What is a healthy coral? Tissue-loss diseases affecting staghorn corals in the Upper Florida Keys

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Knauss Fellow Brown Bag Series
15 September 2016

Photo: K. Patterson

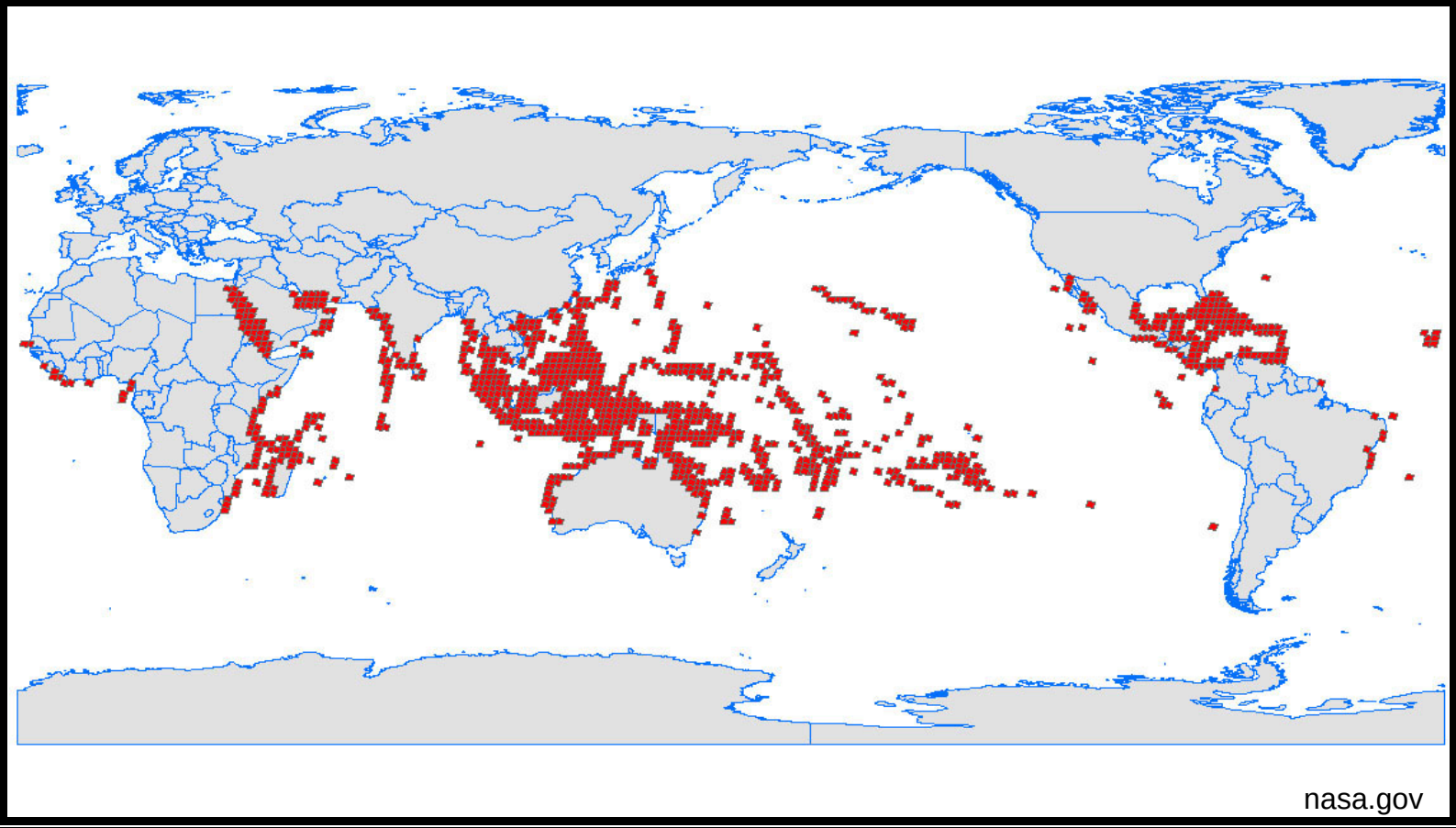
Overview

- Background information:
  - Introduction to coral reefs
  - Introduction to coral disease
- Research
  - Hypotheses
  - Methods
  - Results
  - Conclusions
- Project significance
- Acknowledgements

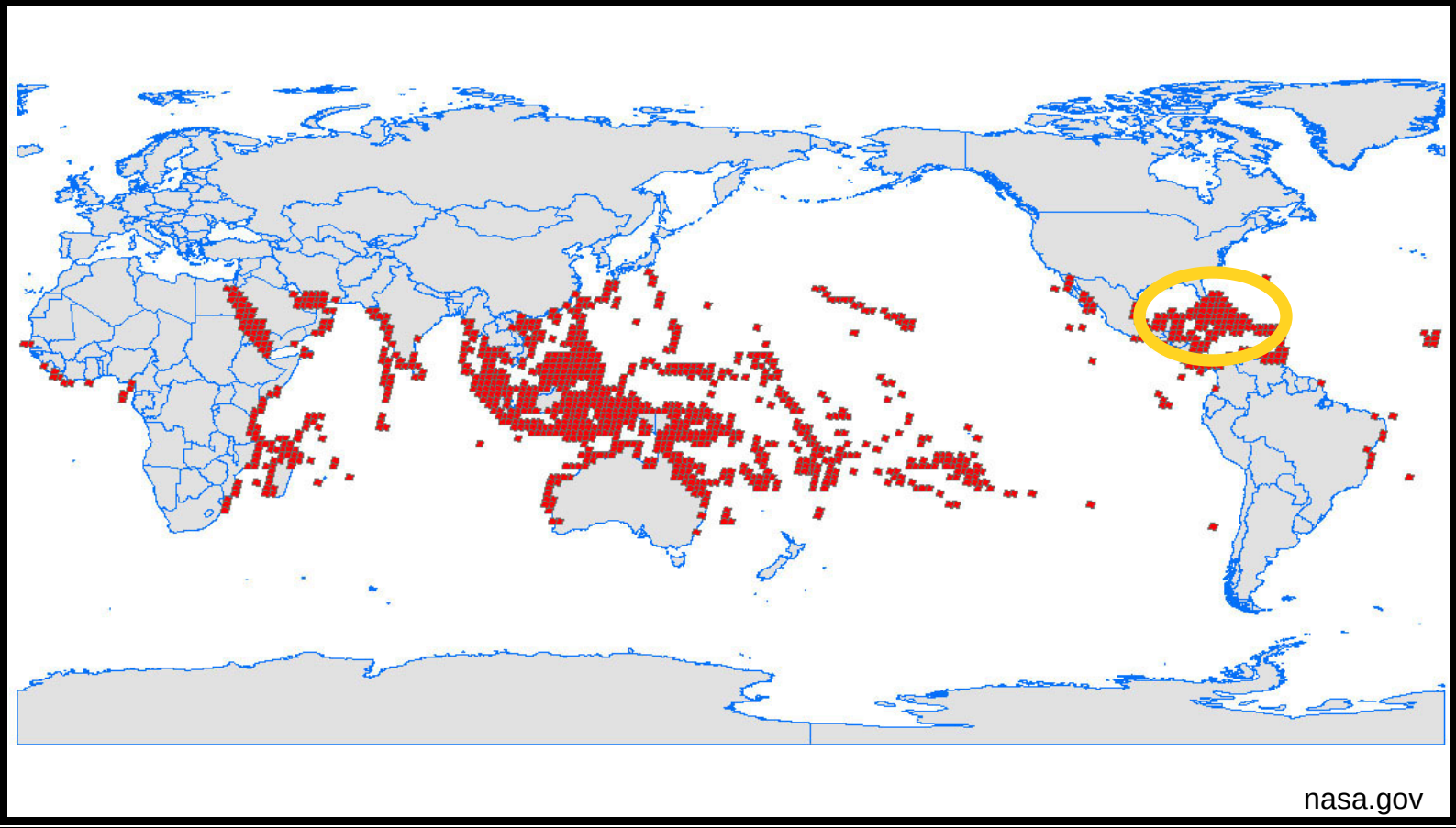
INTRODUCTION

Introduction to coral reefs

The world’s coral reefs



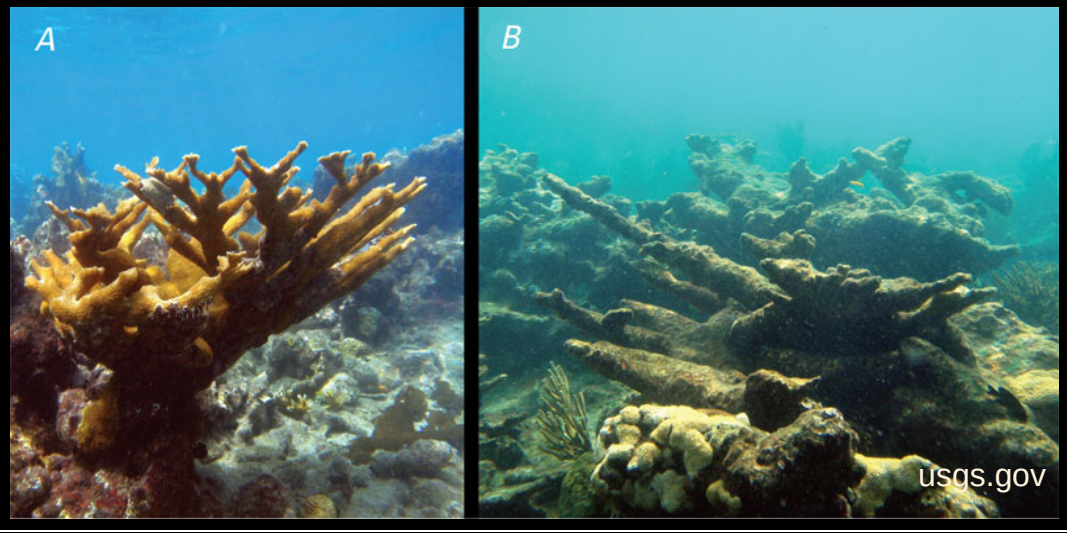
The world’s coral reefs



What is coral disease?

- DISEASE: any interruption, cessation, deviation, proliferation or other malfunction of vital body functions, systems, or organs

(Sutherland et al. 2004)



Coral diseases

usgs.gov

whoi.edu

noaa.gov

noaa.gov

The conundrum of tissue-loss diseases

- White-band disease (WBD) (Type I and II)
- Rapid tissue loss (RTL)
- White-plague disease (Types I, II, and III)
- White-pox disease
- White Syndrome affecting Indo-Pacific acroporids
- *Vibrio shiloi*-induced bleaching
- *Vibrio coralliilyticus*-induced bleaching
- Skeletal eroding band
- Shut-down reaction

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Caribbean acroporids

Caribbean acroporids

White-band disease (WBD)

A. cervicornis

A. palmata

Rapid Tissue Loss (RTL)

Coral disease research

- Presumption: If no lesion is present, a coral colony is assumed to be “healthy”
- Predominately focused on identifying a bacterial pathogen
  - Culture-dependent techniques
  - Culture-independent techniques
- In most cases, pathogens have not been isolated
- Interdisciplinary investigations lacking

HYPOTHESES

Hypotheses

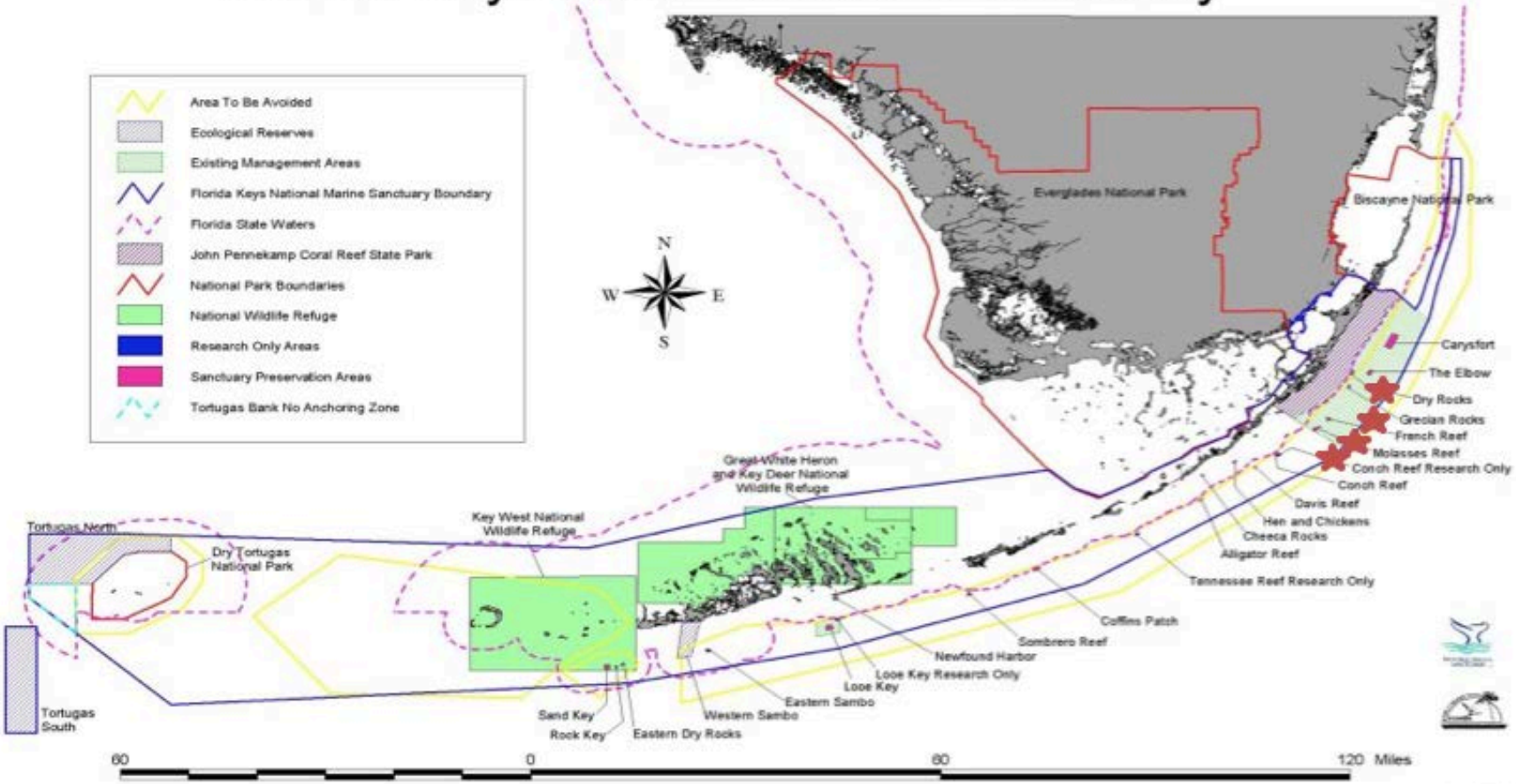
- $H_0$1: The bacterial communities associated with apparently healthy *A. cervicornis* tissues are different than the bacterial communities associated with diseased coral tissue.
- $H_0$2: Rickettsiales abundance is different between apparently healthy and diseased *A. cervicornis* tissue.
- $H_0$3:Vibrionales abundance is different between apparently healthy and diseased *A. cervicornis* tissue.
- $H_0$4: Bacteria in the surrounding sediment are a source of potential pathogens.

