**Supplementary Material**

**Flipping for Food: The use of a methane seep by Tanner Crabs (*Chionoecetes tanneri*)**

**Sarah Seabrook\*, Fabio C. De Leo, Andrew R. Thurber**

**\* Correspondence:** Corresponding Author

E-mail: seabroos@oregonstate.edu

**1 Supplementary Data**

**1.1 Sequencing Pipeline:**

pair\_fastqfiles:

mothur "#make.contigs(file=stability.NA072.txt, trimoverlap=T, processors=1)"

summary\_paired:

mothur "#summary.seqs(fasta=stability.NA072.trim.contigs.fasta)"

screen:

mothur "#screen.seqs(fasta=stability.\*\*.trim.contigs.fasta, group=stability.\*\*.contigs.groups, summary=stability.\*\*.trim.contigs.summary, maxambig=0, maxlength=256, minlength=245)"

summary\_screened:

mothur "#summary.seqs(fasta=stability.\*\*.trim.contigs.good.fasta)"

split\_groups:

mothur "#split.groups(fasta=stability.\*\*.trim.contigs.good.fasta, group=stability.\*\*.contigs.good.groups)"

add\_qiime\_labels:

add\_qiime\_labels.py -i qiime –m qiime/mothur\_to\_qiime\_map\_\*\*.txt -c InputFileName -o qiime

concatenate:

cat combined\_seqs.fna \

\*\*/qiime/all\_seqs.fna \

> all\_seqs.fna' -o concatenate

derep:

/local/cluster/\*USER\*/usearch -threads $(PROCS) -derep\_fulllength all\_seqs.fna -fastaout all\_seqs.derep.fasta -sizeout

cluster:

/local/cluster/\*USER\*/usearch -cluster\_otus all\_seqs.derep.fasta -otus all\_seqs.derep.cluster.fa -uparseout out3.up -minsize 2 -relabel OTU

label:

fasta\_number.py all\_seqs.derep.cluster.fa OTU > all\_seqs.derep.cluster.relabel.fasta

map:

/local/cluster/\*USER\*/usearch -usearch\_global all\_seqs.fna -db all\_seqs.derep.cluster.relabel.fasta -strand plus -id 0.97 -uc all\_seqs.derep.cluster.relabel.map.uc -threads

otu\_table:

python /\*USER\*/uc2otutab\_mod.py all\_seqs.derep.cluster.relabel.map.uc > all\_seqs\_OTU\_table.txt

convert:

biom convert -i all\_seqs\_OTU\_table.txt -o all\_seqs\_OTU\_table.biom --table-type="OTU table" --to-json

taxonomy:

assign\_taxonomy.py -m rdp -c 0.8 --rdp\_max\_memory 24000 -i all\_seqs.derep.cluster.relabel.fasta -t /\*\*/silva123\_97\_taxonomy\_7\_levels.txt -r /\*\*/silva123\_97\_otus\_16S.fasta -o rdp\_assigned\_taxonomy

addtax:

biom add-metadata -i all\_seqs\_OTU\_table.biom -o all\_seqs\_OTU\_table\_tax.biom --observation-metadata-fp rdp\_assigned\_taxonomy/all\_seqs.derep.cluster.relabel\_tax\_assignments.txt --observation-header OTUID,taxonomy --sc-separated taxonomy

convertraw:

biom convert -i all\_seqs\_OTU\_table\_tax.biom -o all\_seqs\_OTU\_table\_tax.txt --to-tsv --header-key taxonomy

removeCMEU(cyanobacteria, mitochondria, eukaryotes, unidentified[@kingdom level]:

filter\_taxa\_from\_otu\_table.py -i all\_seqs\_OTU\_table\_tax.biom -o all\_seqs\_OTU\_table\_tax\_noCMEU.biom -n \_\_Chloroplast,\_\_mitochondria,Eukaryota,Unclassified

convertremoved:

