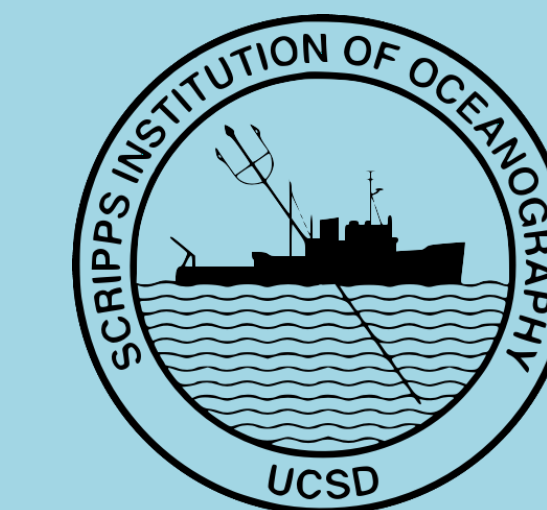# Molecular Analysis of Microbiome Diversity and Diet in Mesopelagic Fish

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Mesopelagic fish

- Majority of the biomass of plankton-feeding fish
- Important prey items for larger fish
- Diel migrators
- Important part of the biological carbon cycle
- Difficult to study *in situ*
- Understanding these fish is an important first step in understanding the greater mesopelagic ecology

Study Significance

- Little is known about the microbial diversity associated with mesopelagic fish despite their ecological significance
- Diet studies have exclusively focused on visual investigations, molecular diet studies may provide higher resolution into trophic diversity

Goal

Utilize molecular methods to determine feeding ecology of mid-water fishes and gut-associated microbial communities
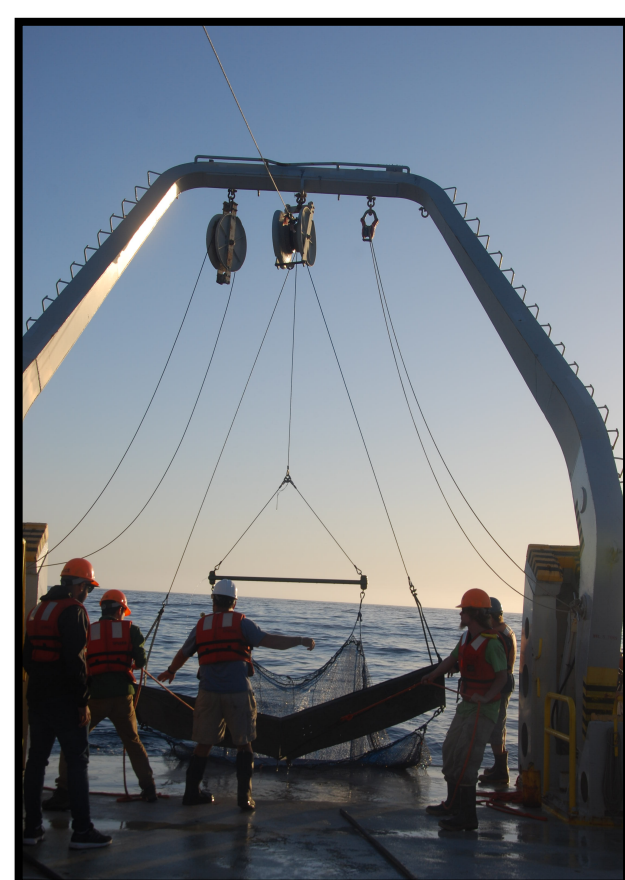
Fish from families Myctophidae & Sternoptychidae collected on multiple cruises

Myctophidae

- Diel migrations
- Bioluminescent
- 32 genera, +250 species
- Account for >50% deep sea biomass
- Considered critical to marine ecosystems worldwide

Sternoptychidae

- Diel migrations
- Small (< 10cm)
- Bioluminescent
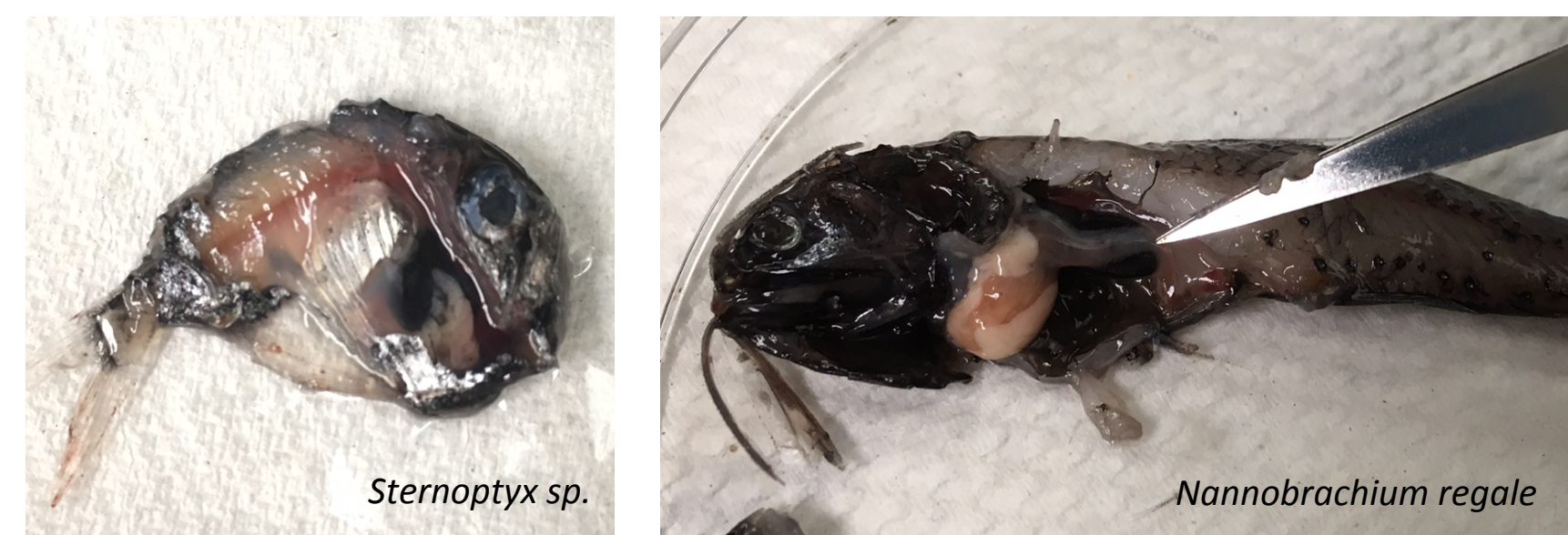- 10 genera, 75 species in two subfamilies
- Temperate-tropical, worldwide.

