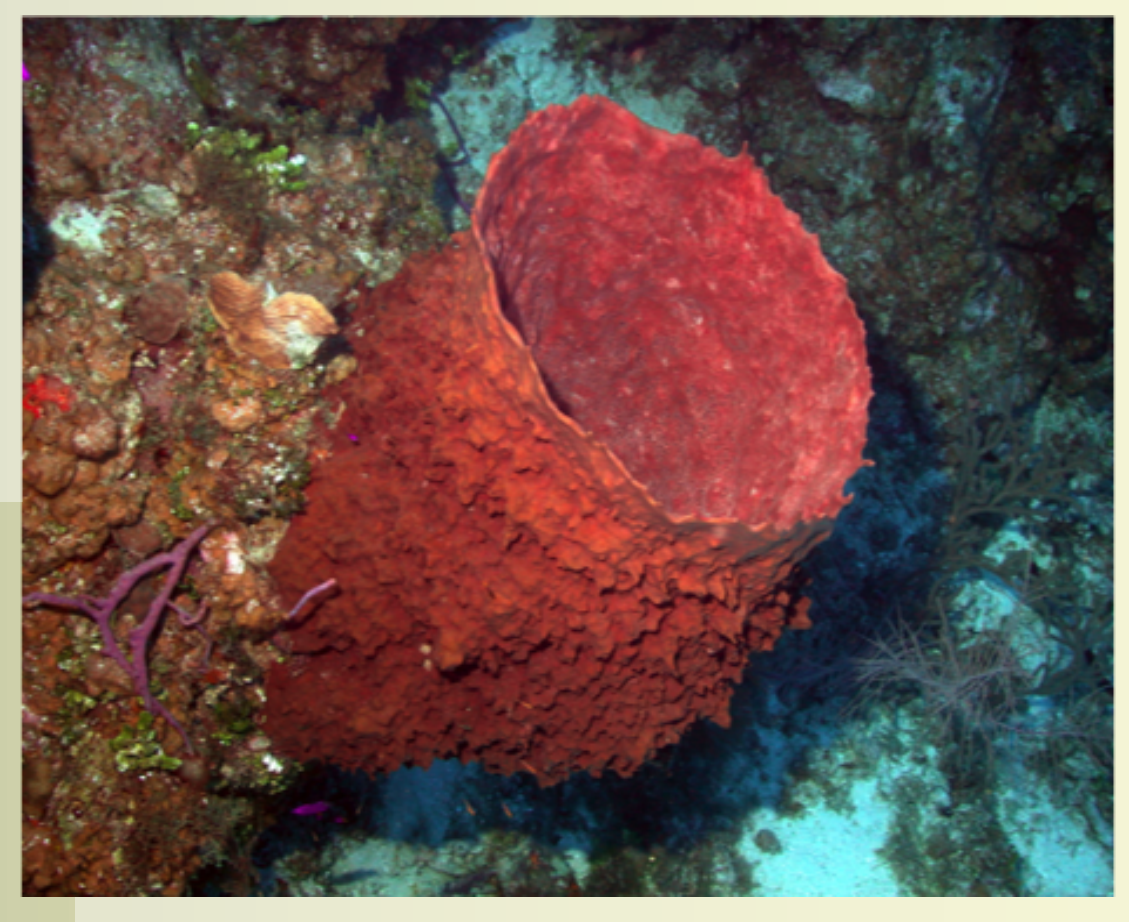


Are changes in the microbial community of the giant barrel sponge, *Xestospongia muta*, environmentally driven over a mesophotic depth gradient?

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Most Abundant Taxa

Lee Stocking Island

Little Cayman

Methods

Samples of *X. muta* were collected from Little Cayman (LC) and Lee Stocking Island (LSI) using open circuit technical diving at 6 depths (n = 3) from 10-90 m. Abiotic parameters were measured at each depth, including irradiance, temperature, and nutrients.

Tissue samples from each sponge were freeze-dried and acidified to remove residual carbonates for bulk stable isotopic analyses (SIP) of C13 and N15 at the marine biological laboratory, Woods Hole, MA

Genomic DNA was extracted from preserved sponges using a [CTAB, proteinase-K, phenol:chloroform] standard protocol, samples were amplified and barcoded for 16S rRNA gene-targeted pyrosequencing on the Roche/454 GS FLX+ platform using primers designed to target the Bacteria and Archaea HV6 region (789F, 1068R) at the University of Illinois W.M. Keck Center for Comparative and Functional Genomics.

Data was analysed using the QIIME pipeline; Aligned to SILVA using the SINA plug-in; Taxa assigned using the RDP classifier (cutoff 0.8). Data were square-root transformed and Bray-Curtis distance values calculated based on normalized OTU tables. PCoA was conducted in PRIMER V6. CCA conducted using vegan in R.

Introduction

Coral reefs are commonly studied within the upper photic zone (2-30 m), although diverse biological communities are supported well into the mesophotic realm (30-150 m), potentially providing refugia from climate stressors.

Deep reefs are technically difficult to access, thus little is known about host-microbial relationships across mesophotic depth gradients. Sponges host abundant and diverse communities of bacteria and are found on both shallow and deep reefs, providing an ideal opportunity to study changes in host-microbial ecology as organisms adapt to living along depth gradients.

The giant barrel sponge, *X. muta*, is a high microbial abundance (HMA) bacteriosponge that supports a diverse community of bacteria that varies in abundance across depth (particularly at Little Cayman), but includes a core group of representatives from the: *Chloroflexi*, *Proteo-*, *Cyano-*, *Actino-*, *Acido-*, and *Poribacteria*, as well as *Archaea* within the *Thaumarchaeota*. This community has the potential to play an extensive role in carbon and nitrogen cycling, including but not limited to nitrogen fixation, nitrate/nitrite reduction, methylotrophy, and chemolithotrophic ammonia oxidation

Discussion

Core members of *X. muta* microbial communities at LC and LSI are similar but only demonstrate significant variability with depth at LC

Nutrients (NOx)

Nitrate + nitrite measurement steadily increased with depth at LSI but not at LC, and may play a role in stabilizing the microbial communities at LSI

Why are the microbial communities more variable at LC?

Little Cayman

Microbial communities were significantly different. A ~50% dissimilarity between 10 & 90 m was driven largely by an:

Increase in the relative abundance of OTU's related to two bacteriosponge-specific phyla, *Chloroflexi* and *Poribacteria*, as well as OTUs related to the class *Thaumarchaeota*, within the phylum *Crenarchaeota*, that play an important role in N and C cycling within *X. muta*.

Decline in photoautotrophs at LC: Cyanobacteria-related OTUs decreased by 60%, and Alphaproteobacteria-related OTUs decreased by 70%, largely driven by *Rhodobacteraceae* and *Rhodospirillaceae* -likely responding to the 100-fold decline in irradiance.