biom convert -i all\_seqs\_OTU\_table\_tax\_noCMEU.biom -o all\_seqs\_OTU\_table\_tax\_noCMEU.txt --to-tsv --header-key taxonomy

addmetadata:

biom add-metadata -i all\_seqs\_OTU\_table\_tax\_noCMEU.biom -o \*\*.biom --sample-metadata-fp metadata\_\*\*.txt

librarystats:

biom summarize-table -i all\_seqs\_OTU\_table\_tax\_noCMEU.biom -o rich\_sparse\_otu\_table\_summary.txt

align:

align\_seqs.py -i all\_seqs.derep.cluster.relabel.fasta -o all\_seqs.derep.cluster.relabel.align -t /\*\*/core\_alignment\_SILVA123.fasta

filteraligned:

filter\_alignment.py -i all\_seqs.derep.cluster.relabel.align/all\_seqs.derep.cluster.relabel\_aligned.fasta -o all\_seqs.derep.cluster.relabel.align -g 0.80 -e 0.10

tree:

make\_phylogeny.py -i all\_seqs.derep.cluster.relabel.align/all\_seqs.derep.cluster.relabel\_aligned\_pfiltered.fasta

rarefy:

single\_rarefaction.py -i all\_seqs\_OTU\_table\_tax\_noCMEU.biom -o all\_seqs\_OTU\_table\_tax\_noCMEAU\_\*\*\*\*.biom -d 16801

convertrarefied:

biom convert -i all\_seqs\_OTU\_table\_tax\_noCMEAU\_16801.biom -o all\_seqs\_OTU\_table\_tax\_noCMEU\_\*\*\*\*.txt --to-tsv --header-key taxonomy

**2 Supplementary Figures and Tables**

2.1 Supplementary Table 1

Summary of sites sampled with push cores within the Ocean Networks Canada Array System

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Site | Latitude  (N) | Longitude  (W) | Water Depth  (m) | Oxygen  (umol/L) | Temp.  (∘C) | Geologic Setting | Description | Sampled |
| Clayoquot Slope / CORK | 48°40’15” | -126°50’52” | 1250 | 19.2 | 2.85 | Continental Slope | Muddy sediments, bubbles, extensive microbial mats, clam beds (*Calyptogena spp..),* gas hydrates | 2015\*, 2016\* |
| Barkley Canyon Axis and Hydrates | 48°18’59” | -126°03’03” | 870-985 | 12.20 | 3.61 | Submarine Canyon | Microbial mats, (*Calyptogena spp..),* gas hydrates | 2015, 2016 |
| Cascadia Basin | 47°45’45” | -127°45’32” | 2660 | 72.56 | 1.78 | Abyssal plain | Fine-grained sediment | 2015 |

