Supplementary Material

Sampling and processing methods impact microbial community structure and potential activity in a seasonally anoxic fjord: Saanich Inlet, British Columbia

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1. Background

Over the past twenty years a wide range of sample collection and processing methods has been used to generate multi-omic (DNA, RNA, protein and metabolite) data sets without standardization. Here we briefly compiled some literature sample collection and processing methods used in marine systems including OMZs. We show a timeline (1996-2015) as reference of the filtration strategies commonly used to generate multi-omic information to be used in the field of marine molecular microbial ecology (**Supplementary Fig. 1A**). Of note the wide range of pore sizes in the filters used regardless the objective of biomass collection (**Supplementary Fig. 1B**). This compilation represents a literature framework from which we can extract useful information to better design collection and processing methods in marine molecular microbial ecology that are reproducible and comparable among environments.

2. Results

Saanich Inlet microbial community monitoring time-series

SSU rRNA gene amplicon sequencing (pyrotags) was used to describe Saanich Inlet microbial community structure along water column (oxygen) O_2 gradients between 2006 to 2011. Resulting data sets revealed consistent microbial community partitioning into discrete clusters associated with oxic (>90 μM O_2), dysoxic (90-20 μM O_2)/ suboxic (20-1 μM O_2) and anoxic (<1μM) water column conditions (**Supplementary Fig. 2**). This pattern is commonly observed in stratified ecosystems where O_2 deficiency is associated with redox-driven niche partitioning among and

between microorganisms (Alldredge and Cohen, 1987;Shanks and Reeder, 1993;Wright et al., 2012). The core microbial community or abundant "biosphere" (relative abundance >1%) (Rappé and Giovannoni, 2003) consisted of operational taxonomic units (OTUs) affiliated with SAR11, SAR324, Nitrospina, SUP05, and Marine Group A within the bacterial domain, and Thaumarchaeota within the archaeal domain (Zaikova et al., 2010;Walsh and Hallam, 2011). Because of their abundance and distinctive metabolisms these microbial groups have been posited to play integral roles in coupled carbon, nitrogen and sulfur cycling in marine OMZs and other oxygen depleted waters (Field et al., 1997;Fuhrman and Davis, 1997;Rappé and Giovannoni, 2003;Brown and Donachie, 2007;Tripp et al., 2008;Lam et al., 2009;Walsh et al., 2009;Zaikova et al., 2010;Walsh and Hallam, 2011;Hawley et al., 2017).

2.2 Filtering conditions effect on microbial community diversity

To investigate the effect of different filter combinations, filtration methods and sample volumes on microbial diversity we evaluated both the alpha and Shannon indices for 29 SSU rDNA pyrotag samples from 165 and 185m. Diversity values changed in relation to filtration timing (*in situ* vs. on ship) and filter pore size. Overall, 0.4 µm *in situ* samples showed higher alpha diversity values (≥150) than those collected from bottles and filtered on ship, regardless of sampling depth. However, Shannon diversity values were evenly distributed among samples, ranging from 4 to 5, for 0.4 and 2.7 µm pre-filtered samples regardless of filtration time or sampling depth (**Supplementary Fig. 3**). Filtered water volume had a limited effect on diversity values between 165 and 185 m depth intervals and Friedman block test results indicated that sample volume differences in community structure were not significant. However, qualitative differences were observed in samples from 165 m when <1 L was filtered through on-ship 0.22 µm filters with in-line 0.4 µm pre-filters. These samples tended to have higher diversity than 1.5 or 2 L filter volumes. Samples from 185 m showed a similar effect when <2 L was filtered through on-ship 0.22 µm filters with in-line 2.7 µm pre-filters.