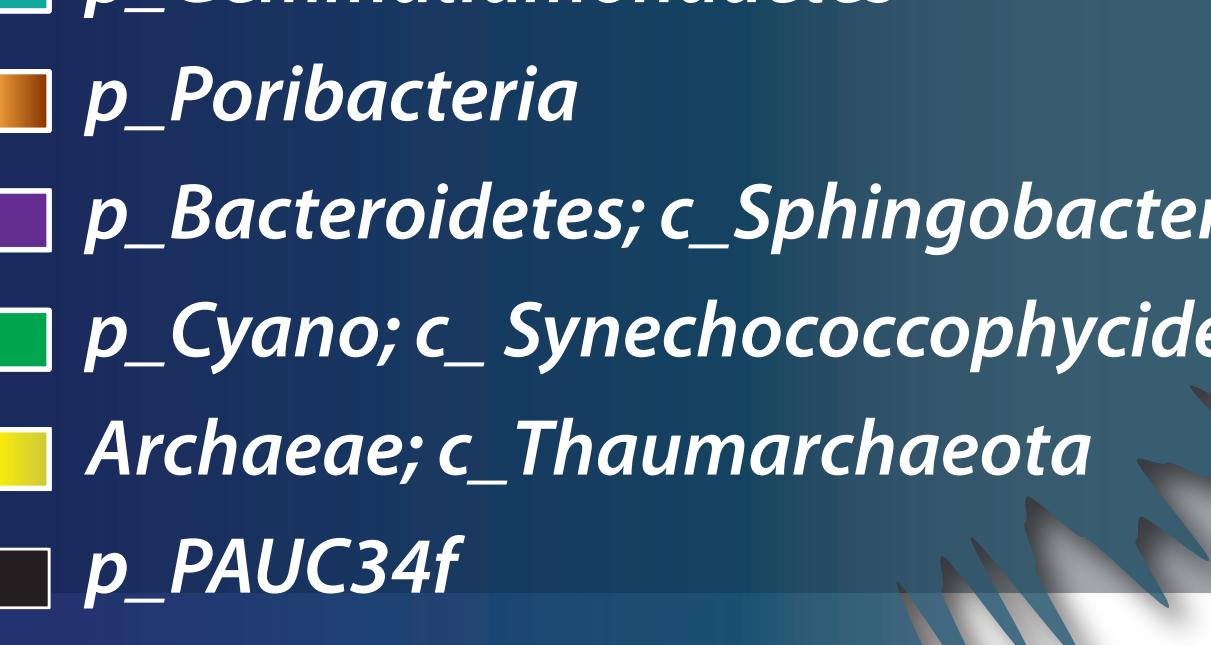
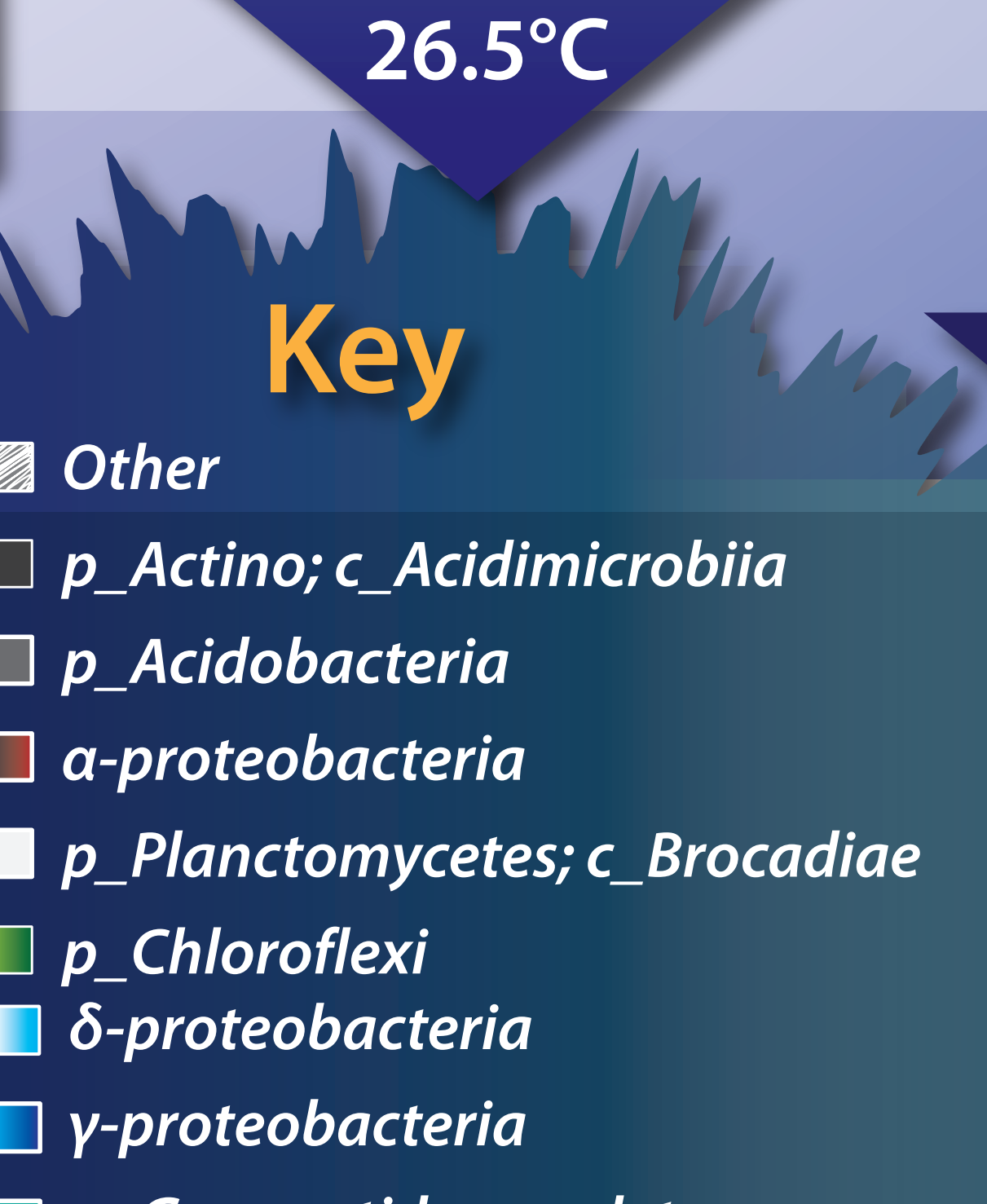