METHODS

Methods

1. Sample collection and preservation
2. Histopathological examination
3. Molecular analysis
4. Bioinformatic analysis

Florida Keys National Marine Sanctuary



Field sites

Field Site	Site type	Depth (m)	Latitude (N)	Longitude (W)
CRF Nursery	Nursery	10	24° 58.940	80° 26.153
KLDR	Outplant	6–7	25° 7.280	80° 17.798
Molasses Reef	Outplant	8–9.5	25° 0.599	80° 22.373
Aquarius	Outplant	16	24° 57.000	80° 27.217
French Reef	Outplant	10	25° 7.314	80° 17.851
Conch Shallow	Outplant	5–7	24° 57.083	80° 27.594
Little Conch	Naturally recruited	5.5	24° 56.780	80° 28.210

Fieldwork

A.
S. Galloway

B.
D. Allen

C.
U33-31
Peters / Miller

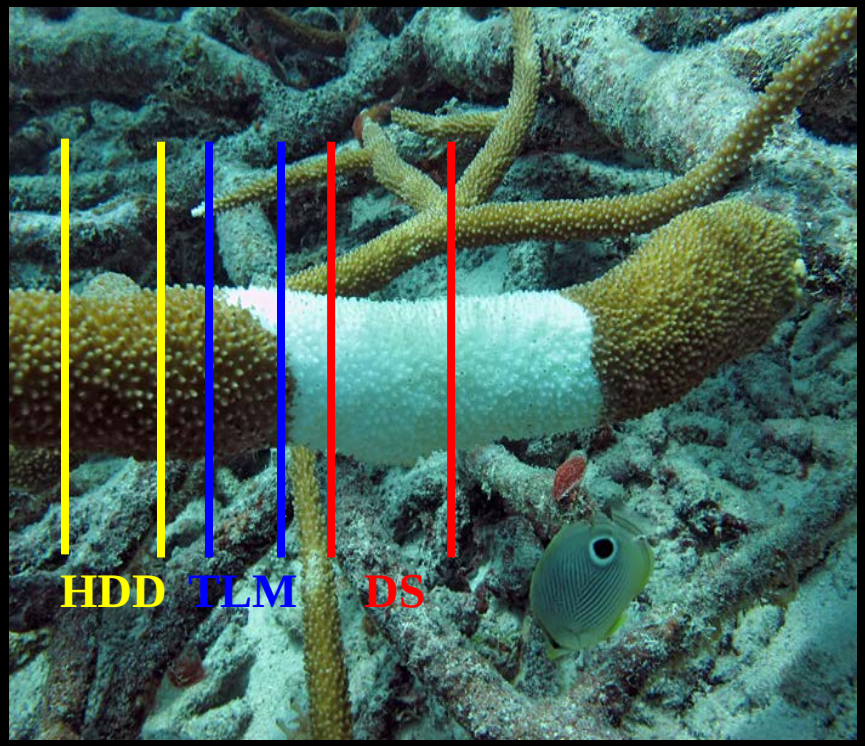
D.
P. Gillevet

E.
Peters / Miller

F.
Peters / Miller

G.
K. Patterson

Coral sample collection



- Apparently healthy colonies
  - One AH colony sampled
- Diseased colonies
  - Three colonies sampled
    - 1 healthy-on-diseased coral fragment (HD) per colony
    - 1 x diseased coral fragment per colony
- Histology: Z-Fix™ + seawater
- Molecular: RNALater®

Sediment sample collection

HISTOPATHOLOGICAL ANALYSIS

Histology methods

Sample fixation in Z-Fix™ + seawater

↓

Decalcification using EDTA

↓

Enrobing of all samples with exposed skeleton

↓

Sample processing & embedding in paraffin

↓

Sectioning

↓

Staining (H&E, Giemsa, & MMP)

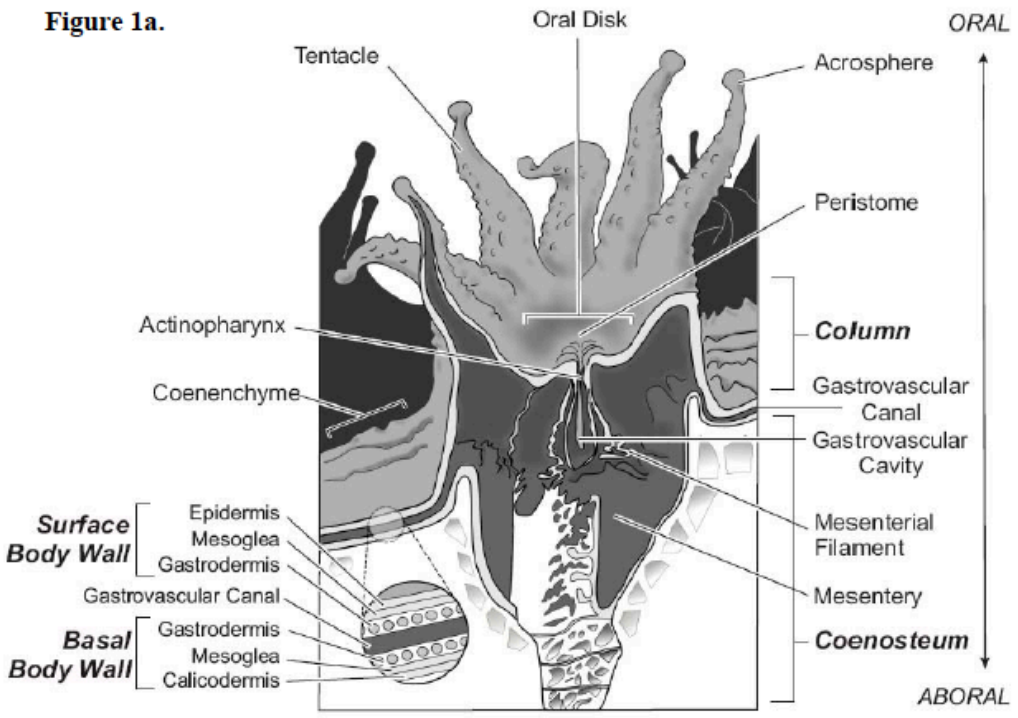
↓

Coverslipping

↓

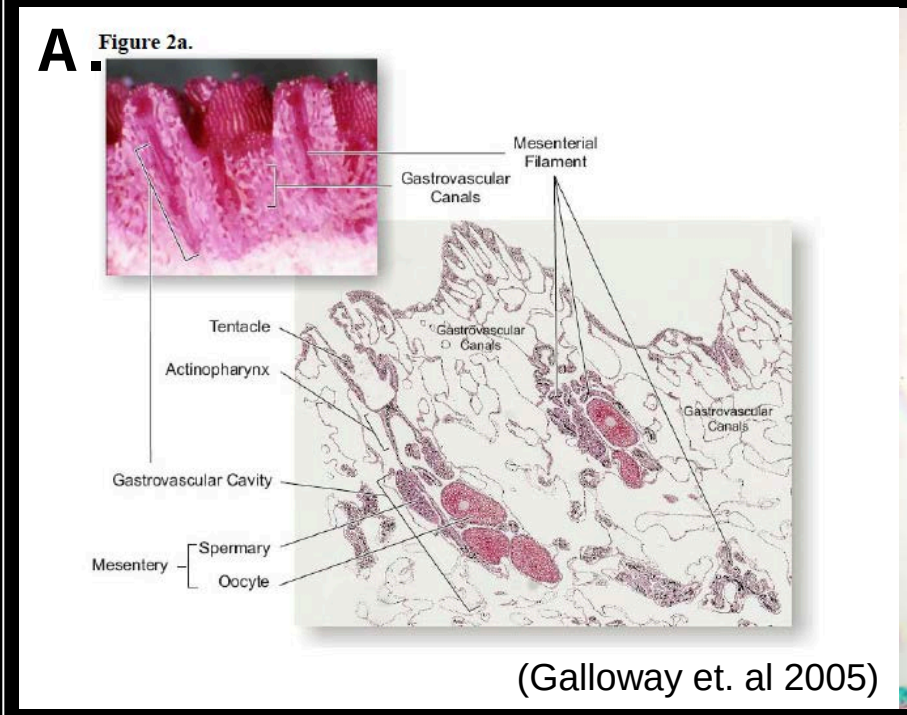
Slide reading

Coral anatomy



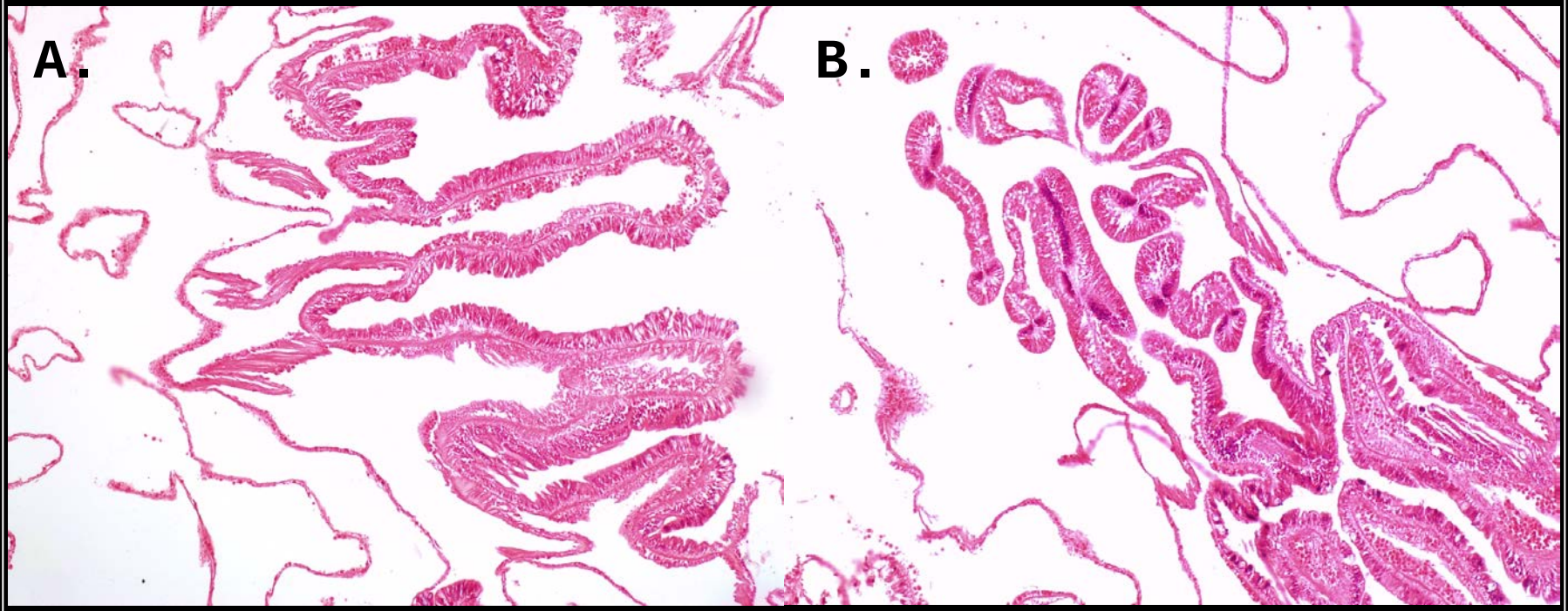
(Galloway et. al 2005)

