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FROM THE COVER

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Microbiota composition and diversity of multiple body sites vary according to reproductive performance in a seabird

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Abstract

The microbiota is suggested to be a fundamental contributor to host reproduction and survival, but associations between microbiota and fitness are rare, especially for wild animals. Here, we tested the association between microbiota and two proxies of breeding performance in multiple body sites of the black-legged kittiwake, a seabird species. First we found that, in females, nonbreeders (i.e., birds that did not lay eggs) hosted different microbiota composition to that of breeders in neck and flank feathers, in the choanae, in the outer-bill and in the cloacae, but not in preen feathers and tracheae. These differences in microbiota might reflect variations in age or individual quality between breeders and nonbreeders. Second, we found that better female breeders (i.e., with higher body condition, earlier laying date, heavier eggs, larger clutch, and higher hatching success) had lower abundance of several *Corynebacteriaceae* in cloaca than poorer female breeders, suggesting that these bacteria might be pathogenic. Third, in females, better breeders had different microbiota composition and lower microbiota diversity in feathers, especially in preen feathers. They had also reduced dispersion in microbiota composition across body sites. These results might suggest that good breeding females are able to control their feather microbiota—potentially through preen secretions—more tightly than poor breeding females. We did not find strong evidence for an association between reproductive outcome and microbiota in males. Our results are consistent with the hypothesis that natural variation in the microbiota is associated with differences in host fitness in wild animals, but the causal relationships remain to be investigated.

KEYWORDS
bacteria, black-legged kittiwake, feathers, fitness, individual quality, microbiota, reproductive success

1 | INTRODUCTION

Host-associated bacterial communities are emerging as fundamental modulators of host phenotype (Clemente et al., 2012). The gut microbiota, for instance, can facilitate digestion, occupy the niche space of pathogens, prime the development of the immune system or influence learning and memory (McFall-Ngai et al., 2013). The microbiota is therefore likely to contribute to host reproduction and survival (Heil et al., 2019; Sison-Mangus et al., 2014), and is, thus, increasingly hypothesized to play an important role in host evolution (Bordenstein & Theis, 2015; Moeller & Sanders, 2020; Simon et al., 2019). However, in contrast to humans and several animal models (Barko et al., 2018; Raymann & Moran, 2018; Rosshart et al., 2017), the importance of the microbiota has not been well-established in wild animals. In natural populations, while a growing number of studies has associated microbiota with ecological or host traits (e.g.,

Leclaire et al., 2019; Mallott et al., 2018; Teyssier et al., 2018), direct associations between microbiota and proxies of fitness are rare (but see Ambrosini et al., 2019; Antwis et al., 2019; Stothart et al., 2021; Suzuki, 2017; Videvall et al., 2019).

Variations in fitness between hosts are expected to be associated with several characteristics of the microbiota. First, variations in fitness can stem from individual or environmental factors that displace sensitive bacterial taxa and thus produce deterministic changes in the composition of microbiota assemblage. For instance, age, immunogenetic characteristics, food availability and pollution levels can affect the ability to invest in reproduction (e.g., Ariyomo & Watt, 2012; Kowalczyk et al., 2014; Nol & Smith, 1987), and are often associated with variations in microbiota composition (Bolnick et al., 2014; Fontaine et al., 2018; Jin et al., 2017). Second, variation in fitness may be related to heterogeneity in bacterial community, if it is associated with stressors that destabilise microbial community. This hypothesis lies within the framework of the "Anna Karenina principle", which suggests that dysbiotic individuals have less stable microbiota, and thus vary more in microbial community composition, than eubiotic individuals (Ma, 2020; Zaneveld et al., 2017). For instance, decreased fitness may be related to disruption in immunity (Eraud et al., 2009; Reaney & Knell, 2010; Uller et al., 2006), which may release the constraints exerted by immunity on microbiota (Belkaid & Harrison, 2017). This is expected to produce stochastic variations in microbiota composition and to result in high interindividual variability in microbiota. Finally, although recently debated, it is widely assumed that more diversity in microbial community is better (Johnson & Burnet, 2016; Reese & Dunn, 2018). Therefore, individuals with higher fitness may be expected to have microbiota with different composition (β-diversity), lower heterogeneity (β-dispersion) and higher diversity (α-diversity) compared to individuals with lower fitness.

Most of the work on wild microbiota has focused on the gut. However, several studies have shown that other parts of the body are also inhabited by a multitude of microorganisms that are essential to the maintenance of physiological homeostasis (Dickson et al., 2016; Grice & Segre, 2011). For instance, the microbiota of the reproductive tract can affect semen quality and pregnancy outcome and thus can have major impact on host fertility (Baud et al., 2019; Green et al., 2015; reviewed in Rowe et al., 2020; Weng et al., 2014). The skin microbiota affects immune barrier response and epithelial integrity and is therefore an important modulator of skin health (Chen et al., 2018). Accordingly, in bats and amphibians, skin bacteria have a role in the defence against fungal pathogens (Harris et al., 2009; Hoyt et al., 2015). In birds, feathers harbour feather-degrading bacteria, that can impair bird thermoregulation, flight and signalling and ultimately fitness (Burtt & Ichida, 1999; Gunderson, 2008; Leclaire et al., 2014), while the uropygial secretions (also called preen secretions) can harbour bacteria with antimicrobial properties (Martin-Vivaldi et al., 2010; Soler et al., 2010) that may be crucial for protecting the embryo from bacterial infection (Martín-Vivaldi et al., 2014). To better appreciate the role of the microbiota in the evolutionary biology of the host, there is therefore a strong need for investigations on the relationship between fitness and bacterial communities harboured in various parts of the body in wild animals (Comizzoli & Power, 2019; Hird, 2017; Ross et al., 2019).

Here, we investigated associations between microbiota of multiple body sites and host reproductive outcome in the black-legged kittiwake (*Rissa tridactyla*). This colonial seagull is a sexually monogamous species (Helfenstein et al., 2004) with biparental care. Males compete for nest sites, participate in nest building, provide most of the food to females during egg formation and share incubation and parental care with their mates (Helfenstein et al., 2003; Leclaire et al., 2010). To characterize the reproductive outcome of individuals, we used two proxies: breeding status (breeders vs. nonbreeders) and an index of breeder reproductive performance that combines several traits associated with reproduction, including body condition, laying date, egg mass, clutch size and hatching success. Nonbreeding may be an adaptive strategy to offset reproductive costs on survival (Cubaynes et al., 2011) and hence maximise lifetime reproductive success (Desprez et al., 2018; Griffen, 2018). However, in kittiwakes, although there are various causes of nonbreeding (Cadiou et al., 1994; Cam et al.,1998; Danchin & Cam, 2002; Desprez et al., 2011), nonbreeders show lower survival probability than breeders (Cam et al., 1998), suggesting that skipping breeding is generally a determinant of fitness in this species, as found in common guillemots (*Uria aalge*; Reed et al., 2015) and southern elephant seals (*Mirunga leonina*; Desprez et al., 2018). We focused on microbiotas sampled during the prelaying period (i.e., the copulation and nest-building period). To characterize the microbiota, we used high-throughput sequencing and focused on multiple body sites, including the cloaca, flank feathers, neck feathers, preen feathers (i.e., the small feathers that surround the duct pores of the preen gland and that are commonly saturated with preen gland secretions), upper respiratory tract (choana and trachea), and outer bill.

2 | MATERIALS AND METHODS

2.1 | Study site

The study was carried out in 2016 and 2017 breeding seasons in a population of black-legged kittiwakes nesting on an abandoned U.S. Air Force radar tower on Middleton Island (59°26'N, 146°20'W), Gulf of Alaska. Artificial nest sites created on the upper walls were observed from inside the tower through sliding one-way windows (Gill & Hatch, 2002). This enabled us to easily capture and monitor breeders. We captured birds during the prelaying period (7 May 2016–12 June 2016 and 29 April 2017–14 June 2017; mean ± SE: 20 ± 1 days before laying, range: 42–4 days before laying). Six birds were sampled both in 2016 and 2017. To reduce interdependency (Results S1 in Supporting Information), we decided to exclude their 2016 samples from the analyses. The sample size after filtering (see below for details about filtering) is given in Table 1 (total: 94 birds in 2016 and 67 birds in 2017). At capture, the microbiotas were sampled, birds were weighed to the nearest 5 g with a Pesola scale, and

TABLE 1 Sample size per sample type. All samples were collected in 2017, except cloacal samples which were collected both in 2016 and 2017

	Nonbreeders			Breeders		
	Females	Males	Number of pairs in which the two members were captured	Females	Males	Number of pairs in which the two members were captured
Flank feathers	7	11	5	15	20	14
Neck feathers	7	10	4	14	20	13
Preen feathers	6	8	5	15	14	12
Outer-bill	6	10	4	12	18	12
Choanae	6	10	0	7	9	0
Trachea	6	10	0	7	10	0
Cloaca 2016	32	18	8	21	17	9
Cloaca 2017	11	13	5	18	21	13

head-bill length was measured to the nearest millimetre with a calliper. Studied birds were sexed based on molecular methods (Merkling et al., 2012) or sex-specific behaviours (copulation and courtship feeding) (Jodice et al., 2000).

All nest sites were checked twice daily (at 9:00 and 18:00) to record bird presence, and events such as egg laying and egg loss. When an egg was laid, it was weighed to the nearest 0.01 g with an electronic scale, and marked with nontoxic waterproof ink within 12 h of laying.

2.2 | Reproductive outcome

We defined two indices of reproductive outcome. First, we considered breeding status, that is, breeders versus nonbreeders. Individuals were considered nonbreeders when they were regularly seen occupying a nesting site, but did not lay eggs. In our study, all nonbreeders had mating partners and attempted to breed. In contrast, breeders built a nest and laid one or two eggs. All birds that were captured at the tower but never observed again were excluded from the analyses, as their breeding status was unknown since they may have bred elsewhere on the island.

Second, we focused on breeders, and defined an index of reproductive performance. We conducted a principal component analyses (PCA) on several life-history traits commonly related to individual reproductive performance: body condition, laying date, clutch size (one or two eggs), egg mass of the first-laid egg and hatching success. These measures are related to individual fitness in numerous bird species, including kittiwakes (e.g., Coulson & Porter, 1985; Moe et al., 2002; Verhulst & Nilsson, 2008). Body condition was estimated as the residuals of a linear regression between body mass and head-bill length performed within each sex. We used head-bill length, as it is known to correlate better with mass than other structural features in kittiwakes (Jodice et al., 2000). Clutch size was calculated as the number of eggs laid. Although two eggs is the typical clutch size in this population of kittiwakes (Gill & Hatch, 2002), 2016 and 2017 were two very poor breeding seasons and an unusually high number of birds laid either no eggs or a single egg. In the studied population, the mean clutch size was <1 egg in 2016 and 2017, while it was >1.3 egg from 2000 to 2015 (S. A. Hatch, personal observation). Hatching success was calculated as the proportion of eggs that hatched. The egg of two breeders was not weighed, and these two breeders were thus excluded for the analyses on reproductive performance.

Breeder reproductive performance was described by the first two principal components of the PCA (hereafter refereed as $PC1_{RepPerf}$ and $PC2_{RepPerf}$). $PC1_{RepPerf}$ and $PC2_{RepPerf}$ accounted for 35 and 24% of the variance observed among life-history traits, respectively. Individuals with higher $PC1_{RepPerf}$ were in higher body condition, laid earlier, had heavier eggs and larger clutches, and had higher hatching success (eigenvector: +0.54, −0.56, +0.60, +0.50 and +0.75). Individuals with higher $PC2_{RepPerf}$ laid earlier, but had lighter eggs and smaller clutches (eigenvector: −0.63, −0.60 and −0.54). $PC2_{RepPerf}$ was not associated with microbiota composition, dispersion or diversity in any body sites, and the results are thus not reported in the manuscript.

2.3 | Microbiota sampling

Birds were caught by the leg using a metal hook and were manipulated with new clean latex gloves, which were changed between each capture. Feather microbiotas were collected from standardized positions of the neck and flank regions (Figure S1), and from the preen tuft. For each of these body regions, we cut a few feathers using scissors and tweezers sterilized with 70% ethanol between each capture. Feathers were placed in sterile 2 ml plastic tubes. Feathers were always held with tweezers. We detected similarity in feather microbiota within pairs (see Section 3 below) and within individuals (see results S1 in Supporting Information), as well as large differences between preen feathers and other feathers (see Section 3 below), suggesting that potential transfers of bacteria between birds or within birds due to poor equipment sterilization or the gloves touching various bird body sites were low. We sampled outer bill, choanal and tracheal bacteria

using sterile plain cotton swabs (Copan Italia). We sampled cloacal bacteria by flushing the cloaca with 1 ml of sterile phosphate-buffered saline solution. This was performed by gently inserting the tip of a sterile needleless syringe 5 mm into the cloaca, injecting the saline solution and drawing it out again. We also collected control samples by collecting empty sterile tubes, blank swabs or by injecting saline solution directly into sterile tubes to control for any possible contamination coming from the manipulation or the material. All samples were stored at −20°C until molecular analyses. In 2017, all types of microbiota were sampled, while in 2016, only the cloacal microbiota was sampled. In addition, due to other experiments run during the 2017 breeding season, not all body sites were sampled on each single individual. This led to disparate numbers of samples among body sites (Table 1).

2.4 | Molecular analysis of microbiota

Because we originally wanted to study viruses in respiratory tracts, we used two different DNA extraction protocols. DNA was extracted from feather, outer bill and cloacal samples using the Qiagen blood and tissue DNA extraction kit and the standard protocol designed for purification of total DNA for gram-positive bacteria (Qiagen). In contrast, DNA was extracted from tracheal and choanal swabs using the nucleospin RNA virus kit following the manufacturer's instructions (Macherey-Nagel).

To characterize the bacterial communities present in each sample, we performed 16S rRNA amplicon high-throughput sequencing. PCR amplifications were performed in 20 μl mixtures containing 2 μl of diluted DNA extract, 1 × AmpliTaq Gold 360 master mix (Applied Biosystems), 0.5 μm of each primer and 3.2 μg of bovine serum albumin (Roche Diagnostics). PCR conditions consisted of an initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation (at 95°C for 30 s), annealing (at 57°C for 30 s) and elongation (at 72°C for 90 s). A final elongation step was run at 72°C for 7 min. We used universal primers that specifically amplified the V5–V6 region (~295 bp in length) of the bacterial 16S rRNA gene (BACTB-F: GGATTAGATACCCTGGTAGT and BACTB-R: CACGACACGAGCTGACG; Fliegerova et al., 2014). To discriminate between samples after sequencing, the 5′ end of both forward and reverse primers included a combination of two different 8-bp tags. The PCR products were purified using the MiniElute PCR purification kit (Qiagen), and then pooled (4 μl of each PCR product in the pool). Amplicons were sequenced with an Illumina MiSeq platform, using the 2 × 300-bp protocol (Fasteris SA). Samples were included in three Illumina runs: feather, bill and cloacal samples from 2017 were included in the first run, tracheal and oral microbiota were included in the second run, and cloacal samples from 2016 were included in the third run. We included sampling controls, extraction controls and PCR blank controls, as well as unused tag combinations, in the sequenced multiplexes to detect and withdraw potential contaminants (Salter et al., 2014).