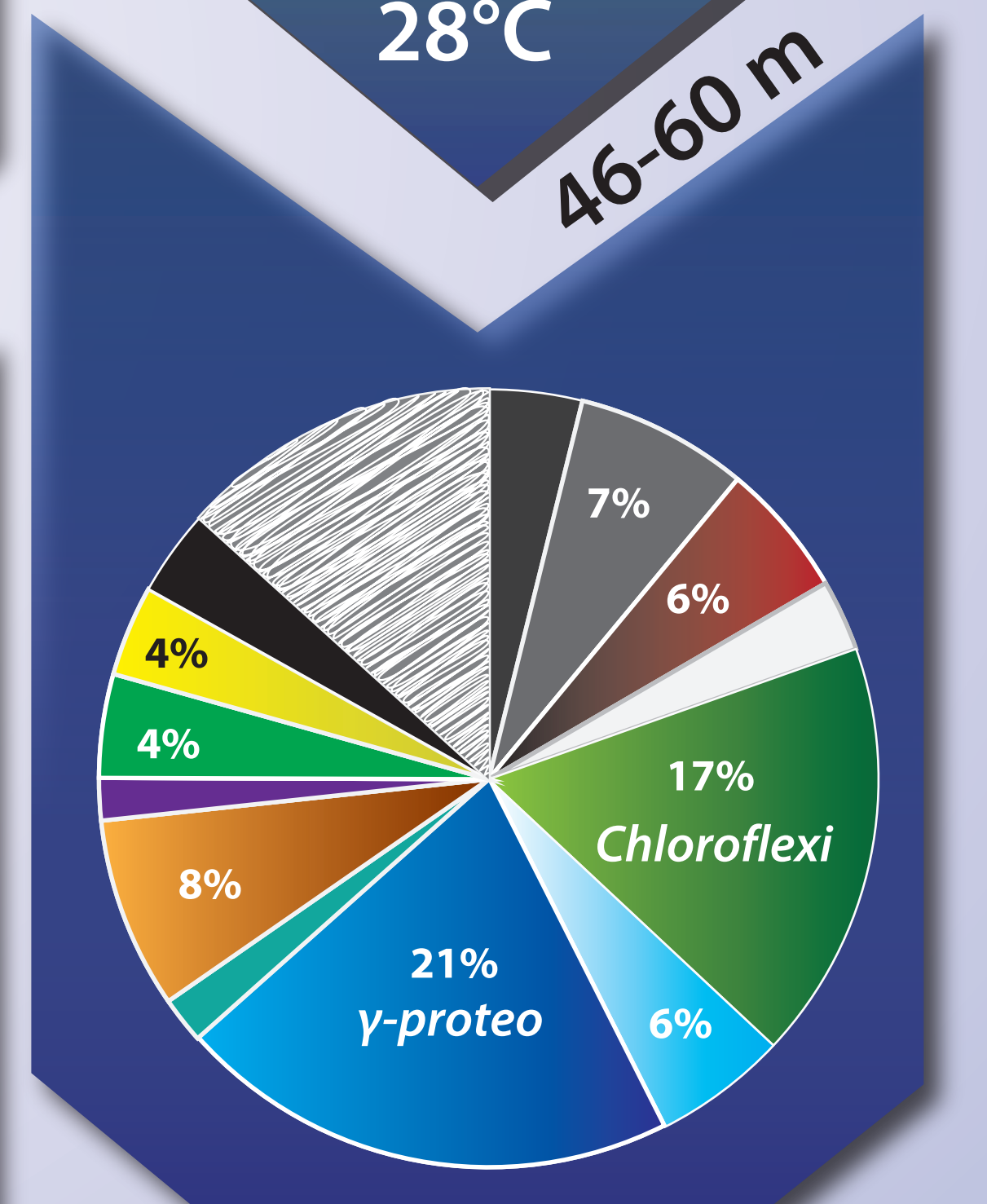
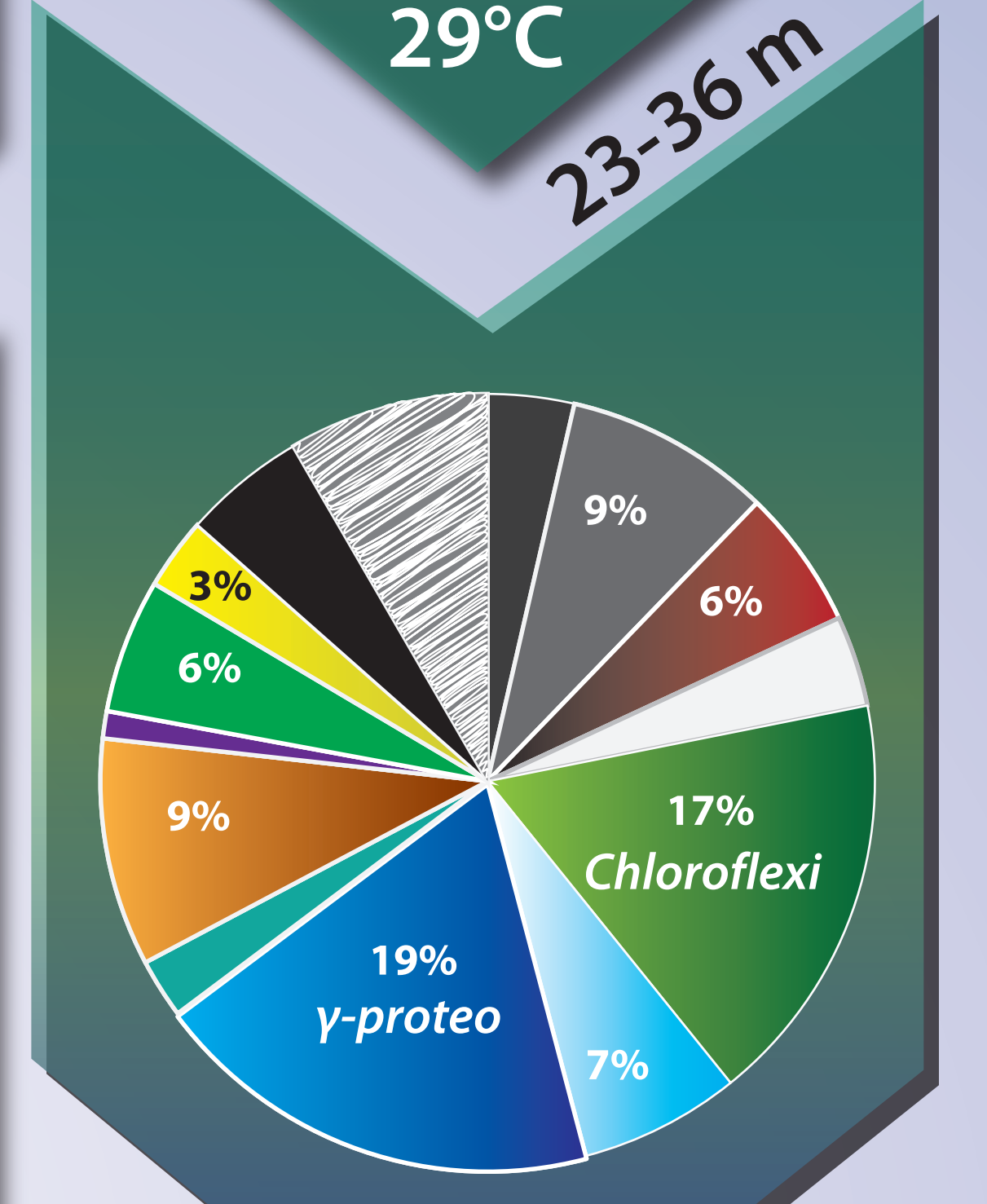