Specimens collected on 11/14/2015, 5/7/2016, and 5/14/2016 with Isaacs-Kidd midwater trawl (IKMT) on research ships operated by the Scripps Institution of Oceanography (SIO)

Argyropelicus affinus

Sternoptyx sp.
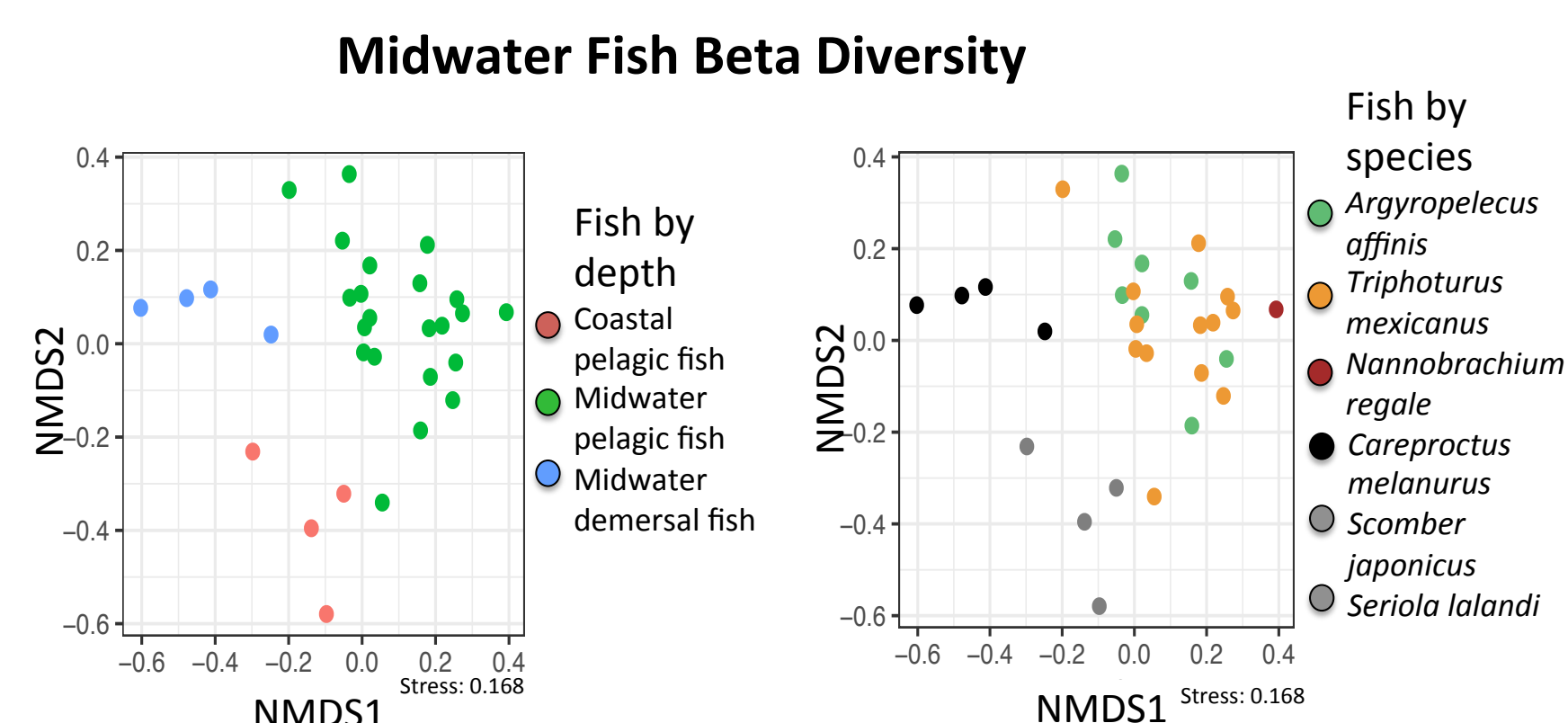
Nannobrachium regale

Methodology

- During dissection of representative fish samples, the intestine was removed and alternately washed with 70% EtOH and water
- Sampled intestines and tissue vouchers stored at -80ºC
- Extracted DNA with standard phenol:chloroform method
- Sequenced marker-gene PCR reactions on the Illumina MiSeq platform