Histological orientation

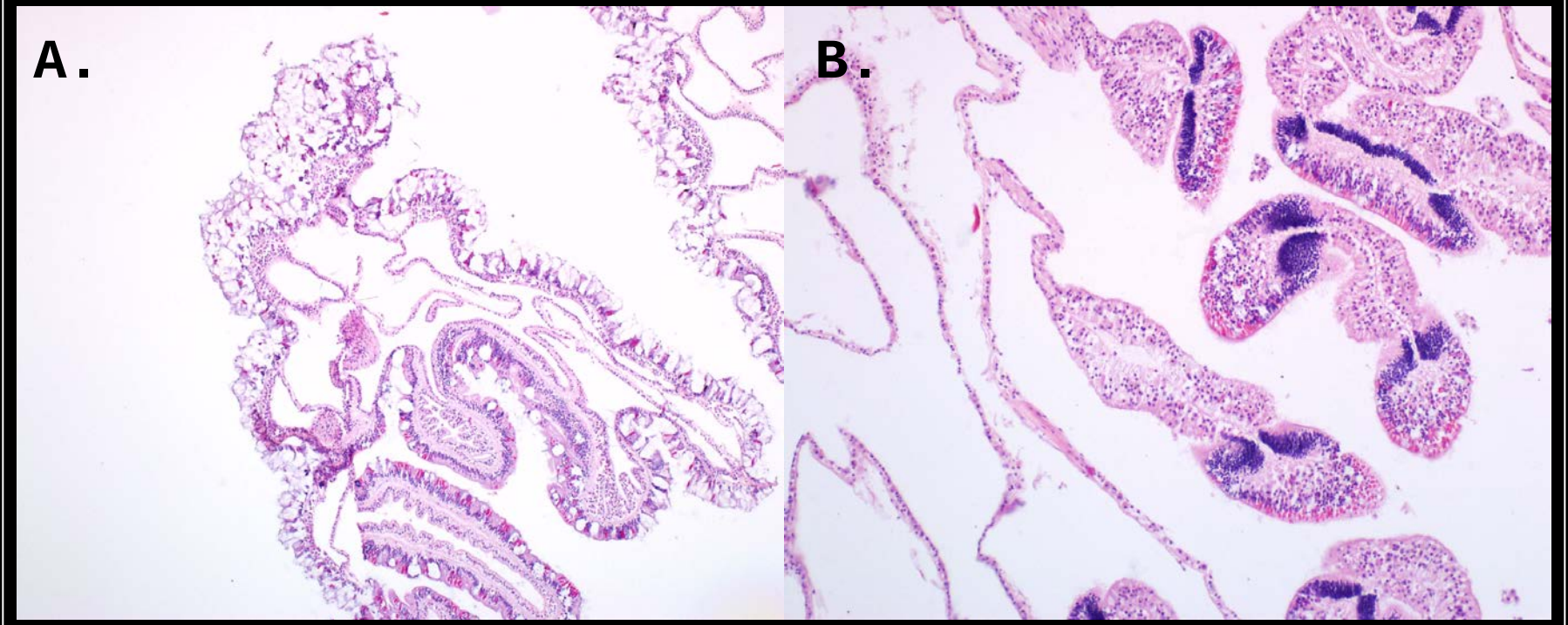


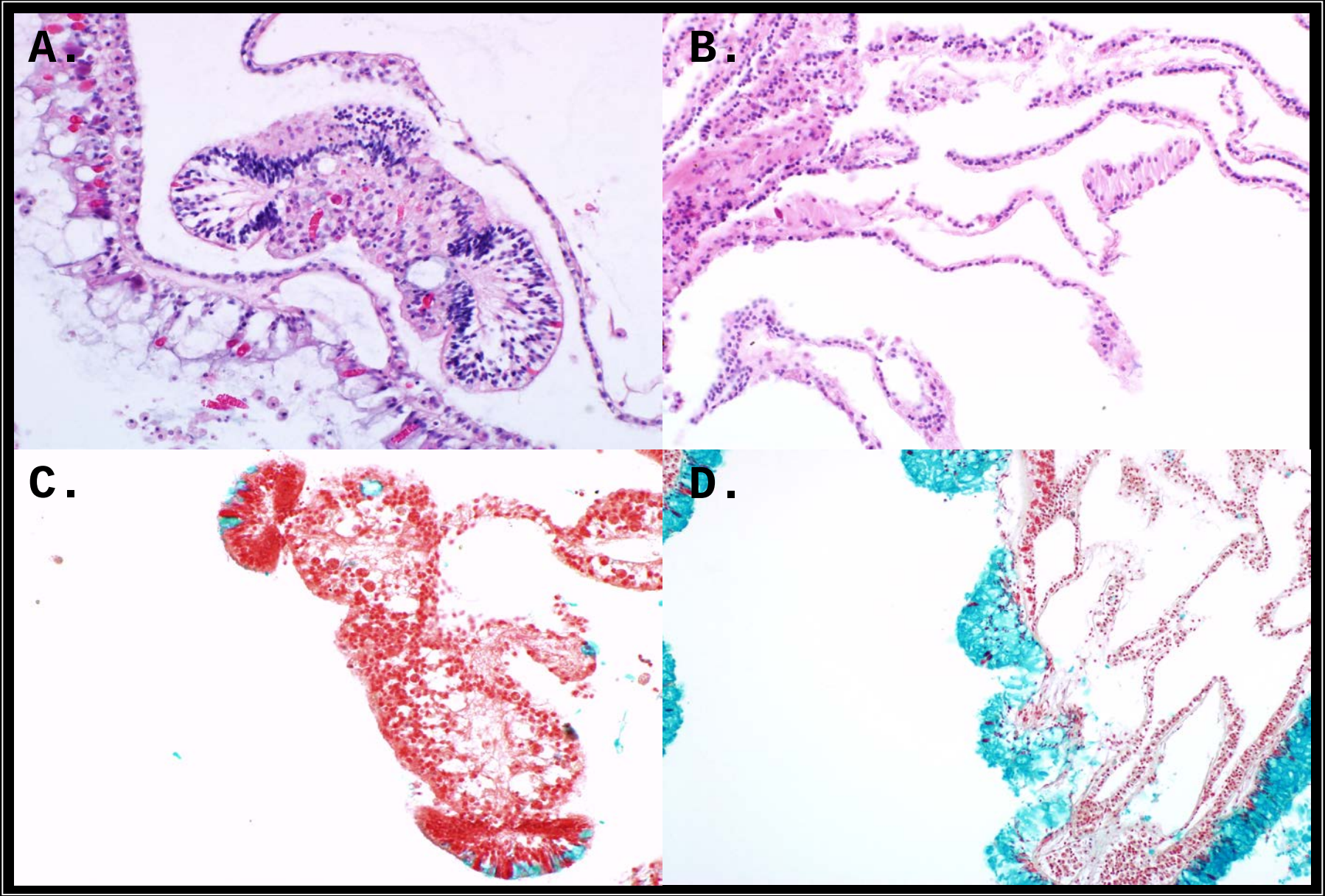
(Galloway et. al 2005)

AH *A. cervicornis* of the past…

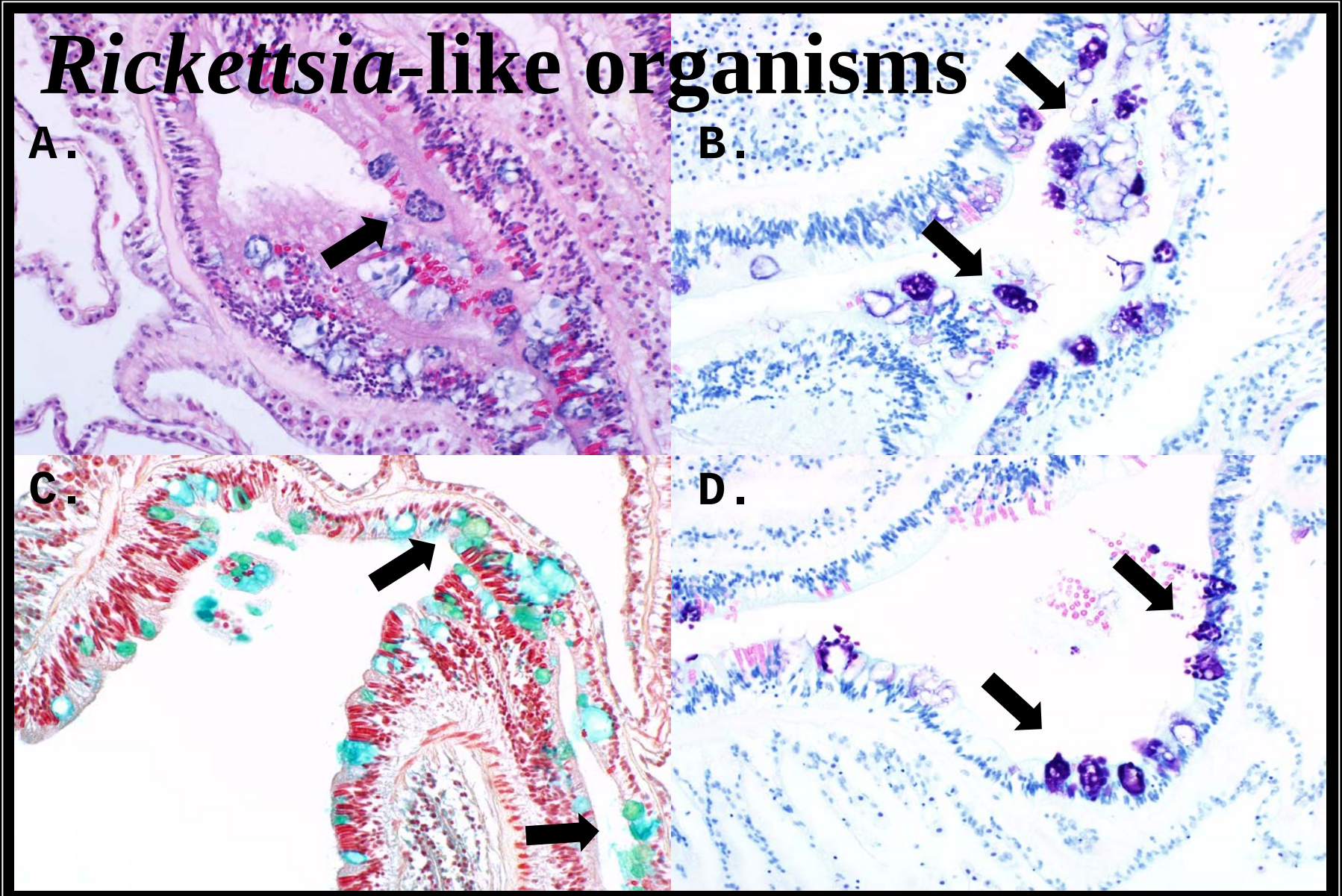


AH today




A.
B.
C.
D.

Rickettsia-like organisms



Histopathological findings

- Apparently healthy *A. cervicornis* exhibited to moderate to severe signs of stress including:
  - Hypertrophy of epidermal mucocytes
  - Dissociation of mesenterial filaments
  - Necrosis of cnidoglandular bands
  - Atrophy of the gastrodermis and calicodermis
  - Presence of *Rickettsia*-like organisms
- It is questionable whether truly “healthy” corals are present in the Upper Florida Keys

MOLECULAR ANALYSES

Molecular methods

DNA Extraction using a FastPrep Soil Kit

↓

Quantitation on an EtBr stained agarose gel

↓

Polymerase Chain Reaction (PCR)

↓

Quantitation on an EtBr stained agarose gel

↓

LH-PCR

↓

PCR Clean-up

↓

Emulsion PCR

↓

Next-generation sequencing (NGS)

↓

Bioinformatic Analysis

Sequence Analysis

Coral

- 802,910 sequences
- 46 coral fragments
  - 6 AH
  - 5 HD
  - 6 HDD
  - 14 TLM
  - 15 DS

Sediment

- 795,195 sequences
- 31 sediment samples
  - 9 AH
  - 10 HD
  - 12 D

A total of 1,616,397 sequences

Average of 17,352 reads per sample

RDP 10 Analysis

Coral

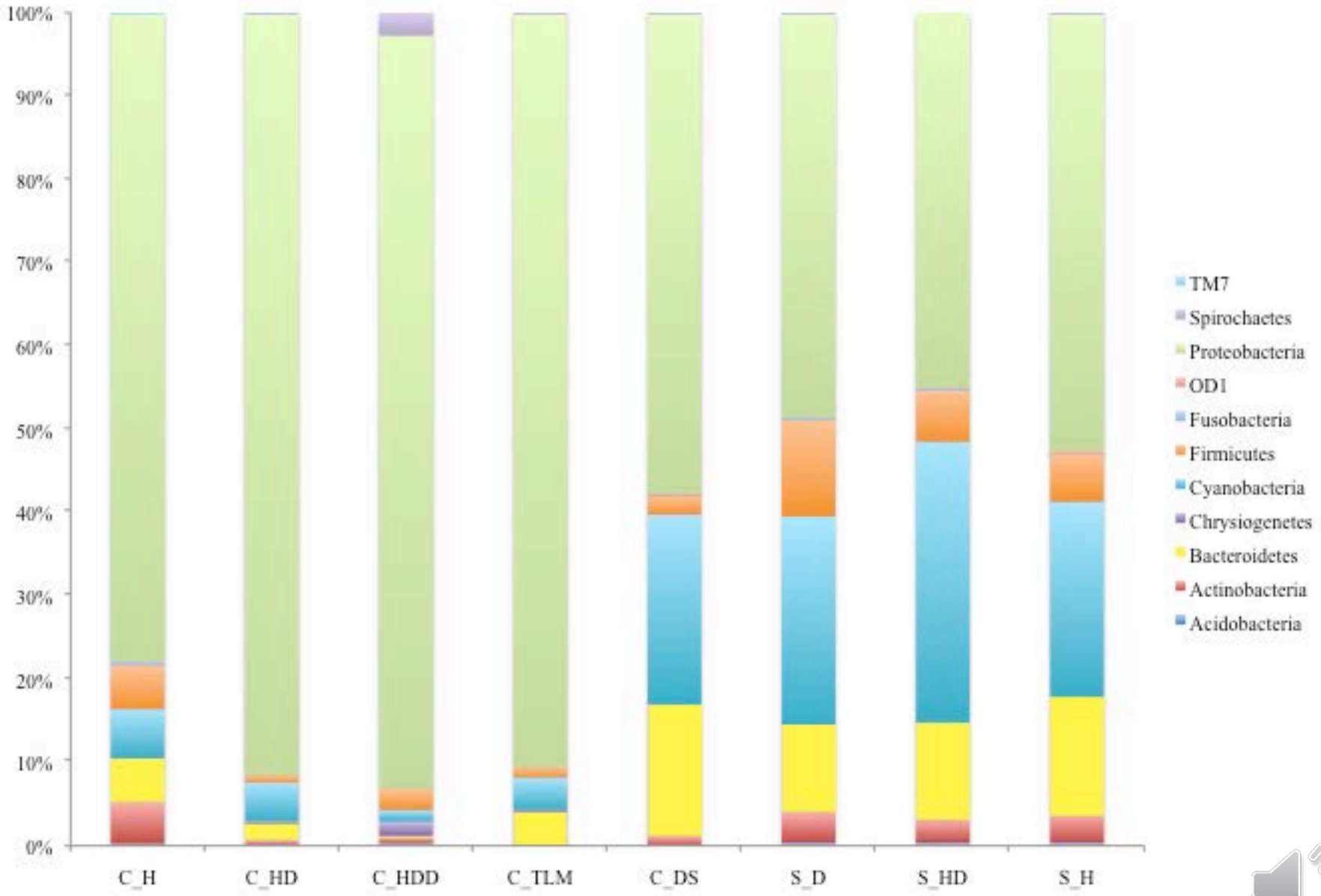
- 11 Phyla
- 19 Classes
- 43 Orders
- 89 Families
- 153 Genera

Sediment

- 9 Phyla
- 14 Classes
- 31 Orders
- 59 Families
- 88 Genera

(Bootstrap P = 0.80, Cutoff = 0.01)

Histogram of RDP 10 taxa abundance (phylum level)



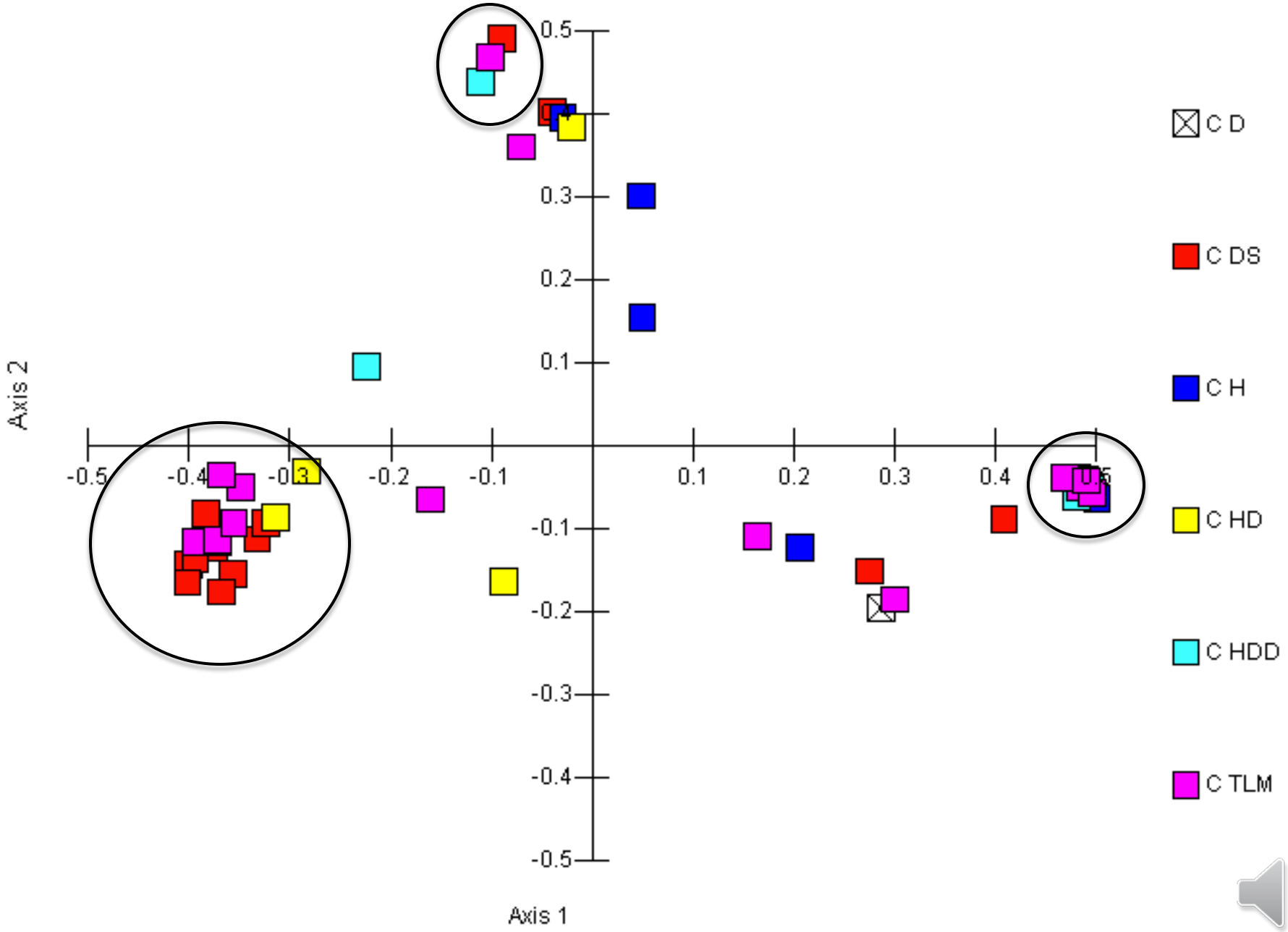
Comparison of the percent abundance (%) of bacterial communities associated with each coral sample type at the class level (Bootstrap P = 0.80, cutoff = 0.01).

