

Supplementary Material

Early sexual dimorphism in the developing gut microbiome of northern elephant seals

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Supplementary Material 1 – Tables and Figures

| Kingdom | Phylum | Class | Order | Family | Genus | Species | Mean rel. abundance % |
|----------|----------------|----------------------|-------------------|-----------------------|-----------------------|------------|-----------------------|
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 17.22 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | NA | 7.84 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | mortiferum | 2.04 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | mortiferum | 1.99 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | fragilis | 1.67 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 1.22 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Anaerococcus | NA | 1.04 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | NA | NA | 0.97 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Odoribacter | NA | 0.93 |
| Bacteria | Proteobacteria | Gammaaproteobacteria | Aeromonadales | Succinivibrionaceae | Anaerobiospirillum | NA | 0.89 |
| Bacteria | Proteobacteria | Gammaaproteobacteria | Enterobacteriales | Enterobacteriaceae | Escherichia/Shigella | NA | 0.72 |
| Bacteria | Proteobacteria | Gammaaproteobacteria | Aeromonadales | Succinivibrionaceae | Anaerobiospirillum | NA | 0.60 |
| Bacteria | Firmicutes | Negativicutes | Selenomonadales | Acidaminococcaceae | Phascolarctobacterium | NA | 0.51 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Peptoniphilus | NA | 0.49 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Peptostreptococcaceae | Peptoclostridium | NA | 0.49 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 0.48 |
| Bacteria | Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae | Dialister | NA | 0.44 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Anaerotruncus | NA | 0.35 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Lachnospiraceae | Blautia | NA | 0.14 |
| Bacteria | Actinobacteria | Coriobacteriia | Coriobacteriales | Coriobacteriaceae | Collinsella | NA | 0.11 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Parabacteroides | merdae | 0.08 |

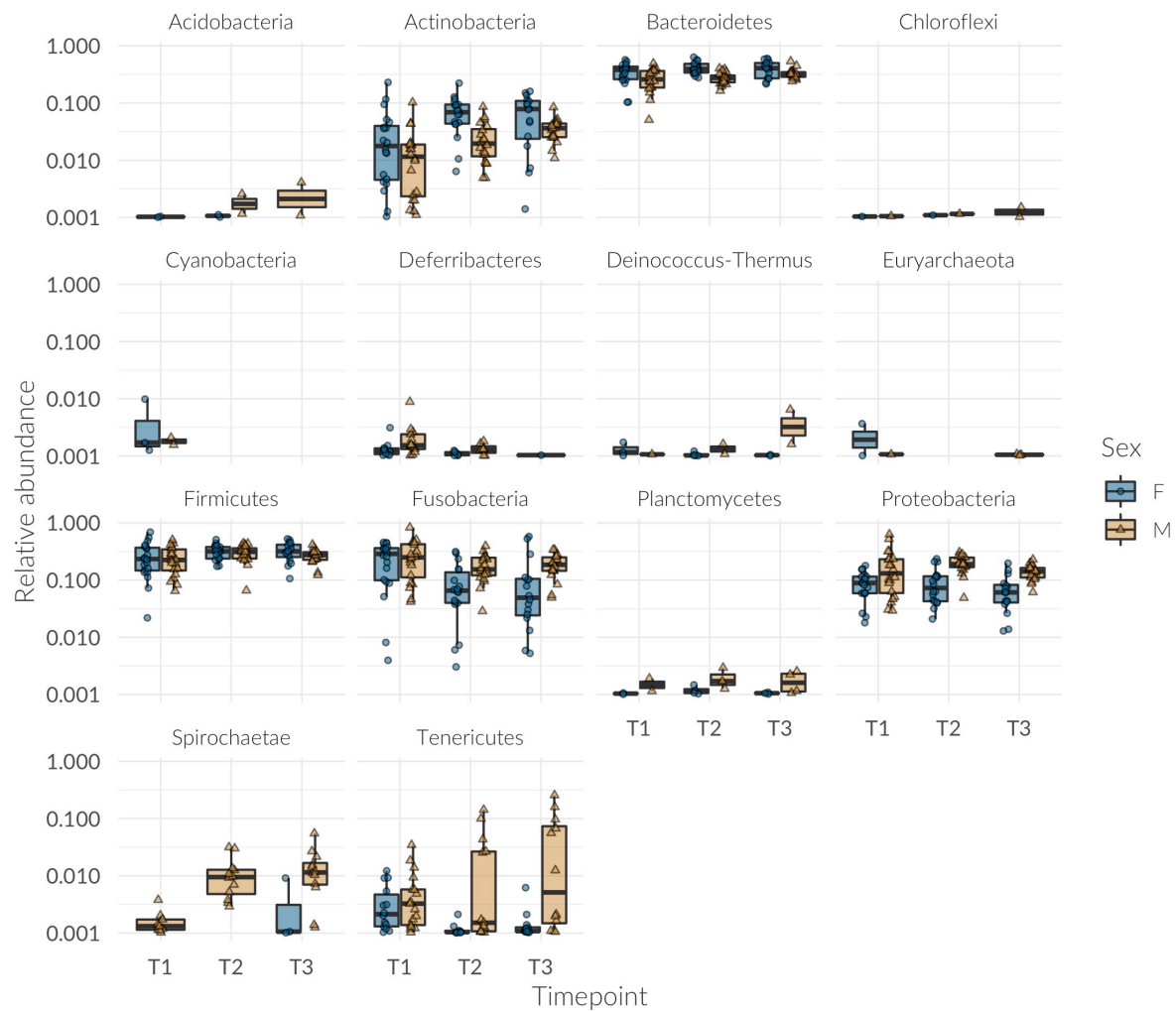
Supplementary Table 1: Core microbiome (ASVs) shared among at least 95 % of individuals during sampling time point one (T1). In some cases, a taxonomic level could not be assigned (NA). Shown is also the mean relative abundance of each core ASV across all samples at T1.