We first analysed the sequence reads using the OBITools package (Boyer et al., 2016). Briefly, after assembly of paired-end reads, we assigned reads to their respective samples and excluded reads with low assembly scores or that contained ambiguous bases (i.e., "N"). We dereplicated strictly identical reads using the "obiuniq" algorithm (Boyer et al., 2016), and excluded sequences with fewer than 10 reads. We then analysed the sequences using the FROGS pipeline (Escudié et al., 2018). Briefly, amplicons were clustered into operational taxonomic units (OTUs) using the "swarm" method and an aggregation distance of 3 (Mahé et al., 2014), and chimeras were removed using VSEARCH (Rognes et al., 2016). Taxonomic assignment was then performed using blast affiliation and the SILVA v138 database (Quast et al., 2012). Finally, we used the controls to remove potential contaminants from the data set. We considered a potential contaminant each OTU for which the mean number of reads across control samples was higher than the mean number of reads across noncontrol samples (n = 125 OTUs). We also considered a contaminant each OTU for which the highest total number of reads per sample was in a control sample (n = 10 additional OTUs). We excluded samples that had fewer than 500 sequences (n = 19 samples), and removed OTUs with fewer than 50 reads (n = 1818 OTUs). We obtained mean ± SE per sample: 14,328 ± 584 sequences, 19,091 ± 1667 sequences, 16,177 ± 833 sequences, 19,522 ± 2556 sequences, 13,540 ± 1980 sequences, 21,279 ± 2137 sequences, 23,061 ± 1440 sequences in cloacal, neck feather, flank feather, preen feather, outer-bill, tracheal and choanal samples respectively.

To analyse β-diversity, we used the phylogenetic isometric log-ratio transform (PhiLR) which allows analysis of compositional data where the parts can be related through a phylogenetic tree (Silverman et al., 2017). PhiLR has been suggested to be a replacement for the weighted UniFrac distances when accounting for the compositional nature of the data is needed (Gloor et al., 2017). We calculated the relative OTU abundance per sample (also known as total sum scaling; McKnight et al., 2019). OTU sequences were aligned using Decipher (Wright, 2015). Then a maximum-likelihood tree was constructed using the package phangorn (Schliep, 2011). This tree was used as a reference from which the Euclidean distances were calculated on phylogenetic isometric log-ratio transformed OTU abundance (i.e., the PhILR transform) between samples (Egozcue et al., 2003; Gloor et al., 2017) using the package PhiLR (Silverman et al., 2017). In the PhiLR transform, we added a pseudocount of 0.1 reads.

α-diversity was measured as the weighted phylogenetic diversity (Faith's PD) based on the nonrooted phylogenetic tree and using the picante package (Kembel et al., 2010). Faith's PD is the sum of total phylogenetic length of OTUs in each sample. Before α-diversity analysis, we rarefied each sample to 5000 sequences. Results were similar when including or excluding the samples with <5000 sequences, and we report results when not excluding samples with <5000 sequences. The number of reads per sample was lower in outer-bill than in other body sites. For the analysis of α-diversity of

outer-bill microbiota, we therefore set the rarefaction threshold to 1000 sequences.

2.5 | Statistical analysis

We performed all statistical analyses using the R statistical software (R Core Team, 2020). To analyse differences in microbiota composition between breeding status (breeder vs. nonbreeder) or breeder performance ($PC1_{RepPerf}$), we performed PERMANOVAs ("Adonis2" function in the VEGAN package in R, with 5,000 permutations and the "margin" option), based on the PhILR transform distance matrix. In the models, breeding status and date of sampling, or $PC1_{RepPerf}$ and the difference between laying date and capture date, were included as predictors. To account for the nonindependence of the two members of a pair (see Section 3 below), we carried out PERMANOVAs within each sex. The two members of a breeding pair were never both sampled for tracheal or choanal microbiota, but tracheal and choanal microbiotas of females were sampled later during the breeding season than those of males (Kruskal–Wallis test: $\chi^2_1 = 10.42$, $p = .001$ and $\chi^2_1 = 9.92$, $p = .002$). Therefore, to avoid confounding the sex and date effects, we carried out PERMANOVAs within each sex for tracheal and choanal microbiota also. We did not test for associations between tracheal and choanal microbiota composition and $PC1_{RepPerf}$ as the sample size within each sex was very low (Table 1). Cloacal samples collected in 2016 were analysed in a different Illumina run compared to the cloacal samples collected in 2017. The year effect is therefore confounded with a sequencing run effect, and in the analysis of cloacal microbiota, we constrained the permutation within years of sampling.

To predict the OTUs that were best associated with breeding status and breeding performance, we used two different procedures. First, we used correlation indices with the point biserial coefficient of association (multipatt procedures in the indicspecies package in R; De Cáceres & Legendre, 2009) to determine a small set of OTUs that best characterize breeding status or breeding performance. The multipatt procedure cannot associate OTU to a continuous variable. Therefore, $PC1_{RepPerf}$ was split into two categories of equal sample size. In the multipatt analyses, we did not adjust for multiple comparisons, as the purpose of these analyses was exploratory in order to generate, rather than confirm, hypotheses (Bender & Lange, 2001; Ranstam, 2019). We also used the selbal procedure, which compares the average abundance of two groups of microbial species (selbal package in R) (Rivera-Pinto et al., 2018). In the multipatt and selbal procedures, we considered OTUs that were present in at least 10% of the individuals and whose the mean relative abundance was >0.025% across samples. Correlation indices and Selbal cannot be compared as they rely on two different concepts. In correlation indices, microbial signature is defined as a combination of individual taxa, while in selbal, it is defined as a compositional balance between two groups of taxa.

To determine whether α-diversity varied with breeding status or breeder performance, we used linear mixed models with reproductive outcome (breeding status or $PC1_{RepPerf}$), sex, date (sampling date or the difference between laying date and sampling date), year (for the analysis on cloacal microbiota only) and the interaction between reproductive outcome and sex as fixed effects and we included nest identity as a random factor. For tracheal and choanal microbiota, we used linear models. Normality of the residuals was checked using Shapiro-Wilk normality tests. Homogeneity of variance was checked visually (for continuous variables) or using Levene's tests (for dichotomous variables). α-diversity in cloacal microbiota was square-root transformed in the analyses of breeding success.

Dispersion in microbiota composition was calculated as the average distance to group centroids using the "betadisper" function in the VEGAN package. We studied both interindividual dispersion within each body site and intraindividual dispersion across body sites. For the calculation of interindividual dispersion, groups were defined according to breeding status or hatching success (i.e., a categorical variable related to $PC1_{RepPerf}$). The association between interindividual dispersion and breeding status or hatching success was tested using permutation tests (permutest function in the VEGAN package in R). These interindividual dispersion analyses were also needed to judge the adequacy of PERMANOVA results, as PERMANOVA tests may confound location and dispersion effects, especially for unbalanced designs (Anderson & Walsh, 2013).

For the calculation of intraindividual dispersion across body sites, each group was defined as a bird individual. Because not all body sites were sampled on each single individual, we restricted the intraindividual dispersion analyses on individuals for which cloaca, flank feathers, neck feathers, preen feathers and outer-bill were sampled ($n = 36$ and 21 individuals for the analysis on breeding status and reproductive performance respectively). We did not include the tracheal or choanal samples, as it would drop the sample size to fewer than nine individuals. The association between intraindividual dispersion and breeding status or $PC1_{RepPerf}$ was tested using Kruskal-Wallis tests or Spearman correlation tests.

To determine whether microbial composition varied with body sites (i.e., cloaca, flank feathers, neck feathers, outer bill, preen feathers, trachea and choanae) and bird identity, we performed PERMANOVAs, with body site, bird identity, and date as predictors. We restricted the analyses to the 2017 samples. We did not include sex in the models as PERMANOVAs carried out within body sites (see below) did not show significant differences in microbiota composition between males and females (see Section 3 below). Pairwise post-hoc PERMANOVAs with Bonferroni correction were carried out to verify that all body sites were different from one another. To determine the OTUs that were preferentially associated with each body site, we used correlation indices with duleg option =FALSE to consider group combinations (De Cáceres et al., 2010). We restricted these correlation index analyses on OTUs whose mean abundance was at least >5% in one of the body sites ($n = 15$ OTUs).

Because the two members of a pair share the same nesting environment, preen each others, share food and copulate, microbial composition may be more similar within pairs than between pairs.

For each body site, we therefore tested similarity in microbiota composition between the two members of a pair using PERMANOVAs with nest identity and date as predictors. We constrained the PERMANOVA permutations within breeding status. We restricted the analyses on pairs in which both members were captured. Similarity between mates in tracheal and choanal microbiota could not be tested as the two members of a pairs were never both captured. To test differences between males and females in microbiota composition, we carried out PERMANOVAs within body sites with sex and date as fixed effects and constrained the permutations within each nest.

3 | RESULTS

3.1 | Association between microbiota and breeding status

The composition of cloacal microbiota varied with breeding status in females ($F_{1,79} = 1.74$, $R^2 = .02$, $p = .025$; Figure 1a), and was marginally associated with breeding status in males ($F_{1,66} = 1.58$, $R^2 = .02$, $p = .053$; Figure S2a). Correlation indices did not identify many OTUs as being characteristics of nonbreeders or breeders in cloaca (Table S1) and the discrimination value of selbal is relatively low (Figure S3), suggesting that the cloacal microbiota of breeders and nonbreeders cannot be well-discriminated using a few OTUs only.

The composition of flank feather microbiota and neck feather microbiota varied with breeding status in females ($F_{1,19} = 1.89$, $R^2 = .08$, $p = .004$ and $F_{1,19} = 1.62$, $R^2 = .08$, $p = .018$; Figure 1b,c), but not in males ($F_{1,28} = 1.17$, $R^2 = .04$, $p = .18$ and $F_{1,27} = 1.18$, $R^2 = .04$, $p = .13$; Figure 2b,c). Correlation indices showed that several of the OTUs best representing the differences between breeders and nonbreeders in females belonged to the families *Lachnospiraceae* and *Salinisphaeraceae* and were enriched in nonbreeders (Table S1). To determine whether, more generally, breeding status was associated with higher relative abundance of *Lachnospiraceae* and *Salinisphaeraceae*, we summed the relative abundance of all *Lachnospiraceae* ($n = 16$ OTUs) or *Salinisphaeraceae* OTUs ($n = 12$ OTUs) within each sample and carried out Kruskal–Wallis tests. We found that, compared to breeding females, nonbreeding females had higher abundance of *Lachnospiraceae* in flank feathers ($\chi^2_1 = 12.67$, $p < .001$; Figure 2a) and had marginally higher abundance of *Lachnospiraceae* in neck feathers ($\chi^2_1 = 3.43$, $p = .067$; Figure 2a). Nonbreeding females had also higher abundance of *Salinisphaeraceae* in flank and neck feathers ($\chi^2_1 = 6.26$, $p = .012$ and $\chi^2_1 = 4.36$, $p = .037$ respectively; Figure 2b).

The composition of outer-bill and choanal microbiota varied also with breeding status in females ($F_{1,16} = 1.72$, $R^2 = 0.10$, $p = .013$ and $F_{1,10} = 1.89$, $R^2 = 0.14$, $p = .035$; Figure 1d,e), but not in males ($F_{1,26} = 0.87$, $R^2 = .03$, $p = .69$ and $F_{1,17} = 0.85$, $R^2 = .05$, $p = .71$; Figures S2d,e). Indicator value showed that the some of the OTUs best representing the differences between breeders and nonbreeders in the outer-bill microbiota of females belonged to the genus *Salinisphaera* (enriched in nonbreeders; Table S1). Indicator value showed that the OTUs best representing the differences between breeders and nonbreeders in choanal microbiota belonged to diverse families (Table S1), but interestingly, both indicator value and selbal identified *Pasteurellaceae* OTUs as being enriched in nonbreeders compared to breeders (Table S1). However, in females, interindividual dispersion in outer-bill and choanal microbiota was higher in nonbreeders than breeders (see below; Figure 1d,e). These differences in dispersion might thus have contributed to the significant differences obtained in PERMANOVAs (Anderson & Walsh, 2013), especially for outer-bill

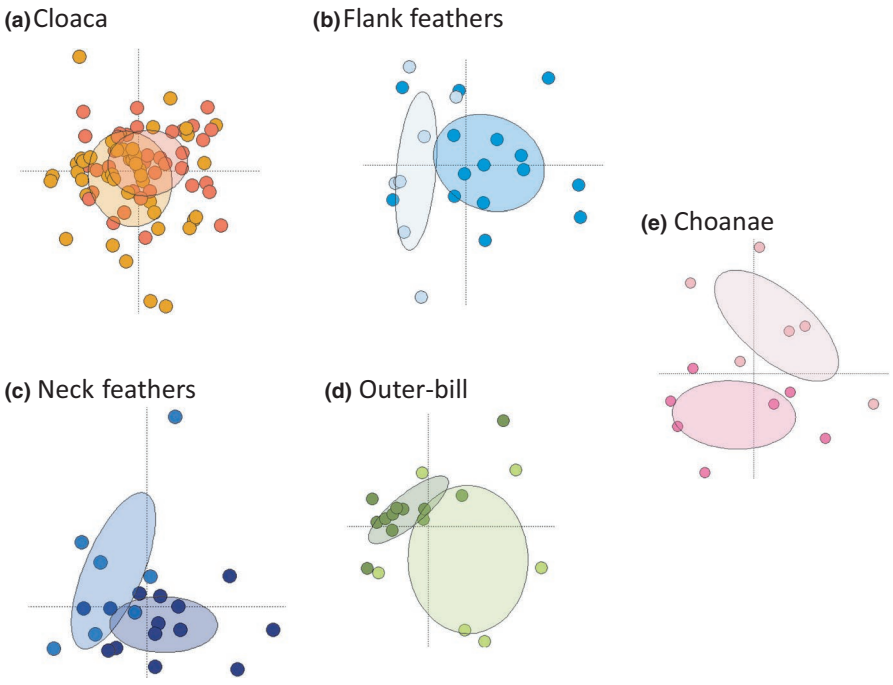

FIGURE 1 Unconstrained distance-based redundancy analysis plots showing differences between nonbreeders and breeders in the microbiota of (a) cloaca, (b) flank feathers, (c) neck feathers, (d) outer bill and (e) choanae in females. In each plot, the lighter colour represents nonbreeders, while the darker colour represents breeders. For all microbiota shown, breeding and nonbreeding females had different bacterial composition (all $p < .035$)