Phylum	Class	C_H	C_HD	C_HDD	C_TLM	C_DS
Acidobacteria	Holophagae	0.00	0.00	0.21	0.00	0.00
Actinobacteria	Actinobacteria	5.09	0.93	0.64	0.39	1.06
Bacteroidetes	Bacteroidia	1.01	0.20	0.12	0.17	0.08
	Flavobacteria	1.24	0.60	0.06	2.21	10.49
	Sphingobacteria	2.26	0.97	0.06	1.14	5.10
Chrysiogenetes	Chrysiogenetes	0.00	0.00	1.72	0.14	0.00
Cyanobacteria	Cyanobacteria	6.07	4.79	1.41	4.10	23.58
Firmicutes	Bacilli	1.37	0.00	0.11	0.08	0.03
	Clostridia	3.74	1.02	2.38	0.79	1.60
	Erysipelotrichi	0.00	0.00	0.00	0.17	0.14
Fusobacteria	Fusobacteria	0.45	0.00	0.00	0.00	0.00
OD1	OD1_	0.00	0.00	0.00	0.00	0.18
Proteobacteria	Alphaproteobacteria	**67.98**	12.28	**85.68**	**48.05**	23.79
	Betaproteobacteria	1.15	0.11	0.30	0.05	0.07
	Deltaproteobacteria	1.53	1.17	0.30	1.64	2.41
	Epsilonproteobacteria	0.00	0.00	0.00	0.32	0.00
	Gammaproteobacteria	8.00	**77.78**	4.25	40.64	**31.39**
Spirochaetes	Spirochaetes	0.00	0.14	2.76	0.12	0.08
TM7	TM7_	0.10	0.00	0.00	0.00	0.00
	Total reads:	**68,917**	**42,899**	**89,830**	**238,727**	**337,925**

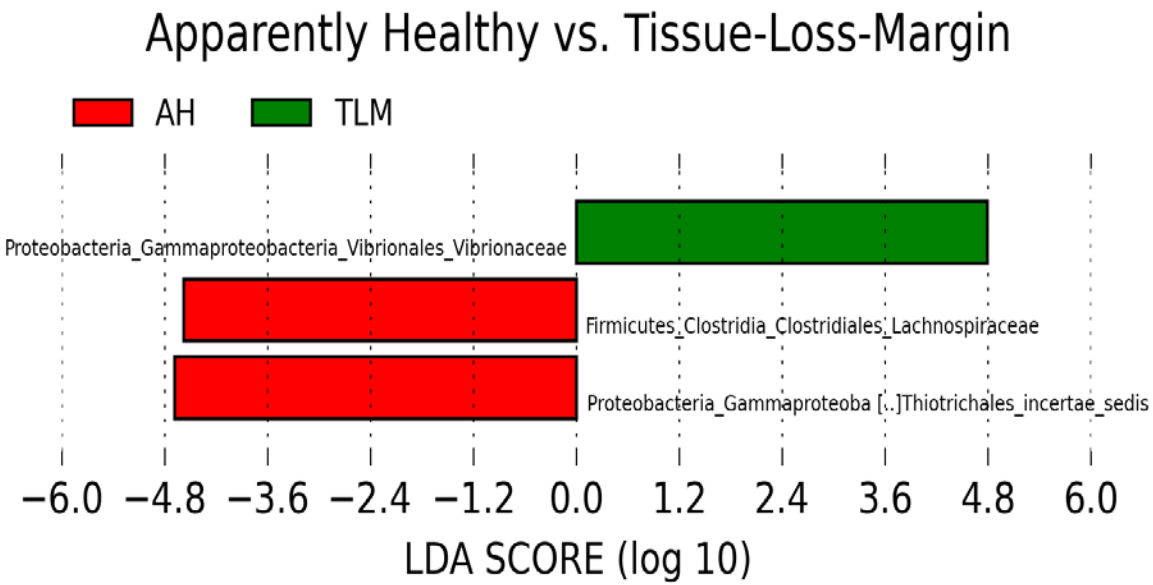
Comparison of the percent abundance (%) of bacterial communities associated with each coral sample type at the class level (Bootstrap P = 0.80, cutoff = 0.01). The most dominant classes are highlighted for each sample type with the most abundant in bold.

Phylum	Class	S_H	S_HD	S_D
Acidobacteria	Acidobacteria_Gp22	0.00	0.00	0.08
Actinobacteria	Actinobacteria	3.26	2.62	3.85
Bacteroidetes	Bacteroidia	1.14	1.02	0.92
	Flavobacteria	5.66	4.63	4.12
	Sphingobacteria	7.18	5.60	5.38
Cyanobacteria	Cyanobacteria	**23.94**	**34.91**	24.83
Firmicutes	Clostridia	5.66	6.38	11.01
Fusobacteria	Fusobacteria	0.05	0.36	0.27
OD1	OD1_	0.03	0.00	0.00
Proteobacteria	Alphaproteobacteria	22.28	21.56	**26.12**
	Betaproteobacteria	0.04	0.00	0.00
	Deltaproteobacteria	7.78	5.01	5.82
	Gammaproteobacteria	22.97	17.93	17.41
Spirochaetes	Spirochaetes	0.00	0.00	0.18
	Total reads:	**265,508**	**245,196**	**262,938**

PCO case scores (Bray Curtis): coral only (genus, 0.1%)



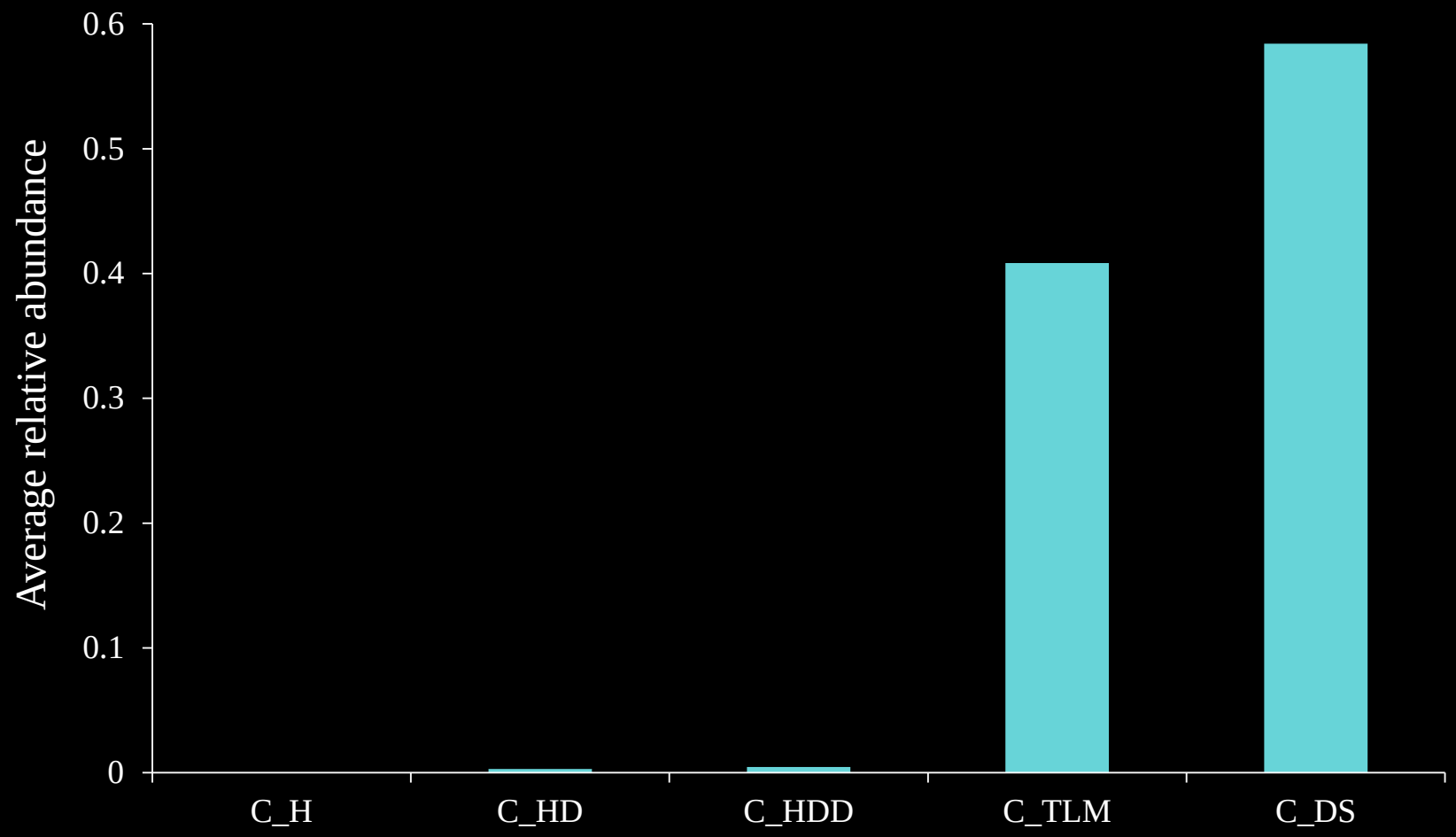
Histogram of LEfSe Results



Bacteria families most depleted or enriched in AH and TLM coral samples as identified by LEfSe. Bacterial taxa enriched in TLM are indicated with positive LDA scores (green), and taxa depleted in TLM are indicated with negative scores (red). Only taxa that met the significant LDA threshold of 3.6 are shown (a=0.01).