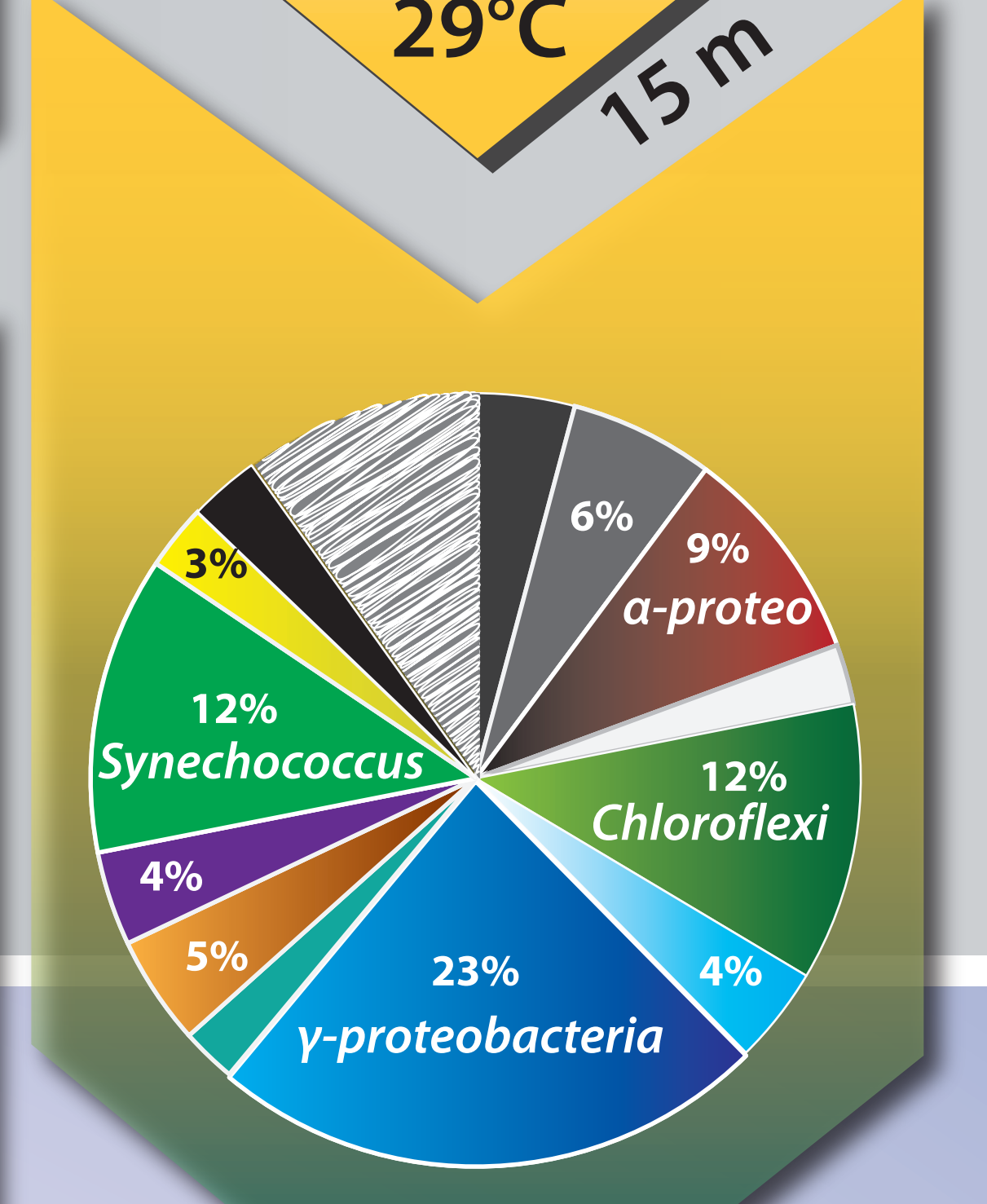
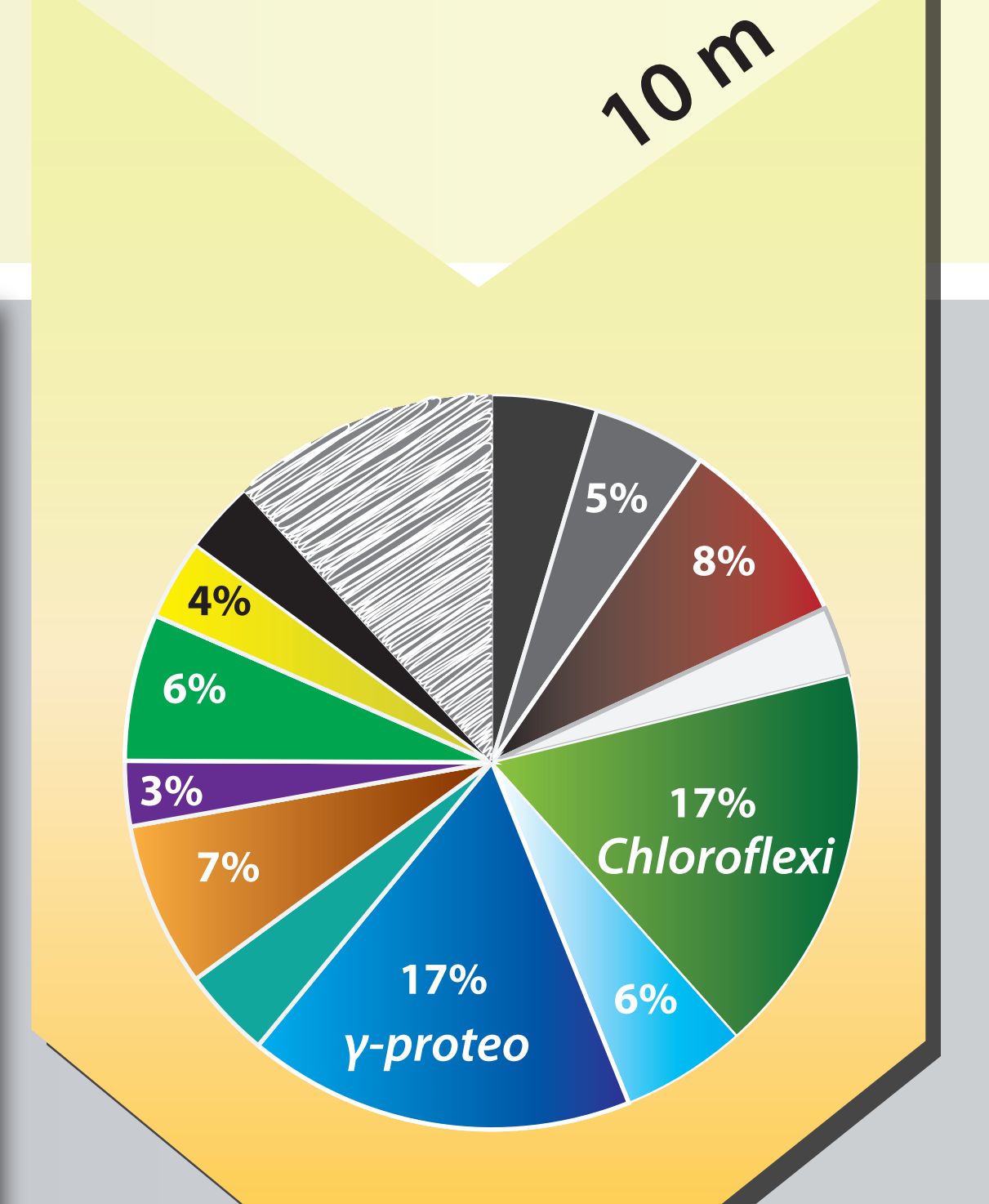