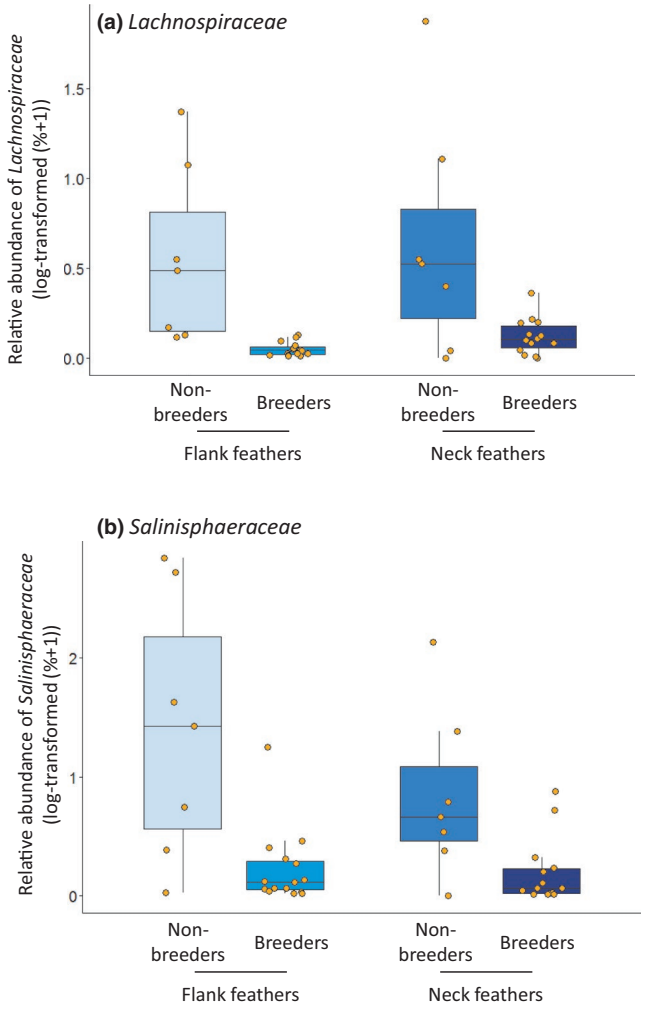

FIGURE 2 Boxplots showing the relative abundance of the operational taxonomic units belonging to the families (a) *Lachnospiraceae* and (b) *Salinisphaeraceae* in the flank and neck feathers of nonbreeding and breeding females. Compared to breeding females, nonbreeding females had higher abundance of *Lachnospiraceae* in flank feathers ($\chi^2_1 = 12.67$, $p < .001$) and *Salinisphaeraceae* in flank and neck feathers ($\chi^2_1 = 6.26$, $p = .012$ and $\chi^2_1 = 4.36$, $p = .037$). They tended also to have higher abundance of *Lachnospiraceae* in neck feathers ($\chi^2_1 = 3.43$, $p = .067$)

whose the sample size was not balanced for (six nonbreeding females and 12 breeding females). Although plots (Figure 1d,e) suggest that both composition (group centroid) and dispersion were different between breeding and nonbreeding females, further studies including larger sample size are required to ascertain the effect of breeding status on outer-bill and choanal microbiota composition in females.

The composition of tracheal and preen feather microbiota did not vary with breeding status in males and females (trachea: $F_{1,18} = 1.34$, $R^2 = .07$, $p = .15$ and $F_{1,11} = 1.31$, $R^2 = .11$, $p = .12$; preen feathers: $F_{1,20} = 1.15$, $R^2 = .05$, $p = .19$ and $F_{1,19} = 1.18$, $R^2 = .06$, $p = .15$).

α-diversity did not vary with breeding status in any of the microbiota types (all $p > .14$). In flank feathers, however, α-diversity was marginally associated with breeding status in interaction with sex ($F_{1,27} = 3.92$, $p = .058$). However, when considering each sex separately, α-diversity was significantly different between nonbreeders and breeders neither in males (mean ± SE: 47.5 ± 3.4 vs. 52.7 ± 2.4, respectively; $F_{1,28} = 1.74$, $p = .20$), nor in females (mean ±SE: 54.8 ± 2.0 vs. 49.4 ± 3.6, respectively; $F_{1,19} = 0.69$, $p = .42$).

In females, interindividual dispersion in outer-bill and choanal microbiota was higher in nonbreeders than breeders ($F_{1,16} = 7.81$, $p = .013$ and $F_{1,11} = 7.43$, $p = .016$; Figure 1d,e). Interindividual dispersion was also found to be higher in breeding males than in nonbreeding males in preen feather microbiota ($F_{1,20} = 6.55$, $p = .016$). Intraindividual dispersion in microbiota across body sites was not different between breeders and nonbreeders either in males or in females ($\chi^2_1 = 2.15$, $p = .14$, $n = 20$ and $\chi^2_1 = 2.31$, $p = 0.13$, $n = 16$).

3.2 | Association between microbiota and breeding performance

The composition of cloacal microbiota varied with $PC1_{RepPerf}$ in females ($F_{1,36} = 2.18$, $R^2 = .06$, $p = .004$; Figure 3) but not in males ($F_{1,34} = 0.73$, $R^2 = .02$, $p = .98$). Correlation indices showed that the majority of the OTUs that best characterized $PC1_{RepPerf}$ belonged to the family *Corynebacteriaceae* and were enriched in the cloacae of females with low $PC1_{RepPerf}$ (Figure 3; Table S2). Selbal analysis identified a balance of OTU_73 (*Clostridia*) and OTU_320 (*Corynebacteriaceae*) versus OTU_12 (*Psychrobacter*) as being associated with $PC1_{RepPerf}$ in female cloaca (Table S2; Figure S4a).

The composition of preen feather microbiota varied with $PC1_{RepPerf}$ in females ($F_{1,12} = 1.61$, $R^2 = .12$, $p = .018$), while the composition of neck and flank feather microbiota was marginally associated with $PC1_{RepPerf}$ in females ($F_{1,10} = 1.37$, $R^2 = .11$, $p = .066$ and $F_{1,11} = 1.50$, $R^2 = .11$, $p = .053$). The composition of preen, neck and flank feather microbiota did not vary with $PC1_{RepPerf}$ in males ($F_{1,11} = 1.01$, $R^2 = .08$, $p = .38$, $F_{1,16} = 0.95$, $R^2 = .05$, $p = .57$ and $F_{1,16} = 0.84$, $R^2 = .04$, $p = .80$ respectively). Among the OTUs that best correlated with $PC1_{RepPerf}$ in the preen feathers of females, both indicator value and selbal analyses showed that one OTU (OTU_123; *Porphyromonas*) was enriched with low $PC1_{RepPerf}$ (Table S2; Figures S4d and S5). The composition of outer bill did not vary with $PC1_{RepPerf}$ (all $p > .69$).

In none of the body sites, interindividual dispersion in microbiota composition varied with hatching success (*all* $p > .10$). Intraindividual dispersion in microbiota decreased with $PC1_{RepPerf}$ in females ($S = 216$, $R^2 = -.80$, $p = .014$, $n = 9$; Figure 4), while it did not vary with $PC1_{RepPerf}$ in males ($S = 392$, $R^2 = -0.37$, $p = .24$, $n = 12$; Figure 4).

α-diversity of preen feather microbiota decreased with $PC1_{RepPerf}$ in both sexes ($F_{1,25} = 11.48$, $p = .002$; sex*$PC1_{RepPerf}$: $F_{1,23} = 1.16$, $p = .29$; Figure 5a). α-diversity of flank feathers decreased with $PC1_{RepPerf}$ in females ($F_{1,1} = 7.13$, $p = .020$; Figure 5b), while it decreased marginally with $PC1_{RepPerf}$ in males ($F_{1,1} = 3.70$, $p = .073$; sex*$PC1_{RepPerf}$: $F_{1,10} = 7.42$, $p = .021$; Figure 5b). α-diversity of neck feather, outer-bill and cloacal microbiota did not vary with $PC1_{RepPerf}$ in males or females (all $p > .26$).

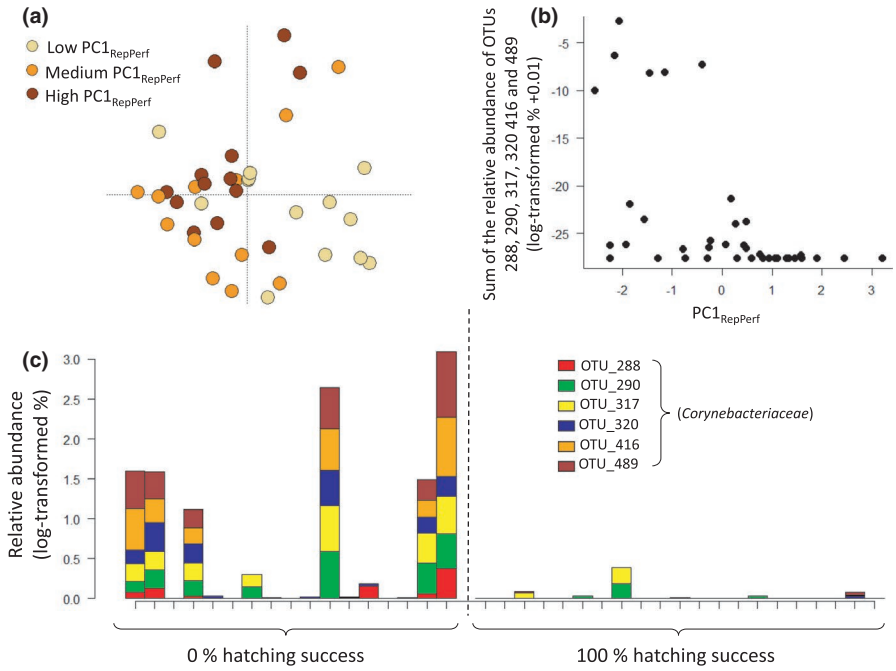

FIGURE 3 Plots showing variations in cloacal microbiota according to reproductive performance in female breeders. (a) Unconstrained distance-based redundancy analysis plots showing differences in female cloacal microbiota according to $PC1_{RepPerf}$. Female cloacal microbiota composition varied with $PC1_{RepPerf}$ in females ($F_{1,36} = 2.19$, $R^2 = .06$, $p = .004$). Although $PC1_{RepPerf}$ was analysed as a continuous variable (see text), it is displayed here as a categorical variable for illustrative purposes. We categorized $PC1_{RepPerf}$ into three groups of equal sample size. Axes 1 and 3 are shown. (b) Sum of the relative abundance of the six *Corynebacteriaceae* operational taxonomic units (OTUs) that correlated with $PC1_{RepPerf}$ with $p < .02$ in the correlation index analysis. (c) Barplot representing the relative abundance of these *Corynebacteriaceae* OTUs in each female according to hatching success

3.3 | Body-site specific microbiota and sex-variation

Composition of the microbiota varied with body sites ($F_{1,247} = 27.97$, $R^2 = .30$, $p < .001$; all pairwise comparisons: $p < .0004$, with Bonferonni-corrected $\alpha = .002$; Figure 6). When considering the OTUs with mean relative abundance > 5% in at least one body site, correlation indices showed that cloacal microbiota was characterized by high levels of two *Firmicutes*: OTU_1 (genus: *Catellicoccus*) and OTU_3 (genus: *Lactobacillus*; Figure 6). Cloacal microbiota, neck feather and flank feather microbiota were characterized by high levels of the *Fusobacteriota* OTU_16 (genus: *Cetobacterium*). Flank feather, neck feather and outer-bill microbiotas were characterized by high levels of the proteobacterium OTU_12 (genus: *Psychrobacter*), the actinobacterium OTU_21 (genus: *Arthrobacter*) and the firmicute OTU_24. Preen feather microbiota was characterized by high levels of the actinobacterium OTU_10 (genus: *Rothia*), while tracheal microbiota was characterized by high levels of the proteobacterium OTU_6 (genus: *Pasteurella*) and the *Bacteroidota* OTU_20 (genus: *Porphyromonas*) and choanal microbiota by high levels of the proteobacterium OTU_7 (genus: *Cardiobacterium*). Preen feather, tracheal and choanal microbiotas were all characterized by high levels of the *Bacteroidota* OTU_2 (genus: *Ornithobacterium*), and the *Proteobacteria* OTU_4 (genus: *Cardiobacterium*). Choanal and preen feather microbiotas were characterized by high levels of the *Bacteroidota* OTU_5 (genus: *Flavobacterium*) and the Bacteroidota OTU_11 (genus: *Riemerella*).

Despite overall differences among body sites, bacterial composition of the different body sites was more similar within than between individuals ($F_{16,247} = 1.72$, $R^2 = .20$, $p < .001$). Results were similar when restricting the analyses to the 13 individuals for which we had samples from six or seven different body sites ($F_{12,64} = 1.83$, $R^2 = .15$, $p < .001$; Figure S6). In addition, we detected similarity between mates in flank, neck and preen feather microbiota and in cloacal microbiota ($F_{1,18} = 1.361$ $R^2 = 0.53$, $p < .001$, $F_{1,16} = 1.36$, $R^2 = 0.55$, $p < .001$, Figure S7, $F_{1,16} = 1.16$, $R^2 = 0.52$, $p = .026$ and $F_{1,35} = 1.32$, $R^2 = 0.56$, $p = .002$, respectively). In contrast, we did not find similarity between mates in outer-bill microbiota ($F_{1,15} = 0.97$, $R^2 = 0.47$, $p = .65$).

Microbiota composition varied with sex in cloaca ($F_{1,149} = 1.12$, $R^2 = .01$, $p = .048$), but not in other body sites (*all* $p > .10$, except in preen feathers where sex was marginally significant: $F_{1,41} = 1.14$, $R^2 = .03$, $p = .058$). Indicator values showed that OTU_31 (genus *Coynebacterium*), OTU_94 (family *Hungateiclostridiaceae*), OTU_71 (genus *Parvimonas*) and OTU_129 (genus *Atopobium*) were enriched in female cloaca compared to male cloaca.

4 | DISCUSSION

Using high-throughput sequencing, we investigated the association between fitness-related traits and microbiota in black-legged kittiwakes. We found that the microbiota of several body sites prior to laying was associated with breeding status (breeders vs. nonbreeders) and reproductive performance in females.