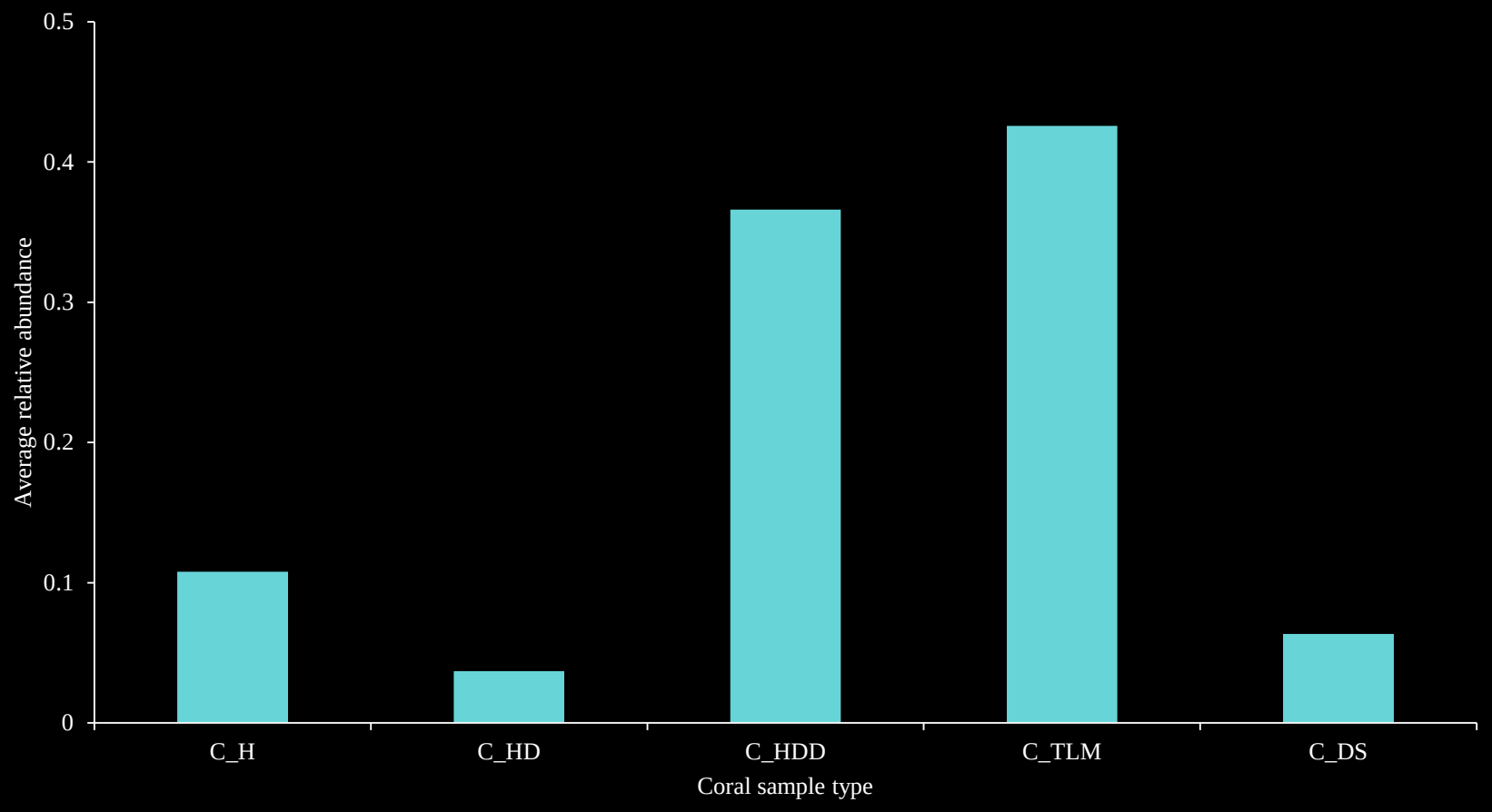
Family Vibrionaceae

Bootstrap P = 0.80, cutoff = 0.01

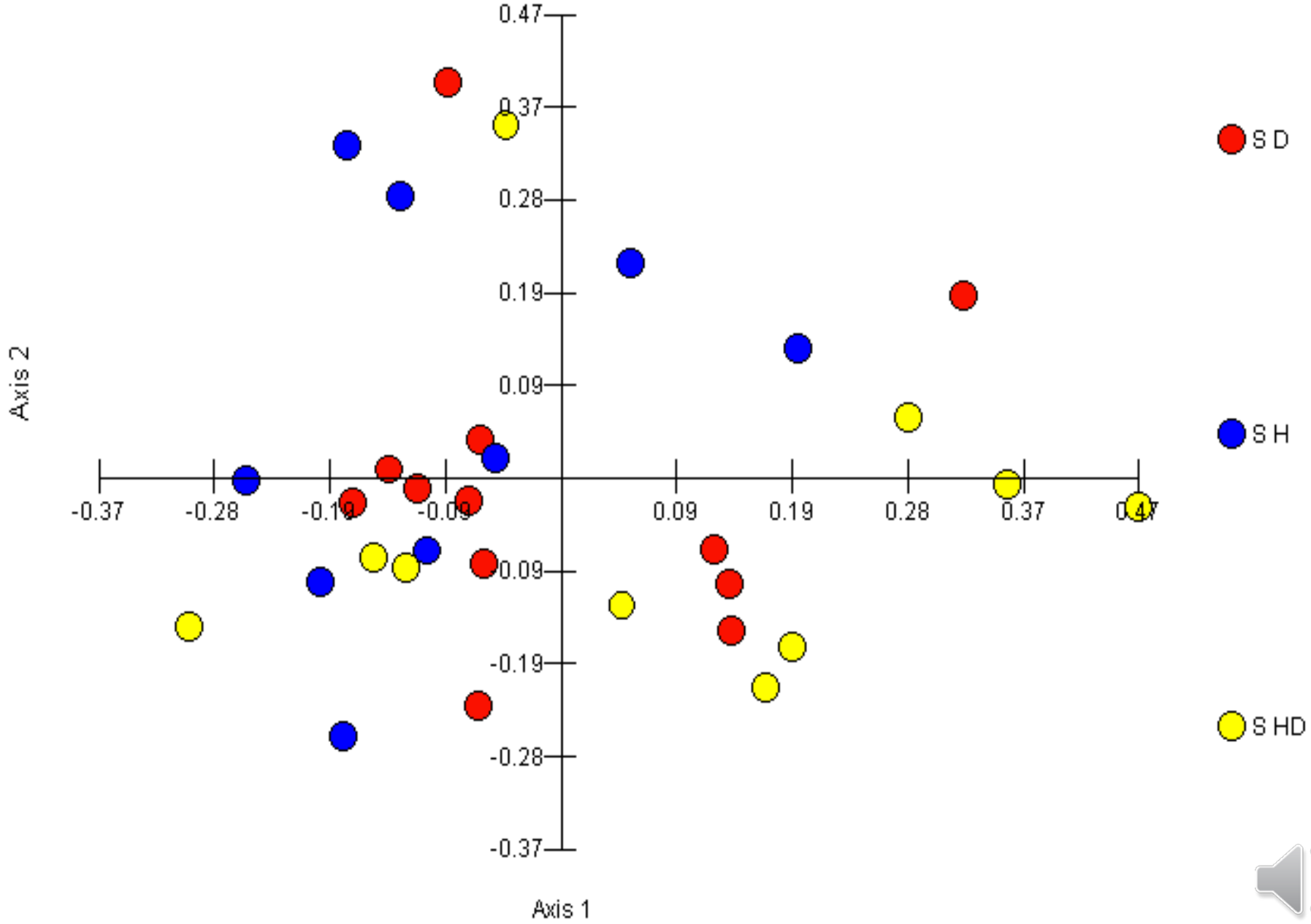


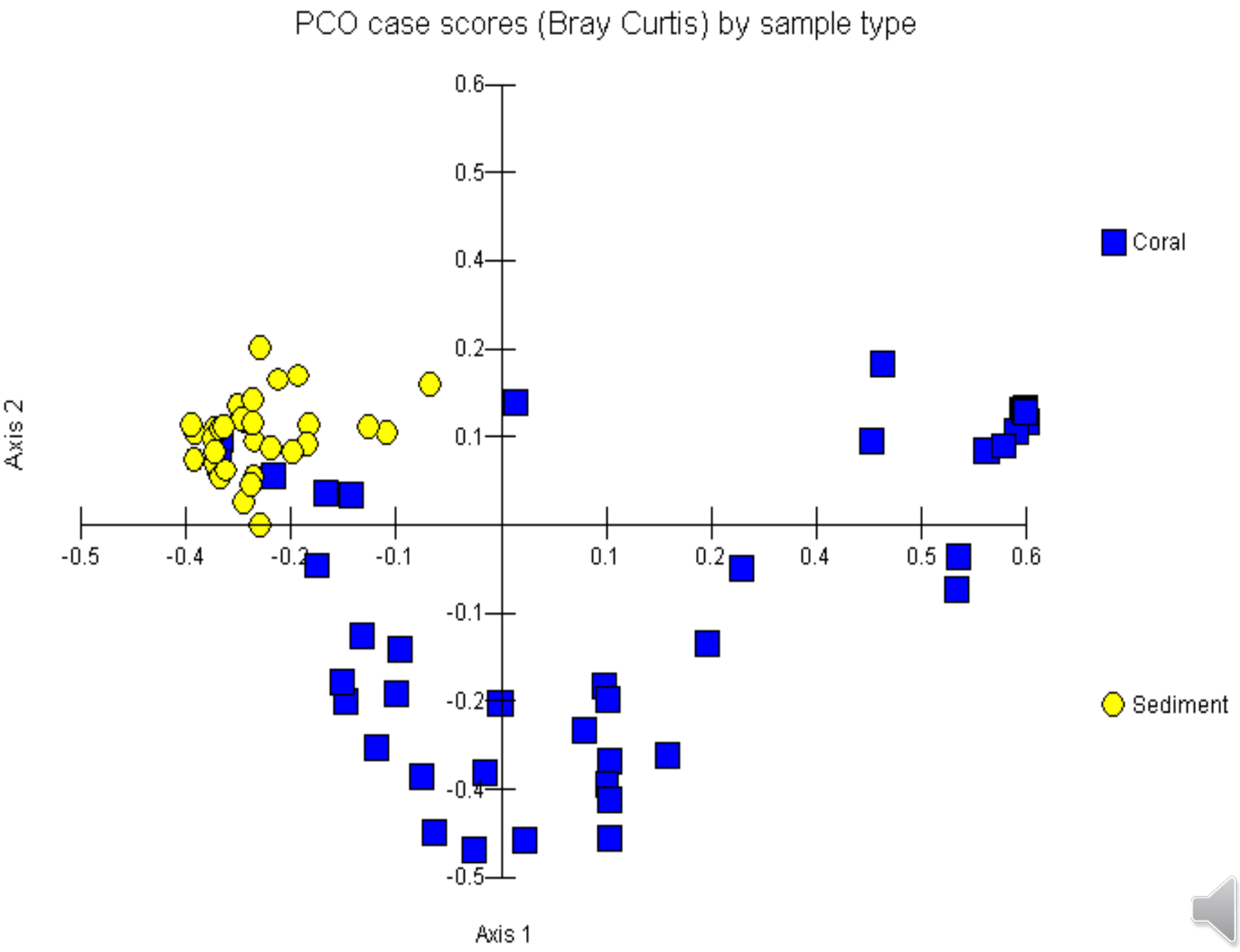
Family Rickettsiaceae

Bootstrap P = 0.80, cutoff = 0.01

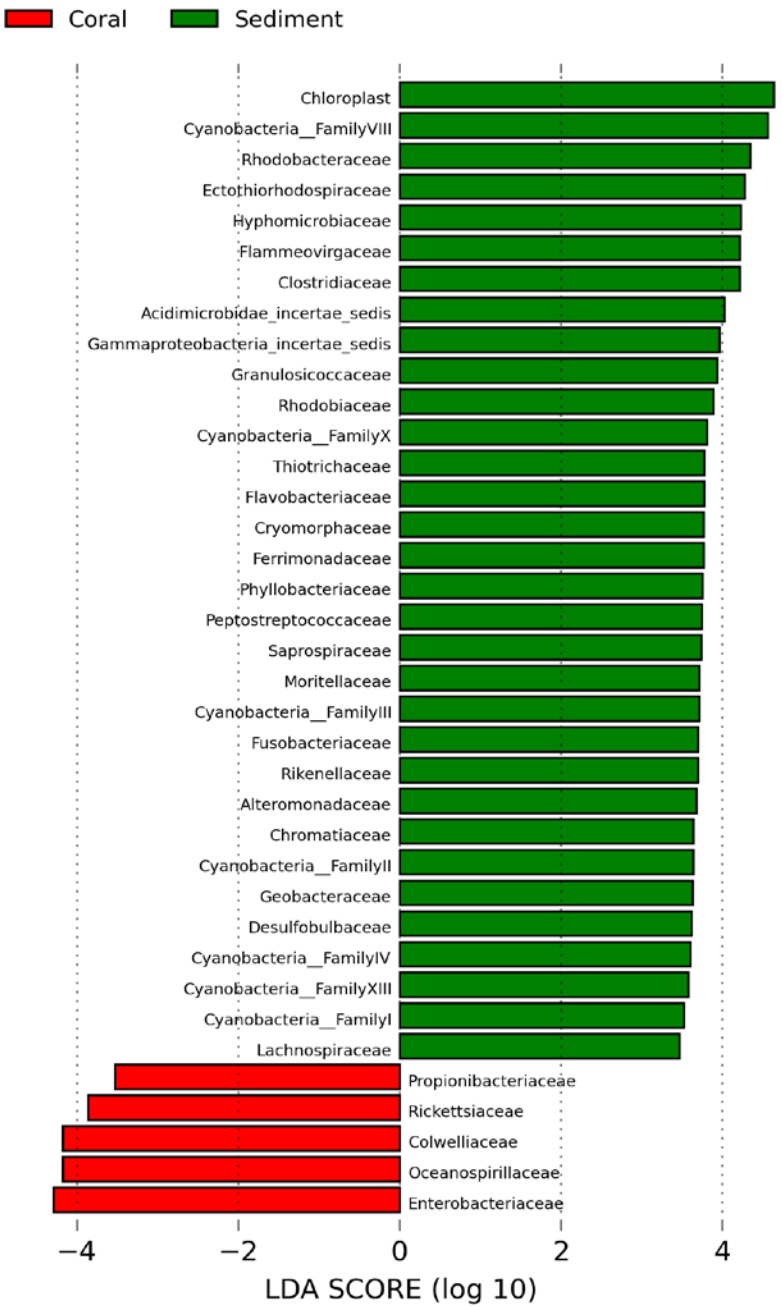


PCO case scores (Bray Curtis): sediment samples by sampling location

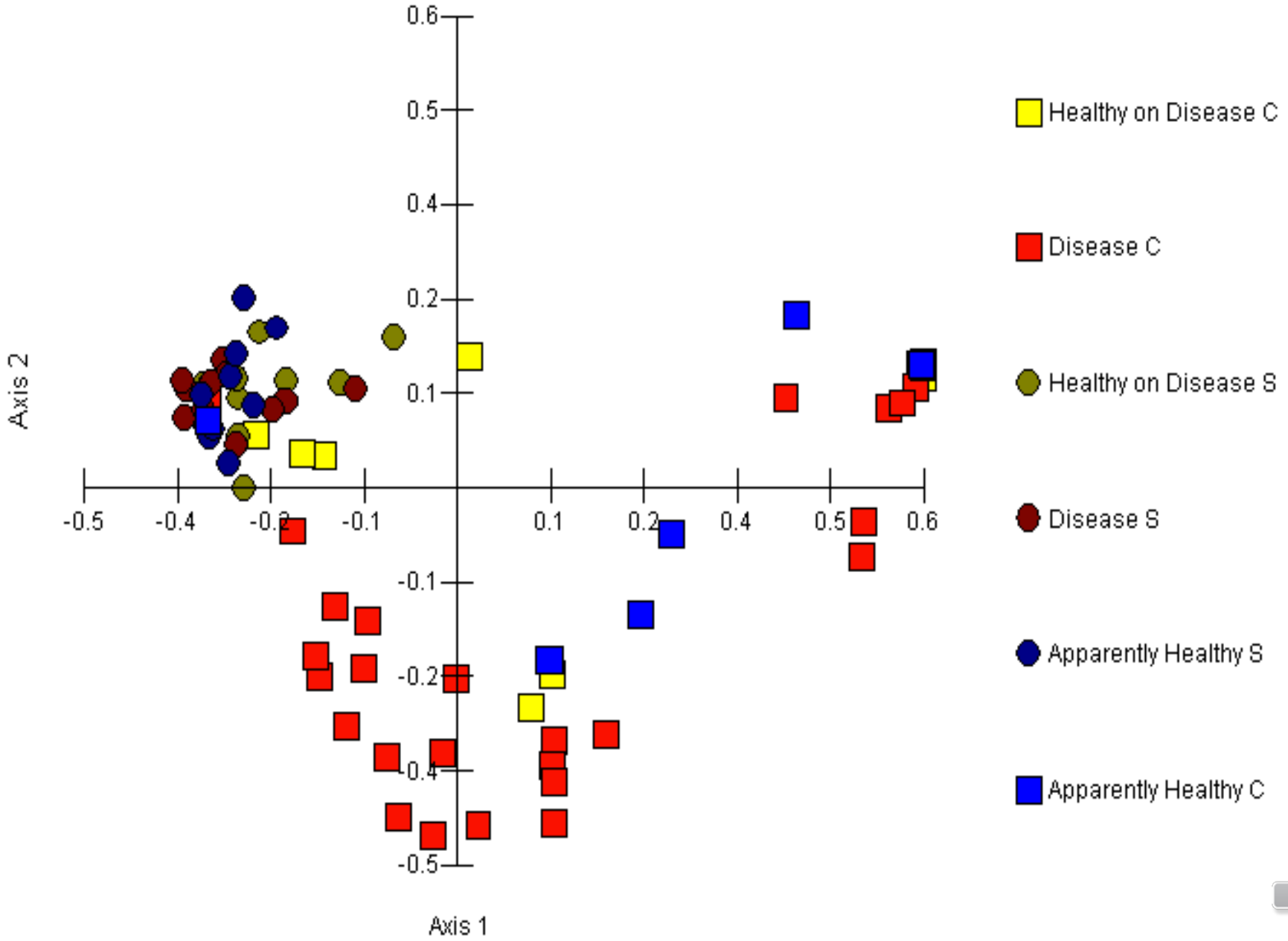



PCO case scores (Bray Curtis) by sample type
Axis 2
Axis 1
0.6
0.5
0.4
0.2
0.1
-0.1
-0.2
-0.4
-0.5
-0.5
-0.4
-0.2
-0.1
0.1
0.2
0.4
0.5
0.6
Coral
Sediment

Coral and sediment associated microbial comparison



PCO case scores (Bray Curtis) by sample location



Phylogentic analysis using QIIME

Taxonomic assignment method	Phylum	Class	Order	Family	Genus
RDP (1% abundance cut-off)	11	19	43	89	153
RDP (0.1% abundance cut-off)	24	42	86	192	352
UCLUST consensus taxonomy assigner	50	151	300	524	947

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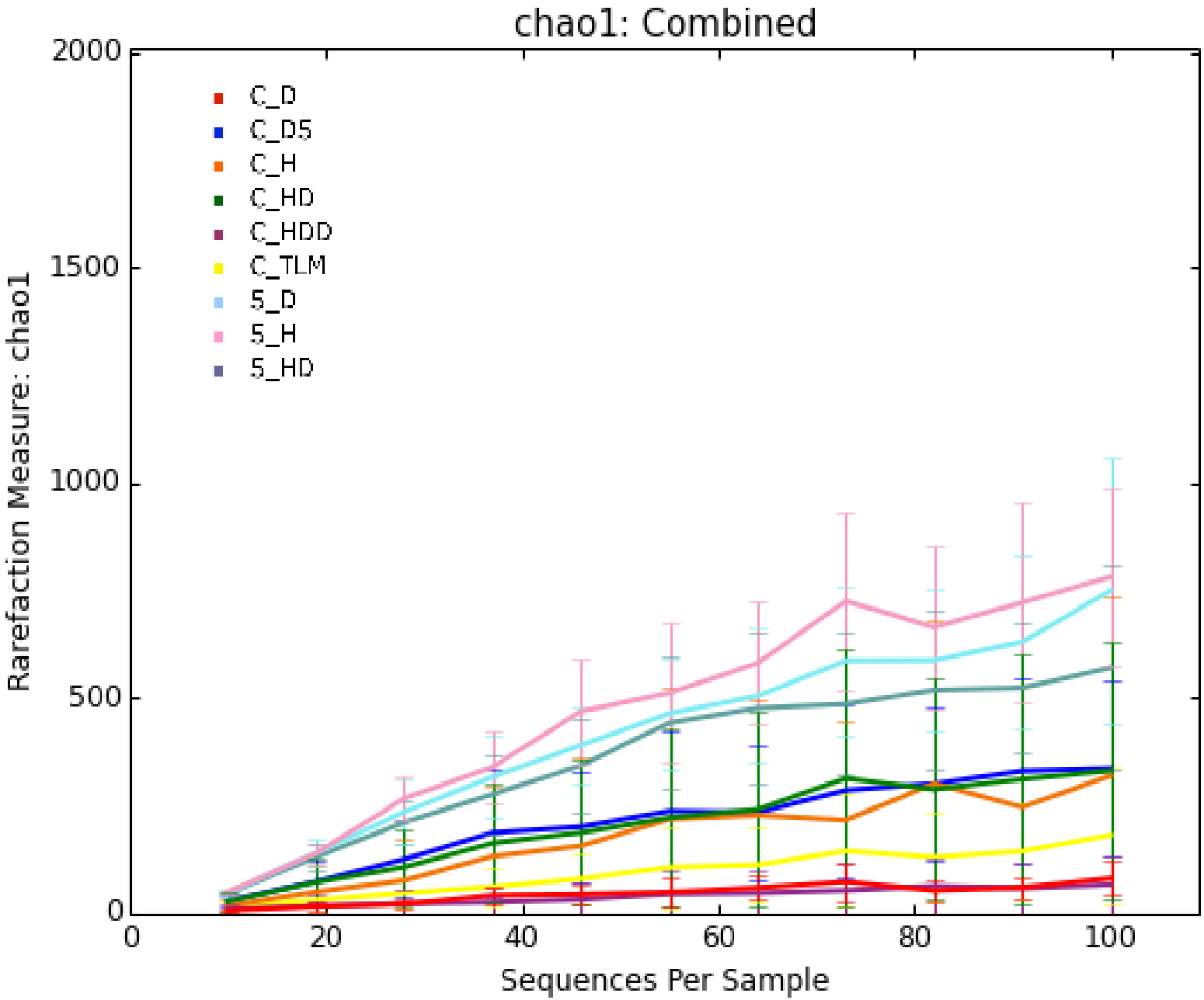

chao1: Combined
C_D
C_D5
C_H
C_HD
C_HDD
C_TLM
5_D
5_H
5_HD
Rarefaction Measure: chao1
Sequences Per Sample
0
500
1000
1500
2000
0
20
40
60
80
100

