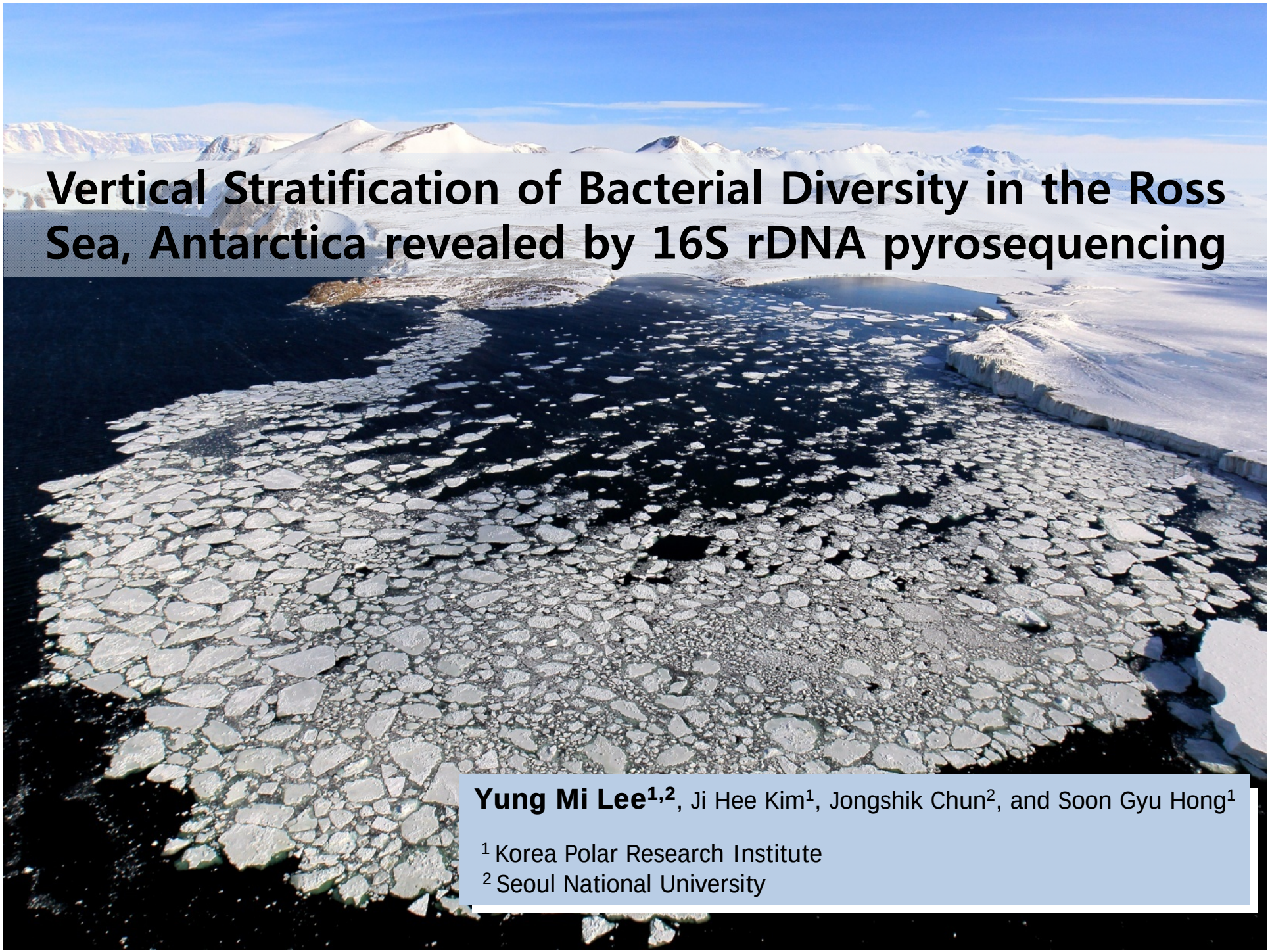


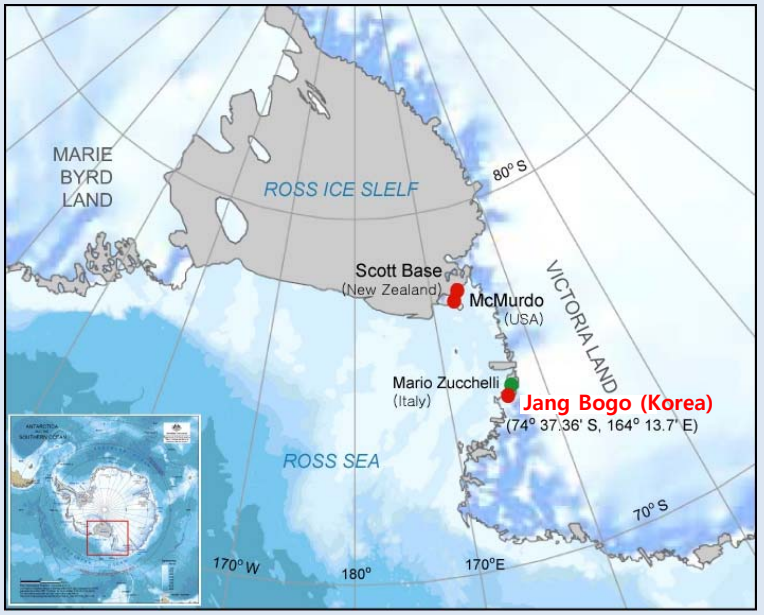
Vertical Stratification of Bacterial Diversity in the Ross Sea, Antarctica revealed by 16S rDNA pyrosequencing

Yung Mi Lee[1,2], Ji Hee Kim[1], Jongshik Chun[2], and Soon Gyu Hong[1]

[1] Korea Polar Research Institute
[2] Seoul National University

Ross Sea

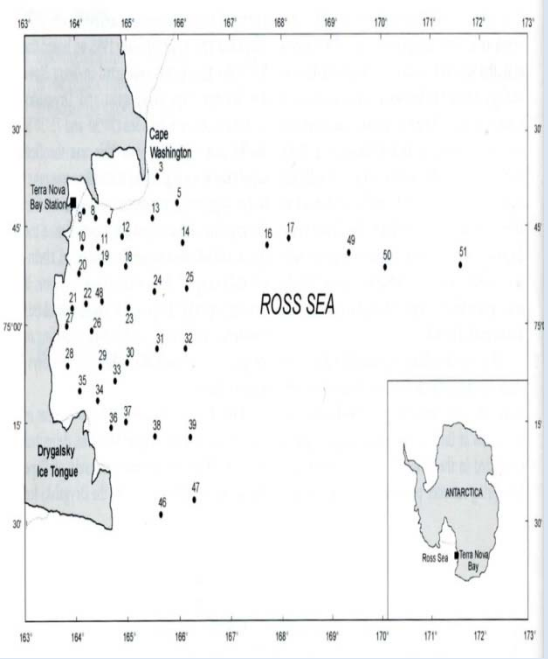
- Jang Bogo Station will be constructed in Terra Nova Bay (2012-2014)

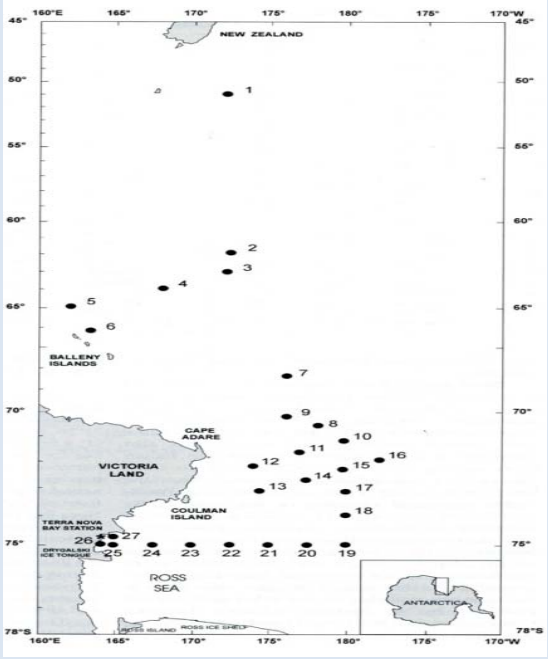


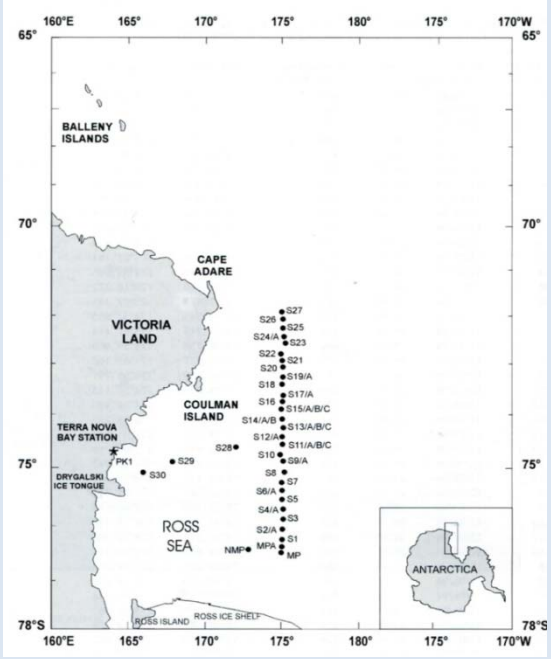
- One of the most productive regions in the Antarctica (Arigo *et al.* 1998)
- Represents a unique habitat
- One of the places of extensive bottom water formation in the Antarctica.