2.2 Supplementary Table 2

Bulk isotope data from the *Chionoecetes tanneri* specimen with tissue type indicated.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample ID | Date Collected | δ15N | δ13C | δ34S | Tissue Type |
| C\_1 | May-16 | 15.48 | -18.22 |  | Walking\_Leg |
| C\_2 | May-16 | 15.53 | -18.20 |  | Walking\_Leg |
| C\_3 | May-16 | 15.05 | -19.12 | 20.30 | Walking\_Leg |
| C\_3\_1 | May-16 | 11.82 | -18.61 |  | Gut |
| C\_3\_2 | May-16 | 13.72 | -20.05 |  | Gill |
| C\_4 | May-16 | 15.76 | -18.41 |  | Walking\_Leg |
| C\_4\_1 | May-16 | 10.70 | -18.91 |  | Gut |
| C\_5 | May-16 | 15.77 | -18.01 |  | Walking\_Leg |
| C\_5\_1 | May-16 | 12.80 | -18.07 |  | Gut |
| C\_6 | May-16 | 14.61 | -18.59 | 20.60 | Walking\_Leg |
| C\_6 | May-16 | 10.93 | -19.29 |  | Gut |
| C\_7 | May-16 | 15.15 | -18.11 | 20.24 | Walking\_Leg |
| C\_7 | May-16 | 12.59 | -18.17 |  | Gut |
| C\_7\_2 | May-16 | 13.36 | -18.99 |  | Gill |
| C\_8 | May-16 | 14.75 | -18.36 |  | Walking\_Leg |
| C\_9 | May-16 | 15.33 | -18.45 |  | Walking\_Leg |
| C\_10 | May-16 | 15.31 | -18.86 | 18.67 | Walking\_Leg |
| C\_11 | May-16 | 15.14 | -18.17 | 20.11 | Walking\_Leg |
| C\_2\_1 | May-17 | 16.06 | -17.53 |  | Walking\_Leg |
| C\_2\_2 | May-17 | 15.66 | -17.80 | 19.47 | Walking\_Leg |
| C\_2\_3 | May-17 | 15.33 | -17.83 |  | Walking\_Leg |
| C\_2\_3\_1 | May-17 | 13.57 | -19.11 |  | Gut |
| C\_2\_4 | May-17 | 15.29 | -18.12 | 17.88 | Walking\_Leg |
| C\_2\_5 | May-17 | 15.92 | -17.62 | 19.44 | Walking\_Leg |
| C\_2\_5\_1 | May-17 | 13.10 | -18.87 |  | Gut |
| C\_2\_5\_2 | May-17 | 15.50 | -18.96 |  | Gill |
| C\_2\_6 | May-17 | 14.79 | -18.58 |  | Walking\_Leg |
| C\_2\_7 | May-17 | 15.94 | -17.62 |  | Walking\_Leg |
| C\_2\_8 | May-17 | 15.19 | -17.36 |  | Walking\_Leg |
| C\_2\_8\_1 | May-17 | 13.79 | -18.74 |  | Gut |
| C\_2\_8\_2 | May-17 | 14.21 | -19.14 |  | Gill |
| C\_2\_9 | May-17 | 15.56 | -17.82 | 19.42 | Walking\_Leg |
| C\_2\_10 | May-17 | 14.69 | -18.31 | 19.35 | Walking\_Leg |
| C\_2\_11 | May-17 | 15.73 | -17.59 |  | Walking\_Leg |
| C\_2\_11\_2 | May-17 | 14.87 | -19.21 |  | Gill |
| C\_2\_12 | May-17 | 14.91 | -18.73 |  | Walking\_Leg |
| C\_2\_12\_1 | May-17 | 12.65 | -18.58 |  | Gut |
| C\_2\_12\_2 | May-17 | 13.59 | -19.72 |  | Gill |

2.3 Supplementary Table 3

Carapace width of *Chionoecetes tanneri* specimen analyzed in this study, per year.

|  |  |  |  |
| --- | --- | --- | --- |
| ID | 2015 width (cm) | ID | 2016 width (cm) |
| C1 | 8.2 | C21 | 6 |
| C2 | 8 | C22 | 6 |
| C3 | 7.5 | C23 | 5 |
| C4 | 11 | C24 | 6 |
| C5 | 8.25 | C25 | 6 |
| C6 | 7.5 | C26 | 6.2 |
| C7 | 9 | C27 | 5.5 |
| C8 | 8 | C28 | 7.2 |
| C9 | 8.25 | C29 | 6 |
| C10 | 9.5 | C210 | 6 |
| C11 | 8.5 | C211 | 6.5 |
|  |  | C212 | 6.5 |