Investigating intestinal microbial diversity
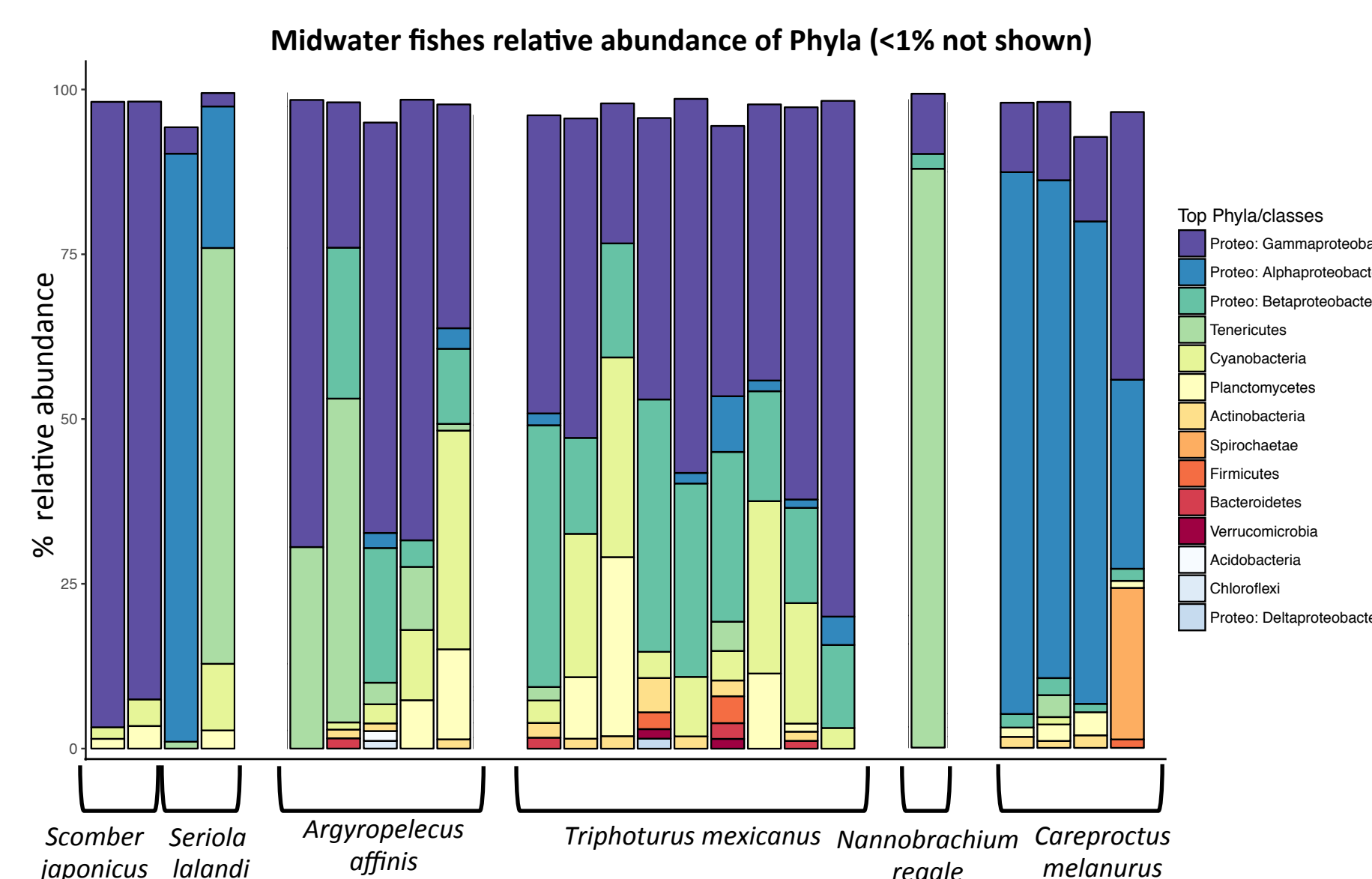
High throughput sequencing of 16S rRNA gene

Midwater Fish Beta Diversity

Fish by depth: Coastal pelagic fish, Midwater pelagic fish, Midwater demersal fish

Fish by species: *Argyropelecus affinis*, *Triphoturus mexicanus*, *Nannobrachium regale*, *Careproctus melanurus*, *Scomber japonicus*, *Seriola lalandi*

Intestinal microbial communities within fish cluster by pelagic zone.

Microbial communities between midwater fish families appear similar.