Previous studies in the Ross Sea

1987-1988 → 1989-1990 → 1994-1995







Ross Sea Ecology (2000)

Marine Bacteria

- **Number and biomass of marine prokaryotes** (Whitman *et al.* 1998)

Environment	No. of prokaryotic cells ($X10^{28}$)	Pg of C in prokaryotes
Aquatic habitats	12	2.2
Oceanic subsurfaces	**355 (55%~87%)**	**303**
Soil	26	26
Terrestrail subsurfaces	25-250	22-215
Total	**415-640**	**353-546**

- The **vast metabolic diversity of marine microorganisms** enables them to play key roles in **marine food webs** and is responsible for the **nutrients cycling** (Suhelen *et al*. 2008)

Previous studies in the Ross Sea

▣ Bacterial diversity study

- **Biodiversity of cultivable psychrotrophic marine bacteria** isolated from Terra Nova Bay (Ross Sea, Antarctica) (2004)
- **Bacterial and viral abundance in Ross Sea** summer pack ice communities (2004)
- **Crude oil-induced structural shift of coastal bacterial communities** of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria (2004)
- Study of **bacterial communities in Antarctic coastal waters** by a combination of 16S rRNA and 16S rDNA sequencing (2006)

▣ Biotechnological application study

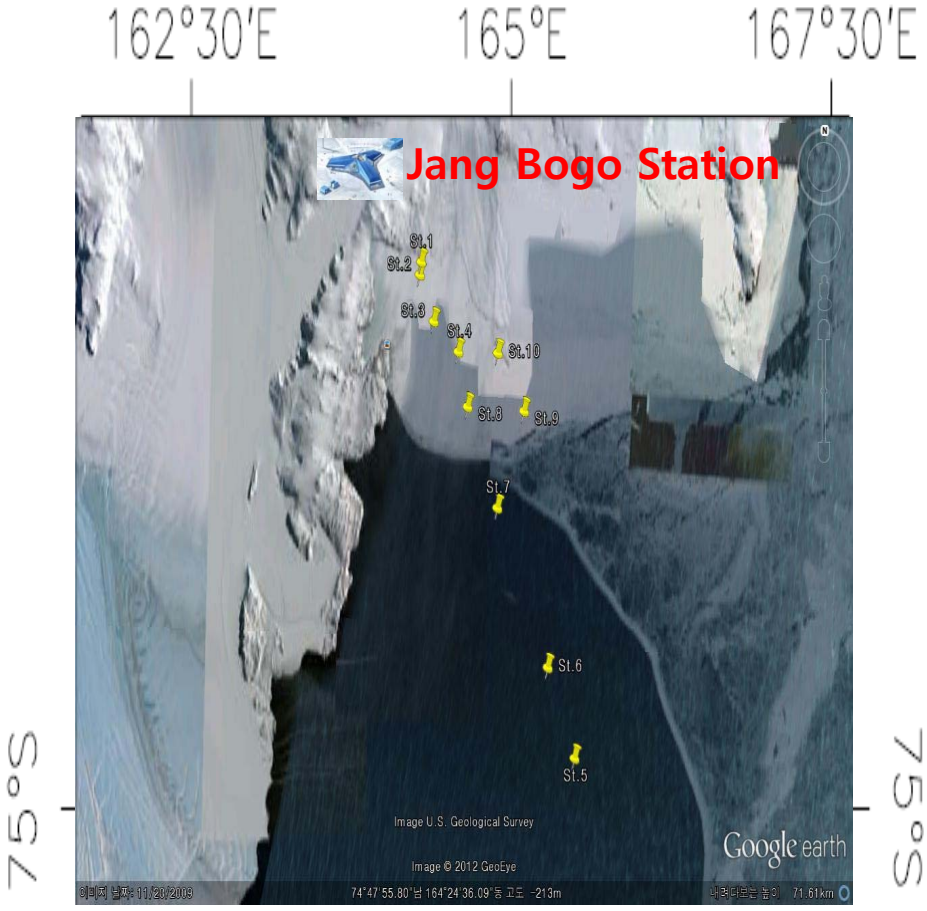
- **Diesel oil and PCB-degrading psychrotrophic bacteria** isolated from Antarctic seawaters (Terra Nova Bay, Ross Sea) (2004)
- **Lipolytic activity** of Antarctic cold-adapted marine bacteria (Terra Nova Bay, Ross Sea) (2006)
- **Detection, expression and quantification of the biodegradative genes** in Antarctic microorganisms using PCR (2010)

Objectives of this study

1. Spatial distribution of physicochemical properties and bacterial community structure in the Ross Sea

2. Correlation of physicochemical properties with bacterial communities

Sampling



Googleearth.com

- From February 6th to 12th, 2011
- Ten stations using Niskin bottles

Depth (m)	Note
0	In all stations
10	
20	
30	
50	
75	
100	
150	
200	
.	100m interval upto bottom depth
.	
.	

Methods

Collection of seawater using Niskin bottles

Microbial community analysis

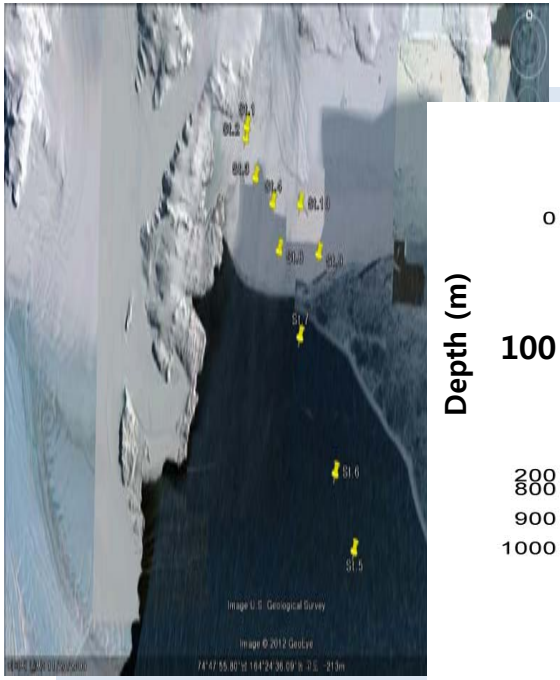
- Filtration (3 μm cellulose acetate)
- Filtration (0.2 μm polyethersulfone)
- Storage (-80℃)
- gDNA Extraction
- PCR and Pyrosequencing
- Trimming by PyroTrimmer
- Clustering by TBC
- Taxonomic assignment by Ez-Taxon E
- PCA analysis