| Kingdom | Phylum | Class | Order | Family | Genus | Species | Mean rel. abundance % |
|----------|----------------|----------------|-------------------|--------------------|-------------------------|------------|-----------------------|
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Ezakiella | NA | 4.37 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 3.22 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | NA | 2.75 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | fragilis | 2.30 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Odoribacter | NA | 1.40 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | mortiferum | 1.02 |
| Bacteria | Actinobacteria | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Lawsonella | NA | 0.97 |
| Bacteria | Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae | Dialister | NA | 0.94 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Peptoniphilus | NA | 0.86 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | mortiferum | 0.73 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Anaerococcus | NA | 0.69 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Ruminococcaceae_UCG-005 | NA | 0.50 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Anaerococcus | NA | 0.48 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Faecalibacterium | NA | 0.35 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Rikenellaceae | Alistipes | NA | 0.24 |

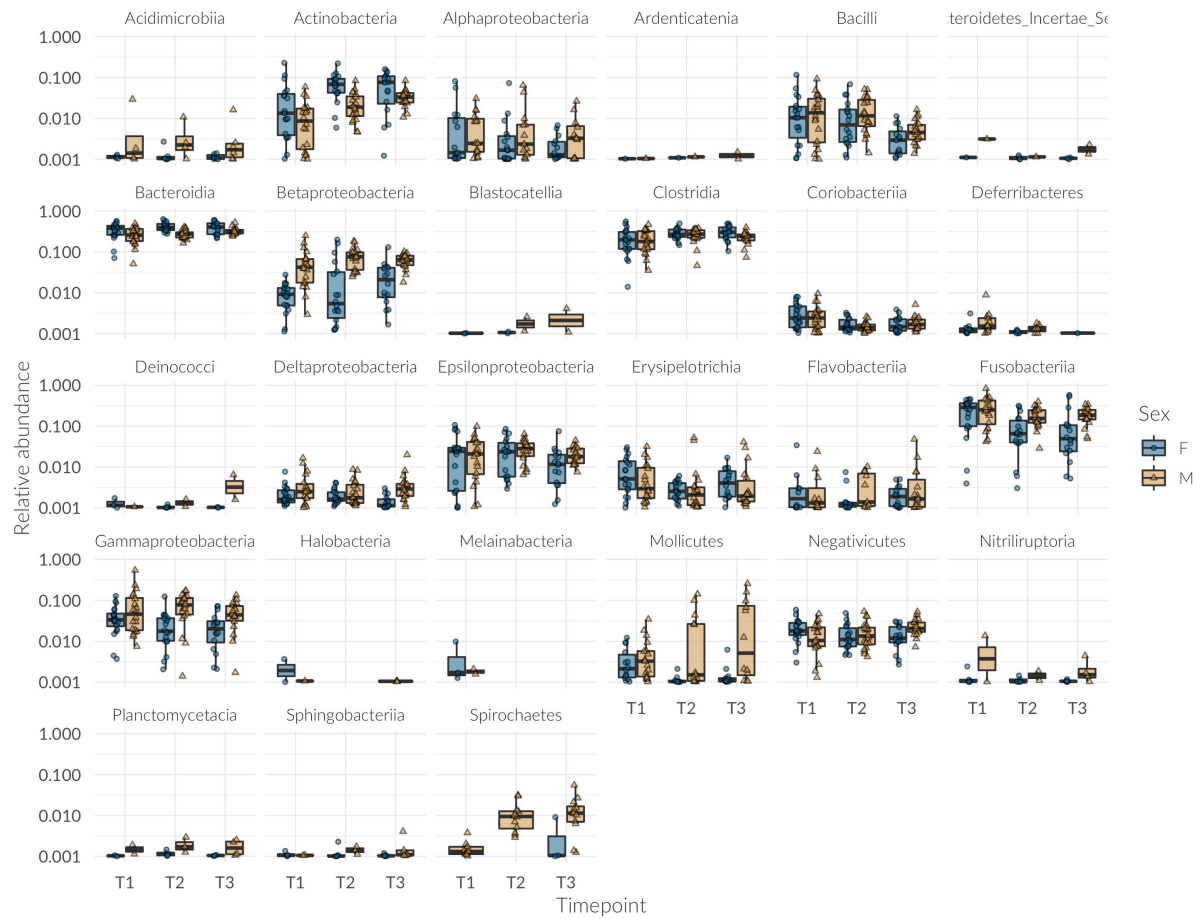
Supplementary Table 2: Core microbiome (ASVs) shared among at least 95 % of individuals during sampling time point two (T2). In some cases, a taxonomic level could not be assigned (NA). Shown is also the mean relative abundance of each core ASV across all samples at T2