4.1 | Association between microbiota and breeding status

In females, nonbreeders hosted different microbiota to that of breeders in neck and flank feathers, and in the outer-bill, the choanae and the cloaca. In kittiwakes, the causes of skipping breeding are various and may include young age (Cadiou et al., 1994), low number of previous breeding episodes (Desprez et al., 2011), recent dispersal (Danchin & Cam, 2002), poor individual quality (Cam et al., 1998) and high pollutant levels (Tartu et al., 2013). In our study, nonbreeding females were younger than breeding females (mean ± SE: 5.5 ± 0.5 years old vs. 7.4 ± 0.7 years old; $\chi^2_1 = 6.21$, $p = .013$; see Results S2 in Supporting Information). However, age was not a driver of variation in microbiota composition in female kittiwakes (see Results S2 in Supporting Information). Differences in microbiota composition between breeders and nonbreeders may therefore reflect physiological or behavioural differences that are not strictly related to age. In our study, most nonbreeders started building a nest, but did not complete it (Personal Observation). Nests are made of mud, grass and mosses collected from humid soil. Shorter time spent in nest-building may thus limit the transfer of soil bacteria on feathers and gut. In addition, in kittiwakes, nonbreeders spend usually less time attending the nest (Hatch & Hatch, 1988), and consequently might spend more time at sea, potentially increasing the acquisition of bacteria from the marine environment. Accordingly, several OTUs belonging to the family *Salinisphaeraceae*, whose members are commonly isolated from marine and high-salinity environments (Vetriani et al., 2014), were enriched in feathers and outer-bill of nonbreeders.

Nonbreeding may also result from physiological constraints (Cam et al., 1998; Reed et al., 2015) associated with poor foraging ability or poor health. Accordingly, in our study, nonbreeding females had lower body mass than breeding females (Kruskal–Wallis test: $\chi^2 = 8.17$, $p = .004$; mean ± SE: 390 ± 5 g vs. 412 ± 5 g). Poor condition is commonly associated with dysbiotic microbiota (Clemente et al., 2012; Tizard & Jones, 2018). Some OTUs that best represented nonbreeding status in feathers of kittiwakes belonged to the family *Lachnospiraceae*. *Lachnospiraceae* are usually isolated from the gastrointestinal tract of mammals and birds, where they can influence short-chained fatty-acid production and thus be associated with gut health and metabolic syndromes (Stanley et al., 2016; reviewed in Vacca et al., 2020). Although *Lachnospiraceae* are often suggested to promote health, different genera and species of this family are

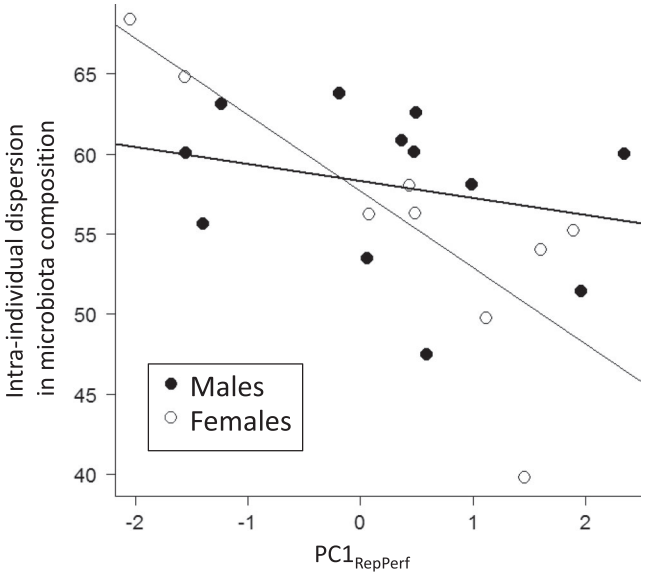

FIGURE 4 Intraindividual dispersion in microbiota composition according to $PC1_{RepPerf}$ in males (black dots and bold line) and females (white dots and thin line). Because not all body sites were sampled on each single individual, we restricted the analyses on individuals for which cloaca, flank feathers, neck feathers, preen feathers and outer-bill were sampled. Intraindividual dispersion varied with $PC1_{RepPerf}$ in females, but not in males ($S = 216$, $r^2 = -.80$, $p = .014$ and $S = 392$, $r^2 = -.37$, $p = .24$)

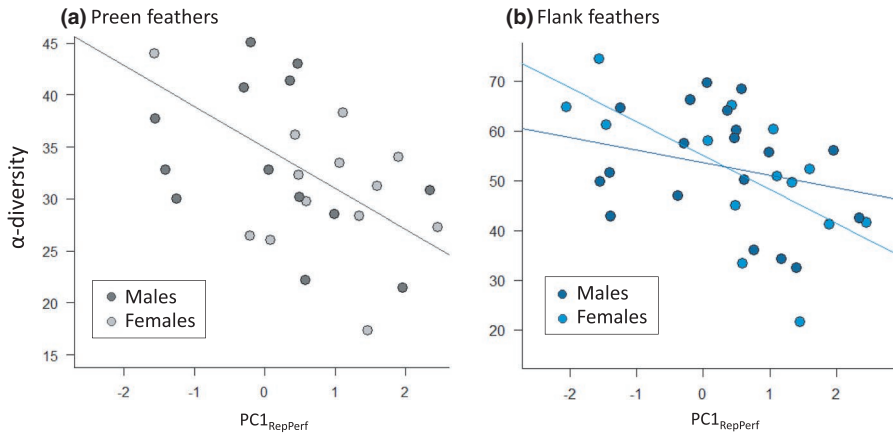

FIGURE 5 α-diversity of (a) preen feather microbiota and (b) flank feather microbiota in relation to $PC1_{RepPerf}$ in males and females. α-diversity (Faith's PD index) was calculated on rarefied samples. In preen feathers, the relationship between α-diversity and $PC1_{RepPerf}$ did not vary with sex ($PC1_{RepPerf}$*Sex: $F_{1,23} = 1.16$, $p = .29$; $PC1_{RepPerf}$: $F_{1,25} = 11.48$, $p = .002$), thereby a single regression line is shown, while in flank feathers, the relationship between α-diversity and $PC1_{RepPerf}$ varied with sex ($PC1_{RepPerf}$*Sex: $F_{1,10} = 7.42$, $p = .021$), thereby sex-specific regression lines are shown (females: $F_{1,1} = 7.13$, $p = .020$ and males: $F_{1,1} = 3.70$, $p = .073$)

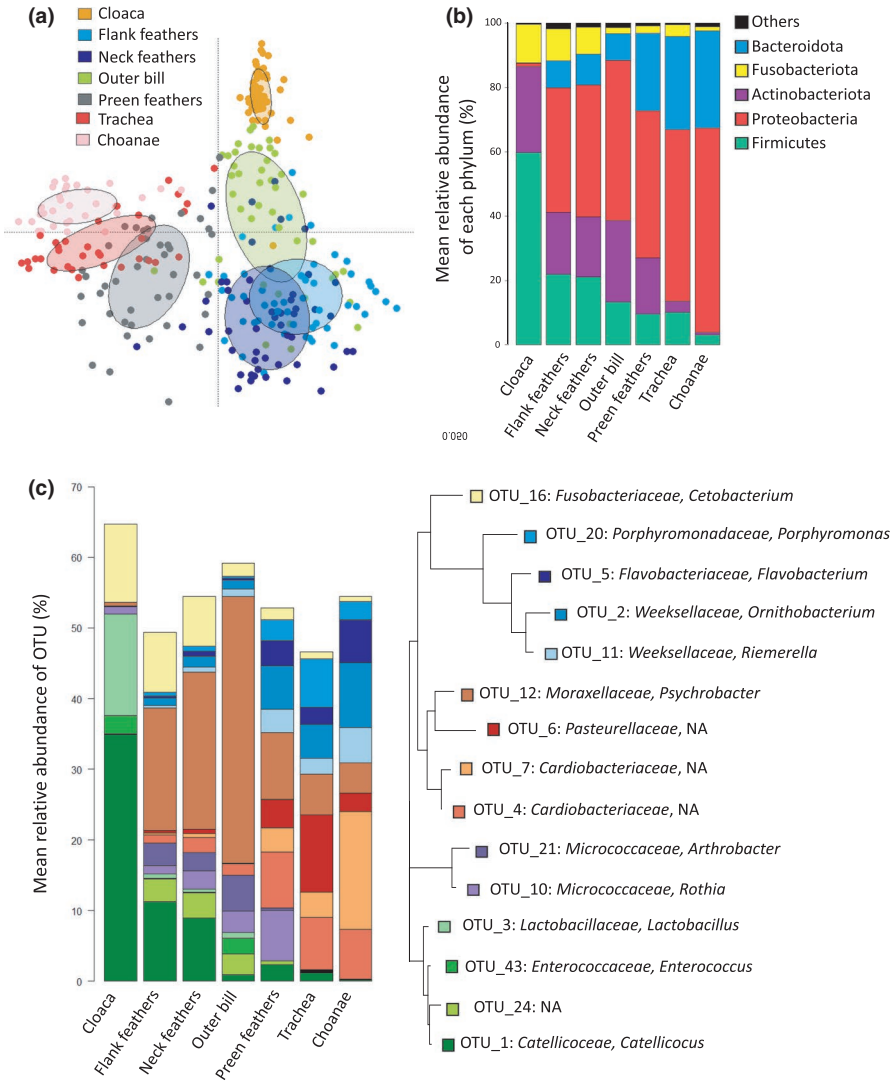

FIGURE 6 Plots showing differences in microbiota between body sites. (a) Unconstrained distance-based redundancy analysis plots showing separation of microbiota by body sites. (b) Mean relative abundance of the most abundant phylum in the different body sites. (c) Mean relative abundance of the most abundant operational taxonomic units (OTUs) in the different body sites and neighbour-joining phylogenetic tree based on the V5–V6 16S rRNA sequences of these most abundant OTUs (NA, not affiliated). Correlation indices showed that all the OTUs shown in (c), except OTU_43, differed significantly between body sites (all $p < .0002$)

increased in diseases (Vacca et al., 2020). For instance, in kittiwakes, the family *Lachnospiraceae* contains mainly the genera *Tyzzerella* and *Lachnoclostridium*, which, in other species, are enriched in individuals with high lifetime risk of cardiovascular diseases (Kelly et al., 2016), Crohn's disease (Olaisen et al., 2021) or colon-associated cancer (Liang et al., 2020; Chun-Sai-Er Wang et al., 2018).

The composition of preen feather microbiota did not vary with breeding status. If differences in microbiota between breeding status is mostly caused by variations in the acquisition of environmental bacteria, the lack of association between microbiota and breeding status in preen feathers might arise from preen feathers being protected from the external environment by large body feathers, and therefore being less prone to environmental contamination than other body sites. Findings about the acquisition of environmental bacteria by preen feather or preen secretion microbiota are mixed (Díaz-Lora et al., 2019; Martínez-García et al., 2016; Pearce et al., 2017). In contrast, preen feather microbiota may be highly prone to the quantity and composition of the lipid-rich and energetically costly preen secretion (Moreno-Rueda, 2017). The lack of association between preen feather microbiota and breeding status suggests therefore that nonbreeding birds can invest similarly in preen secretion as breeding birds. Further studies are required to determine whether there is no intrinsic differences between nonbreeding and breeding females in the ability to invest in preen secretion or whether lower investment in reproduction allows nonbreeding females to save energy to invest in preen secretion as much as breeding females.

4.2 | Association between microbiota and reproductive performance in breeders

When considering breeders only, the composition and diversity of feather microbiota was associated with variation in reproductive performance (as represented by a principal component related to body condition, date of laying, clutch size, egg mass and hatching success) in females. The role of feather microbiota on host fitness has been scarcely studied. Some feather bacteria can alter feather structure and thus reduce fitness, via the alteration of thermoregulation, flight and signalling (Clayton, 1999; Leclaire et al., 2014).

To limit the spread of feather-degrading bacteria (Alt et al., 2020; Moreno-Rueda, 2017), birds spread preen secretions containing antimicrobial bacteria, lipids or immune components (Bandyopadhyay & Bhattacharyya, 1996; Bodawatta et al., 2020; Carneiro et al., 2020; Martin-Vivaldi et al., 2010) onto their plumage. However, investment in preen secretions and preening is energetically costly (reviewed in Moreno-Rueda, 2017). Therefore, better female breeders, being in better physiological state, may be able to invest in preen secretion, and thus control their feather microbiota, more than poorer female breeders. This hypothesis is supported by two results. First, we found that the associations between reproductive performance and microbiota composition or diversity were stronger and more consistent for preen feathers compared to other feather types. Compared to other feathers, preen feathers are usually saturated with higher quantity of preen secretions (Leclaire et al., 2019), and their microbiota might thus be more sensitive to variation in the ability of the host to invest in preen secretions. Second, we found reduced intraindividual dispersion in microbiota composition across body habitats in females with higher reproductive performance, suggesting that good breeders are able to "leash" their microbiota better than poor breeders (Foster et al., 2017).

The "Anna Karenina principle" suggests that stressors affect bacterial community stochastically, resulting in greater variation among dysbiotic bacterial communities compared to eubiotic communities (Zaneveld et al., 2017). However, in contrast to this hypothesis, we did not find strong evidence for greater interindividual dispersion in microbiota composition in poorer breeders. Although several studies in humans or wild animals supported this principle (Altabtbaei et al., 2021; Stothart et al., 2021), others have found the opposite pattern (Lavrinienko et al., 2020; Ma, 2020). In kittiwakes, the lack of association between interindividual dispersion in feather microbiota and breeding performance may stem from the fact that lower quantity of preen secretions, by increasing the spread of feather-degrading bacteria, may not affect feather microbiota composition stochastically.

We found that the diversity of preen and flank feather microbiota decreased with increasing reproductive performance, especially in females. Although numerous studies have shown that high diversity of gut microbiota is pivotal for host fitness (Larsen & Claassen, 2018; Mosca et al., 2016), it has recently been suggested that more diversity is not always better (Reese & Dunn, 2018). Interestingly, in blue petrels (*Halobaena caerulea*), lower microbiota diversity in preen feathers is associated with higher major histocompatibility complex diversity (Leclaire et al., 2019), which correlates with higher fitness in several vertebrates, including in kittiwake chicks (Agudo et al., 2012; Pineaux et al., 2020; Smith et al., 2010; Thoss et al., 2011). Lower bacterial diversity in feathers is also associated with increased body condition in pied flycatchers (*Ficedula hypoleuca*; Saag et al., 2011) and brighter coloration in great tits (*Parus major*; Kilgas et al., 2012). Higher investment in preen secretions and preening in birds with high reproductive performance may decrease bacterial niche availability on feathers and therefore reduce bacterial diversity. Further studies are needed to evaluate whether, in contrast to gut microbiota, lower diversity in feather microbiota is generally associated with enhanced fitness in birds.