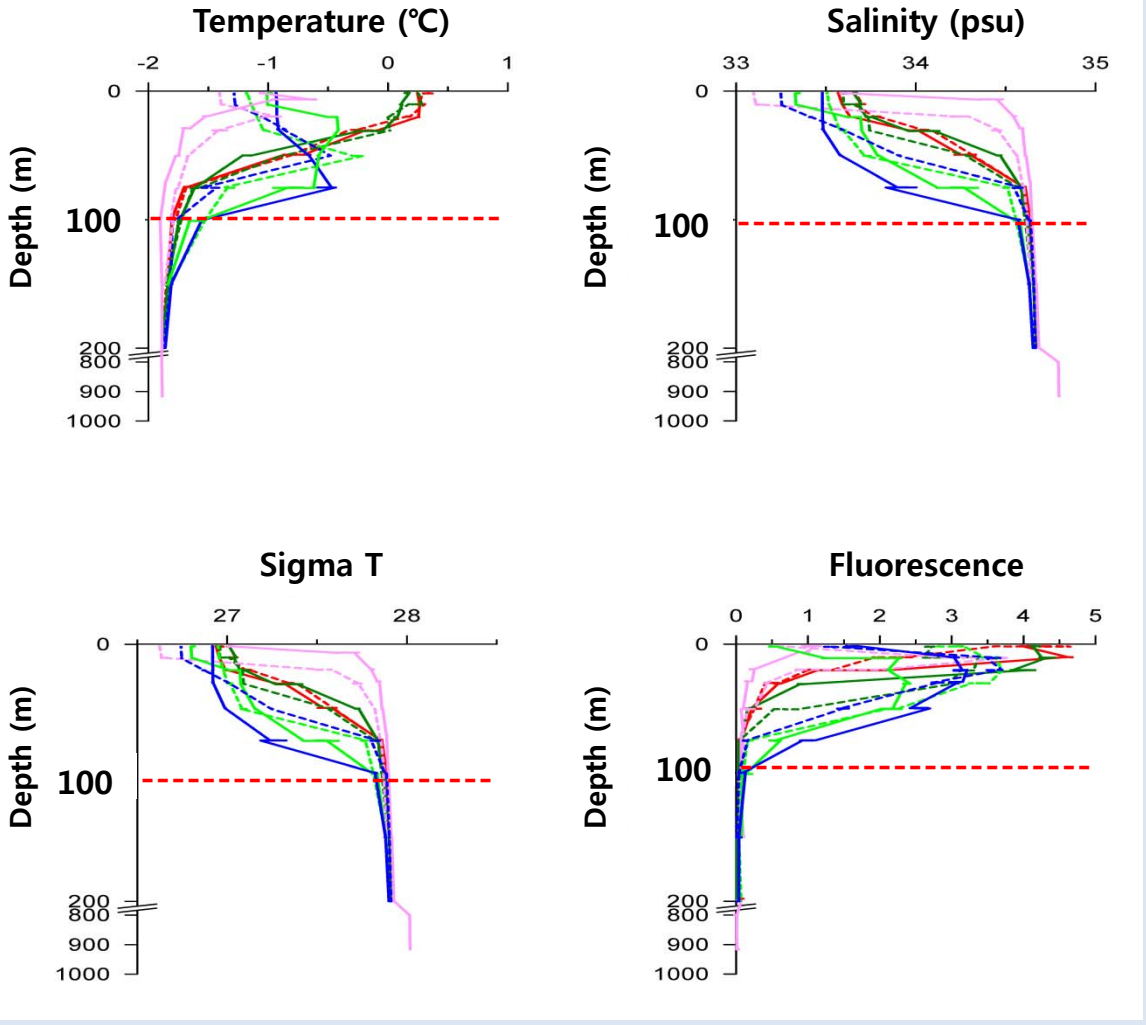
Nutrient analysis

- No treatment
- Storage (-20℃)
- PO_4^{3-}, NO_2^-, NO_3^-, NH_4^+, Si

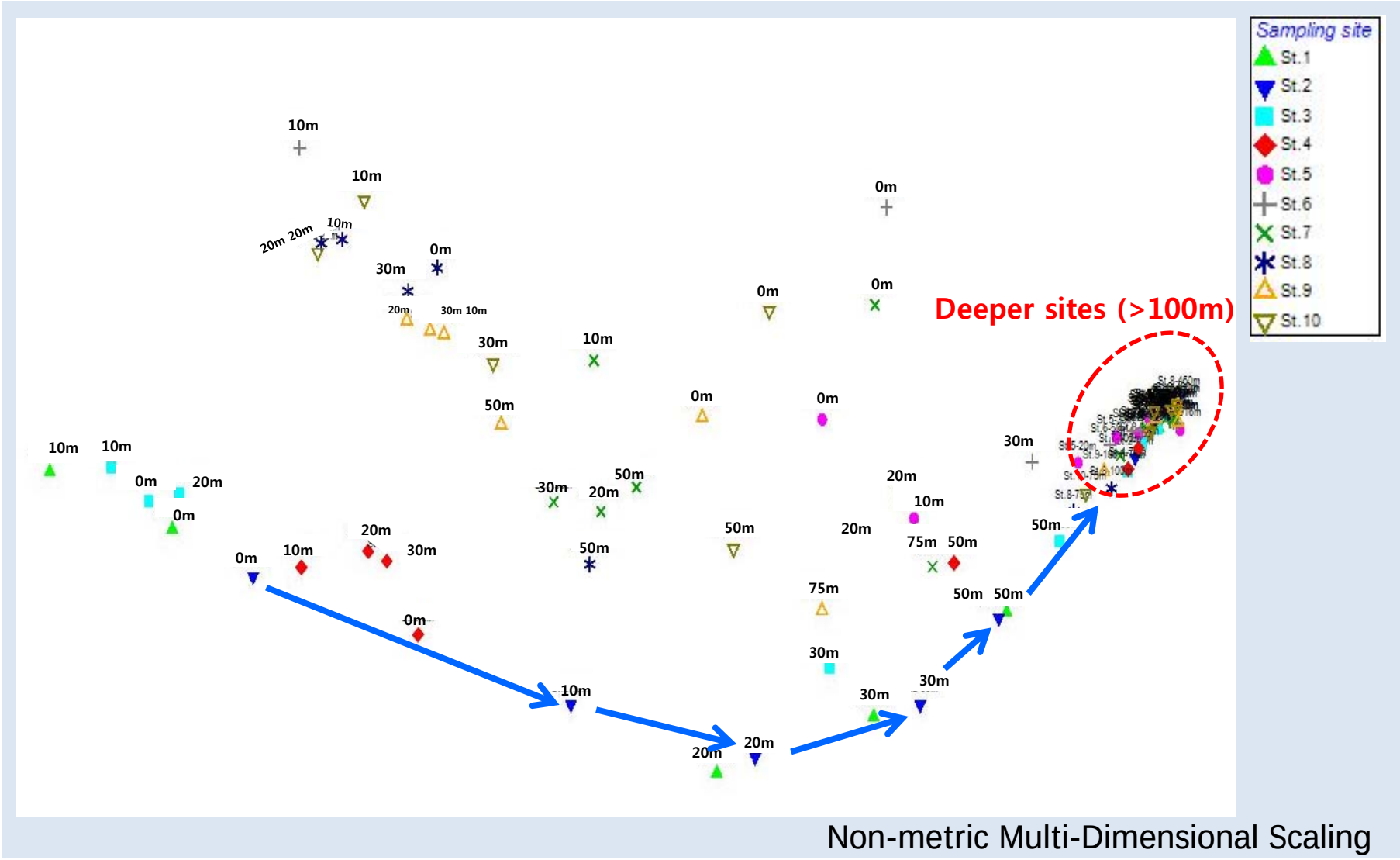
Spatial distribution of physicochemical properties and bacterial community structure