**Microbial taxa**
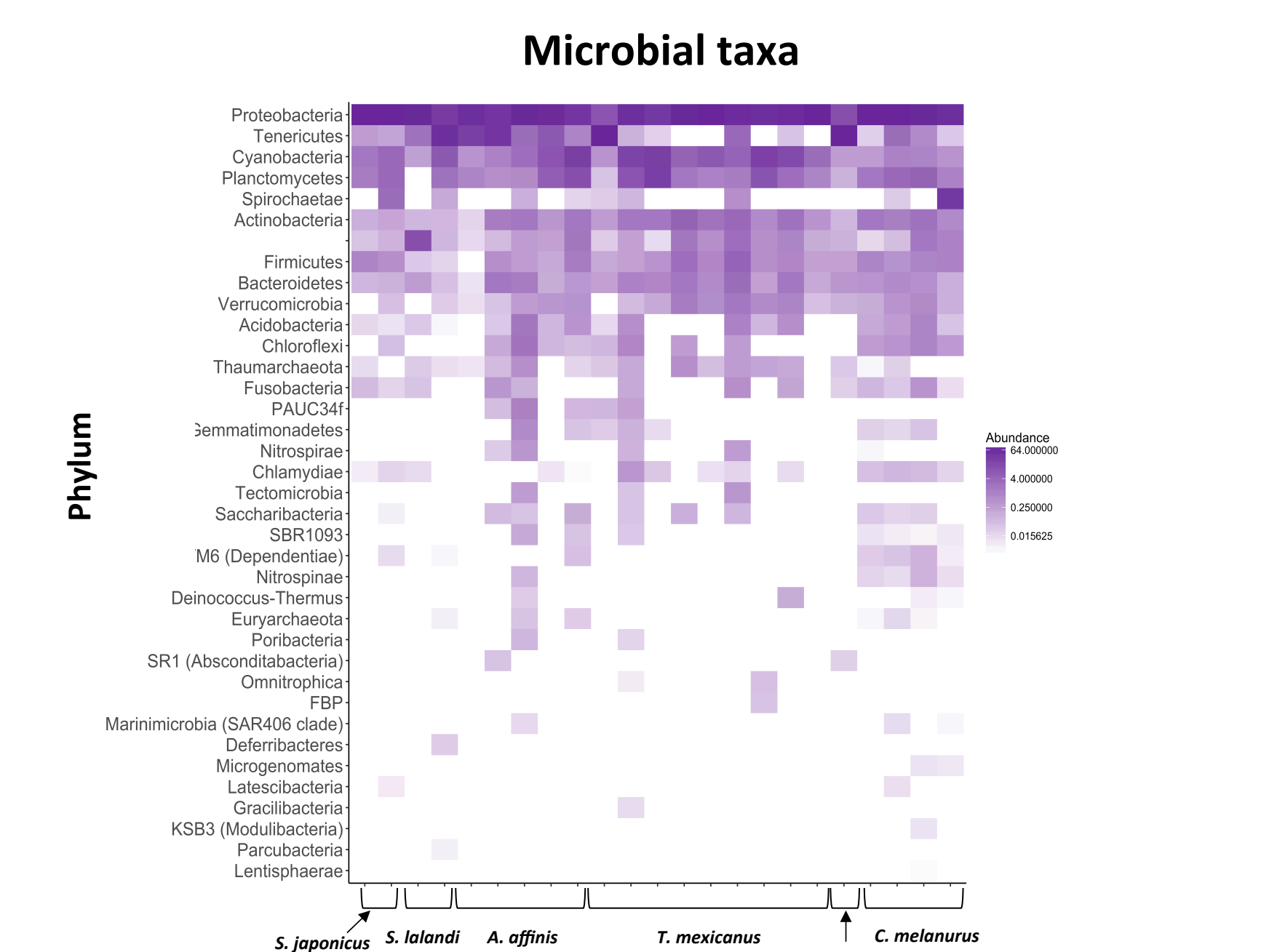
Proteobacteria is top Phyla found in midwater fish. Tenericutes, Cyanobacteria, and Planctomycetes are prominent in midwater fish.

Midwater fishes relative abundance of Phyla (<1% not shown)