| Kingdom | Phylum | Class | Order | Family | Genus | Species | Mean rel. abundance % |
|----------|----------------|---------------------|--------------------|---------------------|-------------------------|------------|-----------------------|
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Ezakiella | NA | 4.23 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Prevotellaceae | Prevotella | NA | 4.22 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Porphyromonas | NA | 3.05 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Proteiniphilum | NA | 3.02 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 2.85 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | NA | 2.68 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Porphyromonas | NA | 2.00 |
| Bacteria | Actinobacteria | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Lawsonella | NA | 1.37 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | NA | 1.11 |
| Bacteria | Firmicutes | Negativicutes | Selenomonadales | Veillonellaceae | Dialister | NA | 0.90 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Ruminococcaceae_UCG-005 | NA | 0.90 |
| Bacteria | Actinobacteria | Actinobacteria | Corynebacteriales | Corynebacteriaceae | Lawsonella | NA | 0.86 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Anaerococcus | NA | 0.86 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Anaerococcus | NA | 0.83 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Porphyromonadaceae | Odoribacter | NA | 0.79 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | mortiferum | 0.75 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Ruminococcaceae_UCG-005 | NA | 0.71 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | mortiferum | 0.66 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Peptoniphilus | NA | 0.51 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Bacteroidaceae | Bacteroides | fragilis | 0.48 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | NA | NA | 0.46 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Anaerotruncus | NA | 0.43 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Ruminococcaceae_UCG-005 | NA | 0.40 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Ruminococcaceae_UCG-005 | NA | 0.30 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 0.28 |
| Bacteria | Firmicutes | Erysipelotrichia | Erysipelotrichales | Erysipelotrichaceae | NA | NA | 0.27 |
| Bacteria | Bacteroidetes | Bacteroidia | Bacteroidales | Rikenellaceae | Alistipes | NA | 0.14 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Anaerotruncus | NA | 0.11 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Ruminococcaceae | Ruminococcaceae_UCG-005 | NA | 0.11 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XIII | NA | NA | 0.10 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Family_XI | Peptoniphilus | NA | 0.10 |
| Bacteria | Firmicutes | Clostridia | Clostridiales | Lachnospiraceae | Blautia | NA | 0.10 |
| Bacteria | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Psychrobacter | NA | 0.09 |
| Bacteria | Proteobacteria | Gammaproteobacteria | Pseudomonadales | Moraxellaceae | Psychrobacter | NA | 0.09 |
| Bacteria | Fusobacteria | Fusobacteriia | Fusobacteriales | Fusobacteriaceae | Fusobacterium | NA | 0.07 |