2.4 Supplementary Table 4

Bulk isotope data from the sediment samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample ID | Date Collected | δ15N | δ13C | Site - Vertical Fraction |
| cb01 | May-16 | 5.42 | -22.15 | Clayoquot 0-1 |
| cb02 | May-16 | 5.16 | -22.06 | Clayoquot 1-2 |
| cb03 | May-16 | 6.09 | -21.75 | Clayoquot 2-3 |
| cb04 | May-16 | 5.13 | -23.03 | Clayoquot 3-5 |
| cr01 | May-16 | 5.80 | -22.14 | Clayoquot 0-1 |
| cr02 | May-16 | 5.45 | -22.56 | Clayoquot 1-2 |
| cr03 | May-16 | 5.60 | -21.87 | Clayoquot 2-3 |
| cr04 | May-16 | 5.17 | -22.21 | Clayoquot 3-5 |
| h1493 1 | May-17 | 5.99 | -22.59 | Clayoquot 0-1 |
| h1493 2 | May-17 | 5.56 | -22.77 | Clayoquot 1-2 |
| h1493 3 | May-17 | 4.58 | -23.08 | Clayoquot 2-3 |
| h1493 4 | May-17 | 4.86 | -22.89 | Clayoquot 3-5 |
| h1503 1 | May-17 | 6.36 | -22.41 | Clayoquot 0-1 |
| h1503 2 | May-17 | 6.73 | -21.65 | Clayoquot 1-2 |
| h1503 3 | May-17 | 6.95 | -21.86 | Clayoquot 2-3 |
| h1503 4 | May-17 | 6.53 | -21.23 | Clayoquot 3-5 |
| bcp1 1 | May-16 | 7.25 | -21.70 | Barkley 0-1 |
| bcp1 2 | May-16 | 7.38 | -21.81 | Barkley 1-2 |
| bcp1 3 | May-16 | 6.57 | -21.83 | Barkley 2-3 |
| bcp1 4 | May-16 | 7.89 | -22.57 | Barkley 3-5 |
| h1497 1 | May-17 | 5.66 | -22.34 | Barkley 0-1 |
| h1497 2 | May-17 | 6.93 | -23.43 | Barkley 1-2 |
| h1497 3 | May-17 | 5.23 | -22.47 | Barkley 2-3 |
| h1497 4 | May-17 | 6.64 | -22.81 | Barkley 3-5 |
| h1509 1 | May-17 | 6.03 | -22.48 | Barkley 0-1 |
| h1509 2 | May-17 | 6.43 | -22.20 | Barkley 1-2 |
| h1509 3 | May-17 | 6.36 | -22.14 | Barkley 2-3 |
| h1509 4 | May-17 | 6.54 | -22.15 | Barkley 3-5 |
| j201 | May-16 | 6.62 | -21.54 | Cascadia 0-1 |
| j202 | May-16 | 6.50 | -21.62 | Cascadia 1-2 |
| j203 | May-16 | 6.46 | -21.49 | Cascadia 2-3 |
| j204 | May-16 | 4.72 | -21.76 | Cascadia 3-5 |
| cas3 2 | May-16 | 5.35 | -21.13 | Cascadia 1-2 |
| cas3 3 | May-16 | 4.81 | -21.47 | Cascadia 2-3 |
| cas3 4 | May-16 | 5.41 | -21.45 | Cascadia 3-5 |
| cas1 01 | May-16 | 6.73 | -21.30 | Cascadia 0-1 |
| cas1 02 | May-16 | 4.88 | -21.71 | Cascadia 1-2 |
| cas1 03 | May-16 | 5.14 | -21.34 | Cascadia 2-3 |
| cas1 04 | May-16 | 5.80 | -21.85 | Cascadia 3-5 |
| cas2 01 | May-16 | 6.55 | -22.43 | Cascadia 0-1 |
| cas2 02 | May-16 | 7.31 | -21.92 | Cascadia 1-2 |
| cas2 04 | May-16 | 6.67 | -21.40 | Cascadia 2-3 |
| cas2 03 | May-16 | 3.61 | -22.52 | Cascadia 3-5 |

2.5 Supplementary Table 5

Relative abundance of bacterial taxa in the sediment that were dominant in *C. tanneri* gut contents. For other community data see Seabrook et al., 2018. Color coding represent the greater abundance of the taxa in seep sediment when compared to non-seep.

