertinent in the Seychelles warbler as they glean insects from the undersides of leaves whilst in flight, and therefore require high flight efficiency. A study on great tits also failed to find a direct association between nestling body mass and GM alpha diversity, but identified a time-lagged relationship whereby nestling weight at day eight was negatively associated with GM alpha diversity at day fifteen [33]. It is possible that such a relationship exists in the Seychelles warbler, however a lack of faecal samples from nestlings and difficulties in catching the same individual within a short timeframe meant that it was not possible to test for this. Since individuals in ill health may also be less active, and thus more difficult to catch in mist nets, we also acknowledge that individuals in very poor condition, which may experience more extreme GM deviations, may not be represented in the dataset.

The relationship between microbial diversity metrics and emergent properties of microbial communities, such as functional capacity, productivity and stability, can be highly complex [40, 86]. For example, microbial communities with very different alpha diversities can have similar functional capacities [86]. Similarly, greater numbers of transitionary microbes could add to GM diversity but contribute very little in terms of long-term benefits to the host, such as increasing energy availability or enhancing host immunity [87]. It has been suggested that the reduced complexity and specificity of the bird digestive system, compared to mammalian species, may increase the abundance and variety

577 of transitionary gut microbes [84, 87, 88]. In support of this, a study on New Guinean birds
578 demonstrated that the GM of smaller passerine species was less stable and more heterogenous than
579 that of larger species, presumably because shorter guts and faster retention times can result in stronger
580 ecological drift and a higher turn-over of bacterial species acquired from environmental sources [89].
581 As Seychelles warblers are insectivorous, bacterial species could be readily acquired from their insect
582 prey [90] as well as from the surrounding environment. As such, differences in GM diversity across
583 individuals could potentially reflect variable uptake from these sources. The significant influence of
584 sampling period on GM composition in the Seychelles warbler and substantial variation in territory
585 qualities across seasons further indicates that this could be the case. Thus, the expectation that high
586 GM alpha diversity is beneficial is over-simplified and may not always extend from laboratory
587 studies, in which environmental conditions are highly controlled and homogenous [40, 91].
588
589 Functional redundancy can also complicate relationships involving beta diversity, since GM
590 communities with different compositions may be capable of performing the same set of functions and,
591 as such, could influence the host phenotype in similar ways [92]. Such complexities could be hidden
592 in analyses involving beta diversity metrics. An assessment of bacterial function, via metagenomic
593 sequencing, may give further insight into whether GM functional diversity, rather than differences in
594 the number or identity of species, is a more important metric for determining host condition. Other
595 measures of host condition could also be incorporated into future analyses. For example, deviations in
596 white blood cell populations have previously been used to assess host health status in a study on
597 northern elephant seals (*Mirounga angustirostris*); these were in turn linked to differences in GM
598 diversity [23]. Haematocrit (the proportion of blood comprising of erythrocytes) has previously been
599 shown to be linked to the condition and survival of Seychelles warblers [93] and, thus, could be a
600 useful alternative metric in future studies.
601
602 **GM variation and survival**

603 Consistent with the body condition analysis, we found no relationship between GM alpha diversity
604 and survival in the Seychelles warbler. However, small differences in GM composition and
605 phylogenetic structure were identified between individuals that survived to the next breeding season
606 and those that did not. Furthermore, 15 ASVs were significantly, differentially abundant between the
607 two groups.
608
609 Few studies have investigated the role that particular bacterial species play in the GM of wild
610 vertebrates and so extrapolating the function of differentially abundant taxa is often difficult and
611 highly speculative [94–96]. However, there were several differentially abundant taxa that are known
612 to be common members of the vertebrate gut and may potentially play a role in host health and
613 functioning. For example, several members of the order *Clostridiales* were more abundant in the GM
614 of individuals that survived, including species in the families *Lachnospiraceae* and *Ruminococcaceae*.
615 Members of the order *Clostridiales* are abundant in the GM of many vertebrate taxa, including other
616 insectivorous passerine species [97, 98] and have previously been linked to an increase in
617 immunological resistance to nest parasites in eastern bluebirds (*Sialia sialis*) [99]. A study on captive,
618 juvenile ostriches (*Struthio camelus*), also showed that the abundance of ASVs in this order was
619 reduced in the hindgut of diseased individuals that subsequently died, suggesting that they may be
620 linked to host health and survival [10]. Species in the order *Clostridiales* play a role in carbohydrate
621 and protein fermentation (for example during the digestion of insect prey) as well as the degradation
622 of toxic by-products from this process [97, 100]. The short-chain fatty acids produced from
623 fermentation can be directly absorbed across the intestinal wall and used as an energy source by the
624 host [94]. Butyrate is one such end-product and plays an important role in maintaining colonic health
625 in humans and other laboratory organisms [101]. A member of the genus *Desulfovibrio* was also
626 enriched in Seychelles warblers that survived. *Desulfovibrio* are sulphate-reducing bacteria that are
627 common in the human gut microbiome [102]; they consume hydrogen, which is a by-product of
628 protein fermentation and, in doing so, increase the energy yields achieved from this process [102,
629 103].
630