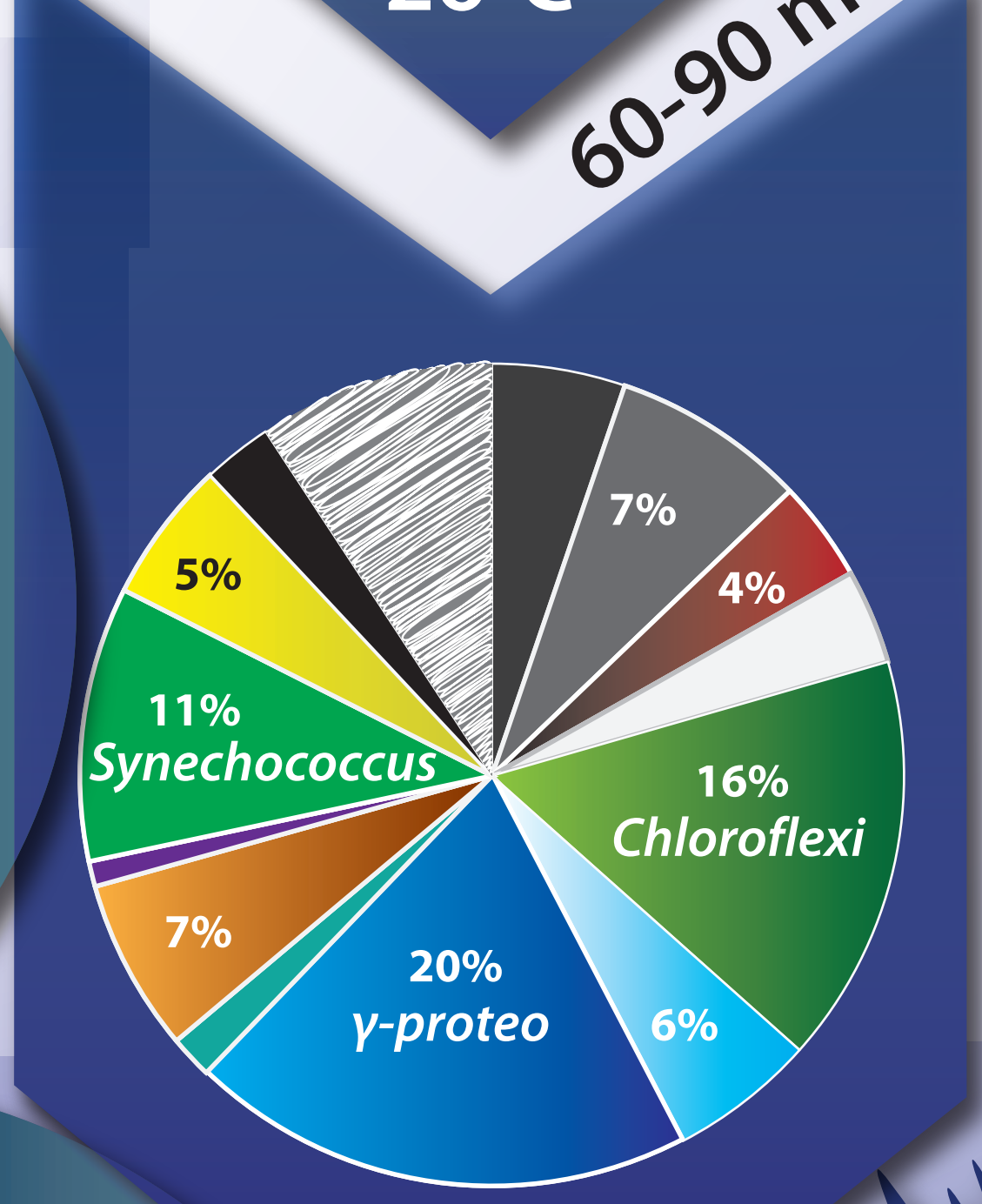
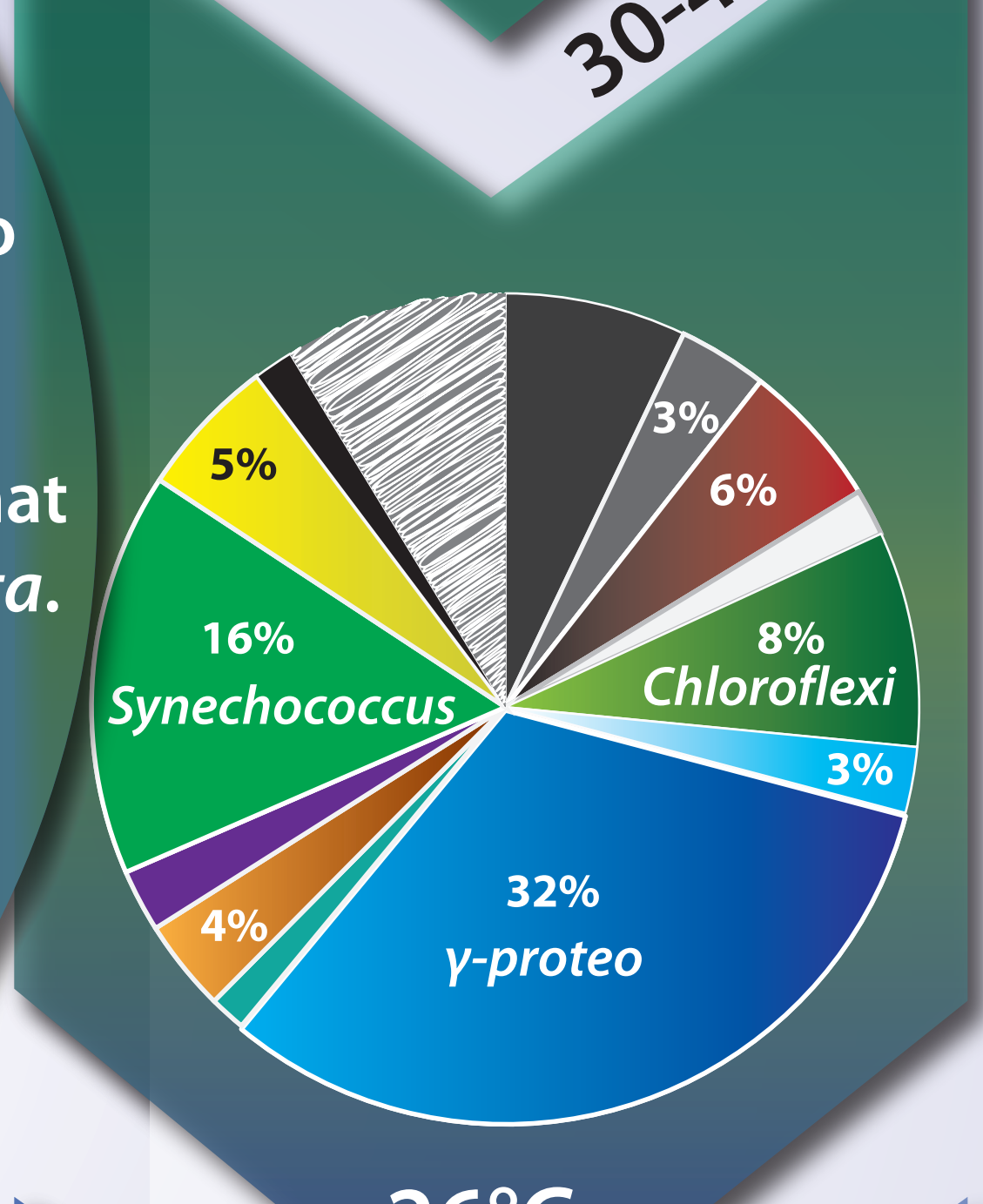