|  |  |  |  |
| --- | --- | --- | --- |
| Bacterial Taxa | Clayoquot | Barkley | Cascadia |
| Desulfobulbus | 2.118±0.237 | 3.158±0.592 | 0.007±0.012 |
| Desulfocapsa | 0.134±0.112 | 0.025±0.011 |  |
| Methylococcales | 0.119±0.024 | 0.052±0.035 | 0.001±0.007 |
| SEEP-SRB1 | 0.611±0.506 | 0.172±0.038 | 0.036±0.021 |
| SEEP-SRB2 |  |  |  |
| SEEP-SRB4 | 0.011±0.011 | 0.003±0.003 |  |
| Sulfurovum | 0.269±0.267 | 0.014±0.011 | 0.003±0.014 |
| Thiotrichales | 0.026±0.026 | 0.007±0.001 | 0.001±0.007 |
| Entomoplasmatales | 0.003±0.003 | 0.010±0.002 | 0.004±0.008 |
| Flavobacteriaceae | 0.077±0.032 | 0.141±0.044 | 0.097±0.088 |
| Fusobacteriaceae | 0.042±0.014 | 0.125±0.062 | 0.002±0.007 |
| Mycoplasmatales |  |  |  |
| Acidimicrobiales | 0.271±0.095 | 0.187±0.016 | 0.196±0.223 |
| Alteromonadales | 0.019±0.004 | 0.071±0.011 | 0.055±0.088 |
| Rhodobacteraceae | 0.114±0.014 | 0.221±0.132 | 0.126±0.042 |
| Rubritalea | 0.066±0.036 | 0.028±0.017 | 0.108±0.337 |
| 100% greater | 50% greater | 25% greater | 10% greater |

2.6 Supplementary Table 6

Relative abundance of archaeal taxa in the sediment that were dominant in *C. tanneri* gut contents. For other community data see Seabrook et al., 2018. Color coding represent the greater abundance of the taxa in seep sediment when compared to non-seep.

|  |  |  |  |
| --- | --- | --- | --- |
| Archaeal Taxa | Clayoquot | Barkley | Cascadia |
| ANME-1 | 0.023±0.023 | 0.119±0.072 | 0.018±0.062 |
| ANME-1b |  | 0.145±0.145 |  |
| ANME-2c |  |  |  |
| Thaumarchaeota | 10.413±3.872 | 5.715±1.529 | 57.914±60.125 |
| Woesearchaeota | 39.974±6.342 | 44.461±2.060 | 27.971±26.320 |
| 100% greater | 50% greater | 25% greater | 10% greater |

2.7 Supplementary Table 7

SIMPER results for *C. tanneri* gut contents and seep sediment.

|  |  |  |
| --- | --- | --- |
| SIMPER: Crab gut contents and seep sediment  Mean dissimilarity per OTU: 0.04, Total OTU’s: 1847  Top 10 contributors to 94.33% average dissimilarity: | | |
| Phylum\_Order | Contribution (%) to dissimilarity | Number of OTU's within Order |
| Proteobacteria\_Desulfobacterales | 4.88 | 107 |
| Proteobacteria\_Sh765B-TzT-29 | 4.25 | 90 |
| Proteobacteria\_Xanthomonadales | 2.75 | 41 |
| Proteobacteria\_DTB120 | 1.45 | 26 |
| Chloroflexi\_Anaerolineales | 1.27 | 35 |
| Tenericutes\_Mycoplasmatales | 1 | 4 |
| Planctomycetes\_Brocadiales | 0.85 | 14 |
| Chlorobi\_Ignavibacteriales | 0.57 | 10 |
| Tenericutes\_Entomoplasmatales | 0.38 | 2 |
| Proteobacteria\_Syntrophobacterales | 0.27 | 2 |

2.8 Supplementary Table 8

SIMPER results for *C. tanneri* gut contents and non-seep sediment.