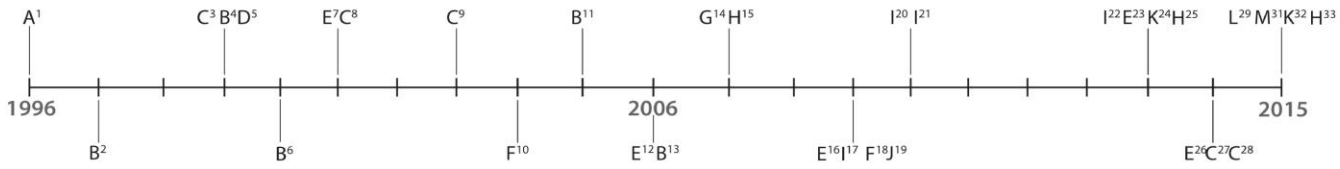
Community richness estimates based on count data for selected *in situ* and on-ship samples (**Supplementary Table 2; Supplementary Fig .4**) indicate that on-ship processed samples have fewer number of OTUs than *in situ* samples. This pattern is consistent with the idea that current bottle collection methods impact community

structure by decreasing the number of particle-associated community members due to a combination of particle settling and turbulence associated with collection and filtration processing steps.

2.0 Supplementary Figures and Table

2.1 Supplementary Figures

A



B

Letter ID	Pre-filter 1 (μm)	Pre-filter 2 (μm)	End filter (μm)
A	10		0.22
B	1		0.22
C	3		0.22
D	10	3	0.22
E	1.6		0.22
F	0.8		0.22
G			2.7
H	3	0.8	0.22
I	2.7		0.22
J	60	1	0.22
K			0.22
L	30	1.6	0.22
M	20-3	3-0.22	

Number of studies 1 3 5

Figure 1. A) Sample collection and processing methods used over a decade (1996-2015) for the generation of multi-omic datasets to determine microbial community structure, function and activity in relation to physical, chemical and biological oceanographic processes. Letters (A-M) indicate filtration strategy used, and numbers (1-33) indicate reference survey ((1) Stein et al., 1996, (2) Massana et al., 1997, (3) Acinas et al., 1999, (4) Murray et al., 1999, (5) Crump et al., 1999, (6) Hollibaugh et al., 2000, (7) Suzuki et al., 2001 , (8) Moeseneder et al., 2001, (9) LaMontagne and Holden, 2003, (10) Venter et al., 2004, (11) DeLong et al., 2006, (12) Hewson and Fuhrman, 2006, (13) DeLong et al., 2006, (14) Waidner and Kirchman, 2007, (15) Rusch et al., 2007, (16) Brown et al., 2009, (17) Walsh et al., 2009, (18) Ghiglione et al., 2009, (19) Kellogg and Deming, 2009, (20) Seth et al., 2009, (21) Zaikova et al., 2009, (22) Ganesh et al., 2013, (23) Hurwitz et al., 2013, (24) Rodriguez-Mora et al., 2013, (25) Smith et al., 2013, (26) Parris et al., 2014, (27) D'Ambrosio et al., 2014, (28) Mohit et al., 2014, (29) Ganesh et al., 2015, (30) Padilla et al., 2015, (31) Brum et al., 2015, (32) Pesant et al., 2015, (33) Orsi et al., 2015). **B)** Description of filtration

strategy (A-M) indicating filters combination and size pore. Bars depict the total number of surveys following each filtration strategy.