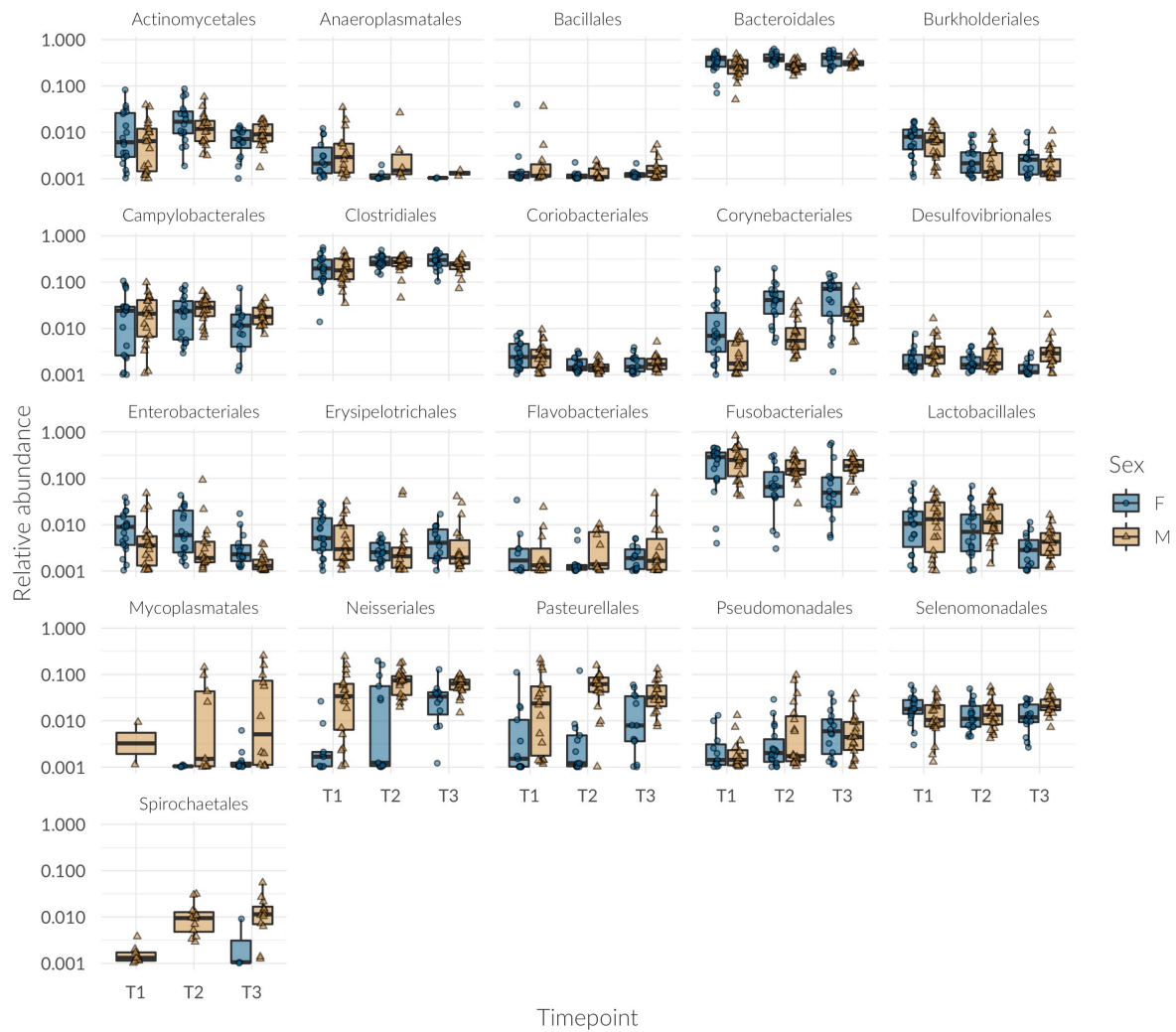
Supplementary Table 3: Core microbiome (ASVs) shared among at least 95 % of samples during sampling time point three (T3). In some cases, a taxonomic level could not be assigned (NA). Shown is also the mean relative abundance of each core ASV across all samples at T3.



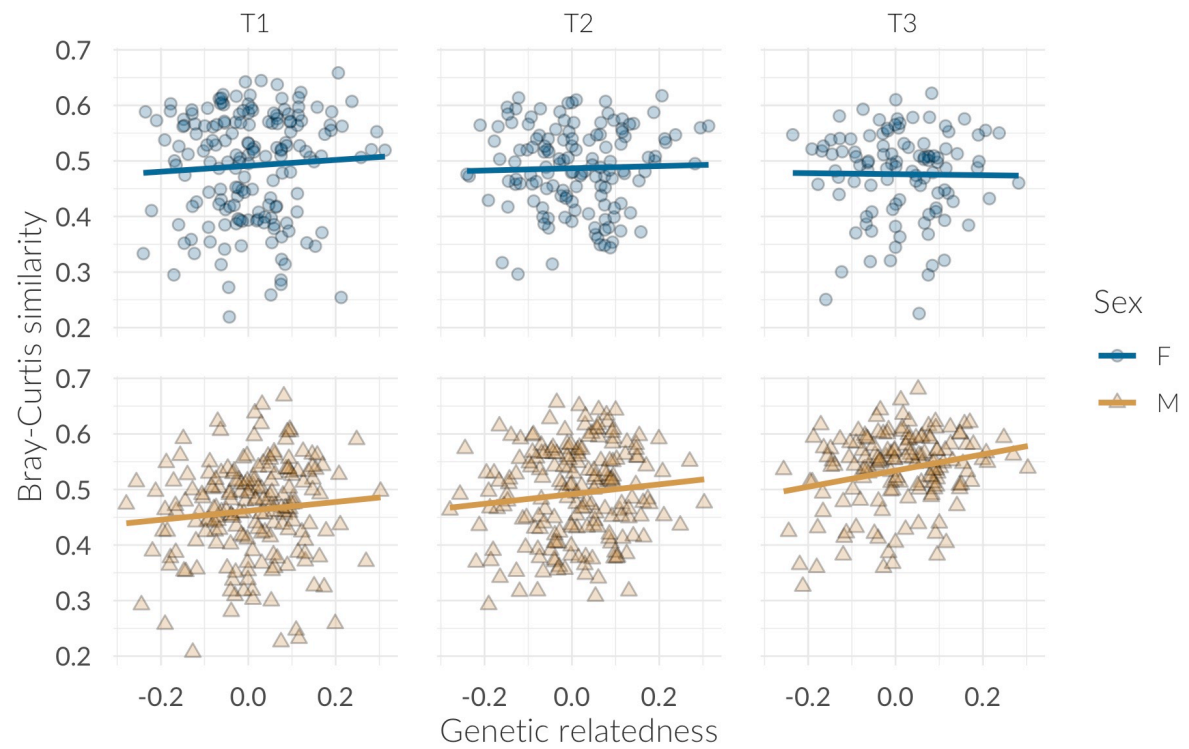
Supplementary Figure 1: Relative abundance of all bacterial taxa analysed in this study at the *Phylum* level across time points and colored by sex. Before visualization on the log scale, taxa with zero abundance were discarded and 0.001 was added to all remaining relative abundances.