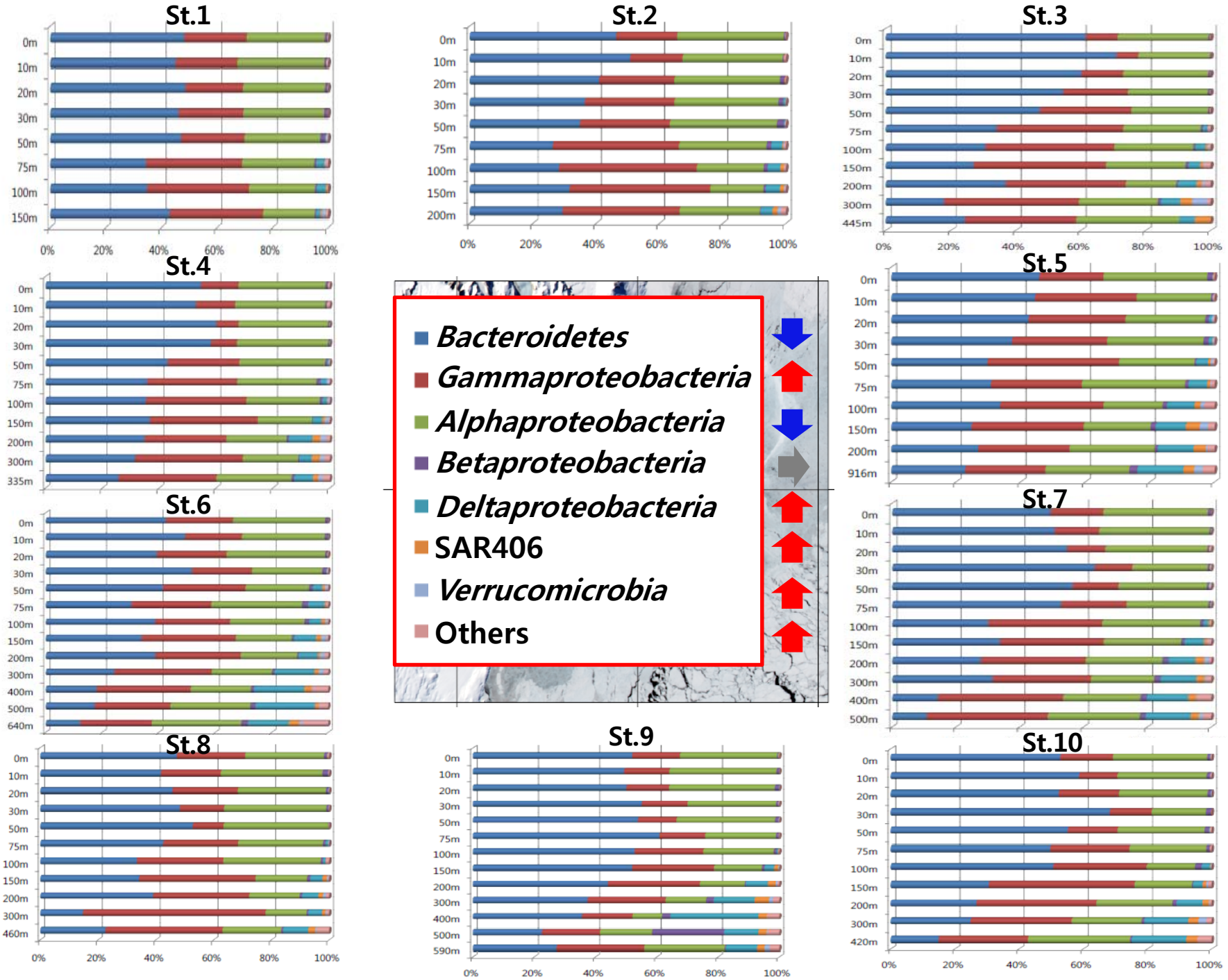
Physicochemical properties





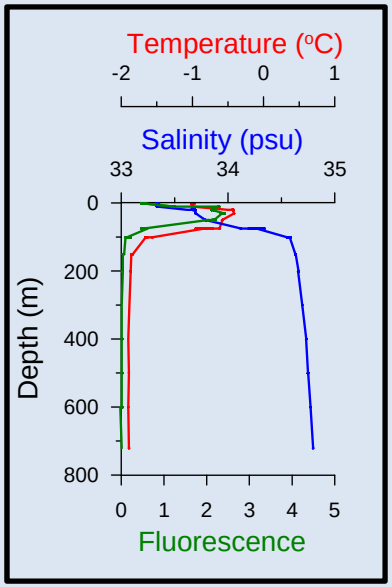
Relationship of physicochemical factors (MDS)




St.1
St.2
St.3
St.4
St.5
St.6
St.7
St.8
St.9
St.10
Bacteroidetes
Gammaproteobacteria
Alphaproteobacteria
Betaproteobacteria
Deltaproteobacteria
SAR406
Verrucomicrobia
Others

Physicochemical properties (St.7)

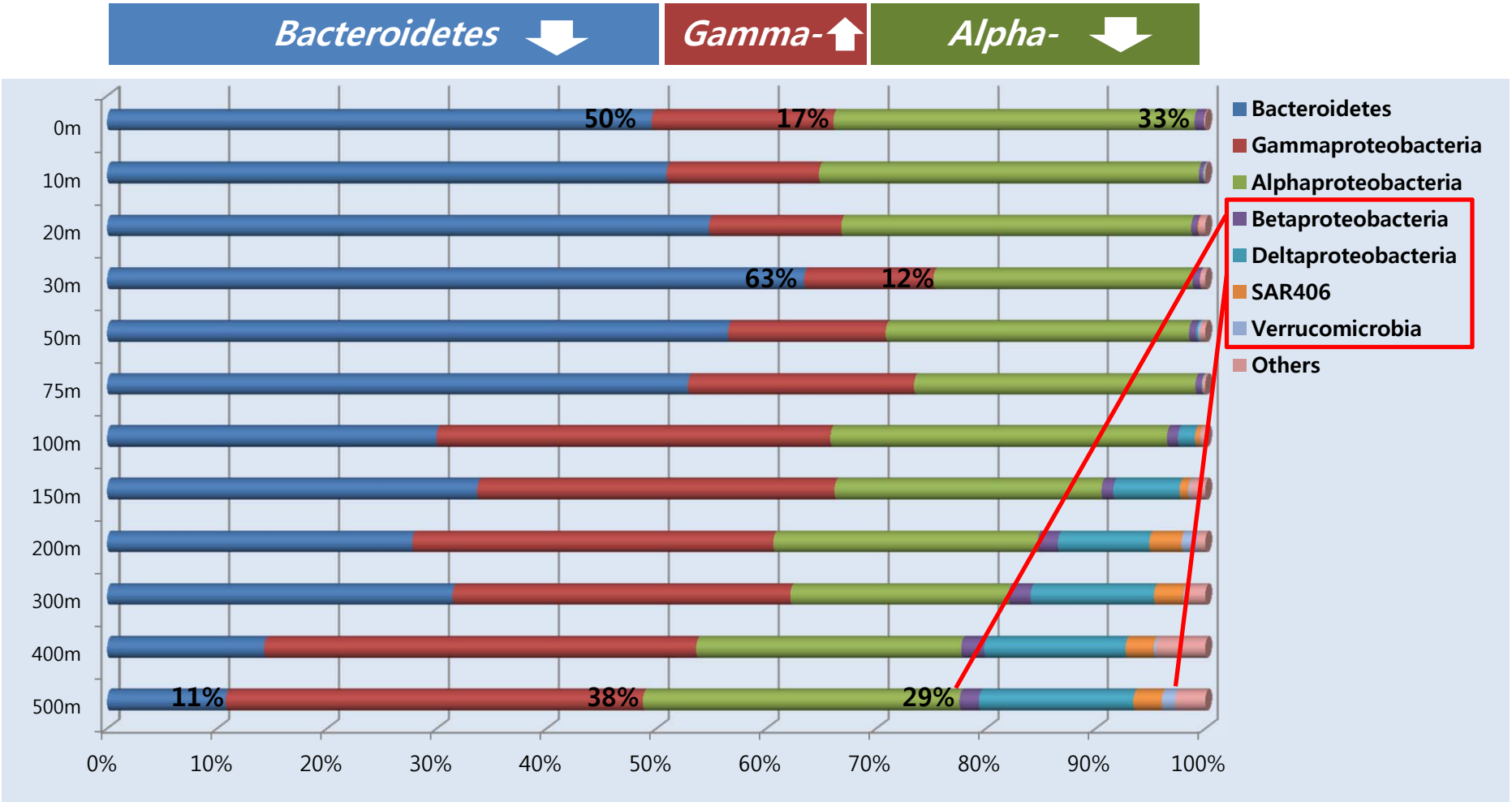
Depth (m)	PO_4^{3-} (μM)	NO_2^- (μM)	NO_3^- (μM)	NH_4^+ (μM)	Si (μM)	Nitrogen (μg/L)	Carbon (μg/L)	C/N ratio
0	0.4	0.3	10.5	1.7	21.6	45.5	172.1	4.4
10	0.3	0.3	10.3	0.0	22.2	44.0	262.2	6.9
20	0.5	0.3	10.3	0.7	23.0	35.3	292.2	9.7
30	0.7	0.3	10.9	0.4	25.9	46.6	94.6	2.4
50	0.7	0.3	13.5	0.3	30.7	34.2	203.8	7.0
75	1.0	0.3	15.6	0.8	37.4	-	-	-
100	1.9	0.2	24.5	0.3	63.3	88.4	466.7	6.2
150	2.0	0.3	29.8	nd	67.2	27.8	84.0	3.5
200	2.0	0.2	24.1	nd	68.4	25.3	124.4	5.7
300	2.0	0.2	30.1	nd	68.6	-	-	-
400	2.1	0.2	29.4	nd	67.8	-	-	-
500	2.1	0.2	29.3	nd	69.2	-	-	-



Summary of sequences and diversity indices (St.7)