Top Phyla/classes: Proteo: Gammaproteobacteria, Proteo: Alphaproteobacteria, Proteo: Betaproteobacteria, Tenericutes, Cyanobacteria, Planctomycetes, Actinobacteria, Spirochaetae, Firmicutes, Bacteroidetes, Verrucomicrobia, Acidobacteria, Chloroflexi, Proteo: Deltaproteobacteria
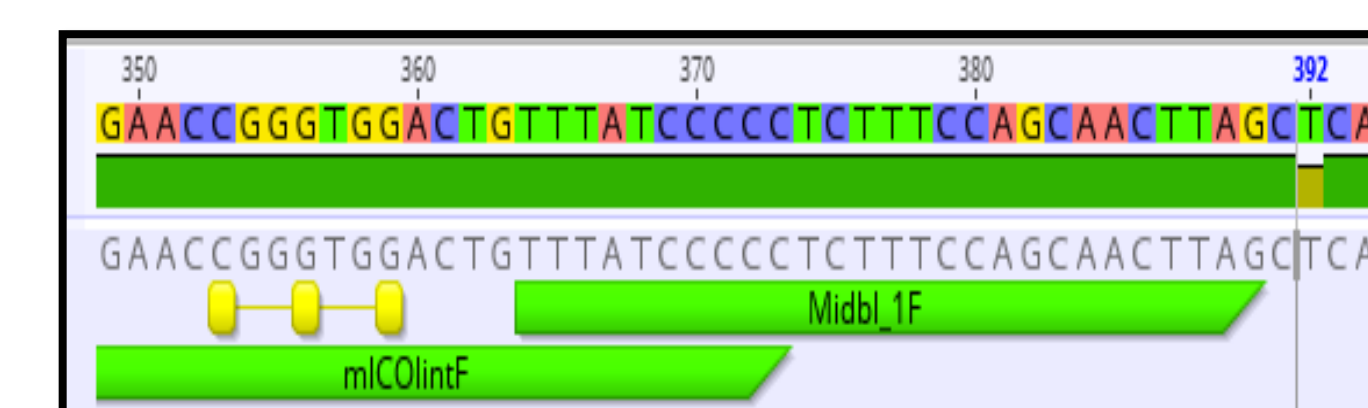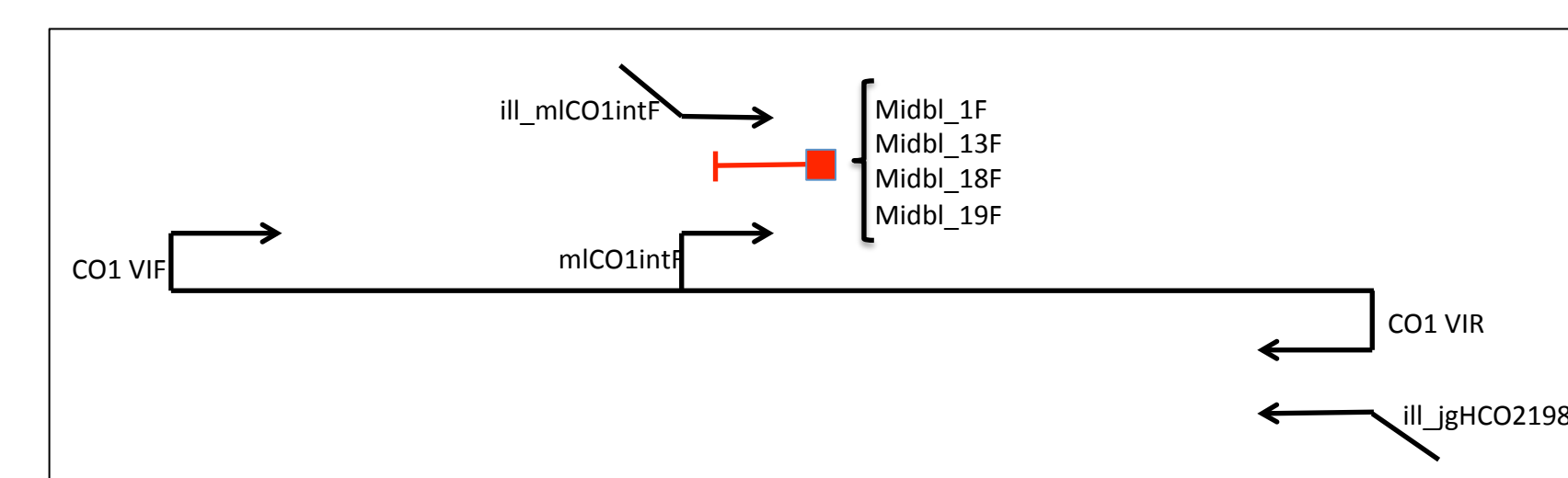
Betaproteobacteria appear enriched in midwater fish but not in epipelagic or benthic fish.

Exploring diet through molecular barcodes

Developing blocking primers for high throughput sequencing of CO1 gene

Triphoturus mexicanus

Nannobrachium regale

Argyropelicus affinis

Sternoptyx sp.
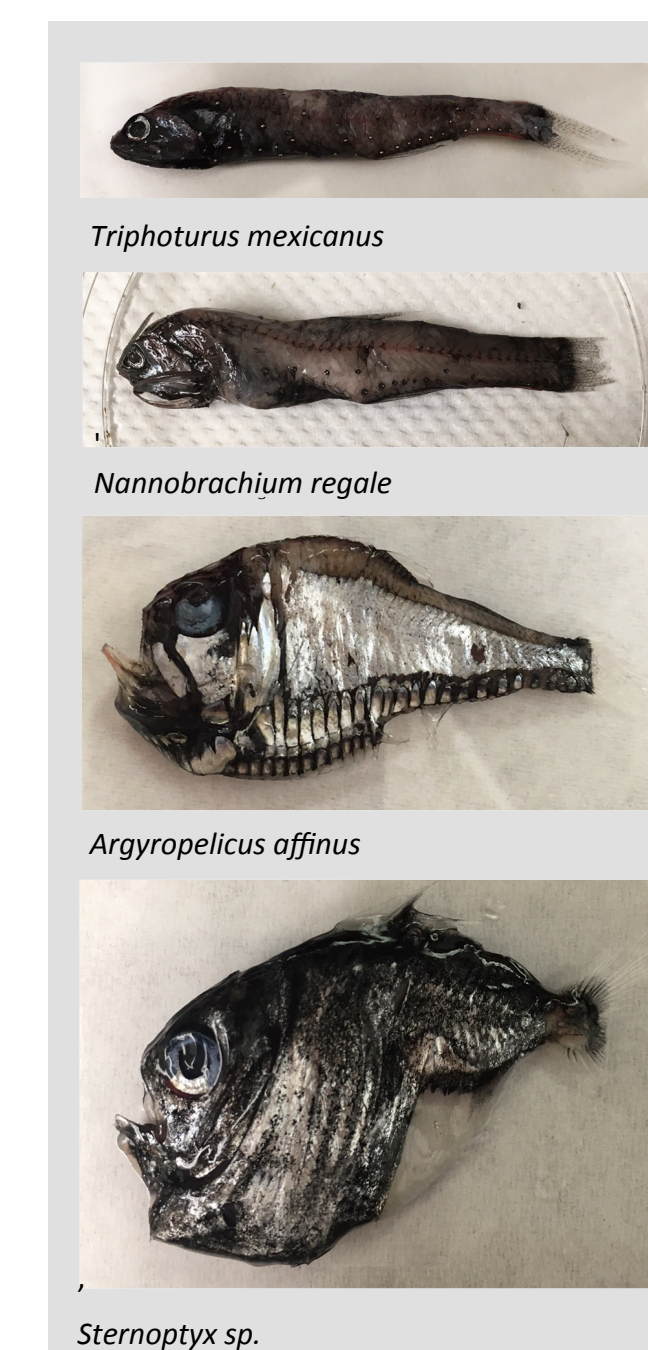
Goal: To block host DNA while allowing non-host (prey) DNA to amplify.