|  |  |  |
| --- | --- | --- |
| SIMPER: Crab gut contents and non-seep sediment  Mean dissimilarity per OTU: 0.04, Total OTU’s: 1616  Top 10 contributors to 95.67% average dissimilarity: | | |
| Phylum\_Order | Contribution (%) to dissimilarity | Number of OTU's within Order |
| Proteobacteria\_Sh765B-TzT-29 | 5.32 | 107 |
| Proteobacteria\_Xanthomonadales | 3.34 | 38 |
| Planctomycetes\_Brocadiales | 1.13 | 12 |
| Proteobacteria\_Chromatiales | 1.03 | 13 |
| Tenericutes\_Mycoplasmatales | 0.98 | 4 |
| Gemmatimonadetes\_BD2-11terrestrialgroup | 0.89 | 16 |
| Acidobacteria\_Subgroup21 | 0.58 | 7 |
| Tenericutes\_Entomoplasmatales | 0.37 | 2 |
| Fusobacteria\_Fusobacteriales | 0.24 | 1 |
| Proteobacteria\_Nitrosomonadales | 0.23 | 2 |

2.9 Supplementary Table 9

SIMPER results for *C. tanneri* gut walls and seep sediment.

|  |  |  |
| --- | --- | --- |
| SIMPER: Crab gut walls and seep sediment  Mean dissimilarity per OTU: 0.04, Total OTU’s: 1879  Top 10 contributors to 96.55% average dissimilarity: | | |
| Phylum\_Order | Contribution (%) to dissimilarity | Number of OTU's within Order |
| Proteobacteria\_Desulfobacterales | 4.87 | 107 |
| Proteobacteria\_Sh765B-TzT-29 | 4.34 | 93 |
| Proteobacteria\_Xanthomonadales | 2.94 | 43 |
| Proteobacteria\_DTB120 | 1.47 | 26 |
| Chloroflexi\_Anaerolineales | 1.36 | 42 |
| Tenericutes\_Mycoplasmatales | 1.17 | 4 |
| Proteobacteria\_Myxococcales | 1.1 | 44 |
| Planctomycetes\_Brocadiales | 0.86 | 14 |
| Chlorobi\_Ignavibacteriale | 0.58 | 10 |
| Proteobacteria\_Syntrophobacterales | 0.28 | 2 |