In preen feathers, the relative abundance of a *Porphyromonas* OTU was associated with higher reproductive performance in females. *Porphyromonas* are well-known residents of the oral microbiota of animals and humans, where they can cause diseases (Acuña-Amador & Barloy-Hubler, 2020; Fournier et al., 2001; Mysak et al., 2014). Recently, some *Porphyromonas* were also detected in other body sites, including lipid-rich glandular secretions of mammals and birds (Leclaire et al., 2017; Rodríguez-Ruano et al., 2015; Theis et al., 2012), but their physiological functionalities are unknown.

Variations in reproductive performance in female breeders were also associated with variations in the composition of cloacal microbiota. In particular, female breeders with lower reproductive performance harboured a higher abundance of several strains of the genus *Corynebacterium*. Corynebacteria are widespread in animal microbiota (Callewaert et al., 2013; Hird et al., 2015; Leclaire et al., 2017), and include commensals, as well as pathogens (Bernard, 2012; Kanmani et al., 2017). Several *Corynebacteria* are associated with infertility in humans and other mammals (Alonso et al., 1992; Othman et al., 2016; Riegel et al., 1995). In birds, the cloaca is the opening of the reproductive tract. Some Corynebacteria detected in female cloacas may thus infect their reproductive system and impair egg formation and development. Consistently with our finding, a previous study in kittiwakes found that a sexually-transmitted *Corynebacterium* (named C34) was associated with decreased hatching success in females, but not in males (van Dongen et al., 2019). In our study, C34 (OTU_31) was not one of the OTUs best representing breeding performance. Still, its relative abundance was negatively correlated with breeding performance in females (Spearman's correlation test: $r = -.51$, $p = .007$). Higher reproductive performance may be associated with better immunity that acts as an ecological filter to limit the spread of the *Corynebacteriaceae* that are potentially pathogenic. As found for C34 (van Dongen et al., 2019), we did not detect an association between cloacal microbiota and reproductive performance in males, suggesting that *Corynebacteria* does not impact male fertility.

4.3 | Sex-specific association between microbiota and reproductive outcome

The variations in microbiota with breeding status or reproductive performance were observed in females, but not in males. Interestingly, in kittiwakes, sex-specific differences between breeders and nonbreeders are observed at the physiological level also (Goutte et al., 2010). Nonbreeding females have higher corticosterone levels and lower luteinizing hormone (LH) levels than breeding females, while there are no differences in hormonal levels between breeding and nonbreeding males (Goutte et al., 2010). It has been suggested that females with high corticosterone levels may have lower foraging skills and thus unable to cope with the energetic requirement for egg formation, and that low LH levels may be due to

weak social interactions with their mate (Goutte et al., 2010). In addition, in our study, in contrast to females, there was no difference in body mass between nonbreeding and breeding males (Kruskal–Wallis test: $\chi^2 = 0.18$, $p = .67$; mean ± SE: 419 ± 4 g vs. 424 ± 5 g, respectively). Altogether, these results suggest that, in kittiwake pairs, reproductive outcome from the decision to lay eggs to hatching is mainly due to females. Accordingly, in several monogamous birds with biparental care including kittiwakes (Jacobsen et al., 1995; Leclaire et al., 2010), females have been suggested to be more determinant of reproductive performance than males (Peralta-Sánchez et al., 2020; Slagsvold & Lifjeld, 1990).

Although the variation in feather microbiota according to reproductive outcome are sex-specific, we did not detect sex-differences in feather microbiota composition. In birds, evidence for sex-differences in microbiota is mixed (Ambrosini et al., 2019; Goodenough et al., 2017; Grieves et al., 2021; Pearce et al., 2017; Saag et al., 2011). Sex-differences in avian microbiota have been attributed to sex-differences in behaviour or hormonal levels (Corl et al., 2020; Leclaire et al., 2019). In kittiwakes, during the prelaying period, males and females differ in numerous behavioural and physiological traits, such as nest attendance (Helfenstein, 2002) and the chemical composition of preen secretions (Leclaire et al., 2011). However, we found great similarity between mates in the microbiota of most body sites. High rates of bacterial transfer between pair-mates due to allo-preening and nest sharing (Kulkarni & Heeb, 2007; Whittaker et al., 2016) might therefore mask any potential pre-existing sex-differences in feather microbiota. In kittiwakes, similarity in cloacal microbiota between mates has been shown to be due to the transfer of cloacal bacteria during copulation (White et al., 2010). However in contrast to feather microbiota, we found a weak sex-difference in cloacal microbiota. Interestingly, female cloacas were enriched with OTUs associated with lower reproductive performance in female kittiwakes (e.g., the C34 bacterium; van Dongen et al., 2019 and OTU_74 [this study]) or belonging to genera (i.e., *Parvimonas* and *Atopobium*) associated with vaginal infection in women (Brotman et al., 2012; Raimondi et al., 2021), further suggesting that female cloaca host female-specific bacteria that might impact fertility.

4.4 | Differences in microbiota across body sites and sexes

We found that the microbiota, although specific to individuals, varies across body habitats in black-legged kittiwakes, which is consistent with other studies on animal microbiomes (Alfano et al., 2015; Costello et al., 2009; Ferretti et al., 2018). In adult kittiwakes, cloacal microbiota was dominated by bacteria belonging to the phyla *Firmicutes* and *Actinobacteria*. The bacterial phyla composition of bird cloaca or gut varies greatly across species (Hird et al., 2015, 2018; Laviad-Shitrit et al., 2019), but our result is in line with a previous study on the same population of kittiwakes (van Dongen et al., 2013). In addition, the most abundant OTU detected in cloaca of kittiwakes was *Catellicoccus marimammalium* (OTU_1), which is also the most common bacteria in the cloaca of other gull species from North America, South Africa and Europe (Koskey et al., 2014; Laviad-Shitrit et al., 2019; Lu et al., 2008; Merkeviciene et al., 2017). A *Lactobacillus* (OTU_3) was found to be the second most abundant OTU in the cloaca of kittiwakes, which is common for the gastrointestinal tract of birds and other vertebrates (Grond et al., 2018; Maurice et al., 2015). Finally, a *Cetobacterium* (OTU_16) was also enriched in the cloaca of kittiwakes, and members of this genus had already been isolated from the gut of other marine animals (Foster et al., 1995; Godoy-Vitorino et al., 2017).

Flank feather, neck feather and outer-bill microbiota of kittiwakes were characterized by high levels of an OTU belonging to the genus *Psychrobacter* (OTU_12). *Psychrobacter* are commonly isolated from cold and saline habitats (Bowman et al., 1996; Rodrigues et al., 2009), and high abundance has also been detected in the feathers of other arctic or subantarctic seabirds (Leclaire et al., 2019; Shawkey et al., 2006). Interestingly, the microbiota of outer-bill was more similar to that of neck and flank feathers, than that of other body regions. Similarity in microbiota might be due to the outer-bill being a keratinized structure like feathers, and being frequently in contact with feathers during preening and sleeping, when birds tuck their bill into their feathers.

Some of the most abundant OTUs in kittiwake trachea and choanae belong to the genera *Ornitobacterium* (OTU_2), *Riemerella* (OTU_11) and *Pasteurella* (OTU_6), and to the family *Cardiobacteriaceae* (OTU_7 and OTU_4). Some members of these genera and this family are commonly present in the upper part of the respiratory tract of birds or mammals (Barbosa et al., 2020; Holman et al., 2017), and can cause diseases. For instance, *Ornithobacterium rhinotracheale*, *Riemerella anatipestifer*, *Pasteurella multocida* and *Sutonella ornithocola* are well-known respiratory avian pathogens, that can cause economic loss when infecting poultry and be major threat for endangered wild birds (Jaeger et al., 2018; Lawson et al., 2011; Sandhu, 2008; Van Empel & Hafez, 1999). Many infectious bacteria are low-abundance residents and cause diseases only when dysbiosis occurs. Further studies are needed to determine the infection potential of these bacteria in kittiwakes.

We found that the major OTUs of preen feathers were more similar to those of the upper respiratory tract than to those of other feather types. Respiratory microbiota was analysed using different DNA extraction protocols and sequencing runs compared to the microbiota of other body sites. Similarity in respiratory and preen feather microbiota might thus be an artefact due the methods used for respiratory microbiota analyses specifically enriching the OTUs detected in preen feathers. Alternatively, similarity in respiratory and preen feather microbiota might come from the transfer of bacteria between the preen feathers and the inner bill (whose microbiota might be similar to that of the respiratory tract), when birds collect preen secretion with their bill before preening their feathers. Previous studies characterizing the microbiota of preen feathers or preen secretions seem to show strong variations across species (e.g., Leclaire et al., 2019; Videvall et al., 2021; Whittaker & Theis, 2016). For instance, similarly

to kittiwakes, in Leach's storm-petrels (*Oceanodroma leucorhoa*), preen gland microbiota is dominated by *Proteobacteria*, and includes high abundance of *Moraxellaceae* (Pearce et al., 2017). In contrast, preen microbiota is dominated by the phylum *Firmicutes* (mainly the class *Clostridia*) in hoopoes (Rodríguez-Ruano et al., 2018), and by *Actinobacteria* in blue petrels (Leclaire et al., 2019).

5 | CONCLUSION

Altogether, our findings are consistent with the idea that natural variation in the microbiota is associated with differences in host fitness in wild birds. Our study is correlative, and the causal relationship between fitness and microbiota is unknown. Further studies are therefore required to determine whether abnormal microbiotas cause dysbioses that impair reproductive performance, or whether differences in microbiota are due to reproductive-performance associated variations in diet, physiology or behaviour. A better understanding of the association between fitness and microbiota in wild animals is important to appreciate the evolution of several behavioural or physiological traits. For instance, bacteria with deleterious effect on fitness can select for the evolution of antibacterial defences, including immune and behavioural mechanism such as preening. In addition, an association between fitness and the abundance of bacteria affecting plumage coloration and odours of the host (Archie & Theis, 2011) might lead colours or odours to evolve as sexual ornaments.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Sarah Leclaire conceived and designed the study, performed the statistical analyses and wrote the manuscript. Maxime Pineaux and Sarah Leclaire collected the data and carried out the molecular analyses. Scott A. Hatch is responsible of the long-term monitoring on Middleton Island and gave access to the study area. All authors contributed comments to the manuscript and gave final approval for publication.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study (OTU count tables, metadata, OTU phylogenetic tree, and OTU sequences) have been archived in the publicly accessible repository "Open Science Framework". https://doi.org/10.17605/OSF.IO/WHCXN.

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REFERENCES

Acuña-Amador, L., & Barloy-Hubler, F. (2020). *Porphyromonas* spp. have an extensive host range in ill and healthy individuals and an unexpected environmental distribution: A systematic review and meta-analysis. *Anaerobe*, *66*, 102280.

Agudo, R., Carrete, M., Alcaide, M., Rico, C., Hiraldo, F., & Donázar, J. A. (2012). Genetic diversity at neutral and adaptive loci determines individual fitness in a long-lived territorial bird. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1741), 3241–3249. https://doi.org/10.1098/rspb.2011.2606

Alfano, N., Courtiol, A., Vielgrader, H., Timms, P., Roca, A. L., & Greenwood, A. D. (2015). Variation in koala microbiomes within and between individuals: Effect of body region and captivity status. *Scientific Reports*, *5*, 10189. https://doi.org/10.1038/srep10189

Alonso, J. L., Simon, M. C., Girones, O., Muzquiz, J. L., Ortega, C., & Garcia, J. (1992). The effect of experimental infection with *Corynebacterium pseudotuberculosis* on reproduction in adult ewes. *Research in Veterinary Science*, *52*(3), 267–272. https://doi.org/10.1016/0034-5288(92)90023-U

Alt, G., Mägi, M., Lodjak, J., & Mänd, R. (2020). Experimental study of the effect of preen oil against feather bacteria in passerine birds. *Oecologia*, *192*(3), 723–733. https://doi.org/10.1007/s00442-020-04599-8

Altabtbaei, K., Maney, P., Ganesan, S. M., Dabdoub, S. M., Nagaraja, H. N., & Kumar, P. S. (2021). Anna Karenina and the subgingival microbiome associated with periodontitis. *Microbiome*, *9*(1), 97. https://doi.org/10.1186/s40168-021-01056-3

Ambrosini, R., Corti, M., Franzetti, A., Caprioli, M., Rubolini, D., Motta, V. M., Costanzo, A., Saino, N., & Gandolfi, I. (2019). Cloacal microbiomes and ecology of individual barn swallows. *FEMS Microbiology Ecology*, *95*(6), fiz061. https://doi.org/10.1093/femsec/fiz061

Anderson, M. J., & Walsh, D. C. I. (2013). PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecological Monographs*, *83*(4), 557–574. https://doi.org/10.1890/12-2010.1

Antwis, R. E., Edwards, K. L., Unwin, B., Walker, S. L., & Shultz, S. (2019). Rare gut microbiota associated with breeding success, hormone metabolites and ovarian cycle phase in the critically endangered eastern black rhino. *Microbiome*, *7*(1), 27. https://doi.org/10.1186/s40168-019-0639-0

Archie, E. A., & Theis, K. R. (2011). Animal behaviour meets microbial ecology. *Animal Behaviour*, *82*(3), 425–436. https://doi.org/10.1016/j.anbehav.2011.05.029

Ariyomo, T. O., & Watt, P. J. (2012). The effect of variation in boldness and aggressiveness on the reproductive success of zebrafish. *Animal Behaviour*, *83*(1), 41–46. https://doi.org/10.1016/j.anbehav.2011.10.004

Bandyopadhyay, A., & Bhattacharyya, S. (1996). Influence of fowl uropygial gland and its secretory lipid components on growth of skin

surface bacteria of fowl. *Indian Journal of Experimental Biology*, *34*(1), 48–52.

Barbosa, E. V., Cardoso, C. V., Silva, R. D. C. F., Cerqueira, A. D. M. F., Liberal, M. H. T., & Castro, H. C. (2020). *Ornithobacterium rhinotracheale*: An update review about an emerging poultry pathogen. *Veterinary Sciences*, *7*(1), 3. https://doi.org/10.3390/vetsci7010003

Barko, P. C., McMichael, M. A., Swanson, K. S., & Williams, D. A. (2018). The gastrointestinal microbiome: A review. *Journal of Veterinary Internal Medicine*, *32*(1), 9–25. https://doi.org/10.1111/jvim.14875

Baud, D., Pattaroni, C., Vulliemoz, N., Castella, V., Marsland, B. J., & Stojanov, M. (2019). Sperm microbiota and its impact on semen parameters. *Frontiers in Microbiology*, *10*(234), https://doi.org/10.3389/fmicb.2019.00234

Belkaid, Y., & Harrison, O. J. (2017). Homeostatic immunity and the microbiota. *Immunity*, *46*(4), 562–576. https://doi.org/10.1016/j.immuni.2017.04.008

Bender, R., & Lange, S. (2001). Adjusting for multiple testing—When and how? *Journal of Clinical Epidemiology*, *54*(4), 343–349. https://doi.org/10.1016/S0895-4356(00)00314-0

Bernard, K. (2012). The genus *Corynebacterium* and other medically relevant coryneform-like bacteria. *Journal of Clinical Microbiology*, *50*(10), 3152–3158. https://doi.org/10.1128/Jcm.00796-12

Bodawatta, K. H., Schierbech, S. K., Petersen, N. R., Sam, K., Bos, N., Jønsson, K. A., & Poulsen, M. (2020). Great tit (*Parus major*) uropygial gland microbiomes and their potential defensive roles. *Frontiers in Microbiology*, *11*, 1735. https://doi.org/10.3389/fmicb.2020.01735

Bolnick, D. I., Snowberg, L. K., Caporaso, J. G., Lauber, C., Knight, R., & Stutz, W. E. (2014). Major histocompatibility complex class IIb polymorphism influences gut microbiota composition and diversity. *Molecular Ecology*, *23*(19), 4831–4845.