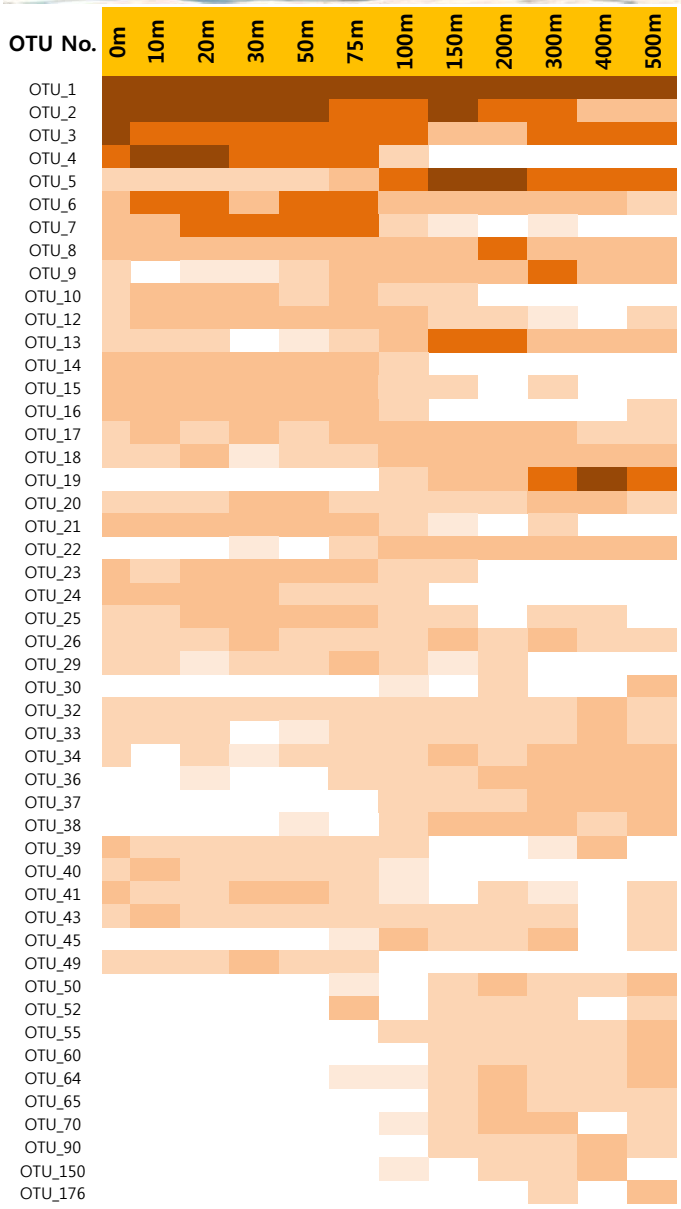
Depth (m)	16S rDNA sequences			Diversity indices				
	Total reads	No. of bacterial reads	No. of OTUs	Richness		Evenness		Coverage
				Chao	ACE	Shannon	Simson	
0	912	912	91	131	152	3.14	0.08	0.96
10	867	864	71	167	278	3.14	0.08	0.95
20	1183	1179	70	127	171	3.21	0.07	0.97
30	1037	1037	84	142	147	3.26	0.06	0.96
50	1220	1219	88	159	190	3.34	0.06	0.97
75	1467	1462	94	205	234	3.56	0.05	0.96
100	1294	1291	88	384	594	3.67	0.07	0.92
150	1113	1110	102	232	247	3.45	0.07	0.94
200	649	648	127	257	268	3.46	0.06	0.92
300	1007	1005	189	176	182	3.67	0.05	0.95
400	431	427	138	104	105	3.53	0.05	0.94
500	735	732	98	193	231	3.65	0.06	0.93
Total	**11,915**	**11,886**	**1,240**					

Bacterial communities at St.7 (Phylum level)



Others: 4P001579_p, Acidobacteria, Actinobacteria, AD3, ANW, Armatimonadetes, Bacteria_uc, BRC1, Caldithrix_p, CD12, Chlorobi, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, DSMV, Elusimicrobia, EU245879_p, Fibrobacteres, Firmicutes, Fusobacteria, Gemmatimonadetes, GN02, GN04, JS1, LD1, Lentisphaerae, Nitrospirae, NKB19, OD1, OP1, OP11, OP3, OP8, Planctomycetes, Poribacteria_p, Epsilonproteobacteria, SM2F11, Spirochaetes, SR1, Tenericutes, Thermobaculum_p, TM6, TM7, WS1, WS3

Abundant OTUs at St.7

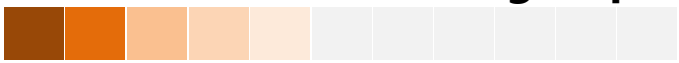


▣ Total 49 OTUs (>1%)

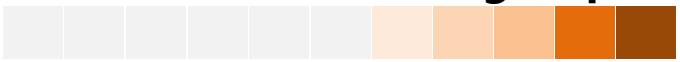
▣ 4 Groups of abundant OTUs

I. OTUs remained at the constant level regardless of depth

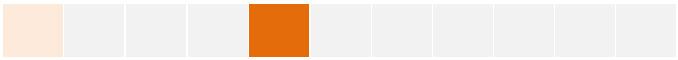
II. OTUs decreased along depth



III. OTUs increased along depth

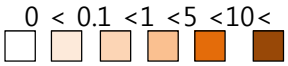


IV. Unclear or random pattern



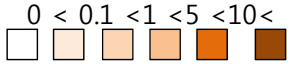
Characteristic abundant OTUs (I)

OTU No.	Depth												Taxonomy					
	0m	10m	20m	30m	50m	75m	100m	150m	200m	300m	400m	500m	Phylum	Class	Order	Family	Genus	Species
OTU_1	17.1	19.7	17.4	14.2	13.5	12.1	19.7	17	15.4	11.4	11	16	Proteobacteria	Alpha-	SAR11	SAR11-1_f	EU800386_g	EU800040_s
OTU_3	10.4	5.8	5.9	6.8	6.8	9.6	8.4	4.4	4.3	5.8	9.6	9.2		Gamma-	Arenicella_o	AM402959_f	AM402959_g	AM402959_s
OTU_17	0.4	1.2	0.8	1.3	1	1.9	1.1	4	3.4	2.5	0.9	0.8	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	DQ395867_g	DQ395867_s
OTU_20	0.8	0.8	0.8	1.1	1.8	0.8	0.9	0.7	0.8	1.1	1.4	0.4			Flavobacteriales	Flavobacteriaceae	AY794203_g	AY794203_s



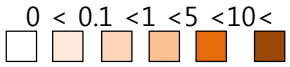
Characteristic abundant OTUs (II)

OTU No.	Depth												Taxonomy					
	0m	10m	20m	30m	50m	75m	100m	150m	200m	300m	400m	500m	Phylum	Class	Order	Family	Genus	Species
OTU_2	16.9	13.7	11.8	12.3	13.5	8.3	5.6	11.5	8.8	8.1	3	2.2	Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae	Polaribacter	Polaribacter irgensii
OTU_4	8.6	11.2	11.2	9.6	9.2	6	0.8	0	0	0	0	0				Flavobacteriaceae	Polaribacter	Polaribacter_uc
OTU_7	2.4	4.3	6.2	7.7	6.6	7	0.9	0.1	0	0.1	0	0				Cryomorphaceae	AY697869_g	DQ372845_s
OTU_10	1	1.3	1.2	1.3	0.5	2.2	0.2	0.3	0	0	0	0				Brumimicrobiaceae	Brumimicrobium	Brumimicrobium_uc
OTU_12	0.7	2.5	3.5	3.1	2.5	3.3	1	1	0.5	0.1	0	0.4				Flavobacteriaceae	ABVV_g	ABVV_s
OTU_14	3.9	1.9	3.4	4.1	2.1	1.8	0.9	0	0	0	0	0				Flavobacteriaceae	Ulvibacter	AY794084_s
OTU_15	3.4	2.2	2.5	3.7	2.4	3.4	0.4	0.3	0	0.6	0	0				EU934238_f	4P000929_g	4P000929_s
OTU_23	1.9	0.8	2.6	4.5	3	2.4	0.2	0.3	0	0	0	0				Flavobacteriaceae	Kordia	Kordia_uc
OTU_24	1.4	1.7	1.2	1.3	0.5	1	0.3	0	0	0	0	0				Flavobacteriaceae	ABVV_g	AM279169_s
OTU_29	0.1	0.3	0.1	0.3	1	1.2	0.4	0.1	0.2	0	0	0				Cryomorphaceae	DQ009083_g	EU005728_s
OTU_41	1.1	0.7	0.7	1.3	1.4	0.4	0.1	0	0.2	0.1	0	0.1				Cryomorphaceae	DQ395037_g	4P000056_s
OTU_6	4.3	6.4	5.8	4.1	5.4	6.6	4.6	2.2	2.8	1.2	1.6	1	Proteobacteria	Alpha-	Rhodobacterales	Rhodobacteraceae	Tropicibacter	EU799811_s
OTU_16	3.4	2.7	3.6	2.1	3.9	1.8	0.2	0	0	0	0	0.1				Rhodobacteraceae	Loktanella	EU795106_s
OTU_21	1.8	2.2	2	1.2	2.2	2	0.3	0.1	0	0.2	0	0				Rhodobacteraceae	Sulfitobacter	EU795102_s
OTU_40	0.4	1	0.5	0.5	0.9	0.9	0.1	0	0	0	0	0		Gamma-	Chromatiales	Chromatiaceae	EU652543_g	EU652543_g_uc