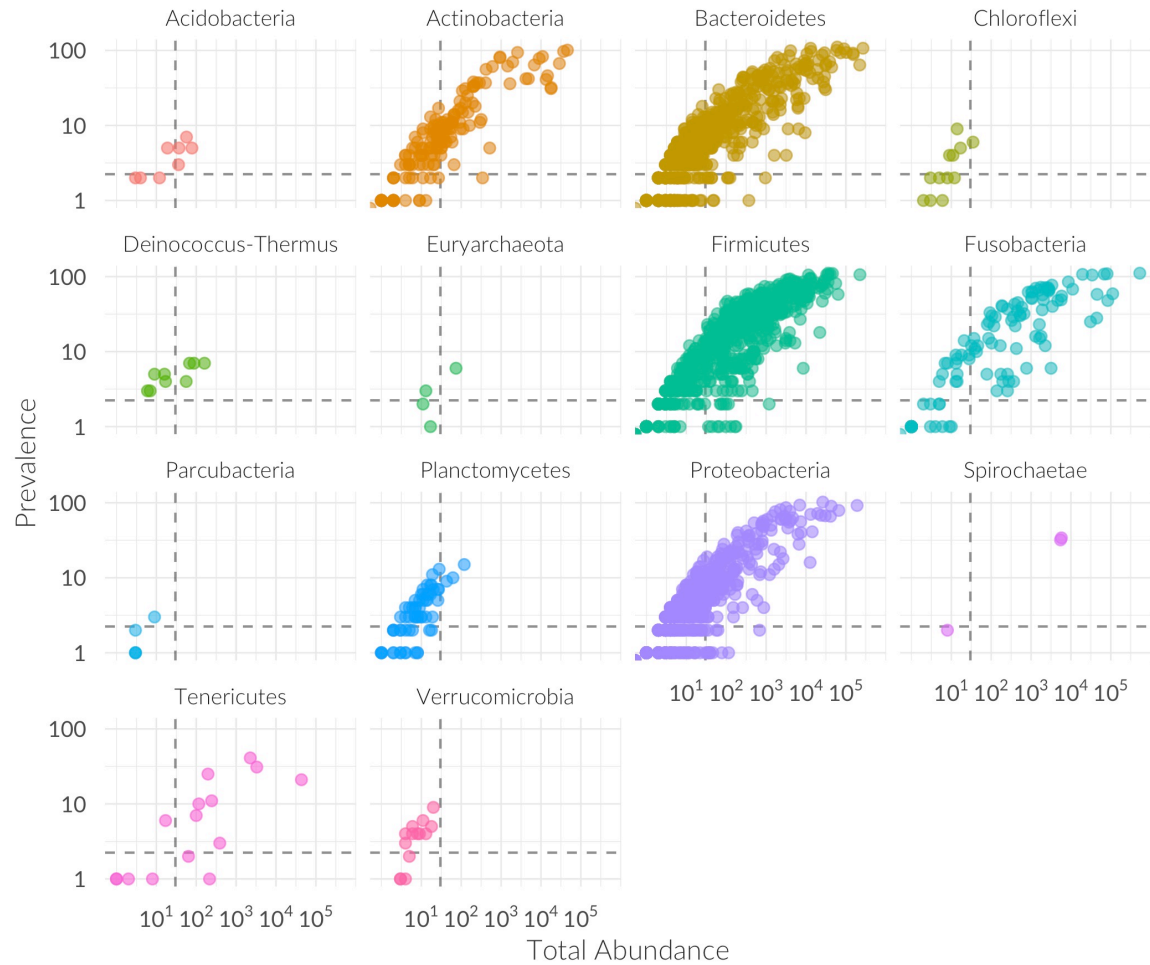
Supplementary Figure 2: Relative abundance of all bacterial taxa analysed in this study at the *Class* level across time points and colored by sex. Before visualization on the log scale, taxa with zero abundance were discarded and 0.001 was added to all remaining relative abundances.