Beta diversity using UniFrac

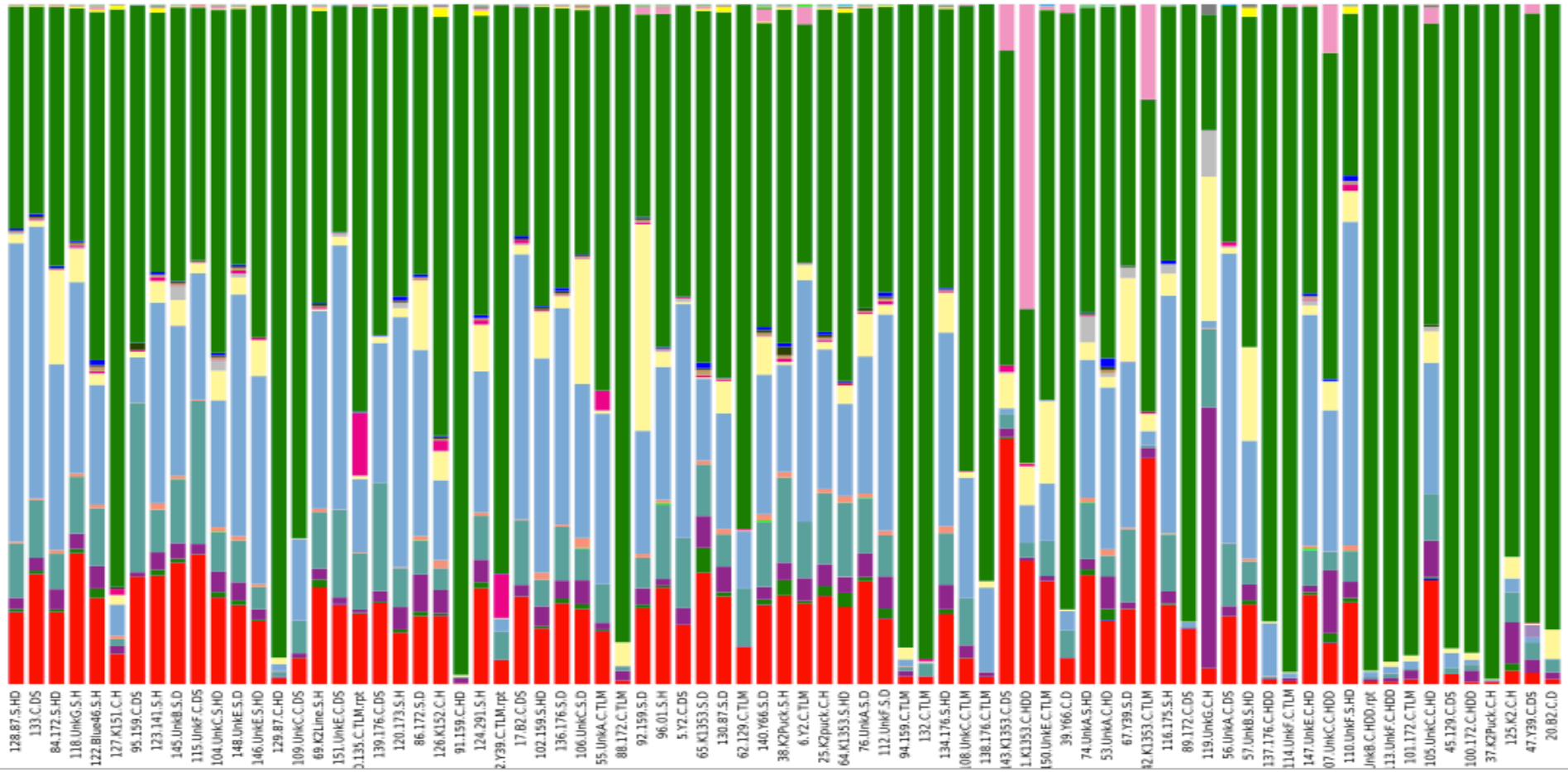
Classes compared

- All field sites
- Colonies of origin
- Colony type (nursery, outplant, or wild)
- Depth
- Sample year (2011/2012)

Findings

- Unweighted UniFrac
  - All classes were significantly different
- Weighted UniFrac
  - No significant differences found between classes

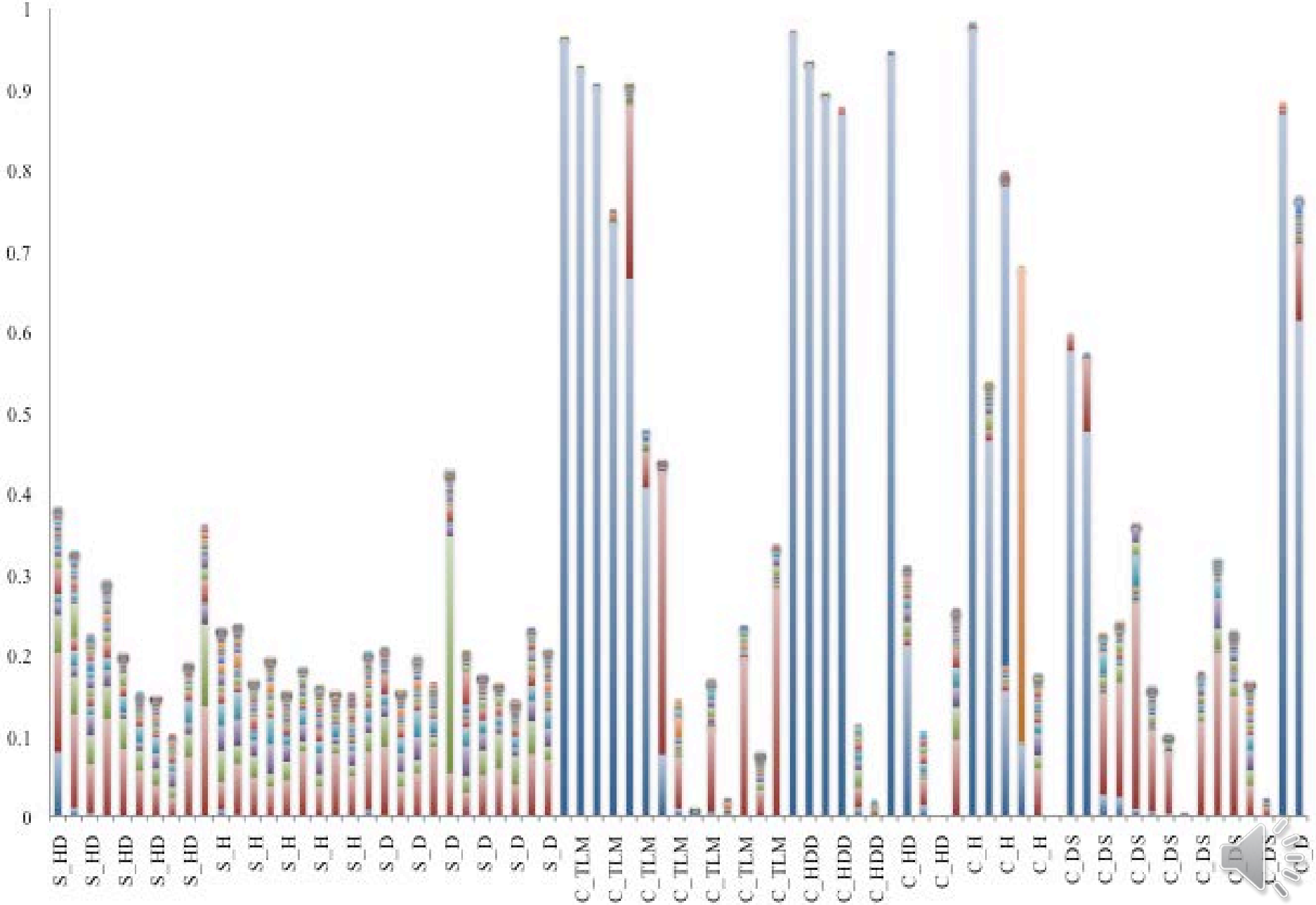
Histogram of QIIME taxa abundance
(phylum level)



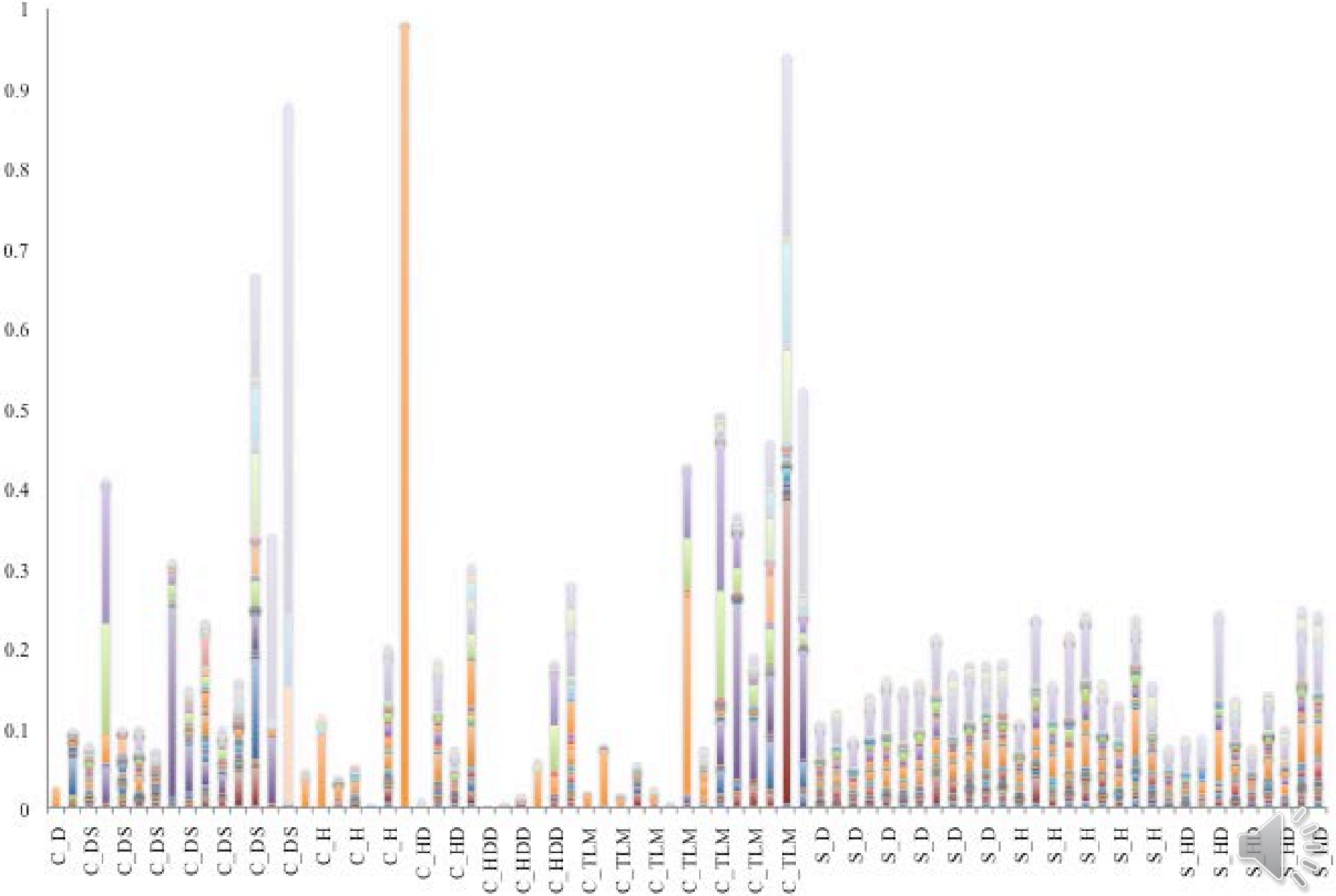
QIIME results were similar to RDP 10

OTUs at the class level	C_H	C_HD	C_HDD	C_TLM	C_DS	S_H	S_HD	S_D
Unassigned;Other;Other	12.03%	5.63%	7.78%	15.68%	7.72%	11.54%	7.25%	7.76%
k__Bacteria;p__Actinobacteria;c__Acidimicrobiia	2.24%	0.74%	1.64%	1.01%	1.51%	1.54%	1.15%	1.37%
k__Bacteria;p__Actinobacteria;c__Actinobacteria	6.52%	1.71%	0.15%	0.38%	1.16%	0.12%	0.40%	0.51%
k__Bacteria;p__Bacteroidetes;c__[Saprospirae]	0.92%	0.47%	0.56%	0.79%	0.61%	1.15%	0.31%	1.13%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia	1.89%	0.97%	0.22%	0.94%	0.49%	0.23%	0.44%	0.10%
k__Bacteria;p__Bacteroidetes;c__Cytophagia	2.14%	0.83%	1.65%	1.70%	1.24%	2.73%	1.42%	1.01%
k__Bacteria;p__Bacteroidetes;c__Flavobacteriia	2.30%	0.90%	1.65%	2.52%	2.48%	3.86%	2.83%	3.18%
k__Bacteria;p__Cyanobacteria;c__Chloroplast	7.53%	5.39%	7.93%	9.63%	9.55%	9.39%	5.81%	13.13%
k__Bacteria;p__Cyanobacteria;c__Oscillatoriophycideae	9.71%	4.95%	7.78%	5.20%	6.49%	3.52%	2.80%	2.07%
k__Bacteria;p__Cyanobacteria;c__Synechococcophycideae	2.92%	1.47%	2.82%	3.92%	1.87%	1.69%	1.27%	1.56%
k__Bacteria;p__Cyanobacteria;Other	1.90%	0.42%	0.82%	0.85%	0.54%	0.44%	0.20%	0.59%
k__Bacteria;p__Firmicutes;c__Bacilli	2.37%	0.51%	0.07%	0.52%	0.21%	0.10%	0.35%	0.19%
k__Bacteria;p__Firmicutes;c__Clostridia	4.04%	1.61%	1.98%	3.09%	3.29%	2.65%	5.90%	1.62%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia	1.33%	0.11%	0.03%	0.36%	0.26%	0.62%	0.04%	0.15%
k__Bacteria;p__GN02;c__BD1-5	0.00%	0.00%	0.05%	0.13%	0.00%	1.74%	0.01%	0.30%
k__Bacteria;p__Proteobacteria;c__Alphaproteobacteria	**16.63%**	**62.36%**	**39.45%**	**25.26%**	**37.48%**	20.86%	**38.97%**	**40.58%**
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	2.00%	0.36%	0.20%	0.43%	0.47%	0.91%	0.17%	1.07%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	3.74%	1.10%	1.73%	2.25%	2.71%	3.78%	2.67%	3.04%
k__Bacteria;p__Proteobacteria;c__Epsilonproteobacteria	0.06%	0.02%	0.04%	0.11%	0.11%	0.37%	0.02%	0.13%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	15.33%	7.40%	21.11%	17.46%	18.83%	**29.11%**	26.30%	16.55%
k__Bacteria;p__Spirochaetes;c__Spirochaetes	0.14%	0.02%	0.02%	5.26%	0.74%	0.15%	0.21%	0.48%