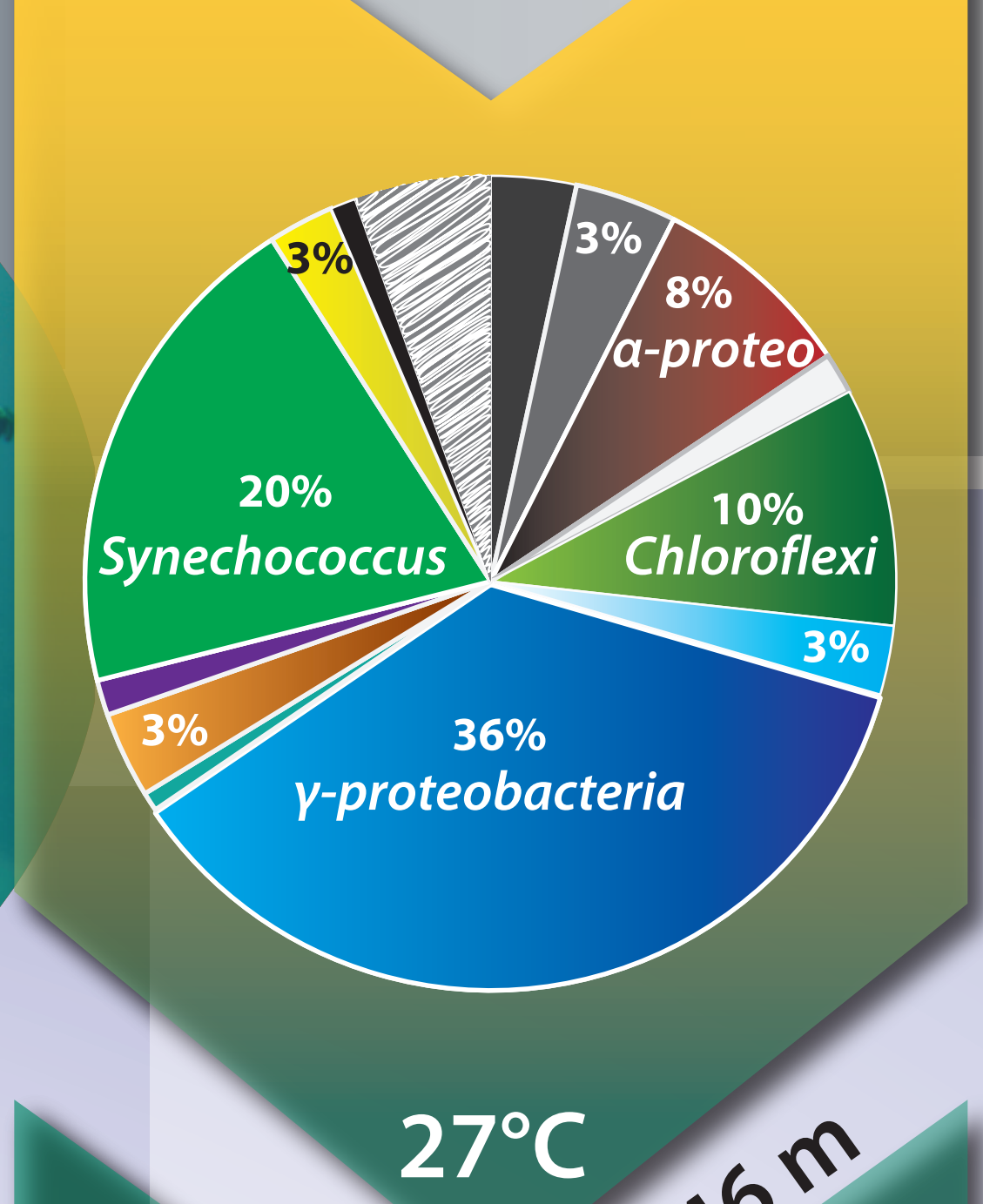
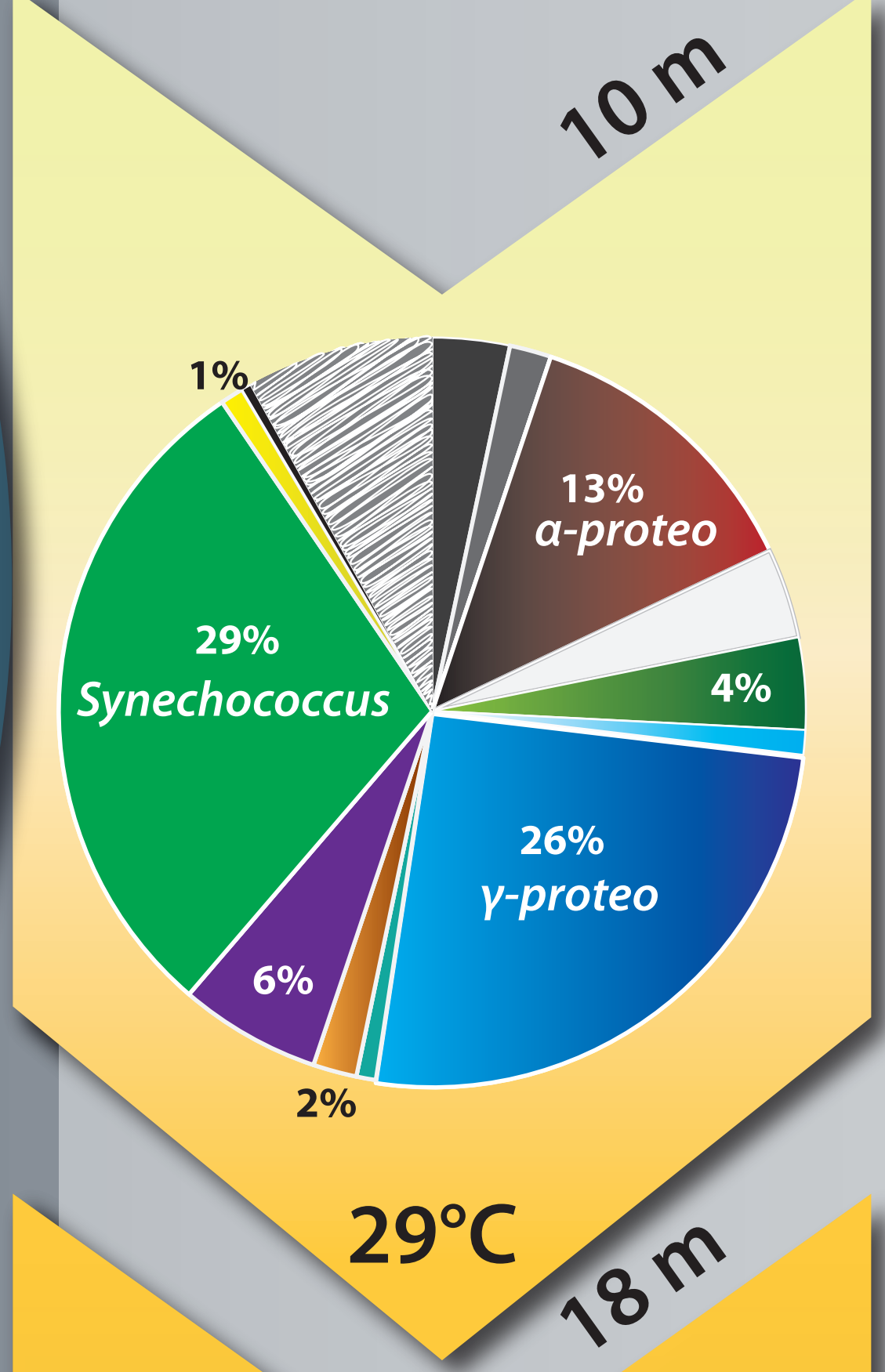
Lee Stocking Island