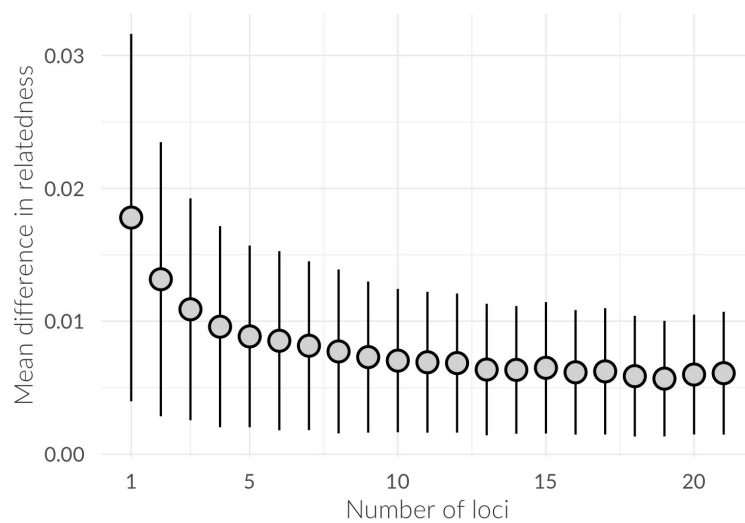
Supplementary Figure 3: Relative abundance of bacterial taxa analysed in this study at the *Order* level across time and colored by sex. Before visualization on the log scale, taxa with zero abundance were discarded and 0.001 was added to all remaining relative abundances. Shown is a subset of bacterial orders with interesting patterns and/or high prevalence across samples.



Supplementary Figure 4: Correlations between microbial similarity and genetic relatedness at three time points, split by sex.



Supplementary Figure 5: Prevalence and total abundance of taxa split by phylum. The horizontal and vertical dashed lines represent the cut-offs for filtering, with taxa present in fewer than three individuals and/or with an overall read count lower than 30 being discarded.



Supplementary Figure 1: Sensitivity of the Loiselle relatedness estimator to the number of loci used. Plotted are the mean and standard deviation (SD) of differences in pairwise genetic relatedness against the number of loci used. SDs were calculated from 1000 bootstrap replicates per locus number.

Supplementary Material 2 – Differential abundances of specific taxa

Differential abundance of specific taxa with age

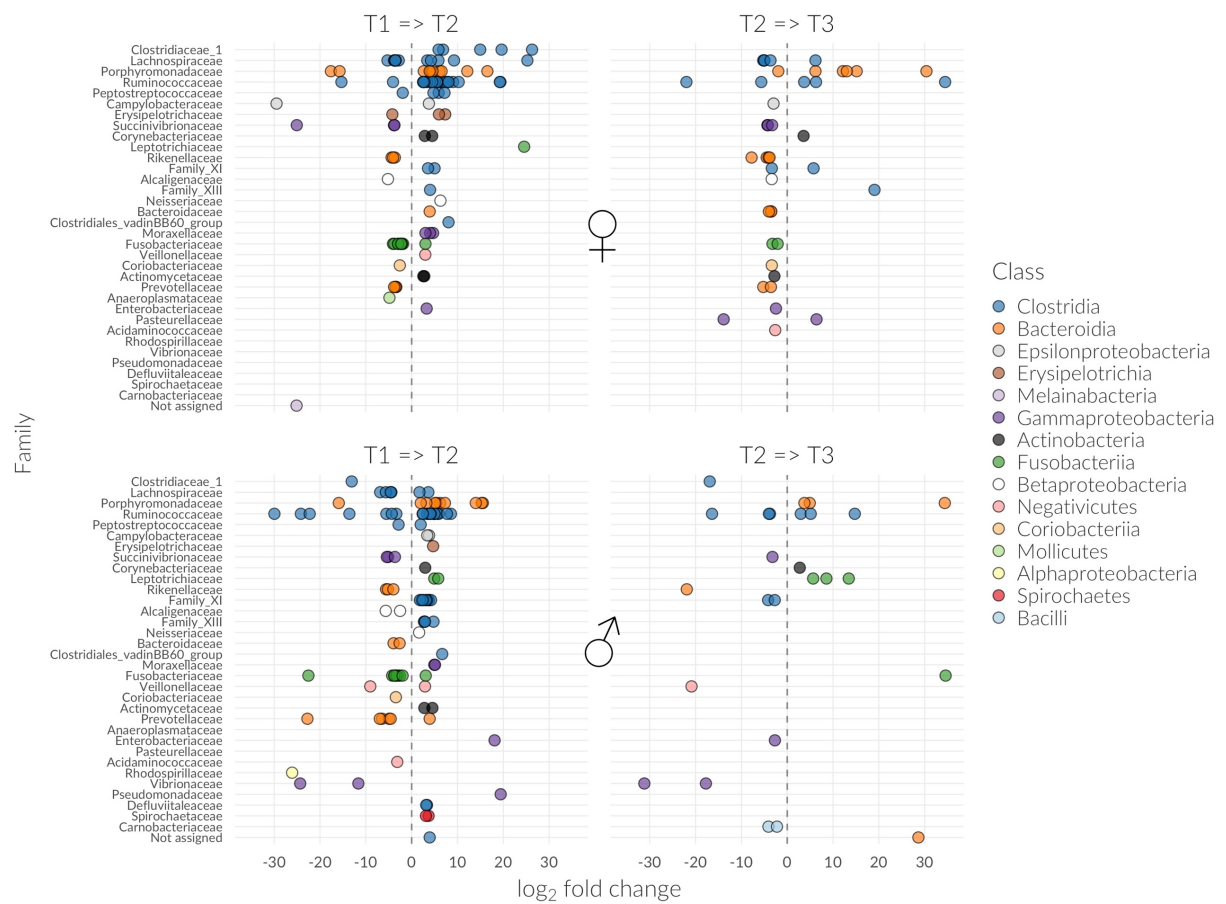
Despite the apparent similarity of phyla across all three time points (Figure 1), on a finer scale a large number of bacterial taxa changed in abundance over time (Supplementary Figure 7 and 8). Most significant changes happened early on, with a large number of taxa varying from T1 to T2 for each sex (F: n = 100, M: n = 106) followed by a smaller number of significantly different abundances of taxa between T2 and T3 (F: n = 43, M: n = 26). On a taxonomic scale, most bacterial classes changed substantially (Supplementary Figure 7). Between T1 and T2, most of the bacteria that changed abundance belonged to the Clostridia in both sexes (F: 47%, M: 44%), followed by the Bacteroidia (F: 18%, M: 20%) and Fusobacteria (F: 13%, M: 12%), a pattern that is very similar for the transition between T2 and T3 in males (Clostridia 35%, Bacteroidia 19%, Fusobacteria 15%), while in females the Bacteroidia (37%) changed substantially, more so than the Clostridia (30%) and Gammaproteobacteria (14 %). Several interesting changes were also apparent in some of the less abundant bacterial classes. While *Deferribacteres* went extinct over time, the *Spirochaetes* increased in relative abundance, mainly in males (Supplementary Figure 3), and started to colonise females at T3. The *Bacilli* and *Fusobacteria* depleted rapidly over time, while the *Actinobacteria* increased in their relative abundances by nearly ten-fold in females and by more than five-fold in males (Supplementary Figure 2).