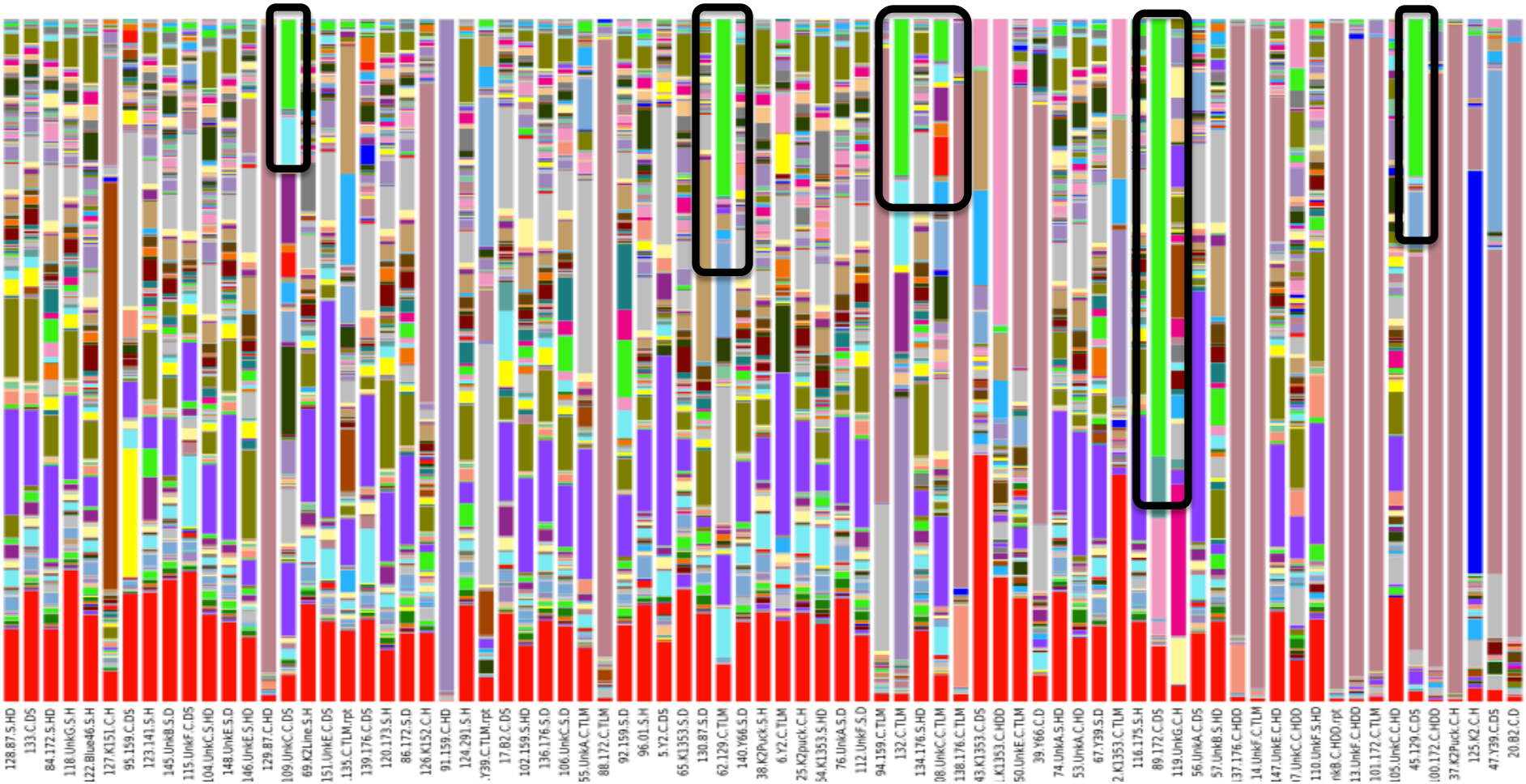
Alphaproteobacteria by sample type



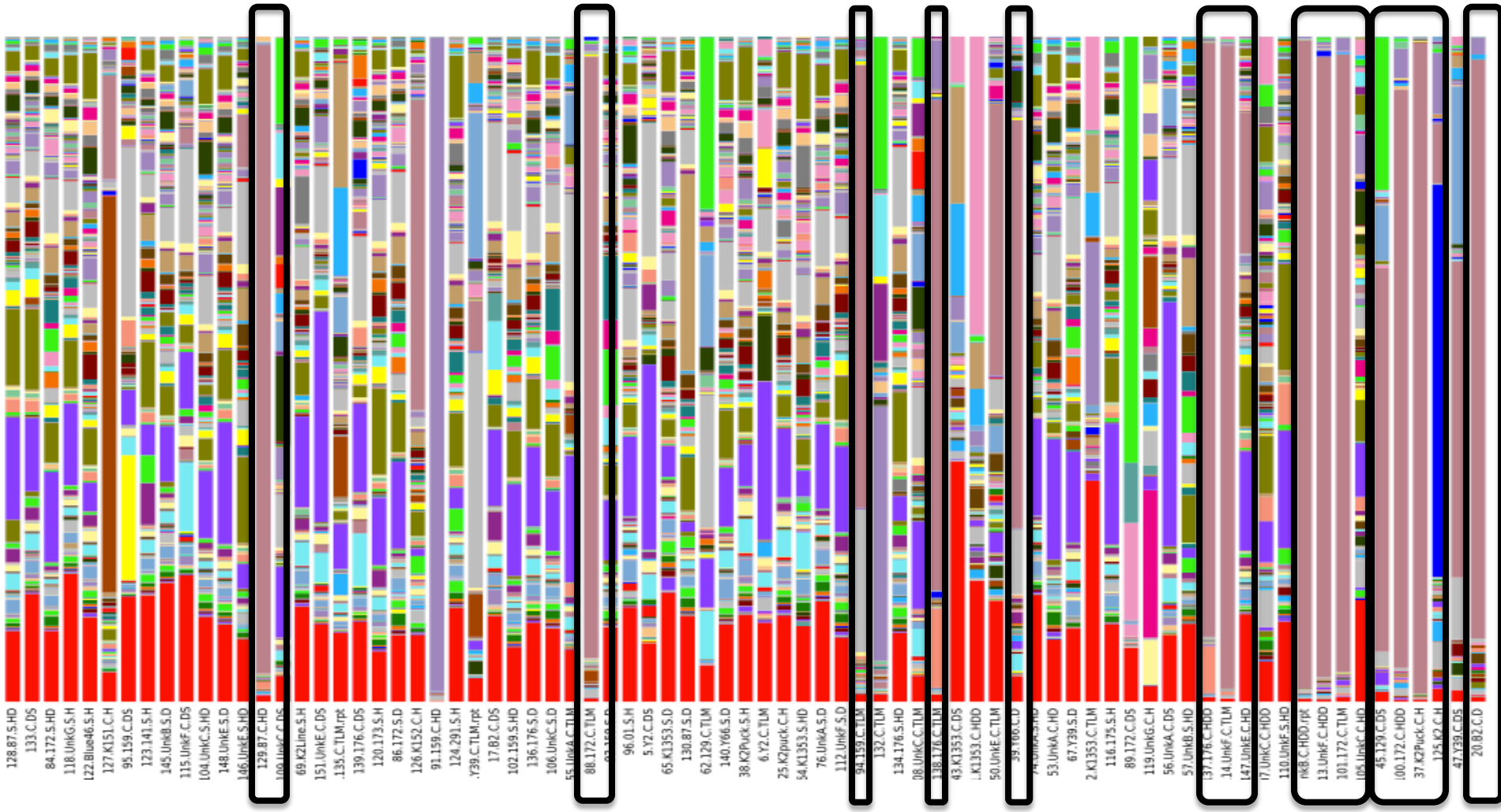
Gammaproteobacteria by sample type



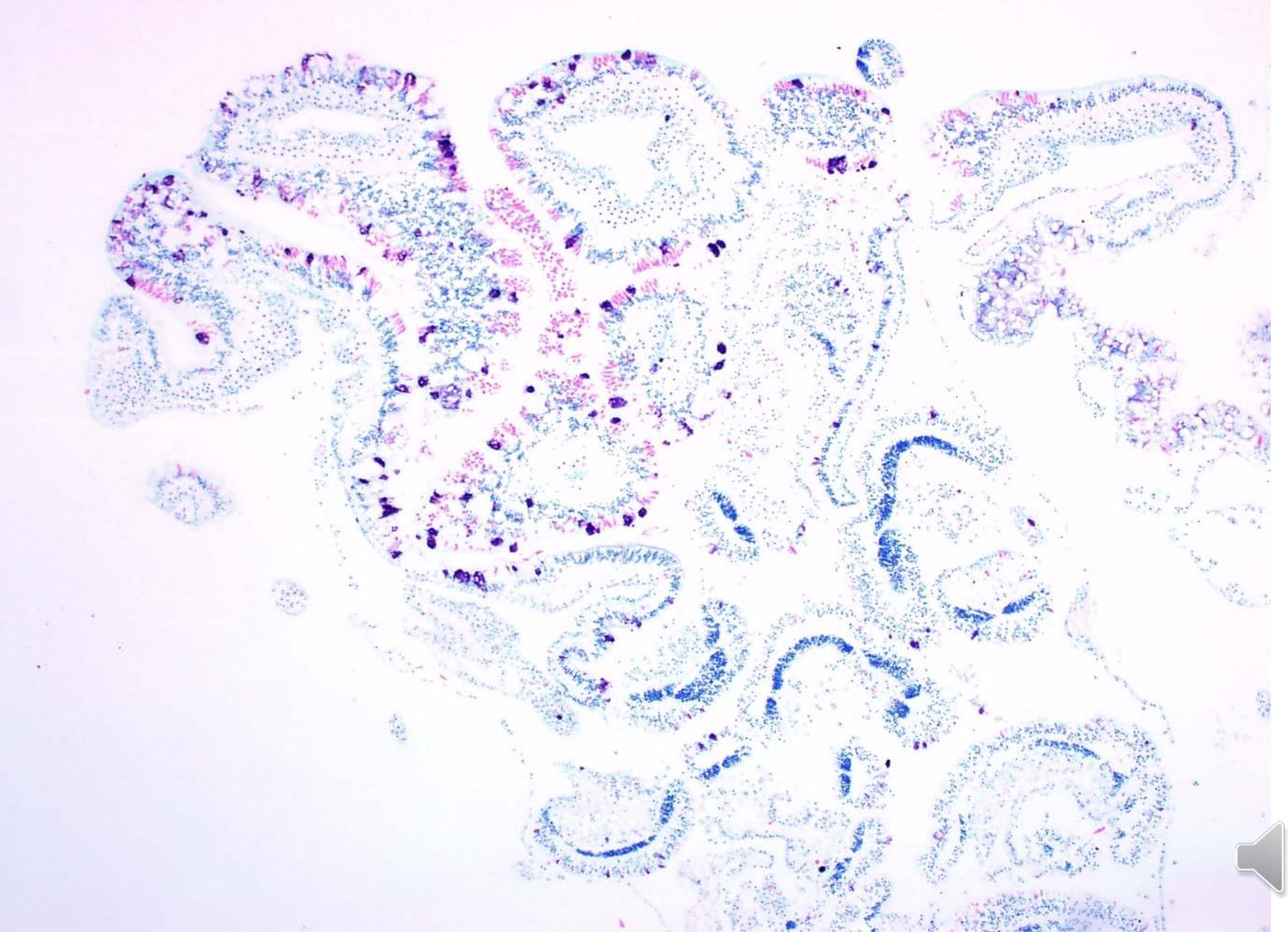
Histogram of QIIME taxa abundance with Vibrionales highlighted (genus level)



Histogram of QIIME taxa abundance with Rickettsiales highlighted (genus level)