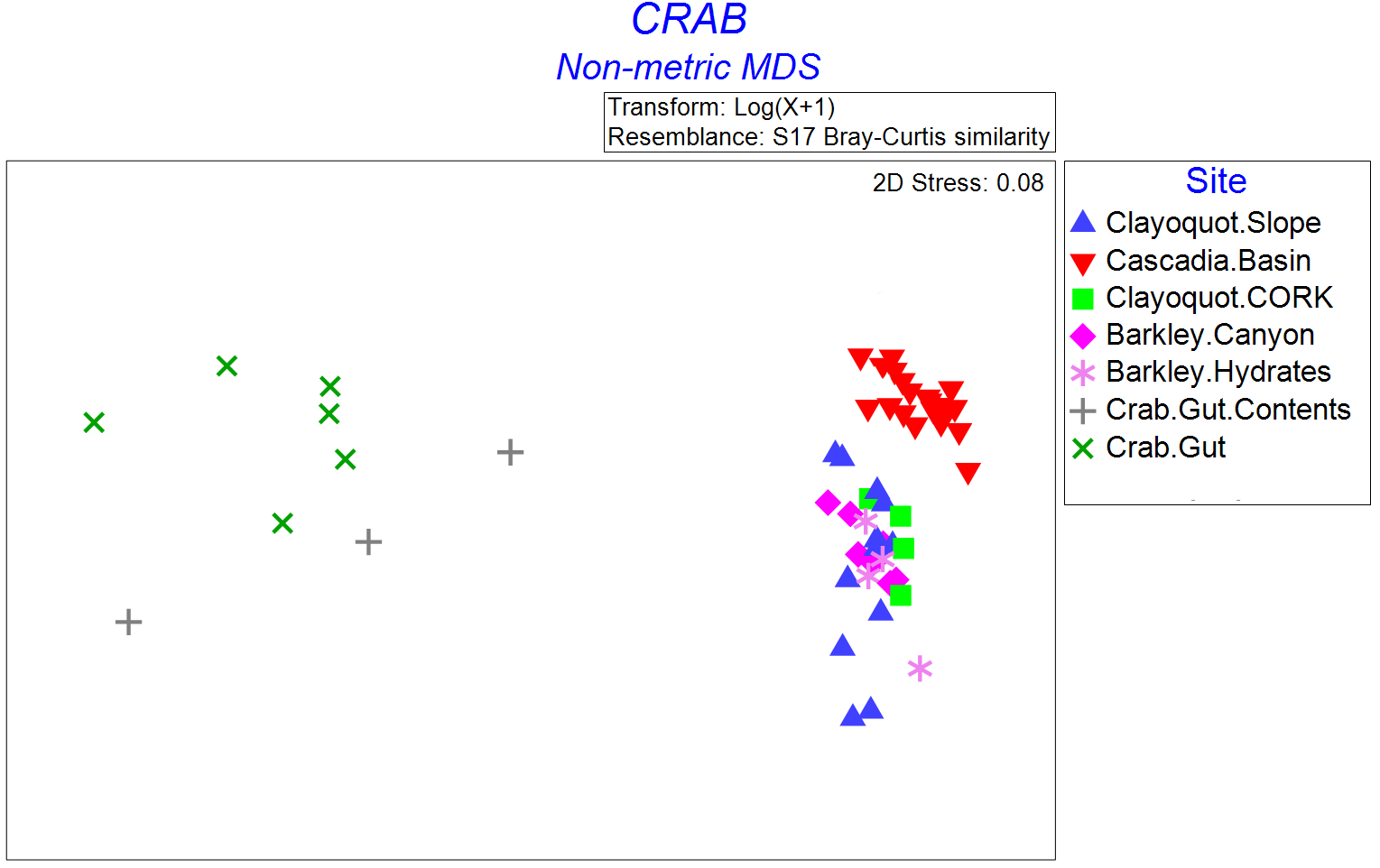
2.10 Supplementary Table 10

SIMPER results for *C. tanneri* gut walls and non-seep sediment.

|  |  |  |
| --- | --- | --- |
| SIMPER: Crab gut walls and non-seep sediment  Mean dissimilarity per OTU: 0.04, Total OTU’s: 1615  Top 10 contributors to 97.09% average dissimilarity: | | |
| Phylum\_Order | Contribution (%) to dissimilarity | Number of OTU's within Order |
| Proteobacteria\_Sh765B-TzT-29 | 5.54 | 109 |
| Proteobacteria\_Rhodospirillales | 4.7 | 96 |
| Proteobacteria\_Xanthomonadales | 3.58 | 40 |
| Tenericutes\_Mycoplasmatales | 1.16 | 4 |
| Planctomycetes\_Brocadiales | 1.14 | 12 |
| Proteobacteria\_Chromatiales | 1.08 | 13 |
| Gemmatimonadetes\_BD2-11terrestrialgroup | 0.93 | 19 |
| Acidobacteria\_Subgroup21 | 0.66 | 9 |
| Proteobacteria\_Nitrosomonadales | 0.22 | 1 |
| Proteobacteria\_Syntrophobacterales | 0.2 | 1 |

2.11 Supplementary Figure 1

Nonmetric multidimensional scaling plot showing the oridination of the microbial community present within all seep (Clayoquot and Barkley) and non-seep (Cascadia) sediment and gut content (crab.gut.contents) and gut wall (crab.gut) samples. The axis are meaningless, the ordination of the points in 2-D space illustrate the similarity and dissimilarity between samples. Stress value indicated (accepted if stress value is <0.17).



2.12 Supplementary Figure 2

Cluster diagram representing the similarity of the seep (Clayoquot and Barkley) and non-seep (Cascadia) sediment and gut content (crab.gut.contents) and gut wall (crab.gut) samples, with percentage similarity shown on the y-axis.