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biology*, *13*(8), e1002226. https://doi.org/10.1371/journal.pbio.1002226

Bowman, J. P., Cavanagh, J., Austin, J. J., & Sanderson, K. (1996). Novel *Psychrobacter* species from Antarctic ornithogenic soils. *International Journal of Systematic and Evolutionary Microbiology*, *46*(4), 841–848.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176–182.

Brotman, R. M., Bradford, L. L., Conrad, M., Gajer, P., Ault, K., Peralta, L., Forney, L. J., Carlton, J. M., Abdo, Z., & Ravel, J. (2012). Association between *Trichomonas vaginalis* and vaginal bacterial community composition among reproductive-age women. *Sexually Transmitted Diseases*, *39*(10), 807–812. https://doi.org/10.1097/OLQ.0b013e3182631c79

Burtt Jr., E. H., & Ichida, J. M. (1999). Occurrence of feather-degrading bacilli in the plumage of birds. *The Auk*, *116*(2), 364–372. https://doi.org/10.2307/4089371

Cadiou, B., Monnat, J. Y., & Danchin, E. (1994). Prospecting in the kittiwake, *Rissa tridactyla*: Different behavioural patterns and the role of squatting in recruitment. *Animal Behaviour*, *47*(4), 847–856. https://doi.org/10.1006/anbe.1994.1116

Callewaert, C., Kerckhof, F.-M., Granitsiotis, M. S., Van Gele, M., Van de Wiele, T., & Boon, N. (2013). Characterization of *Staphylococcus* and *Corynebacterium* clusters in the human axillary region. *PLoS One*, *8*(8), e70538. https://doi.org/10.1371/journal.pone.0070538

Cam, E., Hines, J. E., Monnat, J.-Y., Nichols, J. D., & Danchin, E. (1998). Are adult nonbreeders prudent parents? The kittiwake model. *Ecology*, *79*, 2917–2930. https://doi.org/10.2307/176526

Carneiro, D., Czirják, G. Á., & Rowe, M. (2020). Innate and adaptive immune proteins in the preen gland secretions of male house sparrows. *Journal of Avian Biology*, *51*(11). https://doi.org/10.1111/jav.02556

Chen, Y. E., Fischbach, M. A., & Belkaid, Y. (2018). Skin microbiota–host interactions. *Nature*, *553*(7689), 427–436. https://doi.org/10.1038/nature25177

Clayton, D. H. (1999). Feather-busting bacteria. *The Auk*, *116*(2), 302–304. https://doi.org/10.2307/4089364

Clemente, J. C., Ursell, L. K., Parfrey, L. W., & Knight, R. (2012). The impact of the gut microbiota on human health: An integrative view. *Cell*, *148*(6), 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Comizzoli, P., & Power, M. (2019). Reproductive microbiomes in wild animal species: A new dimension in conservation biology. In P. Comizzoli, J. L. Brown, & W. V. Holt (Eds.), *Reproductive sciences in animal conservation* (pp. 225–240). Springer.

Corl, A., Charter, M., Rozman, G., Toledo, S., Turjeman, S., Kamath, P. L., Getz, W. M., Nathan, R., & Bowie, R. C. K. (2020). Movement ecology and sex are linked to barn owl microbial community composition. *Molecular Ecology*, *29*(7), 1358–1371. https://doi.org/10.1111/mec.15398

Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., & Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science*, *326*(5960), 1694–1697.

Coulson, J. C., & Porter, J. M. (1985). Reproductive success of the kittiwake *Rissa tridactyla* – The roles of clutch size, chick growth-rates and parental quality. *Ibis*, *127*(4), 450–466. https://doi.org/10.1111/j.1474-919X.1985.tb04841.x

Cubaynes, S., Doherty, P. F., Schreiber, E. A., & Gimenez, O. (2011). To breed or not to breed: A seabird's response to extreme climatic events. *Biology Letters*, *7*(2), 303–306. https://doi.org/10.1098/rsbl.2010.0778

Danchin, E., & Cam, E. (2002). Can non-breeding be a cost of breeding dispersal? *Behavioral Ecology and Sociobiology*, *51*(2), 153–163. https://doi.org/10.1007/s00265-001-0423-5

De Cáceres, M., & Legendre, P. (2009). Associations between species and groups of sites: Indices and statistical inference. *Ecology*, *90*(12), 3566–3574. https://doi.org/10.1890/08-1823.1

De Cáceres, M., Legendre, P., & Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. *Oikos*, *119*(10), 1674–1684. https://doi.org/10.1111/j.1600-0706.2010.18334.x

Desprez, M., Gimenez, O., McMahon, C. R., Hindell, M. A., & Harcourt, R. G. (2018). Optimizing lifetime reproductive output: Intermittent breeding as a tactic for females in a long-lived, multiparous mammal. *Journal of Animal Ecology*, *87*(1), 199–211. https://doi.org/10.1111/1365-2656.12775

Desprez, M., Pradel, R., Cam, E., Monnat, J.-Y., & Gimenez, O. (2011). Now you see him, now you don't: Experience, not age, is related to reproduction in kittiwakes. *Proceedings of the Royal Society B: Biological Sciences*, *278*(1721), 3060–3066.

Díaz-Lora, S., Martín-Vivaldi, M., Juárez García-Pelayo, N., Azcárate García, M., Rodríguez-Ruano, S. M., Martínez-Bueno, M., & Soler, J. J. (2019). Experimental old nest material predicts hoopoe *Upupa epops* eggshell and uropygial gland microbiota. *Journal of Avian Biology*, *50*(9). https://doi.org/10.1111/jav.02083

Dickson, R. P., Erb-Downward, J. R., Martinez, F. J., & Huffnagle, G. B. (2016). The microbiome and the respiratory tract. *Annual Review of Physiology*, *78*(1), 481–504. https://doi.org/10.1146/annurev-physiol-021115-105238

Egozcue, J. J., Pawlowsky-Glahn, V., Mateu-Figueras, G., & Barcelo-Vidal, C. (2003). Isometric logratio transformations for compositional data analysis. *Mathematical Geology*, *35*(3), 279–300.

Eraud, C., Jacquet, A., & Faivre, B. (2009). Survival cost of an early immune soliciting in nature. *Evolution*, *63*(4), 1036–1043. https://doi.org/10.1111/j.1558-5646.2008.00540.x

Escudié, F., Auer, L., Bernard, M., Mariadassou, M., Cauquil, L., Vidal, K., Maman, S., Hernandez-Raquet, G., Combes, S., & Pascal, G. (2018). FROGS: Find, rapidly, OTUs with Galaxy solution. *Bioinformatics*, *34*(8), 1287–1294. https://doi.org/10.1093/bioinformatics/btx791

Ferretti, P., Pasolli, E., Tett, A., Asnicar, F., Gorfer, V., Fedi, S., Armanini, F., Truong, D. T., Manara, S., Zolfo, M., Beghini, F., Bertorelli, R., De Sanctis, V., Bariletti, I., Canto, R., Clementi, R., Cologna, M., Crifò, T., Cusumano, G., … Segata, N. (2018). Mother-to-infant microbial transmission from different body sites shapes the developing infant gut microbiome. *Cell Host & Microbe*, *24*(1), 133–145.e135. https://doi.org/10.1016/j.chom.2018.06.005

Fliegerova, K., Tapio, I., Bonin, A., Mrazek, J., Callegari, M. L., Bani, P., Bayat, A., Vilkki, J., Kopečný, J., Shingfield, K. J., Boyer, F., Coissac, E., Taberlet, P., & Wallace, R. J. (2014). Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe*, *29*, 80–84. https://doi.org/10.1016/j.anaerobe.2013.09.015

Fontaine, S. S., Novarro, A. J., & Kohl, K. D. (2018). Environmental temperature alters the digestive performance and gut microbiota of a terrestrial amphibian. *Journal of Experimental Biology*, *221*(20). https://doi.org/10.1242/jeb.187559

Foster, G., Ross, H., Naylor, R., Collins, M., Ramos, C. P., Garayzabal, F. F., & Reid, R. (1995). *Cetobacterium ceti gen. nov.*, sp. *nov.*, a new Gram-negative obligate anaerobe from sea mammals. *Letters in Applied Microbiology*, *21*(3), 202–206.

Foster, K. R., Schluter, J., Coyte, K. Z., & Rakoff-Nahoum, S. (2017). The evolution of the host microbiome as an ecosystem on a leash. *Nature*, *548*(7665), 43–51.

Fournier, D., Mouton, C., Lapierre, P., Kato, T., Okuda, K., & Ménard, C. (2001). *Porphyromonas gulae* sp. *nov.*, an anaerobic, gram-negative coccobacillus from the gingival sulcus of various animal hosts. *International Journal of Systematic and Evolutionary Microbiology*, *51*(3), 1179–1189.

Gill V. A., Hatch S. A. (2002). Components of productivity in black-legged kittiwakes *Rissa tridactyla*: Response to supplemental feeding. *Journal of Avian Biology*, *33*(2), 113–126. https://doi.org/10.1034/j.1600-048x.2002.330201.x

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V., & Egozcue, J. J. (2017). Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology*, *8*(2224). https://doi.org/10.3389/fmicb.2017.02224

Godoy-Vitorino, F., Rodriguez-Hilario, A., Alves, A. L., Gonçalves, F., Cabrera-Colon, B., Mesquita, C. S., Soares-Castro, P., Ferreira, M., Marçalo, A., Vingada, J., Eira, C., & Santos, P. M. (2017). The microbiome of a striped dolphin (*Stenella coeruleoalba*) stranded in Portugal. *Research in Microbiology*, *168*(1), 85–93. https://doi.org/10.1016/j.resmic.2016.08.004

Goodenough, A. E., Stallwood, B., Dandy, S., Nicholson, T. E., Stubbs, H., & Coker, D. G. (2017). Like mother like nest: Similarity in microbial communities of adult female Pied Flycatchers and their nests. *Journal of Ornithology*, *158*(1), 233–244. https://doi.org/10.1007/s10336-016-1371-1

Goutte, A., Angelier, F., Chastel, C. C., Trouvé, C., Moe, B., Bech, C., Gabrielsen, G. W., & Chastel, O. (2010). Stress and the timing of breeding: Glucocorticoid-luteinizing hormones relationships in an arctic seabird. *General and Comparative Endocrinology*, *169*(1), 108–116. https://doi.org/10.1016/j.ygcen.2010.07.016

Green, K. A., Zarek, S. M., & Catherino, W. H. (2015). Gynecologic health and disease in relation to the microbiome of the female reproductive tract. *Fertility and Sterility*, *104*(6), 1351–1357. https://doi.org/10.1016/j.fertnstert.2015.10.010

Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature Reviews Microbiology*, *9*(4), 244–253. https://doi.org/10.1038/nrmicro2537

Grieves, L. A., Gloor, G. B., Kelly, T. R., Bernards, M. A., & MacDougall-Shackleton, E. A. (2021). Preen gland microbiota of songbirds differ across populations but not sexes. *Journal of Animal Ecology*, *90*(9), 2202–2212. https://doi.org/10.1111/1365-2656.13531

Griffen, B. D. (2018). Reproductive skipping as an optimal life history strategy in the southern elephant seal, *Mirounga leonina*. *Ecology and Evolution*, *8*(18), 9158–9170. https://doi.org/10.1002/ece3.4408

Grond, K., Sandercock, B. K., Jumpponen, A., & Zeglin, L. H. (2018). The avian gut microbiota: Community, physiology and function in wild birds. *Journal of Avian Biology*, *49*(11), e01788. https://doi.org/10.1111/jav.01788

Gunderson, A. R. (2008). Feather-degrading bacteria: A new frontier in avian and host–parasite research? *The Auk*, *125*(4), 972–979. https://doi.org/10.1525/auk.2008.91008

Harris, R. N., Brucker, R. M., Walke, J. B., Becker, M. H., Schwantes, C. R., Flaherty, D. C., Lam, B. A., Woodhams, D. C., Briggs, C. J., Vredenburg, V. T., & Minbiole, K. P. C. (2009). Skin microbes on frogs prevent morbidity and mortality caused by a lethal skin fungus. *The ISME Journal*, *3*(7), 818–824. https://doi.org/10.1038/ismej.2009.27

Hatch, S. A., & Hatch, M. A. (1988). Colony attendance and population monitoring of Black-legged Kittiwakes on the Semidi Islands, Alaska. *The Condor*, *90*(3), 613–620. https://doi.org/10.2307/1368350

Heil, B. A., Paccamonti, D. L., & Sones, J. L. (2019). Role for the mammalian female reproductive tract microbiome in pregnancy outcomes. *Physiological Genomics*, *51*(8), 390–399. https://doi.org/10.1152/physiolgenomics.00045.2019

Helfenstein, F. (2002). *Stratégies de reproduction et conflits sexuels. Le cas d'une espèce coloniale: la mouette tridactyle* Rissa tridactyla. PhD thesis, Université Pierre et Marie Curie, Paris.