83.UnkB.C.HDD



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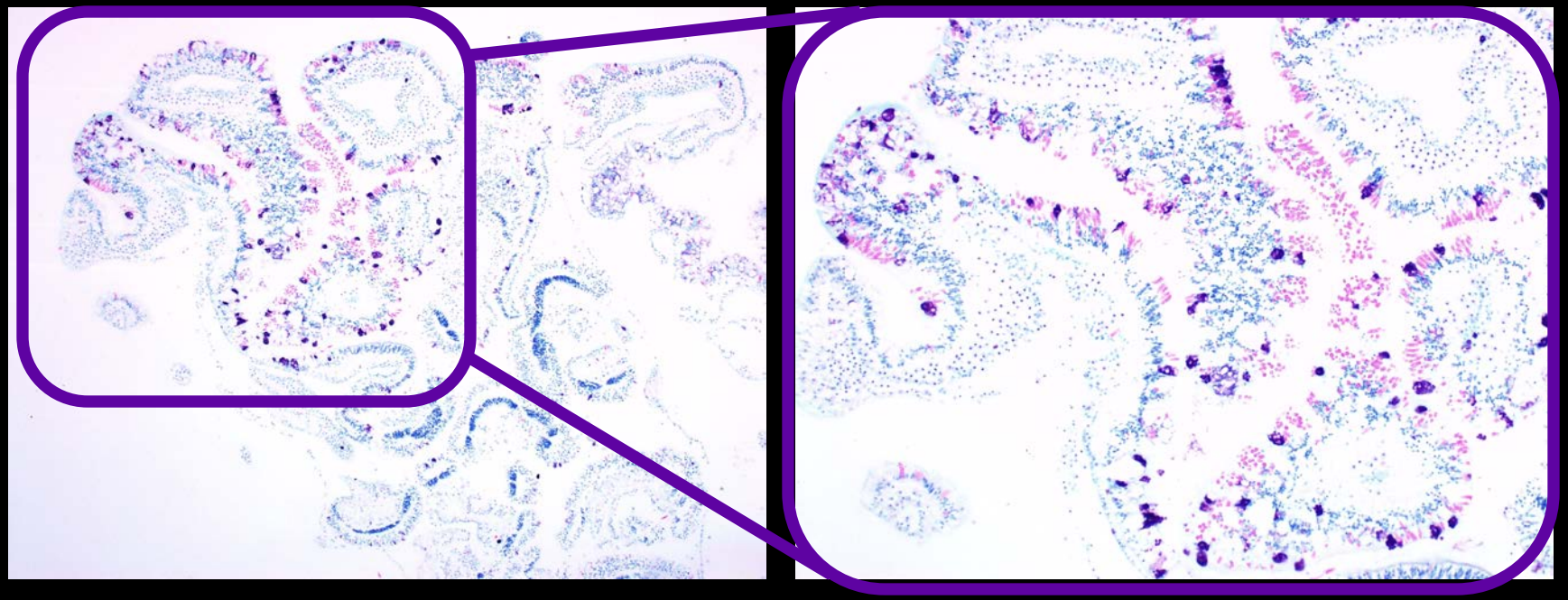
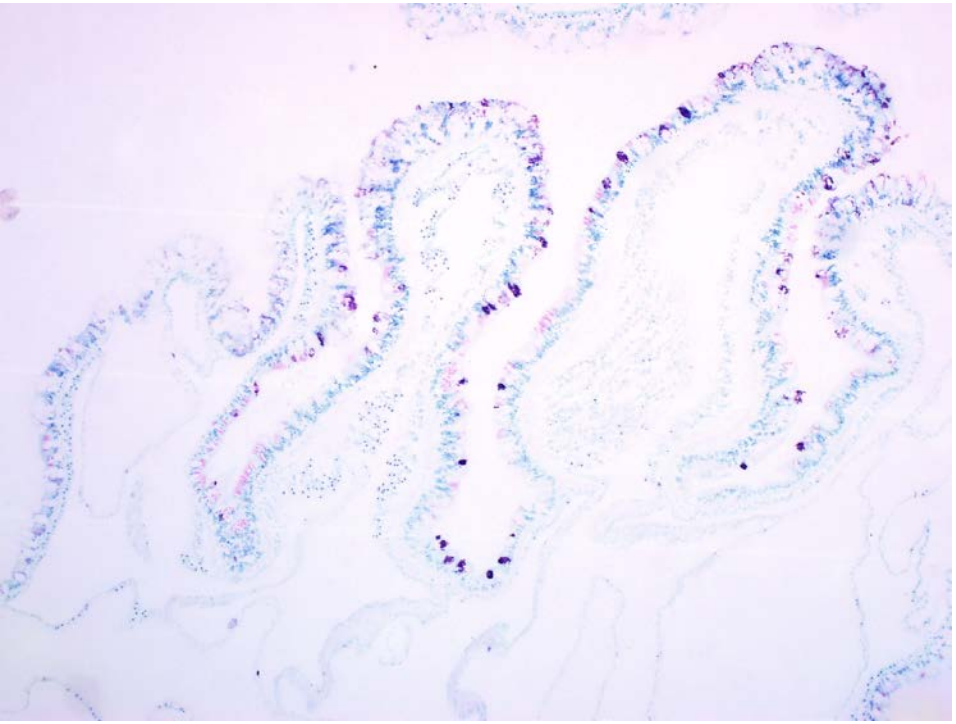
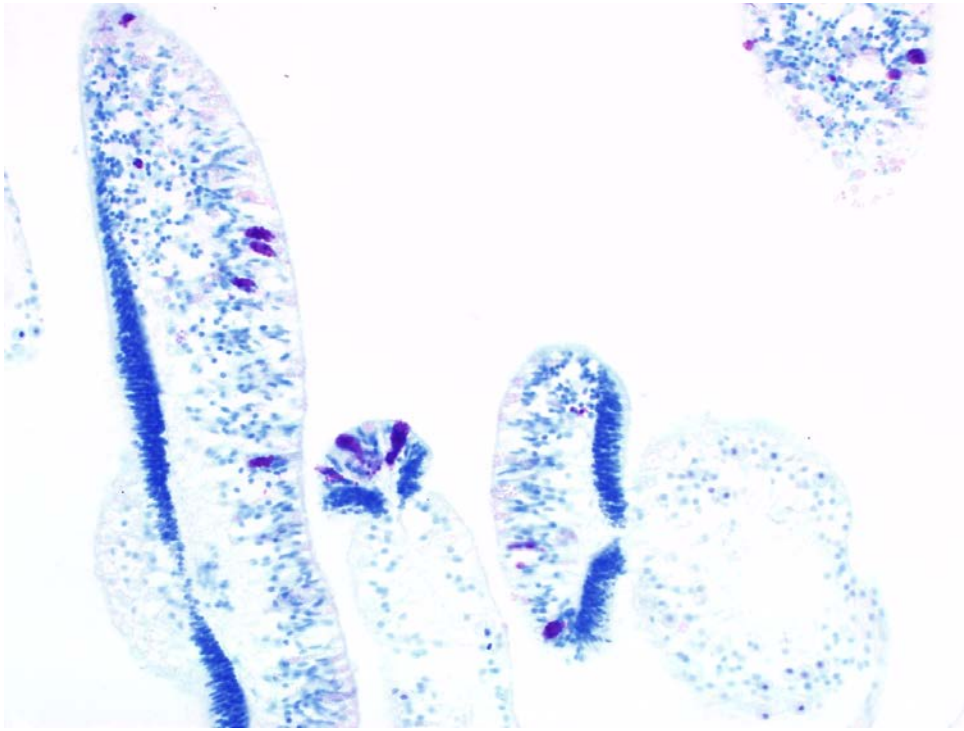
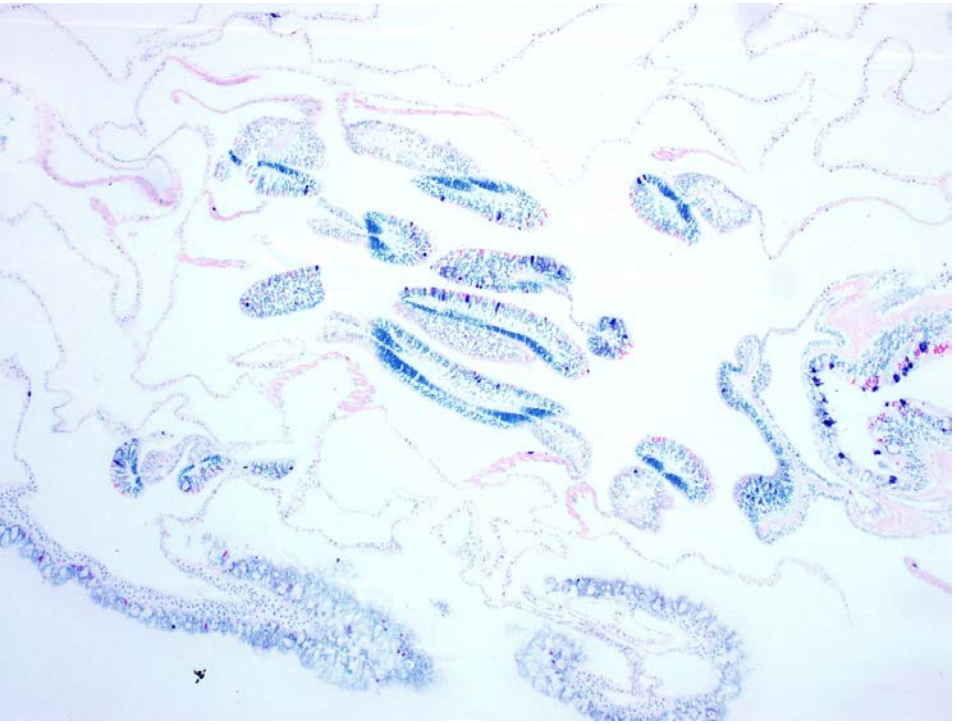


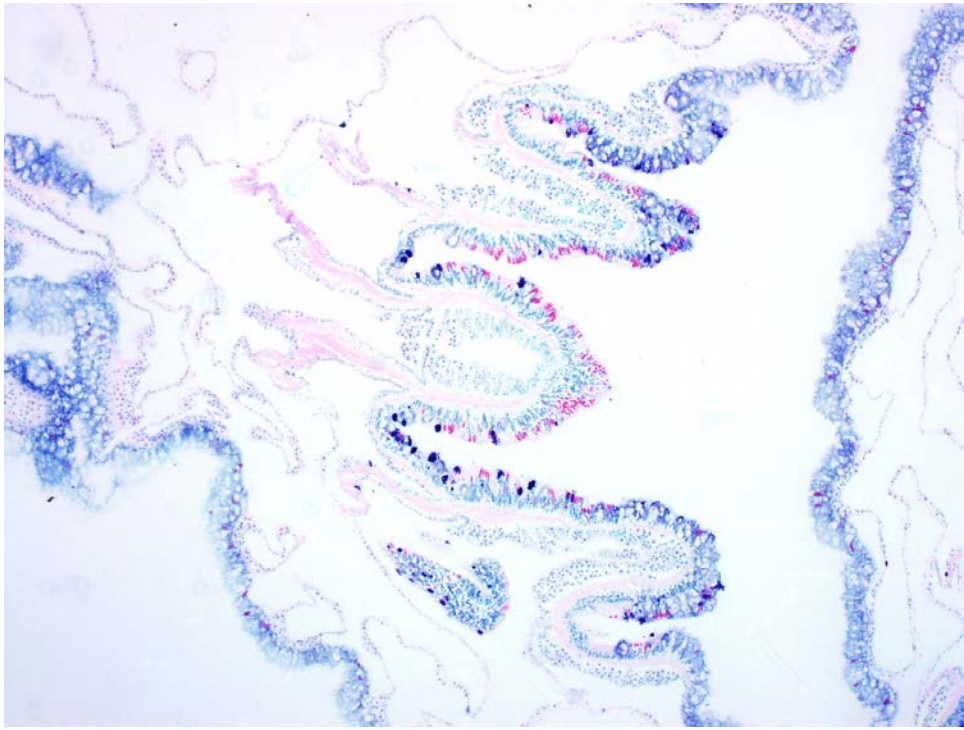
113.UnkF.C.HDD





137.176.C.HDD





Next steps

- Select all Vibrionales and Rickettsiales sequences and run these sequences through BLAST
- The data need to be analyzed at a finer taxonomic level (e.g. genus level) to look for differences between healthy and diseased communities.
- Perform laser capture microdissection on the coral tissue to isolate the *Rickettsia*-like organisms for sequencing.

Next steps

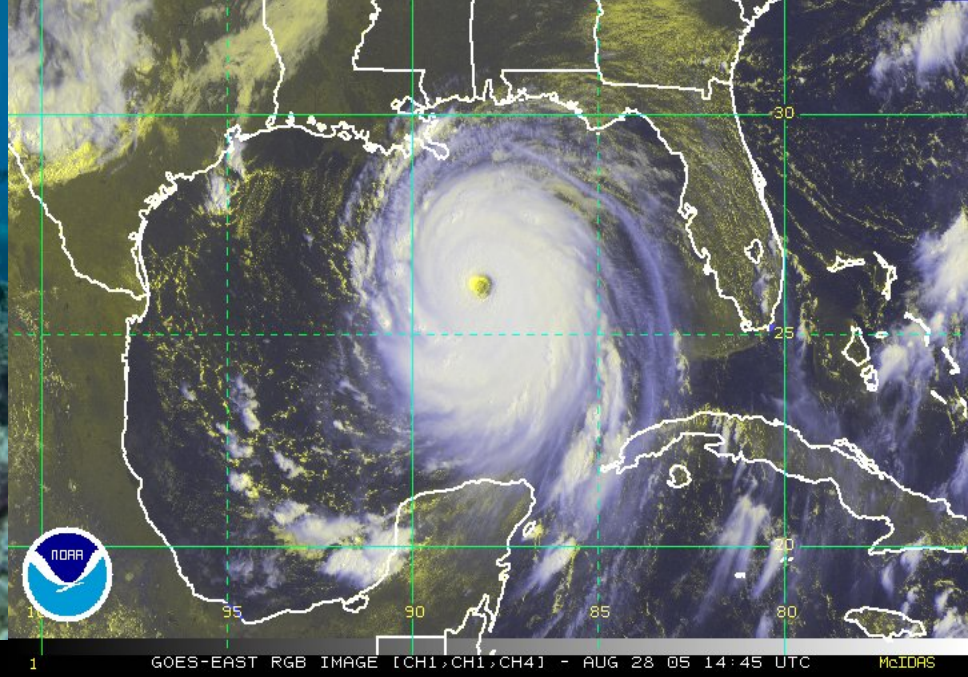
- Now that the microbial communities associated with apparently healthy and diseased *A. cervicornis* have been defined at the finest resolution to date, future studies should examine the physiology and metabolism of these OTUs.

Conclusions

- The results of taxonomic and phylogentic analyses were comparable.
- The microbial communities associated with AH coral tissue were more diverse than those of diseased tissues.
- Sediment microbial communities were unique from coral-associated bacteria.
- No correlations between marine sediment and coral tissue loss, or coral disease, could be observed using PCO analyses.

Conclusions

- The presence of marine bacteria previously associated with disease, Rickettsiales and Vibrionales, were confirmed in this study.
  - The increase in bacteria from family Vibrionaceae may serve as a biomarker of tissue-loss diseases affecting *A. cervicornis.*
  - Rickettsiales-like bacteria appear to be a potential putative pathogen of tissue-loss diseases affecting *A. cervicornis.*
- Histopathological and molecular evidence showed that apparently healthy coral tissue is abnormal, which indicates pathological changes.



Why do we care?

Acknowledgements

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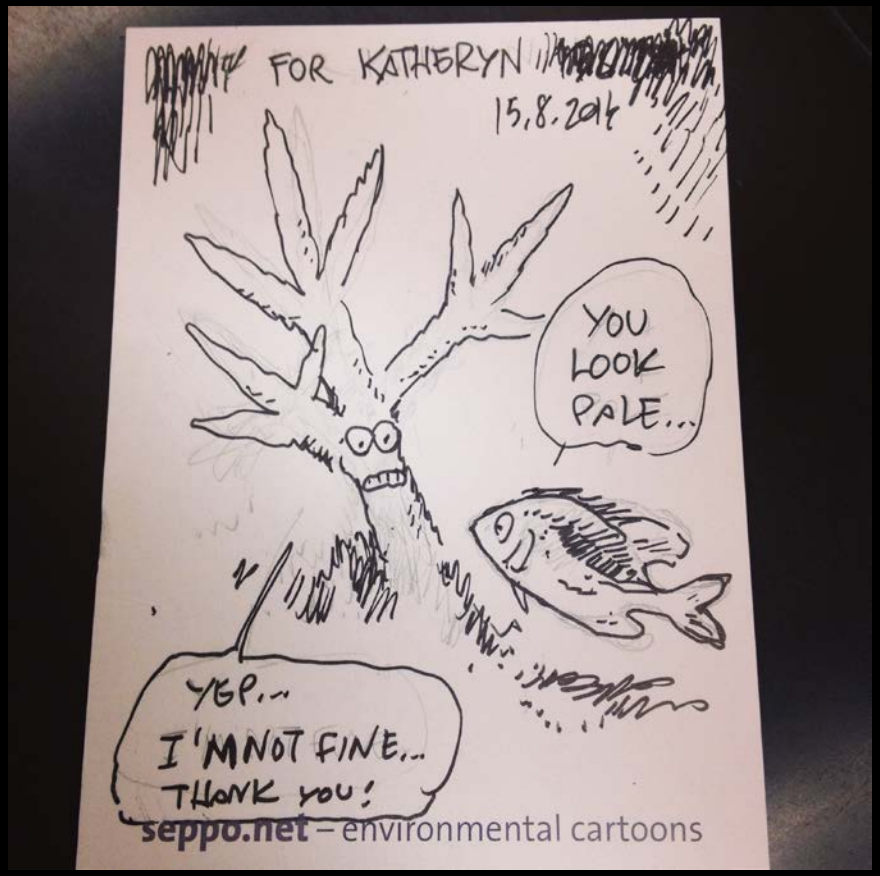
- Sampling collected under National Oceanic and Atmospheric Administration, Office of National Marine Sanctuaries permit number FKNMS-2011-032

Questions?


FOR KATHERYN
15.8.2016
YOU LOOK PALE...
YEP...
I'M NOT FINE...
THANK YOU!
seppo.net – environmental cartoons