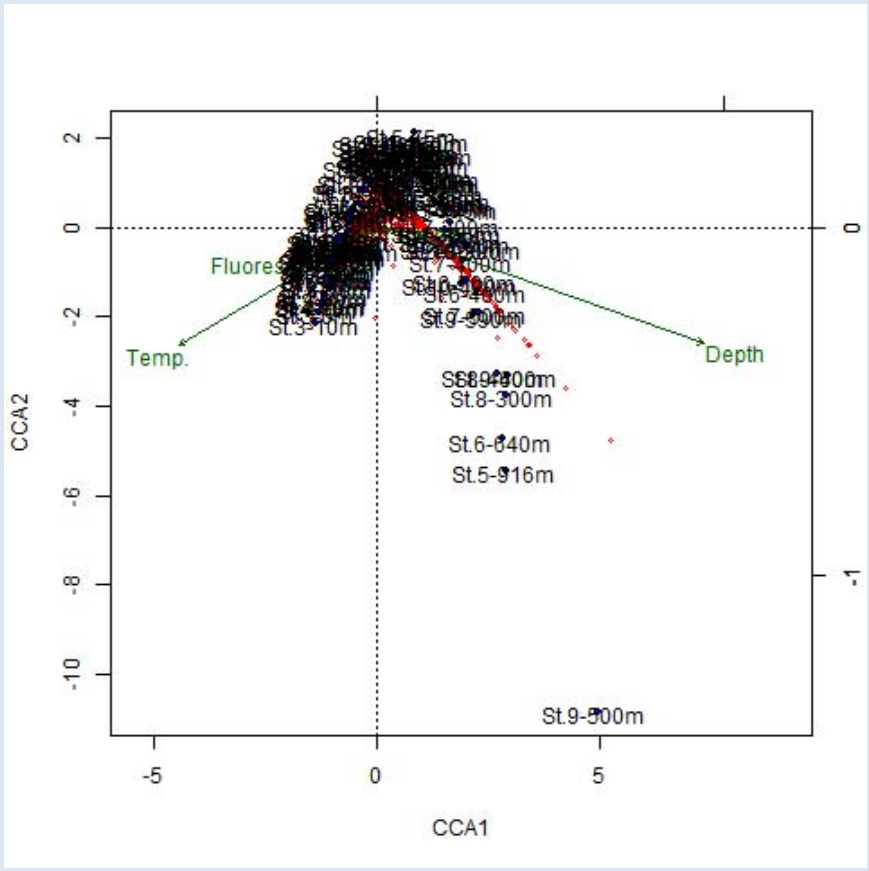
Characteristic abundant OTUs (III)

OTU No.	Depth												Taxonomy					
	0m	10m	20m	30m	50m	75m	100m	150m	200m	300m	400m	500m	Phylum	Class	Order	Family	Genus	Species
OTU_9	0.5	0	0.1	0.1	0.5	1.3	3.9	2.4	2.9	6.6	2.8	2.2	Bacteroidetes	Flavobacteria	Flavobacteriales	Cryomorphaceae	DQ513081_g	EU570869_s
OTU_13	0.1	0.5	0.3	0	0.1	0.6	3.6	5.5	5.7	2.8	2.3	1.5			Flavobacteriales	Flavobacteriaceae	EF575148_g	AM279176_s
OTU_90	0	0	0	0	0	0	0	0.3	0.5	0.3	2.3	0.1	Chloroflexi	SAR202	AY534095_o	AY534095_f	EU491325_g	EU491325_g_uc
OTU_34	0.4	0	0.3	0.1	0.2	0.1	0.7	1.3	0.2	1.5	2.1	1.2	Proteobacteria	Alpha-	SAR11	SAR11-2_f	AY033299_g	AF353223_s
OTU_50	0	0	0	0	0	0.1	0	0.5	1.5	0.8	0.5	1.1			Rhodobacterales	Rhodobacteraceae	Planktomarina	DQ396045_s
OTU_55	0	0	0	0	0	0	0.3	0.5	0.5	0.6	0.7	1.2			Rhodobacterales	Rhodobacteraceae	Pseudoruegeria	EU259801_s
OTU_70	0	0	0	0	0	0	0.1	0.4	1.2	1.2	0	1		Beta-	Nitrosomonadales	Nitrosomonadaceae	Nitrosospira	AJ630750_s
OTU_19	0	0	0	0	0	0	0.8	3	4.2	7.7	10.5	9.2		Delta-	SAR324_o	SAR324_f	SAR324_g	DQ396005_s
OTU_38	0	0	0	0	0.1	0	0.4	2.3	1.9	1.3	0.2	1.4			SAR324_o	SAR324_f	SAR324_g	EU795215_s
OTU_60	0	0	0	0	0	0	0	0.2	0.9	0.5	0.9	1.9			Nitrospinaceae_o	Nitrospinaceae	DQ009478_g	DQ396124_s
OTU_5	0.3	0.8	0.6	0.6	0.5	1.9	9.7	11.6	10.2	9.4	9.8	7.8		Gamma-	Ruthia_o	Ruthia_f	Ruthia	AB193913_s
OTU_8	1.5	2.5	2	1.4	2.5	2.9	4.5	3.8	7.3	3.8	3	4.5			Alteromonadales	SAR92	EF574016_g	AY386340_s
OTU_22	0	0	0	0.1	0	0.2	2.2	3.6	2.3	1.8	3.5	1.6			SAR86	AY552545_f	AY552545_g	AF001651_s
OTU_36	0	0	0.1	0	0	0.2	0.4	1	1.1	1.2	1.2	1.1			Alteromonadales	DQ009153_f	DQ009153_g	DQ009153_g_uc
OTU_37	0	0	0	0	0	0	0.4	1	0.5	2.7	2.8	2			SAR86	DQ396070_f	DQ906753_g	DQ906753_s
OTU_64	0	0	0	0	0	0.1	0.1	0.5	1.2	0.6	0.7	1.6			Xanthomonadales	FJ228294_f	EU652518_g	EU035842_s
OTU_18	0.5	0.3	1	0.1	0.2	0.5	2.4	1.2	2.2	1.8	2.1	1.4			SAR86	AY552545_f	AY552545_g	AY552545_s
OTU_65	0	0	0	0	0	0	0	0.5	1.5	0.3	0.7	0.7	SAR406	SAR406_c	SAR406_o	SAR406_f	SAR406_g	AB292141_s