Helfenstein, F., Tirard, C., Danchin, E., & Wagner, R. H. (2004). Low frequency of extra-pair paternity and high frequency of adoption in black-legged kittiwakes. *The Condor*, *106*, 149–155. https://doi.org/10.1650/7337

Helfenstein, F., Wagner, R. H., Danchin, E., & Rossi, J. M. (2003). Functions of courtship feeding in black-legged kittiwakes: Natural and sexual selection. *Animal Behaviour*, *65*, 1027–1033. https://doi.org/10.1006/anbe.2003.2129

Hird, S. M. (2017). Evolutionary biology needs wild microbiomes. *Frontiers in Microbiology*, *8*, 725. https://doi.org/10.3389/fmicb.2017.00725

Hird, S. M., Ganz, H., Eisen, J. A., & Boyce, W. M. (2018). The cloacal microbiome of five wild duck species varies by species and influenza A virus infection status. *mSphere*, *3*(5). doi:https://doi.org/10.1128/mSphere.00382-18

Hird, S. M., Sánchez, C., Carstens, B. C., & Brumfield, R. T. (2015). Comparative gut microbiota of 59 neotropical bird species. *Frontiers in Microbiology*, *6*, 1403. https://doi.org/10.3389/fmicb.2015.01403

Holman, D. B., Timsit, E., Amat, S., Abbott, D. W., Buret, A. G., & Alexander, T. W. (2017). The nasopharyngeal microbiota of beef cattle before and after transport to a feedlot. *BMC Microbiology*, *17*(1), 70. https://doi.org/10.1186/s12866-017-0978-6

Hoyt, J. R., Cheng, T. L., Langwig, K. E., Hee, M. M., Frick, W. F., & Kilpatrick, A. M. (2015). Bacteria isolated from bats inhibit the growth of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome. *PLoS One*, *10*(4), e0121329. https://doi.org/10.1371/journal.pone.0121329

Jacobsen, K. O., Erikstad, K. E., & Saether, B. E. (1995). An experimental-study of the costs of reproduction in the kittiwake *Rissa tridactyla*. *Ecology*, *76*(5), 1636–1642. https://doi.org/10.2307/1938164

Jaeger, A., Lebarbenchon, C., Bourret, V., Bastien, M., Lagadec, E., Thiebot, J.-B., Boulinier, T., Delord, K., Barbraud, C., Marteau, C., Dellagi, K., Tortosa, P., & Weimerskirch, H. (2018). Avian cholera outbreaks threaten seabird species on Amsterdam Island. *PLoS One*, *13*(5), e0197291. https://doi.org/10.1371/journal.pone.0197291

Jin, Y., Wu, S., Zeng, Z., & Fu, Z. (2017). Effects of environmental pollutants on gut microbiota. *Environmental Pollution*, *222*, 1–9. https://doi.org/10.1016/j.envpol.2016.11.045

Jodice, P. G. R., Lanctot, R. B., Gill, V. A., Roby, D. D., & Hatch, S. A. (2000). Sexing adult black-legged kittiwakes by DNA, behavior, and morphology. *Waterbirds*, *23*(3), 405–415. https://doi.org/10.2307/1522177

Johnson, K.-V.-A., & Burnet, P. W. (2016). Microbiome: Should we diversify from diversity? *Gut Microbes*, *7*(6), 455–458. https://doi.org/10.1080/19490976.2016.1241933

Kanmani, P., Clua, P., Vizoso-Pinto, M. G., Rodriguez, C., Alvarez, S., Melnikov, V., Takahashi, H., Kitazawa, H., & Villena, J. (2017). Respiratory commensal bacteria *Corynebacterium pseudodiphtheriticum* improves resistance of infant mice to respiratory syncytial virus and *Streptococcus pneumoniae* superinfection. *Frontiers in Microbiology*, *8*, 1613. https://doi.org/10.3389/fmicb.2017.01613

Kelly, T. N., Bazzano, L. A., Ajami, N. J., He, H., Zhao, J., Petrosino, J. F., Correa, A., & He, J. (2016). Gut microbiome associates with lifetime cardiovascular disease risk profile among bogalusa heart study participants. *Circulation Research*, *119*(8), 956–964. doi:https://doi.org/10.1161/CIRCRESAHA.116.309219

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kilgas, P., Saag, P., Mägi, M., Edenberg, M., Tilgar, V., & Mänd, R. (2012). Variation in assemblages of feather bacteria in relation to plumage color in female great tits. *The Condor*, *114*(3), 606–611. https://doi.org/10.1525/cond.2012.110103

Koskey, A. M., Fisher, J. C., Traudt, M. F., Newton, R. J., & McLellan, S. L. (2014). Analysis of the gull fecal microbial community reveals the dominance of *Catellicoccus marimammalium* in relation to culturable Enterococci. *Applied and Environmental Microbiology*, *80*(2), 757–765. https://doi.org/10.1128/AEM.02414-13

Kowalczyk, N. D., Chiaradia, A., Preston, T. J., & Reina, R. D. (2014). Linking dietary shifts and reproductive failure in seabirds: a stable isotope approach. *Functional Ecology*, *28*(3), 755–765. https://doi.org/10.1111/1365-2435.12216

Kulkarni, S., & Heeb, P. (2007). Social and sexual behaviours aid transmission of bacteria in birds. *Behavioural Processes*, *74*(1), 88–92. https://doi.org/10.1016/j.beproc.2006.10.005

Larsen, O. F., & Claassen, E. (2018). The mechanistic link between health and gut microbiota diversity. *Scientific Reports*, *8*(1), 1–5. https://doi.org/10.1038/s41598-018-20141-6

Laviad-Shitrit, S., Izhaki, I., Lalzar, M., & Halpern, M. (2019). Comparative analysis of intestine microbiota of four wild waterbird species. *Frontiers in Microbiology*, *10*, 1911. https://doi.org/10.3389/fmicb.2019.01911

Lavrinienko, A., Tukalenko, E., Kesäniemi, J., Kivisaari, K., Masiuk, S., Boratyński, Z., Mousseau, T. A., Milinevsky, G., Mappes, T., & Watts, P. C. (2020). Applying the Anna Karenina principle for wild animal gut microbiota: Temporal stability of the bank vole gut microbiota in a disturbed environment. *Journal of Animal Ecology*, *89*(11), 2617–2630. https://doi.org/10.1111/1365-2656.13342

Lawson, B., Malnick, H., Pennycott, T. W., Macgregor, S. K., John, S. K., Duncan, G., Hughes, L. A., Chantrey, J., & Cunningham, A. A. (2011). Acute necrotising pneumonitis associated with Suttonella ornithocola infection in tits (Paridae). *The Veterinary Journal*, *188*(1), 96–100. https://doi.org/10.1016/j.tvjl.2010.03.010

Leclaire, S., Helfenstein, F., Degeorges, A., Wagner, R. H., & Danchin, E. (2010). Family size and sex-specific parental effort in black-legged kittiwakes. *Behaviour*, *147*, 1841–1862. https://doi.org/10.1163/000579510X538872

Leclaire, S., Jacob, S., Greene, L. K., Dubay, G. R., & Drea, C. M. (2017). Social odours covary with bacterial community in the anal secretions of wild meerkats. *Scientific Reports*, *7*(1), 3240. https://doi.org/10.1038/s41598-017-03356-x

Leclaire, S., Merkling, T., Raynaud, C., Giacinti, G., Bessière, J. M., Hatch, S. A., & Danchin, E. (2011). An individual and a sex odor signature in kittiwakes? Study of the semiochemical composition of preen secretion and preen down feathers. *Naturwissenschaften*, *98*, 615–624. https://doi.org/10.1007/s00114-011-0809-9

Leclaire, S., Pierret, P., Chatelain, M., & Gasparini, J. (2014). Feather bacterial load affects plumage condition, iridescent color, and investment in preening in pigeons. *Behavioral Ecology*, *25*(5), 1192–1198. https://doi.org/10.1093/beheco/aru109

Leclaire, S., Strandh, M., Dell'Ariccia, G., Gabirot, M., Westerdahl, H., & Bonadonna, F. (2019). Plumage microbiota covaries with the major histocompatibility complex in blue petrels. *Molecular Ecology*, *28*(4), 833–846. https://doi.org/10.1111/mec.14993

Liang, J. Q., Li, T., Nakatsu, G., Chen, Y.-X., Yau, T. O., Chu, E., Wong, S., Szeto, C. H., Ng, S. C., Chan, F. K., & Fang, J. Y. (2020). A novel faecal Lachnoclostridium marker for the non-invasive diagnosis of colorectal adenoma and cancer. *Gut*, *69*(7), 1248–1257.

Lu, J., Santo Domingo, J. W., Lamendella, R., Edge, T., & Hill, S. (2008). Phylogenetic diversity and molecular detection of bacteria in gull feces. *Applied and Environmental Microbiology*, *74*(13), 3969–3976. https://doi.org/10.1128/AEM.00019-08

Ma, Z. S. (2020). Testing the Anna Karenina principle in human microbiome-associated diseases. *Iscience*, *23*(4), 101007. https://doi.org/10.1016/j.isci.2020.101007

Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2014). Swarm: Robust and fast clustering method for amplicon-based studies. *PeerJ*, *2*, e593. https://doi.org/10.7717/peerj.593

Mallott, E. K., Amato, K. R., Garber, P. A., & Malhi, R. S. (2018). Influence of fruit and invertebrate consumption on the gut microbiota of wild white-faced capuchins (*Cebus capucinus*). *American Journal of Physical Anthropology*, *165*(3), 576–588. https://doi.org/10.1002/ajpa.23395

Martínez-García, Á., Martín-Vivaldi, M., Rodríguez-Ruano, S. M., Peralta-Sánchez, J. M., Valdivia, E., & Soler, J. J. (2016). Nest bacterial environment affects microbiome of Hoopoe eggshells, but not that of the uropygial secretion. *PLoS One*, *11*(7), e0158158. https://doi.org/10.1371/journal.pone.0158158

Martin-Vivaldi, M., Pena, A., Manuel Peralta-Sanchez, J., Sanchez, L., Ananou, S., Ruiz-Rodriguez, M., & Jose Soler, J. (2010). Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1678), 123–130. https://doi.org/10.1098/rspb.2009.1377

Martín-Vivaldi, M., Soler, J. J., Peralta-Sánchez, J. M., Arco, L., Martín-Platero, A. M., Martínez-Bueno, M., Ruiz-Rodríguez, M., & Valdivia, E. (2014). Special structures of hoopoe eggshells enhance the adhesion of symbiont-carrying uropygial secretion that increase hatching success. *Journal of Animal Ecology*, *83*(6), 1289–1301. https://doi.org/10.1111/1365-2656.12243

Maurice, C. F., Knowles, S. C., Ladau, J., Pollard, K. S., Fenton, A., Pedersen, A. B., & Turnbaugh, P. J. (2015). Marked seasonal variation in the wild mouse gut microbiota. *The ISME Journal*, *9*(11), 2423–2434. https://doi.org/10.1038/ismej.2015.53

McFall-Ngai, M., Hadfield, M. G., Bosch, T. C. G., Carey, H. V., Domazet-Lošo, T., Douglas, A. E., Dubilier, N., Eberl, G., Fukami, T., Gilbert, S. F., Hentschel, U., King, N., Kjelleberg, S., Knoll, A. H., Kremer, N., Mazmanian, S. K., Metcalf, J. L., Nealson, K., Pierce, N. E., … Wernegreen, J. J. (2013). Animals in a bacterial world, a new imperative for the life sciences. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(9), 3229–3236. https://doi.org/10.1073/pnas.1218525110

McKnight, D. T., Huerlimann, R., Bower, D. S., Schwarzkopf, L., Alford, R. A., & Zenger, K. R. (2019). Methods for normalizing microbiome data: An ecological perspective. *Methods in Ecology and Evolution*, *10*(3), 389–400. https://doi.org/10.1111/2041-210X.13115

Merkeviciene, L., Ruzauskaite, N., Klimiene, I., Siugzdiniene, R., Dailidaviciene, J., Virgailis, M., & Ruzauskas, M. (2017). Microbiome and antimicrobial resistance genes in microbiota of cloacal samples from European herring gulls (*Larus argentatus*). *Journal of Veterinary Research*, *61*(1), 27–35.

Merkling, T., Leclaire, S., Danchin, E., Lhuillier, E., Wagner, R. H., White, J., Hatch, S. A., & Blanchard, P. (2012). Food availability and offspring

sex in a monogamous seabird: Insights from an experimental approach. *Behavioral Ecology*, *23*, 751–758. https://doi.org/10.1093/beheco/ars023

Moe, B., Langseth, I., Fyhn, M., Gabrielsen, G. W., & Bech, C. (2002). Changes in body condition in breeding kittiwakes *Rissa tridactyla*. *Journal of Avian Biology*, *33*(3), 225–234.

Moeller, A. H., & Sanders, J. G. (2020). Roles of the gut microbiota in the adaptive evolution of mammalian species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *375*(1808), 20190597. https://doi.org/10.1098/rstb.2019.0597

Moreno-Rueda, G. (2017). Preen oil and bird fitness: A critical review of the evidence. *Biological Reviews*, *92*(4), 2131–2143. https://doi.org/10.1111/brv.12324

Mosca, A., Leclerc, M., & Hugot, J. P. (2016). Gut microbiota diversity and human diseases: Should we reintroduce key predators in our ecosystem? *Frontiers in Microbiology*, *7*(455). https://doi.org/10.3389/fmicb.2016.00455

Mysak, J., Podzimek, S., Sommerova, P., Lyuya-Mi, Y., Bartova, J., Janatova, T., Prochazkova, J., & Duskova, J. (2014). *Porphyromonas gingivalis*: Major periodontopathic pathogen overview. *Journal of Immunology Research*, *2014*. https://doi.org/10.1155/2014/476068

Nol, E., & Smith, J. N. (1987). Effects of age and breeding experience on seasonal reproductive success in the song sparrow. *The Journal of Animal Ecology*, *56*(1), 301–313. https://doi.org/10.2307/4816

Olaisen, M., Flatberg, A., Granlund, A. V. B., Røyset, E. S., Martinsen, T. C., Sandvik, A. K., & Fossmark, R. (2021). Bacterial mucosa-associated microbiome in inflamed and proximal noninflamed ileum of patients with Crohn's disease. *Inflammatory Bowel Diseases*, *27*(1), 12–24. https://doi.org/10.1093/ibd/izaa107

Othman, A., Abba, Y., Jesse, F., Ilyasu, Y., Saharee, A., Haron, A., Zamri-Saad, M., & Lila, M. (2016). Reproductive pathological changes associated with experimental subchronic corynebacterium pseudotuberculosis infection in nonpregnant boer does. *Journal of Pathogens*, *2016*. https://doi.org/10.1155/2016/4624509

Pearce, D. S., Hoover, B. A., Jennings, S., Nevitt, G. A., & Docherty, K. M. (2017). Morphological and genetic factors shape the microbiome of a seabird species (*Oceanodroma leucorhoa*) more than environmental and social factors. *Microbiome*, *5*(1), 146. https://doi.org/10.1186/s40168-017-0365-4

Peralta-Sánchez, J. M., Colmenero, J., Redondo-Sánchez, S., Ontanilla, J., & Soler, M. (2020). Females are more determinant than males in reproductive performance in the house sparrow *Passer domesticus*. *Journal of Avian Biology*, *51*(2).