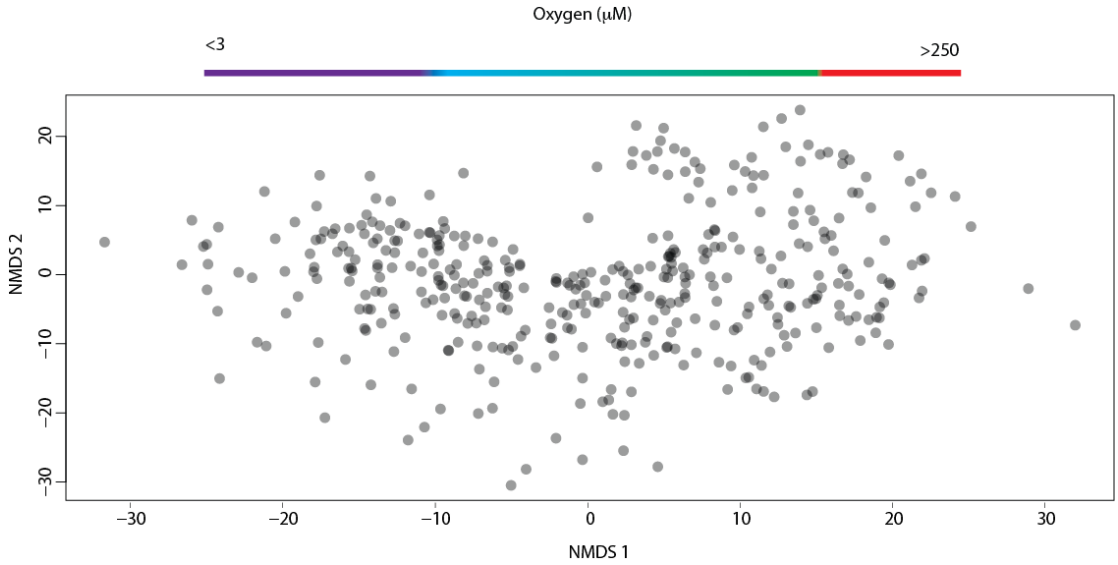


Figure 2. Non-metric multidimensional scaling plot for Saanich Inlet Time-Series SSU rDNA amplicon sequences between 2006-2011 based on Manhattan distance (1000 iterations). Oxygen gradient from oxic (>250 µM; red) to anoxic (<3 µM; purple) is shown on top and pyrotag samples are depicted as gray dots.

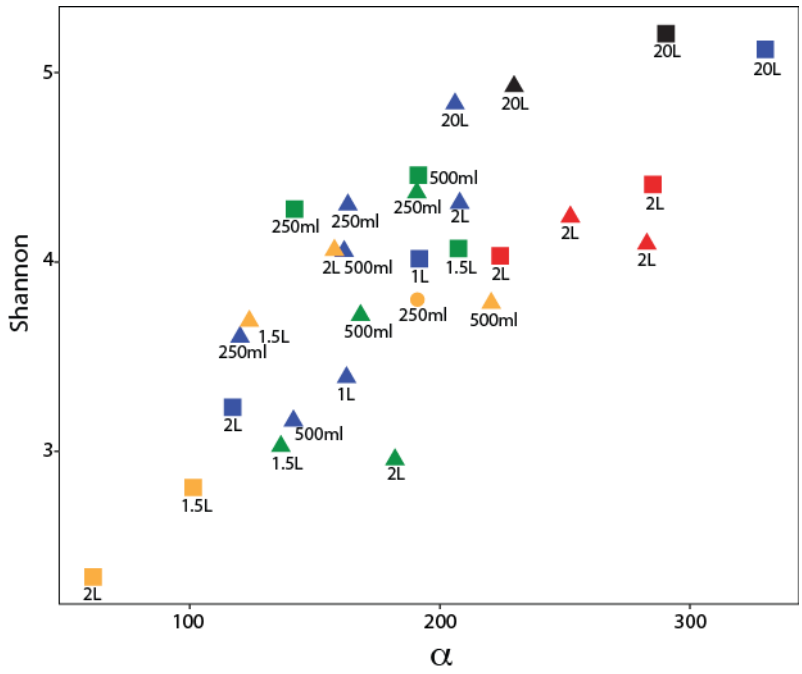


Figure 3. Shannon and alpha (α) diversity indexes for SSU rDNA amplicon sequences generated from 165 (square) and 185 (triangle) m samples collected during the SCOR workshop. Filtering conditions used include PPS *in situ* 0.4 µm (red) and MasterFlex peristaltic pump (MPP) on-ship including 0.4 µm (green), 0.22 µm with in-line 0.4 µm prefilters (yellow), 0.22 µm with in-line 2.7 µm prefilters (blue), and time-series 0.22 µm (black).