Sex specific patterns of changes

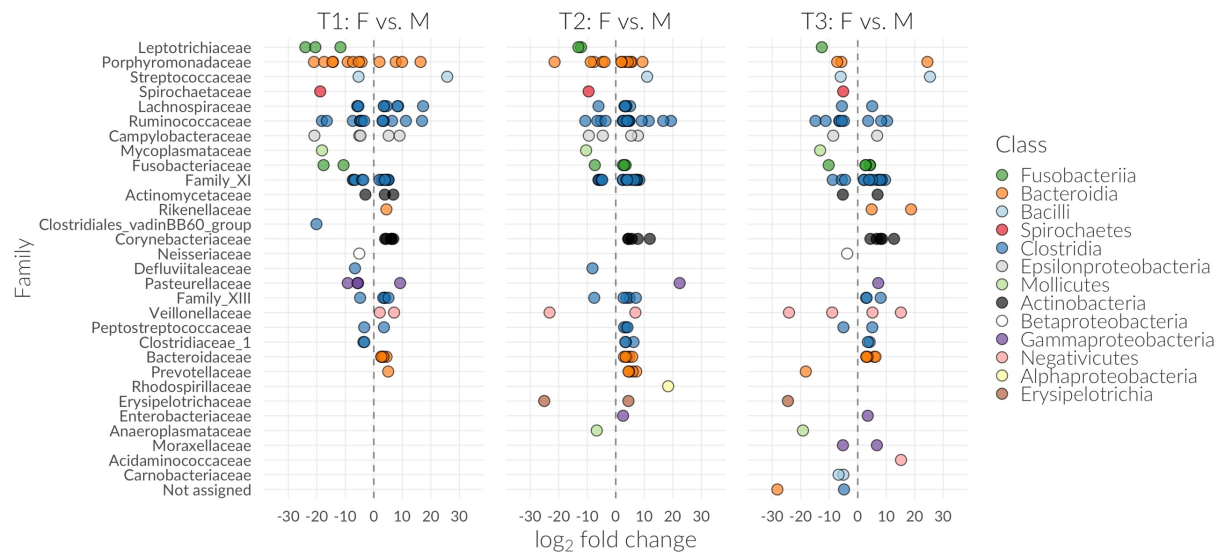
Bacterial communities in both sexes showed similar dynamics throughout the weaning period, although the 'baseline' abundances of many species differed substantially (Supplementary Figures 1-3, Supplementary Figure 8). On the phylum level, the microbial shift from T1 to T2 in both females and males consisted mostly of taxa belonging to the Firmicutes (F: 51%, M: 48%) followed by Bacteroidetes (F: 18%, M: 20%) and Fusobacteria in males (13%) but Proteobacteria in females (14 %). Interestingly, a few bacterial families underwent large changes in abundance from T1 to T2 and made up a major part of the significantly different taxa, especially the Ruminococcaceae (F: 22%, M: 19%) followed by the Fusobacteriaceae (F: 12%, M: 10%) and Lachnospiraceae in females (12%) but the Porphyromonadaceae in males (9%). Bacterial community composition changes between T2 and T3 mainly occurred in the phyla Bacteroidetes (37%), Firmicutes (32%) and Proteobacteria (19%) in females and in the Firmicutes (46%), Bacteroidetes (19%), Fusobacteria (15%) and Proteobacteria (15%) in males. The majority of differentially abundant taxa belonged, similarly to the first transition, to the Ruminococcaceae (F: 12%, M: 23%), Porphyromonadaceae (F: 16%, M: 12%) and the Lachnospiraceae (12%) in females as well as the Leptotrichiaceae (12%) in males.