Pineaux, M., Merkling, T., Danchin, E., Hatch, S., Duneau, D., Blanchard, P., & Leclaire, S. (2020). Sex and hatching order modulate the association between MHC-II diversity and fitness in early-life stages of a wild seabird. *Molecular Ecology*, *29*(17), 3316–3329. https://doi.org/10.1111/mec.15551

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., & Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research*, *41*(D1), D590–D596. https://doi.org/10.1093/nar/gks1219

R Core Team. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Raimondi, S., Candeliere, F., Amaretti, A., Foschi, C., Morselli, S., Gaspari, V., Rossi, M., & Marangoni, A. (2021). Vaginal and anal microbiome during *Chlamydia trachomatis* infections. *Pathogens*, *10*(10), 1347. https://doi.org/10.3390/pathogens10101347

Ranstam, J. (2019). Hypothesis-generating and confirmatory studies, Bonferroni correction, and pre-specification of trial endpoints. *Acta Orthopaedica*, *90*(4), 297. https://doi.org/10.1080/17453674.2019.1612624

Raymann, K., & Moran, N. A. (2018). The role of the gut microbiome in health and disease of adult honey bee workers. *Current Opinion in Insect Science*, *26*, 97–104. https://doi.org/10.1016/j.cois.2018.02.012

Reaney, L. T., & Knell, R. J. (2010). Immune activation but not male quality affects female current reproductive investment in a dung beetle. *Behavioral Ecology*, *21*(6), 1367–1372. https://doi.org/10.1093/beheco/arq139

Reed, T. E., Harris, M. P., & Wanless, S. (2015). Skipped breeding in common guillemots in a changing climate: restraint or constraint? *Frontiers in Ecology and Evolution*, *3*, 1. https://doi.org/10.3389/fevo.2015.00001

Reese, A. T., & Dunn, R. R. (2018). Drivers of microbiome biodiversity: A review of general rules, feces, and ignorance. *MBio*, *9*(4), e01294–e11218. https://doi.org/10.1128/mBio.01294-18

Riegel, P., Ruimy, R., de Briel, D., Prévost, G., Jehl, F., Bimet, F., & Monteil, H. (1995). *Corynebacterium seminale* sp. *nov*., a new species associated with genital infections in male patients. *Journal of Clinical Microbiology*, *33*(9), 2244–2249.

Rivera-Pinto, J., Egozcue, J. J., Pawlowsky-Glahn, V., Paredes, R., Noguera-Julian, M., & Calle, M. L. (2018). Balances: A new perspective for microbiome analysis. *mSystems*, *3*(4). https://doi.org/10.1128/mSystems.00053-18

Rodrigues, D. F., da C Jesus, E., Ayala-del-Río, H. L., Pellizari, V. H., Gilichinsky, D., Sepulveda-Torres, L., & Tiedje, J. M. (2009). Biogeography of two cold-adapted genera: *Psychrobacter* and *Exiguobacterium*. *The ISME Journal*, *3*(6), 658–665. https://doi.org/10.1038/ismej.2009.25

Rodríguez-Ruano, S. M., Martín-Vivaldi, M., Martín-Platero, A. M., López-López, J. P., Peralta-Sánchez, J. M., Ruiz-Rodríguez, M., Soler, J. J., Valdivia, E., & Martínez-Bueno, M. (2015). The hoopoe's uropygial gland hosts a bacterial community influenced by the living conditions of the bird. *PLoS One*, *10*(10), e0139734. https://doi.org/10.1371/journal.pone.0139734

Rodríguez-Ruano, S., Martín-Vivaldi, M., Peralta-Sánchez, J., García-Martín, A., Martínez-García, Á., Soler, J., Valdivia, E., & Martínez-Bueno, M. (2018). Seasonal and sexual differences in the microbiota of the hoopoe uropygial secretion. *Genes*, *9*(8), 407. https://doi.org/10.3390/genes9080407

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Ross, A. A., Rodrigues Hoffmann, A., & Neufeld, J. D. (2019). The skin microbiome of vertebrates. *Microbiome*, *7*(1), 79. https://doi.org/10.1186/s40168-019-0694-6

Rosshart, S. P., Vassallo, B. G., Angeletti, D., Hutchinson, D. S., Morgan, A. P., Takeda, K., Hickman, H. D., McCulloch, J. A., Badger, J. H., Ajami, N. J., Trinchieri, G., Pardo-Manuel de Villena, F., Yewdell, J. W., & Rehermann, B. (2017). Wild mouse gut microbiota promotes host fitness and improves disease resistance. *Cell*, *171*(5), 1015–1028.e1013. https://doi.org/10.1016/j.cell.2017.09.016

Rowe, M., Veerus, L., Trosvik, P., Buckling, A., & Pizzari, T. (2020). The reproductive microbiome: An emerging driver of sexual selection, sexual conflict, mating systems, and reproductive isolation. *Trends in Ecology & Evolution*, *35*(3), 220–234. https://doi.org/10.1016/j.tree.2019.11.004

Saag, P., Mänd, R., Tilgar, V., Kilgas, P., Mägi, M., & Rasmann, E. (2011). Plumage bacterial load is related to species, sex, biometrics and fledging success in co-occurring cavity-breeding passerines. *Acta Ornithologica*, *46*(2), 191–201. https://doi.org/10.3161/000164511X62596

Salter, S. J., Cox, M. J., Turek, E. M., Calus, S. T., Cookson, W. O., Moffatt, M. F., Turner, P., Parkhill, J., Loman, N. J., & Walker, A. W. (2014). Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology*, *12*(1), 87. https://doi.org/10.1186/s12915-014-0087-z

Sandhu, T. S. (2008). *Riemerella anatipestifer* infection. *Diseases of Poultry*, 758–764.

Schliep, K. P. (2011). phangorn: Phylogenetic analysis in R. *Bioinformatics*, *27*(4), 592–593. https://doi.org/10.1093/bioinformatics/btq706

Shawkey, M. D., Hussain, M. J., Strong, A. L., Hagelin, J. C., Vollmer, A. C., & Hill, G. E. (2006). Use of culture-independent methods to compare bacterial assemblages on feathers of crested and least Auklets (*Aethia cristatella and Aethia pusilla*) with those of passerines. *Waterbirds*, *29*(4), 507–511. https://doi.org/10.1675/1524-4695(2006)29%5B507:UOCMTC%5D2.0.CO;2

Silverman, J. D., Washburne, A. D., Mukherjee, S., & David, L. A. (2017). A phylogenetic transform enhances analysis of compositional microbiota data. *Elife*, *6*, e21887.

Simon, J.-C., Marchesi, J. R., Mougel, C., & Selosse, M.-A. (2019). Host-microbiota interactions: From holobiont theory to analysis. *Microbiome*, *7*(1), 1–5. https://doi.org/10.1186/s40168-019-0619-4

Sison-Mangus, M. P., Mushegian, A. A., & Ebert, D. (2014). Water fleas require microbiota for survival, growth and reproduction. *The ISME Journal*, *9*, 59. https://doi.org/10.1038/ismej.2014.116

Slagsvold, T., & Lifjeld, J. T. (1990). Influence of male and female quality on clutch size in tits (*Parus Spp*.). *Ecology*, *71*(4), 1258–1266. https://doi.org/10.2307/1938263

Smith, S., Mang, T., De Bellocq, J. G., Schaschl, H., Zeitlhofer, C., Hacklaender, K., & Suchentrunk, F. (2010). Homozygosity at a class II MHC locus depresses female reproductive ability in European brown hares. *Molecular Ecology*, *19*(19), 4131–4143.

Soler, J. J., Martín-Vivaldi, M., Peralta-Sánchez, J. M., & Ruiz-Rodríguez, M. (2010). Antibiotic-producing bacteria as a possible defence of birds against pathogenic microorganisms. *The Open Ornithology Journal*, *3*(1), 93–100. https://doi.org/10.2174/1874453201003010093

Stanley, D., Hughes, R. J., Geier, M. S., & Moore, R. J. (2016). Bacteria within the gastrointestinal tract microbiota correlated with improved growth and feed conversion: Challenges presented for the identification of performance enhancing probiotic bacteria. *Frontiers in Microbiology*, *7*, 187. https://doi.org/10.3389/fmicb.2016.00187

Stothart, M. R., Greuel, R. J., Gavriliuc, S., Henry, A., Wilson, A. J., McLoughlin, P. D., & Poissant, J. (2021). Bacterial dispersal and drift drive microbiome diversity patterns within a population of feral hindgut fermenters. *Molecular Ecology*, *30*(2), 555–571. https://doi.org/10.1111/mec.15747

Suzuki, T. A. (2017). Links between natural variation in the microbiome and host fitness in wild mammals. *Integrative and Comparative Biology*, *57*(4), 756–769. https://doi.org/10.1093/icb/icx104

Tartu, S., Goutte, A., Bustamante, P., Angelier, F., Moe, B., Clément-Chastel, C., Bech, C., Gabrielsen, G. W., Bustnes, J. O., & Chastel, O. (2013). To breed or not to breed: endocrine response to mercury contamination by an Arctic seabird. *Biology Letters*, *9*(4), 20130317. doi:https://doi.org/10.1098/rsbl.2013.0317

Teyssier, A., Rouffaer, L. O., Hudin, N. S., Strubbe, D., Matthysen, E., Lens, L., & White, J. (2018). Inside the guts of the city: Urban-induced alterations of the gut microbiota in a wild passerine. *Science of the Total Environment*, *612*, 1276–1286. https://doi.org/10.1016/j.scitotenv.2017.09.035

Theis, K. R., Schmidt, T. M., & Holekamp, K. E. (2012). Evidence for a bacterial mechanism for group-specific social odors among hyenas. *Scientific Reports*, *2*, 615. https://doi.org/10.1038/srep00615

Thoss, M., Ilmonen, P., Musolf, K., & Penn, D. J. (2011). Major histocompatibility complex heterozygosity enhances reproductive success. *Molecular Ecology*, *20*(7), 1546–1557. https://doi.org/10.1111/j.1365-294X.2011.05009.x

Tizard, I. R., & Jones, S. W. (2018). The microbiota regulates immunity and immunologic diseases in dogs and cats. *Veterinary Clinics: Small Animal Practice*, *48*(2), 307–322. https://doi.org/10.1016/j.cvsm.2017.10.008

Uller, T., Isaksson, C., & Olsson, M. (2006). Immune challenge reduces reproductive output and growth in a lizard. *Functional Ecology*, *20*(5), 873–879. https://doi.org/10.1111/j.1365-2435.2006.01163.x

Vacca, M., Celano, G., Calabrese, F. M., Portincasa, P., Gobbetti, M., & De Angelis, M. (2020). The controversial role of human gut *Lachnospiraceae*. *Microorganisms*, *8*(4), 573. https://doi.org/10.3390/microorganisms8040573

van Dongen, W. F., White, J., Brandl, H. B., Leclaire, S., Hatch, S. A., Danchin, É., & Wagner, R. H. (2019). Experimental evidence of a sexually transmitted infection in a wild vertebrate, the black-legged kittiwake (*Rissa tridactyla*). *Biological Journal of the Linnean Society*, *127*(2), 292–298. https://doi.org/10.1093/biolinnean/blz009

van Dongen, W. F. D., White, J., Brandl, H. B., Moodley, Y., Merkling, T., Leclaire, S., Blanchard, P., Danchin, É., Hatch, S. A., & Wagner, R. H. (2013). Age-related differences in the cloacal microbiota of a wild bird species. *BMC Ecology*, *13*, 11. https://doi.org/10.1186/1472-6785-13-11

Van Empel, P., & Hafez, H. (1999). *Ornithobacterium rhinotracheale*: A review. *Avian Pathology*, *28*(3), 217–227.

Verhulst, S., & Nilsson, J.-Å. (2008). The timing of birds' breeding seasons: A review of experiments that manipulated timing of breeding. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *363*(1490), 399–410. https://doi.org/10.1098/rstb.2007.2146

Vetriani, C., Crespo-Medina, M., & Antunes, A. (2014). The Family *Salinisphaeraceae*. In E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, & F. Thompson (Eds.), *The prokaryotes: Gammaproteobacteria* (pp. 591–596). Springer.

Videvall, E., Marzal, A., Magallanes, S., Fleischer, R. C., Espinoza, K., & García-Longoria, L. (2021). The uropygial gland microbiome of house sparrows with malaria infection. *Journal of Avian Biology*, *52*(2). https://doi.org/10.1111/jav.02686

Videvall, E., Song, S. J., Bensch, H. M., Strandh, M., Engelbrecht, A., Serfontein, N., Hellgren, O., Olivier, A., Cloete, S., Knight, R., & Cornwallis, C. K. (2019). Major shifts in gut microbiota during development and its relationship to growth in ostriches. *Molecular Ecology*, *28*(10), 2653–2667. https://doi.org/10.1111/mec.15087

Wang, C.-S.-E., Li, W.-B., Wang, H.-Y., Ma, Y.-M., Zhao, X.-H., Yang, H., Qian, J.-M., & Li, J.-N. (2018). VSL# 3 can prevent ulcerative colitis-associated carcinogenesis in mice. *World Journal of Gastroenterology*, *24*(37), 4254. https://doi.org/10.3748/wjg.v24.i37.4254

Weng, S.-L., Chiu, C.-M., Lin, F.-M., Huang, W.-C., Liang, C., Yang, T., Yang, T.-L., Liu, C.-Y., Wu, W.-Y., Chang, Y.-A., Chang, T.-H., & Huang, H.-D. (2014). Bacterial communities in semen from men of infertile couples: Metagenomic sequencing reveals relationships of seminal microbiota to semen quality. *PLoS One*, *9*(10), e110152. https://doi.org/10.1371/journal.pone.0110152

White, J., Mirleau, P., Danchin, E., Mulard, H., Hatch, S. A., Heeb, P., & Wagner, R. H. (2010). Sexually transmitted bacteria affect female cloacal assemblages in a wild bird. *Ecology Letters*, *13*(12), 1515–1524. https://doi.org/10.1111/j.1461-0248.2010.01542.x

Whittaker, D. J., Gerlach, N. M., Slowinski, S. P., Corcoran, K. P., Winters, A. D., Soini, H. A., Novotny, M. V., Ketterson, E. D., & Theis, K. R. (2016). Social environment has a primary influence on the microbial and odor profiles of a chemically signaling songbird. *Frontiers in Ecology and Evolution*, *4*, 90. https://doi.org/10.3389/fevo.2016.00090

Whittaker, D. J., & Theis, K. R. (2016). Bacterial communities associated with junco preen glands: Preliminary ramifications for chemical signaling. In B. A. Schulte, T. E. Goodwin, & M. H. Ferkin (Eds.), *Chemical signals in vertebrates 13* (pp. 105–117). Springer International Publishing.

Wright, E. S. (2015). DECIPHER: Harnessing local sequence context to improve protein multiple sequence alignment. *BMC Bioinformatics*, *16*(1), 322. https://doi.org/10.1186/s12859-015-0749-z

Zaneveld, J. R., McMinds, R., & Thurber, R. V. (2017). Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology*, *2*(9), 1–8. https://doi.org/10.1038/nmicrobiol.2017.121

SUPPORTING INFORMATION

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