631 In contrast, several species in the order *Thermomicrobiales* (phylum *Chloroflexi*) were enriched in the
632 GM of individuals that died by the next breeding season. Although many species in the phylum
633 *Chloroflexi* are poorly characterised, they are distributed across a wide range of environments
634 including freshwater, brackish and marine habitats [104]. Members of this phylum have also been
635 identified at low abundances in the mammalian GM and, in some cases, have been shown to
636 proliferate in diseased humans [104, 105]. Since the ASVs in the order *Thermomicrobiales* were also
637 present at lower abundances in warbler individuals that subsequently survived to the next breeding
638 season, these ASVs may have proliferated in individuals which died shortly after sampling. However,
639 further functional characterisation will be needed to confirm the role that these bacteria play in the
640 GM of avian host species.
641
642 One member of the order *Propionibacteriales* was also more abundant in individuals that died. While
643 members of this order occur in a diverse range of habitats, and are commensals in the GM of various
644 vertebrate species, they are also facultative parasites, at least in humans [106]. Similarly, one ASV in
645 the genus *Aureimonas* (order Rhizobiales) was enriched in individuals that died; members of this
646 genus have primarily been isolated from environmental sources, but there are indications that certain
647 species can be pathogenic to humans [107, 108]. It is important to note here, that changes in ASV
648 abundances within the GM of the Seychelles warbler (e.g., increased abundances of pathogenic
649 species, or reduced abundances of beneficial species) could be causally linked to the death of
650 individuals. However, equally, observed differences could be the outcome of GM perturbations
651 resulting from a decline in health, or changes in host physiology, close to death. Thus, an enrichment
652 of certain ASVs could be a by-product of the processes linked to death, rather than a cause of death,
653 although these are not necessarily mutually exclusive. Functional evaluation of bacterial species and
654 experimental manipulation of the microbiome would be needed to confirm which of these was the
655 case.
656
657 Although there were significant differences between the GM of individuals that survived and those
658 that died, survival only explained a small percentage (0.6-0.9%) of the overall variation in GM

composition across individuals. For 28 of the individuals that died by the next breeding season, the date of GM sampling was the last time they were observed in the population. However, the remaining 16 individuals that died were observed (but not sampled) again in the same breeding season as GM sampling took place; in some cases, this was up to eleven weeks after their last GM sample was taken suggesting they had remained alive for a substantial period following sampling. Additionally, there was a median period of five months between the point when GM samples were taken, and when the population was next censused to assess survival. Thus, it is possible that some of the individuals were sampled up to five months before their point of death, when only small differences, or imbalances, in the GM may have been detectable. A study on survival in juvenile, captive ostriches (*Struthio camelus*) showed that, although there was a correlation between the diversity of the GM during the first weeks of life and the probability of survival beyond six weeks of age, the relationship was strongest in the weeks closest to death [10]. Thus, greater differences might be expected in the months or weeks immediately before death, either as a result of pathogen proliferation or further GM disruption caused by a decline in health. However, we should also acknowledge that the primary cause of death in the Seychelles warbler is largely unknown and so such a relationship may not be the case, or could be further diluted, if death was the result of stochastic events for most individuals, such as entanglement with *Pisonia* seeds or injury.