- Host-specific primer with Spacer C3 at 3' end blocks amplification of host DNA.
- Allows non-host (prey) DNA to amplify.
- Designed to anneal perfectly on an overlapping region of the gene where the universal primer (mlCOIintF) would otherwise anneal.
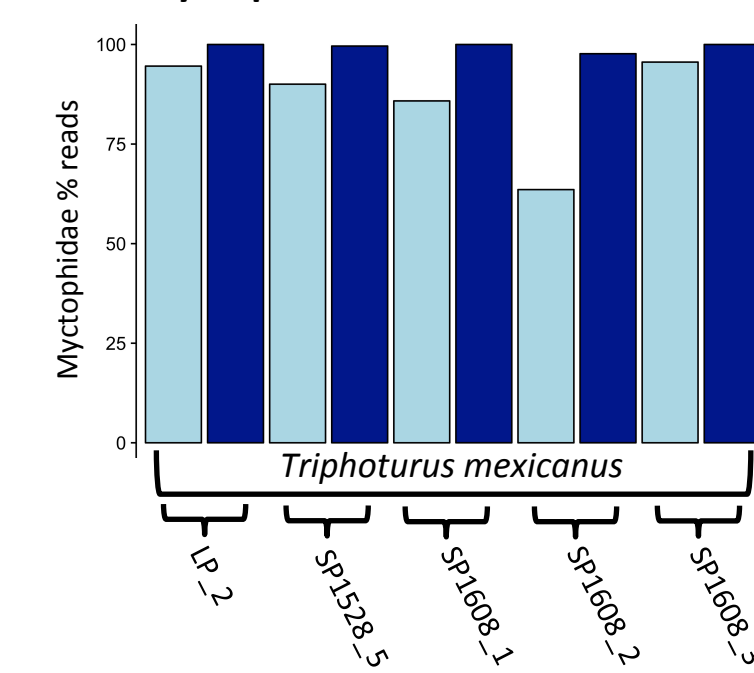
Blocking primers block host DNA
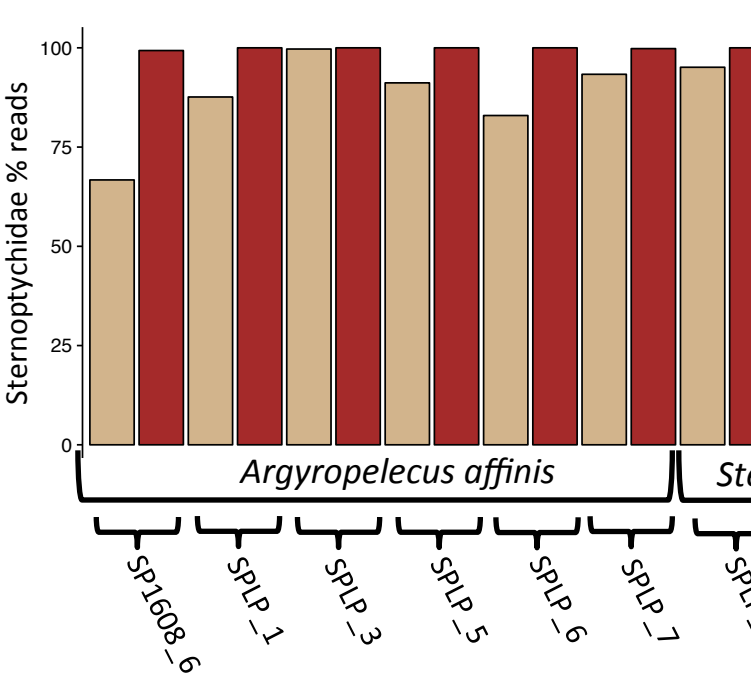
Blocking primer validation on host tissue
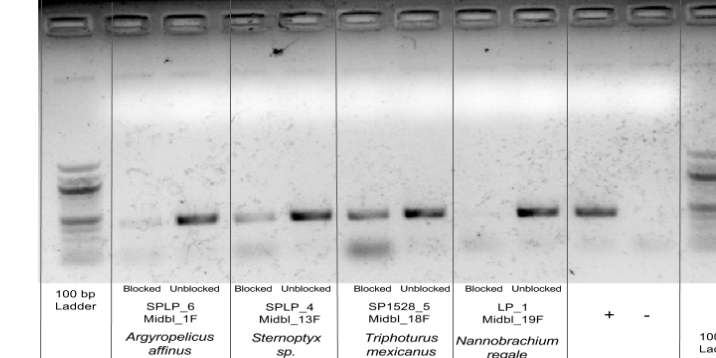
Gel electrophoresis shows less amplification in wells with blocked host.