Communities remained relatively stable with depth, and only exhibited 28.5% dissimilarity between 10 and 60 m (SIMPER analysis).

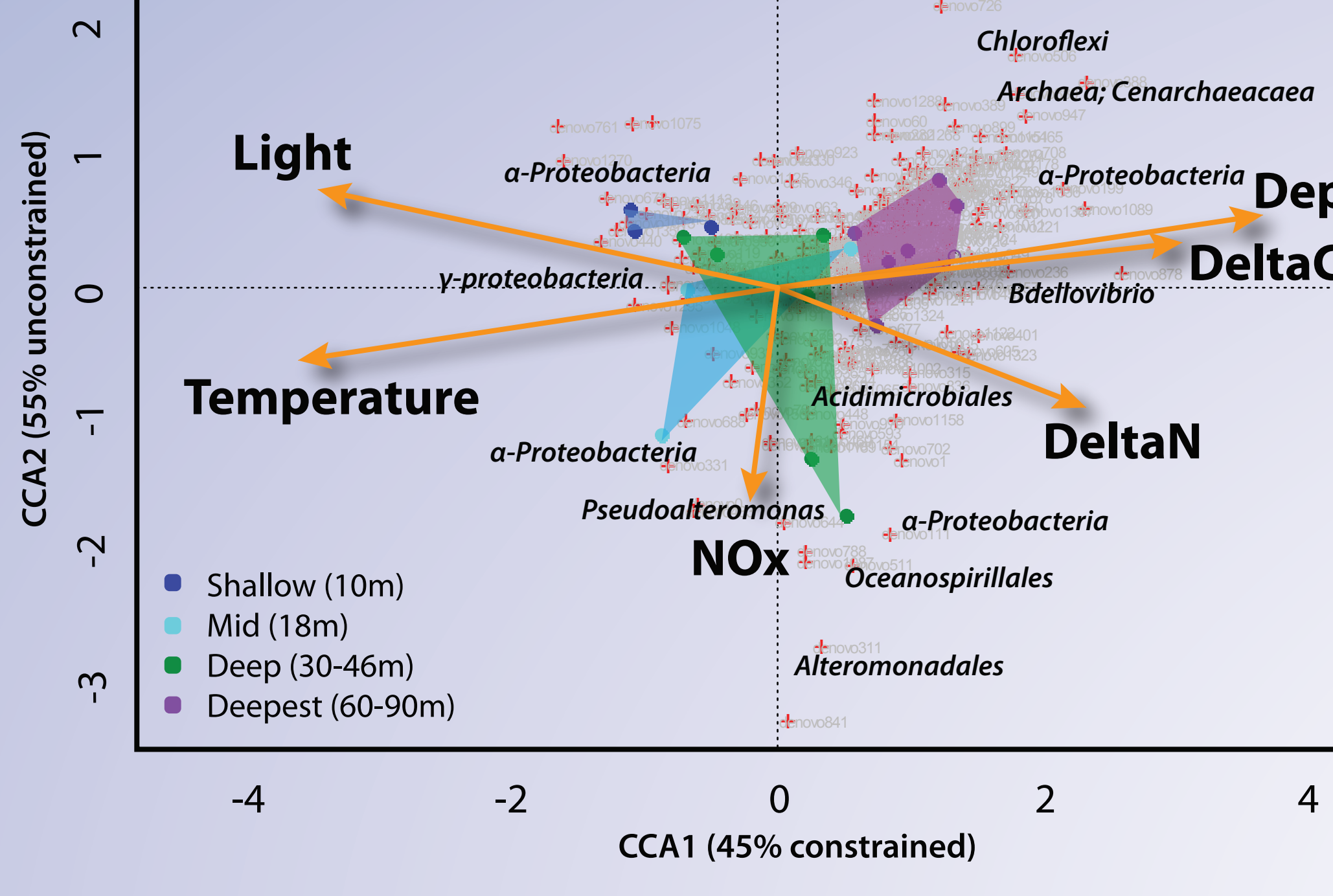
Stable Isotopes

Carbon (13C) and nitrogen (15N) bulk stable isotopic variability was higher at LC (Increase in ΔN; decrease in ΔC), than LSI (variable ΔN, and stable ΔC)