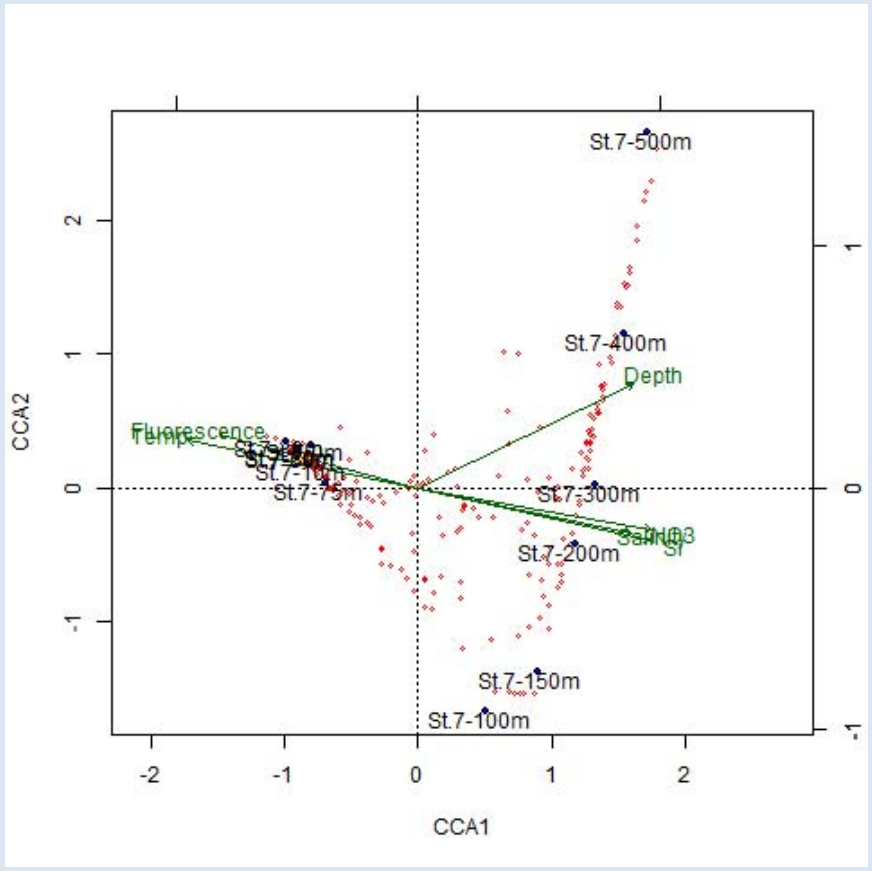


Correlation of bacterial community with physicochemical factors

Correlation



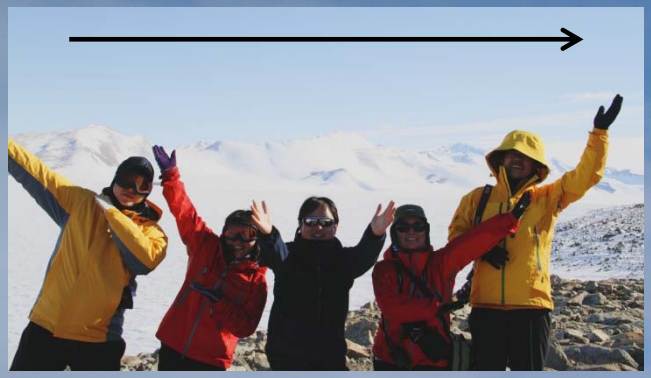
All samples



St.7

Summary

- The physicochemical factors in the mixing layer and thermocline varied among stations while little variation was observed in the mesopelagic zone.
- Vertical stratification of bacterial communities in the water column was remarkably distinguishable.
- Bacterial community was significantly affected by physicochemical factors.
- As a preliminary study, these findings will help understanding overall distribution of marine bacteria in the Ross Sea and further efforts to link between bacterial community structure and their implications for microbial ecology is necessary.



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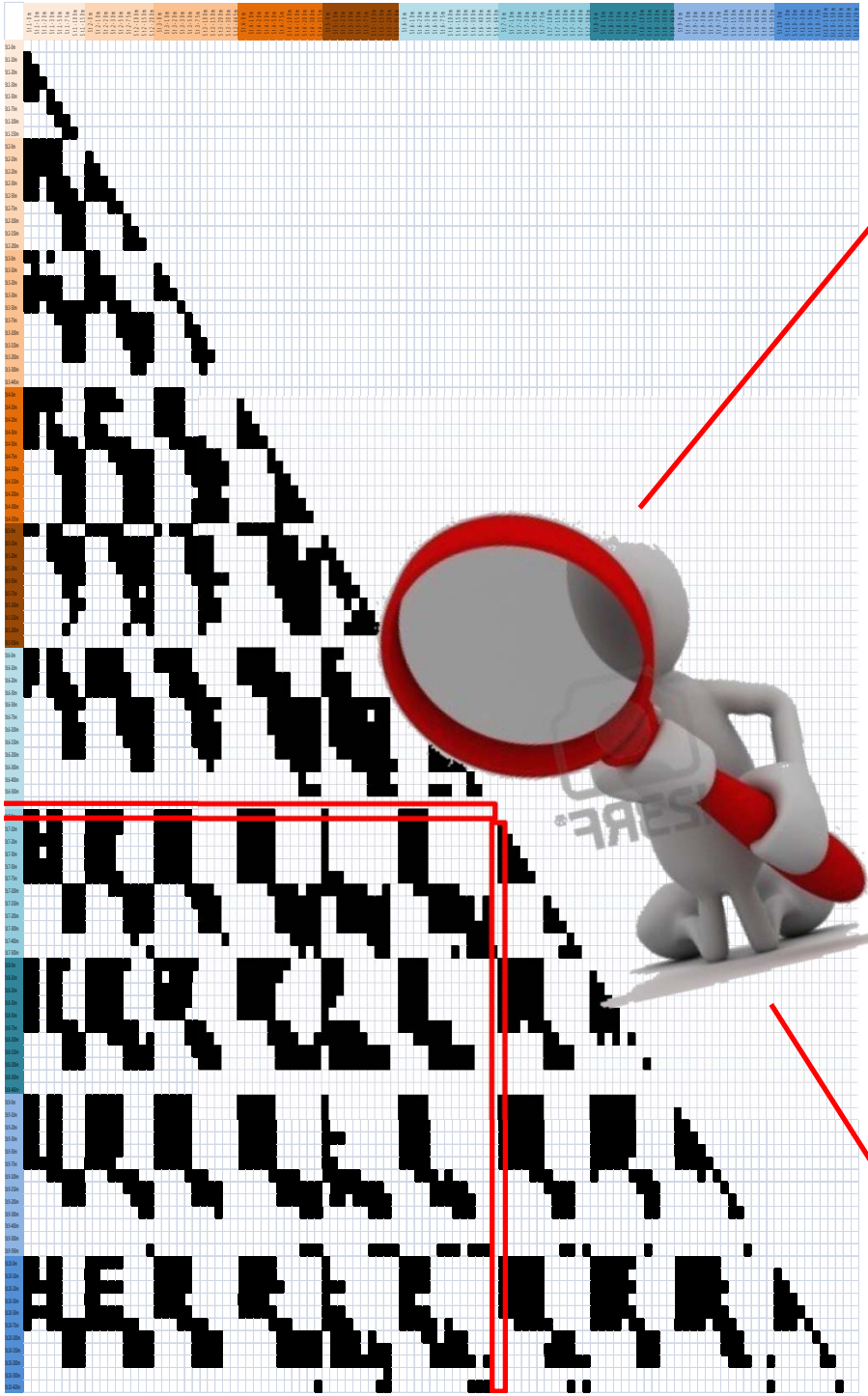
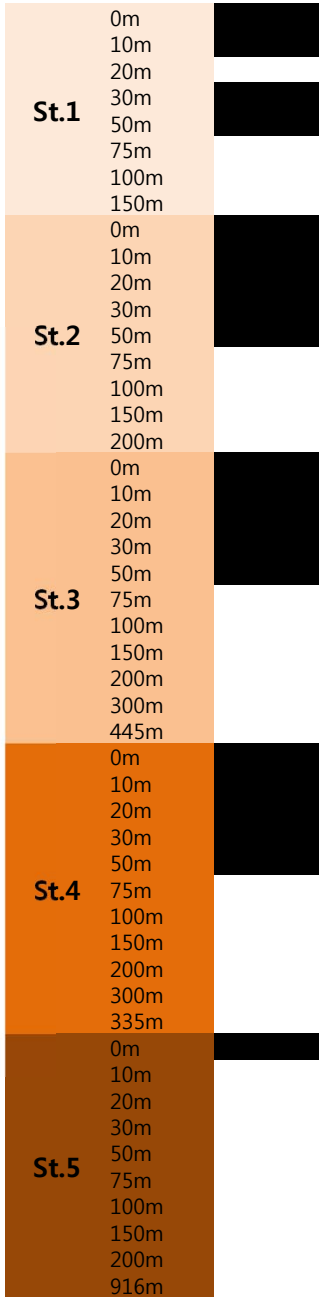
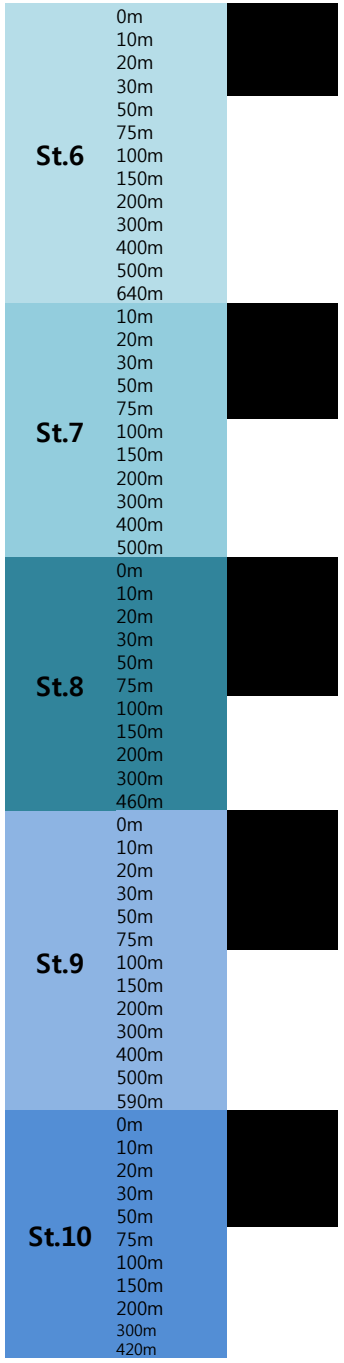
Thank you~^^!!
(ymlee@kopri.re.kr)