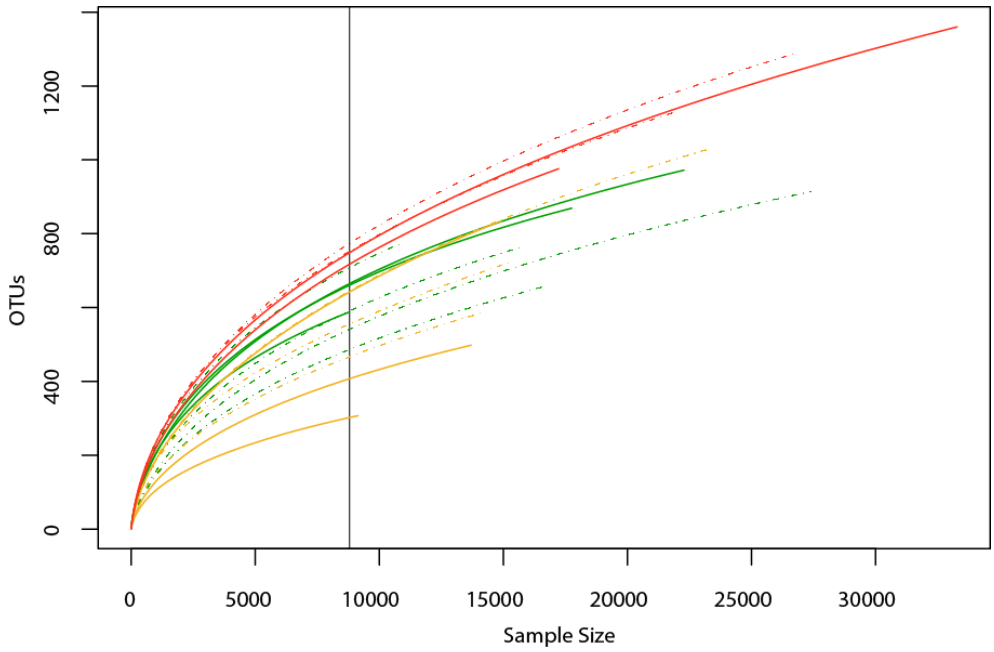


Figure 4. Richness estimates based on count data indicated that overall on-ship processed samples had fewer OTUs than *in situ* samples. Filtering conditions used include PPS *in situ* 0.4 µm (red), MPP on-ship 0.4 µm (green), 0.22 µm with in-line 0.4 µm prefilters (yellow) on 165 m (solid) and 185 m (dashed) water samples.