**Myctophidae host read abundance**
Triphoturus mexicanus

Sternoptychidae host read abundance
Argyropelecus affinis, *Sternoptix spp.*

Host intestine, blocked and unblocked with host-specific primers. The efficacy of the blocking primes varies.
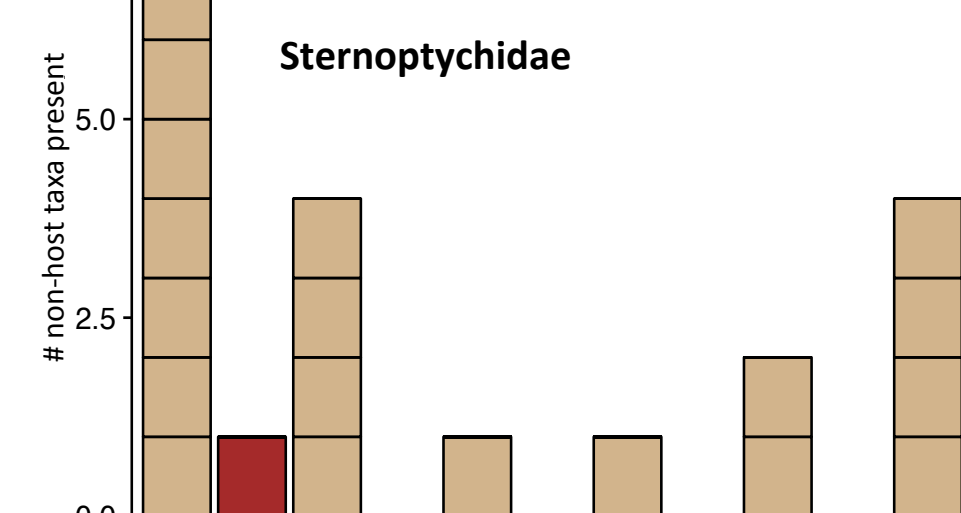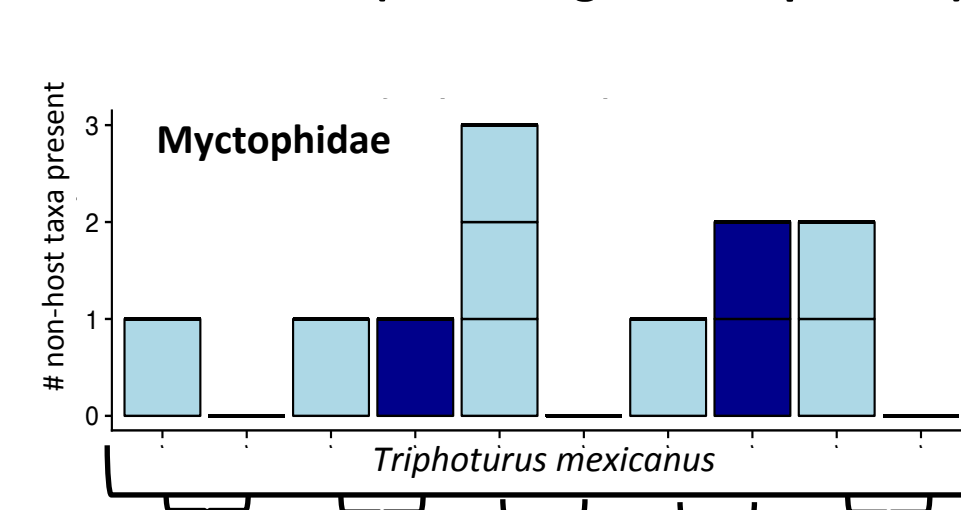
Blocking primers may block some non-host DNA