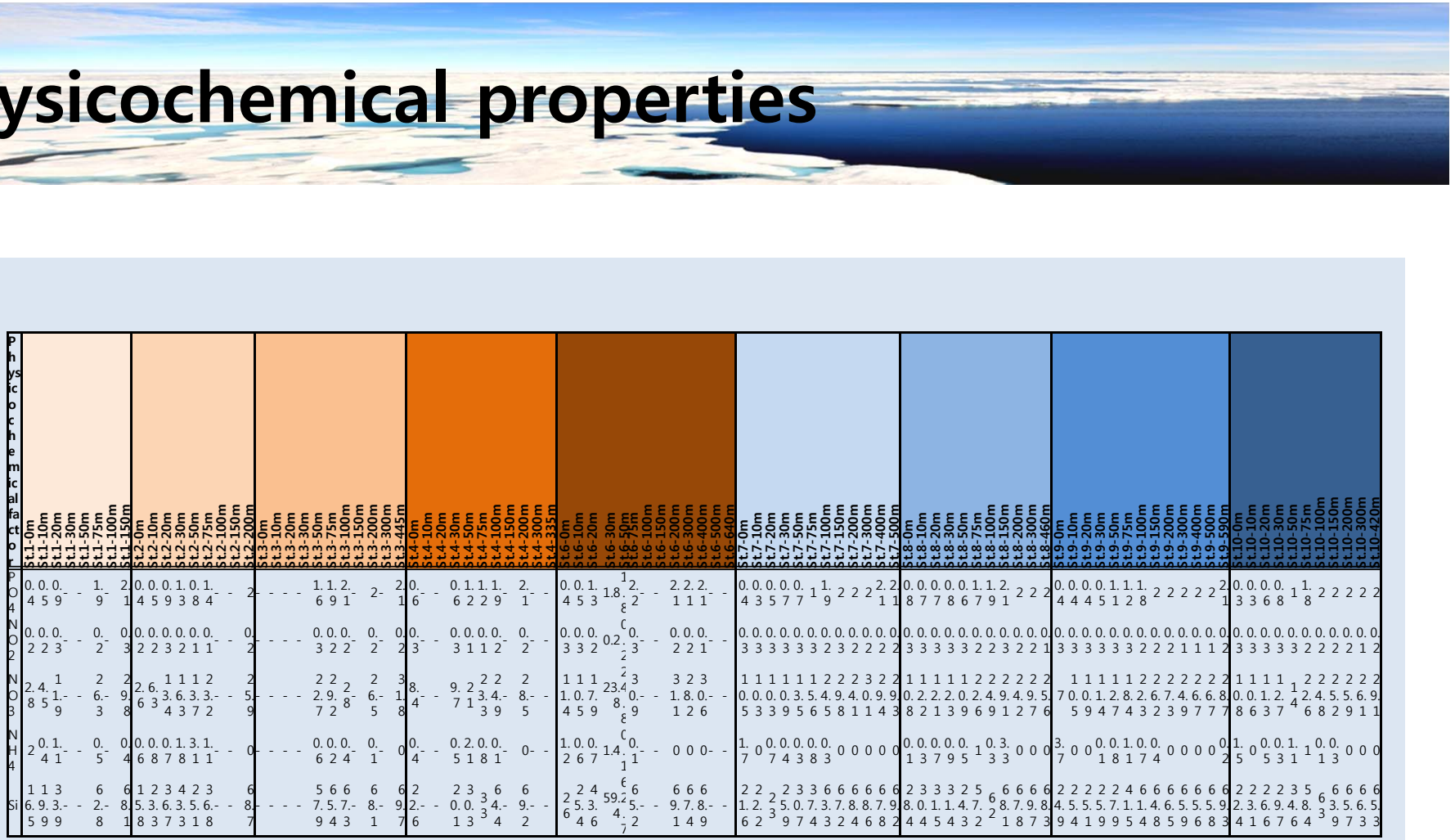
Similarity comparison with St.7 – 0m

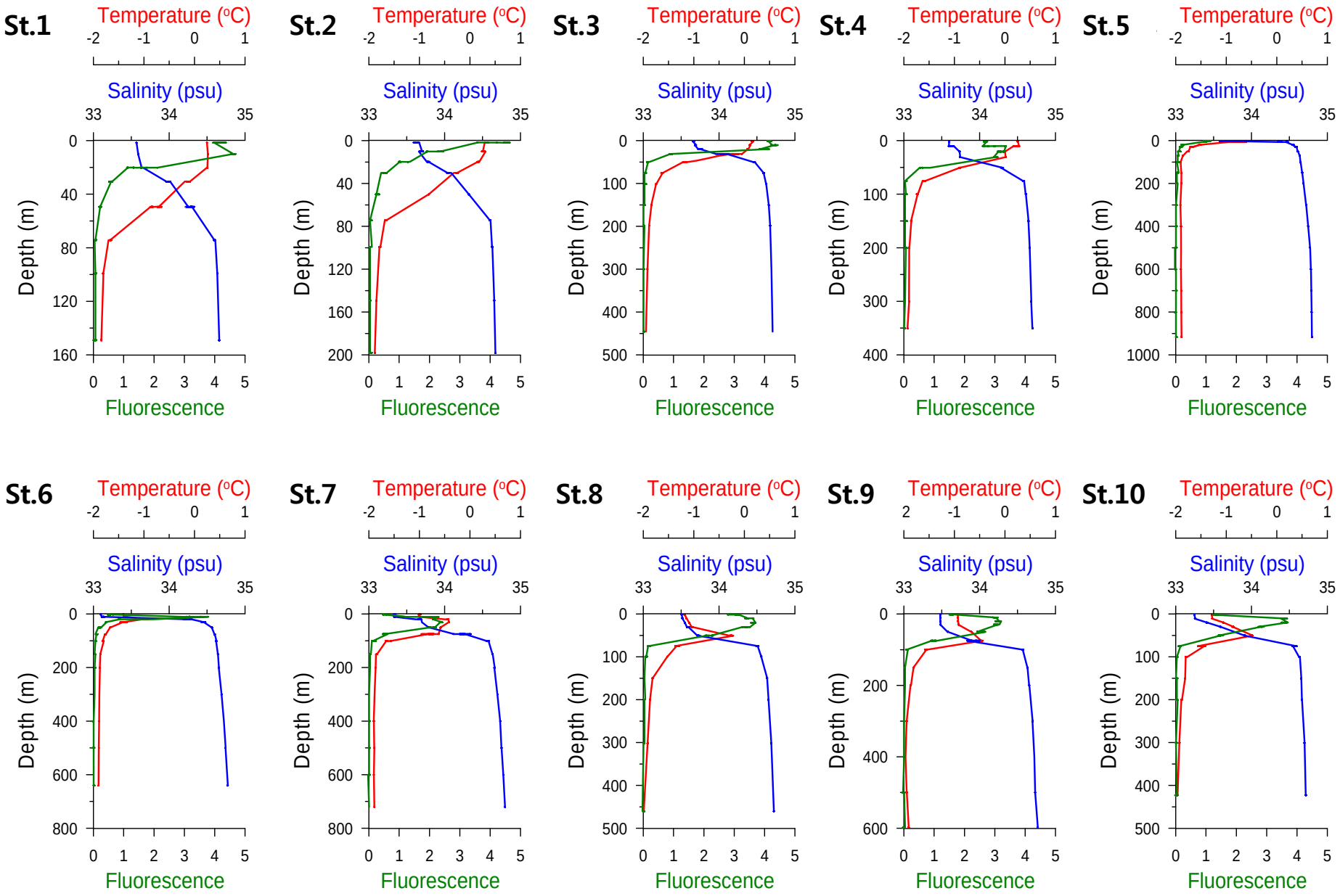






Physicochemical properties




St.1
St.2
St.3
St.4
St.5
St.6
St.7
St.8
St.9
St.10
Temperature (°C)
Salinity (psu)
Depth (m)
Fluorescence

Rarefaction curve (St.7)