Transition from dependency on photoautotrophy to heterotrophy with depth

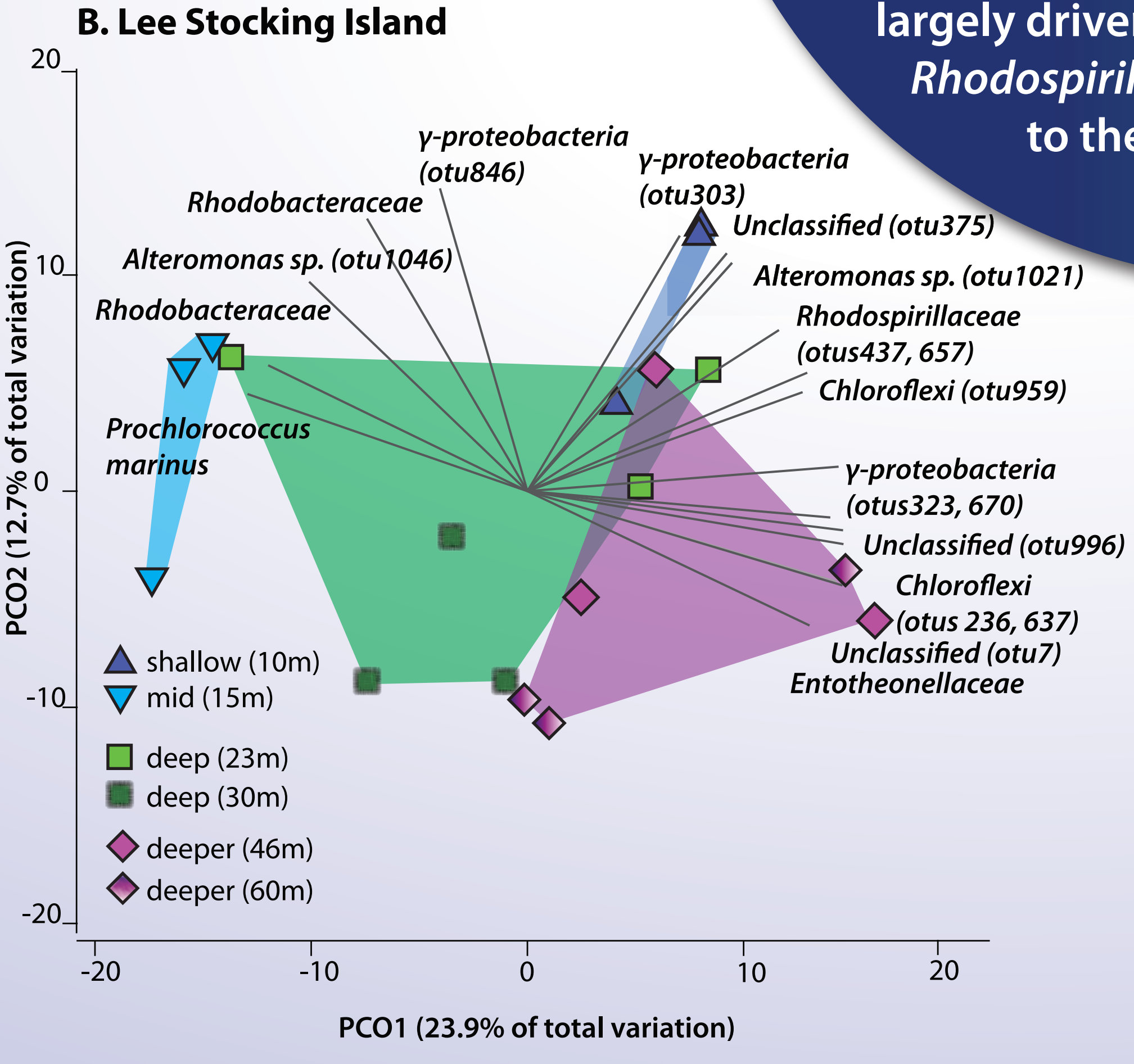
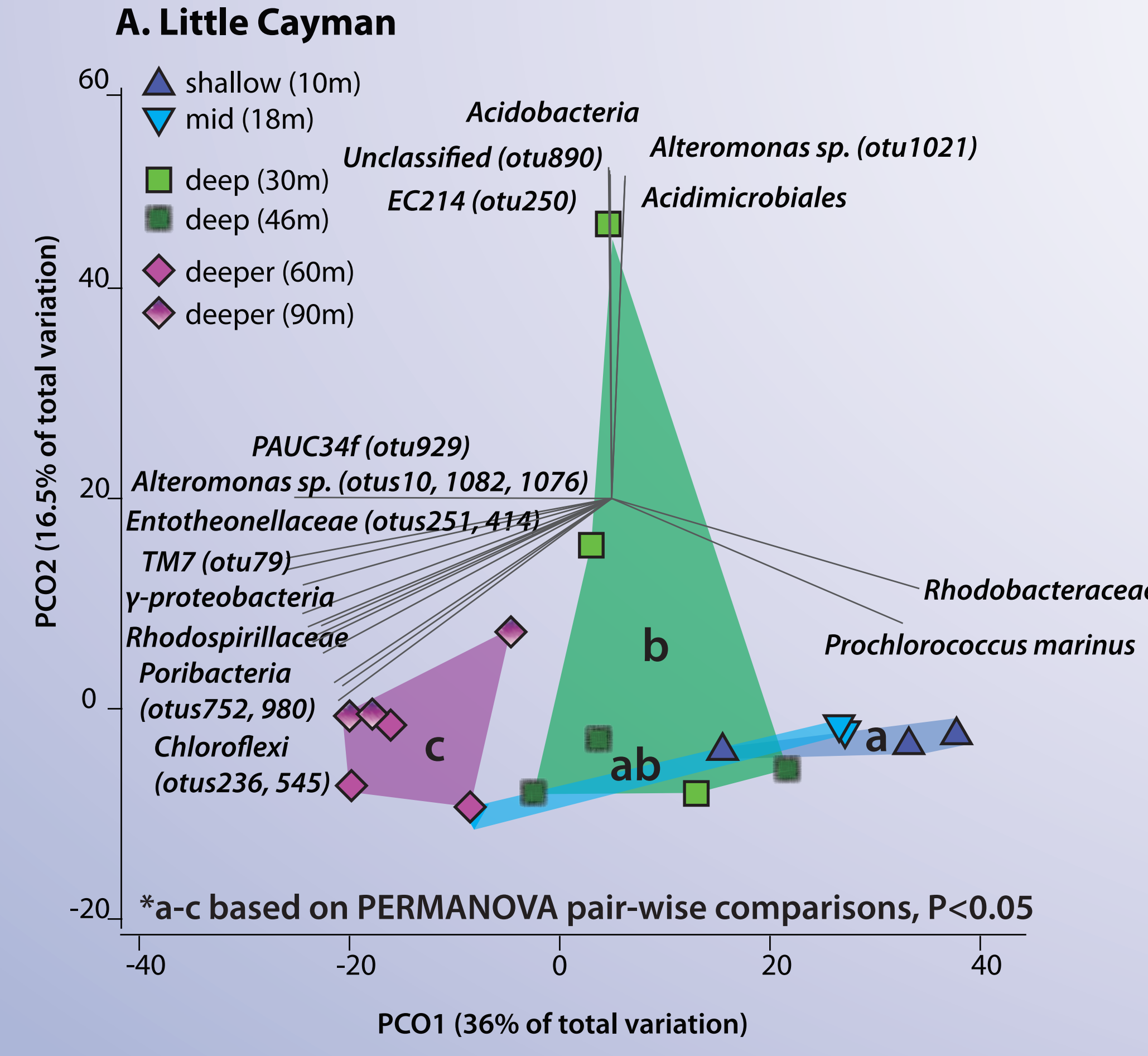


Canonical Correspondance Analysis (CCA) for Little Cayman (F = 1.49, P = 0.04)



Principal Coordinate Analyses (PCoA)

Square root Transform; Bray-Curtis similarity; Pearson Correlations >0.8



Conclusions

Although *Xestospongia muta* appears to support a core community of resident microbes, environmental parameters that change with depth facilitate shifts in microbial assemblages that may impact the organismal performance and productivity of sponges (SIP data). Higher levels of nutrients and upwelling at Lee Stocking are hypothesized to support a more stable microbiome in comparison to Little Cayman, where parameters such as light and temperature, in the absence of nutrient concentrations similar to LSI, drive distinct changes in the photoautotroph community (CCA, PCoA). Results provide additional support for contemporaneous studies across varying locations in the field of applied environmental microbiology prior to drawing organism or ecosystem-wide conclusions.

Acknowledgements



100 fold decline in irradiance from 1000 to 10 μmol quanta m⁻² s⁻¹

Key

- Other
- p_Actino; c_Acidimicrobiia
- p_Acidobacteria
- α-proteobacteria
- p_Planctomycetes; c_Brocadiae
- p_Chloroflexi
- δ-proteobacteria
- γ-proteobacteria
- p_Gemmatidmonadetes
- p_Poribacteria
- p_Bacteroidetes; c_Sphingobacteriia
- p_Cyano; c_Synechococcophycidae
- Archaea; c_Thaumarchaeota
- p_PAUC34f