- Tested *in silico* against 336 CO1 reference sequences from marine organisms.
- Tested against a standard containing both eukaryotic and bacterial DNA, as well as against seawater and guts blocked
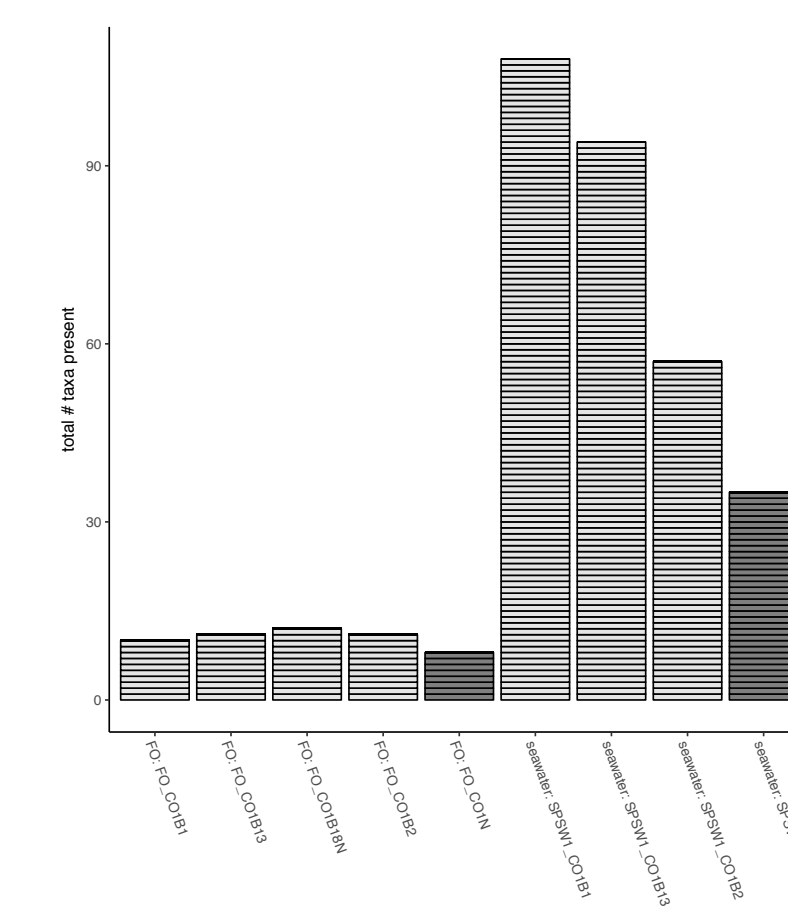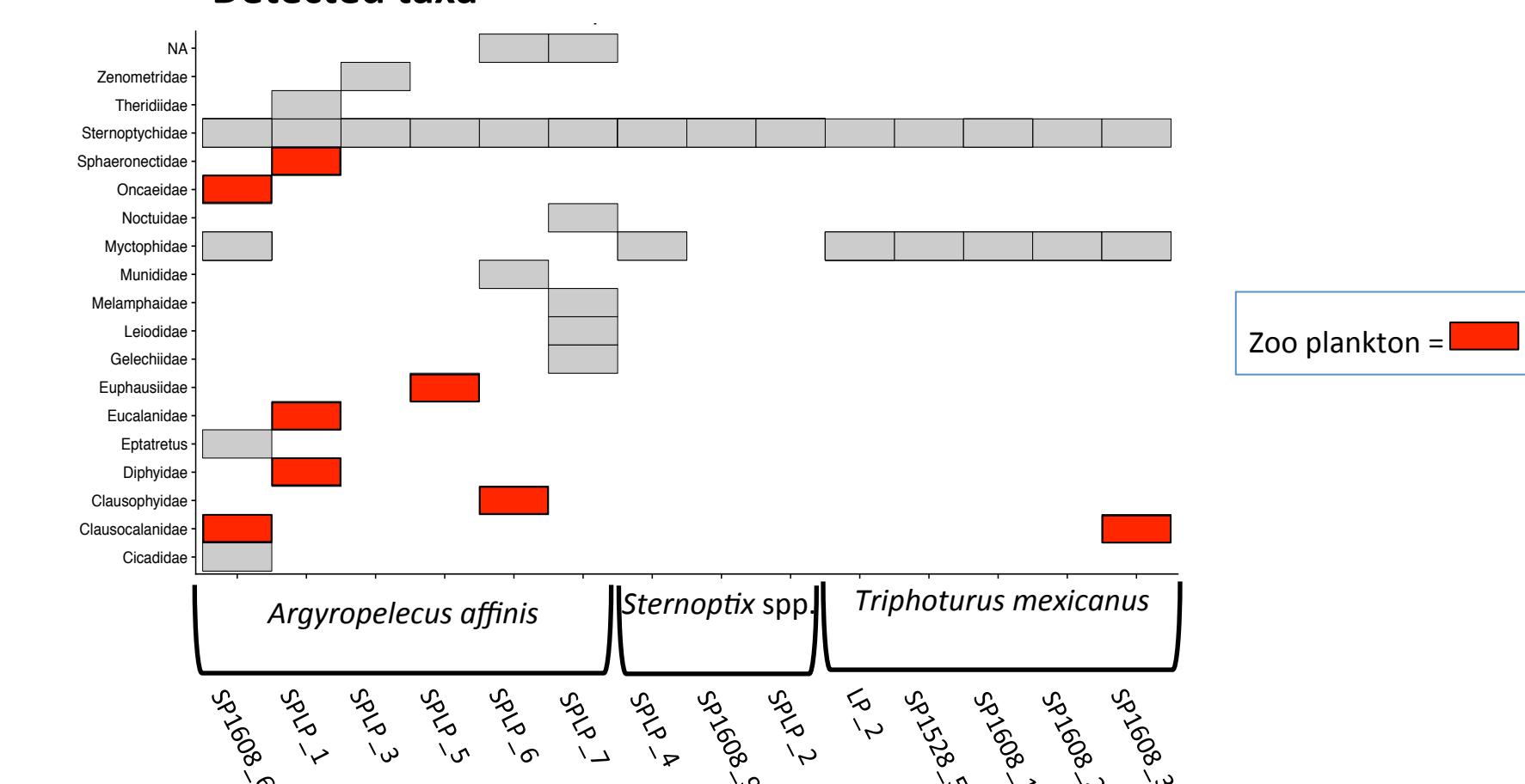
Blocked host samples show more taxa diversity than unblocked host samples.

Taxa detected (excluding host sequences)

Myctophidae — *Triphoturus mexicanus*

Sternoptychidae — *Argyropelecus affinis*, *Sternoptix spp.*

Detected taxa

Argyropelecus affinis, *Sternoptix spp.*, *Triphoturus mexicanus*

Zoo plankton =

Future Directions

- Complete study with more comprehensive sampling (in progress).
- Compare midwater fish intestinal microbiomes with intestinal microbiomes of fish from differing pelagic zones.
- Incorporate isotopic study – does trophic niche space correlate with microbiome signature?
- Comparisons to current diet studies.

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Tarnecki, A.M., Burgos, F.A., Ray, C.L. and Arias, C.R. (2017), Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. J Appl Microbiol, 123: 2–17. doi:10.1111/jam.13415