Introduction
While sponges are generally known to host a wide range of microbial associates, the composition and specificity of the microbial communities in carnivorous sponges are poorly understood. We used 16S-rRNA gene data and supporting experiments to examine and compare the bacterial communities associated with distinct anatomical regions of two carnivorous sponge species from the Canadian arctic.

Study goals
- Investigate similarities and differences in the bacterial communities associated with Chondrocladia grandis and Cladorhiza oxeata (Fig. 1), and determine which factors best explain assemblage composition (Fig 2, 3 & 4).
- Identify bacterial biomarkers for each anatomical region (Fig. 5) of these carnivorous sponges (i.e. taxa that are consistently and significantly enriched).
- Characterize the oligotype stability of common and biomarker taxa across host specimens from various geographical regions and depths (Fig 6).

Sample collection
Chondrocladia grandis (N=7) and Cladorhiza oxeata (N=2) were sampled from Baffin Bay and the Gulf of Maine (Fig. 1).

Microbiome richness
Large differences in bacterial diversity and evenness were observed between the two sponge species (Fig. 2). Microbial communities are not evenly distributed amongst anatomical regions. Specifically, within C. grandis the root and root-tip samples displayed the most diverse communities, while axis, sphere and stem where markedly less diverse.

Comparison of microbiomes
Systematic differences between the bacterial communities of the two sponge species were observed (Fig. 3). In C. grandis, a significant difference in bacterial composition among anatomical regions was also seen, with unique assemblages of bacteria within the root and root tip samples setting these regions apart from the remainder of the sponge body. Geographical location and host individual also influenced the composition of the microbiome (Figure 3B), albeit with a smaller effect size. Clustering of C. oxeata samples within the ordination analysis (Fig. 3C) could not be explained by available variables within our dataset and appeared more diffuse.

Bacterial community composition
Bacterial communities (Fig. 4) in both sponge species included a high abundance of Flavobacteria, γ-proteobacteria and α-proteobacteria. At family level, differences between C. grandis and C. oxeata were observed in terms of bacterial abundance and richness. In C. oxeata, the only ubiquitous family was the Flavobacteriaceae, whose members represented the majority of the community. C. grandis showed a larger diversity, with abundance values distributed amongst the most common families including the Flavobacteriaceae, Rhodobacteraceae, Haliceaeae, Colwelliaceae and Hyphomicrobiaceae.

Biomarker detection
While no biomarker taxa were found for Cladorhiza oxeata, the association between specific bacterial taxa and anatomical regions does occur within Arctic C. grandis (Fig. 5), in agreement with what previously observed (Verhoeven et al., 2017). These associations may constitute symbiotic relationships, although further functional studies are needed to fully confirm this hypothesis.

Oligototype specificity
Oligotyping of genera common to both sponge species (Tenacibaculum, Candidatus Branchiomonas and Fulvivirga) as well as biomarker genera, showed a host species-specific pattern of bacterial oligotypes, consistent throughout the entire sponge body. Within C. grandis this specificity remains unchanged even across a wide geographical distance (Fig 6).

Discussion
The two investigated sponge species hosted distinct bacterial communities, with taxonomic diversity being greater in C. grandis. Some bacterial taxa (including particular oligotypes) were consistently recovered in multiple host individuals from geographically distant sites, suggesting specificity. Within C. grandis, several bacterial taxa were enriched in particular anatomical regions, suggesting functional roles in carnivorous sponge metabolism or other biological processes. The observed differences in bacterial community composition and diversity between C. grandis and C. oxeata may reflect differences in trophic adaptability, specialization and overall reliance on associated bacteria.

References
Verhoeven, J.T.P. and Dufour, S.C., 2017. Microbiomes of the Arctic carnivorous sponges Chondrocladia grandis and Cladorhiza oxeata suggest a specific, but differential involvement of bacterial associates. Arctic Science