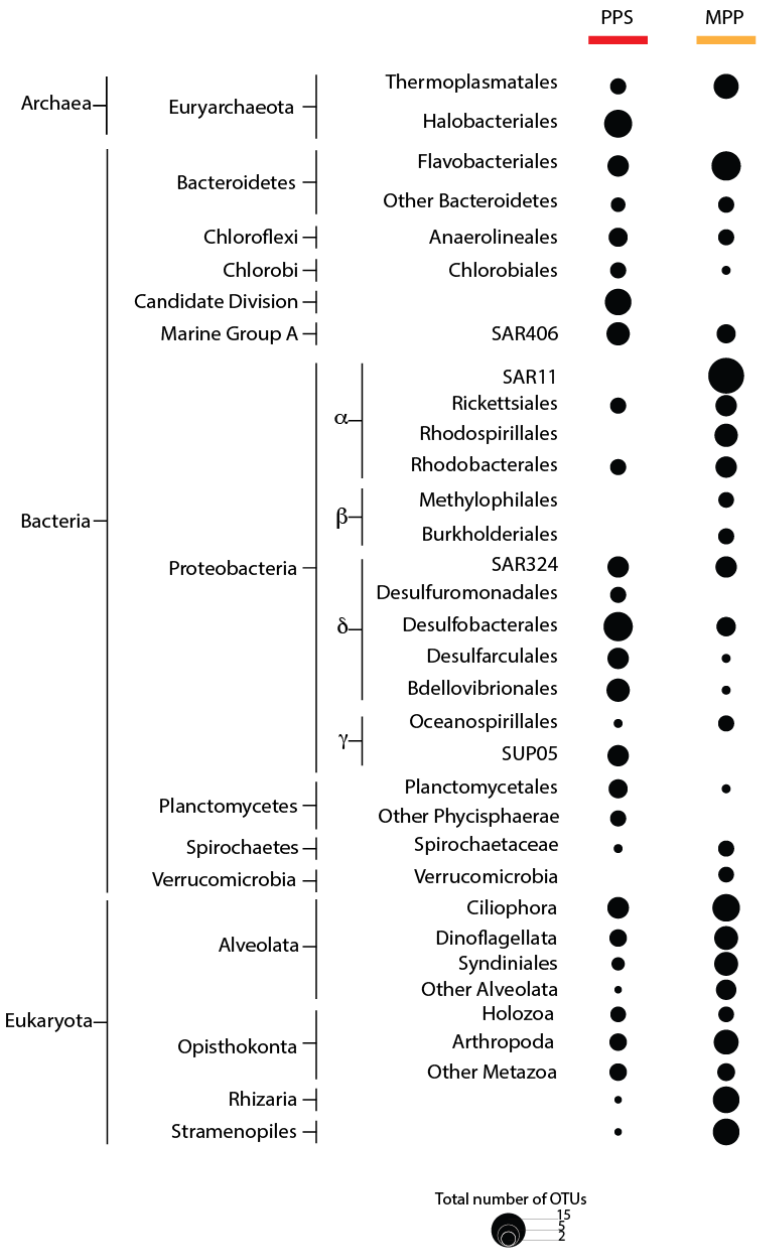


Figure 5. Active microbial community composition based on rRNA:rDNA ratio (>1) for PPS *in situ* 0.4 µm (red) and MPP on-ship 0.22 µm with in-line 0.4 µm prefilters (yellow). The size of dots depicts the total number of OTUs affiliated with a specific taxonomic group.

2.2 Supplementary tables and legends

Supplementary Table 1. Sample size (number of reads and OTUs) and diversity indexes (Shannon and alpha (α)) for SSU rDNA amplicon samples generated during the SCOR workshop. Samples from 165 and 185m are divided by filtering conditions including, PPS *in situ* (0.4μm) and MPP on-ship 0.4μm and 0.22 μm with in-line 0.4 μm prefilters.

Sample			Number of reads	Number of OTUs	α	Shannon
In situ (0.4 μm)	165	(A) 2	17229	976	224.10	4.03
		(B) 2	33287	1360	285.22	4.41
	185	(A) 2	21836	1128	252.20	4.25
		(B) 2	26890	1291	282.79	4.10
0.4 μm	165	0.25	8800	588	141.92	4.28
		0.5	17756	869	191.37	4.46
		1.5	22278	972	207.44	4.07
	185	0.25	10952	776	190.79	4.37
		0.5	15883	767	168.28	3.73
		1.5	16585	656	136.42	3.03
		2	27403	914	182.04	2.96
0.4 μm + 0.2 μm	165	0.25	14931	835	191.01	3.80
		1.5	13703	498	101.34	2.81
		2	9142	307	61.25	2.34
	185	0.5	23321	1030	220.53	3.79
		1.5	14299	589	123.79	3.69
		2	15347	724	157.82	4.07

Supplementary Table 2. Water column chemical properties during SCOR workshop on July14th, 2014. Oxygen (O2), Phosphate (PO^{-3}_4), Silicic acid (SiO_2), Nitrate (NO^-_3), Nitrite (NO^-_2), Ammonium (NH^+_4) and Hydrogen Sulfide (H_2S) concentrations (µM) for 150, 165 and 185 m depth intervals.

Depth (m)	**O_2 (µM)***	**PO^{-3}_4 (µM)**	**SiO_2 (µM)**	**NO^-_3 (µM)**	**NO^-_2 (µM)**	**NH^+_4 (µM)**	**H_2S (µM)**
150	3.803	4.576	86.007	11.952	0.639	0.094	0.000
165	2.355	5.116	90.789	2.311	0.000	3.969	8.139
185	1.923	5.890	108.296	NaN**	0.000	6.108	13.950

**Cadmium column closed

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