Host and environmental factors influencing GM beta diversity

In addition to survival status, other factors were also found to significantly influence GM beta diversity across Seychelles warblers in our study, including the age of the individual. Fledglings had a significantly different GM composition compared to other age classes, despite the fact that they are still reliant on food from their parents and remain in their natal territory. Development has been shown to strongly influence GM composition in humans and other primates [28, 109] and, although few studies have investigated changes in the GM across the life course of birds, several studies have identified differences between the GM of nestlings versus adult individuals [77, 110]. Sub-adult Seychelles warblers also had a more variable GM community compared to other age classes. Birds in this age class are no longer dependent upon their parents for food, but instead are learning to feed

687 themselves. They may also leave their natal territory at this point [111]. Thus, differences in their GM
688 could reflect a reduction in food quality as they become independent, or greater exposure to
689 environmental variation [39].
690
691 We also identified significant differences in GM phylogenetic structure between the sexes in the
692 Seychelles warbler. Reproductive physiology differs between male and female animals and this can
693 manifest in different GM profiles [94]. For example, the reproductive hormone testosterone is thought
694 to be an immunosuppressant [112]. As such, the concentration of circulating testosterone has been
695 shown to positively correlate with bacterial diversity and the relative abundance of *Chlamydia* species
696 in the cloacal microbiome of male rufous-collared sparrows (*Zonotrichia capensis*) [113]. Although
697 there were significant dissimilarities in GM structure between the sexes in the Seychelles warbler, sex
698 only explained a small percentage (< 0.4%) of the overall variation in GM composition across
699 individuals. The extent to which sex drives differences in the GM varies substantially across wild
700 vertebrate populations [20, 25, e.g. 114], but greater differences are often seen in highly dimorphic
701 species [23]. Male and female Seychelles warblers share the same diet and exhibit relatively low
702 levels of morphological and behavioural dimorphism, potentially explaining the relatively weak
703 contribution of sex to GM variation in this system.
704
705 Sampling period explained the largest proportion of variation (4.9-7.0 %) in GM composition across
706 the individuals sampled in this study. As territory qualities also differed substantially across
707 consecutive sampling periods, the observed differences in GM structure could be linked to variation
708 in the abundance, type, or quality of insect prey as well as climatic variables. Season has been
709 identified as an important factor driving differences in the GM of many other wild animal species
710 [24–26] and may be particularly important in avian species that have fast intestinal retention times
711 and a higher turn-over of transitionary bacterial species that are acquired from their environment [89].
712 Dietary differences have been identified as a key driver of seasonal variation in mammalian species
713 [26] and can lead to significant shifts in the GM composition of birds [97, 99, 115].
714

Conclusions

Few studies have investigated the association between the GM and fitness components in wild animal populations, yet such studies are necessary if we are to assess the evolutionary role of the GM. The Seychelles warbler represents an excellent system in which to study the relationship between GM variation and fitness, since survival and life history parameters can be accurately measured for individuals across all age classes. In our study, we show that GM variation was not associated with body condition in the Seychelles warbler. However, while GM alpha diversity was not associated with survival, we identified significant differences in the composition of the GM between individuals that survived, versus those that died. Individuals that died carried reduced abundances of potentially beneficial bacterial taxa but had greater abundances of bacterial taxa that have previously been identified as opportunistic pathogens in other systems. To our knowledge, this is the first time that GM differences associated with survival have been fully characterised for a wild vertebrate species, across multiple age groups and seasons. Future assessments of the functional diversity of the GM will be crucial for understanding the potential contribution of differentially abundant bacterial taxa to avian health. Studying the link between GM characteristics and other fitness components, such as reproductive success, will also provide further insight into the evolutionary significance of GM variation.

Declarations

Ethics approval and consent to participate

Fieldwork was carried out in accordance with local ethical regulations and agreements. The Seychelles Department of Environment and the Seychelles Bureau of Standards approved the fieldwork.

Consent for publication

Not applicable

Availability of data and material

All 16S rRNA gene amplicon sequences have been submitted to the European Nucleotide Archive (ENA) database under the study accession numbers PRJEB45408 (samples taken in 2017 and 2018) and PRJEB47095 (samples taken in 2019 and 2020).

The scripts and metadata to reproduce all analyses and figures can be accessed via the GitHub repository, https://github.com/Seychelle-Warbler-Project.

Competing interests

Not applicable

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Authors' contributions

The study was conceived by SFW and DSR. SFW, CSD, DSR and TB performed fieldwork. SFW, CSD and MEM conducted laboratory work. SFW conducted bioinformatics and statistical analyses with input from DSR. DSR, HLD, JK and TB managed the Seychelles warbler project. All authors read and approved the final manuscript.

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