Differential abundance of taxa across the sexes

Despite showing similar dynamics over time, many taxa were significantly differentially abundant in males and females within all three time points (T1: n = 96, T2: n = 102, T3: n = 80, see Figures 3 and 5). Although many phylogenetically different taxa contributed to these sex-specific differences, three families contributed disproportionately. The Clostridiales Family XI contributed 15% of differentially abundant taxa at T1, 16% at T2, and 18% at T3. The Ruminococcaceae contributed 15% of the taxa at T1, 19 % at T2 and 13 % at T3. The Porphyromonadaceae differed considerably at T1 (13%) and T2 (12%) but less so at T3 (4%).



Supplementary Figure 7: Differential abundance of taxa between sampling points, split by sex.



Supplementary Figure 8: Differential abundance of microbes between sex, split by sampling points

Supplementary Material 3 - Genotyping methods

Total genomic DNA of 40 *Mirounga angustirostris* samples was extracted from each sample using silica-gel membrane technology (DNeasy Blood and Tissue kit, Qiagen) and genotyped at 21 previously developed microsatellite loci (see Supplementary Table 4 for details). The microsatellite loci were amplified in singleplex or multiplex reactions. The following PCR profile was used: one cycle of 3 min at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at T_a °C and 40 s at 72 °C; 8 cycles of 30 s at 94 °C, 30 s at 47 °C and 40 s at 72 °C; and one final cycle of 10 min at 72 °C (see Supplementary Table 14 for T_a). Magnesium concentrations varied among the PCR mastermixes as shown in Supplementary Table 14. Fluorescently labelled PCR products were resolved by electrophoresis on an ABI 3730xl capillary sequencer and allele sizes were scored automatically using GeneMarker v1.85. To ensure high genotype quality, all traces were manually inspected and any incorrect calls were adjusted accordingly.

| Locus | Literature source | Mg (mM) | T _a (°C) |
|------------|-----------------------------------|---------|---------------------|
| 71HDZ441 | Huebinger et al. (2007) | 1.5 | 54 |
| Hg4.2 | Allen et al. (1995) | 1.5 | 56 |
| Lw-8 | Davis et al. (2002) | 1.5 | 47 |
| ZcCgDh4.7 | Hernandez-Velazquez et al. (2005) | 1.75 | 56 |
| PV9 | Goodman (1997) | 2 | 53 |
| ZcCgDh3.6 | Hernandez-Velazquez et al. (2005) | 2 | 39 |
| HI-8 | Davis et al. (2002) | 2 | 53 |
| PVC1 | Garza (1998) | 1.5 | 52 |
| 71HDZ301 | Huebinger et al. (2007) | 1.5 | 42 |
| ZcCgDh1.8 | Hernandez-Velazquez et al. (2005) | 1.5 | 42 |
| ZcwA12 | Hoffman et al. (2007) | 1.75 | 49 |
| ZcwF07 | Hoffman et al. (2007) | 1.75 | 49 |
| Ag-9 | Hoffman et al. (2008) | 2 | 57 |
| ZcwC01 | Hoffman et al. (2007) | 2 | 57 |
| ZcwE04 | Hoffman et al. (2007) | 2 | 52 |
| ZcwG04 | Hoffman et al. (2007) | 2 | 52 |
| Mango01 | (Sanvito et al., 2013) | 1.5 | 55 |
| Mango44 | (Sanvito et al., 2013) | 1.5 | 55 |
| Mango43 | (Sanvito et al., 2013) | 1.5 | 55 |
| Mango35 | (Sanvito et al., 2013) | 1.5 | 53 |
| Mango06 | (Sanvito et al., 2013) | 1.5 | 55 |
| Mango09E19 | (Sanvito et al., 2013) | 1.5 | 52 |
| PV9.1 | This study | 1.5 | 53 |

Supplementary Table 4: Microsatellite loci genotyped in the northern elephant seal. “Mg” denotes the concentration of magnesium used in the PCR mastermix and “T_a” denotes the annealing temperature used.